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UNDERSTANDING BIMATURISM:
THE INFLUENCE OF SOCIAL CONDITIONS, ENERGY INTAKE, AND
ENDOCRINOLOGICAL STATUS ON FLANGE DEVELOPMENT
IN BORNEAN ORANGUTANS (*Pongo pygmaeus wurmbii*)

by
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ABSTRACT OF THE DISSERTATION

Understanding Bimaturism:

The Influence of Social Conditions, Energy Intake, and Endocrinological Status
on Flange Development in Bornean Orangutans (*Pongo pygmaeus wurmbii*)

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Bimaturism in orangutans is defined as two different secondary sexual characteristics of a mature male, unflanged and flanged. Bimaturism has been hypothesized to be condition-dependent, which includes how social interactions, energetic status, and physiological status regulate flange development. Yet we still have little understanding of what triggers the development of secondary sexual characteristics in adult males and why there is such of variation in the timing of flanging. This study examined relationships between social behavior, energy intake, and endocrinological hormones to flange development in Bornean orangutans (*Pongo pygmaeus wurmbii*). By quantifying the orangutans' flange size from three different

male's developmental stages (unflanged, developing flange, and flanged), I observed that 1) Bimaturism in orangutans is provoked by social interactions. Social interactions were related to the size of the orangutans' flange such that males with smaller flanges tended to have greater interactions. Moreover, the amount of social interactions was related to the cortisol production in males with greater flange size. 2) Bimaturism in orangutans is not nutrient-dependent. Total energy intake did not differ among developmental stages and by accounting body mass per Kg, flanged males tended to have the lowest energy intake compared to other stages. 3) Bimaturism in orangutans seems to be influenced by endocrinological status. High testosterone levels were correlated to the flange development, and elevated cortisol levels were highest in unflanged males and may have inhibited flange development.

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1. Chapter I. General Introduction

Bimaturism in wild orangutans has received a lot of attention, yet we know very little about the proximate mechanisms that lead bimaturism in orangutans. Bimaturism was first acknowledged by Maple (1980b) who stated that the presence of a flanged male could increase the stress hormone which suppressed the development growth in adult unflanged male orangutans. The presence of flanged male would initiate high intrasexual competition, which was hypothesized to produce indeterminate male growth (i.e., unflanged male) (Leigh, 1992; Leigh, 1995; Leigh & Shea, 1995). Since these earlier papers, these hypotheses were not tested until Utami-Atmoko et al. (2002) described two different male morphology in Sumatran orangutans i.e., 'fatted' and 'no-fatted' male in terms of the secondary sexual traits development; and the study found that the no-fatted male also seems to have similar opportunities to sire offspring. The research was then adapted for Bornean orangutans and similar results were observed (Banes et al., 2015).

The question focusing on how bimaturism could exist in orangutans remain open, specifically research on the differences in flange development throughout the male's different maturity stages. There are several proposed mechanisms that may lead to flange development in male orangutans. The first mechanism is that social interactions influence flange development, such that the development of flange depends on the occurrences of have more flanged males relative to unflanged males in the same area. Dunkel et al. (2013) found that delayed flange development is correlated with the number of flanged males and their dominance status. Dominancy by flanged

male led to these male monopolizing receptive females, but this also increased the degree of intrasexual competition (Mitra Setia et al., 2009; Utami-Atmoko, Singleton, et al., 2009). This resulted in subordinate adult males playing an alternative mating strategy by delaying their flange development in addition to other secondary sexual traits (Pradhan et al., 2012). However, there is still a lack of information on if rates of competition among flanged males influences arrested flange development in Bornean orangutans.

The second proposed mechanism is that flange development is determined by environmental variation in the existing food resource (i.e., condition-dependent). In a study on the relationship between energetic status and flange development, Emery-Thompson (2017b) argued that the nutritional-stressed condition would slow down reproductive maturation in males but may actually accelerate it in females. However, this hypothesis has yet to be tested in relationship to flange development. The third proposed mechanism is related to the orangutan's physiological status, which has attracted the most attention by researchers. The studies related to stress hormones and bimaturism supported Maple (1980a)'s hypothesis that undeveloped flanged males tend to have higher cortisol concentrations compared to the flanged males – that is, they are more stressed (Maggioncalda et al., 2002; Marty et al., 2015). In addition, studies of androgen hormones found that flange development is positively correlated with testosterone levels (Emery-Thompson et al., 2012; Maggioncalda et al., 1999; Marty et al., 2015; Muller, 2017). However, all of these studies thus far analyzed a small sample

size for the number of individuals from each developmental stage and had low predictive power, leading to uncertainty in their conclusions and interpretations.

The objective of this thesis was to examine those three mechanisms (i.e., social interactions, nutrient dependency, and physiological status) on the influence of bimaturism in orangutans. By analyzing a greater sample size from three different male developmental stages (i.e., unflanged, developing flange, and flanged males) this study brings us closer to understand the proximate mechanism of flange development and bimaturism in Bornean orangutans.

1.1. Structure of the Dissertation

This dissertation is structured into 5 chapters: the first chapter is a general introduction, three main research chapters, and the last chapter summarizes the findings. In the first chapter I introduced the background information related to ecology of orangutans and bimaturism. In the bimaturism section, I summarize the developmental stages in orangutans, which focuses on the definition of adult male's stages (i.e., unflanged, developing flange, and flanged male). I briefly discussed the differences in flange size for each male's stages, which is important for this dissertation. At the end of the introduction chapter, I described the research design, which includes the information on the research site and sample distribution.

Three main chapters (chapter 2 to 4) were constructed as manuscripts that I intend to submit for publication. However, some methodologies and results overlapped between chapters; for example, the result of flange size measurements was used in all chapters' discussion. In chapter two, I examined the influence of social interactions on

flange size differentiation among the male's developmental stages. The hypotheses for this chapter was that the number of flanged males at the same location does not lead to differences in social interactions among males of the different developmental stages. In addition, I predicted that increasing social interactions would not trigger cortisol production and hypothesized that elevated stress hormone would be correlated with flange development. I predicted nonviolent and nonthreatening environments could reduce flange development disruption in matured male orangutans.

In chapter three, I discussed nutrient-dependency in influencing orangutan flange development. I examined the interaction between energetic status and flange size in the three different male stages. The hypothesis proposed in this chapter is that due to regular food supplies, orangutans would be in a positive energy balance, which is indicated from the absence of ketone bodies in urine samples. At the same time, energy intake and urinary C-peptide among male developmental stages would be similar and does not affect flange development. Chapter four brought a different overview to the discussion of the influence of orangutan physiological status on the flange development. I examined the profiles of urinary cortisol and testosterone hormones from different male developmental stages (i.e., unflanged, developing flange, and flanged) that live both in the wild and captivity. In line with previous findings, I hypothesized that there would be no difference in cortisol levels among male developmental stages in both wild and captive populations, due to the elimination of stress in captivity. Moreover, I hypothesized testosterone levels among different male developmental stages would be varied. I predicted high testosterone levels would be found in the developing flange

males, and the lowest level would be in unflanged males. Finally, I conclude the dissertation with chapter 5, which summarized the results from previous chapters and synthesized them as the final conclusion.

1.2. Introduction

1.2.1. Bio-Ecology Orangutans

1.2.1.1. Species and Distribution

In the family of Hominidae, there are two distinctive sub-families that are known as Ponginae and Homininae. The subfamily Ponginae only has one genus (i.e., *Pongo* (orangutans)), while Homininae contains two non-human primate's genera i.e.: *Gorilla* (Gorillas) and *Pan* (Chimpanzees) and one genus of *Homo* (Human). Gorillas and chimpanzees can be found in Africa, whereas orangutans are distributing only in the South-east Asia (Groves, 2016; Roos et al., 2014).

From the early Pleistocene to the Holocene era, orangutans were widely distributed in the mainland of South-east Asia from Southern China, Vietnam, Laos, Thailand, Cambodia, Borneo, Sumatra, and Java (Spehar et al., 2018). Nowadays, the extant orangutan populations are limited to two islands, Sumatra and Borneo (Delgado & van schaik, 2000; Goossens et al., 2009; Spehar et al., 2018). Sumatran populations can be found only in the northern part of the island from the Aceh district to North Sumatra, whereas Bornean populations are dispersed across the entire island (Goossens et al., 2009; Utami-Atmoko et al., 2017). For decades, Sumatran and Bornean orangutans were classified as one species, known as *Pongo pygmaeus* (Groves, 1971). However, based on the differentiation of their morphology, cytogenetic from

chromosome 2, and molecular data specifically mitochondria DNA (*mt*-DNA), the two different populations were categorized into two different subspecies known as *Pongo pygmaeus abelii* for the Sumatran population and *Pongo pygmaeus pygmaeus* for the Bornean population (Mackinnon, 1974b; Warren et al., 2000; Xu & Anarson, 1996; Zhang et al., 2001). Based on several publications, the two different orangutan species were later announced as *Pongo abelii* for Sumatran and *Pongo pygmaeus* for Bornean population (Goossens et al., 2009; Singleton et al., 2004). Due to high polymorphisms of *mt*-DNA of Bornean populations, three subspecies were classified as: *P.p. pygmaeus* were limited to north-west of Borneo island, *P.p. wurmbii* that mainly inhabited from the west-central Kalimantan, and *P.p. morio* which were found in the East Kalimantan and Serawak-Malaysia (Goossens et al., 2009; Singleton et al., 2004; Warren et al., 2000). Recently, a new orangutan species has been discovered in North Sumatra known as the *Pongo tapanuliensis* (Nater et al., 2017). The current wild orangutan distribution and reintroduction orangutan's population can be seen in the Figure 1.1.

1.2.1.2. Population and Conservation Status

According to the orangutan Population and Habitat Viability Analysis (PHVA) 2016 report, there are 14,290 wild orangutans living in Sumatra; the population mainly consists of the *Pongo abelii* species (Utami-Atmoko et al., 2017) and no more than 800 *Pongo tapanuliensis* orangutans are living in the Batang Toru forest (Nowak et al., 2017). The total estimated population of Bornean orangutans is around 57,350 orangutans, which were divided into three sub-species such as *Pongo pygmaeus wurmbii* with 38,200 orangutans, and *Pongo pygmaeus morio* known as the second largest population

with 14,630 orangutans, and the *Pongo pygmaeus pygmaeus* with a population of 4,520 orangutans (Utami-Atmoko et al., 2017). Since the rehabilitation and reintroduction of orangutans was initiated in 1960s, approximately 180 *Pongo abelii* orangutans were released into Sumatran forests and more than 800 *Pongo pygmaeus* orangutans were sent back to the Borneo forests (Russon, 2009; Utami-Atmoko et al., 2017).

Due to the continued decrease in orangutan populations, the International Union for Conservation of Nature and Natural Resources (IUCN) declared all three orangutan species into the Critical Endangered (CR) conservation status (Ancrenaz et al., 2016; Nowak et al., 2017; Singleton et al., 2017). In just fifteen years since 1999, twenty-five percent of Bornean orangutan populations have declined due to habitat loss from the high demand of agriculture, human-orangutan conflicts, anthropogenic activities, and habitat fragmentation (Santika et al., 2017). Another 65 – 80% of the decline in orangutan populations in Borneo was caused by high levels of selective forest logging between 1999-2015. The remaining population that reside in the logged forest have also declined by about 75% due to high levels of forest conversion for plantations purposes (e.g., oil palm) (Voigt et al., 2018).

The current Sumatran orangutan population census has now reported increases compared to the previous assessment (Singleton et al., 2004; Wich et al., 2016). However, this increase in population size was caused by the different survey methods used, which used less survey coverage area and a lower altitude limit in the model (Wich et al., 2016). Similar to the declining orangutan populations in Borneo, the population of the Sumatran orangutans are decreasing because of high forest fragmentation and

habitat loss caused by plantation expansion (Nowak et al., 2017; Singleton et al., 2017; Wich et al., 2016).

1.2.2. Life History

Following Wich et al. (2009), which compared the life histories among the members of the Family Hominidae, orangutans have a slower life history than chimpanzees and gorillas. The average of age at first reproduction in orangutans is 14.5 years old (van Noordwijk et al., 2018; Wich et al., 2009); whereas gorillas could produce offspring at 10.1 years old and chimpanzees at 13.5 years old (Wich et al., 2009). The interbirth interval (IBI) between Sumatran and Bornean orangutans was identified at average 7.6 years (van Noordwijk et al., 2018). The new finding differed from the number reported by Kuze et al. (2012) and Wich et al. (2004), who both reported the IBI of wild orangutans to be between 8.08 – 9.3 years. Because orangutans have a very slow life span compared to others ape, their longevity could reach 50 years old or more. However, this number remains unclear due to inefficient data (van Noordwijk et al., 2018).

The life span of rehabilitant orangutans tend to be faster than wild populations (Russon, 2009). The average age of reproduction for the rehabilitant orangutans was 11.6 years old which was earlier than wild populations even when compared to the orangutans that live in the zoo/captivity (16 years) (Kuze et al., 2012). Similar trends were also reported on the interval of birth with rehabilitant orangutans that will give birth in an average of 6.6 years (Kuze et al., 2012). This proved that energetic status was

related to the reproductive success in orangutans, meaning that positive energy would cause earlier maturity and faster birth interval (Knott, 2001).

1.3. Bimaturism in Orangutans

Bimaturism is the development of two different characteristics of mature individuals that are formed by sequential traits and stages that can be reversible or irreversible (Jarman, 1983). In orangutans, bimaturism is found in adult males (i.e., capable of siring offspring) and is characterized by two different morphological forms; one morph is an adult male with flanges, a large throat pouch used for long-call vocalization, long hair, and increased body mass; and the second morph being an adult male that lacks the traits but is still capable of reproduction. Studies have shown that adult females were more willing to mate with flanged males than unflanged males. Orangutans are the only non-human primate in which forced copulations by unflanged males are regularly observed (Knott, 2009). Some studies have shown that flanged males sire more offspring compared to unflanged males, even though unflanged males sire a small percentage of offspring (Banes et al., 2015; Utami-Atmoko et al., 2002). Even though the flanged males are able to attract females by making a long-call vocalization that can travel for up to 1 km, the unflanged males, on the other hand, cannot make the vocalization (or prefer not) and instead use a sneaker strategy to acquire copulations (Delgado & van schaik, 2000; Mitra Setia & van Schaik, 2007; van Schaik et al., 2013). Unflanged males also tend to avoid conflicts with flanged males and during encounters they would quickly retreat. Most adult male-male contest aggression happens between flanged males (Utami-Atmoko, Singleton, et al., 2009). This therefore shows that the

two male morphs have also evolved to have different mating strategies. For example, the flanged male orangutan advertises his location to females and waits for them to come to him, while the unflanged male wanders around the forest searching for females and uses forced copulations to obtain mating.

1.3.1. Orangutans' Developmental Stages

The term of life stages refers to the patterns of development changes from birth to death. This includes the changing of morphology, biological and physiological function, as well as behavior that could vary among the individual and sexes. In orangutans, the growth and development is divided into four life stages i.e.: infant, juvenile, adolescent, and adult (Mackinnon, 1974a; Rijksen, 1978; van Noordwijk et al., 2018; Wich et al., 2009; Wich et al., 2004) (Table 1.1). Among adult male orangutans, it has been discovered that a bimaturism causes the two forms matured male orangutans (i.e.: unflanged and flanged) which they were capable to sire offspring (Banes et al., 2015; Utami-Atmoko et al., 2002). The life stages are the same between orangutans who lives in the forest and captivity. However, compared to the wild population, the captive orangutans tend to have an earlier maturity age and larger body weight (Kuze et al., 2012; Kuze et al., 2005).

The developmental stages of captive orangutans tend to be faster than the wild population (Kuze et al., 2012; Wich et al., 2009). The facial morphology changes of male orangutans were reported by Kuze et al. (2005) with dark coloration having a positive correlation with the orangutan age and a sign of maturity by the age of 10 years.

According to the face morphometric that was carried out in this research, we identified

more features in the adult male's developmental stage based on the differentiation of their secondary sexual characteristic (Figure 1.2). These differences are shown between the flanged male and the unflanged male, i.e.:

1.3.1.1. Unflanged Male

A matured unflanged male with the average body weight is 56.97 kg (range 40 – 82.5 kg, n= 9) is heavier than the wild body weight that was reported in wild orangutan which is 40.5 kg (Rayadin & Spehar, 2015). Because the lighter body weight had similar weight with the adult female, according to Harrison et al. (2010), it was difficult to identify the differences between both sexes. However, the distinction between them was that the unflanged male has developed a much longer and darker beard, short hairs on the face which is absent in the female, and a throat pouch which supports the orangutan vocalization was more developed. Not every unflanged male have these traits. The unflanged male that developed its throat could produce rough and weak calls compared to the flanged male's calls (Spillman et al., 2016). The other characteristics that distinguishes male's developmental stages are the flange or cheek-pad. Similar with the flange characteristic in young orangutans or some females, the small ridge of flange would be seen in unflanged male (Figure 1.2a). The flange is persistent for couple years and doesn't disappear as demonstrated by juvenile and female orangutans. This fact was reportedly related to their hormonal regulation and social organization (Dunkel et al., 2013; Utami-Atmoko et al., 2002).

Based on the face measurement of 15 unflanged males with ages distribution from 11 to 20 years old, we found the average size of flange from eyes landmark is 4.31 cm (range 2.92 – 6.46 cm).

1.3.1.2. Developing Flange Male

For some males, flanges continue to grow, and they simultaneously develop their secondary sexual characteristics; while other males might be just delayed for a shorter period. For example, Bornean orangutan males tend to have a shorter delay time in the flange development compared to the Sumatran species (Dunkel et al., 2013). The average of developing flange male's body weight is 58.95 kg (range 50 – 68.3 kg) which is likely similar to unflanged males. Moreover, developing flange males gain secondary characteristics such as a large throat poach, hairier face and flange, dark beard, long hairs all over their body that are shiny-dark in color, and this typically demonstrates a starting point to become a "prime" male, which has a bigger flange size compared to unflanged males (Figure 1.2b). Our face measurement from developing males with ages ranging from 12 – 18 years old found that the average size of flange is 5.16 cm (range 4.18 – 6.81 cm).

1.3.1.3. Flanged Male

The average body weight of flanged males in our sample is 77.18 kg (range 55 – 88.9 kg, n= 6) which less than wild orangutans (Harrison et al., 2010). This is twice the size of adult females and larger than the other two male's developmental stages. Similar to developing flanged males, these males have hair that grow all over their body that are long and some individuals in our sample have a hair length that can be as long as

two meters. Flanged males are also characterized by having a throat pouch that is well developed to support their great long call production. The flange itself is fully developed but the flange does not have a lot of hair on it. Based on the face measurement, flanged males have an average flange size of 7.93 cm (range 6.46 – 9.82 cm, Figure 1.2c).

1.4. Study Site

1.4.1. Rehabilitation Center

Built in 1999, Central Kalimantan Orangutan Reintroduction Program is the center that provide cares and rehabilitation for rescued and confiscated orangutans from degraded forests or orangutans that have a high risk of human-animal conflict. Located in Nyarumenteng arboretum, Palangkaraya, Central Kalimantan, Indonesia (2°02'23.4"S and 113°45'06.4"E), the Borneo Orangutan Survival Foundation (BOSF) has managed this center with almost 500 orangutans. Depending on the condition when orangutans have been rescued or confiscated, they will go to three different facilities.

1.4.1.1. Health Care and Quarantine

The first time orangutans arrive in the center, medical examination are conducted following regulations that are reviewed every two years by the orangutan veterinarian group (Commitante et al., 2016). If they pass the health examination check, orangutans are allowed to go to the rehabilitation facility. Unfortunately, for orangutans that are suspected to have one or more of the zoonosis pathogens such as tuberculosis, hepatitis virus, or malaria, the veterinarian places them in the quarantine facility for medical treatment.

1.4.1.2. Rehabilitation

The rehabilitation facility is for healthy orangutans but those that are lacking wild behavior abilities. Typically, confiscated and rescued orangutans are weak in the forest survival and do not have the ability to find and process forest food resources. Following guidelines from the IUCN (Beck et al., 2007), all orangutans at the center are encouraged to learn wild behaviors before they are ready for release to the forest. The center provides facilities for different 'levels' of orangutan wilderness training, i.e.: *i)* The forest school is a center that provides learning experience for infant to juvenile orangutans. In this facility, orangutans learn to process forest foods and their social experiences are encouraged. *ii)* Socialization group (or what is called NM2) is the group of cages that was designed for adult orangutans. Orangutans are placed in the singular cage, and they are provided with harder to process foods, and health and behavior is monitored regularly to identify which individuals can move up to the next facility. *iii)* Orangutan Island is the final stage before release typically. This enclosure provides a small but natural habitat for orangutans but with fewer food resources. Orangutans live like they would in the forest, and the center still regularly provides foods by supplementing what is available in the forest. *iv)* Monitoring group (or so called NM3). This is supposed to be the transition cage for orangutans that are ready to be released to the forest. Selected individuals that are placed in this facility will receive several general scans for health and behavior before being sent to the final stage of rehabilitation, which is the release to the forest.

1.4.1.3. Long-term Sanctuary.

This facility was built for special need orangutans that can't live in the natural forest with other orangutans and thus cannot be released. For example, injured orangutans that could not process food even though they passed the health exams; or old orangutans that might not survive in the forest.

1.4.2. Research Site

The research for this dissertation was carried out at two different facilities: NM2 and NM3. NM2 contains 10 group cages in which every group has 12 singular cages (Figure 1.3). In this group, each cage was separated by solid wall that was built from concrete or a metal-plate to avoid direct or physical contact between orangutans. Only the front and or rear side of the cage was built with a fence, allowing orangutans to see each other. NM3 was designed with 5 different group cages which contains 54 individual cages in total (Figure 1.4). Two group cages were built with connected cages that allowed orangutans to move and interact, but not touch each other.

1.4.3. Orangutans at The Center

At the beginning of this research in 2015, 484 orangutans were managed by the BOS foundation. According to the age's distribution, 3.51% orangutans were infant and similar distributions were found in the juvenile, adolescent, and adult (31.82%, 29.75%, and 34.92% respectively, Table 1.2).

According to the earlier maturation of captive male orangutans (Kuze et al., 2005), this research focused on individuals that were at least 12 years old. Eighty-nine matured male orangutans with ranging in ages of 12-26 years were used for baseline

data. Based on the male developmental stages, 25 matured males were randomly selected as the sample size (Table 1.3). Those males were monitored for their flange development, behavior, energetic status, and physiological status.

1.5. Tables and Figures

Table 1.1. Wild orangutan life stages (Mackinnon, 1974a; Rijksen, 1978; van Noordwijk et al., 2018; Wich et al., 2009; Wich et al., 2004).

Life Stage	Age	Physical characteristics	References
Infant	0 – 2.5 ^{a,b}	Weight between 2-6 kg, bright color around eyes, dark pigmentation on the stomach area, light hair color and standing out. Tiny flange ridge is present but disappears along with age.	^a (Mackinnon, 1974a; Rijksen, 1978; van Noordwijk et al., 2018; Wich et al., 2009; Wich et al., 2004)
Juvenile	2.5 – 10 ^{a,b}	Increase in weight until 15 kg, light color face pigmentation without white-circle around eyes, hairs still standing out and the color become darker especially for Bornean species.	^a (Mackinnon, 1974a; Rijksen, 1978; van Noordwijk et al., 2018; Wich et al., 2009; Wich et al., 2004)
Adolescent	10 – 14 ^{c,d}	Weight between 15-30 kg. Facial pigmentation becomes darker. Grow hair, light color for Sumatran and darker for Bornean. Male and female looks similar	^c van Noordwijk et al., 2018 ^d Wich et al., 2004
Adult	Female 15 + ^{c,d}	Weight up to 50 kg. Some females grew its beard and develop bumped	^c van Noordwijk et al., 2018 ^d Wich et al., 2004

		forehead. Mammary gland is fully developed following by larger nipple sizes.	
Male	14 + ^{c,d}	Weight up to 90 kg. There are two male's morphs that called bimaturism with completely different characteristics (see section 2 for more detail explanation).	^c van Noordwijk et al., 2018 ^d Wich et al., 2004

Table 1.2. Distribution of orangutans in the Nyarumenteng rehabilitation center based on their developmental stages.

Developmental Stages	Age (year)	Sex	Number of Orangutans
Infant	0 - 2	Male	11
		Female	6
Juvenile	3 - 10	Male	76
		Female	78
Adolescent	11 - 14	Male	69
		Female	75
Adult	14 +	Male	92
		Female	77

Table 1.3. The distribution of male orangutans that used as sample size in the study.

No	Orangutan	Developmental Stage	Cage Group	Age (Yrs)	Body Mass (Kg)
1	Casper	Unflanged	NM 2	14	82.5
2	Ibut	Unflanged	NM 3	12	52.5
3	Kevin	Unflanged	NM 3	15	49.6
4	Lomon	Unflanged	NM 3	13	49
5	Lopus	Unflanged	NM 2	16	56.97
6	Nyun nyun	Unflanged	NM 2	13	52
7	Paiman	Unflanged	NM 2	13	40
8	Rambo	Unflanged	NM 3	13	53.6
9	Stuart	Unflanged	NM 2	13	47
10	Edoy	Developing Flange	NM 2	17	58.95
11	Joni	Developing Flange	NM 2	14	58.95
12	Juky	Developing Flange	NM 2	14	65
13	Kisar	Developing Flange	NM 3	15	64
14	Marwoto	Developing Flange	NM 3	18	68.3
15	Palingkau	Developing Flange	NM 2	13	58.95
16	Soni	Developing Flange	NM 3	17	50
17	Bento	Flanged	NM 3	24	87.4
18	Charles	Flanged	NM 2	20	75
19	Danny	Flanged	NM 3	20	71.8
20	Gayo	Flanged	NM 2	19	75
21	Inou	Flanged	NM 3	12	77.18
22	Lucky Thai	Flanged	NM 2	19	77.18
23	Sabun	Flanged	NM 3	14	88.9
24	Saswoko	Flanged	NM 3	19	55
25	Uncui	Flanged	NM 2	14	85

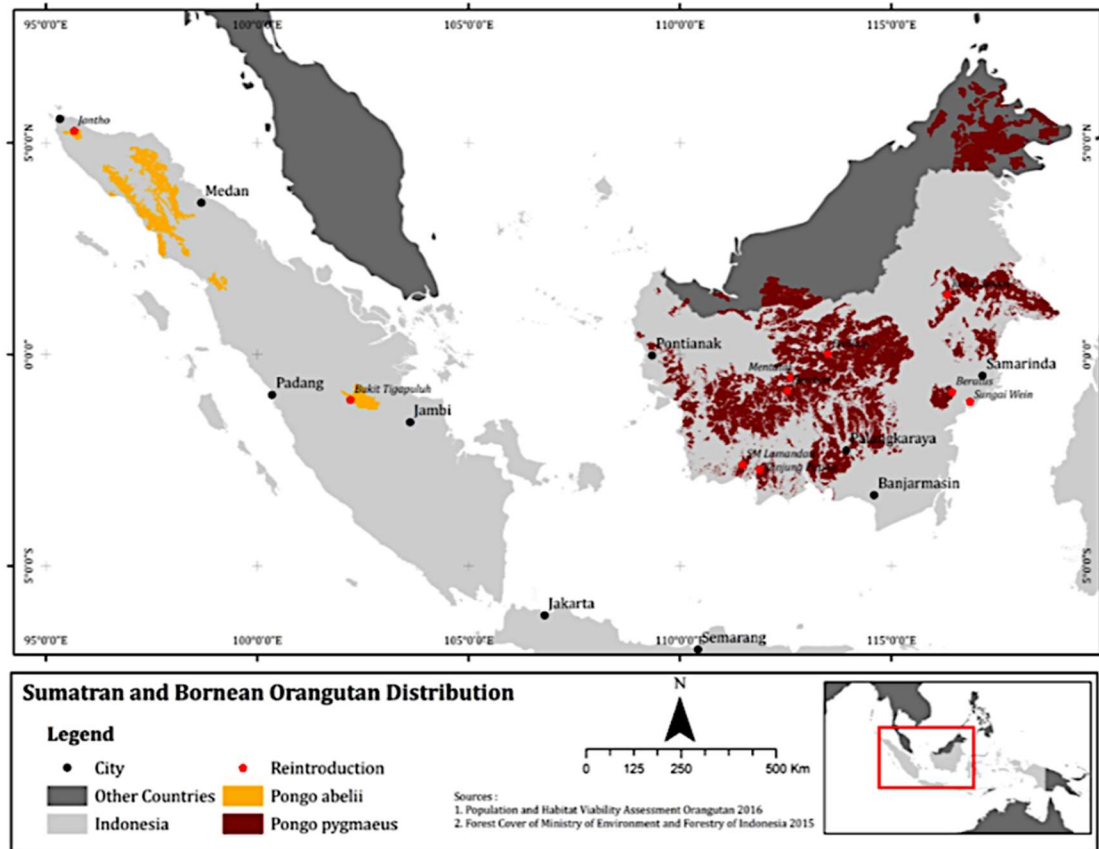


Figure 1.1. Orangutan distribution based on Orangutan Population and Habitat Viability Analysis-PHVA 2016 (Utami-Atmoko et al., 2017).

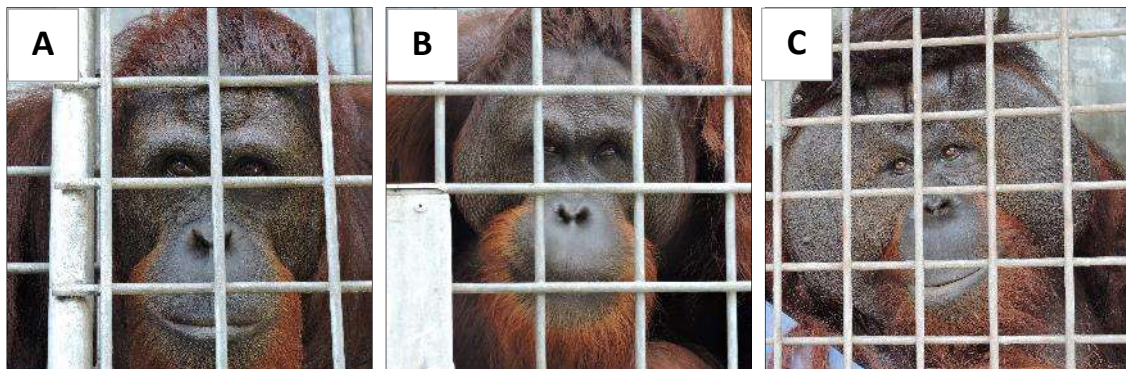


Figure 1.2. Three different developmental stages in male orangutans: (a) unflanged, (b) developing flange, and (c) flanged.

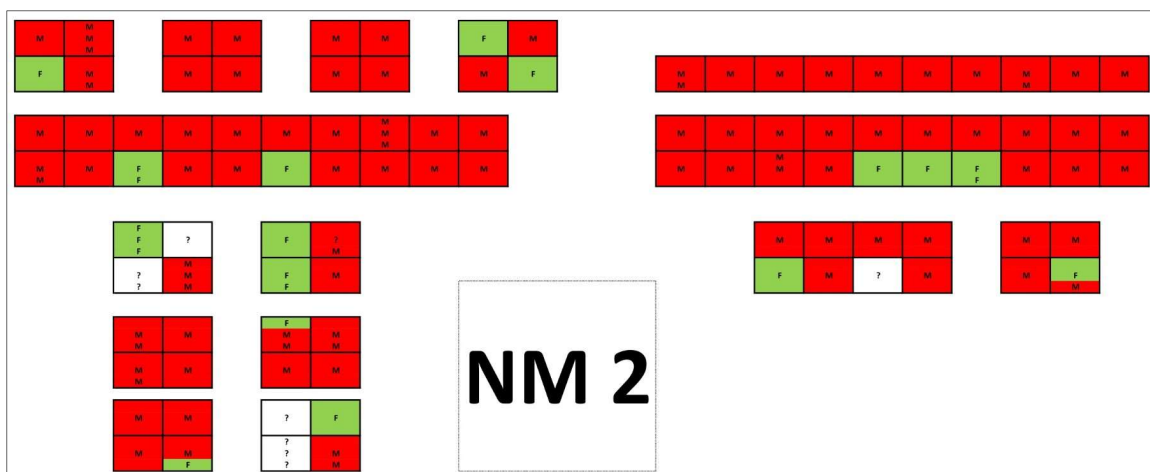


Figure 1.3. Orangutans distribution in NM 2 cage facility. Red block represents male, green block represents female, and the white block represents empty cages.

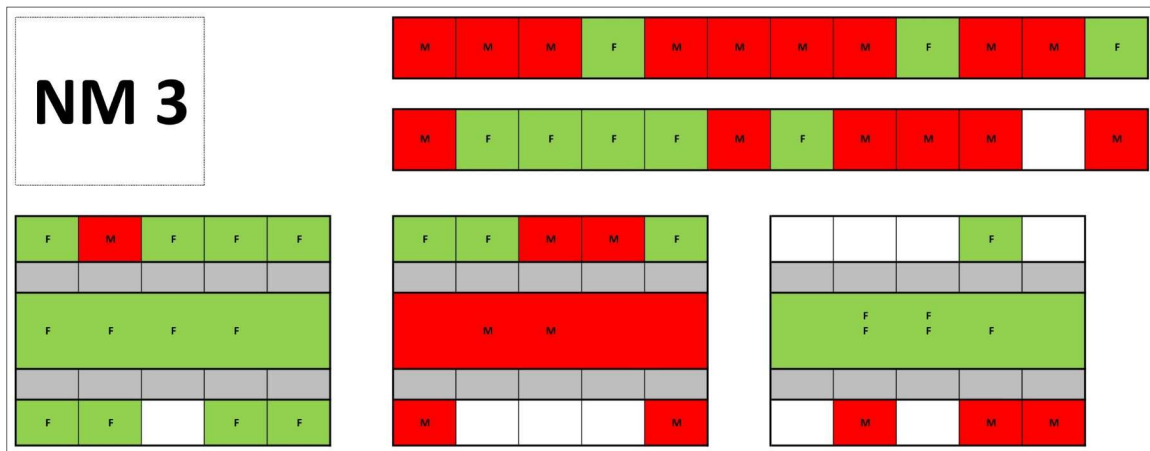


Figure 1.4. Orangutans distribution in NM 2 cage facility. Red block represents male, green block represents female, and the white block represents empty cages, and the grey block represents connecting cages which allowed orangutans move to other cages.

1.6. References

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2. Chapter II. High Social Pressures Provoke Bimaturism in Orangutans

2.1. Introduction

The development of secondary sexual characteristics (SSCs) is influenced by the environmental variability. This includes how environmental state, social interactions, and energetic status regulate trait development (Andersson, 1986; Birkhead et al., 1999; Bjorksten et al., 2000; Borgia, 2006; Jarman, 1983; Leary & Knapp, 2014; Rosenthal & Hebets, 2015). Condition-dependent SSCs will lead to plastic traits, which can be developed sequentially or simultaneously (Taborsky & Brockmann, 2010; Taborsky et al., 2009). When the development occurs sequentially, the SSCs can be either reversible (i.e., the condition which trait could be switched depending on the current or predicted environment) or irreversible (i.e., the trait is fixed) (Setchell, 2008). Adult male orangutans (*Pongo sp.*) provide an excellent example of an irreversible trait through developing cheek-pads or “flanges” once they are reproductively mature (Mackinnon, 1974a) although some individuals can delay the development (Utami-Atmoko et al., 2002). In addition to flange development, these males also experience rapid hair growth and accumulate both fat and muscle mass (Winkler, 1989; Zihlman et al., 2011). The sequential development of the irreversible flange trait in adult male orangutans has intrigued researchers for decades, yet we still have little understanding of what triggers flanging and why there is such variation in the timing of flanging. The timing of flanging is thought to be driven by the evolutionary costs and benefits of developing flanges,

including variation in reproductive success (i.e., attracting females) and intra-sexual competition.

Bimaturism is when there are two different characteristics of a mature individual that are formed by sequential traits and those stages can be reversible or irreversible (Jarman, 1983). In orangutans, bimaturism is found in adult males that are characterized by two different morphological forms. The first morph, called “flanged male”, is an adult male with flanges, a large throat pouch, long hair, and increased body mass. The second morph is an adult male that lacks those traits and is classified as “unflanged male” (Mackinnon, 1974a; Rijksen, 1978). Both male morphs have similar access to receptive females. However, previous studies have shown that adult females are more willing to mate with flanged males than unflanged males (Utami-Atmoko, Mitra Setia, et al., 2009). Indeed, orangutans are the only non-human primate species in which forced copulations by unflanged males are regularly observed (Knott, 2009). As the result, flanged males could sire more offspring compared to unflanged males (Banes et al., 2015; Utami-Atmoko et al., 2002).

There are several proposed mechanisms that may lead to flange development in male orangutans. The first proposed mechanism is social interactions among males. Dunkel et al. (2013) hypothesized that flange development in male orangutans correlated with the number of flanged males and their monopolization over the receptive females. This finding was supported by Pradhan et al. (2012) who modelled the flexible development of flanges and found that delayed development could be linked by the high rate of despotism among flanged males. Compared to Bornean

orangutans, Sumatran male orangutans tend to have fewer flanged males relative to unflanged males (Dunkel et al., 2013). In addition, flanged male dominance in Sumatran orangutans is more stable and they are more likely to monopolize receptive females for longer periods of time (Mitra Setia et al., 2009; Utami-Atmoko, Singleton, et al., 2009). As a result of monopolization, competition over fertile females among flanged males is higher in Sumatran compared to Bornean orangutans (Utami-Atmoko, Singleton, et al., 2009). This situation results in other adult males playing an alternative mating strategy by delaying their flange development (Pradhan et al., 2012).

The second mechanism that has been proposed is the regulation of hypothalamic-pituitary-adrenal (HPA) axis to development of flanges. During periods of physiological stress, the hypothalamus will release neurohormones to stimulate the pituitary gland to produce adrenocorticotrophic hormone (ACTH) which alters the production of cortisol hormone (Rogers, 2012). If the stressor is constantly present, the sympathetic nervous system will produce epinephrine and norepinephrine to initiate the hypothalamus to release corticotropin releasing hormone (CRH) and alter the production of ACTH in the pituitary gland. Then prolactin releasing hormone and gonadotropin hormones immediately initiate the pancreas to produce glucagon which is used to breakdown fat and protein in the muscle as glucose (Sapolsky et al., 2000).

The study of glucocorticoid hormones may allow us to understand correlations between environmental change, behavior, and physiological response. Elevated cortisol has been observed to be correlated to social status, aggression events, energy balance disruption, and the development of secondary sexual characteristic. For example, during

pair separation of adult monogamous marmoset monkey (*Callithrix penicillata*), locomotion and long calls rates were increased, and urinary cortisol levels were increased which indicated stress event. When the pair was reunited, the cortisol levels reverted to the normal range (Duarte et al., 2018). Another example was demonstrated by the male bonobos (*Pan paniscus*) during aggression event. The appearance of a female in estrous initiated high aggression rates among high ranking males. As the result, high cortisol levels were found among them (Surbeck, Deschner, Weltring, et al., 2012). A similar finding was also reported in olive baboons (*Papio anubis*) where high cortisol levels were found among closed high ranking males (Sapolsky, 1992). Increasing cortisol levels has also been observed in the suppressing development of SSCs. Previous studies focusing flange development in orangutans found that high cortisol levels are related to the delayed flanged development in unflanged males (Maggioncalda et al., 2002; Marty et al., 2015).

This study focused on two main questions related to flange development in Bornean captive orangutans. The first question is: *Does the amount of social interactions relate to flange development?* This question is examined in two facilities that are characterized by a difference in the numbers of males present in each facility. The appearance of flanged males plays an important role in the regulation of competition and SSCs development (Dunkel et al., 2013; Maggioncalda et al., 1999). High numbers of flanged males is thought to lead to two developmental strategies for adult males as they mature: 1) develop his flange and compete with other flanged males (“take a chance”), or 2) remain unflanged and play another mating strategy (“play it safe”) (Maggioncalda

et al., 1999). In the wild, male orangutans demonstrate an explicit dominance status, especially in the Sumatran species, with flanged males tending to have higher social rank than unflanged males (Utami-Atmoko et al., 2002). Flanged males have increased access to mates and food resources (Utami-Atmoko et al., 1997), but have to deal with high competition, that can become very aggressive and sometimes lethal, with other males (Mitra Setia & van Schaik, 2007). In Sumatra, the rate of competition between flanged males and unflanged males increased in the presence of females (Utami-Atmoko, Singleton, et al., 2009). Due to high competition among mature males over females, some males delay flange development and play an alternative mating strategy, (i.e., sneaky copulation) (Delgado & van schaik, 2000; Knott, 2009). In captivity, adult orangutans are usually housed individually, and physical contact might be very rare. However, indirect interactions might be present through visual and verbal displays (e.g., vocalizations like long calls, kiss-squeaks and visual displays). Previously, it was hypothesized that these visual and verbal interactions in male orangutans resulted in delayed flange development (Kuze et al., 2005).

The second question that I asked in this study is: *Does the amount of social interactions result in increased stress levels, as measured by urinary cortisol?* Animals that live in the captivity generally face stressful events more often compared to the wild populations, partially because they do not have control of their surrounding environments. This is a result of cage environments, restricted diet options, and forced social proximity and visibility with other animals. This stress response in animals can be observed through their behavioral changes, physiological responses, and growth rates

(Hogan et al., 2012; Mason, 2010; Morgan & Tromborg, 2007). As an indicator of the physiological response during stressful events, elevated glucocorticoid/cortisol hormone levels are observed in the bloodstream, which can be identified in saliva, urine, fecal, or stored in hair's follicle (Ash et al., 2018; Carlitz et al., 2014; Yehuda & Sapolsky, 1997). Increasing stress hormones have been shown to be related to delayed flange development in male orangutans (Maggioncalda et al., 2002; Marty et al., 2015).

To explore these two questions, I hypothesized that:

- 1) Because in the wild, unflanged males are more social than flanged males (Galdikas, 1985; Singleton & van Schaik, 2002; van Schaik, 1999), I hypothesized that flange developmental stage (i.e., unflanged, developing flange, flanged) would be related to the amount of social interactions individuals experience. I predicted that unflanged males would be the most social, followed by developing males, and then flanged males.
- 2) Because males are housed individually in cages, the number of males within the same facility may not influence the amount of observed social interactions. Thus, I predict in the two housing facilities (NM2 and NM3), there will be similar levels of social interactions, despite there being more adult males present in NM2.
- 3) Because males are housed individually in cages and are never in physical contact, I hypothesized the number of males in the facility will not be related to flange development (e.g., flange size).
- 4) Because males are never in physical contact, I hypothesize that cortisol levels should not differ between NM2 and NM3, despite there being more adult males present in

NM2. However, if hearing long-calls and kiss-squeaks (audible signals) results in greater levels of stress, I predict that cortisol may be related to the number of audible signals heard.

2.2. Methods

2.2.1. Study Site and Sample Size

This study was carried out at an orangutan rehabilitation center called Nyarumenteng in Palangkaraya, Central Kalimantan, Indonesia (2°02'23.4"S and 113°45'06.4"E). The Borneo Orangutan Survival Foundation (BOSF) has managed this center since 1999 and more than 500 rescued and confiscated orangutans were placed into two different facilities: 1) quarantine that focuses on the process to improve the health of orangutans, and 2) rehabilitation, which is the next step before the orangutans can be released into the wild. The study focused on 157 orangutans (96 males and 61 females) who live in the rehabilitation facility with ages ranging from 1 to 26 years. The rehabilitation facility is divided into two different sites. The first one is the socialization facility (NM2) which has 10 group cages and each group contains 12 individual cages (Figure 1.3). In this group, every cage (and hence individual) is separated by a solid border made from concrete or a metal-plate to avoid physical contact between orangutans. Only the front and back side of the cages were built with a grid border that allows orangutans to see each other, although all individuals can hear each other. At this facility, orangutans were housed for long time periods, until they are ready to be released at which time they are moved into NM3. The second site is the monitoring

facility (NM3) or sometimes called a “transit cage” that has 5 different group cages which contain 54 individual cages (Figure 1.4). There are 3 connected cages with grid borders in NM3 that allow orangutans to interact, whereas two other groups are separated by solid borders. Depending on the logistics of the release process, orangutans that live in this facility could be stay for short (1 month) or long time periods (2 years).

Prior to data collection, I randomized the sample size based on location, sex class, and orangutans’ age. This information was available in the Nyarumenteng orangutans’ data base which recorded their arrival or birth date, the origin where they were rescued/confiscated, the location where they were housed, their sex, age, body mass, and maturity. All data were updated by the veterinarians during regular health examination. This study focused on matured male orangutans which could be selected from their age and secondary sexual traits. I used the criteria that has previously applied by Kuze et al. (2005) in which male’s secondary sexual traits begin to develop at 11 years old. Twenty-five adult matured males with the age range 11-24 years were selected as a sample size which contained 9 males in the unflanged stage, 7 males is the developing their flange stage, and 9 flanged males (Table 2.1).

Moreover, I divided the sample through different categories based on their flange development (i.e., unflanged male (UFM), developing flange male (DFM), and flanged (FM) (Figure 2.1)). UFM can be identified by having an undeveloped flange or throat pouch, having a similar body size as adult females but smaller than FM. DFM has begun to grow his flanges although the size is not fully developed, often has a small

throat-pouch, and the body size approaches that of FM. FM is characterized by thick and round-shape flange, a large and fully developed throat-pouch, and large body size. See Chapter 1 for a more detailed explanation.

2.2.2. Flange Morphometrics

To quantify flange size, facial morphometric followed the methodology that was previously used by asymmetry facial studies in human and non-human primates (Boulton & Ross, 2013; Little et al., 2012; Penton-Voak et al., 2001). A full-face frontal photograph of orangutans with a neutral facial expression was captured once a month. Occasionally, a physical measurement of flange with calipers was also conducted during health examinations when the orangutans had been anesthetized. Two different landmarks were used to measure flange size (Figure 2.2). The first is the eyes' landmark, which measured the length of flange from the tip of right (A1) and left (A2) eye to the edge of flange. The second is the nostril's landmark which measured the length of flange from the tip of nose to the edge of right (B1) and left (B2) flange. Flange size was determined by the sum of each landmark measurement.

Images were captured with Nikon Coolpix P600 camera. To avoid a worm's-eye view which caused a taller image, the camera was positioned at the same height as orangutan's face by using a monopod. In addition, the distance between the camera and orangutan's face was calculated with Leica® DISTO™ E7100i laser distance measurer. I calibrated the camera by capturing 20 known actual size/length objects (i.e., 10 cm) from different distances. A correction factor was calculated by using equation (2.1, which correction factor (c) was gained by multiplying known object size (s) with the

fraction of image focal length (f) to the distance between object and the camera (d).

With this calculation, the correction factor for image measurement is 0.00164 ($n=20$, Means \pm SE = 9.55 ± 0.14).

$$c = s \frac{f}{d} \quad (2.1)$$

To obtain the actual size from the image (o), I used three different approaches. First, I used equation (2.2, where d is the distance from camera to object, p is pixels of the image, and f is the focal length of the image.

$$o = \left(\frac{d \cdot p}{f} \right) \times c \quad (2.2)$$

The second measurement method was by using a scale comparison from known object size to the flange size image. In this method I used the actual cage grid as the scale. Because the cage was built with almost similar grid size, the length of the grid could be used as a comparison scale to the image. Following the protocol in the IMAGEJ2 software, the actual image size could be quantified (Schindelin et al., 2015). The third approach is the actual flange measurement which was measured with calipers opportunistically during health examinations.

2.2.3. Orangutan Distribution and Social Interaction

I compared two rehabilitation facilities (NM2 and NM3) in which orangutan distribution was clustered under sex class and developmental stage. In addition, the

male sex ratio and the percentage of males from each facility were calculated. To observe orangutan behavior, a standardized focal animal sampling was applied in two-minute instantaneous interval, starting from 07:00 AM to 17:00 PM. Every two minutes, I observed and recorded orangutan activity continuously by using four general activities (i.e., feeding, moving, resting, and social activity). In addition, any interesting behaviors related to the social context (e.g., vocalizations or physical contact) were recorded by using ad-libitum sampling method (Altmann, 1974). To quantify social interactions among orangutans, I focused only on audible interactions that were emitted to- and received from other males.

According to www.aim.uzh.ch/orangutannetwork, long-calls are described as the loud sounds which start with the grumbles sound as introduction, then pulses sound as the main part of the long calls, and ends with several bubbles sound. Long-calls could be emitted very fast (15 seconds) which are called fast-calls, and slow long-calls which can last up to 4 minutes long; this audible sound can be heard for up to 1500 meters (Delgado et al., 2009). Kiss-squeaks are defined as trumpet-lips sounds which can be emitted by both sexes and all developmental stages (Mackinnon, 1974a). Kiss-squeaks are commonly produced by the mouth, but sometimes are facilitated by the use of hands, fingers, leaves, and other materials (van Schaik et al., 2009).

Moreover, I analyzed audible signals and flange development from all stages into three different responses: 1) the number of received long-calls and kiss-squeaks from other male(s) to flange size, 2) the number of emitted long-calls and kiss-squeaks to

flange size, and 3) the number of responses to long-calls and kiss-squeaks after receiving signals to flange size. Similar analyses were also carried out in the context of cortisol.

2.2.4. Urine Sampling and Preservation

A non-invasive sampling method was applied to collect orangutan's urine on a daily basis. I prioritized morning urine that was secreted between 06:00 AM – 10:00 AM to minimize a circadian rhythm's effect (Fuleihan et al., 1997). Circadian rhythms have been shown to affect the chemical compound excretions, often with concentrations increasing in the morning (Devine & Wolf, 2016), and then decreases throughout the day (Czekala et al., 1994; Muller & Lipson, 2003). Right after collection, urine samples were stored in a double-wall insulated jar at low temperatures (approximately $< 5^{\circ}$ Celsius) then moved them to the camp's freezer at -15° Celsius. At the same time, 200 μ L urines were aliquoted to 2-3 Whatman® protein saver cards as a backup. Eventually, all collected samples were transported to -80° Celsius in the Laboratory for Primate Dietary Ecology and Physiology, Department of Anthropology, Rutgers University for further analyses and long-term storage.

Qualitative clinical test was carried out with Roche Chemstrips™ for human analysis which recorded the levels of bilirubin, blood, glucose, ketone, leukocyte, nitrite, pH, protein, specific gravity and urobilinogen. In addition, a quantitative test of specific gravity (SG) value was conducted in the laboratory by using an AtagoPAL® refractometer. The value was used as a correction factor for potential variation in

orangutans' hydration status. Following Miller et al. (2004), the corrected SG value was calculated by: *Corrected SG = ((average SG population – 1.000)) / ((SG sample – 1.000))*.

2.2.5. Hormonal Analysis

Cortisol hormone was assayed with DetectX™'s cortisol enzyme immunoassay kit (K003-H1) which is designed for analyzing dried fecal extracts, saliva, urine, serum, plasma, and tissue culture media samples (Assays, 2017). The kit was previously validated for orangutan's urine with the sensitivity levels for the assay is 27.6 pg/mL, intra-assay coefficient of variation 6.77 n= 255, inter-assay control coefficient of variation 5.37% (low) and 5.51% (high) with n= 11 plates, and inter-assay standard coefficient of variation 7.81% with n= 11 plates.

Urinary cortisol and testosterone concentrations depend on the hydration status and the time of last urination (Miller et al., 2004). I adjusted the hormones concentration with corrected urinary specific gravity (SG) instead of urinary creatinine. The SG value is related to the amount of secreted chemical particles in the urine and can be used as an indicator for dehydration or even the failure of organs function such as cardiovascular and kidney (Lee et al., 2013; Wang et al., 2015). By delineating the water component, the pure urine concentration would be gained and can be used to adjust the urinary hormones concentration (Miller et al., 2004).

2.2.6. Statistical Analysis

Generalized additive mixed model's (GAMM) were used for almost all analysis in this study. As an extension from generalized linear model, GAMM involves a sum of

smooth terms functions as the predictor variables that give the relaxation on parametric assumptions as well as could expose non-linearity data. To run the GAMM, I used *mgcv* R package (Wood, 2018) with orangutan ID as a random effect and random slopes were added to minimize type 1 errors. The formula used for analyses was:

Model = gamm(y ~ s(x) + x + .., data = data, family = gaussian, random = list(ID=~1))

In addition, I used Kruskal-Wallis rank test to compare data which contains more than two independent variables, and when significant result appears, pairwise comparison test between group (multi-comparison test) was conducted to identify which group shows higher difference. Multi-comparison tests after Kruskal-Wallis were run with *pgimerss* R package (Giraudoux et al., 2018). All analyses were conducted in R Studio 1.1.442 (R-Core-Team, 2013).

2.3. Results

2.3.1. Orangutans Distribution and Activity Budget

Eighty-six mature male orangutans were housed in NM2 with the sex ratio of males to female as 2:1. This ratio is larger than 15 matured male orangutans that were housed in NM3, which had a male : female sex ratio of 0.79:1. In NM2, the percentage of FMs and UFM were nearly similar (43% FM and UFM), whereas DFMs made up 11.84% of the males. Meanwhile, the distribution of developmental stages in NM3 was dominated by FMs (60%), followed by UFM (33%), and DFM (7%), thus there was a greater percentage of FMs in NM3 (Table 2.2).

From the total 2,266 observation hours, orangutans in the both facilities spent the most of their time resting, followed by feeding, social interactions, and moving (77.74%, 15.90%, 3.88%, and 2.48% respectively). As seen in Table 2.3 and Figure 2.3, feeding activity was not statistically different among developmental stages. FMs, which have biggest body mass, moved less than UFM (GAMM Estimate \pm SE= -2.55 ± 1.26 , $p=0.04$); whereas UFM and DFM were not significantly different. Because FMs moved less than the other development stages, they spent more time resting. I found resting activity in UFM was significantly less frequent than FMs (GAMM Estimate \pm SE= 17.91 ± 6.63 , $p=0.01$), whereas between UFM and DFM did not differ. Although on average flanged males (FMs) demonstrated more social activities compared to DFM and UFM, our GAMM analysis revealed no significant difference. Unflanged males tend to have similar social activity as both developing males and flanged males (Table 2.3 and Figure 2.3).

Moreover, received signals from other orangutans were not statistically different in all male's developmental stages, which may mean that males in the rehabilitation center could hear audible signals (i.e., long-calls and kiss-squeaks) equally or they are equally unaffected (Table 2.3). There was a difference in the number of emitted and responded sounds by FMs compared to UFM (both GAMM model giving $p < 0.001$), whereas DFM and UFM were not statistically different (Table 2.3).

2.3.2. Orangutans Flange Size

The flange size from two different landmarks measurement (i.e., eyes and nostril) showed strong significant differences between what were classified as unflanged

male (UFM), developing flange male (DFM), and flanged male (FM) (Kruskal-Wallis's Eyes: $X^2= 85.96$, $df=2$, $p= 0.001$; Nostril: $X^2= 68.25$, $df=2$, $p= 0.001$). Moreover, the multi-comparison Kruskal-Wallis tests of flange size among those stages demonstrated true differences, indicating that the average of flange sizes were clearly distinguished between how I classified UFM, DFM, and FM (Table 2.4, Figure 2.4).

2.3.3. Cortisol Hormone

On average, FMs have up to two-times greater cortisol levels than the other stages; whereas DFMs tend to have the lowest levels (Table 2.5). Cortisol levels among 28 male orangutans varied (Kruskal-Wallis's: $X^2= 45.518$, $df= 28$, $p= 0.02$). Based on two different facilities, cortisol levels of orangutans in NM3 were significantly higher than orangutans in NM2 (Mean cortisol NM2 and NM3= 32398 pg/ml and 66824 pg/ml respectively, Kruskal-Wallis's: $X^2=12.26$, $df= 2$, $p= 0.002$).

I ran two different models to investigate the differences in cortisol levels among male developmental stages. First, without accounting for energy intake, FMs produced significantly more cortisol compared to other stages (Estimate \pm SE= 0.27 ± 0.09 , $p= 0.01$), whereas UFM's have approximately similar levels as DFMs (Table 2.5). By including energy intake (Kcal/kgMBM) in the model, the results agreed with the previous model; FMs have significantly higher cortisol levels compared to DFMs and UFM's (Table 2.5).

2.3.4. Social Interaction and Flange Size

I examined the relationship between social interactions and flange size using the two different landmarks measurement that is eyes and nostril and controlled for cage

setting (Table 2.6). From the eye landmark measurement, there was no relationship between total number of social interactions and flange size in all orangutan developmental stages (GAMM UFM $p = 0.64$, DFM $p = 0.12$, and FM $p = 0.94$; Figure 2.5a). However, when social interactions were divided into three different responses (i.e., received, emitted, and responded), there were different patterns between signals and flange size (Table 2.6, Figure 2.5 and Figure 2.6). The number of received signals from other males were not different in all stages. Male orangutans with small flange size received similar audible signals compared to male orangutans with bigger flanges (GAMM UFM $p = 0.46$, DFM $p = 0.11$, and FM $p = 0.81$; Figure 2.5b). The number of emitted signals did not vary with flange size in DFMs and FMs, but UFM's showed a negative relationship between signal given and flange size (GAMM UFM $p = 0.001$, DFM $p = 0.28$, FM $p = 0.52$; Figure 2.5c). The number of responses to vocalizations heard at the same time showed a different pattern between male stages. In UFM's, males with greater flange size tends to respond to the signals received more frequently when they were compared to individuals with smaller flange size (GAMM $p < 0.0001$). However, variation in flange size of UFM's is very low – although a significant result was revealed. Flange size in DFMs was not related to the number of responded to signals, however this is because there were not any responded signals recorded during the study. Flange size in FMs was not related to the number of responded signals (GAMM $p = 0.96$; Figure 2.5d).

From the nostril's landmark measurement, the total number of social interactions in all developmental stages was also not related with flange size; small

flange size tended to have similar social interactions as males with bigger flange sizes (Table 2.6). Similar patterns were shown in the two different audible signal responses; flange size was not related to the number of received and emitted signals (Table 2.6, Figure 2.6). Meanwhile, a different pattern was shown in the model that was tested between flange size and responded signals. The number of responded signals to vocalizations heard at the same time showed a different pattern between male stages. Unflanged males with greater flange size responded to the received signal more frequently compared to the males with smaller flange sizes (GAMM $p < 0.0001$). DFMs flange size was not related to the number of responded signal, considering there were zero number of responded signals were recorded during the study. Meanwhile, the flange size in FMs was not related to the number of responded signals (GAMM $p = 0.98$; Figure 2.6d).

2.3.5. Social Interactions and Cortisol Hormone

Total social interactions in FMs with UFM were significantly correlated with cortisol levels; low cortisol levels were found when there were fewer social interactions and increasing the number of social interactions was related to elevated cortisol production (GAMM $p = 0.01$). However, this pattern was not observed in DFMs, in which social interactions were not related to variation in cortisol (Table 2.7, Figure 2.7a). Similar results were found when examining the relationship between cortisol levels and three different signal responses (i.e.: received, emitted, and responded signals). The production of cortisol in FMs was significantly different when few and more frequent received signals were compared; higher cortisol levels were found when greater

numbers of signals were heard (GAMM $p=0.02$, Figure 2.7b). Meanwhile, DFMs did not show variation in cortisol levels with signals. A similar result was found in the analysis of cortisol and the number of emitted signals. I found FMs tend to have higher cortisol productions when they emitted more signals (GAMM $p=0.02$, Figure 2.7c). Once FMs heard a signal from other orangutans, they would respond to it immediately by producing long-calls. I also found that cortisol production in FMs is related to the number of signals responded to; high cortisol levels were significantly different between the low and high number of signals that were responded to (GAMM $p=0.01$, Figure 2.7d). In contrast, this pattern wasn't found in DFMs.

2.4. Discussion

2.4.1. *Is flange development related to the amount of social interactions?*

As predicted, flanged males (FMs) tend to have more frequent social interactions compared to unflanged males (UFMs) and males who are in the developing flange stage (DFMs). This is because only FMs have ability to produce long-calls, meanwhile the other stages couldn't (see chapter 1 for detail). Moreover, although UFMs and DFMs couldn't emit long-calls, audible signals could be present by kiss-squeak sounds. As a result, the social interactions that were clearly targeted at orangutans were not different among the male developmental stages. All orangutans have capability to emit vocalizations, but only flanged males can pronounce long-calls due to their developed throat sacs. My findings reject the hypothesis that social interaction events among the male developmental stages would not vary.

A similar result was shown from the number of received signals, in which all male stages tend to hear similar number of audible signals from other orangutans. The equal opportunity to hear signals from other orangutans was mainly for kiss-squeaks, which may have been affected by environmental setting of the cage. All cages in NM2 and NM3 were built with a grid border that allow orangutans to see and hear other orangutans. Moreover, the short distance between cages could also allow orangutans to receive a low-signals like kiss-squeaks from unflanged males.

The number of FMs in two different facilities (i.e., NM2 and NM3) may have affected the pattern of total social interactions among developmental stages. In NM3, where the ratio of FMs was higher than other male's stages, there were more frequent social interactions in UFM's compared to the DFM's and FM's. However, where the ratio of FMs to UFM's is similar (e.g., NM2), UFM's tend to interact less. In other words, when there was a greater proportion of individuals that were flanged, there were social interactions among the UFM's. Thus, I reject the hypothesis that the number of males within the same facility would not influence the amount of observed social interactions. The high number of FMs in NM3 may have led to greater sociality among UFM's and DFM's; meanwhile, when the number of FMs decreased, FMs were more social than the other male developmental stages.

The current study is the first of its kind to quantify orangutans' flange size. By measuring the size of the flanges, one can understand the intrinsic-extrinsic factors that might influence the flange development. As infants, orangutans of both sexes have been described as having small and tiny skin ridges at the edge of their cheeks (Mackinnon,

1974a). This skin ridge then disappears with aging, when orangutans grow from an infant to the juvenile stage. Thus, since the developmental change from infant to juvenile, female seem to lose this ridge, whereas, male would have two options, either become a flanged or unflanged male at adulthood. Changes in the flange development have been correlated with the social and physiological response (Dunkel et al., 2013; Emery-Thompson et al., 2012; Maggioncalda et al., 1999; Marty et al., 2015; Utami-Atmoko et al., 2002). It is thought that all adult males eventually develop flanges (see chapter 1 for detail), although they do this at different ages and have flanged that differ in size. The current study quantified the size of orangutans' flange in three developmental stages, and thus, found significant size differences between UFM, DFM, and FM. UFM who develop a small flange ridge, had smallest sized flanges compared to other developmental stages, and FM who developed their flanges grew twice as large compared to DFM, and in the middle of those stages is DFM which has a flange that is about half size of FM flanges.

The types and amounts of social interactions were correlated to flange size and development. Social interactions were commonly observed among the males with more developed flanges compared to those with smaller flanges. Moreover, the effect of social interactions highlighted different patterns, when the analysis was conducted between the number of received, emitted, and responded signals. All males with small or bigger flange size tended to receive a similar number in audible signals. Flange size was not related to the number of emitted audible signals that were directed to other

orangutans. Meanwhile, unflanged males with bigger skin-ridges had significantly lower amounts of vocalizations.

Flange size in unflanged and developing flange males was also related to the number of signals that were responded to; males that are developing their flanges engaged in less social behavior. The only vocalization demonstrated by orangutans in the captive, single cage setting was the use of vocalizations, that is kiss-squeaks and long-calls. Perhaps, males that are developing flanges have additional energetic costs and thus avoid other potential costs that could be associated with social behaviors (Chapter 3). It has been suggested that advertising developmental status through vocalization might increase the likelihood of being threatened by fully developed flange males (Mitra Setia & van Schaik, 2007). Based on my findings, I accepted the hypothesis that social interactions, specifically responding to signals, would be related to flange development in the captive male orangutans.

2.4.2. Does the amount of social interactions result in increased stress levels measured by urinary cortisol, which has been shown to suppress flange development?

Animals that live in the captivity generally face more stress as compared to those who live in the wild. This condition is usually caused by the cage's environment, restricted diet options, and interaction with another animal or human. The stressed animals can be observed by their behavioral changes, physiological responses, and growth rates; most of the captive animals demonstrate negative trend and tend to be smaller in captivity (Hogan et al., 2012; Mason, 2010; Morgan & Tromborg, 2007). As an indicator of the physiological response during stressful events, high glucocorticoid

hormone level would be found in the bloodstream, excreted in saliva, urine, fecal, or stored in hair's follicle (Ash et al., 2018; Carlitz et al., 2014; Yehuda & Sapolsky, 1997). The current study found that flanged males produced two times the amount of cortisol compared to unflanged and males that are developing their flanges. This finding contradicted the result from the previous studies, which analyzed the male orangutan cortisol levels. Maggioncalda et al. (2002) found that the highest cortisol levels were found among males who were developing their flanges, whereas flanged males tended to have the lowest cortisol levels when compared with all the developmental stages. Moreover, the mentioned study suggested high cortisol levels in the DFMs was related to the growth and the caused from SSCs displays.

High cortisol levels in flanged males may be due to several stressors that they experience. The first stressor is limited living space. The cage for the captive orangutans was of limited size; the cage was built in 3 x 3 x 4 meters dimension, which is not enough for an orangutan that has a body mass of 76.87 kg. This limited space results in captive orangutans spending their time resting and being less active. Indeed, Bornean flanged males in the wild have been reported to have home ranges of about 237-556 Ha whereas unflanged males about 184-639 Ha (Buckley, 2014; Morrogh-Bernard, 2009). The current study by using different methods (Kernel density) found flanged males approximately have similar home range with the unflanged males (1900 Ha and 2000 Ha respectively) (Buckley, 2014). In the review of the sources of stress in captive animals, Morgan and Tromborg (2007) included restricted movement area as a stressor for animal who lives in the captivity. In line with this assumption, Rangel-Negrín et al.

(2009) compared the cortisol levels of spider monkeys in different environment. The mentioned study found that spider monkeys that lived in the captivity were more stressed compared to a population that lived in the sanctuary or even fragmented habitat. The second potential stressor that the orangutans in this study may have experienced is restricted food and foraging activity. As seen in the chapter 3, the current study found that males with larger body mass tended to have decreased daily energy intake. The findings corresponds with the assumption that was made by Morgan and Tromborg (2007), which posited that limitation of food and foraging behavior limitation acts as a source of stressful events. For example, flanged males were often observed leaving food and not consuming it when they were given the same food repeatedly. Thus, perhaps the lack of food choices created a more stressful environment for flanged males.

The third potential stressor is the amount of social interactions. Orangutans live semi-solitarily in the wild, and this is more pronounced in Bornean species (reference). Weingrill et al. (2011) found that increasing the group size of Bornean orangutans in the zoo, which encouraged the social interactions, was correlated with the high cortisol levels. Meanwhile, the opposite trend was observed in Sumatran orangutans. Increased social interactions also led to higher cortisol concentrations in subordinate chimpanzees during high aggression events (Yamanashi et al., 2016). The finding of the current study do not align with the previously conducted studies, specifically with male orangutans in the undeveloped or developing flange male states. However, the pattern was present by FMs, in which high cortisol levels were positively related to the number of social

interactions. Similar trends were found in the analyses of the number of received, emitted, and responded signals. All of the audible signals did not correlate with cortisol levels in UFMs and DFMs. Cage environment with different number of male's developmental ratio did not correlate with cortisol concentrations in this study. I found that the cortisol concentrations did not differ in the two cage types of NM2 and NM3. This may be because housing orangutans in single cage may minimize the amount of direct aggression from another orangutan; moreover, orangutans might be habituated to the current environment considering the fact that many individuals have lived in such captive situation for more than 10 years.

However, although captive orangutans have a very small chance to interact physically with other individuals, interactions could be encouraged with the help of audible signals. Flanged males have the ability to respond to signals by generating long-calls, whereas developing flange males and unflanged males tend to be quiet and sometimes demonstrate auto-play behavior. In the wild, unflanged and developing flanged male orangutans would avoid long-calls when they are heard, and this is especially true for subordinate males (Mitra Setia & van Schaik, 2007). If the audible signal tends to be a stressor for other orangutans, consistent presence of it would cause a chronic stress condition and produce greater levels of cortisol. This study supports the proposed hypotheses; there was a relationship between social interactions and cortisol levels only in FMs, but a different pattern was observed by UFM and DFM, in which cortisol levels were not influenced by the number of audible signals.

To conclude, living in captivity with single cage setting does not seem to limit orangutans' interaction, although they cannot have direct interactions in terms of physical aggression. Orangutans might interact verbally through vocalizations and visually by displays. Social interactions were related to the size of the orangutans' flange such that males with smaller flanges tended to have greater levels of social interactions. Moreover, the amount of social interactions was related to cortisol production in males with greater flange size. The high cortisol concentration observed in flanged males was related to the amount of observed social interactions; other stressors such as the limited living space, reduced mobility, and the food restrictions may have led to higher cortisol levels in these larger males.

2.5. Tables and Figures

Table 2.1. The distribution of male orangutans used as sample size in this study. Nutrition and urine sample were collected daily and orangutan's face photographs were taken monthly.

No	Orangutan	Developmental Stage	Facility	Age (Yrs)	Body Mass (Kg)	Nutrition (Day)	Flange (Month)	Urine (Day)
1	Casper	UFM	NM 2	14	82.5	11	3	9
2	Ibut	UFM	NM 3	12	52.5	15	2	13
3	Kevin	UFM	NM 3	15	49.6	5	2	5
4	Lomon	UFM	NM 3	13	49	6	1	6
5	Lopus	UFM	NM 2	16	56.97	22	5	23
6	Nyun nyun	UFM	NM 2	13	52	11	5	11
7	Paiman	UFM	NM 2	13	40	5	5	5
8	Rambo	UFM	NM 3	13	53.6	4	2	4
9	Stuart	UFM	NM 2	13	47	1	3	1
10	Edoy	DFM	NM 2	17	58.95	9	4	14
11	Joni	DFM	NM 2	14	58.95	15	6	14
12	Juky	DFM	NM 2	14	65	8	2	7
13	Kisar	DFM	NM 3	15	64	8	4	8
14	Marwoto	DFM	NM 3	18	68.3	5	3	5
15	Palingkau	DFM	NM 2	13	58.95	-	-	3
16	Soni	DFM	NM 3	17	50	1	1	1
17	Bento	FM	NM 3	24	87.4	11	4	10
18	Charles	FM	NM 2	20	75	11	6	11
19	Danny	FM	NM 3	20	71.8	8	4	9
20	Gayo	FM	NM 2	19	75	22	5	24
21	Inou	FM	NM 3	12	77.18	10	4	10
22	Lucky Thai	FM	NM 2	19	77.18	22	6	24
23	Sabun	FM	NM 3	14	88.9	11	5	9
24	Saswoko	FM	NM 3	19	55	10	5	9
25	Uncui	FM	NM 2	14	85	5	4	5

Table 2.2. The distribution of adult males in two different rehabilitation facilities.

Facility	Male's Developmental Stages		
	Unflanged	Developing Flange	Flanged
NM2 _(n=86)	44.74% _(n=34)	11.84% _(n=9)	43.42% _(n=33)
NM3 _(n=15)	33.33% _(n=5)	6.67% _(n=1)	60.00% _(n=9)

Table 2.3. Activity budget among male's developmental stages from 242 days and 2266 hours follow.

Developmental Stages	Activity Budget (minute)							
	Feed (mean ± SE)		Move (mean ± SE)		Rest (mean ± SE)		Social (mean ± SE)	
Unflanged Developing Flange Flanged	41.09 ± 2.15		6.23 ± 1.26		202.57 ± 5.41		1.08 ± 0.49	
	42.62 ± 3.18		7.00 ± 1.40		204.79 ± 7.48		0.19 ± 0.09	
	41.96 ± 1.83		3.68 ± 0.50		220.48 ± 3.98		1.64 ± 0.42	
GAMM Activity Budget								
	Feeding		Feed + Cage		Move		Move + Cage	
	Estimate ± SE	p-value	Estimate ± SE	p-value	Estimate ± SE	p-value	Estimate ± SE	p-value
Intercept	41.09 ± 2.12		46.80 ± 2.34		6.23 ± 0.94		3.86 ± 1.05	
Developing Flange	1.53 ± 3.73	0.68	-2.69 ± 3.67	0.47	0.77 ± 1.66	0.64	2.52 ± 3.67	0.13
Flanged	0.87 ± 2.83	0.76	0.77 ± 2.71	0.78	-2.55 ± 1.26	0.04	-2.51 ± 1.21	0.04
R ² _(Adj.)	-0.01		0.08		0.02		0.09	
	Resting		Rest + Cage		Social		Social + Cage	
	Estimate ± SE	p-value	Estimate ± SE	p-value	Estimate ± SE	p-value	Estimate ± SE	p-value
Intercept	202.57 ± 4.96		198.06 ± 5.72		11.74 ± 1.56		1.08 ± 0.44	
Developing Flange	2.22 ± 8.72	0.80	5.55 ± 8.95	0.54	-8.27 ± 2.84	0.004	-0.89 ± 0.78	0.25
Flanged	17.91 ± 6.63	0.01	18.00 ± 6.61	0.007	-5.77 ± 2.16	0.008	0.56 ± 0.59	0.34
R ² _(Adj.)	0.03		0.03		0.04		0.01	
GAMM Audible Signals								
	Received		Emitted		Responded			
	Estimate ± SE	p-value	Estimate ± SE	p-value	Estimate ± SE	p-value		
Intercept	1.53 ± 0.24		0.18 ± 0.10		0.12 ± 0.07			
Developing Flange	-0.08 ± 0.35	0.81	-0.07 ± 0.15	0.66	-0.10 ± 0.11	0.37		
Flanged	0.07 ± 0.31	0.83	0.66 ± 0.13	<0.0001	0.46 ± 0.09	<0.0001		
CageNM3	-0.12 ± 0.28	0.67	0.04 ± 0.12	0.74	-0.04 ± 0.09	0.64		
R ² _(Adj.)	-0.01		0.13		0.12			

Table 2.4. Flange size (cm) from two different landmarks measurement.

Developmental Stages	Eyes' Landmark		Nostril's Landmark	
	Mean \pm SE	Min/Max	Mean \pm SE	Min – Max
Unflanged (n=34)	8.04 \pm 0.20	5.84/10.02	14.23 \pm 0.41	9.39 – 20.24
Developing flange (n=19)	11.06 \pm 0.33	8.36/13.62	20.50 \pm 1.00	14.42 – 29.46
Flanged (n=55)	16.23 \pm 0.29	11.93/21.26	24.28 \pm 0.43	18.37 – 30.08
Kruskal-Wallis (KW)				
χ^2	85.96		68.25	
df	2		2	
p	0.001		0.001	
KW multi-comparison	DFM-FM*		DFM-FM*	
	DFM-UFM*		DFM-UFM*	
	FM-UFM*		FM-UFM*	

Table 2.5. Cortisol levels among captive males in different developmental stages.

Cortisol (pg/mL)	Mean \pm SE	Number of orangutans
Unflanged	29290.74 \pm 5190.78	9
Developing Flange	26716.50 \pm 4927.50	8
Flanged	61931.52 \pm 13575.53	11
GAMM: Log C ~ Stages	Estimate \pm SE	p-value
Intercept	4.28 \pm 0.07	
Developing Flange	- 0.02 \pm 0.11	0.88
Flanged	0.27 \pm 0.09	0.01
$R^2_{(Adj.)}$	0.08	
N observations	120	
GAMM: Log C ~ Stages + Intake	Estimate \pm SE	p-value
Intercept	4.14 \pm 0.12	
Developing Flange	0.04 \pm 0.13	0.75
Flanged	0.41 \pm 0.12	0.002
$R^2_{(Adj.)}$	0.12	
N observations	83	

Table 2.6. Model analysis social interactions and flange size of three male's developmental stages (n= 32).

Stages	Formula	Smooth terms	$R^2_{(Adj.)}$	edf	Ref.df	F-value	p-value
Unflanged	Eye ~ s(x, by=Stage) + Cage	s(Total Social)	-0.2	1.00	1.00	0.23	0.64
Developing Flange				1.00	1.00	2.55	0.12
Flanged				1.00	1.00	0.01	0.94
Unflanged		s(Received)	-0.15	1.00	1.00	0.57	0.46
Developing Flange				1.00	1.00	2.75	0.11
Flanged				1.00	1.00	0.06	0.81
Unflanged		s(Emitted)	0.46	3.42	3.42	6.95	0.001
Developing Flange				1.00	1.00	1.23	0.28
Flanged				1.00	1.00	0.43	0.52
Unflanged		s(Responded)	0.84	2.97	2.97	50.71	<0.0001
Developing Flange				1.00	1.00	61.71	<0.0001
Flanged				1.00	1.00	0.00	0.96

Stages	Formula	Smooth terms	$R^2_{(Adj.)}$	edf	Ref.df	F-value	p-value
Unflanged	Nostril ~ s(x, by=Stage) + Cage	s(Total Social)	-0.18	1.00	1.00	0.12	0.73
Developing Flange				1.00	1.00	1.93	0.18
Flanged				1.00	1.00	0.22	0.64
Unflanged		s(Received)	-0.15	1.00	1.00	0.01	0.94
Developing Flange				1.00	1.00	2.11	0.16
Flanged				1.00	1.00	0.42	0.52
Unflanged		s(Emitted)	-0.02	1.00	1.00	2.70	0.11
Developing Flange				1.00	1.00	0.04	0.84
Flanged				1.00	1.00	0.06	0.81
Unflanged		s(Responded)	0.78	2.95	2.95	29.76	<0.0001
Developing Flange				1.00	1.00	33.18	<0.0001
Flanged				1.00	1.00	0.00	0.98

Table 2.7. Model analysis of the correlation between social interactions and cortisol levels.

GAMM	Estimate \pm SE	p-value
Model 1. Log. Cortisol ~ Total Social + Cage		
Intercept	4.12 \pm 0.11	<0.0001
Developing Flange	-0.01 \pm 0.15	0.94
Flanged	0.35 \pm 0.14	0.01
$R^2_{(Adj.)}$	0.15	
N Observation	88	
Model 2. Log. Cortisol ~ Received + Cage		
Intercept	4.11 \pm 0.11	<0.0001
Developing Flange	- 0.01 \pm 0.15	0.96
Flanged	0.35 \pm 0.14	0.02
$R^2_{(Adj.)}$	0.15	
N Observation	88	
Model 3. Log. Cortisol ~ Emitted + Cage		
Intercept	4.13 \pm 0.10	<0.0001
Developing Flange	-0.01 \pm 0.15	0.96
Flanged	0.33 \pm 0.14	0.02
$R^2_{(Adj.)}$	0.16	
N Observation	88	
Model 3. Log. Cortisol ~ Responded + Cage		
Intercept	4.14 \pm 0.11	<0.0001
Developing Flange	-0.01 \pm 0.15	0.97
Flanged	0.36 \pm 0.14	0.01
$R^2_{(Adj.)}$	0.15	
N Observation	88	

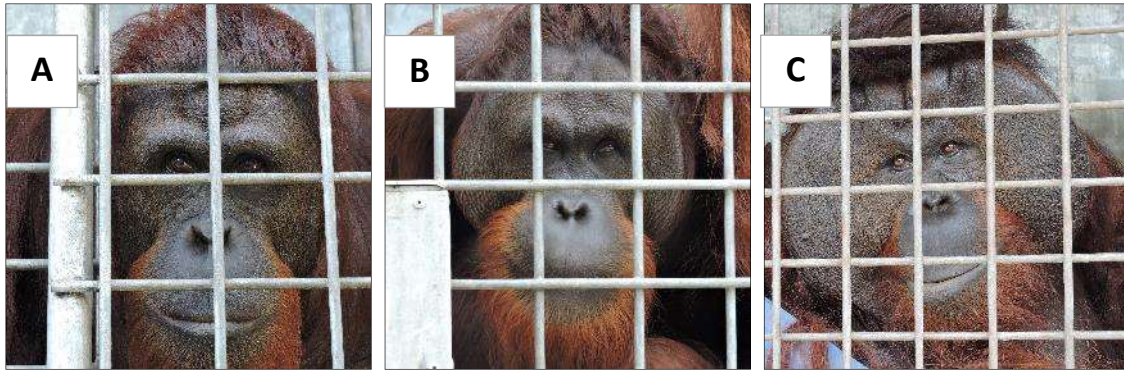


Figure 2.1. Three different developmental stages in male orangutans: (a) unflanged male that characterized by non-developed cheek-pads, (b) developing flange male which the size of flange was half developed, and (c) flanged male which has fully developed cheek-pads.

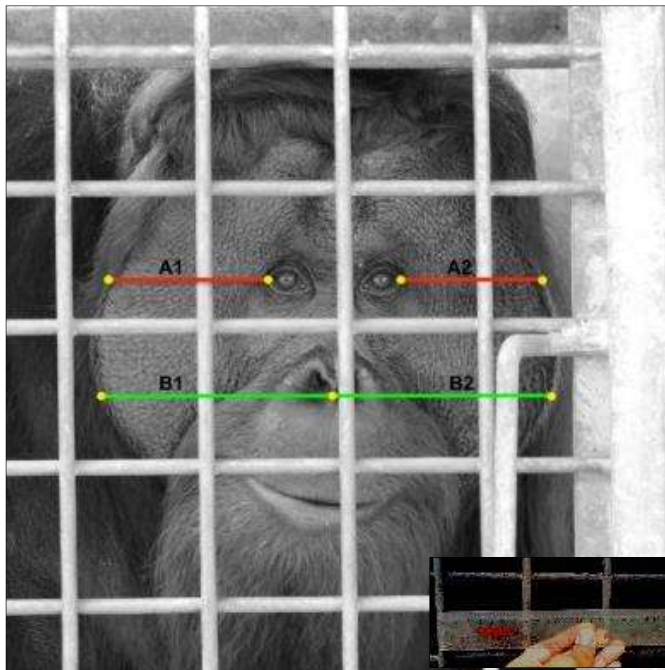


Figure 2.2. Two different landmarks that were used in the flange size measurement: eyes' landmark (A1 and A2) and nostril's landmark measurement (B1 and B2). **insertion image is the scale of the cage grid.*

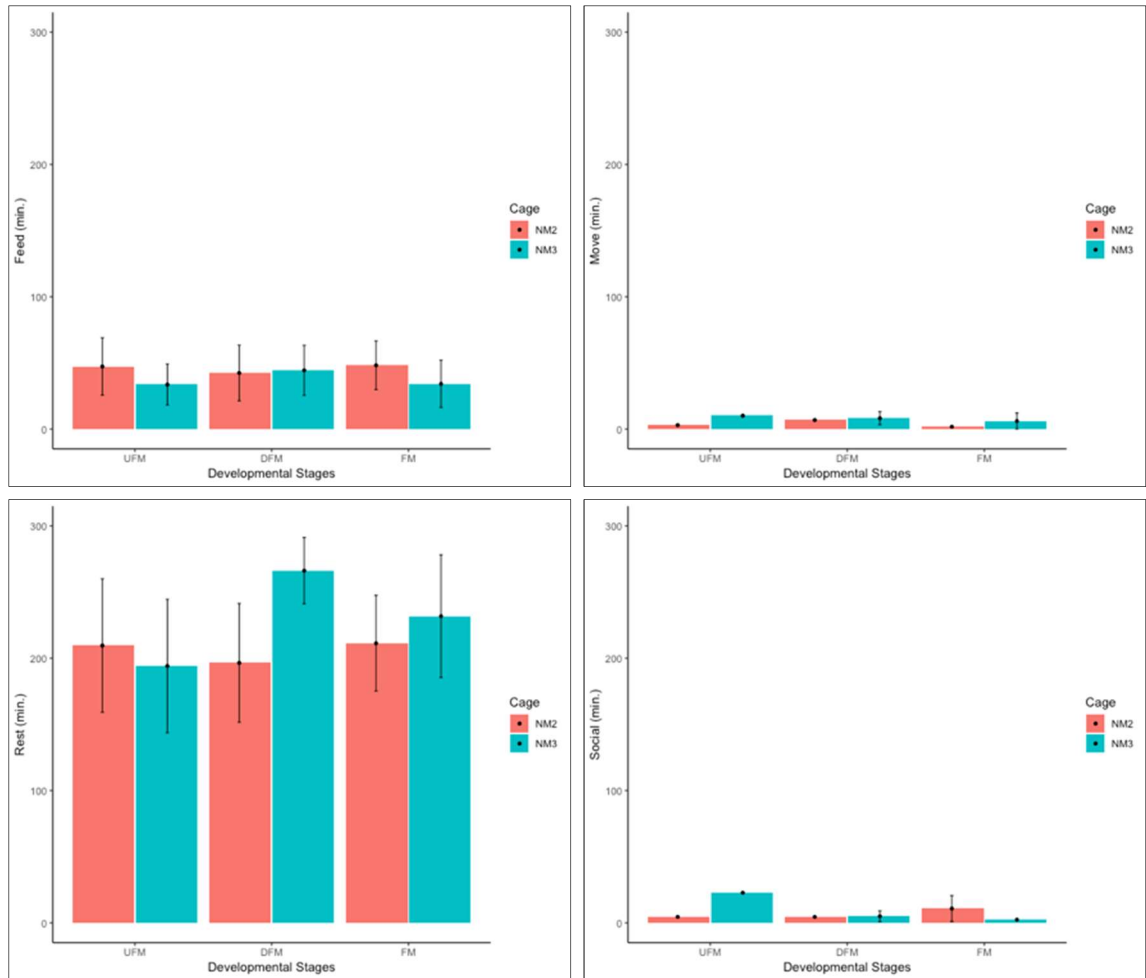


Figure 2.3. The bar plot of activity budgets among different male's developmental stages in NM2 and NM3. The plot determined the average of each activity budget with an error bar showing the minimum and maximum standard deviation. There are no differences in feeding activity among stages in and between cages. Moving activity differed between unflanged males (UFMs) and flanged males (FMs) (GAMM Estimate \pm SE = -2.55 ± 1.26 , $p = 0.04$), whereas UFMs and developing males (DFMs) were similar. FMs tend to rest more frequently than UFMs (GAMM Estimate \pm SE = 17.91 ± 6.63 , $p = 0.01$), meanwhile UFMs and DFMs tend to be the same. Social behavior among stages were significantly different (GAMM UFMs-DFMs Estimate \pm SE = -8.27 ± 2.84 , $p = 0.004$ and UFMs-FMs Estimate \pm SE = -5.77 ± 2.16 , $p = 0.008$).

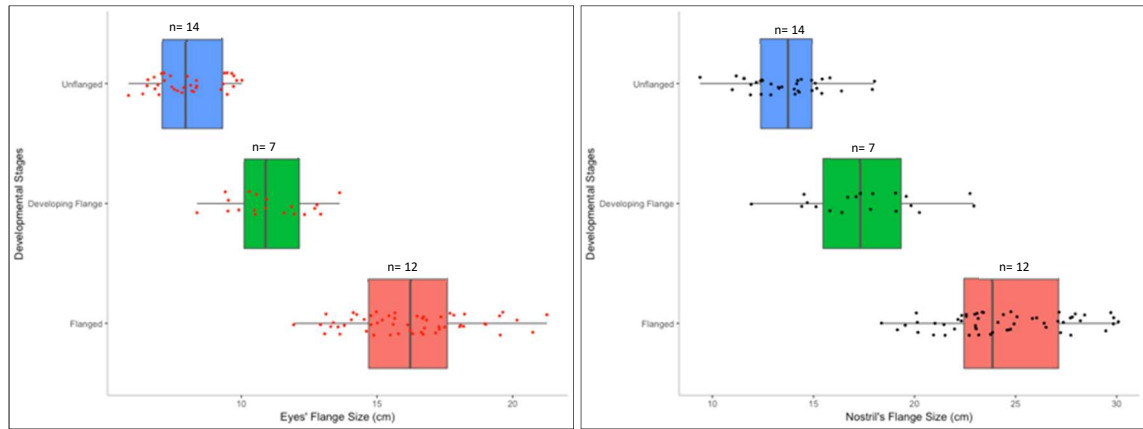


Figure 2.4. Flange size (cm) differentiation among male's developmental stages: Left: the measurement from eyes' landmark showing significantly different between stages (Kruskal-Wallis's $\chi^2 = 85.96$, $df = 2$, $p = 0.001$); and Right: the measurement from nostril's landmark was also showing significantly different between stages (Kruskal-Wallis's $\chi^2 = 68.25$, $df = 2$, $p = 0.001$).

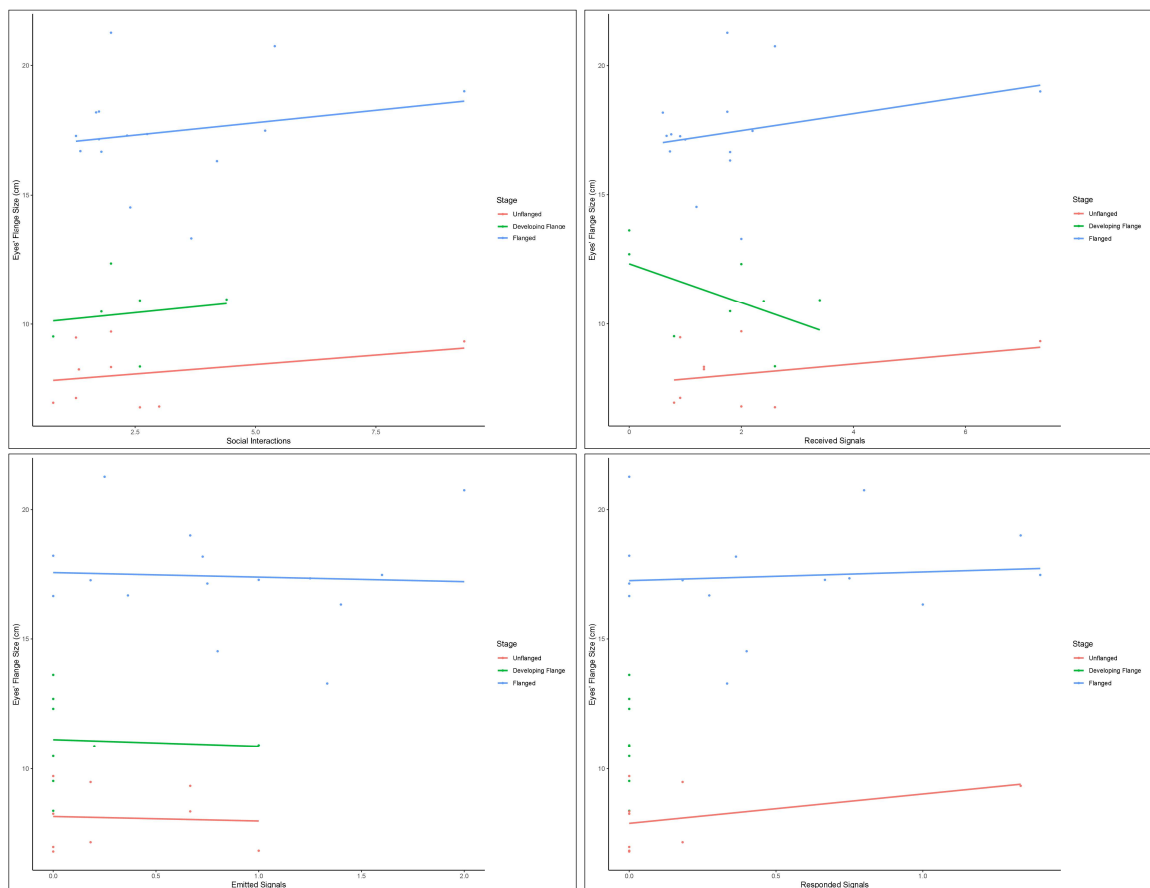


Figure 2.5. Interaction between flange size from eyes' landmark measurement and a. Total social behavior; which shown flange size wasn't related to social interactions in all male's developmental stages. b. The number of received signals which also unrelated

with the flange size; *c. The number of emitted signals* that shown small skin-ridge size in UFM's tend to have higher emitted signals (GAMM UFM's $p = 0.001$); and *d. The number of responded signals* which shown increasing responded signals related to the skin-ridge and flange size in UFM's and DFM's (both GAMM $p < 0.0001$).

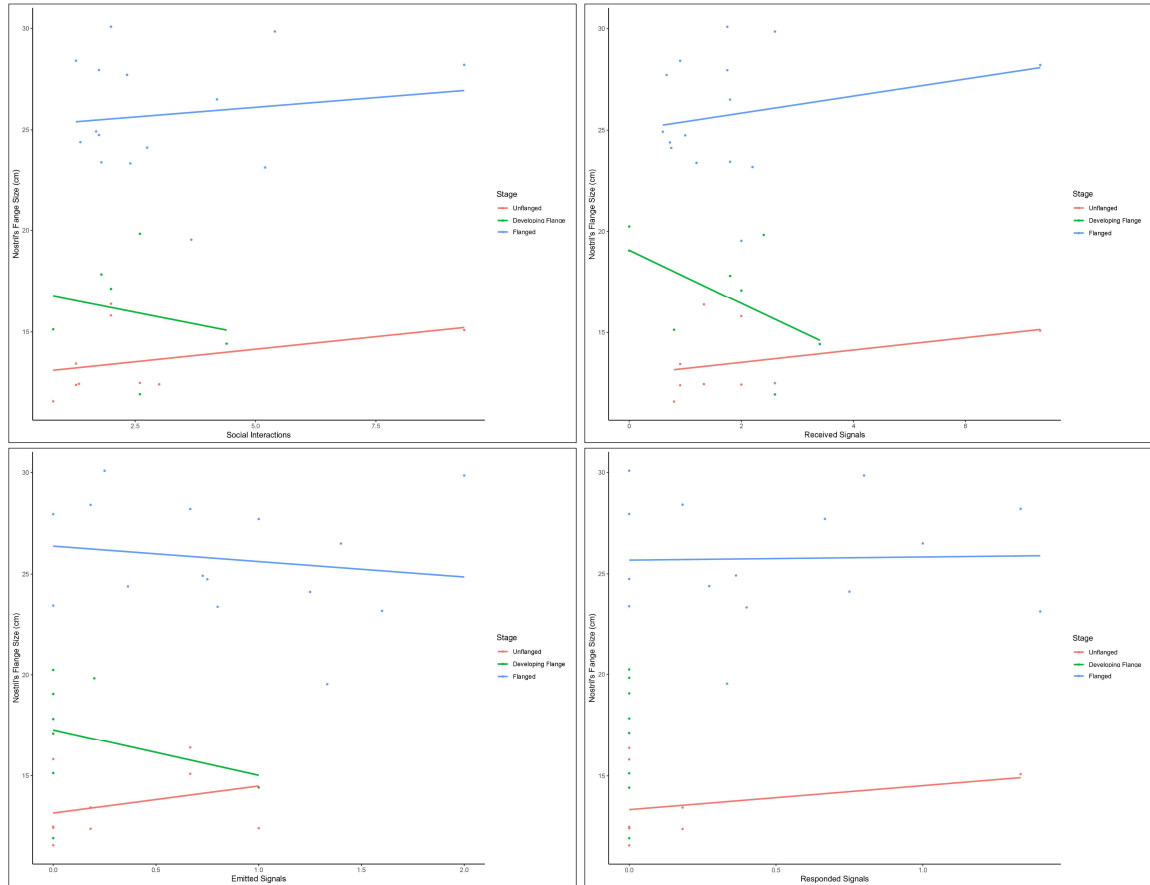


Figure 2.6. Relationships between flange size from nostril's landmark measurement and *a. Total social behavior*; which shown flange size wasn't related to social interactions in all male's developmental stages. *b. The number of received signals* which also unrelated with the flange size; *c. The number of emitted signals* that shown not related to the flange size; and *d. The number of responded signals* which shown increasing responded signals related to the skin-ridge and flange size in UFM's and DFM's (both GAMM $p < 0.0001$).

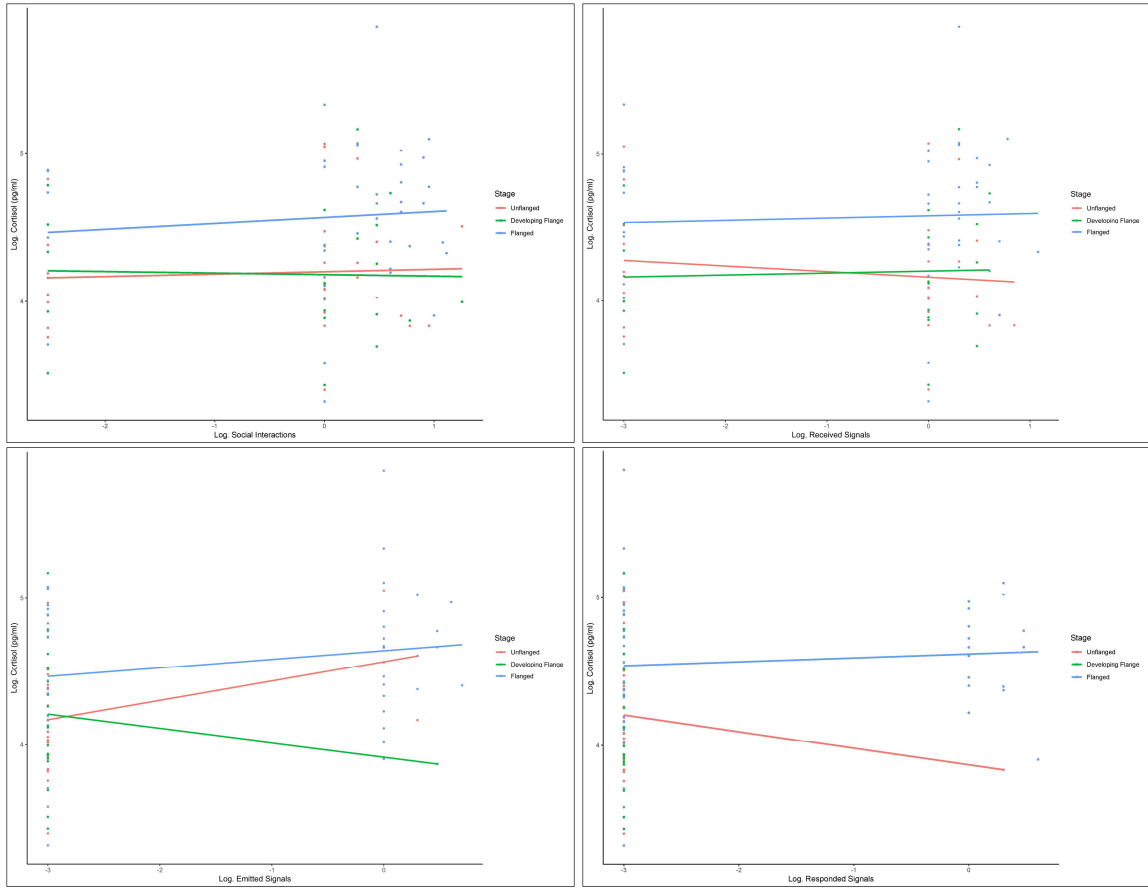


Figure 2.7. Relationships between cortisol concentrations and *a. total social behavior* which shown the more frequent social interactions related to the cortisol productions in FMs but not with DFMs (GAMM FMs $p = 0.01$); *b. the number received signals* that shown high number of received signals related to the elevated cortisol levels in FMs and inversely in DFMs (GAMM FMs $p = 0.02$); *c. the number of emitted signals* which shown increasing the number of emitted signals related to the high stress in FMs (GAMM $p = 0.02$); and *d. the number of responded signals* which also shown increasing cortisol levels related to the high number of responded signals in FMs (GAMM $p = 0.01$).

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3. Chapter III. Nutrient-Dependency in Flange Development of Bornean Orangutans

3.1. Introduction

The sequential development of irreversible cheek-pads or “flanges” in adult male orangutans has intrigued researchers for decades, yet we still have little understanding of what triggers their development and why there is such variation in the timing of flanging. Flanges are only found in males that would be aroused once reproductively matured (Mackinnon, 1974a). Interestingly, some males have delayed their flange development for short or even prolonged periods (Dunkel et al., 2013; Utami-Atmoko et al., 2002). Having these two differing characteristics of maturity is known as bimaturism (Jarman, 1983; Leigh & Shea, 1995). Beside the flange differentiation, bimaturism in male orangutans is also defined by the different size of throat pouch, hair length, and body mass (Utami-Atmoko et al., 2002; Winkler, 1989; Zihlman et al., 2011).

The timing of flanging is thought to be driven by opportunity costs, including variation in reproductive success among these two male morphologies (i.e., attracting females) and energetic status. Emery-Thompson (2017b) hypothesized that individuals in a nutritional-stressed condition would have slower reproductive maturation in males, but it could have the opposite effect on females. As a frugivorous primate, orangutans prefer to consume fruit when it is available (Delgado & van Schaik, 2000; Galdikas, 1988; Mackinnon, 1974a; Russon et al., 2009). Fruit provides greater energetic return compared to fallback foods, such as leaves and bark (Vogel et al., 2017; Vogel et al., 2009). Some evidence has been reported that the irregularity and unpredictability of

fruit availability has led to a suite of physiological adaptations (Knott, 1998; Vogel et al., 2012). It has been hypothesized that the orangutans' reduced their daily energy expenditure ("hypometabolism") may be a physiological adaptation to prevent starvation when they consume low energy foods during episodes of fruit scarcity (Pontzer et al., 2010). As an indicator, ketone bodies production, a by-product of fat catabolism, is found during periods of negative energy balance (Knott, 1998; Vogel et al., 2017).

Under neutral energy balance, the hypothalamus will release gonadotropin-releasing hormone (GnRH) in the pulsatile manner to control the production of luteinizing hormone (LH). The acceleration of LH secretions are controlled by multiple feedback hormones such as insulin, cortisol, and growth hormone (Martin et al., 2008; Rogers, 2012). In times of negative energy balance, the pulsatile of released LH is reduced. In response, the brain will produce agouti-related peptide (AgRP) to stimulate hypothalamic-pituitary-adrenocortical axis to inhibit insulin (Martin et al., 2008; Martin et al., 2007). Insulin is a protein hormone that is released by pancreatic adrenal glands in response to high glucose intake, although carbohydrates, amino acids, lipid, and ketone bodies also can initiate the producing of insulin (Greenstein & Wood, 2006). Increasing the level of insulin will be correlated with the production of connecting peptide (C-peptide) as a by-product of proinsulin conversion. This peptide hormone can be found in the urine and allows researcher to use it as an indicator of energetic status (Emery-Thompson, 2017a).

Several studies have found a correlation between energetic status and the expression of secondary sexual characteristics. For example, a study on male fruit flies (*Drosophila melanogaster*) observed that the appearance of sex combs was regulated by high nutrient intake (Gray et al., 2018). Nutrition restriction was also caused different signal rates in the male crickets (*Gryllus veletis*) (Harrison et al., 2017) and female zebra finches (*Taeniopygia guttata*) (Honarmand et al., 2015), they found that during the low nutrient treatment both species produced lower signaling rates. We know very little about the link between energetic status and flange development in orangutans. A previous study about orangutan's energetic status observed that wild adult male consumed more calories (8422 kcal/day) than adult female (7404 kcal/day) (Knott, 1998). A similar finding was also found in other wild orangutan population, the average of caloric intake in flanged male was slightly higher than unflanged male (1762 kcal/day and 1665 kcal/day respectively) (Harrison et al., 2010). Vogel et al., (2017) found no difference in caloric intake between flanged and unflanged males. However, when they controlled for metabolic body mass, (Vogel et al., 2017) found flanged male consumed significantly lower calories than unflanged male (108.18 kcal/kg and 204.14 kcal/kg respectively).

During the rehabilitation process, orangutans have access to constant energy intake from a regular food supply and thus are not likely to be food limited. Thus, if they are not food limited, orangutans might be in a positive energy balance state and would store body fat and/or build skeletal muscle. I hypothesized that:

- 1) The amount of energy consumed by unflanged, developing flange, and flanged males does not differ, and I predict that all males in the different developmental stages will be in the state of positive energy balance; and
- 2) If individuals are in a positive energy balance, the secretions of the growth hormone, which regulate flange development, would not be disrupted (Martin et al., 2008; Rogers, 2012). Because males are not likely to be nutrient limited, I hypothesize that the development of flanges in male orangutans would not be nutrient dependent, and I predict that flange size will not be correlated with energy intake.

3.2. Methods

3.2.1. Research Site and Sample Size

This study was carried out at an orangutan rehabilitation center called Nyarumenteng in Palangkaraya, Central Kalimantan, Indonesia (2°02'23.4"S and 113°45'06.4"E). Data collection was focused on adult mature male orangutans based on their ages and developmental stages, that is, unflanged, developing flanges, and flanged males (Figure 3.1) The categorization of male developmental stages can be seen in the Chapters 1 and 2. Twenty-five adult mature male orangutans were selected as a sample size that contains nine unflanged males (UFMs), seven males are in the stage of developing their flange (DFMs), and nine fully flanged males (FM) (Table 3.1).

3.2.2. Flange Morphometrics

To quantify flange size, facial morphometric followed the methodology that was previously used by asymmetry facial studies in human and non-human primates

(Boulton & Ross, 2013; Little et al., 2012; Penton-Voak et al., 2001). A full-face frontal photograph with a neutral facial expression was captured once a month. Occasionally, physical flanges measurement was also conducted during health examination when the orangutan had been anesthetized. Two different landmarks were measured to calculate flange size. The first landmark is the eyes' landmark, which measured the length of flange from the tip of right (A1) and left (A2) eye to the edge of flange. The second is the nostril's landmark, which measured the length of flange from the tip of nose to the edge of right (B1) and left (B2) flange. Flange size was determined by the sum of each landmark measurements. For more explanation on how the measurement was conducted see Chapter 2.

3.2.3. Activity Budget and Energy Intake

Activity budgets were calculated from focal follow data collected following the standardized focal animal observation by www.aim.uzh.ch/orangutannetwork. Data collection was carried out by using two-minute interval focal animal instantaneous sampling, beginning at 06:00 AM when individual came out from the sleeping site and ending at 17:00 PM, when they usually went back to the sleeping sites. Four general orangutan activities were recorded including feeding, moving, resting, and social activity. For feeding, I noted the species and the amount of food consumed. In addition, any interesting behaviors specifically in the social context such as vocalizations and physical contact were also recorded by using ad-libitum sampling methods (Altmann, 1974).

In the rehabilitation center, the technician would provide regular food to each orangutan following guidelines from the Orangutan Veterinary Advisory Group (OVAG). Twice a day, in the morning and at noon, orangutans were provided with a large, substantial meal. Small quantities of additional foods, such as honey, seeds, and ice cubes, were served in between these main meals and mostly used to improve the orangutans' foraging skills. Prior to their meals, I weighed all of the food based on the food type (e.g., fruit, vegetable, etc.). Because the cages are built above the ground, leftovers could easily be collected and weighed; however, some orangutans occasionally tried to keep the excess food in their food tray. However, as an established and standardized practice, the cage would be cleaned every single day, which means any leftover and food stored by the orangutans would be collected by technicians. At the end of the day, I collected and weighed any leftover food. The total grams of consumed food were calculated by subtracting the leftover food from the supplied meals.

The nutritional composition of the foods was mainly downloaded from the SMILING D3.5-a -The food composition table for Indonesia-(<http://www.fao.org>) and the USDA food composition databases (<https://ndb.nal.usda.gov>). In addition I also used the report from Nyarumenteng orangutans rehabilitation center (Harrison & Applegate, 2010) and other sources if nutritional composition was not found in the SMILING and USDA databases. Because most of the downloadable sources only provided total energy (Kcal/gr) as fed/wet weight basis, I could directly use those data and multiply them by total grams of consumed food. I used standard physiological fuel values to calculate total energy intake from the database using the equation: $(4 \times \%TNC) + (4 \times \%CP) + (9 \times$

%lipids) + (0.543 x %NDF) for daily intakes of macronutrients, which has been previously used by several studies on wild orangutans (Conklin-Brittain et al., 2006; Harrison et al., 2010; Morrogh-Bernard, 2009; Vogel et al., 2017; Vogel et al., 2015). The NDF physiological fuel value uses assumes low-fermentation metabolizable energy (partial fiber fermentation i.e., 0.543), as this was suggested by Conklin-Brittain et al. (2006) for orangutans.

Following Vogel et al. (2017), I examined variation in total energy intake and energy intake per kilogram of metabolic body mass. Individual body mass was calculated from the latest health examination by the veterinarian, and all data was observed during this study time. Under the supervision of the veterinarian, technician has placed the anesthetized orangutan onto the big weigh scale with the precision sensitivity 0.01 kg. Moreover, if the individual body mass wasn't available, I used the average body mass per developmental stage (Table 3.1). The average body mass of UFM is 54.47 Kg, DFM 61.10 Kg, and FM 76.87 Kg. These numbers differed from the published wild orangutans body mass, which the body mass average of UFM and FM was 40.5 Kg and FM 74 Kg respectively (Rayadin & Spehar, 2015). To examine the latter, I divided individual energy intake by metabolic body mass (MBM), MBM was the body mass of the individual raised to the 0.73 (Pontzer et al., 2014). Daily energy intake was calculated by the sum of total caloric consumed foods in a day. The monthly energy intake was calculated by dividing total energy intake per month by the number of observation day.

To estimate daily energy expenditure (DEE kcal/day), I applied an equation that was previously used by Vogel et al. (2017), that is, $DEE \text{ kcal/day} = [439.32 \times \text{body mass}]$

(kg)]^{0.324}. To calculate the DEE expectation among developmental stages, I entered the average of body mass into this equation.

3.2.4. Urinary C-peptide of Insulin

A non-invasive urine sampling method was applied to collect orangutan urine on a daily basis. We prioritized morning urine (06:00 AM – 10:00 AM) to minimize circadian rhythm's effect (Fuleihan et al., 1997). Right after collection, urine was stored at low temperatures (approximately < 5⁰ Celsius) and transferred to -15⁰ Celsius for longer storage time. All collected samples were transported to the Laboratory for Primate Dietary Ecology and Physiology, Department of Anthropology, Rutgers University for further analysis.

Urinary C-peptide was assayed by using EMD Millipore's Human C-peptide radioimmunoassay kit (HCP-20) that is designed to measure C-peptide concentration (UCP) in human's serum, plasma, and other tissue culture with the limit of assay sensitivity is 0.065 ng/mL (Merck, 2013). This method has been validated in orangutans (Emery-Thompson & Knott, 2008; Knott, 1998), chimpanzees (Emery-Thompson et al., 2009), bonobos (Deschner et al., 2008), and humans (Ellison & Valeggia, 2003). Our Intra-assay coefficient of variation is 4.23% with n= 242, and inter-assay coefficient of variation for low control is 5.05% and high control in 1.98% with a total 4 assay kits.

As a correction of the hydration status, UCP was corrected by specific gravity (SG) following Miller et al. (2004), which is Corrected UCP = UCP x Corrected SG. Corrected SG = ((Average SG population – 1.000)) / ((SG sample – 1.000)). The SG for the population was 1.007. The SG value is related to the amount of secreted chemical particles in the

urine and can be used as an indicator of dehydration or even the failure of organs function such as cardiovascular and kidney (Lee et al., 2013; Wang et al., 2015). By delineating the water component, the pure urine concentration would be obtained and can be used to adjust the urinary hormones concentration.

3.2.5. Data Analysis

Generalized additive mixed models (GAMMs) were used to examine variation in energetic status in all orangutan males and among different developmental stages using *mgcv R package* (Wood, 2018). Orangutan ID was treated as a random effect in each model. Analyses were run with total energy intake and total energy intake/ Kg MBM. Absolute energy intake was controlled by metabolic body mass from each focal; if the information has not available, we used an average body mass for each developmental stage from Nyarumenteng (Table 3.4). The correlation between body mass and total energy intake was carried out with Pearson's pair test.

Kruskal-Wallis and multi-comparison tests were conducted with *pgimerss R package* (Giraudoux et al., 2018)) to identify which stages were greater if there was a significant model. The significance level was set at $\alpha < 0.05$. All statistical analyses were conducted in R Studio 1.1.442 (R-Core-Team, 2013) by using the model formula:

Model= gamm (y ~ x, data = data, family = gaussian, random = list (Orangutan ID= ~1)

3.3. Results

The total of feeding time among male developmental stages did not differ (Kruskal-Wallis's $X^2 = 0.88$, $df = 2$, $p = 0.66$), and there was very little variation among the

stages (Mean \pm SE of UFM= 41.09 ± 2.15 , DFM= 42.62 ± 3.18 , and FM= 41.96 ± 1.83), indicating that once the meals were served, individuals fed immediately until the food was all consumed (Table 3.2).

3.3.1. Orangutans' Energetic Status

As expected, due to regular food supplies, all orangutans were in the positive energy balance. Urinary ketone bodies were not detected in any of the orangutan urine samples throughout the study period (n= 279). Thus, individuals did not enter into a state of fat catabolism as they do in wild populations.

3.3.1.1. *Energy intake*

The average of body mass among developmental stages differed: UFM's had smaller body mass compared to DFM's, and FM's tended to be the heaviest individuals weighing about 20 kilograms more than UFM's (Mean \pm SE of UFM= 54.47 ± 4.09 , DFM= 61.10 ± 5.63 , and FM= 76.87 ± 4.45 , Table 3.4). During the study, twenty-eight food items were supplied to orangutans in the center (Table 3.3). The amount of food supplied to the males did not vary greatly, although it did sometimes vary slightly (Mean Kg \pm SE: UFM= 4.37 ± 0.24 , DFM = 4.87 ± 0.28 , and FM= 4.35 ± 0.18). Based on their body mass, the expected daily energy expenditure (DEE) between UFM's and DFM's was similar (1603.06 Kcal/day and 1663.80 Kcal/day respectively); meanwhile FM's had a greater DEE (i.e., 1792.21 Kcal/day). For this study, there was a significant negative correlation between total daily energy intake and body mass when all stages were included in the analysis (Pearson's: $t = -2.85$, $df = 233$, $p = 0.01$, $cor. = -0.18$), such that

heavier individuals had lower total daily energy intake. As expected, due to the higher amount of supplied foods in DFMs, their total energy intake was slightly higher ($2463.97 \text{ kcal/day} \pm 349.00$), whereas FMs had the lowest caloric intake ($1956.51 \text{ kcal/day} \pm 135.97$ (Table 3.4). However, there was no difference among the male development stages in total energy intake, despite flanged males being larger (Table 3.4, Figure 3.2). However, when the daily energy intake was controlled by metabolic body mass, UFM and DFMs had similar caloric intake (Estimate \pm SE = -1.40 ± 17.67 , $p = 0.94$), whereas UFM had significantly higher caloric intake compared to FMs (Estimate \pm SE = -38.81 ± 14.39 , $p = 0.01$; Table 3.4, Figure 3.3).

3.3.1.2. *Urinary C-peptide*

Urinary C-peptide concentrations among three male's developmental stages did not differ, similar total energy intake (Mean \pm SE of UFM = 1.66 ± 0.16 , DFM = 2.04 ± 0.19 , FM = 1.93 ± 0.17 ; Table 3.5, Figure 3.4).

3.3.2. Energetic Status and Flange Size

The size of flanges among the development stages from two different landmarks measurement (i.e., eyes and nostril) were significantly distinguished; FMs have larger flanges compared to UFM and DFMs (see Chapter 2). From the eyes' landmark measurement, there was no relationship between flange size and energetic status in all orangutans, and there was no relationship between flange sizes and energy intake scaled for metabolic body mass (kcal/kgMBM) or UCP concentrations (Table 3.6, Figure 3.5). There was a similar result when the model was run among developmental stages,

there was no relationship between energetic status and flange size among the different developmental stages (Table 3.6, Figure 3.5).

The same result was found using the nostril landmark measurement. The models that were run in all orangutans and among different developmental stages showed no relationship between the size of flange and the energy intake (kcal/kgMBM) (Table 3.7, Figure 3.6).

3.3.3. Energetic Status and Males Transition from Unflanged to Flanged: a Case Study

Fortunately, during the study period I was able to observe two matured unflanged males that transitioned into developing flange male stage, that is, Joni and Lopus. Both males were in the almost similar age, 14 and 16 years old respectively. I ran two GAMM models to examine variation in energy intake per KgMBM and urinary C-peptide levels during transitioned flange stages. There was no difference in energy intake of individuals as they transitioned from unflanged to developing flange (Estimate \pm SE= -138.60 ± 868.10 , $R^2_{(Adj.)} = -0.03$, $n = 37$, $p = 0.87$). The same was true of energy balance, there was no difference between the unflanged and flanging state in urinary C-peptide in these two males (Estimate \pm SE= 0.02 ± 0.11 , $R^2_{(Adj.)} = -0.03$, $n = 35$, $p = 0.87$; Figure 3.7).

3.4. Discussion

Orangutans who live in the rehabilitation center were supplied with daily foods following the guideline from the orangutans' veterinarians (Commitante et al., 2016). The main course was served twice daily, in the morning and afternoon. In addition, they

were also supplied with a small quantity of “snacks”, intended to improve their wild foraging skills, such as, honeybees, sunflower seeds, and termites log. Feeding times followed a regular schedule, and the technician typically would discard any leftover food at the end of the day. Because orangutans only had a small amount of time to eat once the food was served, they immediately eat and finish the majority of their food during each feeding. As a result, the total daily feeding time for all male developmental stages was similar.

The current study observed the average of body mass in unflanged males being smaller than developing flange males, whereas flanged males tend to have the heaviest body mass than others. Because there was a negative correlation between energy intake (Kcal) and body mass (Kg), orangutans in our study were in a good shape and likely far from being overweight. It appears that the rehabilitation center is providing a monitoring sufficient nutrition to individuals in the center and that they are not being over- or underfed. One study on wild Bornean orangutans in Eastern Kalimantan that was conducted in a poor habitat quality found that the average body mass of unflanged males was 40.5 kg, whereas flanged males weighed 74 kg (Rayadin & Spehar, 2015), and similar body mass range was reported by Markham and Groves (1990). These ranges of body mass are similar to what I found in this study, supporting the assumption that orangutans in the rehabilitation center were in the range of normal weight. Obesity was commonly observed in the zoo orangutans due to unmonitored food supplies (Cocks, 2007).

Total energy intake (kcal/day) did not differ among UFM, DFMs, and FM. This result is in line with previous studies that observed no variation in energy intake among sexes and developmental stages (Harrison et al., 2010; Knott, 1998; Vogel et al., 2017). The current study supported the hypothesis and was likely due to the amount of food provided for individuals – they all received about the same amount of food and consumed what was offered to them, thus it is not surprising that energy intake did not vary. When individual had low caloric intake, it was due to the consumption of low foods quantity. Although not statistically different, developing flange males consumed on average about 500 Kcal/day more than flanged males, and 300 kcal/day more than unflanged males. It is expected that males who are developing flanges required more energy compare to non-flanging males and flanged males, as they are investing energy somatic development and optimizing the hypothalamic function to accelerate growth hormones production (Ellison, 2003; Emery-Thompson, 2017b; Martin et al., 2008). However, based on DEE from body mass alone, UFM and DFM have about the same DEE but flanged males should have greater DEE because of their large body size. This estimate of DEE does not take into account of growth and development. It is also interesting that the flanged males do not eat more as they must support this large body mass. Flanged males moved about 50% less time than males in the other development stages, although this did not differ statistically. However, it is important to note that because of the cage environment, movements were restricted for orangutans. Overall, the activity budgets of the males in different development stages did not vary.

Interestingly, the energy intake per unit $M^{0.73}$ supported the idea that UFM and DFMs consumed significantly higher energy intake per unit body mass compared to FM. This result is in agreement with previous studies from other wild Bornean orangutans, which observed that immature males had the highest intake per unit body mass, and this intake declined as males reached maturity and even more when males were flanged (Vogel et al., 2017). Considering there have been no differences found in total energy expenditure using doubly labeled water between zoo and captive living orangutans (Pontzer et al., 2014), it is not surprising that the results are similar to what has been found in wild populations. First, it was assumed that decreasing energy intake per unit $M^{0.73}$ in FM is part of the strategy in maintaining metabolic homeostasis. In the wild, low caloric intake in FM may be related to conservative energy strategy by reducing its energy expenditure through less activity and decreased travel distances (Harrison et al., 2010; Pontzer et al., 2010; Vogel et al., 2017). Although not statistically different, the FM in our study also reduced their activity (i.e., less movement, slow foraging, and resting more often than UFM and DFM). More advanced examination related to energy expenditure specifically in flanged males is recommended to understand the correlation between their reduced energy intake and how they may maintain homeostasis.

Second, it is possible that the low caloric intake per unit $M^{0.73}$ in flanged males is related to their stored energy reserves. Cheek-pads or flanges are formed by fat that is bound to fibro-fatty tissues (Winkler, 1989) in mature males (Leigh & Shea, 1995; Mackinnon, 1974a). In addition, males that developed flanges also have been observed

to have an increase in body fat; Pontzer et al. (2010) calculated that the body fat in adult males orangutan was 16.3% greater than juvenile males, and 7% greater than adult females. Fat stores as a long-term energy supply are regulated by the feedback loop of the hormone leptin (Frayn, 2010). The abundant of fat stores would lead to higher levels of leptin that, in turn, stimulate the hypothalamus to decrease energy intake and increase energy expenditure (Friedman & Halaas, 1998). One study from human populations observed that daily energy intake was significantly decreased in overweight and obese populations compared to normal-weight populations (Austin et al., 2011). Moreover, a study of leptin profiles in chimpanzees found high leptin levels were associated with increasing body mass in adult males (Bribiescas & Anestis, 2010). Studies of the relationship between energy intake, leptin, and body mass are more complete in humans compared to non-human primates due to limitations of plasma sample collection from wild living animals. However, studies on captive animals would be promising, considering this current study observed similar patterns of energy intake between wild and captive animals.

Although FMs have lower total energy intake per $M^{0.73}$ compared to UFM and DFMs, this current study didn't find a correlation between flange size and energy intake per $M^{0.73}$. This overall result was supported with my observations in two UFM that transitioned into developing flange stage; growing the flange did not require higher energy intake. Perhaps because these males are getting enough energy, it does not influence flange development. It is possible that if these males were not receiving sufficient calories, flange development would have been delayed or slowed down. Thus,

because the males are well fed and not nutrient limited, energy intake does not influence their flange development. Future studies should look at the effect of reducing energy intake by 20 – 30% to examine how caloric restriction affects flange development.

To conclude, this study supports previous studies that total energy intake was not differ among matured male orangutans. Developing flanges required more energy compare to non-flanging males and developed flange males, as they are investing energy somatic development and optimizing the growth hormones production. By accounting body mass, energy intake per unit $M^{0.73}$ was not related to the changed of the developmental stage, flanged males tend to have lowest energy intake compare to other stages. Decreasing energy intake per unit $M^{0.73}$ was assumed as the strategy in maintaining metabolic homeostasis and stored energy reserves.

3.5. Tables and Figures

Table 3.1. Sample distributions which determined orangutan developmental stages i.e., unflanged (UFM), developing flange (DFM), and flanged males (FM). Age and body mass were gained during the study was carried out. Nutrition and urines collection were conducted at the same date with behavior data, whereas images data were taken at the end of each month.

No	Orangutan	Stage	Age	Body Mass (Kg)	Nutrition (Day)	Image (Month)	Urine (Day)
1	Casper	UFM	14	82.5	11	3	9
2	Ibut	UFM	12	52.5	15	2	13
3	Kevin	UFM	15	49.6	5	2	5
4	Lomon	UFM	13	49	6	1	6
5	Lopus	UFM	16	56.97	22	5	23
6	Nyun nyun	UFM	13	52	11	5	11
7	Paiman	UFM	13	40	5	5	5
8	Rambo	UFM	13	53.6	4	2	4
9	Stuart	UFM	13	47	1	3	1
10	Edoy	DFM	17	58.95	9	4	14
11	Joni	DFM	14	58.95	15	6	14
12	Juky	DFM	14	65	8	2	7
13	Kisar	DFM	15	64	8	4	8
14	Marwoto	DFM	18	68.3	5	3	5
15	Palingkau	DFM	13	58.95	-	-	3
16	Soni	DFM	17	50	1	1	1
17	Bento	FM	24	87.4	11	4	10
18	Charles	FM	20	75	11	6	11
19	Danny	FM	20	71.8	8	4	9
20	Gayo	FM	19	75	22	5	24
21	Inou	FM	12	77.18	10	4	10
22	Lucky Thai	FM	19	77.18	22	6	24
23	Sabun	FM	14	88.9	11	5	9
24	Saswoko	FM	19	55	10	5	9
25	Uncui	FM	14	85	5	4	5

Table 3.2. The average of activity budget among male's developmental stages.

Developmental Stages	Activity Budget (minute)			
	Feed (mean \pm SE)	Move (mean \pm SE)	Rest (mean \pm SE)	Social (mean \pm SE)
Unflanged	41.09 \pm 2.15	6.23 \pm 1.26	202.57 \pm 5.41	12.82 \pm 2.41
Developing Flange	42.62 \pm 3.18	7.00 \pm 1.40	204.79 \pm 7.48	4.55 \pm 1.07
Flanged	41.96 \pm 1.83	3.68 \pm 0.50	220.48 \pm 3.98	7.04 \pm 0.82
Kruskal-Wallis H test				
χ^2	0.88	2.76	6.67	1.30
df	2	2	2	2
p-value	0.66	0.25	0.04	0.52

Table 3.3. Food items that were supplied to orangutans in the center. Total energy (Kcal/gr) was obtained from downloadable resource SMILING (<http://www.fao.org>) and the USDA food composition databases (<https://ndb.nal.usda.gov>).

No	Food type	Total Energy (Kcal/gr)	Resource
1	Banana	3.54	USDA
2	Bitter melon	0.17	USDA
3	Breadfruit	1.37	SMILING
4	Cassava	1.56	SMILING
5	Coconut whole	6.75	USDA
6	Common Guava	3.54	USDA
7	Corn	3.52	USDA
8	Cucumber	3.24	USDA
9	Eggplant	3.25	USDA
10	Honeydew	3.54	USDA
11	Jicama	3.83	USDA
12	Long bean	3.64	SMILING
13	Orange (no rind)	3.56	USDA
14	Pomelo	3.50	USDA
15	Papaya	3.49	USDA
16	Peanut	6.05	SMILING
17	Pineapple	3.57	USDA
18	Raisin	3.54	USDA
19	Rambutan	0.79	SMILING
20	Rattan	0.37	Manohara, 2013
21	Squash	3.27	USDA
22	Starfruit	3.60	USDA
23	Sugar syrup	6.26	SMILING
24	Sugarcane	0.94	USDA

No	Food type	Total Energy (Kcal/gr)	Resource
25	Sweet Potato	3.78	USDA
26	Tempeh	2.19	SMILING
27	Tofu	0.89	SMILING
28	Watermelon	3.51	USDA

Table 3.4. The average of body mass (Kg), total energy intake (Kcal/day), and energy intake per kg unit M^{0.73} (Kcal/kgMBM) among male's developmental stages. Pearson test was carried out to observe the correlation between energy intake (kcal) and body mass in all orangutans and per developmental stage. GAMM models were tested the total energy intake (kcal) and energy intake per unit M^{0.73} in all stages.

Developmental Stages	Body Mass (Kg)	Energy Intake (Kcal/day)		Energy Intake (Kcal/kgMBM)
UFM _(n=9)	54.47 ± 4.09	2281.05 ± 267.92		121.88 ± 13.63
DFM _(n=7)	61.10 ± 5.63	2463.97 ± 349.00		120.47 ± 17.26
FM _(n=9)	76.87 ± 4.45	1956.51 ± 135.97		83.06 ± 5.69
Pearson's: Intake (kcal/day) ~ BM	All	UFM	DFM	FM
t	-2.85	-0.20	-1.42	-1.57
df	233	75	47	107
p-value	0.01	0.85	0.16	0.12
Correlation	-0.18	-0.02	-0.20	-0.15
GAMM: Intake (kcal/day) ~ Stages	Estimate ± SE	p-value		
Intercept	1954.54 ± 165.59			
DFM	260.98 ± 265.08	0.33		
FM	1.97 ± 215.15	0.99		
R ² _(Adj.)	0.003			
N Observation	232			
GAMM: Intake (kcal/kgMBM) ~ Stages	Estimate ± SE	p-value		
Intercept	121.88 ± 11.02			
DFM	-1.40 ± 17.67	0.94		
FM	-38.81 ± 14.39	0.01		
R ² _(Adj.)	0.03			
N Observation	235			

Table 3.5. The average of urinary C-peptide (ng/ml) secretions among male's developmental stages. GAMM model was run in all stage over the unflanged males.

C-peptide (ng/ml)	Mean ± SE	
UFM	1.66 ± 0.16	
DFM	2.04 ± 0.19	
FM	1.93 ± 0.17	
GAMM: UCP ~ Stages	Estimate ± SE	p-value
Intercept	1.66 ± 0.19	
Developing Flange	0.38 ± 0.29	0.19
Flanged	0.26 ± 0.24	0.28
R ² _(Adj.)	0.00	
N Observation	240	

Table 3.6. The GAMM models of energy intake (kcal/kgMBM) and urinary C-peptide (ng/ml) over the flange size (cm) from eyes' landmark measurement (n= 31).

GAMM	Smooth Terms	R2(Adj.)	edf	erf.edf	F-value	p-value
Intake (kcal/kgMBM) ~ Flange						
All Stages		0.06	1	1	2.90	0.10
UFM	s(Flange_Eyes)	0.04	1	1	0.29	0.60
DFM			1	1	1.64	0.21
FM			1	1	0.15	0.70
UCP (ng/ml) ~ Flange						
All Stages		-0.01	1	1	1.09	0.30
UFM	s(Flange_Eyes)	-0.01	1	1	0.02	0.89
DFM			1	1	0.19	0.67
FM			1	1	0.82	0.38

Table 3.7. The GAMM models of energy intake (kcal/kgMBM) and urinary C-peptide (ng/ml) over the flange size (cm) from nostril's landmark measurement (n= 31).

GAMM	Smooth Terms	R2(Adj.)	edf	erf.edf	F-value	p-value
Intake (kcal/kgMBM) ~ Flange						
All Stages		0.05	1	1	2.77	0.11
UFM	s(Flange_Nostril)	0.01	1	1	0.14	0.71
DFM			1	1	1.09	0.31
FM			1	1	0.38	0.54
UCP (ng/ml) ~ Flange						
All Stages		-0.03	1	1	0.64	0.43
UFM	s(Flange_Nostril)	-0.09	1	1	0.06	0.81
DFM			1	1	0.60	0.45
FM			1	1	0.07	0.80

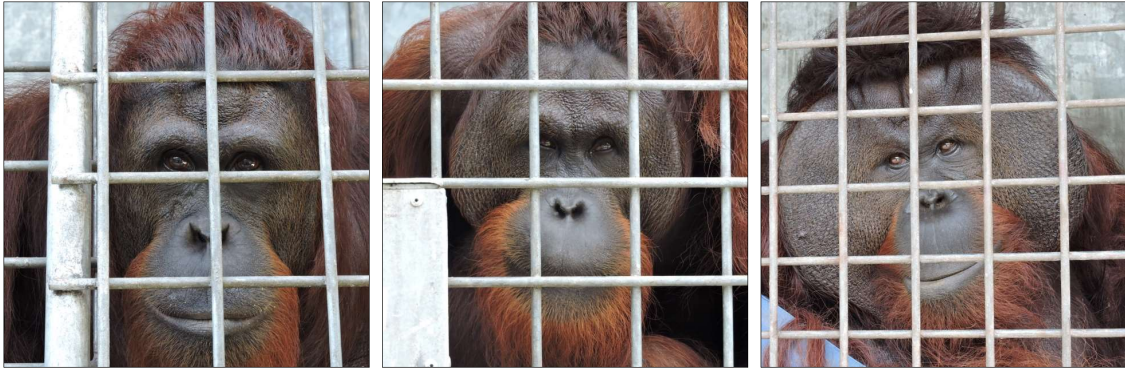


Figure 3.1. Three different developmental stages in male orangutans (*Left*: unflanged male, *Middle*: developing flanges male, and *Right*: fully flanged male).

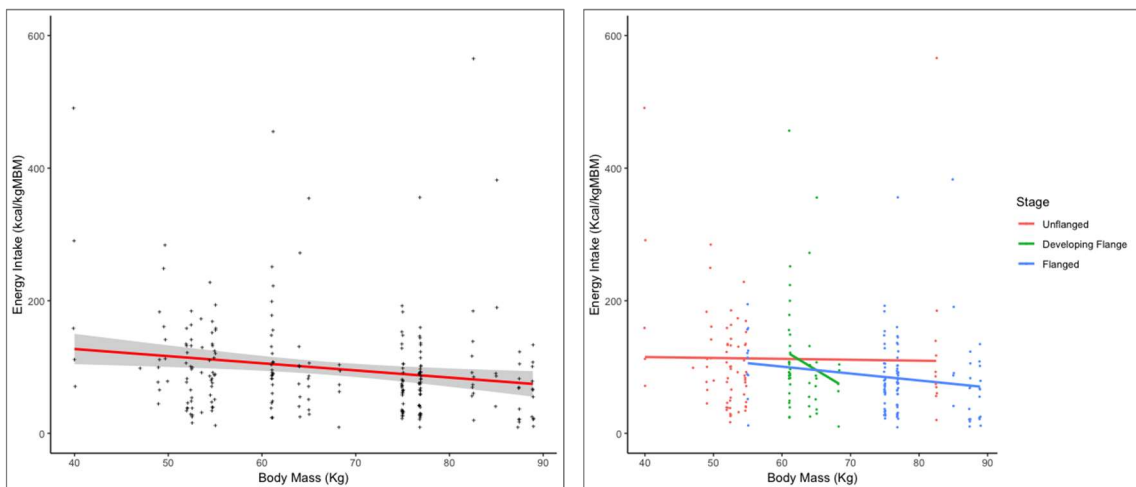


Figure 3.2. Pearson's test between body mass (kg) and absolute energy intake (kcal/day). Left: Increasing body mass was negative correlated with the daily energy intake (Person's $t = -2.85$, $df = 233$, $p = 0.01$, $cor. = -0.18$). Right: Body mass wasn't related to the stage's energy intake.

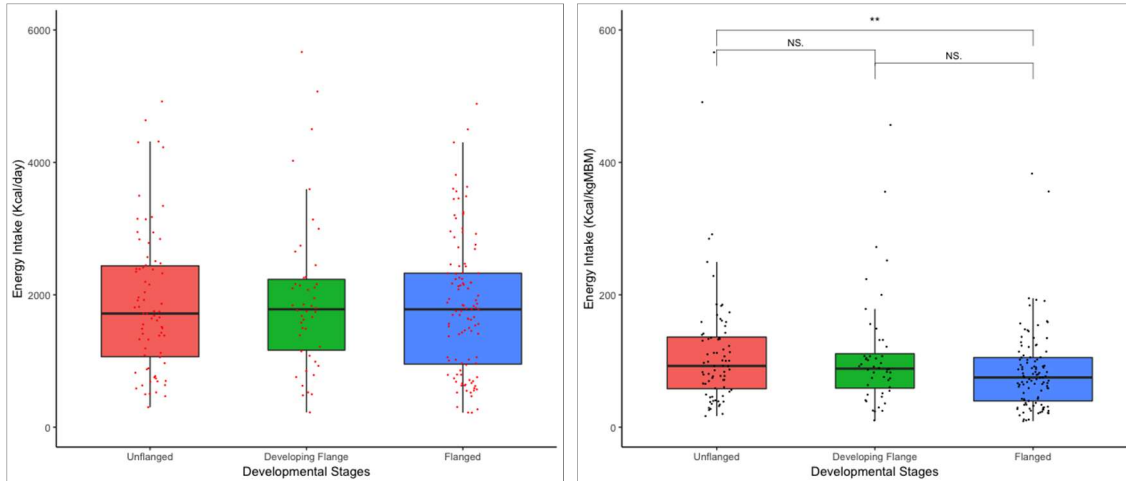


Figure 3.3. Boxplot of; *Left*: Total energy intake (kcal/day) among male's developmental stages which shown no differentiation among them. *Right*: Accounting metabolic body mass, UFM had significantly had higher energy intake per unit $M^{0.73}$ with FM (GAMM Estimate \pm SE= -38.81 ± 14.39 , $p=0.01$), whereas UFM-DFM and DFM-FM were not differed.

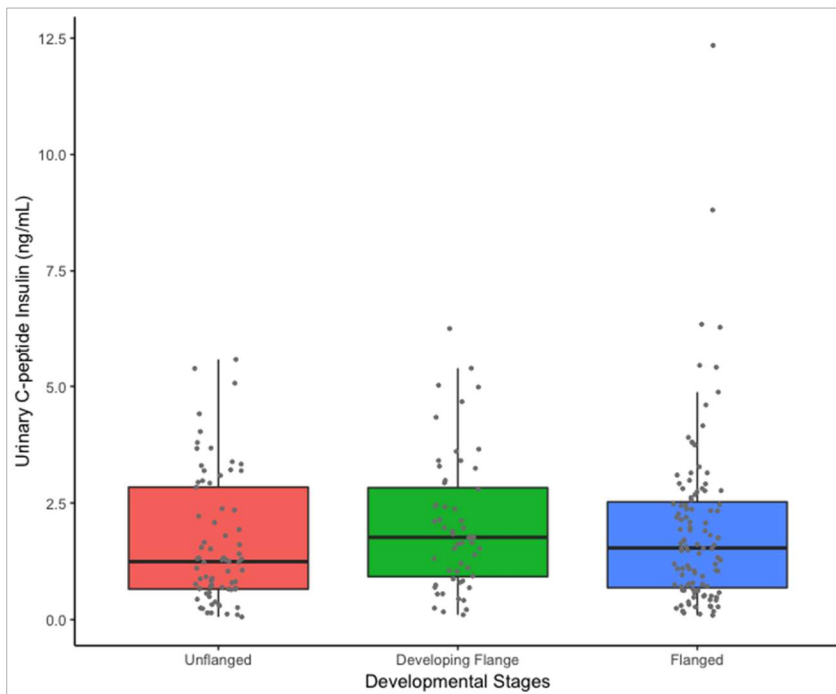


Figure 3.4. Boxplot of urinary C-peptide (UCP) concentrations among male's developmental stages. The GAMM model showed no differentiation among developmental stages.

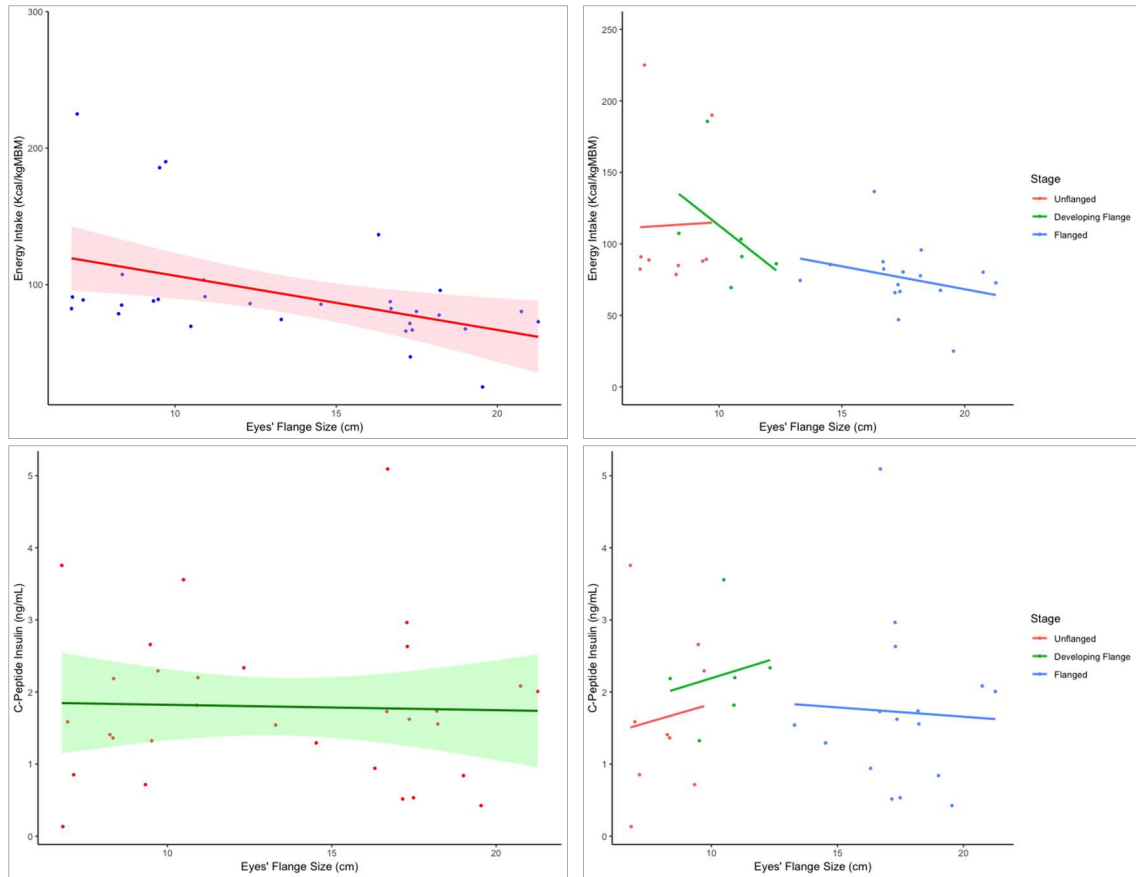


Figure 3.5. Interaction between eyes' flange size and, Top: energy intake (kcal/kgMBM) and Bottom: C-peptide (ng/ml), in all orangutans and among developmental stages. None of the GAMM models shown significant results.

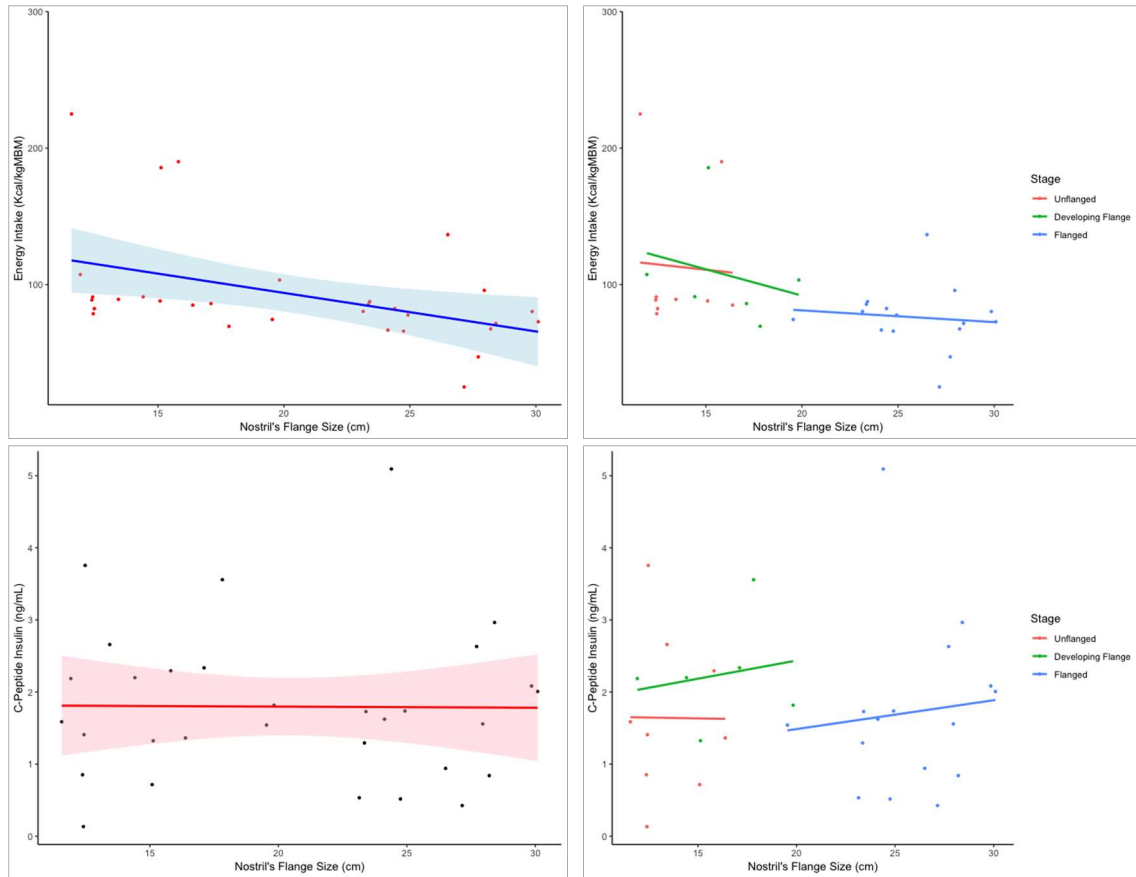


Figure 3.6. Interaction between nostril's flange size and, Top: energy intake (kcal/kgMBM) and Bottom: C-peptide (ng/ml) in all orangutans and among developmental stages. None of the GAMM models shown significant results.

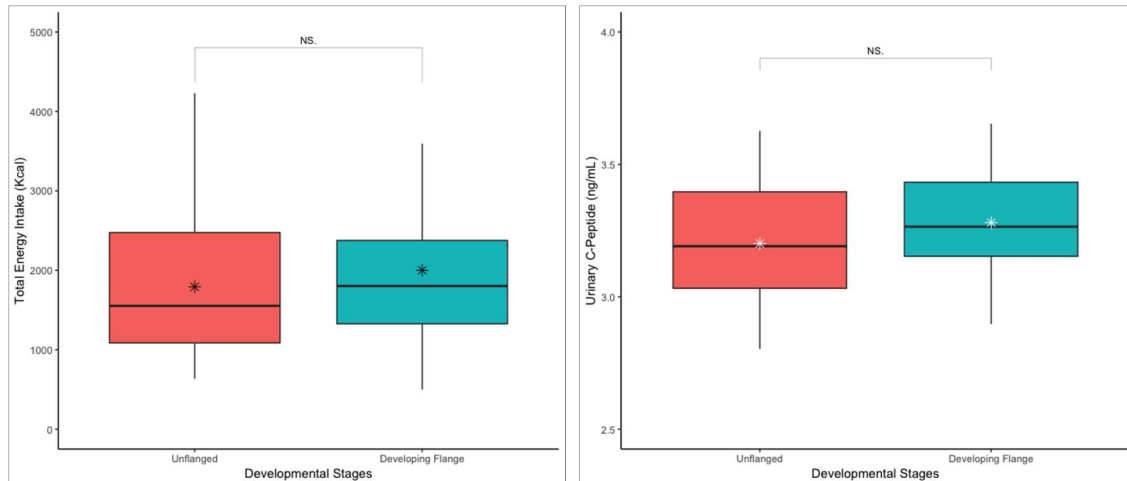


Figure 3.7. Bar plot of, Left: energy intake (kcal/kgMBM) and Right: urinary C-peptide (ng/ml) in transitioned flange stage. The asterisk (*) indicates the average of energy intake and urinary C-peptide in each developmental stage.

3.6. References

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4. Chapter IV. The price of bimaturism: Endocrinological status of wild and captive Bornean orangutans

4.1. Introduction

Bimaturism is defined as two different characteristics of a mature individual, which are formed sequentially; the different stages can be reversible or irreversible (Jarman, 1983). Among orangutans, bimaturism is found in matured males, that are characterized by an irreversible secondary sexual characteristics (SSCs) known as cheek-pads or flanges and a pronounced throat pouch (Mackinnon, 1974; Rijksen, 1978). The development of SSCs is regulated by changes in the physiological status and linked to the response of the hypothalamic-pituitary axis, specifically changes in hormone production by adrenal and testes glands (Rogers, 2012). The feedback loop of hypothalamic-pituitary-glucocorticoid (HPG) axis has effectively explained the manner in which the body responds to the stressor(s) by the activation of epinephrine and norepinephrine hormones. The appearance of these hormones stimulates the pituitary gland to release corticotropin-releasing hormone and initiate the production of adrenocorticotrophic hormone (ACTH). Increasing ACTH in the blood plasma triggers the adrenal gland to produce cortisol, another stress hormone (Rogers, 2012). As a response to the high cortisol levels, the pancreas immediately produces glucagon, which breakdowns body fat and protein present in muscles as glucose (Sapolsky et al., 2000). If the stress event occurs continually during an episode of restricted energy intake, Emery-Thompson (2017b) reviewed that energetic-stress would slow down the developmental

growth specifically infant and juvenile, reduce daily activity, delay reproductive maturation in male, and disrupt the immune system.

The study of cortisol aids in the understanding of physiological responses to changes in behavior, social situation, and during development. For instance, high cortisol levels have been observed to be related to both social status and behavior in a variety of species (Sapolsky, 2004). High cortisol was detected when an adult monogamous marmoset monkey pair was separated (*Callithrix penicillata*), in addition to an increase in their locomotion and long call rates (Duarte et al., 2018). Elevated cortisol was also found in association with high rates of aggression among high ranking male bonobos (*Pan paniscus*) (Surbeck, Deschner, Weltring, et al., 2012) and olive baboons (*Papio anubis*) (Sapolsky, 1992). Moreover, high cortisol levels of female chimpanzees (*Pan troglodytes*) were correlated with their respective age, social dominance, and reproductive status, in which young, low ranking, and lactating individuals exhibited high cortisol concentrations when compared with high ranking females (Emery-Thompson et al., 2010). High cortisol levels were also hypothesized to suppress the development of secondary sexual characteristics in orangutans: undeveloped flanged (unflanged) males had higher cortisol level in comparison to flanged males (Maggioncalda et al., 2002). However, the wild orangutans study conducted by Marty et al. (2015) found a different pattern, flanged males produced similar glucocorticoid levels with unflanged males.

Among males, SSCs are elevated during the onset of puberty, which is furthermore regulated by the expression of hypothalamus-pituitary-testes (HPT)

hormones (Rogers, 2012). The hypothalamus releases gonadotropin-releasing hormone to stimulate the anterior pituitary gland in the production of luteinizing hormone (LH). Increasing LH stimulates the testes to generate testosterone hormone which is critical for the development of SSCs such as facial hair, muscle mass, and libido (Laycock & Karim, 2013; Rogers, 2012). Muller (2017) reviewed the correlation between male reproductive challenge. He hypothesized that elevated testosterone levels were observed in the high social status male and the winner from the aggressive encounters. Moreover, high testosterone was found to be correlated to the development of muscle mass, secondary sexual characteristics, and the vocalization tract. However, the perceived advantages from high testosterone were suggested could decrease pain sensitivity which increase the potential of injury during competition (Muller, 2017).

Orangutans (*Pongo sp.*) provide an excellent opportunity to study the relationship between hormones and the development of SSC, specifically the development of cheek-pads or “flanges”, which has been a constant source of intrigue for the researchers for decades. Despite this interest and intrigue, there is still limited knowledge and studies on the factors that triggers flanging and the presence of variation in the timing of flanging. This is mainly because sample sizes have always been small on adult male orangutans in the wild because they range very far and are often hard to follow (Buckley, 2014; Singleton & Van Schaik, 2001) and the methods for examining hormones in remote conditions have only recently been developed (Behringer & Deschner, 2017). This current study examines variation in urinary cortisol and testosterone from two different types of orangutan populations: wild and captive.

Moreover, I assessed the variation in the three different male's developmental stages (unflanged (UFM), developing flange (DFM), and flanged (FM)) to examine potential hormonal triggers in bimaturism in orangutans. Three main studies in relation to hormonal regulation of orangutan bimaturism has been conducted both in the captive/zoo and wild environment, however, no concrete agreement was formulated (Emery-Thompson et al., 2012; Maggioncalda et al., 1999; Marty et al., 2015) . For example, Maggioncalda et al. (2002) studied zoo orangutans and found that high cortisol concentrations were found in DFMs, which they explained was due to higher social stress events, and the concentrations declined and remained stable when the flange was fully developed. Meanwhile, a study from a wild population (Tuanan) showed different result; Marty et al. (2015) observed similar cortisol levels among UFM and FM, which they explained was due to a stress avoidance strategy used by UFM.

The orangutans in the current study were housed in an individual cage, which was assumed akin to the social setting of the wild population, as male orangutans spend most of their time alone. However, unlike captivity, wild males are often physically very isolated from other males and male-male interactions, although potentially lethal (Marzec et al., 2016), are rare (Spillmann et al., 2016). Thus, in accordance with the assumption, I hypothesized that cortisol levels among the different male developmental stages both in the wild and captivity wouldn't be different. In line with previous findings, I predicted cortisol levels among male's developmental stages that live both in the wild and in captivity would not vary because they are able avoid stressful encounters. I also examined variation in urinary testosterone among males' developmental stages from

the wild and captive populations. Previous studies from the wild and zoo populations have found similar patterns: higher testosterone levels were observed while males develop their flanges (DFM) relative to flanged or unflanged males (Emery-Thompson et al., 2012; Maggioncalda et al., 1999; Marty et al., 2015). Following those findings, I hypothesized that the testosterone levels among different male's developmental stages would vary with DFMs having the highest testosterone levels, Flanged males below the DFM levels, and UFM's would have the lowest levels. Due to different social status, diet, and ecology which known related to the physiological status, this current study wasn't compare cortisol and testosterone levels between wild and captive populations.

4.2. Methods

4.2.1. Study Site and Data Collection

This study was carried out at two sites. The first site was located at an orangutan rehabilitation center called Nyarumenteng in Palangkaraya, Central Kalimantan, Indonesia (2° 02' 23.4" S and 113° 45' 06.4" E). The Borneo Orangutan Survival Foundation (BOSF) has managed this center since 1999; more than 500 rescued and confiscated orangutans were placed into two different groups: 1) quarantine that focuses on the process to improve the health of orangutans, and 2) rehabilitation, which is the next step before the orangutans are released back into the wild. The second site is Tuanan Orangutan Research Program, located at Tuanan, Muara Mangkutub, Central Kalimantan, Indonesia (2° 09' 06" S and 114° 26' 26" E). The Kapuas Forest Management Unit, Ministry of Environment and Forestry of Indonesia, manages the mentioned site

which has one of the highest densities of wild orangutans at 4.25 ind. within the 750 hectares of the research area. This density is the highest in Central Kalimantan and the population is the second largest wild orangutan population in Central Kalimantan after Sebangau National Park (Morrogh-Bernard et al., 2003; van Schaik et al., 2005). The research area is located with the Mawas Conservation Area (MCA) which is about 365,000 hectares.

Data collection focused on adult mature male orangutans based on their developmental stages, which are, unflanged (UFM), developing flange (DFM), and flange (FM) males (Figure 4.1). Adult UFM can be identified by undeveloped flange or throat pouch; their body size is similar to that of adult females, and their size is smaller when compared to FM. DFM has begun to grow flanges, and are often constituted with a small throat pouch, and their body size approaches that of a fully flanged male. FM is characterized by thick and round-shape of flange, big throat pouch, and the largest body mass (see chapter 1 for details). Forty-four adult matured male orangutans were used as the sample size, 28 males from captive and 16 males from wild population (Table 4.1). Three different developmental stages (UFM, DFM, and FM) were identified from the captive samples; whereas the samples from the wild population only contained two different stages (UFM and FM). In the wild, we did not have continuous data from the flanged males as at Tuanan they only follow orangutans for 5 days and then do not follow them again for another month, if they can find the same individual again; sometimes the selected UFM would disappear for a long time and then, when he was seen again, he had transitioned into a flanged male.

4.2.2. Urine Sampling and Preservation

A non-invasive urine sampling method was used to collect orangutan urine daily. I prioritized morning urine that was secreted between 06:00 AM – 10:00 AM to minimize the effect of circadian rhythm's effect (Fuleihan et al., 1997). Right after collection, urine samples were transported from the field at low temperatures (approximately $< 5^{\circ}$ Celsius) to the camp refrigerator at -15° Celsius. In addition, 200 μL of urine samples were aliquoted to 2-3 Whatman® protein saver cards as a backup, in case the frozen samples were damaged. Qualitative clinical information was carried out with Roche Chemstrips™ for human clinical analysis. This test recorded urinary levels of bilirubin, blood, glucose, ketone, leukocyte, nitrite, pH, protein, specific gravity and urobilinogen using a categorical scale. Furthermore, quantitative specific gravity value was conducted in the laboratory using an Atago™ refractometer. All collected samples were transported to a -80° Celsius freezer in the Laboratory for Primate Dietary Ecology and Physiology, Department of Anthropology, Rutgers University for further analyses.

4.2.3. Hormonal Analysis

To avoid the effect of circadian rhythm on hormonal excretions (Czekala et al., 1994; Devine & Wolf, 2016; Muller & Lipson, 2003), I used the first morning urine voids. Cortisol was assayed with DetectX™'s cortisol enzyme immunoassay kit (K003-H1). This kit was designed for analyzing dried fecal extracts, saliva, urine, serum, plasma, and tissue culture media samples (Assays, 2017). The kit was validated for orangutan's urine with sensitivity of the assay at 27.6 pg/mL, recovery 116%, intra-assay coefficient of

variation 6.77 n= 255, inter-assay control coefficient of variation 5.37% (low) and 5.51% (high) from 11 plates, and inter-assay standard coefficient of variation 7.81% from 11 plates.

Testosterone was carried out with DetectX™ enzyme immunoassay kit (K032-H1); the arbor assay company followed the kit protocol. The kit was validated for orangutan's urine with sensitivity of the assay at 9.92 pg/mL, recovery 80.15% with $R^2=0.96$, intra-assay coefficient of variation 6.64% n= 256, inter-assay control coefficient of variation 16.5% (low) and % 9.83(high) with n= 9, and inter-assay standard coefficient of variation = 11.27% with n= 12 plates. To validate the kit, we first wanted to test if we had to perform a deconjugation process to recover the metabolic testosterone in the urine (Emery-Thompson et al., 2012; Muller & Wrangham, 2004; Robbins & Czekala, 1997). Metabolic urine testosterone was deconjugated through ether extraction which had been previously hydrolyzed in overnight 37° C incubation by β -glucuronidase in pH 5 buffer (Robbins & Czekala, 1997). I tested the similarity of testosterone concentrations between conjugated and deconjugated samples; comparison showed no difference among the two methods on the same samples ($t_{(85.773)} = -0.059$, n= 46, p= 0.95). Thus, for all further analyses of testosterone, assays were conducted on urine samples without using the deconjugation process.

I controlled the cortisol and testosterone concentrations with corrected urinary specific gravity (SG) to account for water content in the sample. To gain a corrected SG, I used an equation suggested by Miller et al. (2004), that is, an average SG (1.007) – 1.0 divided by SG sample – 1.0. In other words, I gained the pure urine concentration by

delineating the water component. The SG value is related to the amount of secreted chemical particles in the urine, and can act as a crucial indicator for dehydration or even the failure of organs function, such as cardiovascular and kidney (Lee et al., 2013).

4.2.4. Data Analysis

Generalized additive mixed models (GAMM) were used to test the variation of cortisol and testosterone concentrations in all orangutans and among male's developmental stages by using *mgcv R package* (Wood, 2018). In addition, I used Orangutan ID as a random effect in each model to avoid auto replication. I added individual energy intake (Kcal) as an independent factor to manage the physiological response in respects to the ecological dietary changes. Finally, I used pairwise comparison test among groups (Kruskal-Wallis multi-comparison test with *pgimerss R package* (Giraudoux et al., 2018)) to identify which variable might differ with others in the GAMM was significant; the test was set at the significance level of $\alpha < 0.05$. The interaction of cortisol-testosterone hormones secretions was analyzed by Pearson pair test. The test was carried out in all orangutans, and also among the varied developmental stages. All statistical analyses were conducted in R Studio 1.1.442 (R-Core-Team, 2013) by using the model formula:

Model= gamm (y ~ x, data = data, family = gaussian, random = list (Orangutan ID= ~1)

4.3. Results

4.3.1. Variation Among Classes in Cortisol

For captive orangutans, I found out that FMs had cortisol levels that are two times higher than the other male's stages, whereas DFMs had the lowest concentrations (Table 4.2). Among the 28 captive orangutans, cortisol levels varied (Kruskal-Wallis's $\chi^2=45.52$, $df=28$, $p=0.02$). Moreover, without including energy intake in the model, FMs secreted significantly more cortisol than UFM and DFMs, whereas DFMs secreted similar levels as the UFM (Table 4.2, Figure 4.2a). By controlling cortisol with total energy intake (kcal), the results remained consistent; FMs had significantly higher cortisol levels compared to two other stages (Table 4.2, Figure 4.2a).

In examining the wild population from Tuanan where I could compare flanged and unflanged males only, FMs had significantly higher cortisol concentrations compared to UFM (GAMM Estimate \pm SE = 0.32 ± 0.14 , $R^2_{(Adj.)}=0.03$, $n=124$, $p=0.02$). However, when total daily energy intake (kcal) was included in the model, the differences were no longer significant (GAMM Estimate \pm SE = 0.38 ± 0.23 , $R^2_{(Adj.)}=0.01$, $n=71$, $p=0.10$) (Table 4.3, Figure 4.2b), suggesting that variation in energy intake may be driving the variation in cortisol in the wild population.

4.3.2. Variation in Testosterone Among Male Stages

Among adult mature males, several individuals produced high testosterone levels compared to other individuals. The non-parametric one way ANOVA rank test showed significant difference of testosterone levels among individuals in the captive

orangutan populations (Kruskal-Wallis's $X^2= 71.86$, $df= 28$, $p= 0.001$); a similar result was found in the wild population, which every individual produced different testosterone levels (Kruskal-Wallis's $X^2= 31.99$, $df= 15$, $p= 0.001$). Overall, FMs tend to have higher testosterone levels compared to UFM and DFM. For the captive population, both GAMM models (with and without total energy intake in the model) exhibited significant differences in testosterone levels among the developmental male stages (Table 4.4, Figure 4.3a). A similar result was also found in the wild Tuanan population of orangutans; testosterone levels in FMs were significantly higher compared to UFM (Table 4.5, Figure 4.3b).

4.3.3. Testosterone and Cortisol Hormones in the Transitioning Male Stage

I analyzed samples collected from several UFM who developed flanges, two males from the captive population and four males from the wild population. In captivity, cortisol levels were significantly increased when males were developing flanges (GAMM Estimate \pm SE= -0.51 ± 0.24 , $R^2_{(Adj.)}= 0.15$, $p= 0.05$). Meanwhile, there was no difference in cortisol levels for the four males that transitioned from unflanged to flanged in wild populations (GAMM Estimate \pm SE= 0.33 ± 0.31 , $R^2_{(Adj.)}= -0.02$, $p= 0.29$) (Figure 4.4). Furthermore, testosterone levels between captive and wild population exhibited different results; in captivity, developing flange stage had significantly higher testosterone compared to the unflanged stage (GAMM Estimate \pm SE= -0.33 ± 0.09 , $R^2_{(Adj.)}= 0.52$, $p= 0.003$). Meanwhile, there was no difference in testosterone levels for the males that transitioned from the unflanged to the flanged stage from wild

populations (GAMM Estimate \pm SE= 0.14 ± 0.18 , $R^2_{(Adj.)} = -0.04$, $p = 0.46$) (Table 4.6, Figure 4.5).

4.3.4. Interaction Between Cortisol and Testosterone

I tested for an interaction between cortisol and testosterone concentrations at the same time in the same animal as I ran the assays on the exact same urine samples. Among the captive population, I found that high cortisol concentrations were associated with elevated testosterone levels (*Pearson's*: $t_{(118)} = 3.46$, CI= 95%, $p = 0.0001$, $cor. = 0.30$, Figure 4.6). Similar to the captive orangutans, cortisol levels were positively correlated with testosterone levels in wild orangutans (*Pearson's*: $t_{(121)} = 5.07$, $p = 0.0001$, CI= 95%, $cor. = 0.42$, Figure 4.7). Thus, individuals with higher cortisol also have higher testosterone.

4.4. Discussion

This study is one of the first to examine how cortisol and testosterone vary among developmental stages from a large sample of both captive and wild male orangutans (*Pongo pygmaeus wurmbii*). I rejected the proposed hypothesis, according to which, cortisol levels among male orangutans would be similar due to the elimination of social stress. Flanged males (FMs) produced significantly higher cortisol levels compared to unflanged (UFMs) and developing flange males (DFMs). The finding from captive population support previous study, which observed variation in cortisol levels among developmental stages (Maggioncalda et al., 2002); however, the pattern found in the previous observation was not similar to the results in this current study. Previous

study that was conducted in the zoo found that developing flange males had the highest cortisol levels compared to unflanged and flanged males (Maggioncalda et al., 2002). Although zoo setting was allowed orangutans to physically interact, high cortisol levels wasn't due to the competition with other males, but more related to the stressful behavior while developing flange male displayed his cheek-pads to the flanged male (Maggioncalda et al., 2002; Maggioncalda & Sapolsky, 2002). Because orangutans in my study was housed in single cages, I predicted that increasing cortisol levels in FMs was related to persistent stressor from the cage environment rather than social interactions. I found that social behavior is related to the levels of urinary cortisol and the size of the flange (see Chapter 2). This finding conform previous study that observed high rates of direct and indirect social interactions in captive orangutans were correlated to high hair cortisol concentrations (Carlitz et al., 2014). Furthermore, FMs have the ability to generate long-calls as a signal to other orangutans, while DFMs and UFM are quiet and tend to retreat from the long-calls source. In the wild, subordinate male orangutans (UFMs and DFMs) response to long-calls from FMs by moving away from the source (Mitra Setia & van Schaik, 2007). In Nyarumenteng where this study was conducted, the long-calls rate that was heard by the researcher is 1.60/day which is lower than the long-calls rate from wild population, 4.38/day (Erb.W.personal-communication, 2019; Martines, 2019). If the audible signal tends to be a stressor for other males, I observed frequent signal in captivity could trigger an increase in cortisol, which is an indicator of stress.

Moreover, my results on the wild population did not conform to the previous study conducted by Marty et al. (2015), which was observed on the same population of wild orangutans. They examined fecal glucocorticoids as an indicator of stress and found no difference between flanged and unflanged males. Further they suggested that because of this lack of variation among stages, social stress does not explain arrested development in unflanged males in wild orangutans (Marty et al., 2015). I found that FMs had higher cortisol levels than UFM. However, when I included total energy intake in the model, the difference between UFM and FM was no longer significant and the results did not differ from Marty et al. (2015). Thus, it may be that nutritional intake is tightly linked to variation in cortisol levels and the difference observed between flanged and unflanged males in the previous analysis may be more due to nutritional intake and may not be tightly linked to social stress. However, it is also important to note that in Tuanan, the number of flanged males is much greater than the number of unflanged males (Dunkel et al., 2013). Thus, flanged males are subjected to a greater number of encounters (verbal and visual) with flanged males relative to unflanged males. This could potentially induce a very stressful situation for flanged males. Future studies at Tuanan should focus when male testosterone levels increase after social interactions with other flanged males and compare these levels with testosterone levels from when they have interactions with unflanged males.

Unlike captive orangutans, high stress levels in wild FMs has been observed to be triggered by intra-sexual competition. Utami-Atmoko, Singleton, et al. (2009) observed competition rates among FMs in Tuanan were significantly greater when females were

absent; however, rate of aggression declined if female were nearby. Based on this observation, it was proposed by Dunkel et al. (2013) that the high competition rates among FMs were due to males having highly overlapping home ranges in one area, rather than due to contests for females. This finding was also previously hypothesized by Marty et al. (2015). Although not statistically significant, Marty et al, (2015) found that increasing cortisol levels in FMs was tightly linked to high levels of competition with other flanged males. Since the current study did not analyze the correlation between competition rates and cortisol levels, the conclusion generated by the mentioned studies might be still immature. Indeed, my study suggests that variation in energy intake, a variable never examined previously, may be more important for driving cortisol levels than competition in these males. Further analyses are suggested to see if direct competition could be linked to high cortisol in wild population.

Beside the social conditions, I predicted that high cortisol levels in FMs may be influenced by the stress from having limited living space (see Chapter 2), as adult male orangutans have been documented to have extremely large home ranges (Buckley, 2014; Singleton & Van Schaik, 2001). In a review of the sources of stress in the captivity, Morgan and Tromborg (2007) identified that stress could be a consequence of animals having restricted movement area. There were several publications from different animal taxa that supported this; it has been observed that cortisol levels in male curve-billed thrashers birds (*Toxostoma curvirostre*) increased significantly when they moved from the field to captivity (Fokidis et al., 2011). In Yucatán spider monkeys (*Ateles geoffroyi yucatanensis*), higher cortisol levels were found in the population that live in captivity

rather than sanctuary or fragmented habitat (Rangel-Negrín et al., 2009). A similar result was showed in bonobos (*Pan paniscus*), which posited that hair plucking was a noticeable indicator for stress (Brand et al., 2016) and was commonly observed in indoor instead of outdoor cage setting (Brand & Marchant, 2015). However, based on my results, the differences between captive and free ranging animals could also be a consequence of dietary changes.

Furthermore, the physiological stress in FMs might be due to restricted food and foraging activity, as was suggested by Morgan and Tromborg (2007) in their review. Accounting for cortisol levels with total energy intake in the captive orangutans, I found that FMs had higher cortisol levels than DFMs and UFM. As observed in the chapter 3, I found that males with greater body mass had lower daily energy intake per unit $M^{0.73}$. However, the result was different for wild orangutans in that I found both stages UFM and FM had similar levels of cortisol. Food and foraging space restrictions unlikely trigger stressors among the wild orangutans, as could be the case in captive orangutans. Wild orangutans, specifically Bornean populations, are more adaptive to great variation in diet and energy intake. During episodes of low fruit abundance, wild orangutans in Tuanan reduced their energetic costs by being less active and travelling shorter distance (Harrison et al., 2010; Pontzer et al., 2010; Vogel et al., 2017; Vogel et al., 2009), feeding on both ripe and unripe fruit (Vogel et al., 2009), and by relying on fallback foods such as tougher inner bark (Vogel et al., 2017; Vogel et al., 2008; Vogel et al., 2014). In addition, decreased daily caloric intake in FMs was hypothesized as the strategy in maintaining metabolic homeostasis (see Chapter 3).

Based on the pattern of cortisol levels from the wild and captive orangutans, this study doesn't support earlier hypothesis which posited that secondary sexual characteristic arrest in male orangutans is linked to stress (Maple, 1980a). Evidently, the current data show that cortisol as an indicator of stress does not suppress flange development in male orangutans. From frequent monitoring of two captive males who transitioned from UFM to DFM, I observed that cortisol increased in association with changes in developmental stage. However, the four wild male orangutans that transitioned from UFM to FM, had similar cortisol levels. It is important, however, to mention that these sample sizes are still small and thus the results are preliminary for these transitioning males. It is possible that other factors affect cortisol levels more and perhaps high cortisol is more likely to be observed after males have physical fights, something that is rare but does occur. My result supported the previous study that posited high glucocorticoid hormones were identified only in the DFMs; whereas, UFM and FM have similar cortisol levels (Maggioncalda et al., 2002).

Testosterone levels among male's developmental stages from wild and captive populations exhibited similar pattern to cortisol. I observed that testosterone levels varied among the developmental stages; UFM secreted lower testosterone levels compared to DFMs, while FM had the highest levels. The result from the captive population differed with the results from studies conducted previously, which found that DFMs has the highest testosterone levels (Maggioncalda et al., 1999). Similarly, the pattern observed in the wild population support a previous study on the same population that observed higher testosterone levels (measured by fecal androgens) in

FMs (Marty et al., 2015). Moreover, from observations of six males who were constantly monitored, the testosterone levels of two captive males increased during the transition of development stage, from unflanged to developing flange; whereas in the wild, four males show similar levels of testosterone during the transition from unflanged to flanged stages. Based on the testosterone pattern from the transitioned stage, developing flange tended to produce more testosterone compare other stages which is conform the previous studies by Marty et al. (2015) and Maggioncalda et al. (1999).

Supporting my finding that testosterone levels were increased during developing flange stage, the expression of hypothalamic-pituitary-testes axis in humans specifically men arises during puberty, during which time individuals express high levels of testosterone (Laycock & Karim, 2013; Rogers, 2012). Similar patterns were also reported in the comparative analysis between chimpanzees and bonobos (Behringer et al., 2014), gorillas (Robbins & Czekala, 1997), and capuchin monkeys (Jack et al., 2014); testosterone levels were increased during the young and the puberty stage, peaked at the high rank or alpha state, and become stable when they reached a mature stage. Testosterone regulates the development of secondary sexual characteristics such as facial hair, muscle mass, and libido. In chimpanzees, Muller (2017) examined high testosterone levels correlated to higher social status, winning social contests, increased muscle mass, and developed secondary sexual characteristics. This hypothesis support the notion that elevated testosterone is linked to increases in an animal's social status (Clutton-Brock & Huchard, 2013; Eisenegger et al., 2011).

However, since male bonobos and chimpanzees fail to develop SSCs like orangutans, the reason for the observed increase in testosterone levels in male orangutans even after the complete development of flanges remain unclear. A previous study observed that testosterone levels in captive orangutans were linked to the timing of flanged development (Emery-Thompson et al., 2012). Despite developmental timing in orangutans' flange, I predicted that increasing testosterone levels in FMs is related to social strategy. In the wild, increasing testosterone was predicted to be correlated with social interactions among flanged males, because of the strenuous and stressful nature of the activity. The competition rates among FMs in Tuanan is remarkably higher, even when there are more females present (Utami-Atmoko, Singleton, et al., 2009). Every mature male in Tuanan seems to play a "take a chance" strategy, rather than "play it safe" strategy; they all seem to eventually develop their flanges and compete with other males, although the timing of flanging varies greatly among males of different ages. This hypothesis has been supported by a previous study, which observed that developmental arrest in Tuanan (Bornean) orangutans was less pronounced compared to Suaq (Sumatran) orangutans (Dunkel et al., 2013).

Testosterone might not only be important for the regulation of somatic development, but also for maintenance of the body. Testosterone levels may increase exponentially at the puberty stage (physical exercise can also trigger the secretions (Brownie et al., 2005)), and then decline and then remain stable after post maturity. Considering the human male species, testosterone levels are related to gonadal development, and are increased during puberty and remain stable when males reach a

mature stage (post 40 years) (Kelsey et al., 2014). Supporting the pattern in humans, long-term monitoring in chimpanzees has shown that testosterone levels increased during the puberty stage and declined after the fertility stage -post alpha stage (Muller, 2017). Testosterone plays an important role in the sexual dimorphism musculature as well in chimpanzees (Muller, 2017); high testosterone was related to the increasing muscle mass in chimpanzees (Emery Thompson et al., 2012). If testosterone plays an important role in the orangutans' musculature, the comparison analysis from urinary testosterone and creatinine would be interesting to perform considering the method was well applied (Emery Thompson et al., 2012; Kim et al., 2016; Oterdoom et al., 2009; Oterdoom et al., 2008; Rule et al., 2009).

Nonetheless, the interaction between testosterone and cortisol still remains unclear, particularly in the case of animals. The dual-hormone hypothesis was initially proposed by Mehta and Prasad (2015), when they observed that testosterone and cortisol seem to be vary together. Specifically, this hypothesis posits that when testosterone is high, cortisol will be low. In human studies, the examination of dual-hormone hypothesis seems more advanced, specifically in the correlation with social characteristics and/or behavior. For example, high testosterone and low cortisol levels were related to the dominance/leader status (Mehta & Josephs, 2010), the winner during bargaining job positions (Mehta et al., 2015), or personal empathy (Zilioli et al., 2014). However, not every dual-hormone hypothesis showed a crossed reaction, a positive correlation between testosterone and cortisol levels, which contradicts with dual-hormone hypothesis, was observed in men that are characterized as

psychopath/bad personality (Welker et al., 2014). These two hormones have been examined in several non-human primates' species in the context of social status (Sapolsky, 1992; Surbeck, Deschner, Schubert, et al., 2012; Surbeck, Deschner, Weltring, et al., 2012; Weingrill et al., 2011), trait development (Cox et al., 2008; Emery-Thompson et al., 2012; Marty et al., 2015), and fitness (Beehner & Bergman, 2017; Kelly & Jones, 2013; Traish, 2014). However, the analyses in these studies has not put particular emphasis on the interaction between these hormones. As per my initial findings, the correlation between cortisol and testosterone in male orangutans (captive and wild population) did not support the dual-hormone hypothesis. Instead, my results should that high levels of cortisol correspond to high testosterone concentrations. Further analyses are warranted on this topic; as the human study found that the dual-hormone interactions are related to good and bad personalities.

To conclude, in this current study I observed different endocrinological results compared to previously published studies. Cortisol and testosterone hormones varied with developmental stage, with lower concentrations in unflanged males and increases as males transitioned from developing flanges and a flanged male. Development arrest in unflanged males wasn't regulated by the stress behavior but was positively correlated with low testosterone levels. Further comparison analysis of endocrinological status between wild and captive orangutans seems very interesting to examine. Endocrinological status in captivity there seemed to be greater differences among the male developmental stages compared to the wild populations. Both sample sizes were not large, but the differences were more pronounced in captivity for cortisol,

testosterone, and the interaction between these two hormones as well. This may be because there is more environmental noise in the wild. That is, there is much more variation in travel behavior, interactions with other orangutans (both male and female), diet and nutritional intake, and encounters with humans (e.g., researchers, local people, local hunters). Thus, future studies should explore these factors in relation to varying cortisol and testosterone.

4.5. Tables and Figures

Table 4.1. Orangutan distribution based on their habitat and developmental stages. The number of samples determines the number of observation day(s). The * sign indicate that the male transitioned his flange development during the study period.

No.	Orangutan	Developmental Stage	Population	Number of Samples
1	Casper	UFM	Captive	4
2	Joni*	UFM – DFM	Captive	6
3	Kevin	UFM	Captive	2
4	Klsar	UFM	Captive	3
5	Lomon	UFM	Captive	2
6	Lopus*	UFM – DFM	Captive	10
7	Nyun nyun	UFM	Captive	6
8	Paiman	UFM	Captive	4
9	Rambo	UFM	Captive	2
10	Stuart	UFM	Captive	1
11	Edoy	DFM	Captive	7
12	Ibut	DFM	Captive	4
13	Inou	DFM	Captive	4
14	Juky	DFM	Captive	4
15	Marwoto	DFM	Captive	2
16	Palingkau	DFM	Captive	3
17	Soni	DFM	Captive	1
18	Bento	FM	Captive	3
19	Charles	FM	Captive	4
20	Danny	FM	Captive	4
21	Gayo	FM	Captive	11
22	Jacky	FM	Captive	2
23	Lucky Thai	FM	Captive	12
24	Nilton	FM	Captive	3

No.	Orangutan	Developmental Stage	Population	Number of Samples
25	Sabun	FM	Captive	5
26	Saswoko	FM	Captive	4
27	Umpong	FM	Captive	3
28	Uncui	FM	Captive	4
29	Dayak*	UFM – FM	Wild	20
30	Ekko*	UFM – FM	Wild	3
31	Gismo*	UFM – FM	Wild	11
32	Jip	UFM	Wild	3
33	Momo	UFM	Wild	2
34	Ted	UFM	Wild	3
35	Wodan*	UFM – FM	Wild	28
36	Chaz	FM	Wild	2
37	Dolay	FM	Wild	1
38	Helium	FM	Wild	6
39	Henk	FM	Wild	14
40	Max	FM	Wild	5
41	Niko	FM	Wild	10
42	Otto	FM	Wild	7
43	R1200	FM	Wild	1
44	Tomi	FM	Wild	10

Table 4.2. Cortisol levels among captive males in different developmental stages.

Cortisol (pg/mL)	Mean \pm SE	N orangutan
Unflanged	29290.74 \pm 5190.78	9
Developing Flange	26716.50 \pm 4927.50	8
Flanged	61931.52 \pm 13575.53	11
<i>GAMM: Log C ~ Stages</i>	<i>Estimate \pm SE</i>	<i>p-value</i>
Intercept	4.28 \pm 0.07	
Developing Flange	- 0.02 \pm 0.11	0.88
Flanged	0.27 \pm 0.09	0.006
$R^2_{(Adj.)}$	0.08	
N observations	120	
<i>GAMM: Log C ~ Stages + Intake</i>	<i>Estimate \pm SE</i>	<i>p-value</i>
Intercept	4.14 \pm 0.12	
Developing Flange	0.04 \pm 0.13	0.75
Flanged	0.41 \pm 0.12	0.002
$R^2_{(Adj.)}$	0.12	
N observations	83	

Table 4.3. Cortisol levels among wild males in different developmental stages.

Cortisol (pg/mL)	Mean \pm SE	n orangutan
Unflanged	36863.21 \pm 9788.81	7
Flanged	96892.75 \pm 10614.25	13
<i>GAMM: Log C ~ Stages</i>	<i>Estimate \pm SE</i>	<i>p-value</i>
Intercept	4.33 \pm 0.12	
Flanged	0.32 \pm 0.14	0.02
$R^2_{(Adj.)}$	0.03	
N observations	124	
<i>GAMM: Log C ~ Stages + Intake</i>	<i>Estimate \pm SE</i>	<i>p-value</i>
Intercept	4.36 \pm 0.25	
Flanged	0.38 \pm 0.23	0.10
$R^2_{(Adj.)}$	0.01	
N observations	71	

Table 4.4. Testosterone levels among captive males in different developmental stages.

Testosterones (pg/mL)	Mean \pm SE	Number of orangutans
Unflanged	16709.43 \pm 2295.75	9
Developing Flange	22410.11 \pm 2489.37	8
Flanged	27274.84 \pm 1769.13	11
<i>GAMM: Log T ~ Stages</i>	<i>Estimate \pm SE</i>	<i>p-value</i>
Intercept	4.13 \pm 0.04	
Developing Flange	0.14 \pm 0.06	0.03
Flanged	0.26 \pm 0.05	0.0001
$R^2_{(Adj.)}$	0.16	
N observations	120	
<i>GAMM: Log T ~ Stages + Intake</i>	<i>Estimate \pm SE</i>	<i>p-value</i>
Intercept	4.08 \pm 0.07	
Developing Flange	0.19 \pm 0.08	0.02
Flanged	0.33	0.0001
$R^2_{(Adj.)}$	0.18	
N observations	83	

Table 4.5. Testosterone levels among wild males in different developmental stages.

Testosterones (pg/mL)	Mean \pm SE	Number of orangutans
Unflanged	49732.59 \pm 9735.01	7
Flanged	99666.05 \pm 14128.95	13
<i>GAMM: Log T ~ Stages</i>	<i>Estimate \pm SE</i>	<i>p-value</i>
Intercept	4.61 \pm 0.03	
Flanged	- 0.17 \pm 0.07	0.02
$R^2_{(Adj.)}$	0.03	
N observations	134	
<i>GAMM: Log T ~ Stages + Intake</i>	<i>Estimate \pm SE</i>	<i>p-value</i>
Intercept	4.53 \pm 0.10	
Flanged	- 0.33 \pm 0.11	0.004
$R^2_{(Adj.)}$	0.10	
N observations	76	

Table 4.6. GAMM model for Cortisol and testosterone concentrations before and after transitioning to flange.

1. CAPTIVE POPULATION		
<i>GAMM: Log C ~ Stages + Intake</i>	<i>Estimate \pm SE</i>	<i>p-value</i>
Intercept	4.49 \pm 0.30	
Developing Flange	- 0.51 \pm 0.24	0.05
$R^2_{(Adj.)}$	0.15	
N observations	15	
<i>GAMM: Log T ~ Stages + Intake</i>	<i>Estimate \pm SE</i>	<i>p-value</i>
Intercept	4.45 \pm 0.11	
Developing Flange	- 0.33 \pm 0.09	0.003
$R^2_{(Adj.)}$	0.52	
N observations	15	
1. WILD POPULATION		
<i>GAMM: Log C ~ Stages + Intake</i>	<i>Estimate \pm SE</i>	<i>p-value</i>
Intercept	4.09 \pm 0.38	
Flanged	0.33 \pm 0.31	0.29
$R^2_{(Adj.)}$	-0.02	
N observations	36	
<i>GAMM: Log T ~ Stages + Intake</i>	<i>Estimate \pm SE</i>	<i>p-value</i>
Intercept	4.39 \pm 0.23	
Flanged	0.14 \pm 0.18	0.46
$R^2_{(Adj.)}$	-0.04	
N observations	36	

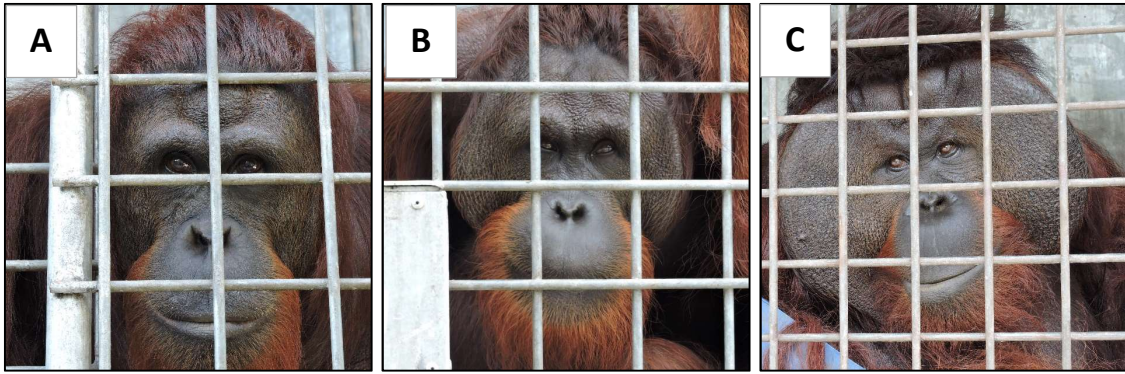


Figure 4.1. Three different developmental stages in male orangutans: a. unflanged male (UFM) was determined by undeveloped flange, b. developing flange male (DFM) which have half-grown flanges, and c. flanged male (FM) which flanges were fully developed.

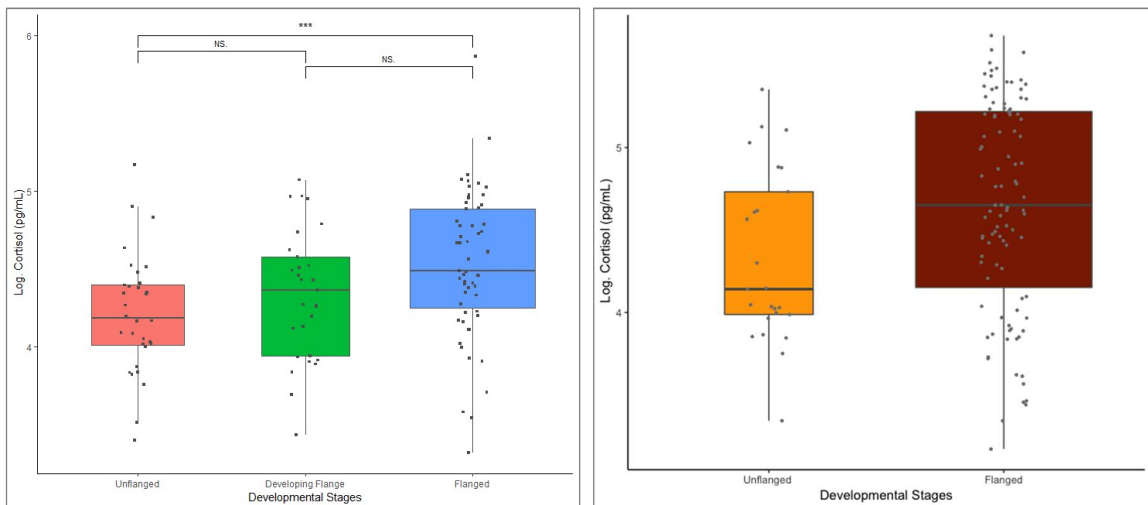


Figure 4.2. Boxplots of cortisol concentrations among male's developmental stages without accounting total energy intake. Left: captive population shows the cortisol levels in FMs were significantly higher than UFM and DFMs ($p=0.006$), whereas UFM and DFMs produced similar level; and Right: In wild population, FMs have significantly higher cortisol levels than UFM ($p=0.02$).

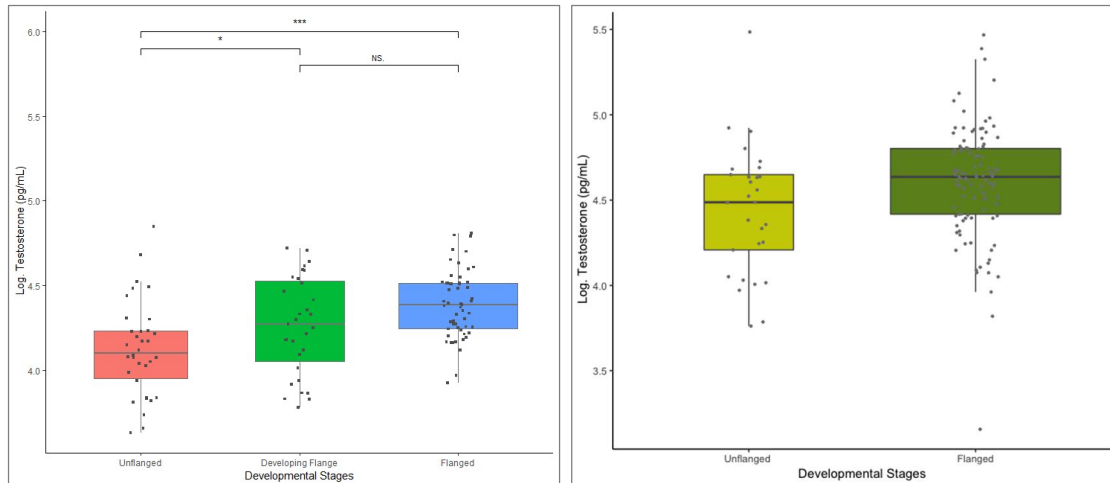


Figure 4.3. Boxplots of testosterone concentrations among male's developmental stages without accounting for total energy intake. Left: the captive population shows testosterone levels in UFM's were significantly lower than DFM's ($p=0.03$) and FM's ($p=0.0001$), whereas DFM's and FM's tend to produce similar testosterone levels; and Right: wild population which shows testosterone levels in UFM's were significantly lower than in FM's ($p=0.02$).

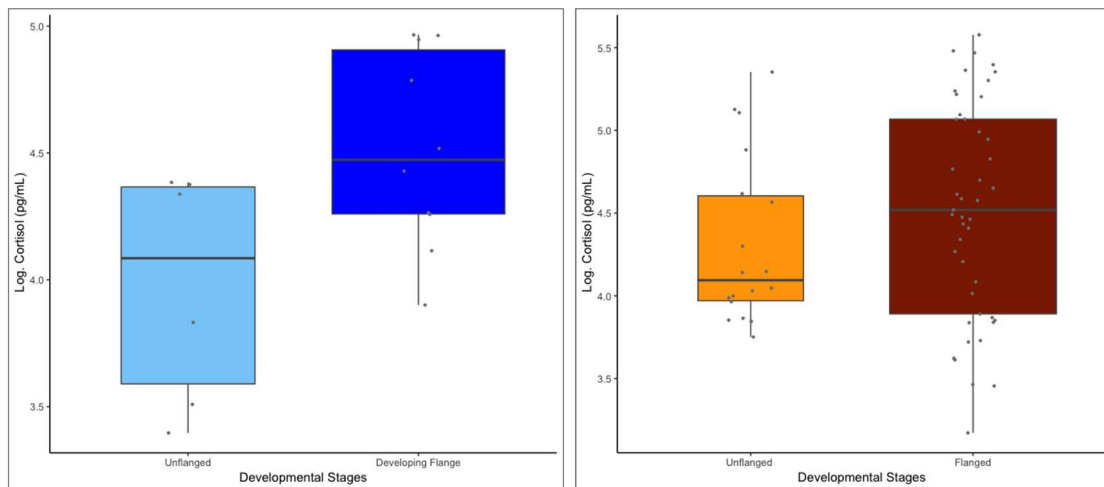


Figure 4.4. Cortisol levels (pg/mL) from transitioned flange males without accounting the total energy intake. Left: The cortisol levels from captive population were significantly greater in DFM's compared to UFM's ($p=0.05$). Right: The cortisol levels from wild orangutan shows similar levels between UFM's and FM's ($p=0.29$).

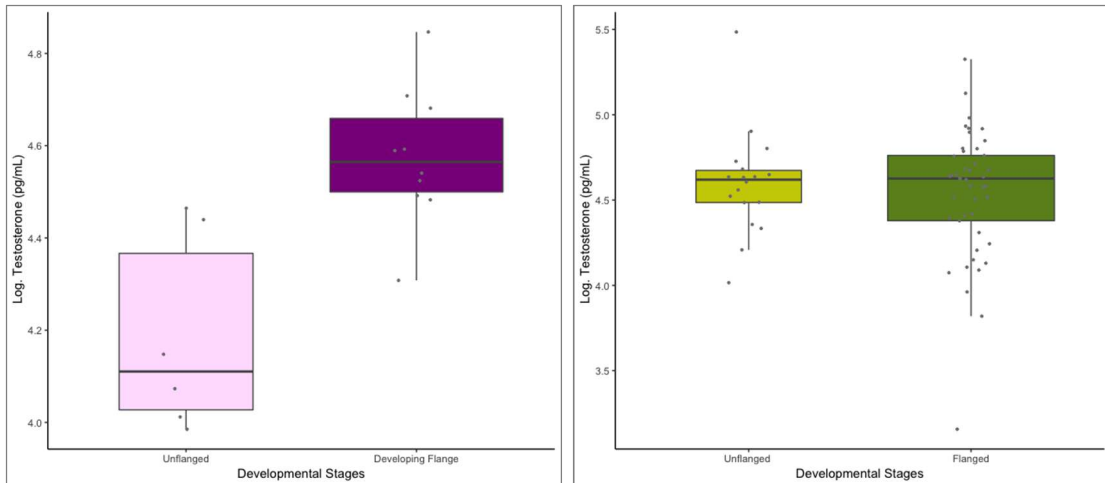


Figure 4.5. Testosterone levels (pg/mL) from transitioned flange males without accounting the total energy intake. Left: Testosterone levels from captive population were significantly increased with the changed of the flange ($p=0.003$). Right: Testosterone levels from wild orangutan shows similar levels between UFM and FM ($p=0.46$).

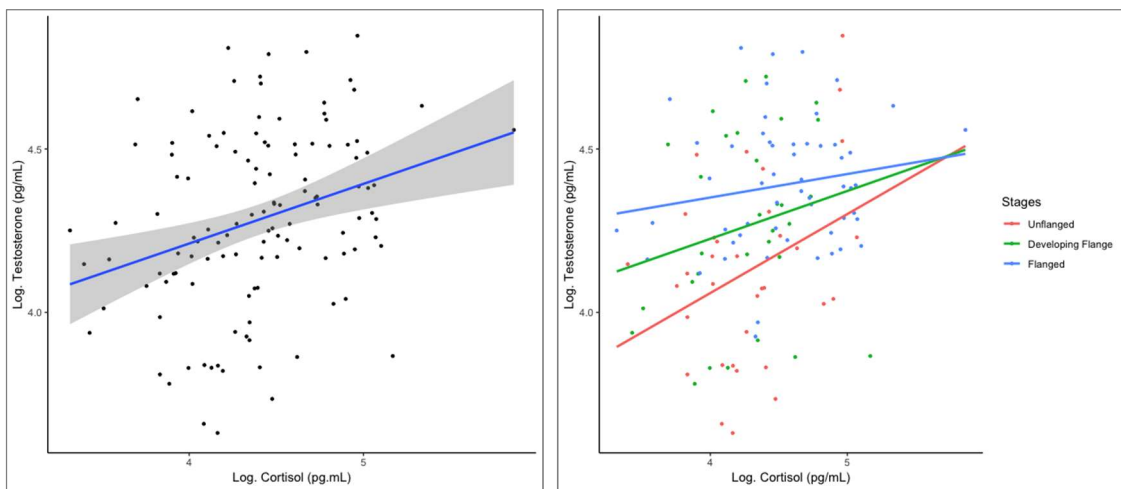


Figure 4.6. Interaction between cortisol and testosterone secretions in all captive orangutans (Left) and among different male's developmental stages (Right).

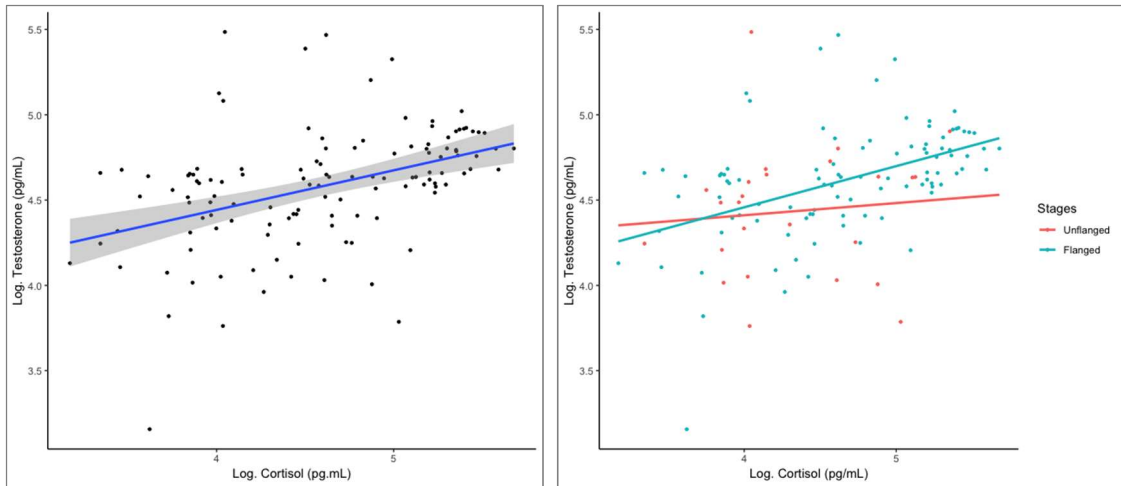


Figure 4.7. Interaction between cortisol and testosterone hormone secretions in all wild orangutans (Left) and among different male's developmental stages (Right).

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5. Chapter V. Conclusion

5.1. Summary

Darwin (1871) stated that *“The females are most excited by, or prefer pairing with, the more ornamented males (p.262)”* which he then observed *“The Male generally more modified than the Female. The great eagerness of the male has thus indirectly led to the much more frequent development of secondary sexual characters...(p.275)”*.

Moreover, Jarman (1983) observed two different male characteristics and described this as bimaturism. In orangutans, bimaturism is found in adult males that are characterized by two different morphological forms; one morph is an adult male with flanges and a large throat-sac; whereas the second morph is an adult male that lacks those traits (Maple, 1980a; Utami-Atmoko et al., 2002). The sequential development of the irreversible flange in adult male orangutans has intrigued researchers for decades, yet we still have little understanding of what triggers flanging and why there is such variation in the timing of flanging. The timing of flanging is thought to be driven by the evolutionary costs and benefits of developing flanges, including variation in reproductive success and intra-sexual competition.

However, there are several proposed mechanisms that may lead to flange development in male orangutans. The first mechanism is social interactions. Dunkel et al. (2013) observed flange development arrest is correlated to the number of flanged males and their dominance status. High-ranking flanged males could monopolize the receptive females, although they have to deal with high intrasexual competition (Mitra

Setia et al., 2009; Utami-Atmoko, Singleton, et al., 2009); meanwhile, the low-rank males will have a different mating strategy by delaying their flange (Pradhan et al., 2012). However, there is still a lack of information if the rates of competition are related to flange development in orangutans. The second proposed mechanism is condition-dependency. In the review of energetic status and development, Emery-Thompson (2017b) hypothesized the nutritional-stressed condition would slow down reproductive maturation in males but may actually accelerate it in females. However, this hypothesis has yet to be tested in relationship to flange development. The third mechanism is physiological status. There were several studies that tested Maple (1980a)'s hypothesis, which undeveloped flange might due to high stress levels. Though the study of cortisol levels from captive (Maggioncalda et al., 2002) and wild (Marty et al., 2015) did not find a concrete conclusion, as well as with the studies of testosterone hormone (Emery-Thompson et al., 2012; Maggioncalda et al., 1999; Marty et al., 2015; Muller, 2017).

Here, I summarized my findings into three different chapters. Chapter two addressed the question about the relationship between social interactions flange development and if stress events influence flange development. I tested three hypotheses; first, the current study supported the hypothesis that flange development was related to the amount of social interactions. Moreover, I rejected the hypothesis about social interactions and the number of males within the same facility. I observed that the greater number of flanged males was associated with greater sociality among unflanged and developing flange males; meanwhile, when the number of flanged males decreased, flanged males were more social than the other developmental stages.

Secondly, I accepted the hypothesis that social interactions would be related to flange development. Flange size in unflanged and developing flange males was related to the amount of social behavior; males that are developing their flanges engaged in less social behavior. Finally, I rejected the hypothesis that cortisol levels among developmental stages would not be different. I found that cortisol levels among developmental stages differed; interestingly, the pattern was related to social interactions. The high cortisol levels found in flanged males may be due to several stressors that previously hypothesized by (Morgan & Tromborg, 2007), including limited living space and restricted food choices.

In chapter three I discussed nutrient-dependency and its influence on flange development. During the rehabilitation process, orangutans were predicted to have access to constant energy intake from a regular food supply. Unflanged males have smaller body mass compared to developing flange males, and flanged males tended to be the heaviest individuals, weighing about 20 kilograms more than unflanged males. The expected daily energy expenditure (DEE) in unflanged males was slightly different from developing flange males, that is, 1603.06 Kcal/day and 1663.80 Kcal/day respectively; meanwhile flanged males have a greater DEE, 1792.21 Kcal/day. There was no difference of total energy intake among development stages; however, when the daily energy intake was controlled by metabolic body mass, unflanged and developing flange males have a similar caloric intake, whereas flanged males tended to have lowest caloric intake compared to others. This current study rejected the hypothesis and observed all developmental stages were in the state of positive energy balance. The

differentiation of total energy intake was not correlated to the flange development, which means the proposed hypothesis was accepted. The flange development in male orangutans was not nutrient-dependent.

Chapter four observed the profile of cortisol and testosterone hormones among male's developmental stages from wild and captive populations. Several studies in relation with orangutans' endocrinological status have been conducted (Emery-Thompson et al., 2012; Maggioncalda et al., 2002; Maggioncalda et al., 1999; Marty et al., 2015); yet, a conclusion wasn't formulated. Orangutans in the current study were housed in individual cages, which was assumed have a similar social setting with the wild population. Thus, in accordance with the assumption, this current study observed flanged males significantly produced high cortisol and testosterone levels as compared to unflanged and developing flange males. The result wasn't conforming previous studies (Maggioncalda et al., 2002; Maggioncalda et al., 1999; Marty et al., 2015). In captivity, high cortisol levels in flanged males were related to persistent stressor from the cage environment; whereas intrasexual competition was assumed related to the cortisol secretions in wild population. Furthermore, high testosterone levels in flanged males were assumed related to social strategy and somatic maintenance.

5.2. Implication for Orangutans Rehabilitation and Conservation

Since orangutan rehabilitation centers were established in Indonesia and several workshops of the Orangutan Veterinary Advisory Group (OVAG) were held to discuss and find better management practices for orangutans during the rehabilitation process,

it is surprising that male bimaturism, flanged and unflanged male individuals, was never considered as one of the criteria for the design of captive orangutan locations, groupings, and cages. Researchers concur that Bornean orangutans are more solitary compared to their close relatives - the Sumatran and Tapanuli species (Rijksen, 1978; van Schaik, 1999). Although it is well known that among Bornean male orangutans they do not have a clear dominance hierarchy (van Schaik, 1999) and female orangutans are more tolerant towards other females than males (van Noordwijk et al., 2012), the Bornean orangutan rehabilitation centers frequently put mature males within same cage groups and also keep females separated from these adult males. The reason for this is to minimize the potential stress experienced by receptive females and to avoid potential physical conflicts among male orangutans.

This study revealed several important results that might be useful to improve the orangutans' rehabilitation process, specifically among the Bornean species. First, I observed that a greater number of flanged males in one area was associated with increased social behavior overall. Increased social behavior was also related to flange development such that males that were not developing their flanges engaged in more social behavior compared to the other developmental stages. My results suggest that housing several flanged males in a similar area creates a more stressful environment among them as well as other male orangutans. This concurs with another study on wild orangutans that found that intra-sexual competition triggers higher stress levels in wild flanged males. Utami-Atmoko, Singleton, et al. (2009) observed that competition rates among flanged males in a wild population (Tuanan-Borneo) were significantly higher

when females were absent; however, the rate of aggression declined if females were nearby. This observation was also supported by Dunkel et al. (2013) and Marty et al, (2015), who both found that increasing cortisol levels in flanged males was tightly linked to high levels of competition with other flanged males. To minimize a stressful environment and potentially improve the management of orangutan rehabilitation in Nyarumenteng, I propose to decrease the number of flanged males in one area and interchange the flanged males with more male orangutans of different developmental stages. In addition, my study supports that an increase in the number of females into the male's cage group might also reduce stressors among flanged males. In fact, the rehabilitation center did begin to conduct a rotation procedure for all orangutans regularly to minimize stressful events; unfortunately, this procedure wasn't implemented during the study period.

Second, I observed daily energy expenditure in flanged males tended to be greater than unflanged and developing males; yet there was no difference in total energy intake among those development stages. Interestingly, when daily energy intake was controlled by metabolic body mass, flanged males tended to have the lowest caloric intake compared to others, similar to what was found in wild orangutans (Vogel et al., 2017). Although this finding does not provide factors affecting flange development in Bornean male orangutans, future studies on energy restriction, specifically in developing males and flanged males, are needed to understand the relationship between energy status and flange size. As previously stated, flanged males tended to have the lowest caloric intake compared to unflanged and developing males, which conform to the

previous finding from wild population (Vogel et al., 2017). This finding is very useful to improve the rehabilitation procedure, as in the captive facility they supplied flanged males with a greater volume of food. To prevent obesity in captive males, the amount of supplied foods should be decreased to match average caloric intake in from wild populations (Vogel et al., 2017) and should be varied depending on male developmental stage.

Finally, the results of this study showed a positive association between social interactions and cortisol levels. Flanged males, which had a greater number of overall social interactions, also had higher levels of cortisol compared to unflanged and developing males. While this is simply an association, this finding suggests that being a flanged males results in greater levels of stress compared to males in other developmental stages. A similar result was shown with testosterone levels among males: flanged males tended to have the highest levels compared to unflanged and developing males. Supporting my finding that testosterone levels increased during the developing flange stage, the expression of hypothalamic-pituitary-testes axis in humans in men arises during puberty, during which time individuals express high levels of testosterone (Laycock & Karim, 2013; Rogers, 2012). Based on these findings, flanged males tend to be more stressed during the rehabilitation process, potentially because they encounter more flanged males than they naturally would in the wild. Thus, I suggest that flanged males are not housed within visual contact of one another in captive settings and that cortisol and testosterone levels should be assessed. In addition, because of their increased cortisol levels, I recommend that flanged male

should spend as little time in rehabilitation centers and their releases into the forest should be prioritized.

5.3. Conclusion

Bimaturism in orangutans was hypothesized had a correlation with social interactions, energetic status, and endocrinological response. By analyzing orangutans from captive and wild populations, this current study concluded that,

1. The amount of social interactions is linked to bimaturism in orangutans. Social interactions were related to flange development, and males with smaller flanges tended to have greater social rates compared to males with larger flanges. In addition, high rates of social interactions were stressful for captive orangutans. The limited living space, reduced mobility, and food choice restrictions may have led to higher cortisol levels in captive orangutans.
2. Bimaturism did not seem to be nutrient-dependent in this study. However, total energy intake did not differ among developmental stages and energy was probably sufficient for these males. Because they were provided with all of their required nutrients near round, it is still possible that males who do not get enough energy in the wild delay flange development. Future studies should examine if caloric restriction reduces flange development and transitions from unflanged to flanged male. Such data would only be possible if long-term data are available on the same individuals in captive or wild settings or if nutritional restriction experiments were conducted on captive males. Accounting for body mass per Kg, energy intake per

unit $M^{0.73}$ was not related to variation among the developmental stages, but as in the wild flanged males tended to have the lowest energy intake compare to other stages. Decreasing energy intake per unit $M^{0.73}$ was assumed as a strategy for maintaining metabolic homeostasis and stored energy reserves.

3. Endocrinological status was related to bimaturism. Flanged males produced significantly higher cortisol and testosterone levels compared to unflanged and developing flange males. In captivity, high cortisol levels in flanged males were likely related to persistent stressors from the caged environment; whereas intrasexual competition was assumed to be related to variation in cortisol levels in the wild population. Furthermore, high testosterone levels in flanged males may be related to social strategy and somatic tissue maintenance.

5.4. References

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