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Vertebrate Scavengers Control Abundance of Diarrhea-causing Bacteria in Tropical Plantations

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Scavenging is a common phenomenon, particularly amongst carnivorous vertebrates. By consuming carrion, vertebrate scavengers reduce resource availability for both pathogenic bacteria and their insect vectors. We investigated the ability of wild vertebrate scavengers to control agents of human diarrheal diseases (specifically *Salmonella* spp. and Shiga toxin-producing *Escherichia coli* [STEC]) in oil palm plantations in Sabah (East Malaysia), and the existence of spillover effect whereby additional vertebrate scavengers from adjacent forest patches result in greater disease control in plantation sections near these forest edges. Experimental carcasses were removed by common scavengers (*Varanus salvator*, *Canis lupus familiaris*, and *Viverra tangalunga*) at different time points, and this determined the length of time that the carcasses persisted in the environment. The amount of pathogenic bacteria on the surfaces of filth flies collected above the experimental carcasses was positively correlated to the duration of carcass persistence, and reduction in pathogenic bacterial abundances was largely due to carcass consumption by these vertebrate scavengers. Instead of a predicted positive spillover effect (greater scavenger activity near forest edges, hence reduced pathogen abundance), we detected a weak inverse spillover effect in which STEC counts were marginally higher in plantation sections near forest patches, and human hunting along the forest-plantation boundaries could explain this. We propose that making oil palm plantations scavenger-friendly could yield great human health benefits for the millions of workers employed in this rapidly-expanding industry, without drastically changing current management practices.

Key words: Carcass removal, Filth flies, *Salmonella*, Shiga toxin-producing *E. coli*, Spillover effect.

BACKGROUND

Scavenging is widespread in nature, and nearly all carnivorous vertebrates and invertebrates scavenge facultatively (DeVault et al. 2004). Despite being integral components of food webs, scavengers are often ignored in many food web studies, resulting in a severe underestimation—up to 16-fold—of the prevalence of scavenging across all systems (Wilson and Wolkovich 2011). Perhaps due to this neglect, there is very limited understanding of how scavengers contribute to ecosystem functions and services, particularly disease regulation. As carcasses are ephemeral and high-quality resources, there is great competition between organisms that utilize this form of resource. At one extreme, microbes can monopolize carcasses by producing substances that are toxic to vertebrates (Janzen 1977).

Not surprisingly, many of those microbes are also

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human pathogens (*e.g.*, *Escherichia coli* O157:H7 and *Salmonella typhi*; DeVault et al. 2004) and can cause diarrhea in humans when ingested. Approximately two million people die from diarrheal diseases each year and 88% of the cases are due to contaminated food or drinking water (WHO 2000). Vertebrate scavengers are large-bodied and highly mobile, and therefore able to locate and consume carrion at a rate much greater than invertebrates (NTL Lim, unpubl. data). As such, vertebrate scavengers have the potential to reduce the prevalence of some human disease by reducing the availability of key resource for such microbes as well as macroinvertebrates that are vectors for similar microbes or diseases (*e.g.*, housefly *Musca domestica*; Levine and Myron 1991; Grübel et al. 1997; Fotedar 2001).

The potential importance of this ecosystem service was exemplified recently in India, where vulture populations (*Gyps bengalensis* and *G. indica*) declined by > 90% due to unintentional poisoning by anti-inflammatory drugs in livestock carcasses (Pain et al. 2003; Prakash et al. 2003; Ogada et al. 2012). A consequence of the vultures' decline was an increase in the number of putrefying animal carcasses in the surroundings and the need to bury or incinerate those carcasses promptly to limit the spread of diseases like anthrax (Prakash et al. 2003).

In this study, we investigated the potential for vertebrate scavengers to control the abundances of necrophagous filth flies (Diptera: families Calliphoridae, Muscidae, and Sarcophagidae) and pathogenic bacteria transmitted externally by these filth flies in oil palm (*Elaeis guineensis*) plantations in tropical Southeast Asia. Palm oil production is a rapidly expanding agricultural industry (Clay 2004); in the two largest palm oil producing countries (Malaysia and Indonesia), conservative estimates of plantation area are 4.0 and 5.5 million ha, respectively (Wicke et al. 2011), with planned expansion of 60–100 thousand ha annually in Malaysia and 10–20 million ha in Indonesia (Colchester et al. 2011). This rapid expansion, and resulting alteration and fragmentation of natural habitats, has received much attention (*e.g.*, Koh and Wilcove 2008, Edwards et al. 2014). Additionally, the industry employs over a million workers in Indonesia alone (Sinaga 2013), most of whom spend most of their day in the plantations and often consume their meals there. To our knowledge, however, the potential impact on human health via altered scavenger assemblages has not been investigated. This study focused on disease transmission, so we did not consider other macroinvertebrates (*e.g.*, nonvolant species, predatory beetles) because filth flies constitute the bulk of the invertebrate biomass in carcasses (Putman 1983) and are strong fliers (*e.g.*, up to 7 km; Nazni et al. 2005) that are often found in high abundance in rural villages (Greenberg 1971, Wolfe and van Ziji 1969). Additionally, because pathogenic bacteria have been known to persist on solid surfaces for 30–60 days (*e.g.*, Maule 2012 for *E. coli* O157 on stainless steel, Solomon et al. 2003 for *E. coli* O157:H7 on lettuce), we believe that filth flies are the main viable vectors to transport pathogenic bacteria on decaying animal carcasses in oil palm plantations to human settlements or workers within the plantations when contaminated flies come into contact with human food.

MATERIALS AND METHODS

Study sites

We conducted sampling in oil palm (*Elaeis guineensis*) plantations in March–July 2013 in three areas of the Malaysian state of Sabah (Borneo): 1) Danum Palm and Kebun Jaya estates adjacent to the Danum Valley Conservation Area (Ulu Segamat; ca. 43,800 ha), 2) Luangmanis and Moynod estates adjacent to the Lungmanis Forest Reserve (Sandakan; ca. 6,700 ha), and 3) Table estate adjacent to the Tawau Hills National Park (Tawau; ca. 27,000 ha) (Fig. 1). The forest reserves were chosen due to their large area and protected status; the vertebrate communities were also recorded to be largely intact (*e.g.*, Payne and Francis 1985; Wells et al. 2005). The monoculture plantations adjacent to these reserves have extensive coverage (*i.e.*, >5,000 ha) and comprise oil palm stands approximately 8 years old.

Field sampling

We established 31 sampling stations in the plantations at increasing distances (range: 10–3280 m) from nearest contiguous forest to examine the spillover effect; we also recorded the distance of the stations from the nearest human settlement or buildings (range: 110–2710 m). The stations were evenly distributed between the three localities (11 at Lungmanis, and 10 each at Danum Valley Conservation Area and Tawau), and they were selected at random within three zones in the plantations: ~50 m; ~1500 m; and ~3000 m from the forest edges. At each station, we deployed one chicken and one rat carcasses about 30 cm apart, tethered to the ground to prevent displacement and removal by small scavengers that may not be able to consume the carcasses completely. We chose chicken and rat carcasses because there are wild analogues at the study sites and they were commercially-available. We also chose two types of carcasses from different taxonomic groups (*i.e.*, a mammal and a bird) to obtain

more generalisable results concerning scavengers, as opposed to having results that only pertain to vertebrates that scavenge on mammals or birds. The animals were euthanised via carbon dioxide overdose (*i.e.*, not chemical euthanasia) to avoid affecting carcass detection by scavengers. One infrared camera trap (HyperFire HC600 and PC900, Reconyx, Wisconsin) was deployed at each station to record the identities and activities of vertebrate scavengers that consumed the carcasses, as well as the time taken for the carcasses to completely rot or be consumed. The camera traps were secured to a tree 1.5 m away from the carcasses and at a height of 50 cm above ground level. Large scavengers that were able to remove the carcasses despite the tethering were assumed to be capable of consuming the carcasses completely upon removal. We also placed a fly cone trap (diameter 19 cm; model #2826, BioQuip Products, California) at each station, suspended 2 cm directly above the tethered carcasses. The cone traps and exterior of the carcasses were sterilized with 70% ethanol solution and ultraviolet lamp prior to deployment, and gloves were used during all handling.

Upon setting up the sampling stations, we collected the trapped macroinvertebrates daily until the carcasses were completely consumed or rotted away (generally within 7 days). We used a battery-powered aspirator (model #2820GA, BioQuip Products, California) with sterilized collecting chamber and extension tube to collect the macroinvertebrates on all occasions. The macroinvertebrates were then killed by placing the collecting chamber in a killing jar with ethyl acetate for 15 min before transferring only filth flies to sterile centrifuge tubes with analytical-grade absolute ethanol. Macroinvertebrates from the same station were pooled for subsequent pathogen quantification.

Microbial identification and quantification

Prior to actual field sample collection, we carried out preliminary trials in which chilled sterile water was used instead of ethanol in the centrifuge tubes to maintain and identify pathogenic bacteria that could be found on the exteriors of the macroinvertebrates at our study sites. We detected the presence of viable *Salmonella* spp. and *E. coli* in the chilled water through standard microbiological protocols in the Bacteriological Analytical Manual (FDA 2013). Subsequent DNA extraction and qPCR reactions (see below) of these samples confirmed the presence of *Salmonella* spp. and pathogenic Shiga toxin-producing *E. coli* (STEC).

For field samples preserved in ethanol, we pooled all samples collected at each respective station before mixing the samples thoroughly with a vortex

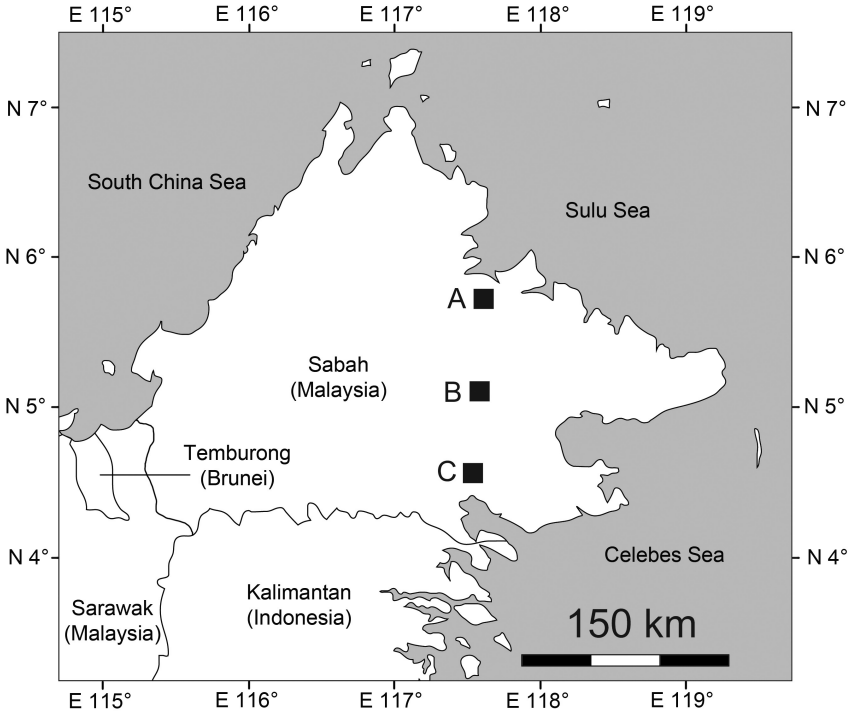

Fig. 1. Locations of the three oil palm plantations, denoted by solid squares, used in this study on how vertebrate scavengers affect pathogenic bacterial abundances on flies within the Malaysian state of Sabah on the northern tip of Borneo island. A: Luangmanis and Moynod estates (Sandakan); B: Danum Palm and Kebun Jaya estates (Ulu Segamat); C: Table estate (Tawau).

mixer. We extracted DNA from 1 ml of the ethanol section of the field samples using the DNeasy Blood and Tissue Kit (Qiagen Inc., California), according to the manufacturer's instructions for gram-negative bacteria. We eluded total DNA in the spin columns using 400 µl of elution buffer in the final step. 30 µl of extracted DNA samples or negative template control was added to each TaqMan®-based qPCR reaction tubes of the MicroSEQ *Salmonella* spp. Detection Kit and RapidFinder STEC Screening Assay (Applied Biosystems Inc., California); the reaction tubes contained lyophilized internal positive controls, primers, and reagents needed to quantify *Salmonella* spp. and STEC on the ABI StepOnePlus and the ABI Prism 7500 FAST sequence detection systems, respectively (Applied Biosystems Inc., California). Recommended thermal cycling conditions were used per the manufacturer's instructions.

Standard curves for both groups of microbes were constructed using ten-fold serial dilutions of pure cultures of *S. typhimurium* and *E. coli* O157:H7 grown overnight in tryptic soy broth at 37°C. In addition to the same DNA extraction and qPCR quantification processes as for the field samples, we estimated the colony-forming units per ml (CFU/ ml) of the pure cultures by streaking on tryptic soy agar plates and estimation by an automated colony counter (aCOLyte, Synbiosis, Maryland). DNA concentrations of the undiluted pure cultures were also determined by UV spectrophotometry (ND-1000, NanoDrop Technologies, Delaware).

We conducted three independent runs of qPCR reactions, each comprising duplicates for standard curve positive control samples and a negative template control, and triplicates for all field samples. We quantified *Salmonella* spp. and STEC of the field samples from the standard curve (*i.e.*, cycle threshold values against CFU/ ml) in each qPCR run before averaging over the three runs and finally multiplying by the amount of ethanol used during field collection. The numbers of *Salmonella* spp. and STEC were expressed as CFU/ ml.

Statistical analyses

We performed modeling to investigate hypothesized relationships between the bacteria count of both bacteria with time to complete carcass removal, and distances to forest edge and/or human settlement. (There was no collinearity between the variables of distance to forest edge and distance to human settlement; $r < 0.7$.) The response variables of bacteria counts were $\log_{10}$-transformed via $\log_{10}$ (bacteria count + 1) to fulfill assumptions of normality and homoscedasticity. We included the locality of the plantation as a random effect in mixed models and allowed the intercept coefficient to vary across each plantation to account for spatial autocorrelation. The candidate models were fitted with lme4 package (Bates et al. 2020) in R statistical software (version 3.6.1; R Core Team 2019). The days to carcass removal by scavengers was also modeled as a non-linear predictor using the Michelis-Menten model, with the V_{max} parameter varying across each plantation to account for spatial autocorrelation. We performed model selection using maximum likelihood and Akaike's information criterion corrected for small sample size (AIC_c; Burnham and Anderson 2002). Finally, we obtained unbiased parameter estimates of the selected models using restricted maximum likelihood methods and performed model averaging for models with $\Delta AIC_c \leq 2$ using Akaike weights (w_i; Burnham and Anderson 2002). Due to interaction effects in the selected models, we performed model averaging at mean values and at mean ± 1 SD to illustrate the influence of particular predictors on the response variable.

RESULTS

Scavenger identities

The common scavengers recorded on the camera traps were *Viverra tangalunga*, *Varanus salvator*, and *Canis lupus familiaris* (Table 1). These species were present throughout the range of distances from forest edges and human settlements sampled (Table 1, Fig. 2), and they are generally recognized as widespread and adaptable species that can persist in both forest and agricultural landscape (*e.g.*, Azlan et al. 2008; Bennett et al. 2010).

Microbial quantification

Pure cultures of *S. typhimurium* and *E. coli* O157:H7 reached a density of 1.3×10^9 and 2.3×10^9 CFU/ ml, respectively. In order to construct standard curves, we covered six orders of magnitude—ranging from 1.3×10^2 to 1.3×10^7 CFU/ ml for *S. typhimurium* and 2.3×10^2 to 2.3×10^7 CFU/ ml for *E. coli* O157:H7. All standard curves had reaction efficiencies of 96.3–100.7% and R^2 values > 0.995. Additionally, internal positive controls indicated an absence of PCR inhibitors while negative template controls confirmed a lack of contamination.

Reading off the standard curves constructed, we determined that field-collected filth fly samples had total *Salmonella* counts of 0.0 to 1.6×10^4 CFU (mean = 2.7×10^3, SD = 3.7×10^3) and total STEC counts of 0.0 to 1.7

$\times 10^6$ CFU (mean = 9.9×10^4; SD = 3.0×10^5) on their exterior surfaces.

Modelling

Prior to the modelling, the bacteria count on the exterior surfaces of flies were found to correlate substantially with the dry biomass of flies collected in the cone traps above the carcasses (STEC: $r = 0.76$; *Salmonella*: $r = 0.46$). Since the bacteria are the actual infectious agents of interest, modelling was conducted for bacterial abundances without considering fly biomass.

When modelling the abundance of STEC in the plantations, the most parsimonious model (*i.e.*, lowest AIC_c) was the model with days to carcass removal (*i.e.*, TIME) as a non-linear predictor (*i.e.*, NL.TIME; $w_i = 48.17\%$); two other models involving TIME and distance from forest edge (*i.e.*, FOREST) had $\Delta AIC_c \leq 2$ (Table 2). In all, these three models had McFadden's pseudo-R^2 values of 0.226–0.265 and a total Akaike weight of 93.57%. Due to interaction effects in one of the selected models, we performed model averaging at mean FOREST values and at mean distances ± 1 SD (Fig. 3a). Similarly, we performed model averaging for the predictor FOREST at mean TIME values and at mean TIME ± 1 SD (Fig. 3b). It was evident that FOREST had a weaker influence on the abundance of STEC compared to TIME.

For models on the abundance of *Salmonella* on filth flies, the most parsimonious model had TIME as the only (non-linear) predictor ($w_i = 98.14\%$, McFadden's pseudo-$R^2 = 0.394$; Table 2, Fig. 3c).

Overall, there was strong empirical support for

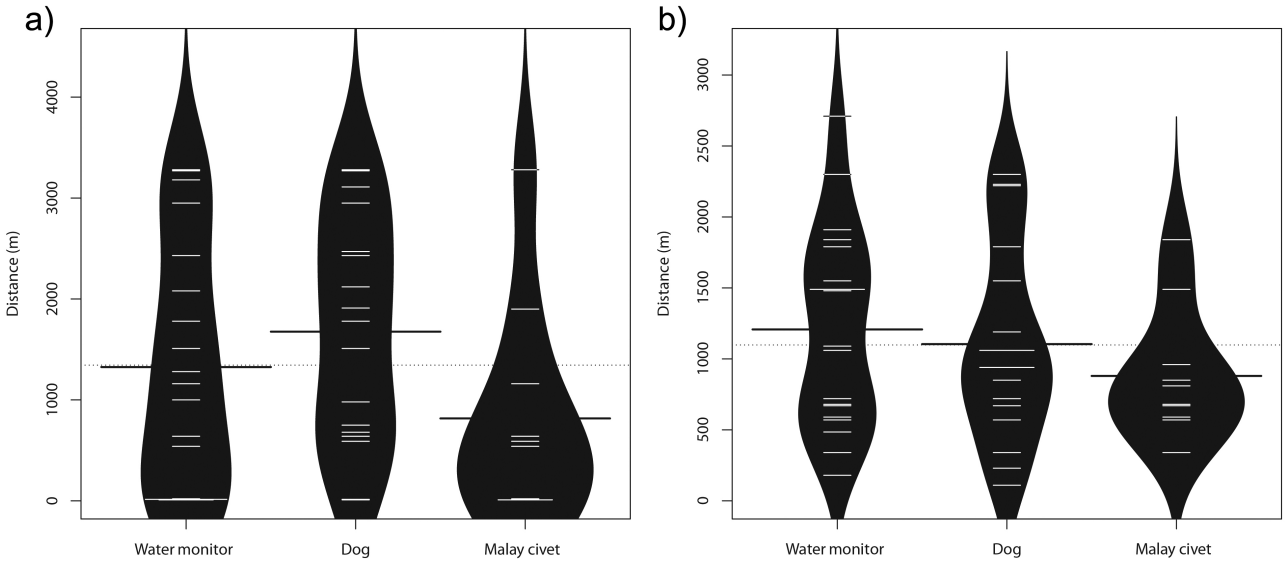

Fig. 2. Beanplot for occurrence of the three common scavenger species (water monitor *Varanus salvator*, dog *Canis lupus familiaris*, and Malay civet *Viverra tangalunga*) recorded on camera traps in oil palm plantations with reference to (a) distance from forest edge or (b) distance from human settlements, where the width of the beanplot denotes the frequency of occurrence at the respective distances. Horizontal black solid bar is the mean distance for individual species; horizontal dashed line is the overall mean distance for all three species; horizonal white bars are observations.

Table 1. List of scavenger species recorded on camera traps, distance of sightings from distance of forest and human settlements, and their IUCN Red List status. Frequency refers to the number of sampling stations from which the species was recorded (out of a total of 31 stations); this avoids over-representation when individuals revisited the same station over consecutive days. Sampling stations were situated 10–3280 m from forest edges and 110–2710 m from human settlements

Species	Common name	Frequency	Mean distance from forest (± SD; m)	Mean distance from human settlements (± SD; m)	IUCN Red List status
Varanus salvator	Water monitor lizard	19	1320 (1200)	1200 (687)	Least Concern
Canis lupus familiaris	Feral dog	18	1680 (1100)	1100 (672)	N.A.
Viverra tangalunga	Malay civet	10	817 (1000)	880 (430)	Least Concern

TIME being an important predictor of the abundance of both groups of bacteria on filth flies. Additionally, the bacteria abundance appeared to have a non-linear relationship with the variable TIME (Fig. 3a, c). The variable of distance from human settlements (*i.e.*, SETTLEMENT) did not feature in any of the selected models (*i.e.*, $\Delta AIC_c > 2$; Table 2).

DISCUSSION

From the data gathered, we found that the experimental carcasses were removed by common vertebrate scavengers (Table 1) and that the pathogenic bacterial abundances were mainly affected by the duration of carcass persistence in the environment. In other words, vertebrate scavengers had a strong effect on the abundance of pathogenic microbes on the exterior surfaces of filth flies. This was shown by the inclusion of the predictor TIME/NL.TIME (*i.e.*, days to carcass removal) in all selected models with $\Delta AIC_c < 2$ (Table 2); additionally, all selected models had McFadden's pseudo-R^2 values of 0.2–0.4 (*i.e.*, "excellent fit"; see McFadden 1979, p. 307). This predictor reflected the carcass removal action by vertebrate scavengers, with low values representing efficient scavenging function. For 15 of the 31 sampling stations, scavengers were able to remove the tethered carcasses immediately or fully consume them within 24 hours of its first appearance, rendering low values for TIME and also pathogenic bacteria abundances at these stations (Fig. 3).

When vertebrate scavengers remove resources from pathogenic bacteria on a carcass, they will ultimately produce feces. However, the scavengers will make use of most of the biomass consumed and only a small amount of undigested matter will be excreted as feces (*e.g.*, ± 5% of nitrogen intake in humans; Bender and Bender 1997), resulting in a huge reduction in resource availability to pathogenic bacteria. Additionally, the gastric acid in vertebrates will inactivate most microbes and feces from these scavengers will not contribute meaningfully to the

Table 2. Mixed models of $\log_{10}$ (STEC count + 1) (a) and $\log_{10}$ (*Salmonella* count + 1) (b) at 31 sampling stations against the various predictors, with the plantation identity as a random effect. Definitions of abbreviations used are as follows: STEC = Shiga-toxin *Escherichia coli*; TIME = time to complete removal of carcass; NL.TIME = TIME as a non-linear predictor; FOREST = distance to nearest forest edge; SETTLEMENT = distance to nearest human settlement; NULL = null model (containing only random effects); -*LL* = negative loglikelihood of fitted model; *k* = number of parameters; AIC_c = Akaike's information criterion corrected for small sample sizes; ΔAIC_c = difference in AIC_c value of each candidate model from the most parsimonous model; w_i = Akaike weight, R^2 = McFadden's pseudo-R^2

(a) STEC

Candidate model	-*LL*	*k*	AIC_c	ΔAIC_c	w_i	R^2
NL.TIME	-46.78	4	103.09	0.00	48.17%	0.226
TIME*FOREST	-44.44	6	104.37	1.28	25.41%	0.265
TIME+FOREST	-46.22	5	104.85	1.76	19.99%	0.235
TIME+FOREST+SETTLEMENT	-46.07	6	107.65	4.56	4.93%	0.238
TIME	-50.51	4	110.57	7.47	1.15%	0.164
TIME+SETTLEMENT	-50.51	5	113.43	10.34	0.27%	0.164
TIME*SETTLEMENT	-50.28	6	116.06	12.97	0.07%	0.168
NULL	-60.43	3	127.75	24.66	0.00%	0.000

(b) *Salmonella*

Candidate model	-*LL*	*k*	AIC_c	ΔAIC_c	w_i	R^2
NL.TIME	-30.12	4	69.78	0.00	98.14%	0.394
TIME*FOREST	-31.14	6	77.78	8.00	1.79%	0.374
TIME+FOREST	-36.70	5	85.80	16.02	0.03%	0.262
TIME	-38.49	4	86.51	16.73	0.02%	0.226
TIME+FOREST+SETTLEMENT	-36.65	6	88.81	19.03	0.01%	0.263
TIME+SETTLEMENT	-38.39	5	89.18	19.40	0.01%	0.228
TIME*SETTLEMENT	-38.37	6	92.24	22.46	0.00%	0.229
NULL	-49.75	3	106.38	36.60	0.00%	0.000

bacterial load in the environment (Martinsen et al. 2005). Therefore, we strongly believe that vertebrate scavengers serve to control the abundance of pathogenic bacteria that can be transmitted to humans via the exterior surfaces of filth flies (*e.g.*, when plantation workers have their meals).

Although TIME was the primary predictor of bacteria abundances, the relationships were not linear (Fig. 3a, c). The saturating curves observed for both STEC and *Salmonella* could be due to the limited resources offered by the chicken and rat carcasses. Even in the absence of vertabrate scavengers, macroinvertebrates, particularly the larvae of filth flies, will completely consume carcasses within about 7 days. As such, faced with the declining amount of resources available to the bacteria with time, it is only logical that the amount of bacteria on the carcasses and exterior surfaces of adult filth flies collected daily will be reduced towards the end of the sampling period, leading to slower increases in the cumulative

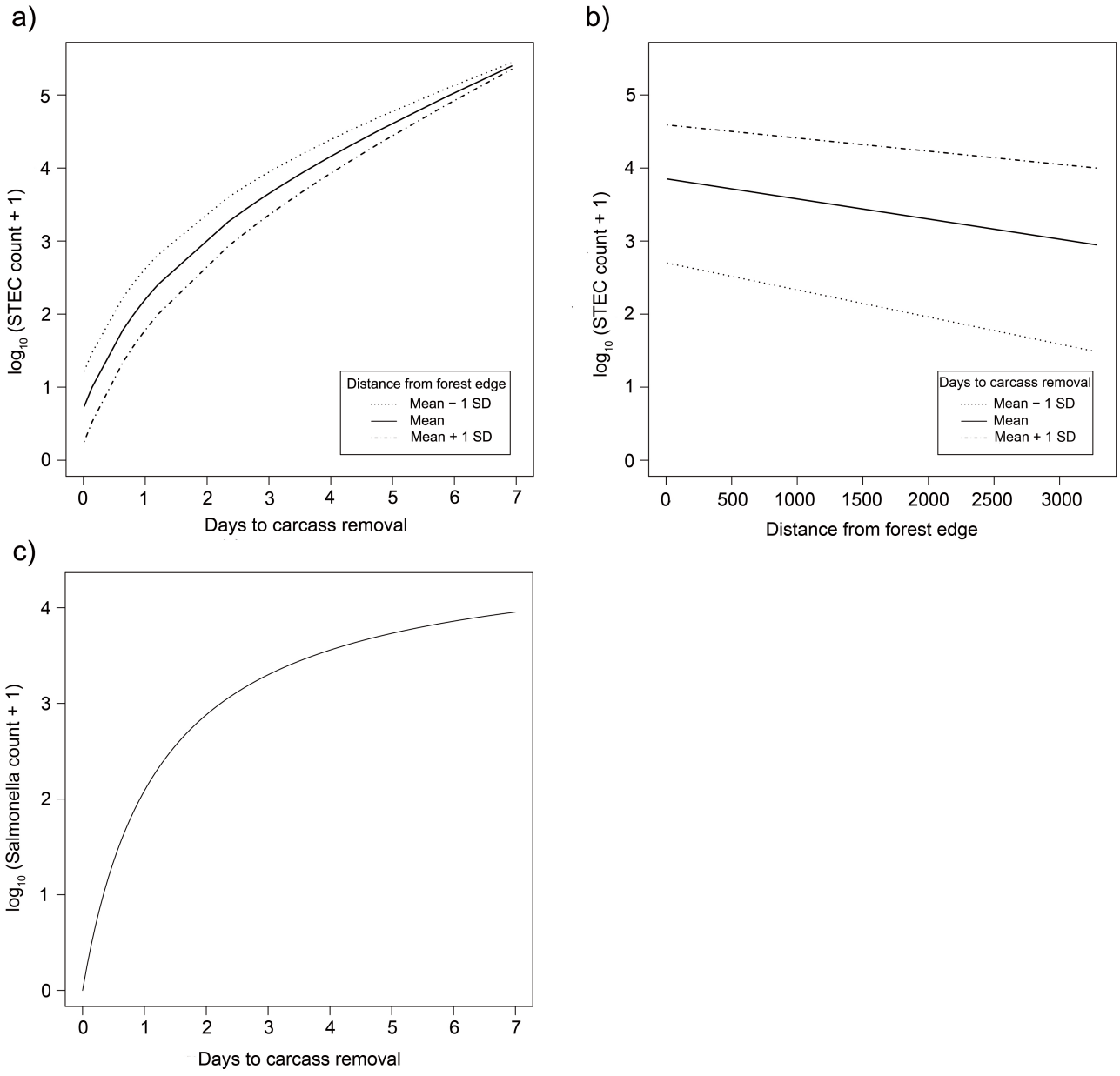

Fig. 3. Averaged models of pathogenic bacterial abundances on filth fly surfaces as a result of scavenging activity by vertebrates: (a) Shiga toxin-producing *Escherichia coli* (STEC) abundance against days to carcass removal by vertebrate scavengers (TIME); (b) STEC abundance against distance from forest edge (FOREST); (c) *Salmonella* abundance against days to carcass removal by vertebrate scavengers (TIME). Due to interactive effects in averaged model for STEC, curves were plotted by controlling the other predictor at mean values and at mean values ± 1 SD.

amount of bacteria detected and the saturating curves observed. Additionally, filth fly larvae are known to secrete antibiotics when competing for resources with micro-organisms (*e.g.*, Jaklič et al. 2008; Thompson et al. 2013); the increasing number of filth fly larvae and accumulation of antibiotic materials over time could also be a factor limiting the bacteria abundances, and this could be another reason for the saturating curves observed.

The predictor of distance from the forest edge (*i.e.*, FOREST) was featured in some of the selected models for STEC abundance, but not for the selected model accounting for *Salmonella* abundance (Table 2). With the averaged STEC model, it was observed that FOREST had a weak negative relationship with STEC abundance (Fig. 3b). Should there be a spillover effect for the action of vertebrate scavengers on microbes, there will be a positive relationship between FOREST and bacteria abundances, whereby there will be lower bacteria counts at sites nearer to forest boundaries. Therefore, we did not detect a spillover effect for the action of vertebrate scavengers on microbe abundances in this study. This finding was also in agreement with the observation that the primary scavengers were detected at all ranges of distance from forest edge (*i.e.*, high SD values in Table 2; Fig. 2).

A possible explanation for the lack of spillover effect and the negative relationship observed could be the presence of hunting activities at the forest boundaries. From the camera-traps monitoring scavenger activities at the sampling stations, we recorded an instance of a man with flashlight and machete, presumably a hunter, at 0200 hrs at the boundary of the plantation and adjacent forest. Wildlife hunting and trade has been reported to be a widespread issue in much of Southeast Asia (*e.g.*, Nijman 2010), and is acknowledged to be the main threat for *Varanus salvator*, one of the primary scavengers in this study (Bennett et al. 2010). Surprisingly, the bearded pig (*Sus barbatus*) was not documented in this study at all, even though it is a common scavenger in forested sites (NTL Lim, unpubl. data); this may be the result of hunting activities within the plantations since this species is highly sought after (*e.g.*, Chin 2003, Luskin and Ke 2017). Anecdotal reports from the plantations also suggested that illegal poaching of wild animals occurs to varying degrees because of the high price that some animals can fetch—for example, a kilogram of pangolin scales costs about $600 (Zhou et al. 2014) while a porcupine bezoar may exceed the annual income of the typical plantation worker. With the opportunity to catch valuable forest species like porcupines and pangolins, hunting is more likely to occur in the plantation sections immediately adjacent to forest patches. The concentration of such hunting activities at boundary areas will most probably lower the species richness and abundance of vertebrates, including species that are less valuable but can be easily found and caught (*e.g.*, monitor lizards and reticulated pythons; see below). As vertebrates are generally mobile and large-bodied, they can locate and consume carrion efficiently. Therefore, hunting along boundaries adjacent to forests will result in depressed scavenging efficiencies compared to the plantation interiors and thus explain the negative relationship observed between bacteria count and FOREST (Fig. 3b).

While the primary scavengers detected at the study sites were not of important conservation statuses (Table 1), they were highly effective in removing carrion that was encountered and providing the ecosystem service of disease control. These species generally do not have a high commercial value, unlike pangolins and porcupines, but they were often harvested because they can be easily found and caught in large numbers. Seizures involving the illegal wildlife trade by national authorities in the region often find scavenging species within the consignment, particularly *Varanus salvator*, of which thousands are harvested annually (Koch et al. 2013), and *Malayopython reticulatus* (reticulated python), of which more than 300,000 are caught annually (Kasterine et al. 2012). Thus, it is most likely that continued illegal harvesting of wild vertebrates, regardless of their conservation statuses, will negatively impact their ability to provide the ecosystem service of disease control.

This ecosystem service of disease control is largely performed by facultative scavengers in Southeast Asia because of the lack of obligate scavengers, particularly vultures. Unlike Neotropical forests, Old World vultures are not found in forested habitats (Corlett and Primack 2011); this is because New World vultures possess the ability to locate carrion by olfaction and sight but their Old World counterparts only rely on sight (Stager 1967). A continuous forest or plantation canopy is a formidable visual obstacle to the forest interiors and thus hinders the vultures' ability to locate carrion. Without obligate scavengers, tropical Southeast Asian forests and plantations will naturally have a lower scavenging efficiency compared to habitats with vultures. Additionally, scavenging can be considered as a chance event (DeVault et al. 2004) because it can only occur if scavengers are in the proximity and conditions are favorable for the scavengers to locate the carrion by smell; this was indicated by the high variability in TIME (65.3 ± 40.2 hours) at stations where vertebrate scavengers were recorded by the camera traps. Due to a naturally lower scavenging efficiency and the fact that scavenging is opportunistic in nature, plantations

in Southeast Asia may be all the more vulnerable to the negative impacts of illegal harvesting of vertebrates when considering the ecosystem service of disease control.

CONCLUSIONS

We found that vertebrate scavengers have a strong effect on the abundance of pathogenic microbes on the exterior surfaces of filth flies and provide the ecosystem service of disease control in oil palm plantations. However, this ecosystem service is most probably negatively impacted by the illegal poaching of vertebrates. Therefore, to promote scavenger-friendly plantations, we recommend that illegal hunting within the plantations, particularly of monitor lizards and wild pigs, be curbed through the efforts of public education campaigns and adequate enforcement (*e.g.*, Bennett and Robinson 2000; Challender and MacMillan 2014). While feral dogs were found to be one of the primary scavengers within the plantations, we caution against the widespread use of feral dogs for carcass removal and disease control; this is because feral dogs are reservoirs for rabies (Wandeler et al. 1993) and are known to disturb or prey on wildlife (*e.g.*, on monitor lizards; Rashid 2004).

Ultimately, we believe that promoting scavenger-friendly plantations will allow residents of oil palm plantations to benefit from the ecosystem service of disease control without greatly altering plantation management practices and workers' lifestyles.

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