

Behavioral ecology of flying foxes:
a cue of disease transmission in the wild

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General Introduction

“People are beginning to understand there is nothing in the world so remote that it can’t impact you as a person.”—William H. Foege [27]. This statement reflects the impact of globalization on human welfare in nowadays. The features of globalization; the increase in movement of goods and peoples, promote the spread of disease vectors around the world. Because the time taken for pathogens to spread across the country border or continent become much quicker by long-international flights. By this reason, public health has become a priority topic on the world stage in this era. The recent survey suggested that the infectious diseases account for 19% of global deaths [72]. And 75% of emerging infectious diseases (EIDs) are zoonoses; the diseases that are transmitted between animals and humans [42]. The zoonotic-disease threats are expanding, and become a global burden. And, this is the challenges that we need to overcome.

“The more we know about a reservoir of infection, the more we can save life”. So, what is the reservoir of infection? Haydon *et al.*, (2002) defined the reservoir as “one or more epidemiologically connected populations or environments in which the pathogen can be permanently maintained and from which infection is transmitted to the defined target population” [52]. In the last few decades, bats of family *Pteropodidae* have been recognized as a reservoir of dangerous zoonotic viruses for humans and domestic animals. For example (I) Henipaviruses, including Hendra virus (HeV) and Nipah virus (NiV), had been emerged in Australia, South-East Asia, and South Asia [19, 23]. The potential pathways of NiV-transmission were from bats to pigs and humans that consumed food contaminated with bat saliva [73]. For HeV-spillovers, the infection had been occurred by horse exposure to urine, saliva and feces of flying foxes in the overlapped-foraging sites [38]. (II) Ebola virus (EboV) caused hemorrhagic fever and massive die-offs of great apes and human in west Africa. Scientists believe that the first patient became infected by direct contact with a natural host (fruit bats) or mediated host (non-human primates)

through hunting activity [70]. (III) Marburg virus caused hemorrhagic fever infection in humans at Africa [15]. The first source of infection to human was African green monkeys from Uganda [79]. These zoonoses can be transmitted from bats to humans by various routes, and the role of bats on some viral transmission remains unclear [52]. And, most studies just focus on surveillance of viruses rather than the role of bats on the transmission. To fill the gap in knowledge, we really need further investigations on bat nature and its interaction with susceptible host in natural habitat.

“*Adaptation is a part of being animal*”. As well as other mammals, bats share a common mammalian features [69]. However, the major characteristic that separates bats from other mammals is “the ability to fly” [45]. This special ability helps bats to survive in a changing environment. Bats usually fly out for foraging at nighttime, and many bat species have long-distance flights during seasonal migrations [45]. For example, *P. alecto* were recorded their long-distance movement between the northern region of Australia and Indonesian Papua [16]. Brierley *et al.* (2016) revealed that bat-borne diseases have been occurred all over the world [14]. This finding raises up the awareness of the potential of zoonotic viral transmissions, in the country at which bats are existing, such as our country “Japan”. Wilkinson *et al.* (2002) suggested that the life span of bats was 3.5 times more than those of non-flying mammals which have similar sizes [121]. Many microbats have life spans up to 35 years, documented for a little brown bat [19], while the flying fox (*P. giganteus*) had been recorded living exceeding 30 years in captivity [121]. However, the evolution theory of aging suggests that predation, disease and accidents shorten life span of bats [121]. The persistence of viral infections among long-lived bats, combination with the gregarious roosting behavior, could increase the chance for intra/interspecific transmission [19]. Normally, bats spend a half of their lives living at daytime roosts wherein they mate, rear young, and hibernate. Microchiroptera have

utilized the internal shelters such as caves, rock crevices, and man-made shelters, while Megachiroptera (including the flying foxes) have adapted to the external roost, such as trees in the forest or even on trees in human community [66]. Even though the different species of bats have different roosting ecologies, most of them congregate in the large colonies with high population density during daytime, sometime over 50,000 individuals [55]. Under this condition the airborne-viral transmission might be occurred, increasing the possibility of intraspecies transmission.

We have a greater responsibility to improve the health problem of animals, humans and environments. To improve this problem, we firstly need to understand the connection among animals, humans and emerging diseases, and/or the complex ecological interactions between natural host and susceptible host, that facilitates zoonotic-spillover events. Therefore, in this study, we mainly focused on the behavioral ecology of host, which drive intra/interspecies viral transmission in the wild. As the important host of zoonotic viruses, the flying fox species were used as the animal model of this study, by which the quantitative observations on daytime behavior, nighttime behavior and population dynamic of flying foxes at their natural habitats, were performed (as described in the following chapters). The information gained from this study would allow us to draw a picture of how the appearance of disease and the expansion of diseases might occur in the future.

In chapter 1, the author quantified daytime behavior of two species of flying foxes; (i) *P. vampyrus* which is a natural host of Nipah virus, in West Java province, Indonesia [73]. (ii) *A. jubatus* which is believed to be a natural host of Ebola reston virus, at Subic bay, the Philippines [56]. We illustrated the behaviors, facilitating the spillover of pathogens within bat population, or to another animal species. Also, the complete quantitative data on daytime activity patterns and daytime activity budget of these flying

foxes were firstly reported by this study. These data are considered as the important parameter for developing the mathematic model of intra/interspecific transmission, which can be used to predict the possibility of bat viral outbreaks in natural habitat of flying foxes.

In chapter 2, the author described nighttime behavior of flying foxes (*P. vampyrus*), living at West Java province, Indonesia. The satellite telemetry and direct observation were performed to obtain the information on movement and foraging of the flying foxes. This information is not only important for the conservation of flying fox population, but it can be used also as databases for developing the flying fox distribution model and management program, to estimate the expansion of disease area, and to reduce the conflicts between humans and flying foxes in Indonesia.

In chapter 3, the author revealed the population dynamics of flying foxes, in terms of mortality rate. This long-term research was conducted on *P. lylei*, living close to humans at Buddhist temple in Thailand. The seasonal variation of mortality rate in this flying foxes were found. And this variation unexpectedly coincided with the prevalence of Nipah virus in the flying foxes, reported by previous study [115]. This indicates that the mortality rate of flying foxes might be one of the main factors influencing on the dynamic of viral infection within bat population. Therefore, the death rate should be taken into account for building up mathematical model of disease outbreaks in the near future.

Chapter 1

Daytime behavior of flying foxes

Topic 1: Daytime behavior of *Pteropus vampyrus* in a natural habitat: the driver of viral transmission

Introduction

The bats of the genus *Pteropus* (commonly known as the flying fox) are the largest fruit bats that congregate in large colonies numbering from thousands to ten thousand in tropical, subtropical and temperate regions, in Asia, Australia, and islands in Indian Ocean and western Pacific Ocean [5]. Flying foxes are believed to navigate using visual and olfactory senses, and they play an important role in keeping native forests healthy, through dispersing seeds and pollinating [40, 91]. However, flying foxes are well-recognized reservoirs of zoonotic viruses, as they host a large number of viruses covering 15 viral families [74]. In addition, they possess special characteristics, such as the long distance flight ability, occupation of large geographic ranges, colonial nature and long life, which facilitate the virus spillover from bats into other animals, including humans [19]. The expansion of agricultural and urban areas should lead to an increase in living proximity of humans and domestic animals to flying foxes. For example, in Queensland, Australia, the habitat ranges of flying foxes have been overlapped with that of equines. This enhances the risk of cross-species transmission through the presence of infectious materials, such as urine and feces, in foraging areas utilized by both animals [39].

The habitat overlapping of humans and flying foxes has raised concerns for public health, since the first human case of lyssavirus infection in Australia was detected. This infection occurred by direct contact between human and flying foxes [3]. In 2013–2014, an Ebola virus outbreak in humans resulted in more than 20,000 human victims. The first report of Ebola virus (EBOV) outbreak in human was in 1976 [98]. In 2005, the evidence of asymptomatic EBOV-infection was found in fruit bats, belonging

to the family Pteropodidae: *Epomops franqueti*, *Hypsignathus monstrosus* and *Myonycteris torquata* [70]. Therefore, these three fruit-bat species are believed to be a reservoir of Ebola virus. Great apes, such as gorillas and chimpanzees, are believed to be an intermediate host for this virus [48]. Nipah virus, a novel paramyxovirus, was reported for its first outbreak at Malaysia in 1998, causing a severe respiratory disease with high mortality rate in humans and pigs [24]. The virus was detected and isolated from three species of flying foxes; *P. hypomelanus*, *P. vampyrus* and *P. lylei* [19]. Furthermore, a previous study discovered that Nipah virus circulated in the population of *P. vampyrus* in Indonesia [107]. This raised up the awareness of Nipah virus outbreaks in this region. However, the potential daytime behaviors circulating the viruses within bat populations and ecological interactions between the infected bat and recipients are still unknown. To improve the understanding of these processes, daytime behavioral observation in the large flying foxes is required. To gain new insights into host-recipient interactions, the daytime behavior of *P. vampyrus* (the large flying fox) and its interactions with wildlife living around the roosting site of the flying foxes were investigated in a natural habitat at Leuweung Sancang conservation area, Indonesia. This study site is considered to be a good model as a hotspot to monitor zoonotic disease, since it contains many species of animals, such as the crested serpent eagle (*Spilornis cheela*), wild boar (*Sus scrofa*), long-tailed macaques (*Macaca fascicularis*) and the endemic species-ebony leaf monkey (*Trachypithecus auratus*) [47, 90]. The understanding of the complex social relationships in roosting bats or between bats and other wildlife will provide evidence to speculate how an emerging infectious disease appears in humans and wildlife populations.

Materials and Methods

Study site

The present study was conducted at the Leuweung Sancang natural conservation area, West Java Island, Indonesia (7° 43' 45.12" S, 107° 54' 10.08" E) (Fig. 1-1). This region is a part of the tropical rain forest, spanning across 2,157 hectares of land [108]. We chose this conservation area, since it is the natural habitat of *P. vampyrus* (the large flying fox) and many other wildlife species, including the endemic species *T. auratus* (ebony leaf monkey) [90]. The dominant plants occupying this area belong to the Family Dipterocarpaceae [109]. In addition, fisherman's village is located along beachside of the conserved area, where local peoples stay nearby two observed roosting sites of the flying foxes (approximately 846 m from roosting site 1 and 451 m from roosting site 2) (see Fig. 1-1). Therefore, it is a human-wildlife-flying foxes interface area that is fit for the study of inter-species interaction. Three species of roosting trees in these two roosting sites were identified by the Faculty of Forestry, Bogor Agricultural University. These are *Artocarpus elasticus*, *Nauclea orientalis* and *Glochidion rubrum*. A preliminary survey was carried out to determine the location of the flying fox's roosting sites and the optimal time for observation. A direct count of the population size of the flying fox in the study site was not available due to dense canopy cover. This study received approval from the Ministry of Environment and Forestry, the Office of West Java province and Garut city, Indonesia.

Instantaneous scan sampling technique

The observations of the daytime behavior of the flying fox were conducted by 3 observers during May 11–25, 2016. To investigate the diurnal variation in activities of the flying fox, one observer conducted instantaneous scan sampling, in which the

behaviors of group were rapidly scanned at regular intervals [4]. Due to the difficulty to access the study site (walking in a deep forest), therefore, observations started after the arrival of observers around 0700 hr to 1700 hr. As it is impossible to observe behaviors of all individuals in the group, due to its large population, the observer randomly selected a subgroup (containing 50 bats) using the acetate sheet method. A clear acetate sheet was divided into squares by gridline, and each square was addressed by numbers. The sheet was placed on the monitor screen of the camera which was tracking a group of the flying foxes. Using two random numbers, the subgroup closest to the center of a chosen square was selected. In the case that the flying foxes moved to another roosting site in deeper forest with dense canopy covers, our ability to see a group of the flying foxes could be limited. Therefore, we needed to choose a visible subject group containing at least 30 adults of both sexes. Each one-hour recording session was divided into 15-min sampling intervals, giving 4 sampling points per one-hour recording session. At each sampling point, the behavior of all individuals in the subject group was recorded one by one. All recorded behaviors were classified according to the behavioral units described in Table 1-1. To minimize the effects of observers, the observers kept enough distance, approximately 15–20 m from the roosting trees, so as not to disturb the behavior of the flying foxes. Environmental measurements of ambient temperature, humidity, light intensity and wind speed, were taken at 2.5 m, above the ground in order to find the relationship between the environmental factors and behavioral patterns.

All-occurrence focal sampling

To compare the diurnal activity budgets; the proportions of time spent to different behavioral categories between adult male and adult female bats, another two observers performed all-occurrence focal sampling parallel with the period of

instantaneous scan sampling. All-occurrence focal sampling is the technique in which one individual is observed for all observation sessions [77]. The focal subjects were randomly chosen by acetate sheet method as described above. In this study, we gathered data from 5 males and 5 females. Each focal subject was observed through all recording sessions (totally 7 hr in a day). The flying foxes living in the wild have some naturally distinctive markings, because of injuries. These features, such as damaged ears, scars or holes on wing membranes, enabled the experienced observers to distinguish the subjects from others. In each one-hour recording session (which was stopped for 30 min between each session), all behaviors of the focal bats were recorded with information about the time of occurrence; the length of time (in seconds) for which a single behavior lasts. The recorded behaviors were characterized according to behavioral units, divided into 5 behavioral categories (Table 1-1). Some behavioral units could be considered as the solitary behavior, if that behavior is performed by a single animal without interaction with another individual, such as sleeping, self-grooming, wing flapping, wing spreading, hanging relax and excretion. The social behavior was defined as the behavior involved by two or more animals, such as mutual grooming, maternal care, mating/courtship, aggression and hanging alert [75]. In the case that the subject went out of sight, a new focal subject was randomly chosen immediately. In addition, the interaction or the action of flying foxes in this colony and other wildlife that has effects on one another was noted throughout the observation period, as the data for assessment of the risk of disease transmission across animal species.

Statistical analysis

For instantaneous scan sampling, the raw data were initially tested to determine, if they showed the normal distribution by the Kolmogorov-Smirnov Test. The result

indicated that the data sets were not normally distributed. Thus, non-parametric statistics were used for this behavioral analysis. The number of bats with each behavioral unit was grouped into one-hour blocks and was analyzed to determine 1) whether bats performing each behavior differ between time of day using the Kruskal-Wallis test and 2) whether there is a relationship between behaviors and the weather conditions, using the Spearman correlation. To calculate the percentage, the average number of bats displaying a particular behavior in every one-hour session was first calculated and then was converted to the percentage. For all-occurrence focal sampling, the total length of time (in sec) which focal bats spent for each behavioral category was tested by Mann-Whitney U Tests, to compare the activity budgets between adult males and females. To calculate the percentage, the total duration (in sec) which observed bats dedicated to each behavioral unit or category was divided by the total observation times and then converted to the percentage. Significant correlation and difference of all tests were determined at the probability of $P < 0.05$ (IBM SPSS 18.0, IBM Corp., New York, NY, U.S.A.).

Results

Instantaneous scan sampling

The most common daytime behavior was sleeping ($53.1 \pm 13.9\%$ of all recorded bats), followed by wing flapping ($23.9 \pm 11.2\%$), self-grooming ($5.7 \pm 2.3\%$), wing spreading ($4.1 \pm 1.6\%$), mating/courtship ($3.6 \pm 1.7\%$), hang relax ($2.8 \pm 2.2\%$), aggression ($2.4 \pm 1.7\%$), movement ($2.3 \pm 1.6\%$), hang alert ($1.9 \pm 4.0\%$) and maternal cares ($<1 \pm 0.2\%$). The number of the bats with sleeping, wing flapping, self-grooming, mating/courtship, showing aggression and movement differed significantly with time of day (Kruskal-Wallis test, $\chi^2=48.26$, $d.f.=9$, $P < 0.001$; $\chi^2=85.06$, $d.f.=9$, $P < 0.001$;

$\chi^2=26.57$, $d.f.=9$, $P=0.02$; $\chi^2=73.03$, $d.f.=9$, $P<0.001$; $\chi^2=55.40$, $d.f.=9$, $P<0.001$; and $\chi^2=37.50$, $d.f.=9$, $P<0.001$, respectively). There was no statistical difference in the number of bats with wing spreading throughout the daytime (Kruskal-Wallis test, $\chi^2=8.33$, $d.f.=9$, $P=0.501$). The highest percentage of sleeping behavior was noted in late evening (1600–1700 hr), and the lowest was recorded in early afternoon (1200–1300 hr, Fig. 1-2). Wing flapping was the most common behavior during 1200–1300 hr and decreased consistently from late afternoon to evening (Fig. 1-2). The number of bats with self-grooming peaked in the early morning (0700–0800 hr) and reached the lowest level in the late evening (1600–1700 hr, Fig. 1-2). The number of bats with mating/courtship, aggression and movement behaviors was highest in early morning (0700–0800 hr) and dropped to the lowest level during 1000–1500 hr (Fig. 1-3). We found two types of roosting positions in the flying foxes: hang relax (HR) and hang alert (HA). HR is a resting state which can be seen throughout the day and was not influenced by time of day (Kruskal-Wallis test, $\chi^2=7.91$, $d.f.=9$, $P=0.543$). HA is the excited state of flying foxes and occurred when the flying foxes were activated by other animals, such as the ebony leaf monkey or unknown gibbon species, unexpectedly. Therefore, HA was not included in statistical analysis for behavioral variability with time of day. Maternal cares, excretion and play behaviors were not analyzed due to its rare occurrence. The correlation analysis between the behavior of flying foxes and environmental factors showed that only wing flapping behavior was strongly correlated with ambient temperature, relative humidity and light intensity (Spearman correlation, $r=0.498$, $P<0.001$; $r= -0.530$, $P<0.001$; and $r=0.637$, $P<0.001$, respectively). This indicates that the frequency of wing flapping increased, when air temperature was higher.

All-occurrence focal sampling

There were significant differences in diurnal activity budgets between adult males and females, except in self-maintenance, thermoregulation, negative social behavior and HA behaviors (Table 1-2). During daylight, females spent more time sleeping ($80.5 \pm 4.5\%$ of total observation time) than males ($61.0 \pm 5.0\%$). Males engaged in sexual activities (including mating, courtship and masturbation) more than females (6.5 ± 1.6 and $0.2 \pm 0.1\%$, respectively). During observation, focal males completely mated with several females, an average 3.4 ± 1.0 females per day (maximum = 5, minimum = 2). Females showed a kind of positive social behavior, such as maternal care, by grooming their adjacent pups for an average of $0.4 \pm 0.1\%$ of their times, while males did not participate in parental care (Table 1-2). Another kind of positive social behaviors, such as mutual grooming and play behaviors, was not found in both male and female bats. There was no significant difference in times spent for negative social behavior between males and females. The proportions of time spent on HR behavior and movement in males was higher than those in females (HR: 13.2 ± 1.7 and $3.5 \pm 0.9\%$; movement: 2.5 ± 0.4 and $0.1 \pm 0.0\%$, respectively) (Table 1-2).

Interspecies interaction

The interactions between the flying foxes and other wildlife were recorded throughout the observation period. The flying foxes often interacted with the non-human primate, *Trachypithecus auratus* (ebony leaf monkey). Ebony leaf monkeys disturbed flying foxes by their vocalization and their intrusion an average of 3.3 ± 0.5 times a day. This caused a roosting shift (changing the location of roosting site) of flying foxes during the daytime an average of 1.8 ± 0.3 times a day. HA behavior was mainly triggered by ebony leaf monkeys, but sometimes by unknown wildlife that we

could not identify to the species level, such as gibbon in the family Hylobatidae. Due to dense tree covers at the observation points, it was unclear if there was direct contact between the bats and the monkeys. Not only did the flying foxes face disturbances by wildlife, but they also faced illegal hunters by human. During observation, we found local peoples hunted the flying foxes. This could lead to the high risk of disease transmission to human, through direct contact with body fluid exchanges between bats and human. The observers recognized other potential predators of flying foxes, such as (i) long-tailed macaque (*Macaca fascicularis*), that invaded into the roosting site of the flying foxes, totally 2 times during observation period. (ii) crested-serpent eagle (*Spilornis cheela*), which flew over the roosting site of flying foxes only 1 time during the observation. However, the observers could not confirm that if these potential predators hunt the flying foxes, or not. Furthermore, there were two nests of wild boars (*Sus scrofa*) under the roosting area, where was highly contaminated with flying fox's feces. According to feeding behavior of wild boars, they will dig up the ground to search for food. Such a behavior could make wild boars exposure to the feces of flying foxes.

Discussion

Daytime activities of *P. vampyrus*

This study is the first report on the complete daytime behavioral patterns of the flying foxes, which almost cover the whole range of daytime hours. It is well known that flying foxes are nocturnal animal, but they are also active during daytime hours. The flying foxes performed both solitary and social behaviors. sleeping was the most common behavior that occurred mostly in the late evening. This finding is consistent with that of a previous study on *P. poliocephalus* [25], which found that the highest

percentage of bats asleep primarily occurred during the evening. Wing flapping was the most common behavior during noon and early afternoon. The flying foxes in the tropical zone are usually exposed to high temperatures with strong sunlight during daytime. They typically lack sweat glands for cooling the skin surface and reducing body temperature [105]. Therefore, wing flapping is considered to be the thermoregulatory behavior that flying foxes use for controlling their body temperature, when the ambient temperature is higher than the range of the thermoneutral zone (the range of environmental temperatures without regulatory changes in metabolic heat production of animal) that lies between 24 to 35°C [59, 88].

Self-grooming was one of the common behaviors which can be seen throughout the day, but mostly occurred in early morning. A similar pattern has been observed in another frugivorous bats, *P. poliocephalus* [25]. Nelson suggested [87] that the function of self-grooming is for cleaning the body and wing membrane or reducing oil secretions. For self-maintenance, it is important to keep the wing soft and flexible by spreading the lipid droplets around the wing membrane by self-grooming [104]. In addition, it is a behavioral strategy for reducing the ectoparasite density, which tends to be higher in rainy seasons [71, 112]. The high level of gene flow in the blood-feeding parasite (*Cyclopodia horsfieldi*) found on the body of flying foxes suggested that there were frequent contacts among the flying fox species living in the South-East Asia region [93]. The contacts between host species were promoted by the encounter during foraging in the same habitat or roosting in dense colony and thus enable ectoparasite movements between hosts [94]. Even though bat flies have high degree of host specificity, the disturbance by host's behavior, such as self-grooming, should lead to abandonment of host and/or host switching by bat flies [30]. Therefore, self-grooming is

the potential behavior that allows the spreading of zoonotic diseases in *Pteropus* species.

The reproductive period of *P. vampyrus* varies geographically and seasonally. The flying foxes living in Peninsular Malaysia have the peak of pregnancy in November and January. In Thailand, pregnancy has been noted in the same period to those in Malaysia, but infants are born during March and early April, respectively. In the Philippines, the parturition occurs during April and May [68]. From this information, we can infer that the peak of mating season of the large flying foxes in these countries would be during November and January, because the gestation period of flying foxes is approximately 4 months [88]. However, fruit bats in the family Pteropodidae have a mechanism to delay embryo implantation or interrupt the embryo development in order to coordinate birth with the period of maximum food availability and to create optimal conditions for raising the young bats [88]. Therefore, we could not expect the period of mating season precisely, without daytime behavioral observation. This study found that males tried to approach females for mating many times in a day. This suggests that breeding season of *P. vampyrus* in Indonesia would cover our observation period in May. The highest frequency of mating/courtship behavior was noted in the early morning, and it tended to decrease gradually toward noon. This result corresponded to variation in testosterone secretion levels of *P. vampyrus*, which was high in the early morning and gradually decreased during the day, but this variation was not significantly different [101]. Before mating, males showed courtship behavior by licking around genital area of female, and this often leads to the aggressive behavior of females to reject males. After being rejected by the first female, males tried to find other females several times, until they could mate with female. This leads to high contact rates with body fluid exchanges between males and females during mating season. The author

therefore suggests that male bats have a higher potential to spread viruses within the population. When females refused males, male bats sometimes groomed their penis, resulting in the erection of the penis. This penile grooming is considered masturbation in flying foxes [87]. Aggression and movement patterns showed similar trends to mating/courtship patterns that peaked in the morning and gradually declined during the day. Aggressive behavior of flying foxes has been shown to be influenced by hormonal regulation [100]. The elevations of glucocorticoid was considered a stress response, which usually occurred in breeding males during mating seasons. And, this is the reason why breeding males are more aggressive than non-breeding males [100]. During fighting, bats bite or scratch each other by their claws which are usually contaminated by saliva during self-grooming, and this is a possible way to transmit viruses. Movement activity mainly occurred when the courtship display by males was initiated. Once males moved closer to females, females initially rejected males by showing aggressive behavior and moving away, resulting in the frequent movement of the flying foxes during the mating/courtship period.

Our finding suggests that sexual activities and related behaviors, such as fighting between sexes and competition between males, are the potential actions that promote intraspecies transmission of viruses through high rates of direct contacts during breeding season. In temperate zone, seasonal variation in prevalence of rabies viruses relates to life history pattern of bats that includes seasonal migration and reproduction [43]. This may related to the seasonal changes in social behaviors affecting the contact rates between the flying foxes, and thus would be a biological mechanism driving disease transmission within the host population. To address this issue, long-term study on seasonal variation in bat behavior is required.

Differences in time-activity budgets between males and females

Adults of both sexes spent the majority of daytime sleeping. However, females spent more time sleeping than males. Demment suggested [29] that the high energetic demand for reproduction affects the activity budgets of females, and this is the reason why females require more time for feeding and resting than males. We found differences in time spent on sexual activities, movement, hanging relax and positive social behaviors between sexes. Males allocated their time to sexual activities, movement and hanging relax more than females during the observation period. Prior to the beginning of mating season, male *P. alecto* marked mating territories by rubbing their necks and shoulders on tree branches [76]. This behavior also had been observed in male *P. vampyrus* living in the Philippines (unpublished data). The breeding territories of flying foxes contain up to six females, referred as a “harem” [60, 88]. The higher activity level of males should be partly involved in harem maintenance that provides more mating opportunities and increases reproductive success for males.

The only positive social behavior found in this study was maternal care, which occurred between mother and its independent child. Normally, the contact rates between lactating female and offspring are increased during early of lactation period [53]. Use of saliva during maternal grooming facilitates infection and enables the parasite movement between bats, similar to the situations of sexual activities and fighting behavior. The positive social behavior, such as mutual grooming, was not observed in this study. However, this behavior was recorded in *P. poliocephalus* and in *Cynopterus sphinx*, which helps to establish the social bonds among group members [87, 99]. Seasonal Nipah virus surveillance on *P. lylei*, in Thailand suggested that the viral genome was found frequently during the reproductive periods: mating, parturition and lactation periods [116]. This supports the role of sexual activities and maternal care behaviors on

the viral transmission within the bat colony. In general, females become immunosuppressed during peri-parturition periods making them susceptible to viral infection [96].

Interspecies interaction: the driver of the emergence of zoonotic disease

This study discovered the risk of viral transmission between bats and other wildlife living in the same habitat. The flying foxes had conflicts with *T. auratus* (ebony leaf monkey) for living space, and this caused a roosting shift of flying foxes in the day. It was unclear why the monkeys invaded the roosting tree of the flying foxes. It is possible that the flying foxes occupied a tree that might be food resources of the monkeys. Ebony leaf monkeys would not be a predator of flying foxes, because they are herbivores [63]. Our questionnaire, for the residents and governmental rangers (over 150 persons) also supported that the monkeys do not eat flying foxes. However, the monkey may be exposed to feces of the flying foxes that deposited on tree branches. This is one possibility that viruses are transmitted to other animals in the wild. The flying foxes in this area still face with human encroachments for commercial purposes, because trading of flying fox is still active in many regions of Indonesia [50]. The human-bat interactions have been taking place on a large scale in Asia and Africa [53] and thus enhance bat-to-human spillover risk. In addition to humans, the long-tailed macaque (*M. fascicularis*) and crested serpent eagle (*S. cheela*) could be considered potential predators of the flying foxes in this study area. Ecological interactions, such as predation, also lead to direct contacts between the predators and the flying foxes that enable the exchange of body fluids, such as saliva and blood. This could be a factor that drives zoonotic disease transmission in the wild. Wild boars (*Sus scrofa*) utilized the habitat under the roosting area, which was contaminated with urine/feces of flying

foxes. As a result, wild boars face a higher chance of exposure to bat waste and become one of the potential recipients of pathogens without any direct contact with the flying foxes. Therefore, the surveillance of bat viruses in the animals and humans that have direct or indirect contacts with flying foxes is necessary for the prevention of viral spillovers in this region.

The existence and propagation of viruses depend on multiple factors: host cell, host itself, host population and host community [95]. In this study, we focused on the levels of host population and host community. The flying foxes, as a natural host, are highly social animals that communicate with each other by vocalization and social behaviors. Social behaviors lead to direct contacts between bat individuals, and this enhances the opportunity for virus sharing within/between bat populations. In the wild, the flying fox's community is surrounded by diverse wildlife communities. This condition enables interspecies interactions and transmission of pathogens. Flying foxes also have great ability to make long distance flights. Therefore, transmission of pathogens also has to be considered at the inter-community level, using a satellite telemetry system as a tool for investigation.

Figures and Tables

Figure 1-1

The map shows the location of Leuweung Sancang conservation area ($7^{\circ} 43' 45.12''$ S, $107^{\circ} 54' 10.08''$ E), in West Java Island, Indonesia. The conserved area consists of fisherman village and two roosting sites of the large flying foxes. The roosting site 1 located at $7^{\circ} 43' 51.60''$ S, $107^{\circ} 50' 59.30''$ E, and the roosting site 2 located at $7^{\circ} 43' 24.50''$ S, $107^{\circ} 50' 20.00''$ E. The distance between these roosting sites is approximately 1.48 km.

Figure 1-2

The pattern of sleeping, wing flapping, and self-grooming behaviors of *P. vampyrus* during 0700–1700 hr.

Figure 1-3

The pattern of mating/courtship, movement, and aggression behaviors of *P. vampyrus* during 0700–1700 hr.

Figure 1-1

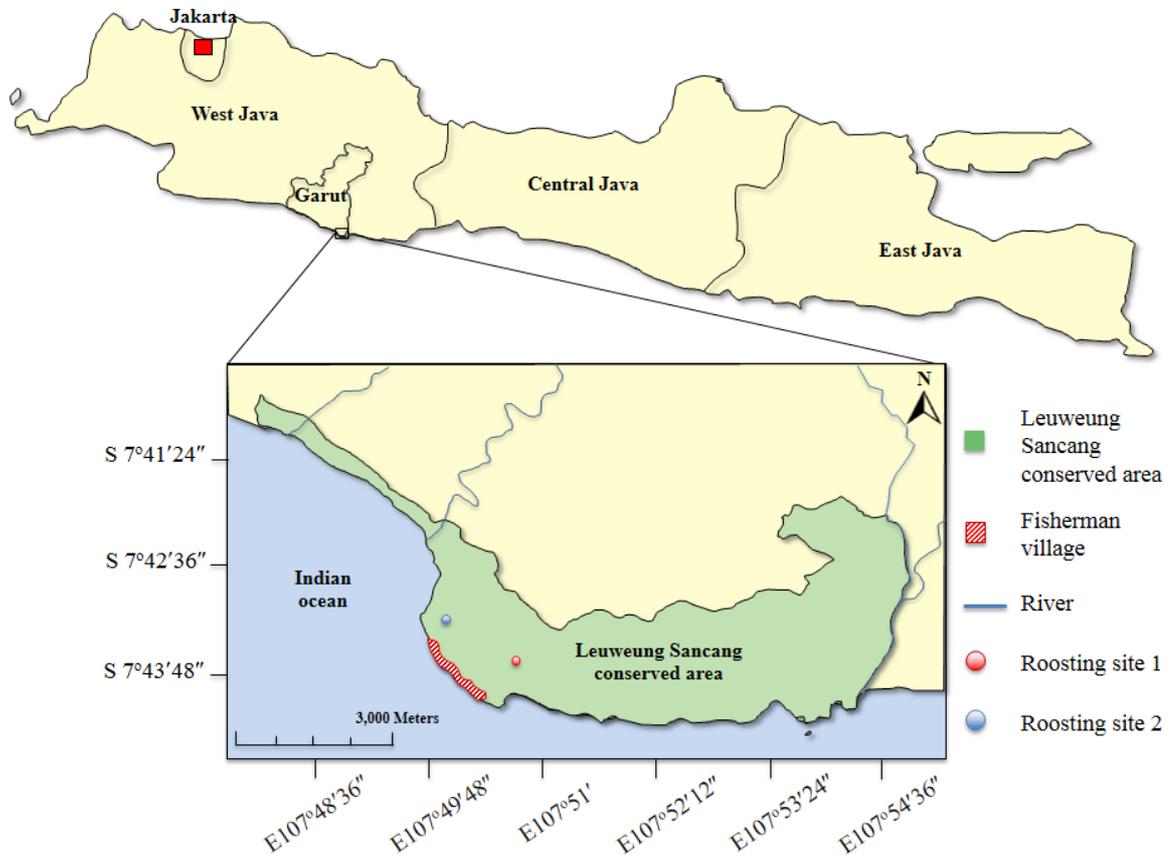


Figure 1-2

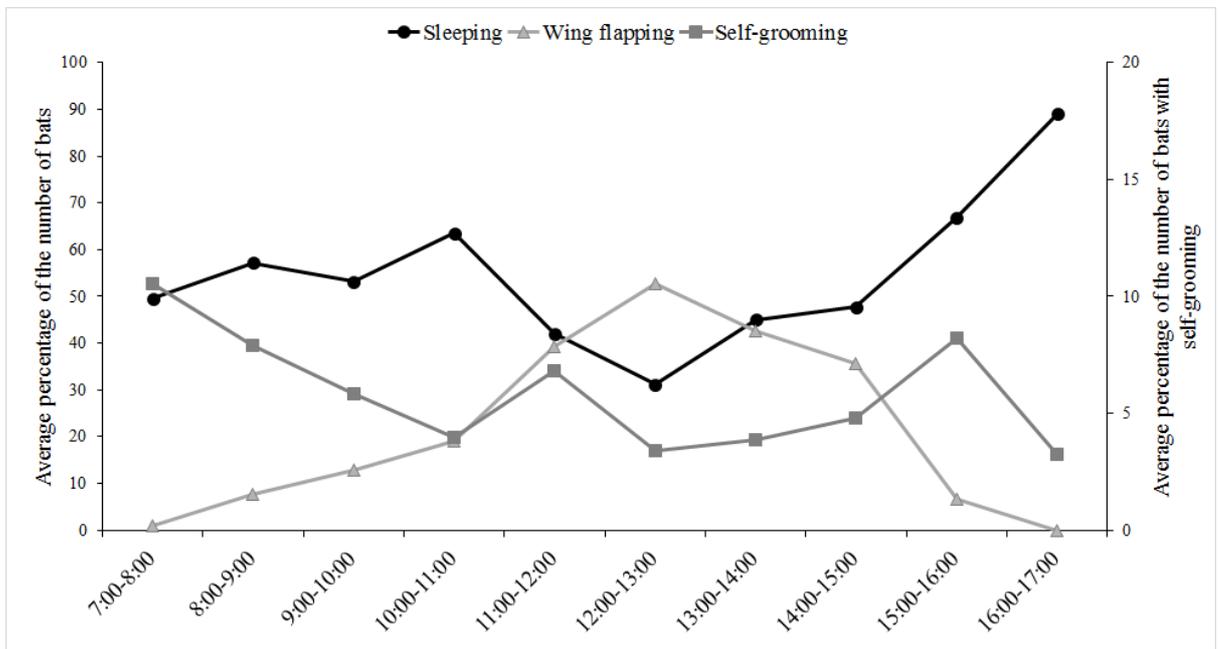


Figure 1-3

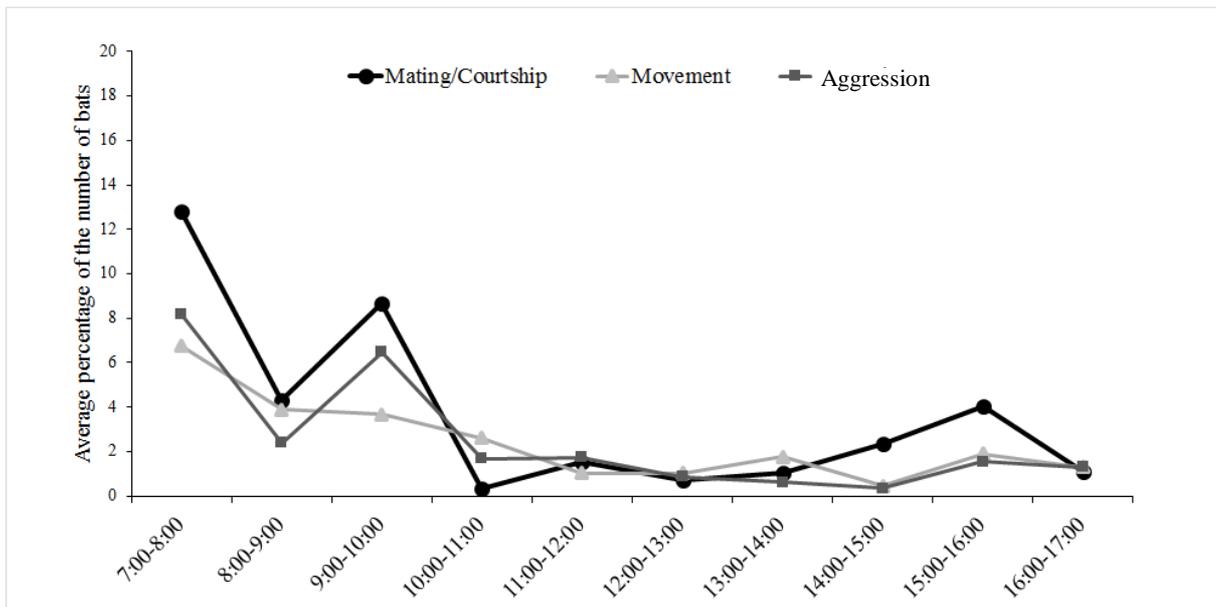


Table 1-1 Ethogram used to score the instantaneous scan sampling and all occurrence focal sampling

Behavioral Category	Behavioral Unit	Description	Context
Sexual activities	Mating	Male grasp-restrains the female from behind, biting female's neck and inserts the penis into the vagina	Reproduction
	Courtship	Male approaches the female and licks genital area of female	Reproduction
	Masturbation	Male starts licking its penis, leading to erection, and continuously licks the erect penis for more than one min, without urination and ejaculation	Serves as masturbatory function, mostly found when male is rejected for mating by a female
Self-maintenance	Self-grooming	Licking wing membrane or occasional bouts of genital tract licking or scratching body part including head	Cleaning function or wing membrane maintenance
Thermoregulation	Wing flapping	Fanning the body with wing membrane	Reducing body temperature
Positive social behavior	Maternal care	Newborn attached to or being carried by female or juvenile grooming by female	Mostly found during lactation period, establishes social bond between the mother and offspring
	Mutual grooming	Licking one another's body, excluding the licking of juvenile by its mother or rubbing of the neck and head	Associated with group recognition, bonding within the group
	Play	Mock-biting or mock-wrestling with an absence of vocalization	Usually occurs among young males
Negative social behavior	Aggression	Aggressive vocalization, wing shaking, chasing, biting and/or fighting between individuals	Self-defense from threats or female rejecting a male
	Hang alert	Hanging bipedally or monopodally with eyes open and ear movement around	Tension state, mostly found when predators or intruders are present
Territorial behavior	Territory defense	Aggressive vocalization, wing shaking, chasing, biting and/or fighting towards invader	Defense of territory that is normally performed by the harem-holding male
	Scent mark	Rubbing neck or shoulder along tree branches	Marking territory
	Wing spreading	Wings widely opened and extended	Related to the defense of territory or threatening display to others, mostly performed by males
Non-categorized	Sleeping	Eyes closed and wing wrapped around the body	
	Hang relax	Hanging bipedally or monopodally with wings folded or wings opened and eyes open looking around	No tension from predators or intruders
	Movement	Moving along a branch or trunk without flying	
	Excretion	Turning the body upright to urinate and/or defecate	

NOTE: These behavioral units were described according to the postures and were grouped into behavioral categories according to their function, following Connell *et al.*, (2006) [25], Nelson (1965) [87], and Markus and Blackshaw (2002) [75,76].

Table 1-2 Average \pm SD proportion of time spent for each activity during 0700 to 1700 hr

Behavioral category	Behavioral Unit	Male (n = 5)		Female (n = 5)		Z(P-value)
		Proportion of time for behavioral unit (%)	Total (%)	Proportion of time for behavioral unit (%)	Total (%)	
Sexual activity	Mating	2.04 \pm 0.7		0.2 \pm 0.1		
	Courtship	4.06 \pm 1.0	6.5 \pm 1.6	0	0.2 \pm 0.1	-4.6(<0.001)
	Masturbation	0.4 \pm 0.2		0		
Self-maintenance	Self-grooming	2.3 \pm 0.4	2.3 \pm 0.4	2.4 \pm 0.5	2.4 \pm 0.5	-0.03(0.97)
Thermoregulation	Wing flapping	11.9 \pm 7.1	11.9 \pm 7.1	10.9 \pm 4.9	10.9 \pm 4.9	-1.6(0.10)
	Maternal care	0		0.4 \pm 0.1		
Positive social behavior	Mutual grooming	0	0	0	0.4 \pm 0.1	-2.4(0.01)
	Play	0		0		
Negative social behavior	Fighting	0.4 \pm 0.2		0.7 \pm 0.5		
	Hang alert	1.9 \pm 1.3	2.1 \pm 0.3	1.3 \pm 1.2	2.0 \pm 0.5	-0.9(0.35)
Territorial behavior	Wing spreading	0.4 \pm 0.0		0		
	Territory defense	0	0.4 \pm 0.0	0	0	-3.6(<0.001)
	Scent mark	0		0		
Non-categorized	Sleeping	61.0 \pm 5.0		80.5 \pm 4.5		-3.2(0.01)
	Hang alert	1.9 \pm 1.3		1.3 \pm 1.2		-1.1(0.23)
	Movement	2.5 \pm 0.4		0.1 \pm 0.0		-3.8(<0.001)
	Excretion	0		0		-

Note: Z = Mann-Whitney U Test; P < 0.05 two-tailed test

Topic 2: Diurnal behavior and activity budget of the golden-crowned flying fox (*Acerodon jubatus*) in the Subic bay forest reserve area, the Philippines

Introduction

With 1,240 identified species, bats constitute approximately 25% of all the mammalian species worldwide [57]. The fruit bats of the family *Pteropodidae*, especially species belonging to the genus *Pteropus*, sometimes aggregate in large populations, with several thousands per colony [83]. Their geographical distribution ranges from tropical and subtropical to temperate regions of Asia, Australia and islands in the Indian Ocean and western Pacific Ocean [5, 91]. Pteropodid bats play a role in propagation of at least 289 plant species, which is important for the maintenance of ecosystem health [40]. However, they are suspected to be hosts of zoonotic viruses, such as filoviruses (Ebola and Marburg virus), which were a serious problem in Africa; henipaviruses (Hendra and Nipah virus), which had outbreaks in Asia and Australia; and lyssavirus (Australian bat lyssavirus), which is widespread in Australia [48]. To estimate the risk of disease emergence, it is necessary to understand the behavioral ecology of wildlife reservoirs. However, knowledge on the behavior of bats is still limited. Only about 5% of all bat studies focus on its ethology [13]. This information highlights the need of a bat behavioral study, especially in a natural condition.

In 2008–2009, filovirus infection was found in domestic pigs and pig farm workers in the Philippines [81]. The serological evidence of Ebola Reston virus infection strongly suggested that the endangered species, *Acerodon jubatus*, is a host of this virus [56]. *A. jubatus* (the golden-crowned flying fox) is a species endemic to the Philippines, with its individual body weight ranging from 0.73 kg to 1.00 kg [28, 113]. Its population has declined by more than 50% in the last few decades, owing to habitat loss [80]. A nocturnal behavioral

study on this species supported the fact that they are nomadic animals with high flight capacity (maximum around 87.04 km per night), and their movement patterns depend on the distribution of food resources [28]. Long-distance flight ability is a key factor in virus transmission, because it enables the bats to come in contact with various animals utilizing in the same foraging area. Comparison between the day- and night-time behavioral data is important for the conservation of bats and the prediction of disease outbreak. However, there have been no studies on the diurnal behavior of the golden-crowned flying fox. Therefore, the activity of *A. jubatus*, living in a mixed-species roost with *P. vampyrus*, at the Subic Bay Freeport Zone, the Philippines, was investigated using instantaneous scan sampling and all-occurrence focal sampling techniques. In most animals, the activity patterns are influenced by environmental factors, such as temperature and/or light intensity [10]. Therefore, the environmental factors were measured in parallel with the behavioral observations.

Materials and Methods

Study site

The field work was undertaken at a mixed-species roost of *A. jubatus* and *P. vampyrus* in the Subic Bay Freeport Zone, the Philippines (14° 47' 13.47" N, 120° 16' 39.25" E) (Fig. 1-4), under the permission of the Subic Bay Metropolitan Authority (Approval number: ECD-RPD-16-0767). The observation period was from 8 to 17 January, 2017, amid the rainy season. The population size of the two bats species was estimated by bounded count method. Estimated population size = $2n_{\max} - n_{\max-1}$, where n_{\max} is the maximum counted number and $n_{\max-1}$ is the second maximum counted number [117]. The majority of the roost was *P. vampyrus* (7,200 individuals), whereas *A. jubatus* comprised a small proportion of the mixed-species population (624 individuals).

Data collection

To examine the variation in diurnal activity of *A. jubatus*, quantitative behavioral data were collected by instantaneous scan sampling—a group scan, during which the behaviors of all individuals in the group were noted in a short period of time [4]. The presence of observer might have an influence on the behavior of the flying foxes. To minimize this, the observation point was set far away from the roosting trees, at over 20 m. We were not able to observe the whole group (containing 624 individuals) within a short time period, and therefore, we selected a subgroup containing 50 bats as the subject group for sampling. The group scans were conducted during 0700–1800 hr on each observation day. Each 1-hr recording session was divided into 20-min sampling intervals, yielding 3 sampling points per recording session. At each sampling point, the behavior of 50 individuals in the subject group was briskly recorded, one by one. By this technique, we got information about the activities of the scanned-group members at a particular period of time. Furthermore, the environmental data, such as ambient temperature, relative humidity, light intensity and wind speed, were measured by Light Meter (LX-2000SD, Custom Corp, Tokyo, Japan) and Hot Wire Anemometer (WS-03SD, Custom Corp), at each sampling point of the scan sampling.

To investigate the sex differences in activity budgets, we performed all-occurrence focal sampling, which concentrates on one individual during a continuous recording period. Data collection was done during 0700–1800 hr, on six adult males and six adult females using binocular as a tool for observation. Sexes of the bats were identified through morphologic observation at genital area. The duration of each recording session was 1 hr, with a 30-min break between consecutive sessions, yielding 7.5 hr of observation period for each focal subject. Capturing and marking of the focal bats might alter the actual behavior

of the flying fox. Therefore, we used naturally distinctive markings, such as damaged ears, scars or holes on wing membranes to distinguish the subjects from one another. In the event of a subject going out of sight, a new focal subject was randomly chosen, immediately. This technique provided the accurate duration of behavioral data, which enables us to compare the activity budget of male and female bats. The behavioral units and categories are described in Table 1-3. Furthermore, the roost-switching events between *P. vampyrus* and *A. jubatus* were recorded to determine the physical inter-species interactions.

Statistical analysis

Normality of the data was examined using Kolmogorov-Smirnov Test. When the distribution of raw data was not normal, non-parametric statistics were used for the analysis of behaviors. The analysis and calculation were conducted separately for each sampling method (scan and focal sampling).

For scan sampling, Kruskal-Wallis Test was performed for nine behavioral units, i.e., sleeping, self-grooming, wing spreading, movement, wing flapping, hanging relaxation, aggression, mating and courtship, to investigate how these behaviors vary with the time of the day. Pearson correlation coefficients were then used to test the possible relationships between the daily variation in daytime behaviors and the environmental factors. The results are presented as average percentage of the number of bats \pm standards errors (SE). To calculate the average percentage of bats for each behavioral unit, the number of bats displaying a particular behavior in a 1-hr recording session was grouped, and then, the average was calculated for each session. The average number of bats for each behavioral unit (n) was divided by the total number of observed bats at each sampling point ($N = 50$) and converted into percentage ($((n/N) \times 100)$).

For focal sampling, Mann-Whitney U Tests were performed for five behavioral categories and the non-categorized behaviors, in order to compare the activity budgets of adult males and females. Results are presented as average percentage of time \pm standards errors (SE). To calculate the average percentage of time spent for each activity, the average duration of time (in sec) that the focal bats allocated to each behavioral category (t) was divided by the total duration of observation (in sec) for one observation day ($T = 27,000$ s) and converted to percentage ($(t/T) \times 100$).

Significant correlations and differences for all tests were determined at $P < 0.05$ level (IBM SPSS 18.0).

Results

Diurnal variations in behavior

The most common behavior was sleeping; on an average, $76.3 \pm 3.0\%$ of the bats exhibited sleeping behavior throughout the day, followed by wing flapping ($5.0 \pm 2.5\%$), self-grooming ($4.2 \pm 0.6\%$), hanging relaxation ($3.4 \pm 0.7\%$), wing spread ($2.9 \pm 0.4\%$), movement ($2.4 \pm 0.5\%$), mating/courtship ($2.4 \pm 0.2\%$), aggression ($1.9 \pm 0.4\%$), hanging alert ($1.2 \pm 0.6\%$), excretion ($0.1 \pm 0.03\%$) and scent marks ($0.05 \pm 0.02\%$).

The statistical analysis showed a significant effect of time on the number of bats performing the following behaviors: sleeping ($\chi^2 = 29.3$, $d.f. = 10$, $P = 0.001$), wing flapping ($\chi^2 = 52.2$, $d.f. = 10$, $P < 0.001$), self-grooming ($\chi^2 = 26.2$, $d.f. = 10$, $P = 0.003$), hanging relaxation ($\chi^2 = 55.3$, $d.f. = 10$, $P < 0.001$), aggression ($\chi^2 = 46.7$, $d.f. = 10$, $P < 0.001$), mating/courtship ($\chi^2 = 40.0$, $d.f. = 10$, $P < 0.001$) and movement ($\chi^2 = 49.6$, $d.f. = 10$, $P < 0.001$) (Fig. 1-5). The highest frequency of sleeping was noted during 1700–1800 hr, whereas the lowest was recorded during 1200–1300 hr. Wing flapping was observed only in late morning and afternoon (1100–1600 hr), and was higher during 1200–1400 hr than

during the rest of this period. Self-grooming and hanging relaxation showed the same trend, peaking during 0800–1000 hr, and then gradually decreasing towards the evening. Mating/courtship, aggression and movement behaviors showed similar temporal patterns during the daytime. The frequencies of these behaviors were the highest during 0900–1000 hr and tended to decrease steadily from this period until the evening (Fig. 1-5).

The analysis showed a significant effect of time on the number of bats exhibiting solitary behaviors ($\chi^2 = 60.6$, $d.f. = 10$, $P < 0.001$), social behaviors with physical contact ($\chi^2 = 50.3$, $d.f. = 10$, $P < 0.001$) and social behaviors with no physical contact ($\chi^2 = 50.9$, $d.f. = 10$, $P < 0.001$). The flying foxes tended to perform the solitary behaviors (including sleeping, self-grooming, movement, wing flap, excretion, hang relax) in the afternoon section, compared with the morning section, whereas the social behaviors with direct contact (including aggressive behavior with biting and/or fighting between individuals and mating/courtship behaviors) and those without physical contact (including aggressive vocal, wing spread, hanging alert and scent marks) were more frequent during the morning, compared with the afternoon (Fig. 1-6).

Hanging alert is an excited behavior, which is seen when the animals were disturbed. In this study, we found that the golden-crowned flying foxes showed hanging alert behavior when they were faced with anthropogenic exposure by tourists and/or predation by aerial predators (bird in the genus *Spilornis*), but there was no disturbance by non-human primates. This is in contrast to my previous study on *P. vampyrus* in Indonesia (In the topic 1), wherein the roosting site of the flying foxes was invaded by non-human primates (*T. auratus*), an average 3.3 ± 0.5 times a day. Therefore, hanging alert was not included in the statistical analysis for behavioral variation with the time of the day. Excretion and scent masking behaviors were not analyzed due to their rare occurrence.

The effect of environmental factors on the behaviors of flying foxes

Self-grooming behavior showed a positive correlation with relative humidity ($r = 0.304$; $P < 0.001$). Wing flapping showed a positive correlation with the ambient temperature ($r = 0.223$; $P = 0.001$) and light intensity ($r = 0.263$; $P < 0.001$), but had a negative relationship with relative humidity ($r = -0.350$; $P < 0.001$). Other correlations among the environmental factors and behaviors were not significant ($P > 0.05$).

Difference in activity budget between males and females

In total, the behavioral data of six males and six females were obtained. The amount of time spent on some behavioral categories was significantly different between adult males and females. Resting state (sleeping behavior) was the most common activity for both adult males and females, with females allocating more time for rest than the males (males = 83.0% and females = 90.0%). Adult males spent more time in sexual activities than the females (males = 1.80% and females = 0%). When a male was rejected by a female, the male chased the female persistently for an average 1.1 ± 0.2 min, until the completion of copulation, which lasted for 1–2 min. The focal males mated with 2.4 ± 0.8 females on the average in a day (maximum = 3, minimum = 1). Furthermore, males spent more time in thermoregulation (males = 1.8% and females = 0.3%), territorial behaviors (males = 2.4% and females = 0%) and movement (males = 0.8% and females = 0.1%) than the females. However, the amount of time spent on self-maintenance, negative social behaviors, hang relax and excretion was not significantly different between males and females. Positive social behavior was not found in either males or females (Table 1-3). The difference in the time spent on thermoregulation seemed related to the location of the observed bats on the roosting trees; males tended to perch on exposed branches (with fewer leaves) and/or on treetops, whereas the females gathered in the center of the trees where they were protected from sunlight.

Roost switching between *P. vampyrus* and *A. jubatus*

The group of golden-crowned flying foxes seemed to select a roosting tree separate from those occupied by the group of *P. vampyrus*. In other words, there was interspecific roost-segregation between these two bats species. However, roost-switching events were observed between these two species. On an average, 30.4 ± 4.7 individuals of both species switched to the roosting trees occupied by the other species, during a day. However, direct contact and interspecific competition between *P. vampyrus* and *A. jubatus* was not clearly observed.

Discussion

The patterns of daytime activity

In this study, the author described the diurnal patterns of activity in the golden-crowned flying foxes, as well as their activity budget in a natural habitat. The most frequently observed activity in the group of flying foxes was sleeping, followed by wing flapping, self-grooming, hanging relaxation, wing spread, movement, mating/courtship, aggression, hanging alert, excretion and scent marks. Connell *et al.* (2006) and my previous study (topic 1) working on *P. poliocephalus* and *P. vampyrus*, respectively, also found that sleeping (resting state) was the most dominant behavior, with the highest frequency in late evening, compared with the other times in a day [25]. *A. jubatus* also showed this tendency.

Wing flapping is a common behavior in the flying fox living in tropical regions, as they are always exposed to severely high ambient temperatures during daytime. This behavior had a strongly positive correlation with the ambient temperature and light intensity, i.e., the flying foxes performed wing flapping more frequently when the ambient temperature and light intensity are higher, especially around noon and early afternoon. Flying foxes lack sweat glands for body temperature regulation [105], and wing flapping is therefore used as

a thermoregulatory behavior to lower body temperature. Ochoa-Acun and Kunz [92] showed that *P. hypomelanus* tended to increase the frequency of wing fanning when the ambient temperature reached 36 °C, thus, exceeding the range of their thermoneutral zone. This gets further support from the fact that wing flapping was rarely observed in the flying foxes living in temperate zones [25].

Bats are known to be parasitized by mites, ticks, bugs, fleas and flies [49]. Bat flies (*Cyclopodia horsfieldi*) are blood-sucking parasites found on the fur and wing membrane of the flying fox species living in South-East Asia [93]. These parasites have negative effects on the physical condition of their hosts, by delaying the timing of reproduction in adults and/or reducing the growth rate of the young ones [82]. In bats, self-grooming is behavioral strategy to reduce parasite loading on body, which tends to be higher during the rainy season [44, 71]. This would explain the positive correlation between the frequency of self-grooming and humidity found in this study. This behavior typically occurred early in the morning, consistent with the study on topic 1 and the study of *P. poliocephalus* in Australia [25].

There is little information available on the mating behavior of the golden-crowned flying foxes. The mating systems have been documented for only 9.2% of the total Pteropid bat species [26]. Most of flying foxes are polygynous, with mating groups that contain a single male and multiple females, also known as harems. In Australian *Pteropus* species (*P. alecto*, *P. gouldi*, *P. poliocephalus* and *P. scapulatus*), males set up their mating territory around the females, just prior to or at the beginning of the breeding season, and the male reproductive success depends on the location of the territory on a roosting tree [78, 87]. To set up mating territories, males rub their neck and shoulder along tree branches, which is referred to as scent-mark behavior. The study in topic 2 found that male bats usually show scent-mark behavior in the late evening before the foraging fly-out, occasionally. Compared with the other behaviors, the frequency of mating/courtship behavior was considerably low.

However, the frequency of mating/courtship behavior seemed to vary during the day, showing the highest rate during early to middle morning, and tended to decrease gradually from noon until the evening. A similar pattern was found in aggressive and movement behaviors. Males moved towards females and then sniffed or licked around the genital area of females. Females always repelled the males by showing aggressive behaviors, such as screaming or wing shaking towards the male and/or moving away. These behaviors normally occurred when mating/courtship behaviors were initiated by males. If a male was rejected by a female, the male chased the female persistently (an average 4.6 ± 1.1 min) until the completion of copulation. This pattern of mating/courtship behavior was also recorded in the Indian flying fox *P. giganteus*; however, males of this bat species spent 20–40 min to chase the females before his copulation was completed [62].

Difference in the activity budget between males and females

Bats spend half their lives living in roosts, where they mate, bear offspring and exhibit a social life. The amount of time spent on these activities has direct effects on the energetic demands and reproductive success of the animals [122]. To balance their energy budgets, bats need to modulate their activity patterns in accordance with the environmental conditions. The golden-crowned flying foxes spend a majority of the daytime sleeping. Due to long-distance flights, the energetic demand for flight in flying foxes is 15 times higher than that of the resting state [18]. This could be the reason why bats need to save the energy for foraging at night by sleeping during the day. We also found significant differences in the activity budget for sleeping, movement, sexual activity, thermoregulation and territorial behaviors between sexes. In polygynous species, the male reproductive success involve male-male competition and/or territory defense [20]. This could be a reason why male bats were more active and allocated a greater proportion of their time for sexual activity and

territorial behavior than the females. This indicates that male would play an important role on viral spreading in the colony. The difference in time spent for thermoregulation behavior could be explained by the roosting position of males and females. The hanging position on branch determined the amount of exposure to sunlight. Males tended to hang on the exposed branches or on treetops, whereas females roosted in more covered or central positions of the colony. Holmes (2002) suggested that the optimal roosting location would be the center of the colony and a shaded place, because it is safer from predation when flying foxes congregate in large numbers [56]. Therefore, males seemed to be exposed to a stronger sunlight and predation risk than the females.

Compared with a previous study on *P. vampyrus* living in Leuweung Sancang conservation area, Indonesia (in the topic 1), *A. jubatus* in this study site spent a greater amount of the daytime sleeping. This could be explained by lesser disturbance by other wildlife in this study area, compared with *P. vampyrus*, which were faced with high disturbance by non-human primates during daytime.

Interspecific interactions in the mixed-species colony

Due to their small population, *A. jubatus* might need to live with the big population of *P. vampyrus*, because living in a large group enhances the protection from predators [106]. Even though the large flying foxes and the golden-crowned flying foxes occupied the same habitat areas, they showed a clear habitat segregation among trees. The roosting segregation between bat species could be a strategy to avoid interspecies breeding and competition. However, a high rate of roosting shifts between the flying fox species was observed in this study site. We could not find direct physical contacts between *P. vampyrus* and *A. jubatus*. However, parasite exchange between species is possible, given the roost shifts. For example, if a parasite detaches from the body of *P. vampyrus*, it is possible for the parasite to re-climb

the tree and attach to *A. jubatus*. Moreover, *A. jubatus* must be exposed to the aerosol derived from the body fluid and/or fecal wastes of *P. vampyrus*, because of their neighboring positions after roost exchange. Therefore, there is a potential risk of the transmission of pathogens between the two bat species in Subic Bay conserved area.

Figures and Table

Figure 1-4

Location of the study site at the Subic bay forest reserve area, the Philippines ($14^{\circ} 47' 13.47''$ N, $120^{\circ} 16' 39.25''$ E).

Figure 1-5

The variation of sleeping (A), wing flapping (B), self-grooming (C), hang relaxation (D), mating/courtship (E), aggression (H) and movement (G) during 0700–1800 hr.

Figure 1-6

The percentage of bats perform solitary behavior, social behavior with direct contact and Social behavior with non-direct contact.

Figure 1-4

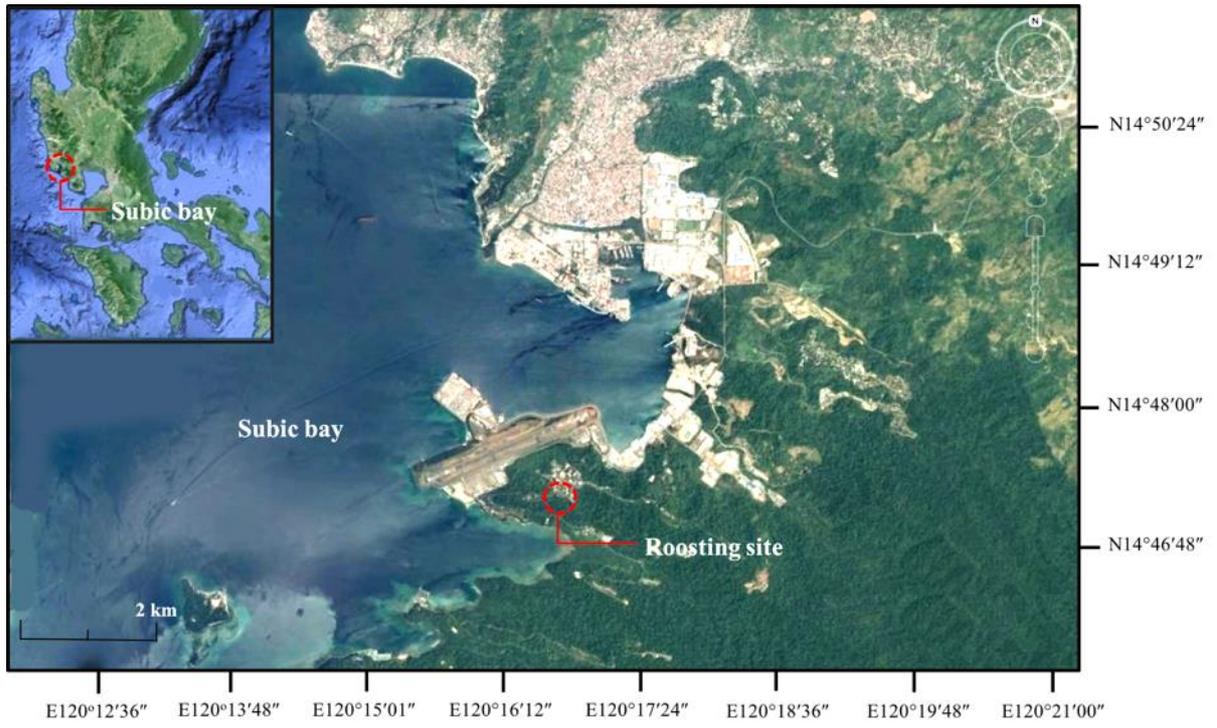


Figure 1-5

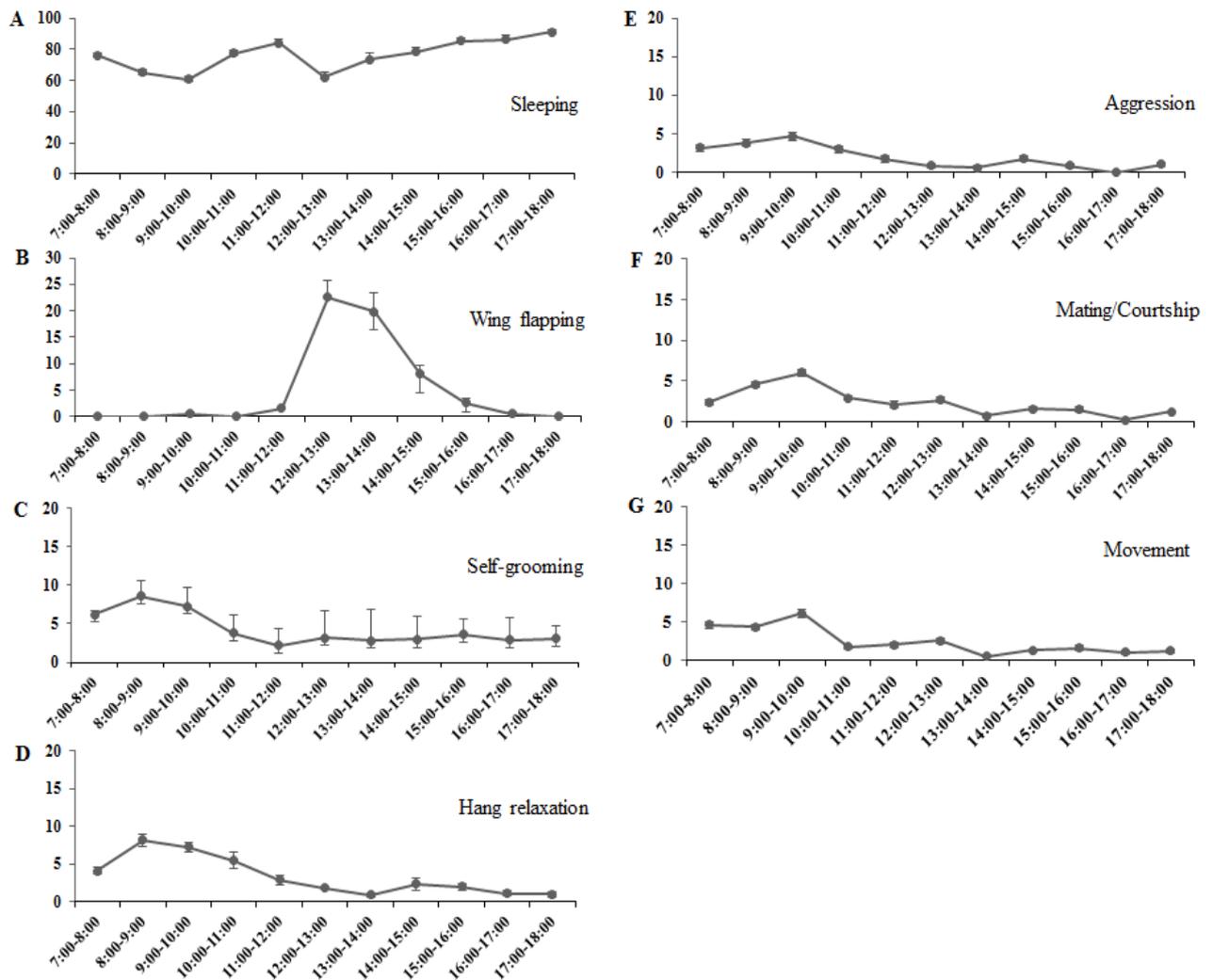


Figure 1-6

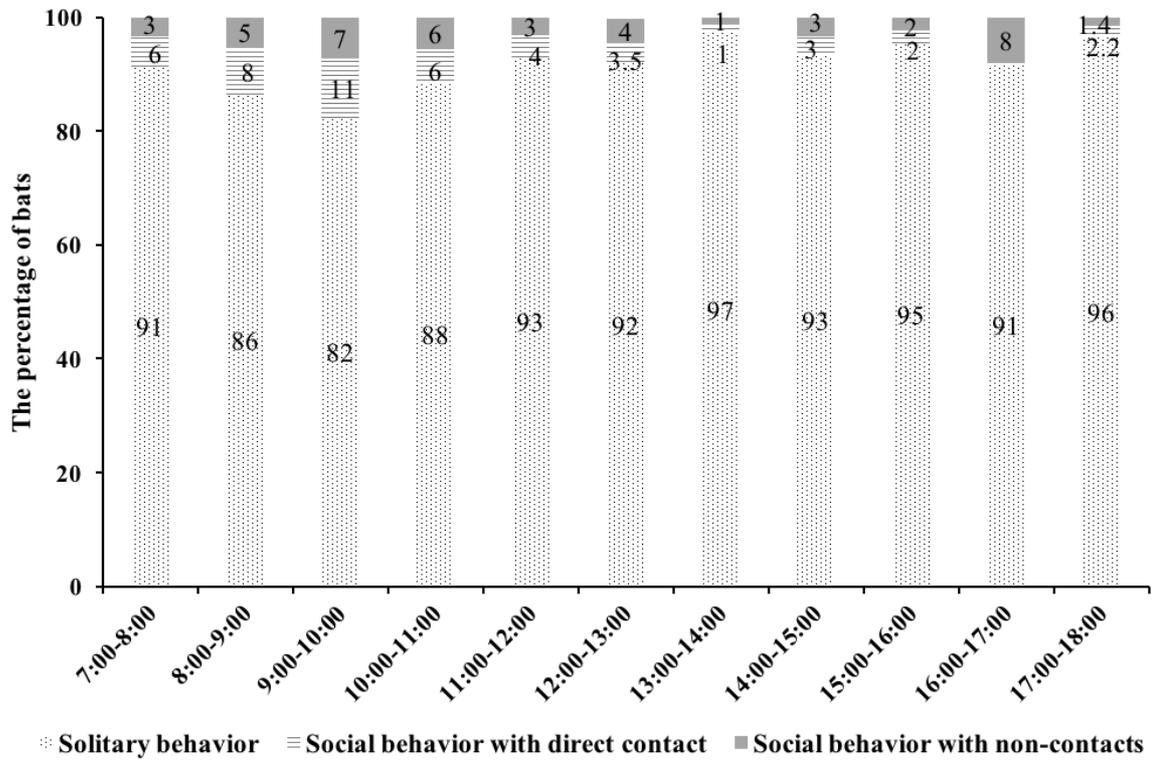


Table 1-3 Average \pm SE proportion of time spent for each activity in male and female bats, during 0700 to 1800

Behavioral Category	Behavioral Unit	Male (n = 5)		Female (n = 5)		Z(P-value)
		Proportion of time for behavioral unit (%)	Total (%)	Proportion of time for behavioral unit (%)	Total (%)	
Sexual activities	Mating	0.16 \pm 0.10		0		
	Courtship	1.04 \pm 0.20	1.80 \pm 0.29	0	0	-3.0 (0.002)
	Masturbation	0.16 \pm 0.10		0		
Self-maintenance	Self-grooming	1.77 \pm 0.30	1.77 \pm 0.30	2.5 \pm 0.40	2.5 \pm 0.40	-1.4 (0.15)
Thermoregulation	Wing flapping	1.8 \pm 0.50	1.8 \pm 0.50	0.3 \pm 0.20	0.3 \pm 0.20	-3.5(0.001)
Positive social behavior	Maternal care	0		0		
	Mutual grooming	0	0	0	0	0
	Play	0		0		
Negative social behavior	Fighting	0.28 \pm 0.06		0.1 \pm 0.06		
	Hang alert	1.09 \pm 0.14	1.37 \pm 0.06	0.5 \pm 0.20	1.6 \pm 0.27	-1.9 (0.055)
Territorial behavior	Territory defense	0.05 \pm 0.04		0		
	Scent mark	0.05 \pm 0.03	2.4 \pm 0.60	0	0	-3.0 (0.002)
	Wing spreading	2.3 \pm 0.60		0		
Non-categorized	Sleeping	83.0 \pm 2.49		90.0 \pm 0.90		-3.2 (0.001)
	Hang relax	6.9 \pm 1.50		5.6 \pm 0.70		-0.4 (0.631)
	Movement	0.8 \pm 0.23		0.1 \pm 0.00		-2.32 (0.02)
	Excretion	0.01 \pm 0.00		0.02 \pm 0.00		-0.6 (0.493)

Note: Z = Mann-Whitney U Test; P < 0.05 two-tailed test

Chapter 2

Nighttime behavior of flying foxes

Topic 1: Nighttime behavioral study of flying foxes on the southern coast of West Java, Indonesia

Introduction

Southeast Asia is home to up to 30 species of flying foxes (family Pteropodidae), which play an important role in ecosystem functions of tropical forests, through pollination and seed dispersion [40, 58]. Based on high deforestation rates in this region, approximately 24% of fruit bat species in the family Pteropodidae may become extinct by the end of this century, due to habitat loss [58]. This anthropogenic disturbance is not only a major threat to flying fox species, but also impacts on humans. For instance, urban areas of eastern Australia have received a large influx of flying fox populations (*P. scapulatus*), causing a nuisance, and public health concerns for the humans living close to them [65]. In general, the movement of flying foxes depends on the distribution of food resources and roosting sites [32]. As natural food resources decline (up to 75% on the east coast of Australia), flying foxes tend to find an alternative food source in residential areas, such as fruit in orchards [97]. The abundance of food resources has attracted the flying foxes, which have set up their colonies in the east coast cities of Australia, with no migration [97]. This increases the conflicts and contacts among flying foxes, horses and humans, leading to Hendra virus (HeV) transmission across species.

The understanding of movement patterns and foraging behaviors of flying foxes is essential for developing conservation methods, as well as for minimizing the conflicts between humans and flying foxes. However, such ecological behaviors of flying foxes are not well understood. To gain this knowledge, we explored the movement pattern of *P. vampyrus*, a species listed as ‘Near Threatened’ by the international IUCN Red List, using satellite telemetry, a powerful research tool for tracking migratory animals [8, 9], and also

performed direct observation on the foraging behavior of fruit bats (*Pteropus* spp. and non-*Pteropus* spp.) in West Java, Indonesia.

Materials and Methods

Study Areas

The studies were performed in the Garut district of the West Java province, Indonesia. For the telemetry study, we captured *P. vampyrus* in their day roosting site at the Leuweung Sancang conservation area (7° 43' 45.12" S, 107° 54' 10.08" E), a home for more than 10,000 flying foxes [7]. For the behavioral study, we observed the foraging behavior of fruit bats at 2 locations. The first location was Mekarsari village (7° 38' 28.9" S, 107° 46' 03.8" E); the second site was Sancang village (7° 41' 44.5" S, 107° 52' 59.2" E) (Fig. 2-1).

Data collection

For the telemetry study, we captured 3 adult males of *P. vampyrus*, for tracking from their roosting site in the Leuweung Sancang conservation area, using a mist net between 1600 and 1730 hours on October 4, 2017. We kept the captured bats in cloth bags until they were anesthetized, using a ketamine and xylazine combination, to reduce stress during handling, following Heard *et al.* (1996) [54]; these processes were approved by Bogor Agricultural University Ethical Committee, as permitted by the Indonesian government. Body mass and forearm length of all bats were recorded. The bats were fitted with tracking collars with a solar-powered satellite Platform Terminal Transmitter (9.5 g Solar PTT-100, Microwave Telemetry, USA) (Fig. 2-2). The mass of the collar with the PTT was 0.8% of the average body mass of a bat (1.31 ± 0.11 kg); this equates to less than 5% of the animal's body weight, as recommended [2]. To reduce stress, all bats were hand-fed with banana before releasing. Each bat was identified by platform ID (164317, 164318, and 164319). The

transmitters were programmed to have 10 hours on and 48 hours off. This enabled the gadgets to save battery, and provided the location data for day and night times. Data were obtained via global satellite-based location, and Data Collection System (DCS). The geolocation data included latitude, longitude, local time, and estimated location errors, which allowed the researcher to estimate the accuracy of locations. We explored the 3 accessible sites where flying fox ID 164319 visited, with an estimated error of < 200 m. The habitat types, fruit trees, and animal species within a 200 m radius of observed points were noted. The data from the first night of tracking were excluded from the analysis, because handling may affect the behavior of flying foxes.

For nighttime behavioral observation, we directly observed flying foxes, *Pteropus* spp. by night vision CCTV camera, from October 5 to 17, 2017. The observation took place at 2 locations, where local people reported that bats had visited. The first point was at a fig tree (*Pittosporum moluccanum*), located on a small hill behind Mekarsari village (Fig. 2-1B). The second point was at a mango tree (*Mangifera indica*), located at Sancang village (Fig. 2-1C). There were other fruit trees such as banana (*Musa* spp.), Sapodilla plum (*Achras sapota*), coconut (*Cocos nucifera*) and soursop (*Annona muricata*) trees around these two locations. Small fruit bats always visited these trees; this allowed us, on occasion, to observe the foraging behavior of non-*Pteropus* bats. Observations were made from 1700 to 0600, which ranged from before sunset and sunrise times. The observation point was set at 20 m away from the selected trees, so as not to disturb the behavior of the bats. The observers noted the number of bats that landed in and around the trees, the duration of bats on the trees, the number of fruit dropping onto the ground, and the behavior of bats during foraging. The behavior of bats was characterized according to the classification in the topic 1.

Analysis

Satellite telemetry study

Day roosts were identified as the recorded locations during sunrise to sunset periods; night roosts were defined as the recorded locations during sunset to sunrise periods. Four values were calculated for each tracked bat: 1) the maximum displacement, which was the distance in km between the day roost at which the bat was captured and the furthest recorded roost; 2) cumulative distance, which was determined as the summation of all distances between consecutive roosts within a 1-day period; 3) the home range, which was specified as the area covering all recorded day/night roosts of the tracked individual; 4) percentage of revisited feeding roosts, which were calculated using:

$$\% \text{ of revisited feeding roosts} = \frac{f}{T} \times 100$$

f = the number of night roosts which the bat had visited more than once throughout the tracking period.

T = the total number of recorded night roosts during the tracking period.

Night time behavioral study

We calculated the average number of flying fox visits, the average duration that the bat stayed on the tree, and the average number of fruit dropping for each location. We also analyzed the difference in number of bat visits at different times of night, using the Kruskal-Wallis test. The relationship between the number of bat visits and the number of fruits dropping at different times were analyzed by Spearman correlation. Significant correlations and differences for all tests were determined at a probability of $P < 0.05$ (IBM SPSS 18, IBM Corporation, New York, USA).

Results

The telemetry study

We downloaded 14 days' data from three satellite-tracked males (ID number: 164317, 164318, and 164319). Unfortunately, the data from bat ID 164318 was lost by unknown reason. The bat might be died, and the body fallen down to ground wherein satellites could not detect the signal from transmitter. For bat ID 164317, we obtained a total of 22 location points for night roosts, and 24 location points for day roosts. Daytime roosting sites were in various habitat types, such as forest protected areas, agricultural areas, and mountainous ranges. The nighttime roosts consisted of forest protected areas, agricultural land, and small wooded plantations in residential areas. The maximum displacement was about 100 km, with the furthest roost being located outside the Garut district, but still in the West Java province. The cumulative travelling distance for a day was 25.5 ± 9.2 km on average ($n = 5$) (Table 2-1). The home range, which encompassed all recorded roosts, was 136,773 ha (Fig. 2-3). Site fidelity of day roosts was low for this individual bat. Three to four day-roost shifts were seen in a single day. For the foraging sites, 22% of all nighttime roosts were repeatedly visited at least twice during the tracking period.

For bat ID 164319, we collected a total of 26 locations for night roosts, and 18 geolocations of daytime roosting sites. The day roosts were found in forest protected areas, agricultural landscapes, and mountain ranges, as for the former bat; the night roosts considered as feeding sites were located in forest protected areas, agricultural areas, mountain ranges, and residential areas. The maximum displacement was approximately 15.6 km, with the furthest roost located within the Garut district. The cumulative displacement for a 1-day period was 8.7 ± 2.3 km on average ($n = 5$). The home range of this bat covered 6,505 ha of Garut city, a much smaller area than the former individual (Fig. 2-4). Roost fidelity was also low for this bat. This flying fox switched day roosts around 3-6 times in a

single day. However, 11.5% of all feeding sites were repeatedly visited during the tracking period (Table 2-1). To estimate the risk of disease transmission, we performed a walking survey at 3 feeding sites of this bat. The first location was agricultural land, 0.9 km away from the nearest village; the food plants were soursop (*Annona muricata*), jackfruit (*Artocarpus heterophyllus*), banana (*Musa* spp.), and fig trees (*Pittosporum moluccanum*). The second foraging place was located in a small village in which domestic animals such as dogs, goats, and chicken were free-living; the fruit trees found in this location were banana (*Musa* spp.), fig trees (*Ficus* spp.), and rambutan (*Nephelium lappaceum*). The third point was located in an *Albizia chinensis* garden, where fruit trees such as *Bridelia tomentosa*, banana, and papaya (*Carica papaya*) were planted. Therefore, these locations were considered to be contact zones for flying foxes, humans, and domestic animals.

Nighttime behavior

Study site 1: Fig tree

During 4 nights of direct observation, the number of bats on and around the tree was an average of 121.0 ± 30.4 per night. At least 39.1% of visiting bat landed on the tree, with 60.9% of bats flying around, or passing by, the tree. Most of the observed bats (83.5%) performed solitary foraging, while 16.5% foraged in a group containing 2–5 individuals. The average duration for a bat to search and consume fruit on the tree was 12.0 ± 1.7 min (maximum = 67 min, minimum = 1 min). Behaviors observed during foraging were wing spreading, excretion, fighting, aggressive calls, movement, hanging relax, and hanging alert. The flying foxes visited the fig tree from 1800 to 0500, which was around 30 min after sunset and 30 min before sunrise. The number of bat visits was significantly different during the night hours (Kruskal-Wallis test, $\chi^2 = 27.18$, $d.f. = 11$, $P = 0.004$). The number of bat visits was highest from 1900 to 2100, and lowest from 2300 to 0000, and 0400 to 0500. We found

a positive correlation between the number of bat visits and the number of fruits dropping (Spearman correlation, $r = 0.379$, $P = 0.008$) (Fig. 2-5). Also, the observers occasionally found domestic animals such as dog, cat, goat, and chicken, searching for food under the observed tree during daytime.

Study site 2: Mango tree

During 4 nights of investigation, the number of bats on and around the tree was an average of 5.7 ± 2.3 per night. 60% of the total number of bats landed on the tree, while 40% just flew around or passed by the tree. All bats visiting the mango tree came alone (solitary foraging). No foraging groups were observed. The average duration for a bat to occupy the tree was 25.0 ± 6.4 min (maximum = 40 min, minimum = 10 min). Behaviors observed during foraging were wing spreading, movement, hanging relax, and hanging alert. As for study site 1, the flying foxes visited the mango tree from 1800 to 0500. The number of bats visiting was not significantly different between the night hours (Kruskal-Wallis test, $\chi^2 = 15.2$, $d.f. = 11$, $P = 0.14$). However, the highest number of bat visits was found from 0000 to 0100. A positive relationship was detected between the number of bat visits and the number of fruits dropping (Spearman correlation, $r = 0.430$, $P = 0.002$) (Fig. 2-6).

Differences in the foraging behavior of *Pteropus* and non-*Pteropus* species

Pteropus and non-*Pteropus* bats have some differences in their foraging behaviors. Flying fox species usually accessed the tree by landing at the top or middle of the canopy. On occasions where the fruit tree was near to people's homes, the flying foxes carefully accessed the fruit tree by landing on the adjacent tree first, and looking around for up to 20 min; after that, they jumped to the feeding tree, and started searching for food. Small fruit bats (non-*Pteropus* spp.) usually flew around, or hovered near, the targeted tree many times

before approaching it. After approaching, they spent short bouts (< 10 sec) eating the chosen fruit, with rest periods in an adjacent tree (< 8 m away from the fruit tree) between bouts. In cases where the fruit or food was smaller than their body size, they would take the food, and move to another tree to eat it.

Discussion

The telemetry study

Most flying fox species (genus *Pteropus*) are highly mobile mammals which commonly forage across wide areas [85]. However, their day/night movements are not well understood. In this study, we provided semi-quantitative data of movement patterns and foraging behavior in *P. vampyrus*, which is recognized as the natural host of Nipah virus (NiV) [23]. Our study suggested several behaviors: (i) Flying foxes show low roosting site fidelity. They tended to shift day roosts frequently (a maximum of 6 times) in a day, as we expected. This supports a previous study in Borneo [46] where *P. vampyrus* occupied multiple daytime roosting sites. It was suggested that the benefits of frequent movement between daytime roosts were the avoidance of disturbance by humans, minimization of commuting distance to feeding areas, reduction of predation, and escape from high population densities of ectoparasites. (ii) Males of *P. vampyrus* are nomadic animals, and the distance moved was unpredictable and varied between bat individuals. For example, the tracked bat ID 164319 exhibited nomadic movement, with 15.6 km of maximum displacement between releasing point to furthest recorded roost (Fig. 2-4). Bat ID 164317 showed a longer nomadic movement, with almost 100 km of maximum displacement (Fig. 2-3). The movement of all species of *Pteropus* bats is greatly dependent on food plants. Since they do not hibernate, they need to consume food all year. To find seasonal food resources, they have developed the capacity for migration or nomadic movement [51]. *P.*

vampyrus in West Java seems to be nomadic rather than migratory, because the southern coast area of the Garut district is covered by evergreen forest and fruit plantations, that can provide food for the flying foxes year-round. Therefore, flying foxes do not need to migrate to other regions far from Garut. Local peoples also mentioned that flying foxes are present in this area all year round. (iii) The home range of *P. vampyrus* covered diverse habitat types. The bats spent time roosting and foraging within or outside the natural forest (Leuweung Sancang conservation area). The majority of habitats were natural conservation areas, forest plantations, agricultural land, and residential areas. *P. vampyrus* showed some degree of foraging site fidelity, by visiting the same feeding locations repeatedly. This result is not surprising because returning to predictable food sites allows bats to save time and energy [114]. However, larger samples including different sexes and reproductive conditions are required, to provide a comprehensive understanding of their movement ecology.

The walking surveys allowed us to identify 8 species of food plant which are also consumed by humans. The most common fruit in the observed locations was banana. This fruit has also been recorded as the most frequently consumed plant species for *P. lylei* in Thailand [119]. Eby (1991) mentioned that fruit bats forage on cultivated fruits when natural food resources become insufficient, making them become an agricultural pest [32]. This conflict between bats and humans causes illegal hunting of flying foxes in several countries of Southeast Asia [36]. The adaptation for foraging in human areas leads to overlap zones between flying foxes, humans, and domestic animals; this factor may drive the spread of infectious disease from bats to humans and domestic animals in this region.

Foraging behavior

During our observations, we found that numerous flying foxes visited the observed trees, especially the fig tree. More than 30 species of fig tree have been recorded in the diet

of pteropid bats in Asia, Australia and Africa [12]. Flying foxes foraged both in groups and alone. This behavior was also observed on *P. giganteus* in India [111]. Group foraging is beneficial for sharing information about feeding locations, that improves foraging efficiency and reduces risk of predation [5, 120].

Feeding behavior of bats varied among bat species and food size. This study found that small fruit bats or non-*Pteropus* spp. performed *ex situ* feeding behavior (carrying fruit to another tree for feeding) when the fruit was smaller than them; they also exhibited *in situ* feeding patterns (feeding on fruit on the same tree), when the fruit was bigger than them. The *Pteropus* bats only showed *in situ* feeding behavior; this result is consistent with a previous study on fruit bats, *Cynopterus sphinx* and *P. giganteus* [86]. To save time and energy, flying foxes not only perform *in situ* feeding, but they also stay on the fruit tree for some period after eating [86]. This enhances the risk of predation and intraspecific interference. In contrast, *ex situ* feeding behavior is considered a foraging strategy that reduces intraspecific competition and predation risks [33]. This reflects trade-offs between predation risks and energy gains, that vary between individuals and species. During feeding, bats dropped partially eaten fruits on the ground. The advantage of this event is to facilitate seed dispersion, but on the other hand, it increases virus-contaminated areas along the feeding route of bats.

Based on our serological survey, the flying fox population in this study site were seropositive for Japanese encephalitic virus (13%), Nipah virus (4%), Zika virus (2%) and Dengue virus (5%) (unpublished data). During foraging, the observed flying foxes displayed various activities such as wing spreading, excretion, fighting, aggressive calls, movement, hanging relax, and hanging alert. Some of these activities probably lead to the transmission of pathogens from flying foxes to other animals. In 1994, chimpanzees fed on a fig tree alongside fruit bats before developing Ebola hemorrhagic fever [37]. This incident supported

the idea that a feeding tree for fruit bats, being a meeting point for different animals, might be an origin for disease transmission. Fighting between bat individuals facilitates physical contact and bodily fluid exchanges among bats, and thus may cause the viral spillover at feeding sites. This study found variation in the number of bat visits at different times of night. Bats started to visit the observed trees around 1800 (30 min after sunset time), leading to the possibility that bats remembered the location of the fruit. The frequency of bat visits at different hours was significantly correlated with the number of fruits dropping. This indicated that the number of fruits dropping might be used as an indicator of bat activity levels during foraging.

In conclusion, our findings have improved the knowledge of the ecological movement and foraging behavior of *P. vampyrus*, and thus will be very useful for developing an alternative method to conserve flying fox populations, as well as managing the conflict between humans and bats in Indonesia.

Figures and Table

Figure 2-1

Map showing the location of capture sites in the Leuweung Sancang conservation area (green area, $7^{\circ} 43' 45.12''$ S, $107^{\circ} 54' 10.08''$ E). **A.** The behavioral study took place in the red star area of the Garut district, Indonesia. There were two study sites for observation: **B.** At a fig tree in Mekarsari village ($7^{\circ} 38' 28.9''$ S, $107^{\circ} 46' 03.8''$ E); **C.** At a mango tree in Sancang village ($97^{\circ} 41' 44.5''$ S, $107^{\circ} 52' 59.2''$ E).

Figure 2-2

P. vampyrus with solar-powered satellite transmitters.

Figure 2-3

The minimum home range of *P. vampyrus* (ID 164317) is represented in the yellow polygon. Green dots represent day roost locations, while yellow dots represent night roost locations. The maximum distance from the release point (orange star) to the furthest roost is shown as a dashed line.

Figure 2-4

The minimum home range of *P. vampyrus* (ID 164319) is represented in the yellow polygon. Green triangles represent day roost locations, while yellow triangles represent night roost locations. The maximum distance from the release point (orange star) to the furthest roost is shown as a dashed line.

Figure 2-5

The number of bat visits and dropping fruits observed at different times of night at the fig tree study site.

Figure 2-6

The number of bat visits and dropping fruits observed at different times of night at the mango tree study site.

Figure 2-1

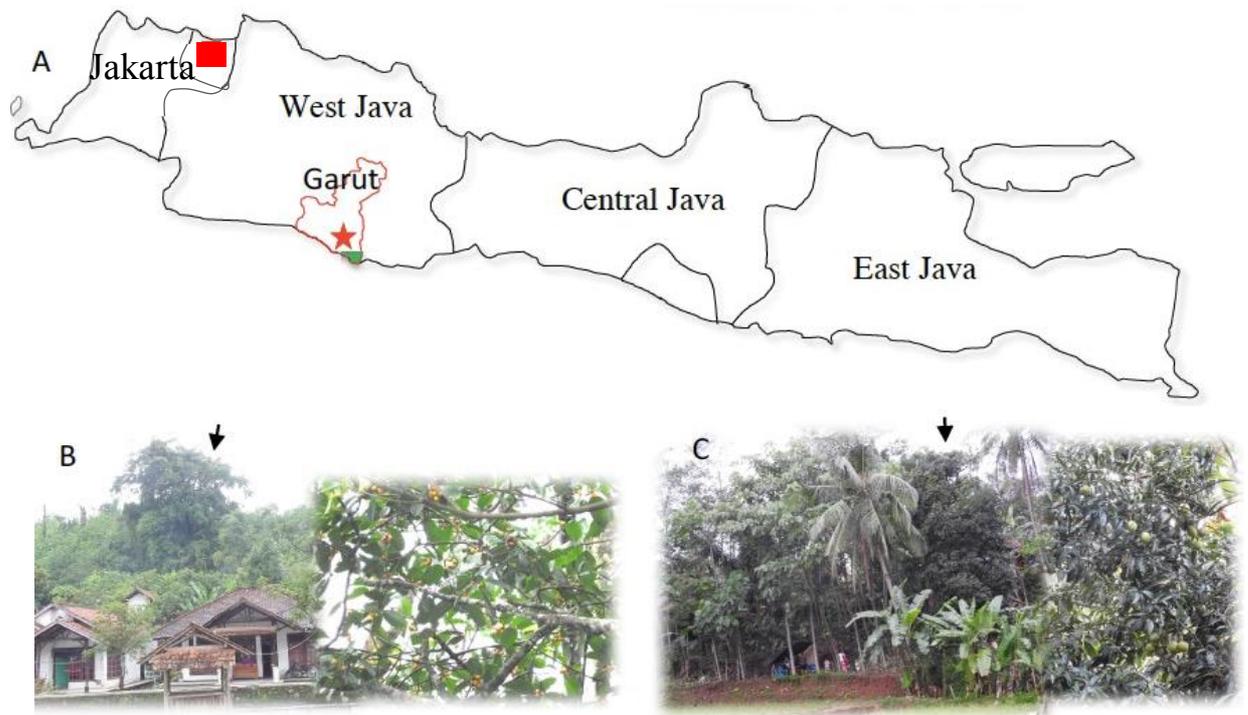


Figure 2-2



Figure 2-3

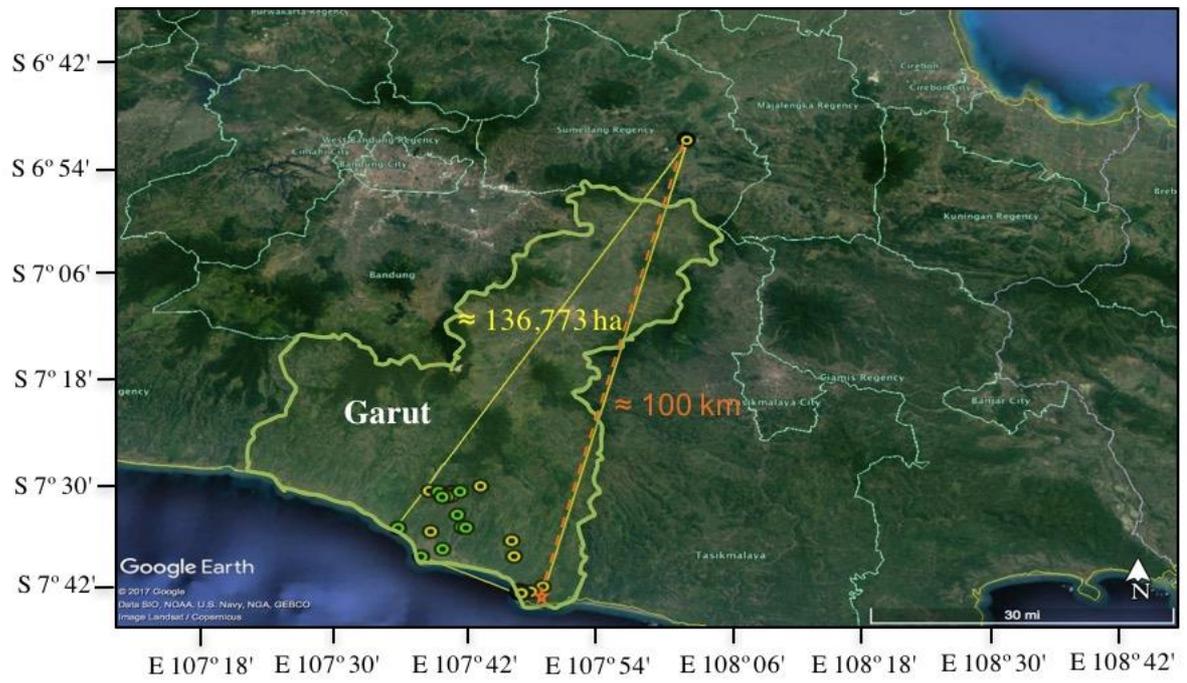


Figure 2-4

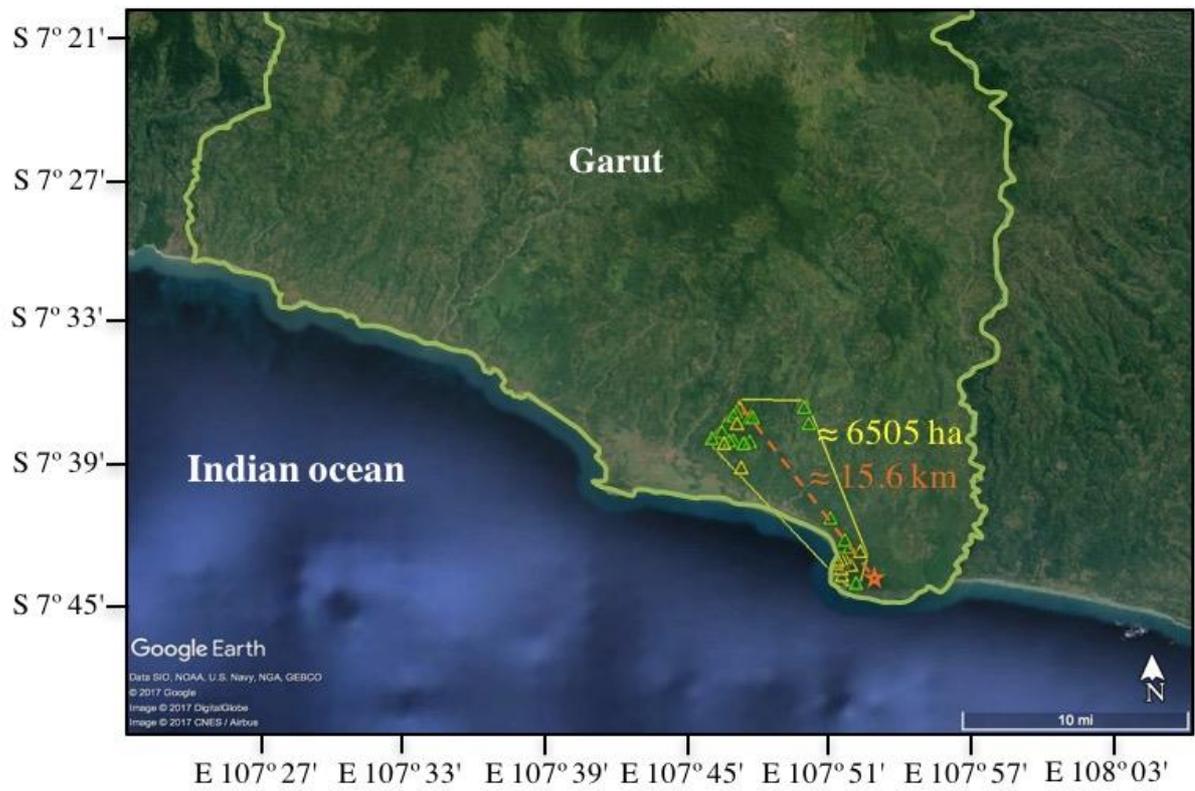


Figure 2-5

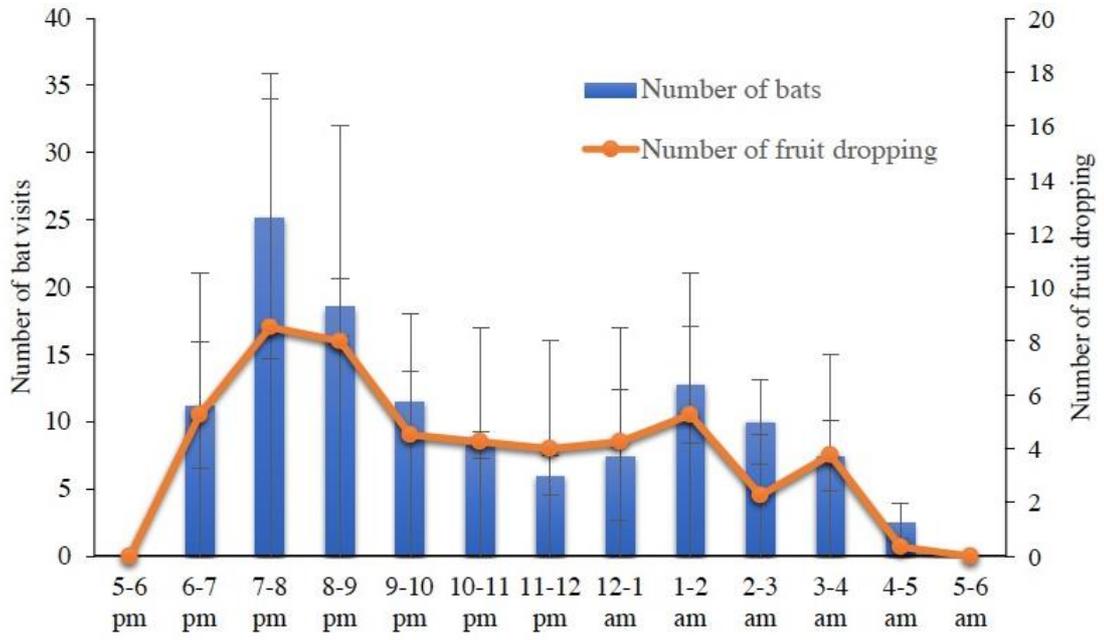


Figure 2-6

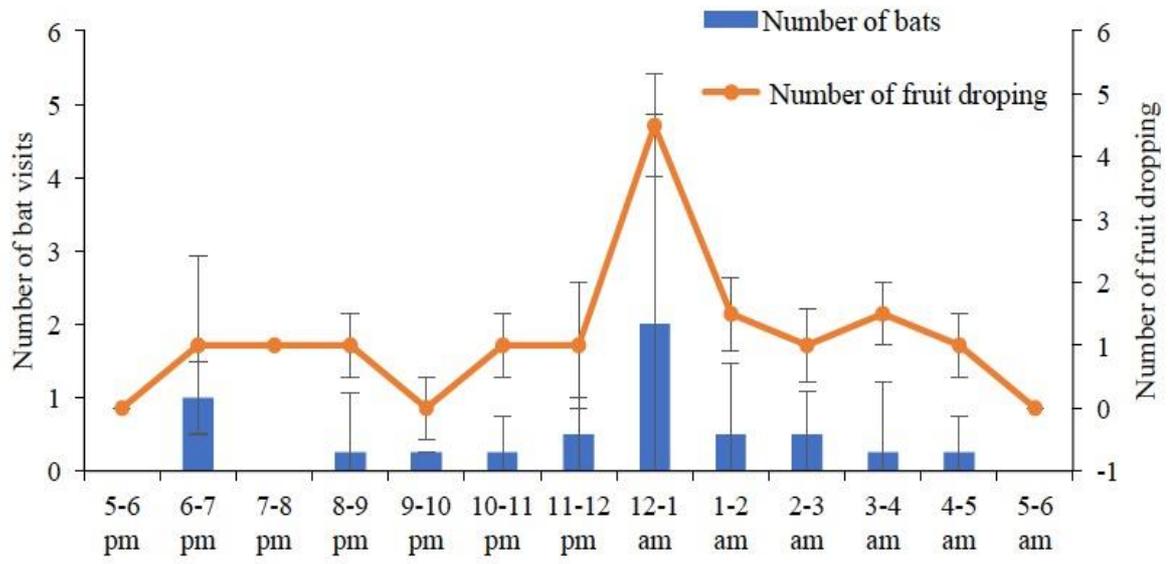


Table 2-1. Data summary of characteristic, distance moved, the number of roosting sites over 14-days of tracking period in *P. vampyrus*

Bat ID	Capture site	Weight (kg)/ Forearm length (cm)	Night roosts	Day roosts	Max displacement (km)	Cumulative distance (km)	% of re-visited night roosts	Home range
164317	Leuweung Sancang	1.45/74	22	24	100	25.5 ± 9.2	22 %	136,773 ha
164319	Leuweung Sancang	1.25/72	26	18	15.6	8.7 ± 2.3	11.5%	6,505 ha

Chapter 3

Population dynamics of flying foxes

Topic 1: Seasonal variation in mortality rates of *Pteropus lylei* in Wat Pho Bang

Khla temple, Thailand

Introduction

Thailand has four species of *Pteropus* bat (*P. hypomelanus*, *P. intermedius*, *P. lylei*, and *P. vampyrus*) [6]. *P. hypomelanus*, *P. intermedius*, and *P. vampyrus* have roosting sites on islands and in forests, while *P. lylei* live in human communities (e.g., temples) where contact between bats and humans occurs [8, 110]. To establish whether a higher contact with humans carries higher risk of disease, therefore, this study focused on *P. lylei* (Lyle's flying fox). Their home ranges include Cambodia, Thailand, Vietnam, and southern China, but their distribution in Thailand is restricted to the central and eastern parts [11, 21]. This bat is classified as "Vulnerable" by the IUCN Red List due to the reduction in their populations [17]. Surveillance of the Nipah virus confirmed that up to 6.7 % of the flying fox population living at the central plain of Thailand were infected with this virus, especially during the reproductive cycle, which lasts for 5 months [115, 116]. This suggested that there was seasonal variation in disease transmission, which might be related to their behavior and/or population dynamics. Mortality rates in their roosting sites are a very important part of their population dynamics. Therefore, we examined the variation in mortality rate of Lyle's flying foxes at their permanent roosting site in Thailand, from 2015–2017, to understand the population dynamics of this bat species.

Materials and Methods

The study was conducted at Wat Pho Bang Khla temple (13° 43' 16.37" N, 101° 12' 06.00" E), Chachoengsao Province, Thailand, which is home to 6,128 Lyle's flying

foxes [21]. The study site is a tourist attraction that covers a large area (17,300 m²). Therefore, it is a potential hotspot for bat-borne disease in Thailand. To evaluate the variation in mortality rate of flying foxes, the number, sex, and age class of dead bats were recorded every day from July 2015 to July 2017. All dead bats were removed after observation, to avoid counting the same individual twice. The temperature at 2 m height aboveground in a grid of 13.75 N, 101.25 E around Wat Pho Bang Khla temple during this period was obtained from the Japanese 55-year Reanalysis (JRA-55) [61]. Non-parametric tests were used for statistical analysis as the raw data were not normally distributed. The seasonal variation and sex/age class difference in mortality rate was examined using the Kruskal-Wallis test. The relationship between the number of bat deaths and ambient temperature was analyzed using Spearman's rank correlation. Significance of all tests was determined at $P < 0.05$ (IBM SPSS 18, IBM Corporation, New York, U.S.).

Results

A significant difference in the mortality rate of Lyle's flying foxes between time of a year was found (Kruskal-Wallis test: $\chi^2 = 44.6$, $d.f. = 11$, $P < 0.001$). The highest mortality rate was recorded during March and April, which was the birthing period, while the lowest was observed from August to February, which was part of the mating season (Fig. 3-1). During March and April, the number of newborn deaths was higher than adults of both sexes. From May to July, the number of adult female deaths was higher than adult males and juveniles. In November and January (mating season), the mortality rates of males were higher than females and juveniles (Fig. 3-2). However, these sex/age class differences were not significant (Kruskal-Wallis test: $P > 0.05$). This might be the effect of a small sample size of only 2 years data. A larger sample size

might have higher power to detect a statistical significance. We found a strong correlation between the number of bat deaths and average ambient temperature in each month (Spearman's rank correlation: $r = 0.73$, $P < 0.001$) (Fig. 3-1).

Discussion

This is the first report of seasonal mortality for *P. lylei* in Thailand. This data suggests that mortality rates of Lyle's flying foxes varied across the different seasons. A higher mortality rate, especially in adult females and juveniles, was observed during the reproductive cycle, specifically the birthing and lactation periods. This can be explained by nutritional stress, high energetic requirements, and immunosuppression associated with pregnancy and lactation. Korine et al., (2004) [64] showed that lactating females increase their metabolic energy intake by up to 80%, compared to non-reproductive females. This demonstrates the high energetic demands of lactation. However, fruit bats consume low-carbohydrate/protein food, so that lactating females might also be deficient in nutrients. To compensate for the increase in such requirements, reproductive bats reduce their energy expenditure for self-maintenance [12]. Furthermore, immunosuppression during pregnancy and lactation has been demonstrated in bats [11]. This may partly explain why lactating females showed higher mortality rates than males from March to July. The high mortality rate in juvenile bats could have resulted from their weak immunity against infection and anthropogenic impacts. Muhldorfer, et al, (2011) [84] suggested that juvenile bats were significantly more susceptible to infection than adults, and this was a major cause of juvenile death in the maternity season. Furthermore, one study showed pesticide residues in the milk of bats after they consumed contaminated foods [67]. Therefore, consuming pesticide-contaminated milk could also influence the mortality rate of juveniles. A higher mortality rate in adult

males during the mating season was also observed in this study. This can be explained by the male-specific behavior of Lyle's flying foxes. Adult males usually perform aggressive behavior (fighting) to protect their mating territories. This may cause injury and even death of males [7]. In addition, the mortality rate of *P. lylei* was positively related with ambient temperature. Previous studies reported that climate change with extreme high temperature affects the survival and mortality rate of fruit bats, because they have low tolerance to high temperature [1].

Figures

Figure 3-1

The relationship between variation in mortality rate of *P. lylei* and temperature. Light-gray, white, and dark-gray areas represent the rainy, cold, and hot seasons, respectively. The mating season last from November to August, while the maternity season last from March to May.

Figure 3-2

The difference in mortality rate of adult male, adult female, and juvenile bats in each month.

Figure 3-1

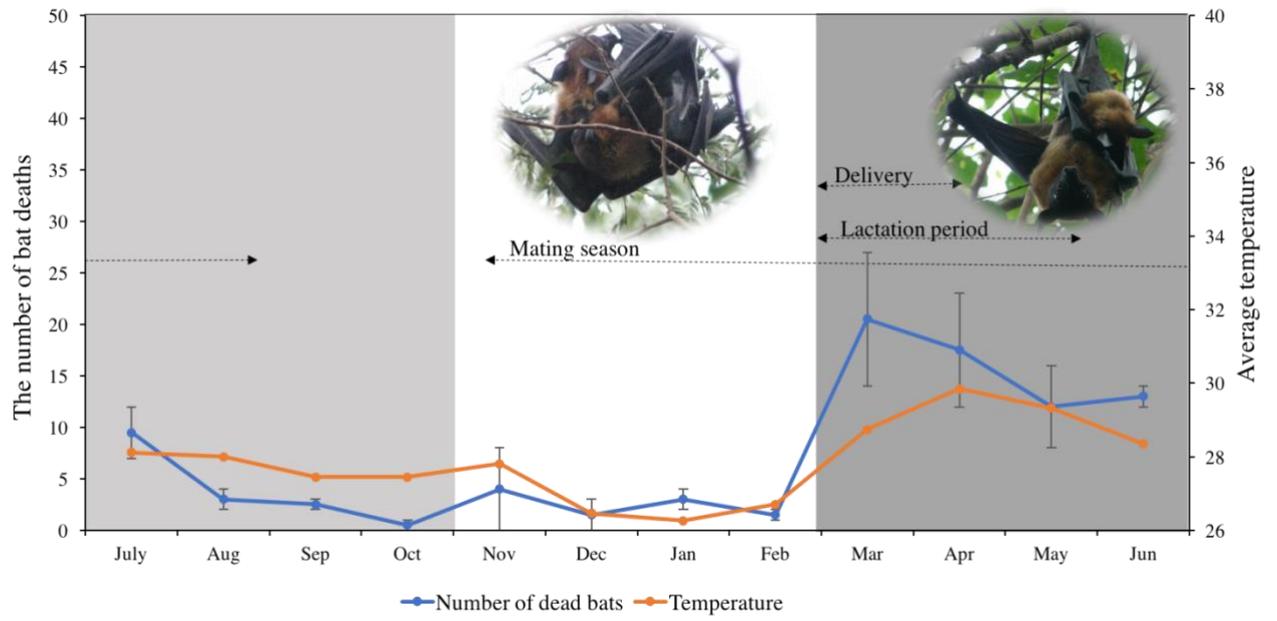
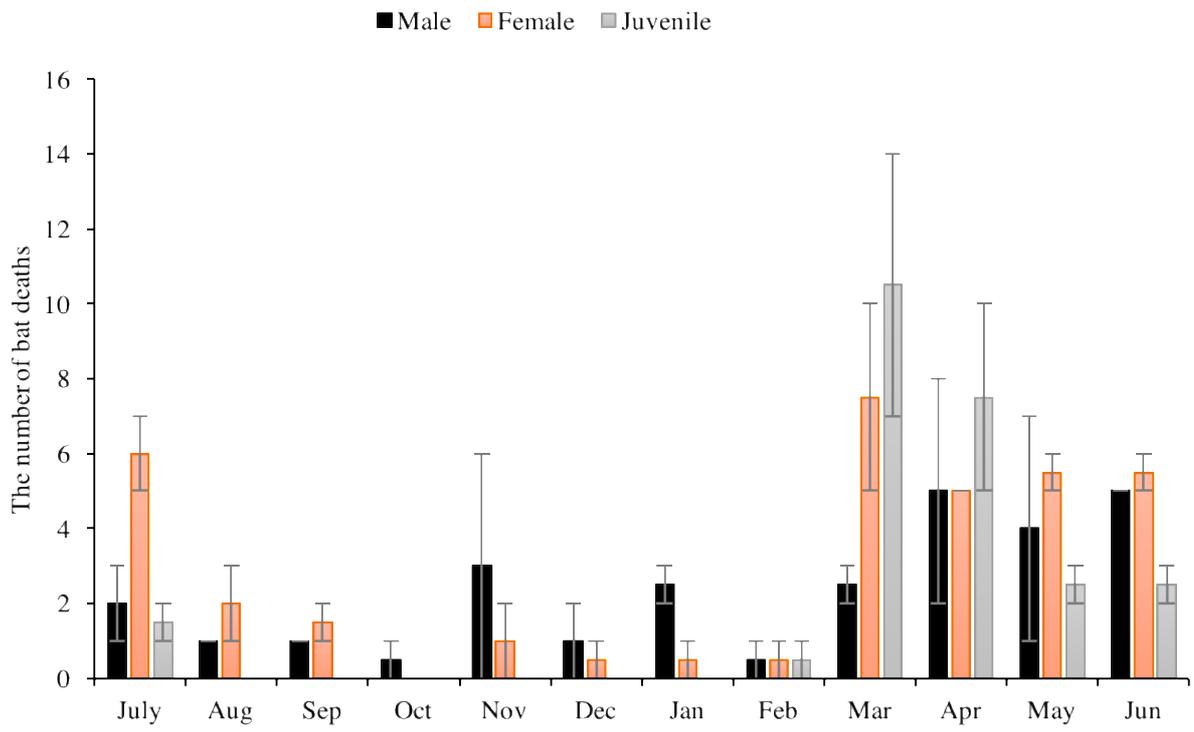


Figure 3-2.



General Discussion

Animal behavior is the ways which animals interact with other individuals of both in the same species and other species, or with their environment [89]. However, these interactions sometime lead to novel infectious diseases spreading from wildlife to human populations. Therefore, this study investigated the ecological behavior of flying foxes, and provided the quantitative data of day/nighttime behaviors of flying foxes, which is very important for developing the mathematical simulation of intra/interspecies viral transmission models. This could lead to the precise prediction of outbreak and pandemic of emerging infectious diseases in the nature.

In chapter 1, the author revealed all of daytime behaviors of flying foxes that can be found at daytime roosts. Based on our findings, we could specify non-active behavior (i.e. sleeping), and active behaviors which promote the contact rate and/or pathogen exchanges between bat individuals (i.e. self-grooming, mutual grooming, mating/courtship, maternal care and aggression). Furthermore, we provided the quantitative data on (i) the frequency of these active behaviors along daytime (ii) the proportion of time spent for these active activities in male and female bats. (iii) The frequency of interspecies interactions at natural habitats of flying foxes. This information would allow scientists to calculate the variation in the contact rate among bats, which is the most important factor on viral transmission among bat individuals [22]. Therefore, further studies which aim to develop the models for infectious disease transmission to predict the expansion of pathogens inside the colonies of flying foxes, could set the value of epidemiological parameters, based on our reliable data.

The movement of organism is one of the most basic character of life on Earth. However, it is also one of the most important factor driving the wide spreads of infectious disease on Earth. Therefore, in the chapter 2, we paid a particular attention to the movement ecology of flying foxes. Based on our well-designed study, we could

estimate (i) distance of movement and size of home range in specific period, (ii) favorite-feeding location of the flying foxes and (iii) the interface area among humans, livestock and flying foxes in Indonesia. This information not only allows us to predict the hotspot areas wherein the bat-viral spillover might occur, but also to use as relevant data for developing the species distribution model. This model are tools used to map the geographical distribution of animals [34]. In addition, it also has been widely used for making prediction on the movement pattern of the animals across the study area. Therefore, it should allow us to predict the areas where flying foxes may move in the future, and thus is very necessary for making the strategies of disease prevention in this region.

Many studies illustrated the strong seasonal variation of virus prevalence in bat populations, such as in the case of rabies and nipah virus infections [43, 115]. This variation of infection appeared to be involved with the complex mixtures, including population dynamic of host species [43]. Therefore, in chapter 3, we examined the population dynamics of flying foxes, including (i) mortality rate of whole population during a year, and (ii) age/sex specific mortality rate in each season. This information is required for calculating the changing of population size along times of a year [31], which is needed to create the dynamic transmission models for this bat species. Furthermore, the mortality rate is ecological factor that regulate the growth of host population [118]. If the growth of host population is constant, the number of infected/susceptible hosts could be limited. And this would allow us to measure and predict the disease expansion in our environments.

In conclusion, this study highlights the necessity of collaboration between mathematicians and biologists, who perform field works and accumulate the data on

animal behavior in the wild, to build up the transmission models, which are essential to understand the disease ecology.

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