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The hormonal and behavioral response to the presence and activities of humans in three co-roosting flying fox species (*Acerodon jubatus*, *Pteropus vampyrus* and *P. hypomelanus*) in Boracay and Mambukal in the Philippines*

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Abstract　Many species of large flying foxes are endangered, mainly due to habitat loss and hunting. Although hunting and logging are prohibited in many areas in the Old World Tropics, numbers of flying foxes are still decreasing. The best compromise for the welfare of both local residents and flying foxes in many areas would be ecotourism. However, the effect of human activities on the welfare of flying foxes is unknown and needs to be investigated. Using non-invasive methods, stress levels of three species of flying foxes (*Pteropus vampyrus*, *Pteropus hypomelanus* and the endangered *Acerodon jubatus*) in two tourist areas in the Philippines were investigated, Boracay with little human activity, and Mambukal with intense and large variations in human activities. Fecal samples were collected in the most disturbed area, Mambukal, while the flying foxes were out foraging and concentrations of glucocorticoid metabolites (GCM) were measured. Stress-related as well as other types of behavior, environmental conditions and human activities were measured. The results suggest that flying foxes have habituated to the presence of humans. (1) We found no overall differences in behaviors between both study sites, which differed significantly in human numbers and activities, and during days with disturbance and days without disturbance. Flying foxes behaved identical in both study sites. (2) No behavior correlated with measured GCM. (3) Despite the large variation in human activities in the study site, we found no effect of human activities on glucocorticoid metabolite concentrations. However, GCM was lower in the center of the colony than in the periphery. (4) Some environmental factors like disturbance and distance from the colony center had an effect on behavioral factors like 'uneasiness', 'body care' and yawning. If disturbance has no effect on flying fox stress levels, ecotourism would be a good solution to preserve the habitat of endangered flying foxes. However, one has to realize that the influence of human presence on bat behavior might be more complex than outlined in this study which is based on visible bahaviors, and that disturbance should always be kept at a minimum [*Acta Zoologica Sinica* 52 (5): 827-837, 2006].

Key words　Flying foxes, Megachiroptera, Glucocorticoid metabolites, Corticosterone, Stress, Behavior, Conservation, Ecotourism

菲律宾 Boracay 岛和 Mambukal 岛三种共栖狐蝠对人类活动的行为和激素反应*

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摘　要　栖息地丧失和捕猎导致许多大型狐蝠濒危。尽管在东半球热带地区已经禁止捕猎和采伐，狐蝠的数量仍然在下降。既能维护当地居民利益又能保护狐蝠的折衷对策是生态旅游。然而，人类活动对狐蝠的影响还是

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未知的。菲律宾 Boracay 和 Mambukal 都是旅游区，前者游人少而稳定，后者游人密集且变异较大。我们用非损伤取样法研究了这两个旅游区三种狐蝠的生理紧张情况。在 Mambukal，当狐蝠外出采食时，采集狐蝠的粪样，实验室分析粪样肾上腺皮质激素代谢物（GCM）浓度。我们观察记录了紧张反应等一些行为，同时记录了环境因素和人类活动情况。结果表明狐蝠已经习惯了游人的活动，表现在：（1）我们发现两个旅游区游人数量和活动强度差异显著，但是两地狐蝠的行为没有明显的差异；并且在有干扰和无干扰的日期之间，狐蝠的行为也无显著差异；两个旅游区的狐蝠行为表达一致；（2）各种行为与测定的粪样 GCM 浓度都不相关；（3）尽管在不同研究地点人类活动变异很大，我们发现人类活动并未影响粪样 GCM 浓度；不过，栖息在狐蝠群中心位置个体的粪样 GCM 浓度低于在群外围个体的 GCM 浓度；（4）一些环境因素（如干扰以及与栖息群的距离）影响狐蝠一些行为（如不安、身体护理和哈欠）的表达。如果干扰没有造成狐蝠紧张反应，那么生态旅游将不失为一种保护濒危狐蝠栖息地的理想方案。不过，我们还应认识到，人类干扰对狐蝠行为的影响可能比本研究观察到的更加复杂。因此，对于保护濒危狐蝠，时刻保持干扰最小是最好的选择［动物学报 52（5）：827－837，2006］。
关键词　狐蝠　大蝙蝠亚目　肾上腺皮质激素代谢物　皮质酮　紧张　行为　保护　生态旅游

Measuring metabolites of stress hormones is the most direct way of determining animal welfare (Möstl and Palme, 2002; Von der Ohe and Servheen, 2002). Stressors, in vertebrates, stimulate the sympathetic nervous system, which results in secretion of norepinephrine and adrenaline. This stress response has a perceptible effect within milliseconds, increasing heart rate and blood glucose and stimulating blood diversion to organs needed for 'fight or flight' (Fox, 1999; Reeder and Kramer, 2005). Reproduction, foraging activity and territorial behavior are suppressed, while escape or avoidance behavior is promoted. At the same time, the hypothalamic-pituary-adrenal axis causes the increased secretion of adrenocorticotropic hormone by the anterior pituary, which stimulates the adrenal cortex to secrete glucocorticoids (Selye, 1950). The adrenocortical stress response acts to provide the energy needed for escape or avoidance behavior over a longer period of time (Reeder and Kramer, 2005). If stress is prolonged or repeated, the adrenocortical stress response and its metabolites will lead to exhaustion and possibly sickness or death (Wingfield et al., 1997). Stress in animals is often measured by extracting glucocorticoids from the animal's blood (Walker et al., 2005). However, catching the animal and extracting blood is a stressor itself and will increase the level of stress hormones at the time of collection. Therefore, it is difficult to determine whether the measured stress levels are induced by handling or by environmental factors. Non-invasive methods for quantifying the amount of stress, which are desirable for conservation biology research, have recently been developed and tested (e.g., Brown et al., 1997; Palme and Möstl, 1997; Wasser et al., 2000; Möstl et al., 2002; Touma and Palme, 2005). In mammals, metabolites of glucocorticoids end up in the feces and urine and can be measured using group specific enzyme immunoassay (Palme and Möstl, 1997). In this study, we measure the effects of human activities and environmental factors on the levels of corticosterone metabolites in feces of flying foxes.

Although one fifth of presently discovered mammals are bats, relatively little conservation research has been done on this order. Flying foxes (Mammalia: Chiroptera: Pteropodidae) are large, primarily fruit-eating bats (Fujita and Tuttle, 1991), occurring in the Old World tropics. Several recent studies have shown the high ecological importance of flying foxes, for they are dispersers of seeds and pollinators of large night-blooming flowers (e.g., Rainey et al., 1995; Banack, 1998; Shilton et al., 1999; Curio et al., 2002). The rapid decline in many species is mainly caused by habitat loss (Jones at al., 2003) and flying foxes are vulnerable to hunting pressure or catastrophes (Pierson and Rainey, 1992). Although in most areas hunting has been prohibited, the mere presence of humans near the roosting site might still be stressful for the animals, and animals living in the periphery might have to cope with more external stressing factors than the relatively more protected animals in the center. The social system of many flying fox species determines the location inside the colony, the most dominant animals staying in the center of the colony (Eby et al., 2001). Most human activities near flying fox roosts are assumed to be stressful for the bats. Given that these activities are virtually always long-lasting, it is most probable that these activities have negative effects on the welfare of the flying foxes.

In the Philippines, the golden-crowned flying fox *Acerodon jubatus* and the Kalong or giant flying fox *Pteropus vampyrus* commonly roosts together with the common island flying fox *Pteropus hypomelanus*. *Acerodon jubatus* is declared endangered by the IUCN and is listed in Appendix Ⅱ of CITES; *P. vampyrus* and *P. hypomelanus* can be found in the larger part of South-east Asia (Hilton-Taylor, 2000). On the Island of Boracay (Panay, West Visayas, Philippines 122′N 12′E), one of the last roosts of these three species can still be found. Development for the tourist sector has stripped off 65% of the island's nature (Trousdale, 1998) and only a small patch of forest in the northern tip of the island (approx. 150 hm^2) can still house at least 2 000 flying foxes. The total population size of the three species of flying foxes on Boracay has declined from approximately 30 000 in the early 1980's (Guadalupe, pers. comm.) to 2 000 (Mildenstein et al., 2003). The proportion of the endangered *A. jubatus* in the island's total population has dropped from 58% in 1997 (Luft and Meier, 1998) to only 12% of the flying fox population in 2002 (Mildenstein et al., 2003[①]). The number of residents on the island has increased from approx. 2 000 in the early 80's to 9 000 in 1995 and in the same period the number of tourists has increased from nearly 0 to up to 16 000 visitors per year (Luft and Meier, 1999), then to 599 000 per year (Dept. of Tourism, pers. comm. 2006) and the number of people on the island is growing still. Because local island residents rely completely on tourism, ecotourism would be the best compromise on Boracay, although ecotourism can have serious effects on the stress levels and life cycles of animals (e.g., Griffiths and van Schaik, 1993; de la Torre et al., 2000; Müllner et al., 2004).

The first aim of this study is to determine how human activities and other environmental conditions influence stress levels in the three species of flying foxes. Fecal samples were collected and concentrations of glucocorticoid metabolites (GCM) were measured. To our knowledge, this non-invasive method for stress quantification was used for the first time in a wild population of flying foxes. The second aim is to determine how environmental and behavioral factors are correlated and which types of behavior are most indicative of stress. The effects of human activity, weather conditions and location in the colony on GCM and behavior are investigated and recommendations are made for future development. If human activity affects stress, we expect Mambukal bats to have higher basic GCM levels than Boracay bats as tourist pressure in the immediate proximity of the flying fox colony is much higher in Mambukal. We also hypothesize that GCM levels are higher on days with many visitors than on quiet days. Furthermore, we expect to observe more stress-related behavior, like collective fly-ups, on these days. Finally, we expect that animals in the centre of the colony are less stressed than animals in the periphery.

1 Materials and methods

1.1 Study area and population

The study was carried out between 4 March and 17 October 2004 in two populations of flying foxes, each comprising of *A. jubatus*, *P. vampyrus* and *P. hypomelanus*. Both study sites, the island of Boracay and the Mambukal resort, are located in the West Visayas, the Philippines (Fig. 1) and characterized by type Ⅲ climate; the seasons are not pronounced, however, it is relatively dry from November to April, and wet between May and October (see also Lorica et al.[②]). The Mambukal colony was located inside a popular recreation area, while the Boracay colony, our control site, was located on the relatively quiet side of Boracay Island, the Philippines' most popular tourist attraction. The bat population in Mambukal Resort (Negros Occidental, West Visayas, Philippines), a 23.6-hectare resort at 366 meters above sea level, characterized by secondary growth forest, consisted of over 3 000 individuals: *A. jubatus* 5%, *P vampyrus* 10% and *P. hypomelanus* 85% (Mildenstein et al., 2003). Data collection posed no difficulties in this area. The population roosting on Boracay consisted of over 2 000 individuals, *A. jubatus* 12%, *P. vampyrus* 85%, *P. hypomelanus* 3% (Mildenstein et al., 2003 and additional data from Cariño, pers. comm.). The flying foxes were always in one group, occupying 1 of 5 roosting sites spread over the northern tip of the island (approx. 150 hm^2). The area was characterized by mainly secondary limestone forest, dominated by pioneer species. GCM levels and behaviors of the Boracay population were compared to the behaviors of the Mambukal population.

1.2 Observations

After quantifying and selecting types of behavior that could be indicative of stress (see below), data gathering took place between 1 April and 17 October 2004. On alternating days, behavior was recorded. On each observation day, nine consecutive periods of 5 min observations were done. There was a 2-min rest interval between all 5 min observation periods, in which no behavior was recorded, the total observation period adding up to 61 min per day. Fly-ups, i.e. communally flying away from the roost, were recorded throughout the period of our presence and were not limited to the 5 min observation periods. Observers were at least 100 meters away from the day roost, to minimize disturbance in the bats. On each observation day a different focal group of at least 6 and at most 10

① Mildenstein T, Stier S, Cariño AB, 2003. Final Report: Bat Count 2002. Unpublished.

② Lorica RP, Curio E, van der Aa PJH, 2005. Behavioral responses of the large flying foxes to human-induced disturbance. Wildlife Conservation Society Philippines. Unpublished.

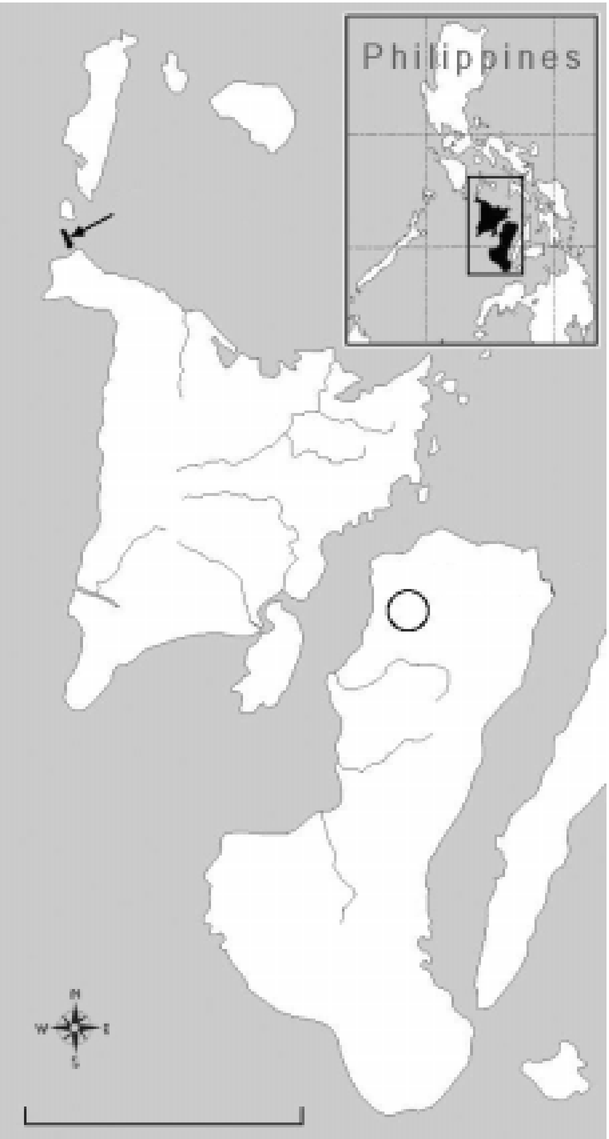

Fig.1 Location of study sites Boracay and Mambukal in the Philippines

individuals was selected from fairly visible clumps of bats and the behaviors of all individuals in this focal group were recorded simultaneously. The following behaviors were recorded: (1) wing stretching, i. e. the stretching of one or both wings fully while hanging in resting position, was believed to be non-stressed behavior, as the bat would have to feel secure enough to shift its center of gravity. It might also be agonistic behavior (Kaiser et al.①); (2) wing flapping, i.e. the movement of one or both wings from and towards the body in resting position, has been described as a cooling mechanism (Ochoa-Acuña and Kunz, 1999); (3) aggression, i.e. hitting or biting another individual, is indirectly related to stress, as position in the colony (Eby et al., 2001), crowded branches or lack of rest could increase the instances of aggression; (4) calls, i.e. loud, high-pitched vocalizations, have been described as a warning mechanism if a threat is near (Nelson, 1964; Wilkinson, 1995); (5) salivating, i.e. the licking of body or wings, was seen as cleaning behavior and might have an indirect relation with stress, although this has not been reported before; (6) scratching was believed to increase with disease or parasites and thus indirectly indicative of stress, as stressed animals are more susceptible to diseases or, the other way around, animals with many parasites might get stressed because of that (Jokela et al., 2005); (7) yawning was often observed together with wing-stretching and therefore might be non-stressed behavior; (8) waste excretion could be indicative of stress, as animals excrete more, but smaller amounts in times of stress (e.g. Okano et al., 2005); (9) branch transfer, i.e. the movement along a branch or between branches; (10) tree transfer, i.e. the movement of less than 10 individuals to another tree, and moreover, (11) roost transfer, i.e. the movement of the whole population to another location, could indicate or cause stress. All behaviors were recorded and analyzed separately, but for clearness' sake they were divided into two groups: 'active' and 'passive'. Passive behavior encompassed all 'routine' behaviors, like grooming and wing flapping, while active behavior included movement, vocalizations and aggression.

During the observation periods (and in the periods of rest in between), occurrence and duration of human activity and of fly-ups were recorded, as well as weather conditions like temperature, rain and wind strength. Day number (Monday=1…, Sunday=7) was also noted. Recorded human activities were sounds produced by vehicles (airplanes, boats, jet skis, cars, motorbikes, claxons), construction (chainsaws, construction equipment) or recreation (voices, music). The duration of these disturbances were recorded separately. Additionally, in Mambukal, the numbers of tourists going into the flying fox roosting area, were recorded by park guards.

1.3 Fecal sample collection and analyses

From 5 May to 12 May 2004, fecal samples were collected on Boracay. After this period, collection was hampered, because all flying foxes roosted on the east side of the island in the *habagat* season, the period in which the wind is coming from the west. This roost was inaccessible. Between June and October 2004, the flying foxes did not stay long e-

① Kaiser D, Welbergen J, Komdeur J. 2005. Territoriality and personalities in the grey-headed flying foxes *Pteropus poliocephalus* in New South Wales. MSc State University of Groningen.

nough in one accessible roosting site to collect samples again. On May 18 – 23, August 30 and 31 and on October 11 – 17 fecal samples were collected in Mambukal. Unfortunately, a fire destroyed all fecal samples from both study sites that were collected until June 2. Rice sacks of 1 × 0.7 m were cut in two pieces of 1 × 0.7 m, and hung with strings to surrounding trees underneath the trees where the flying foxes roosted in. To avoid disturbing the bats, the sacks were hung up and the fecal samples were collected after the bats had left to forage, as they do each night at sunset. Due to dense foliage cover, suitable locations for collection were limited within roosting sites. Wherever fresh fecal samples could be found on the forest floor or a clear way from the flying foxes to the ground was observed, sacks were hung up just above the forest floor. All possible locations throughout the roosting sites were used. Each sack was labeled four times with the location code. The night previous to fecal sample collection, the sacks were cleared of old feces to guarantee that samples collected were no more than 14 h old. Stress metabolites are subject to degrading by fecal bacteria (Kahn et al., 2002). Individual guano pellets with minimal or no contamination with urine were collected. The samples were put in 5 ml plastic vials with lid and were stored in a − 20℃ freezer within 2 h after collection. The distances between sacks and the distances to possible sources of disturbance or other fixed points were estimated using a site map. Fecal samples were collected the evening following the observation periods, within 12 h after observation. Within a day, adrenal hormonal secretion has an overt fluctuation (Fox, 1999). Because of this fluctuation, which differs greatly between individuals, there is much variation between samples collected over a day. However, in our samples this fluctuation is minimized. First, the fecal samples were always collected between 18:30 and 20:00. Second, possible fluctuation in adrenal hormone secretion is much less expressed in fecal samples than in blood (Reeder and Kramer, 2005). It was not possible to determine the exact source of the samples; *A. jubatus*, *P. vampyrus*, or *P. hypomelanus*, nor the sex to which the sample belongs. Collected samples were pooled.

Because faecal samples of flying foxes had never been analysed using Enzyme Immunoassay (EIA), a pilot study was carried out in January 2004 with a captive population of *Rousettus aegyptiacus* at the Allwetterzoo in Münster, Germany. The importance of validation should not be underestimated; the assumptions that hormone metabolite concentrations reflect circulation levels of the actual hormone and that the measured hormone metabolite is indicative of stress need to be properly validated (Buchanan and Goldsmith, 2004; Touma and Palme, 2005; Goymann, 2005). The Münster experiment was set up to validate the biological relation between the measured GCM and stress in flying foxes. Seven fecal samples were collected from three males and two females. The fecal samples were transported in a styrofoam box with dry ice (− 80℃) and stored in a − 20℃ freezer. Several different EIA assays for groups of glucocorticoids were tested, of which the cortisone-EIA used by Rettenbacher et al. 2004 returned the highest immunoreactivity and the best profiles. This assay measures GCM with a 3, 11-dione structure and uses 4-pregnene-17α, 21-diol-3, 11, 20-trione as standard, 4-pregnene-17α, 21-diol-3, 11, 20-trione-21-HS: BSA as antibody and 4-pregnene-17α, 21-diol-3, 11, 20-trione-CMO-biotinyl-LC as label. More details on the essay procedure can be found in Rettenbacher et al. 2004 and Palme and Möstl 1997. The results of this validation experiment are displayed in Fig. 2. Six points represent male samples and one point represents a female sample. The glucocorticoid metabolites measured by the cortisone-EIA occurred in the feces of this flying fox and show an increase some time after handling, followed by a decrease to normal levels, displaying the stress hormone levels in the blood with a 90 min lag time. It needs to be pointed out that this validation experiment was not sufficient. The difference between male and female basic levels might be high (Touma and Palme, 2005), as well as differences between species, so conclusions need to be made with utmost care. The fecal samples collected in the Philippines were extracted and analyzed in the same way (Rettenbacher et al., 2004) in January 2005.

1.4 Data analyses

When calculating effects of different variables on glucocorticoid metabolite (GCM) concentrations, only the behavioral data gathered by P.v.d.Aa on the days preceding the nights on which fecal samples were collected, were used in the analyses. Behavioral data was linked to the collection date and thus behavior was linked to each sample. When comparing behavior between Boracay and Mambukal, only behavior data gathered by P.v.d.Aa was used. In these analyses, averages per day were calculated and presented as Means ± *SE*. GCM concentrations had to be normalized using 10log transformations.

Because many variables were intercorrelated, a Principal Component Analysis (PCA) was carried out to divide all related variables into components. We used a general linear model (GLM) with day number as random factor, and sample mass and each of the PCA components as covariates to determine the main environmental factors causing variation in GCM concentrations. In the GLM with behavioral compo-

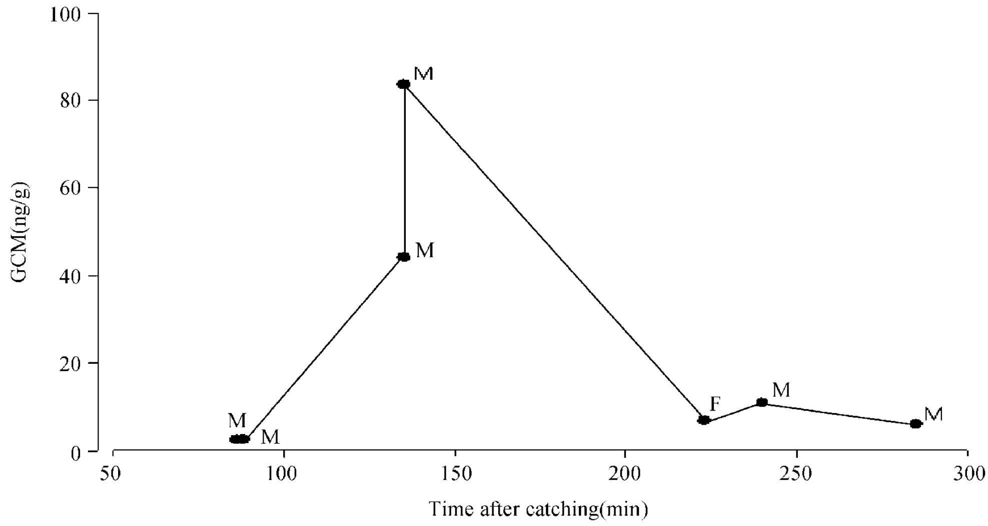

Fig. 2 Glucocorticoid metabolite concentration (GCM) measured in 7 samples from 4 individuals of *Rousettus aegyptiacus* during the pilot study in Münster, Germany and Vienna, Austria, set out against the time after catching (X-axis)

Each point corresponds with one measured value, M = Male, F = Female.

nents, day number was left out of the model because values of PCA components were identical for a given day and thus did not show variation within days. In the GLM analyses all variables were added to the model (full model) and with a backward-procedure removed when least significant. This was repeated until only significant variables were present in the model (minimal adequate model). P values < 0.05 are considered significant and significance tests are two-tailed throughout. The analyses were carried out with SPSS version 12.0.1.

2 Results

2.1 Daily activity of flying foxes on disturbed and undisturbed days

In Mambukal, the number of tourists visiting the roosting area per day was 123 ± 43 (Mean ± *SE*), varying from 2 – 574, with most visitors accessing the roost on Saturdays. From Boracay, no tourist pressure data were available, but we never observed visitors in the roosting area. Observation days were divided into two groups: days with and days without recorded human disturbance. On 85% of all observation days in Mambukal, at least one human activity was recorded, in most cases this was the sound of motorized vehicles (58%), music from a videokebar (16%) and construction activities (16%). The remaining 10% were disturbances caused by people shouting or walking in the near vicinity of the roost. On Boracay, human disturbance was recorded on 38% of the observation days and consisted mostly of the sounds of motorized boats (50%) and planes (47%). The remaining 3% was caused by music, when the bats were roosting near a village.

In Boracay, we found that the flying foxes displayed significantly less yawning and branch transfers on disturbed days as compared to undisturbed days (Table 1). In Mambukal, the flying foxes displayed significantly less branch transfer, wing flapping and wing stretching on disturbed days as compared to undisturbed days. For disturbed and undisturbed days combined, we found no significant differences between the behaviors of Boracay bats and Mambukal bats. In Mambukal, during 55% of the observation periods, fly-ups were recorded and the bats were flying on average for 56 s per observation day. On Boracay, during 47% of the observation periods, fly-ups were recorded, and the bats were flying an average of 53 s per observation day. However, number and duration of fly-ups did not differ significantly between Mambukal and Boracay bats.

2.2 Categorization of behaviors indicative of stress

A total of 129 individual fecal samples from the Mambukal population, ranging from 0.01 g to 0.2 g, could be analyzed and compared with behavioral and environmental data of 9 days in two sessions (30 and 31 August and 11 – 17 October 2004). As many variables were intercorrelated, a PCA (with varimax rotation, a transformation that makes the division between components clearer) was carried out that divided all behavioral variables into 4 components (Table 2). Not all components had clear biological meanings, e.g., the number and duration of fly-ups loaded component 1 together with vocalizations and wing stretching. Fly-ups can be interpreted as flight behavior, vocalizations as a warning mechanism and wing stretching as agonistic behavior. On the other extreme, yawning was the only factor loading component 4. Component 2 could be interpreted as 'uneasiness', being loaded by aggression and tree transfers, component 3 as 'body care', loaded by salivating and wing flapping. None of the 4 components, showed a significant relation with GCM concentrations. The clearest effect on GCM concentrations came from sample mass. In a separate GLM, day number showed no significant effect on GCM ($F = 2.11$, $df = 6$, $P = 0.06$)

Table 1 Comparison of behaviors of three species of flying foxes (*A. jubatus*, *P. vampyrus* and *P. hypomelanus*) on days with and without disturbance in Boracay and Mambukal populations, Philippines

		Boracay (B)				Mambukal (M)				B vs. M
	Behavior	Undisturbed (Mean ± *SE*) ($n = 3$*)	Disturbed (Mean ± *SE*) ($n = 3$*)	*t*-test (*P*)	Overall (Mean ± *SE*) ($n = 6$*)	Undisturbed (Mean ± *SE*) ($n = 1$)	Disturbed (Mean ± *SE*) ($n = 15$)	*t*-test (*P*)	Overall (Mean ± *SE*) ($n = 16$)	*t*-test (*P*)
Active	AG	0.04 ± 0.04	0.00 ± 0.00	0.42	0.02 ± 0.02	0.00	0.06 ± 0.02	0.51	0.06 ± 0.02	0.35
	VOC	*	0.00*	*	0.00*	0.00	0.02 ± 0.06	0.38	0.19 ± 0.06	0.40
	BT	0.13 ± 0.03	0.02 ± 0.02	**0.037**	0.08 ± 0.03	0.19	0.03 ± 0.01	**<0.001**	0.04 ± 0.01	0.14
	TT	0.05 ± 0.05	0.00 ± 0.00	0.42	0.02 ± 0.02	0.00	0.00 ± 0.00	0.67	0.00 ± 0.00	0.40
	RT	0.00 ± 0.00	0.33 ± 0.33	0.42	0.17 ± 0.17	0.00	0.00 ± 0.00	*	0.00 ± 0.00	0.36
	NFLY	0.00 ± 0.00	1.33 ± 0.88	0.21	0.67 ± 0.49	0.00	1.40 ± 0.42	0.42	1.31 ± 0.41	0.39
	DFLY	0.00 ± 0.00	120.00 ± 120.00	0.37	60.00 ± 60.00	0.00	340.00 ± 106.48	0.44	318.75 ± 101.84	0.15
Passive	WF	1.87 ± 0.94	1.25 ± 0.90	0.66	1.56 ± 0.60	1.00	0.10 ± 0.04	**<0.001**	0.15 ± 0.07	0.07
	WS	*	0.04*	*	0.04*	0.31	0.09 ± 0.02	**0.016**	0.10 ± 0.02	*
	SAL	0.32 ± 0.27	0.05 ± 0.03	0.43	0.18 ± 0.14	0.31	0.17 ± 0.02	0.10	0.18 ± 0.02	0.99
	SCR	0.11 ± 0.09	0.10 ± 0.06	0.95	0.10 ± 0.05	0.25	0.15 ± 0.02	0.24	0.16 ± 0.02	0.18
	YAW	0.09 ± 0.03	0.01 ± 0.01	**0.049**	0.05 ± 0.02	0.00	0.05 ± 0.01	0.27	0.05 ± 0.01	0.96
	WASTE	0.07 ± 0.04	0.00 ± 0.00	0.17	0.03 ± 0.02	0.00	0.01 ± 0.00	0.37	0.01 ± 0.00	0.44

Means and standard errors for all types of behavior were calculated from tallied events per individual per observation day. Student's t-tests for equality of means, between days with and days without disturbance and between Boracay and Mambukal. Significant effects are printed in bold.
Active: AG = aggression, VOC = vocalizations, BT = branch transfers, TT = tree transfers, RT = roost transfers, NFLY = number of fly-ups, DFLY = duration of fly-ups. Passive: WF = wing flapping, WS = wing stretching, SAL = salivating, SCR = scratching, YAWN = yawning, WASTE = waste excretion.
* Not enough values to perform statistics.

Table 2 Components from a PCA with behavioral variables and their contribution to a General Linear Model (GLM) analysis with log (GCM) as dependent variable and sample mass and the 4 behavioral components as explanatory variables in three species of flying foxes (*A. jubatus*, *P. vampyrus* and *P. hypomelanus*)

	Component	Highest loading factors	*df*	*F*	*P*
Included	Corrected model	NA	1	45.68	**<0.001**
	Sample mass	NA	1	45.68	**<0.001**
Excluded	1. No unambigous explanation	Number fly-ups (0.917) Duration fly-ups (0.807) Vocalizations (0.886) Wing stretching (−0.780)	1	0.00	0.97
	2. Uneasiness	Aggression (0.911) Tree transfer (0.897)	1	0.03	0.86
	3. Body care	Salivating (0.841) Wing flapping (0.839)	1	2.00	0.16
	4. Yawning	Yawning (0.972)	1	50.18	0.10

All factor loadings above 0.750 were noted down to indicate influences of different variables within a component. The signs of the loadings are arbitrary. Statistics of excluded variables reflect their last contribution to the model, and are shown in order of exclusion. Average values per observation day of behavioral data gathered on the day preceding the night of collection were used ($n = 129$ fecal samples).

2.3 Influence of environmental factors on GCM

A second PCA divided all environmental variables into 3 components (Table 3). Component 1 was loaded by all three recorded distances, component 2 was interpreted as 'disturbance' as it was loaded by number of visitors, number of human activities, but also with sunshine. Component 3 was loaded by wind and the duration of human activities and was difficult to summarize with a single biological explanation. Sample mass and component 1 had a significant effect on GCM. To correct for the spurious effect of sample mass, this variable was kept as a covariate in the analysis. In separate linear regression tests, GCM concentrations showed a decrease with longer distance to the road and to the videokebar, however the association was not significant (Fig.3A, B). However, GCM concentrations showed a significant increase with higher distance from the colony center (Fig.3C).

Table 3 Components from a PCA with environmental variables and their contribution to a General Linear Model (GLM) analysis with log (GCM) as dependent variable and sample mass, day number and the 4 behavioral components as explanatory variables in three species of flying foxes (*A. jubatus*, *P. vampyrus* and *P. hypomelanus*)

	Component	Highest loading factors	*df*	*F*	*P*
	Corrected model	NA	2	30.18	**<0.001**
	Sample mass	NA	1	48.47	**<0.001**
Included	1. Distance	Distance to road (0.955)	1	8.93	**0.003**
		Distance to videokebar (0.924)			
		Distance to colony center (−0.867)			
	3. No unambiguous explanation	Wind (0.987)	1	0.08	0.78
		Duration of human activities (0.809)			
Excluded	2. Disturbance	Number of human activities (0.894)	1	0.52	0.47
		Number of visitors (0.878)			
		Sunshine (−0.764)			
	Day number	NA	6	1.74	0.12

All factor loadings above 0.750 were noted down to indicate influences of different variables within a component. The signs of the loadings are arbitrary. Statistics of excluded variables reflect their last contribution to the model, and are shown in order of exclusion. Average values per observation day of environmental data gathered on the day preceding the night of collection were used ($n = 129$ fecal samples).

2.4 Correlations between environmental and behavioral components

Using the components created in the two PCA's, a cross-correlation test was carried out to find relations between environmental and behavioral variables. 'Distance' showed a negative correlation with 'body care' ($r = -0.30$, $n = 127$, $P < 0.001$). 'Disturbance' was positively correlated with behavioral component 1 ($r = 0.34$, $n = 127$, $P < 0.001$) and negatively correlated with 'uneasiness' ($r = -0.57$, $n = 127$, $P < 0.001$) and 'body care' ($r = -0.67$, $n = 127$, $P < 0.001$). Environmental component 3 was positively correlated with behavioral component 1 ($r = 0.54$, $n = 127$, $P < 0.001$) and negatively correlated with 'yawning' ($r = -0.31$, $n = 127$, $P < 0.001$).

3 Discussion

3.1 Recognizing and predicting stress in flying foxes

Our observation data show that Mambukal bats display less branch transfers on disturbed days than undisturbed days, which supports the findings of Lorica et al. (2006). Boracay bats showed the same effect in this study. Furthermore, in the Mambukal study site we found significant more wing stretching, wing flapping and yawning during 'disturbed' days than during 'undisturbed' days. In both studies, however, no fly-ups were recorded on days without disturbance whereas fly-ups did occur on days with disturbance. Due to low sample size, we found no significant effect.

In both PCA's, the clearest effect on GCM came from sample mass, but this effect is caused by the fact that concentrations and mass are not independent and this effect has no biological value (Goymann, 2005). In the behavioral PCA, we found no relations between behavioral components and GCM. This would mean that all recorded behaviors were not indicative of stress. However, we have to be careful with this conclusion, because it is most likely that significant associations are found in follow-up studies with larger sample sizes and adjustments to the collecting and analyzing methods after a proper validation for the EIA used. The act of flying, for instance, is costly (e.g., Maina, 2000) and therefore fly-ups would most probably have a clear impact on GCM concentrations.

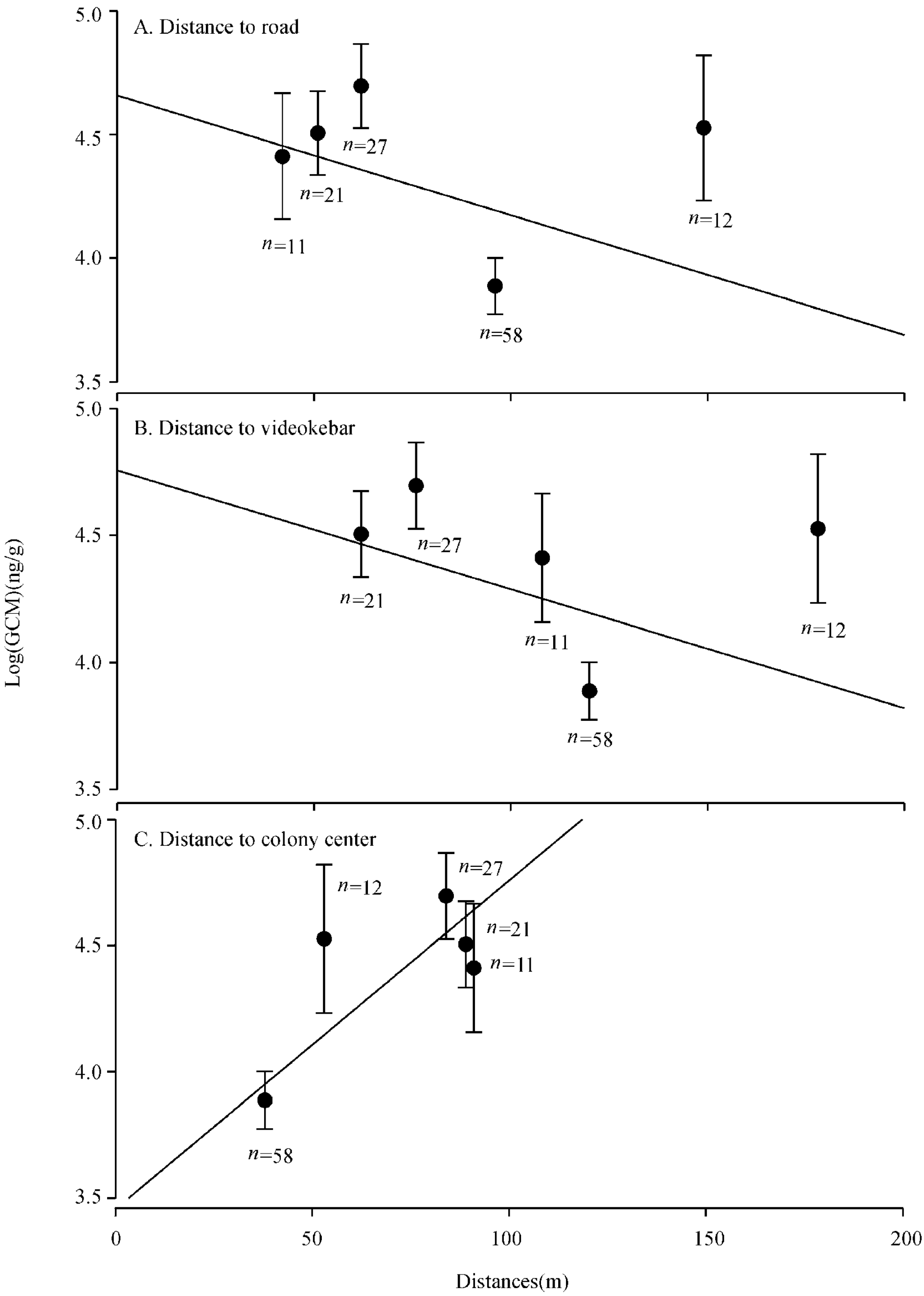

Fig. 3 The influence of location in the colony on GCM concentrations in fecal samples of three species of flying foxes (*A. jubatus*, *P. vampyrus* and *P. hypomelanus*)

A. log (GCM) (Y) decreases with distance of collection site to the road (X): $r^2=0.02$, $n=129$, $P=0.08$; $Y=-4.84\times10^{-3}X+4.66$; B. log (GCM) ($Y$) decreases with distance of collection site to videokebar (X): $r^2=0.03$, $n=129$, $P=0.06$; $Y=-4.69\times10^{-3}X+4.76$; C. log (GCM) ($Y$) increases with distance of collection site to colony center (X): $Y=1.30\times10^{-2}X+3.46$; $r^2=0.11$, $n=129$, $P<0.001$

That this cannot be found in our data could be due to the fact that the variation across days might be compromised by the variation between species, sex and individuals (Buchanan and Goldsmith, 2004). Some previously described relations between environmental factors and behavior, include dominance and place within the colony (Eby et al., 2001), the effects of temperature (Codd et al., 2003), and the effects of seasonal and social changes (e.g., Reeder and Kramer, 2005; Reeder et al., 2006). In this study, we only found a significant negative association between distance of sample collection site to the colony center and GCM concentrations. No other associations between PCA components and GCM concentrations were found. Most likely, there were not enough data from different collecting days to show these effects separately in the PCA's.

The absence of a significant effect of day number (and with that the number of visitors) on GCM might indicate adjustment to the presence of humans. Although this cannot be concluded from our data, the fact that hunting in the Mambukal area is strictly prohibited since 1990 whereas in Boracay hunting was officially prohibited only as from 2001 onwards supports the idea that the Mambukal bats have become habituated to the presence of humans. Bats in Mambukal may no longer associate humans with a direct threat. This is consistent with the findings of e.g. Borkowski et al. (2001) and Schultz and Bailey (1978), where respectively sika deer and elk learned to tolerate people when hunting pressure was low or nihil. The influence of human presence on bat behavior might be much more complex than merely increasing or decreasing activity. Much variation between these days is probably caused by a complex of inter-correlated environmental and behavioral variables and more research should be done on this subject.

In the cross-correlations between environmental and behavioral components, the positive relation between 'distance' and 'body care' might reflect differences in social status through location inside the colony (Eby et al., 2001). 'Body care' and 'uneasiness' decrease with higher 'disturbance', but behavioral component 1, which is loaded by fly-ups, vocalizations and wing stretching increases. This supports the idea that the activities of humans have an effect on flying fox behavior. That this effect was not reflected in GCM does not necessarily indicate that flying foxes are not affected by human activities. The basic GCM levels may generally differ from those of bats less exposed to human activities, but in the absence of fecal samples from the control group, we cannot say much about basic, let alone 'healthy' GCM concentrations.

3.2 Recommendations for area management

Flying foxes appear to be able to adjust to the presence of humans, as long as humans do not pose a direct threat. In Subic Bay, Luzon (Philippines) bats have been roosting along a highway for years and this population appears to be flourishing (Stier, 2003[①]). However, approaching the roost too closely will cause a fly-up, which is of course stressful and energetically unfavorable for the flying foxes. So, ecotourism would be a good way to save the area where the flying foxes live and simultaneously providing livelihood for local residents, but distance to the flying foxes should be observed. The buffer areas within at least 150 m around the roosting sites, as proposed in the development plans for the Boracay area, would give the flying foxes the chance to gradually adjust to human presence and it would give humans a good viewing distance. However, area management does not only apply to the area containing the day roosts. Studies have to be made on the minimum area size needed to sustain the Boracay flying fox colony, that consists of at least 2 000 individuals. Furthermore, logging should be minimized in the area surrounding the day roosts with a radius of at least 15 km. Endemic species like *Acerodon jubatus* have specific needs (Stier, 2003) which need to be taken in consideration.

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