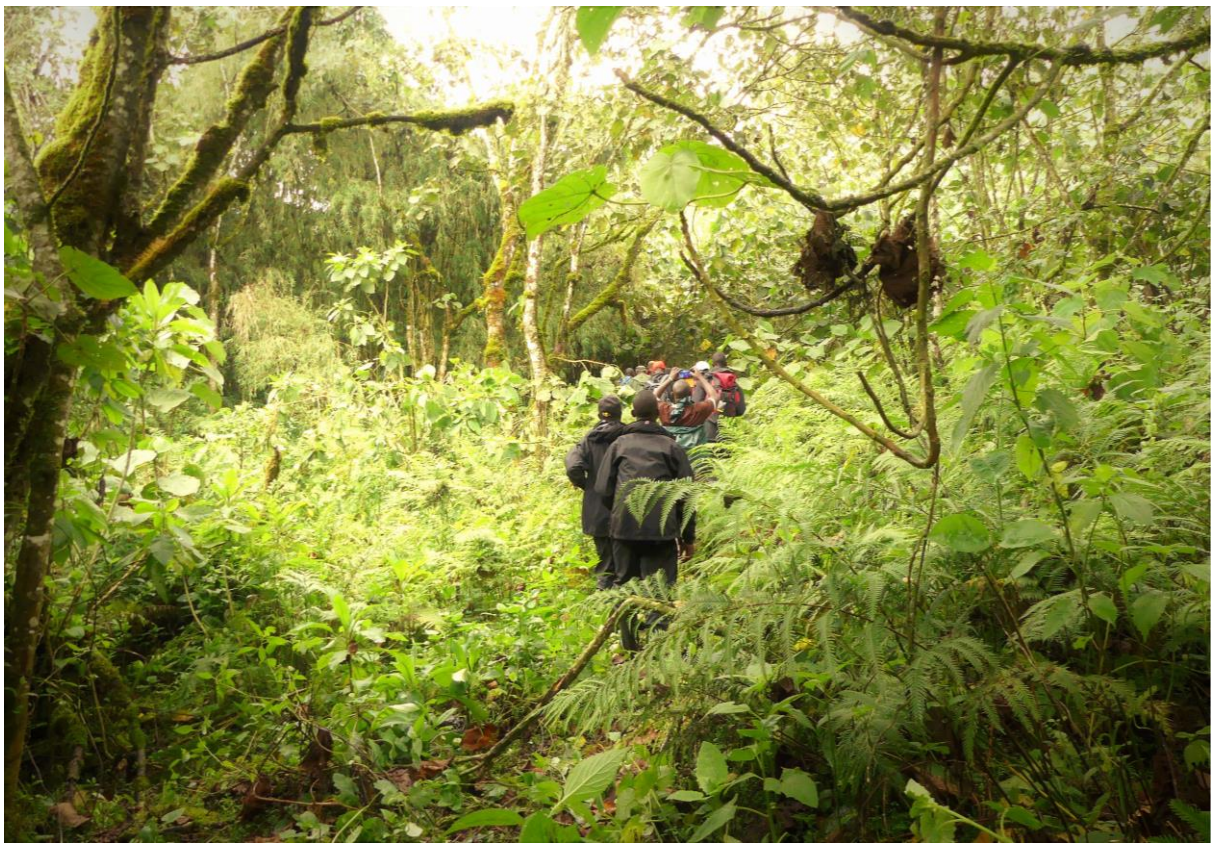
Virunga 2015-2016 Surveys

Monitoring Mountain Gorillas, Other Select Mammals, and Illegal Activities



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FINAL REPORT

April 2019

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Citation[1]: Hickey, J.R., Granjon, A.C., Vigilant, L., Eckardt, W., Gilardi, K.V., Cranfield, M., Musana, A., Masozera, A.B., Babaasa, D., Ruzigandekwe, F., & Robbins, M.M. 2019. Virunga 2015–2016 surveys: monitoring mountain gorillas, other select mammals, and illegal activities. GVTC, IGCP & partners, Kigali, Rwanda.

[1] Cited in Hickey *et al*. (2018) as: Hickey, J.R., Granjon, A.C., Vigilant, L., Eckardt, W., Gilardi, K.V., Babaasa, D., Ruzigandekwe, F., Leendertz, F.H. & Robbins, M.M. 2018. Virunga 2015–2016 surveys: monitoring mountain gorillas, other select mammals, and illegal activities. GVTC, IGCP & partners, Kigali, Rwanda.

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Virunga 2015-2016 Surveys

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Executive Summary

For over two decades, the mountain gorilla (*Gorilla beringei beringei*) was classified as Critically Endangered by the IUCN Red List of Threatened Species™. The most recent IUCN Red List reclassified mountain gorillas as Endangered (Hickey *et al* 2018) and described this subspecies of great ape (Photo 1) as existing in two isolated subpopulations in the Greater Virunga Landscape of Rwanda, Uganda, and the Democratic Republic of Congo (DRC). These subpopulations are commonly referred to as populations in the literature, but here we use the IUCN Red List terminology. The northern subpopulation resides in the Bwindi-Sarambwe ecosystem of Uganda and DRC, whereas the southern subpopulation occurs within an approximately 451-km^2 area collectively referred to as the Virunga Massif that encompasses Volcanoes National Park, Mgahinga Gorilla National Park, and the Mikeno Sector of Virunga National Park. This report describes the survey and results from fieldwork completed in 2015 and 2016 specifically limited to the latter subpopulation in the Virunga Massif. As of June 2016, surveys detected 604 individual gorillas comprised of 41 groups and 14 solitary males, reflecting the highest abundance of gorillas ever recorded in this subpopulation.

As in the previous survey conducted in 2010 (Gray *et al* 2013a,b), field teams walked pre-determined compass bearings through the forest to ensure thorough coverage of all areas that were physically accessible to them, while searching for signs of gorillas, other select mammals, and illegal activities. When fresh gorilla signs were detected, the teams followed the gorilla trail to locate three recent nest sites. At each of these sites, the teams collected fecal samples from nests. We genetically analyzed these samples to determine individual genotypes, which were the basis of the minimum count of unmonitored gorillas, and then added the known number of monitored gorillas. An abundance estimate for the Virunga subpopulation is forthcoming.

Importantly, any comparison of the abundances between the previous survey effort in 2010 and this 2015-2016 effort should be done cautiously, because search effort in 2015-2016 was nearly double that of the 2010 survey and increased effort conveys a concomitant increased opportunity to find more gorillas. That said, the minimum count (604 individuals) from 2015-2016 reflects an increase from the minimum count of 458 (or 480 with correction factors) gorillas in 2010 (Gray *et al* 2013). At the time, the correction factors were estimates of the number of undetected infants, as well as individual genotypes that did not fully amplify, that were added to the minimum count. These

Photo 1. Adult female with infant in Kwitonda Group

factors were not applied to the 2015-2016 estimate in order to stay true to the common definition of a minimum count and to avoid adding uncertainty to that count. The increased number of groups found in 2015-2016 (n=41) compared to 2010 (n=36) is likely due to a combination of group fissions, solitary males acquiring females, and the detection of additional unmonitored groups.

Considering only the minimum count of unmonitored gorillas, three factors likely caused the increased number of detected individuals (106 in 2010 versus 186 in 2015-2016). First, the increased detection may reflect intrinsic growth of these groups. Second, more gorillas dispersed from monitored to unmonitored groups than vice versa (e.g. 39 transfers from monitored to unmonitored versus 2 transfers from unmonitored to monitored). Third, the increase reflects better detection of gorillas because we conducted two sweeps instead of one. The estimated mean annual rate of change for unmonitored gorillas ranged from -2.0 to +5.1% and largely encompassed the estimated growth that occurred between the 2003 and 2010 surveys (0.9%, Gray *et al* 2013). The large range reflects the challenge in estimating growth rates when there are changes in effort, and therefore detection probability. For example, the 5.1% value represents the rate of increase when comparing the minimum count from the single sweep in 2010 to that of the combined 2015 and 2016 sweeps, which benefitted from approximately double the effort of 2010. Alternatively, if the annual rate of increase for the unmonitored subset is calculated between the minimum count in 2010 and that of either one of the two sweeps conducted in 2015 and 2016, respectively, then the annual rate of change appears negative (-2.0% and -1.4% for 2015 and 2016, respectively).

The field sampling effort for each individual sweep from 2010 to 2016 was roughly comparable such that we can assume that a similar proportion of groups and individuals were detected in the single sweep of 2010 as in each individual sweep of 2015 and 2016, and all three sweeps can thus be compared to each other. We found 130 and 134 unique unmonitored gorillas in the 2015 and 2016 sweeps, respectively, whereas 106 were found in 2010, suggesting potential modest growth of the unmonitored subset even when accounting for the increased effort associated with two sweeps.

The gorillas habituated for research and tourism are monitored on a daily basis. Although we cannot conclusively state that the unmonitored subset of gorillas is experiencing intrinsic growth (versus simply an increased detection of unmonitored gorillas), we are able to accurately calculate the growth rate of the monitored subset from the known number of monitored gorillas. The monitored subset continued growing between years 2010 and 2016, from 352 to 418 habituated gorillas, reflecting an annual growth rate of 4.4%. This rate is slightly lower than for the same monitored subset between the 2003 and 2010 (4.7%).

Taken as a whole, the entire subpopulation (monitored plus the detected number of unmonitored individuals) grew at an annual rate of 4.7% since 2010. We attribute the increase in mountain gorillas inhabiting the Virunga Massif to the effectiveness of conservation policies and strategies, notably: veterinary interventions, daily protection and regulated tourism that benefit the monitored gorillas, as well as intensive law enforcement, community conservation projects, and transboundary collaboration among government institutions and NGO actors that benefit all gorillas.

There were no indications of population declines since 2010 for the other mammals surveyed, including elephants. While exercising caution due to the limitations of the study, the information

collected will inform species-distribution models for better understanding of population ecology of not only mountain gorillas, but other mammals as well.

Unfortunately, illegal activities in the transboundary area have not declined since 2010, despite impressive conservation efforts in law enforcement and community engagement. The survey teams destroyed 384 snares during the recent survey. Although these snares usually targeted other mammals, particularly forest antelopes, the survey teams did discover a dead mountain gorilla in a snare, highlighting that these snares also pose a direct threat to mountain gorillas.

This report represents the incredible collaborative effort and investment in human and financial resources necessary to estimate the abundance of mountain gorillas in the Virunga Massif, as well as to monitor illegal activities and other select large mammals. Furthermore, this project provided the pre-requisite baseline data to inform many related studies, from the influences of human activities on wildlife to the production of niche models based on associations between species occurrences, land cover, and other variables. Ultimately, we offer management recommendations for the further conservation of mountain gorillas and their habitat.

Recensements du Virunga 2015-2016

Surveillance des Gorilles de Montagne, autres mammifères sélectionnés et activités illégales

Résumé

Pendant plus de deux décennies, le gorille de montagne (*Gorilla beringei beringei*) était classé comme étant en danger critique sur la Liste Rouge des espèces menacées de l'UICN. La Liste Rouge la plus récente de l'UICN a reclassé les gorilles de montagne comme étant en voie de disparition (Hickey *et al* 2018) et décrit cette sous-espèce de grand singe (Photo 1) comme existant dans deux sous-populations isolées dans le paysage du Grand Virunga du Rwanda, de l'Ouganda et de la République démocratique du Congo (RDC). Ces sous-populations sont communément appelées populations dans la littérature, mais ici nous utilisons la terminologie de la Liste Rouge de l'UICN. La sous-population du Nord réside dans l'écosystème de Bwindi-Sarambwe en Ouganda et en RDC, alors que la sous-population du Sud se trouve dans une zone d'environ 451 km^2 collectivement désignée comme le Massif des Virunga qui englobe le Parc National des Volcans, le Parc National des Gorilles de Mgahinga et le secteur Mikeno du Parc National des Virunga. Ce rapport décrit le recensement et les résultats des travaux de terrain achevés en 2015 et 2016 spécifiquement limités à cette dernière sous-population du Massif des Virunga. En juin 2016, les recensements ont détecté 604 individus dans 41 groupes et de 14 mâles solitaires, reflétant l'abondance la plus élevée de gorilles jamais enregistrée dans cette sous-population.

Comme dans le recensement précédent mené en 2010 (Gray *et al* 2013a,b), les équipes de terrain ont parcouru la forêt sur des sentiers prédéterminés à la boussole, afin de garantir une couverture complète de toutes les zones qui leurs fut physiquement accessibles, tout en recherchant des signes de gorilles, d'autres mammifères sélectionnés et d'activités illégales. Lorsque des signes frais de gorille ont été détectés, les équipes ont suivi la piste des gorilles pour localiser trois sites de nidification récents. A chacun de ces sites, les équipes ont recueilli des échantillons de fèces dans les nids. Nous avons analysé génétiquement ces échantillons pour déterminer les génotypes individuels, qui furent la

base du nombre minimum des gorilles non surveillés, et puis le nombre connu de gorilles surveillés fut ajouté.

Une estimation de l'abondance de la sous-population des Virunga est à venir. Il est important de noter que toute comparaison des abondances entre le recensement précédent de 2010 et cet effort de 2015-2016 devrait être fait prudemment, parce que l'effort du recensement de 2015-2016 était presque le double de celui du recensement de 2010 et l'effort accru traduit une opportunité accrue concomitante de trouver plus de gorilles. Cela dit, le nombre minimum (604 individus) de 2015-2016 reflète une augmentation du nombre minimum de 458 gorilles (ou 480 avec des facteurs de correction) en 2010 (Gray *et al* 2013). A cette époque, les facteurs de correction étaient des estimations du nombre de nourrissons non détectés, ainsi que les génotypes individuels qui n'étaient pas complètement amplifiés, qui furent ajoutés au nombre minimum. Ces facteurs n'ont pas été appliqués à l'estimation de 2015-2016 afin de rester fidèle à la définition commune d'un compte minimum et d'éviter d'ajouter de l'incertitude à ce compte. L'augmentation du nombre de groupes trouvés en 2015-2016 (n = 41) comparativement à 2010 (n = 36) est probablement attribuable à une combinaison de fissions de groupes, de mâles solitaires obtenant des femelles, et détection de groupes additionnels non surveillés.

Considérant seulement le nombre minimum de gorilles non surveillés, trois facteurs ont probablement provoqué l'augmentation du nombre d'individus détectés (106 en 2010 contre 186 en 2015-2016). Premièrement, la détection accrue peut refléter la croissance intrinsèque de ces groupes. Deuxièmement, plus de gorilles se dispersent de groupes surveillés aux groupes non surveillés que vice versa (p. ex. 39 transferts de surveillés aux non surveillés contre 2 transferts de non surveillés aux surveillés). Troisièmement, l'augmentation reflète une meilleure détection des gorilles parce que nous avons effectué deux quêtes au lieu d'une. Le taux de croissance annuel moyen estimé pour les gorilles non surveillés variait de -2,0 à + 5,1% et englobait en grande partie la croissance estimée qui a eu lieu entre les recensements de 2003 et 2010 (0,9%, Gray *et al* 2013). La large gamme reflète le défi dans l'estimation des taux de croissance quand il y a des changements dans l'effort, et donc la probabilité de détection. Par exemple, la valeur de 5,1% représente le taux d'augmentation lorsque l'on compare le nombre minimum de l'unique quête en 2010 à celui des quêtes combinées de 2015 et 2016, qui ont bénéficié d'environ le double de l'effort de 2010. Alternativement, si le taux annuel d'augmentation du sous-groupe non surveillé est calculé entre le nombre minimum de 2010 et celui de l'une des deux quêtes effectuées en 2015 et 2016, respectivement, alors le taux de variation

Photo 2. Femelle adulte avec le nourrisson dans le groupe de Kwitonda

annuel semble négatif (-2,0% et -1,4% pour 2015 et 2016, respectivement).

L'effort d'échantillonnage sur le terrain pour chaque quête individuelle de 2010 à 2016 était à peu près comparable, de sorte que nous pouvons supposer qu'une proportion similaire de groupes et d'individus a été détectée dans l'unique quête de 2010 comme dans chaque quête individuelle de 2015 et 2016, et ainsi les trois quêtes peuvent donc être comparées les unes aux autres. Nous avons trouvé 130 et 134 gorilles uniques non surveillés dans les quêtes de 2015 et 2016, respectivement, alors que 106 ont été trouvés en 2010, suggérant une croissance modeste potentielle du sous-groupe non surveillé, même en tenant compte de l'effort accru associé aux deux quêtes.

Les gorilles habitués à la recherche et au tourisme sont surveillés quotidiennement. Bien que nous ne puissions pas affirmer de manière concluante que le sous-groupe des gorilles non surveillé connaît une croissance intrinsèque (plutôt qu'une détection accrue des gorilles non surveillés), nous sommes en mesure de calculer avec précision le taux de croissance du sous-groupe surveillé à partir du nombre connu de gorilles surveillés. Le sous-groupe surveillé a continué de croître entre les années 2010 et 2016, passant de 352 à 418 gorilles habitués, reflétant un taux de croissance annuel de 4,4%. Ce taux est légèrement plus bas que pour le même sous-groupe surveillé entre 2003 et 2010 (4,7%).

Pris dans son ensemble, la sous-population entière (gorilles surveillés plus le nombre détecté d'individus non surveillés) a augmenté d'un taux annuel de 4,7% depuis 2010. Nous attribuons l'augmentation des gorilles de montagne habitant le Massif des Virunga à l'efficacité des politiques et stratégies de conservation, notamment: les interventions vétérinaires, la protection quotidienne et le tourisme réglementé qui profitent aux gorilles surveillés, ainsi qu'à l'application intensive de la lois, des projets de conservation communautaire et une collaboration transfrontalière entre les institutions gouvernementales et les acteurs des ONG qui profitent à tous les gorilles.

Il n'y avait aucune indication de déclin de la population depuis 2010 pour les autres mammifères recensés, y compris les éléphants. Tout en faisant preuve de prudence en raison des limitations de l'étude, les informations recueillies informeront les modèles de distribution des espèces pour mieux comprendre l'écologie de la population non seulement des gorilles de montagne, mais également d'autres mammifères.

Malheureusement, les activités illégales dans la zone transfrontalière n'ont pas diminué depuis 2010, malgré les efforts de conservation impressionnants dans l'application de la Loi et l'engagement communautaire. Les équipes de recensement ont détruit 384 pièges au cours du recensement récent. Bien que ces pièges ciblent habituellement d'autres mammifères, particulièrement les antilopes, les équipes de recensement ont découvert un gorille de montagne mort dans un piège, soulignant que ces pièges posent également une menace directe aux gorilles de montagne.

Ce rapport représente l'incroyable effort collaboratif et l'investissement dans les ressources humaines et financières nécessaires pour estimer l'abondance des gorilles de montagne dans le Massif des Virunga, ainsi que pour surveiller les activités illégales et d'autres grands mammifères sélectionnés. En outre, ce projet a fourni les données de référence préalables nécessaires pour informer de nombreuses études connexes, des influences des activités humaines sur la faune à la production de modèles sur les niches fondés sur des associations entre les occurrences des espèces, la couverture terrestre et d'autres variables. En fin de compte, nous offrons des recommandations de gestion pour la conservation des gorilles de montagne et de leur habitat.

Introduction

Long-term monitoring of wildlife populations enables the assessment of species status, conservation efforts, and the effects of numerous variables including potential impacts of hunting, land-use change, climate change, and other disturbances on species of interest. The mountain gorillas (*Gorilla beringei beringei)* of the Virunga Massif – a range of volcanoes located within the Greater Virunga Landscape at the nexus of the Democratic Republic of Congo (DRC), Rwanda, and Uganda – are arguably the best-monitored subpopulation of great apes. They have been periodically surveyed since the early 1970s, with ever increasing effort (Harcourt and Groom 1972, Sholley 1991, Stewart *et al* 2001, Gray *et al* 2005, 2009, 2013a, 2013b). In fact, regarding previous estimates of mountain gorilla abundance, Harcourt and Groom (1972) stated that "the numerous estimates amount to guesses based on extrapolation from known numbers in very small areas" and their own survey effort (including that described in Groom 1973) encompassed an area much smaller than subsequent efforts. Of note, survey coverage from the 1980s forward increased, and innovative techniques were periodically added in an effort to improve the accuracy of abundance estimates. Taken on balance, and acknowledging the adjustments in methodology and effort over time, results from the last three decades of these surveys suggest that mountain gorillas are the only subspecies of great ape that is not declining in numbers.

Photo 2. Adult female with infant in Susa Group

As a globally recognized and Endangered subspecies (Hickey *et al* 2018), the mountain gorilla has benefitted from intensive conservation effort (Robbins *et al* 2011) even as it suffers from extreme restriction of habitat (Harcourt and Fossey 1981). Only two mountaintop refugial subpopulations remain of this subspecies, one inhabiting the Virunga Massif and the other inhabiting Bwindi Impenetrable National Park (BINP), Uganda; both of which are entirely isolated by a human-dominated agricultural landscape. Around the Massif, human population densities are among the highest in the world, considering the rural setting. For example, Volcanoes National Park (VNP) is located within the Virunga Massif with areas adjacent to it averaging 590 people per km^2 and Gahunga Sector, in the eastern zone, approaching 1000 people per km^2 (Bush *et al* 2010). Furthermore, native forest in VNP has been whittled away over time, with a combination of deforestation projects occurring as humanitarian relief, as well as illegal encroachments, to make

land available for farming (Harcourt and Fossey 1981). As a result, the 160-km^2 VNP is about one-third its size when originally gazetted (RDB 2017), thereby resulting in a decrease in the amount of area protected within the Massif. These historic habitat losses imposed on a very small mountain gorilla subpopulation, coupled with pressures exerted from high human density and periodic civil unrest (Kalpers *et al* 2003), as well as the threat of disease (Köndgen *et al* 2008; Palacios *et al* 2011), demand vigilant population monitoring, law enforcement, and conservation action. To that extent, periodic full-population surveys of the entire Massif complement routine monitoring and patrolling. These Massif-wide surveys cover virtually the entire area, including remote locations that are rarely patrolled, and provide the opportunity to survey for illegal activities and destroy snares throughout the Massif. Further, a full subpopulation-abundance estimate is made possible by the Massif-wide survey which includes unmonitored gorillas, that is not possible with existing protocols from routine monitoring because those daily patrols only monitor the habituated gorilla groups (Photo 2).

Changes in survey effort and method over time have confounded wildlife-population monitoring for decades. For example, mountain gorilla population surveys began around 1970 with nest counts; then around the turn of the century, researchers began using genetics to identify fecal samples to individual (Guschanski *et al* 2009). Although both types of errors occurred (e.g. non-detection or "missing", and false detection or "double counting", individuals or groups) when using field evidence alone, Guschanski *et al* (2009) demonstrated that the historic approach used for previous mountain-gorilla population surveys would have led to a net over-estimate of the minimum count in their study. The 2011 survey in BINP incorporated genetic capture-mark-recapture (CMR) approaches for the first time in the history of mountain-gorilla abundance estimation (Roy *et al* 2014); which meant approximately doubling survey effort to achieve the minimum requisite of two survey occasions for CMR in order to estimate detection probability. Importantly, Roy *et al* (2014) demonstrated that single-sweep surveys under-estimated the true abundance of individuals (because they did not account for imperfect detection). Therefore, mountain gorilla surveys without genetics may have led to misunderstandings of historic population abundance and trends.

Changes over time in survey effort or method can be difficult to tackle because as scientific approaches advance, it makes little sense to continue using dated methods – with known problems – simply to preserve comparable protocols. If one examines the survey effort (e.g. area surveyed, km walked, number of person-days, etc. depending on how effort was reported) of consecutive Virunga Massif mountain gorilla surveys since the early 1970s, search effort clearly increased with every survey. These increases in survey effort over the decades potentially contributed further to misinterpretations of population trend. A simple yet fundamental rule in wildlife surveys is that the more you search, the more you find – presumably until reaching an asymptote where further increases in effort yield no further increases in individuals detected. Moreover, recent protocols (Guschanski *et al* 2009; Gray *et al* 2013a, b; Roy *et al* 2014) incorporated advancements in science (e.g. swapping the use of field evidence to presume group identity, in favor of genetic approaches that uniquely identify each fecal sample to individual). These modern methods hone the population estimate to more realistic numbers.

In the present survey, the intent was to double the 2010 Virunga Massif survey effort. That is, we meant to follow the CMR approach in the BINP 2011 survey (Roy *et al* 2014) and conduct two full sweeps of the Massif (whereas in Virunga 2010, only one sweep was conducted). However, as likely

unwittingly happened in previous survey efforts, small but relevant adjustments to our protocol may have resulted in a greater-than-intended increase in survey effort. For example, Virunga 2015-2016 was the first full survey of mountain gorillas in this subpopulation to eliminate paper data entry and instead employ electronic devices for recording all data, including geo-referenced positions of every observation. Furthermore, we refined the number and type of select mammal and illegal activity signs that were recorded to reduce data-entry time, as well as to facilitate team movement through the Massif. Hence, it is possible that the encounter rate of these signs in the current study may appear increased since 2010, simply as an artifact of observers being able to more easily focus their search for the signs of primary interest. Finally, in sweep 2, we added a new sector "W" at high elevation (Figure 1), which was not surveyed in previous efforts. Here, we present the minimum count of gorillas in the Virunga Massif as of June 2016, which is the best option for comparison to the 2010 results. A CMR abundance estimate will be produced using a new approach (Hickey and Sollmann 2018) and shared in a scientific publication.

In addition to surveying the Virunga Massif to estimate the abundance of unmonitored gorillas, we documented changes in the number of gorillas in monitored groups, as well as their demographic rates (Robbins *et al* 2011; Gray *et al* 2013), through routine monitoring of the habituated gorillas that has continued since the late 1960s. Since a substantial proportion of the subpopulation is monitored, tracking these changes throughout the year is a relatively low-cost approach to routinely assess population dynamics.

Despite the challenges posed by long-term monitoring and the progressive advances of science, the Virunga 2015-2016 surveys provide another benchmark in ongoing assessments of the status of mountain gorillas and select mammal populations, as well as the illegal activities that continue to occur in the Virunga Massif. This effort provides the pre-requisite baseline data to inform several related studies, from the influences of human activities on wildlife to the production of niche models based on associations between land cover and species occurrences.

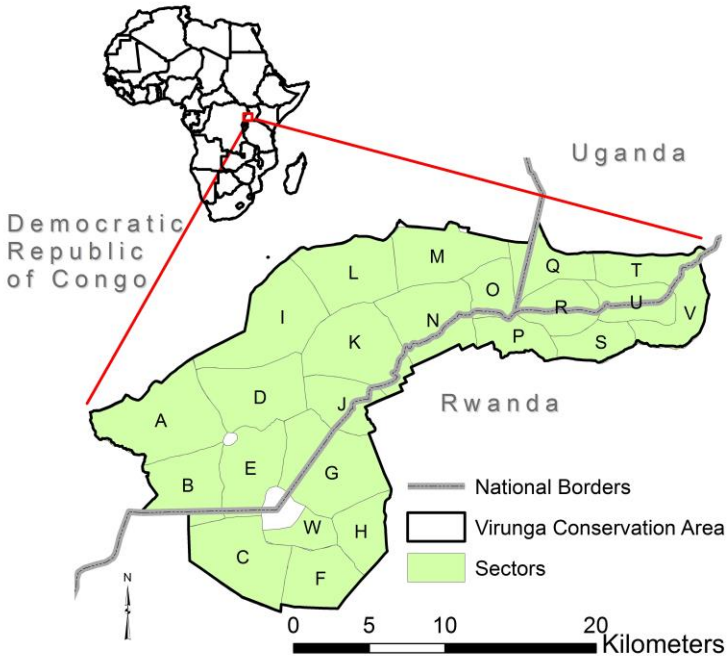

Figure 1. The Virunga Massif encompasses the Mikeno Sector of Virunga National Park in the Democratic Republic of Congo, Volcanoes National Park in Rwanda, and Mgahinga Gorilla National Park in Uganda. The sectors indicated here by letter helped organize field work.

Methods

Study Site

Virunga Massif

The Virunga Massif is comprised of the Mikeno portion of Virunga National Park (ViNP) in the Democratic Republic of Congo (DRC), Volcanoes National Park (VNP) in Rwanda, and Mgahinga Gorilla National Park (MGNP) in Uganda, encompassing approximately 451 km^2 (Figure 1). Elevation ranges from 2000- to 4500-m above sea level (McNeilage 2001). Correspondingly, bamboo or mixed bamboo, *Hagenia* forest, *Hypericum* woodland, herbaceous, meadow, *Mimulopsis*, mixed forest, *Neobutonia* forest, subalpine shrub, and alpine meadow characterize most of the landcover in the conservation area (Grueter *et al* 2013 and this study). Climate in the study site is characterized by two rainy and two dry seasons per year. Usually, the long rainy season spans March through May and the short rainy season spans September to November. For logistical efficiency, we divided this tri-national conservation area into 23 sectors ranging in size from 8 to 34 km^2 (Figure 1). Excluding the rocky alpine zones (~8 to 13 km^2), which were not surveyed due to logistical constraints, the area surveyed encompassed 442 km^2 and 447 km^2 in sweeps 1 and 2, respectively.

Field Methods

Sweeps

The field-survey approach was generally based on past protocols (Sholley 1991, McNeilage *et al* 2001, 2006, Gray *et al* 2009, 2013, Guschanski *et al* 2009) and modified in a similar manner as in Roy *et al* (2014) to collect two occasion histories for genetic mark-recapture abundance estimation.

Starting with the southwestern sectors and progressing toward the northeast, each sector was surveyed by two field teams searching for observations of wildlife and illegal activities (Photo 3). Teams typically included 2 trackers, an armed ranger, and 1 or 2 data recorders, for a total of 4 to 5 members. Often data recorders and rangers also had tracking skills. Once a sector was completed, teams moved to a new sector to resume surveys, until all 23 sectors had been surveyed. Six teams worked in two-week shifts and then rotated out with fresh teams replacing them for the subsequent two weeks, until a single survey of the entire Massif, termed a "sweep", was complete. Teams conducted two sweeps, the first occurred from October to December 2015 (57 days), and the second from March to May 2016 (59 days), both corresponding to rainy seasons.

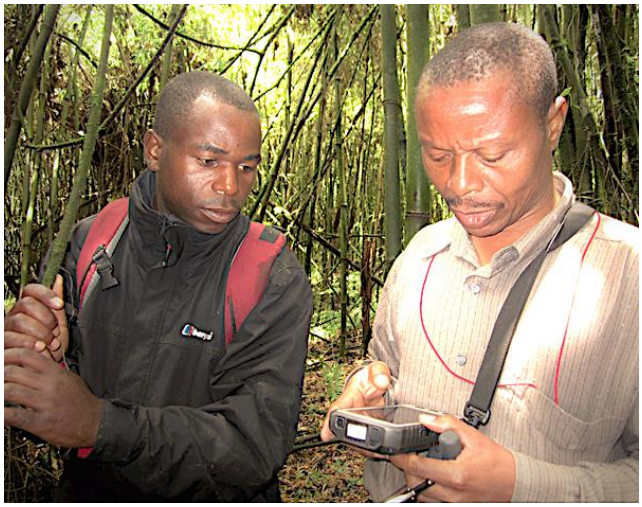

Photo 3. Ignace Hatangimana and Anselme Matabaro record observations during the Virunga 2015-2016 surveys

Table 1. Species, types of observations, and manner of aging signs

Common Name	Observation Type	Age of Sign (days)	Latin Name
Buffalo	Sighting	NA	*Syncerus caffer*
Golden or Blue monkey	Heard Sighting	NA	*Cercopithecus kandti or C. mitis*
Elephant or Carnivore	Dung Heard Tracks Scraping Sighting	Fresh (0-1d) Recent (2-4d) Old (>4d)	*Loxodonta africana, Canis adustus, Canis lupus familiaris, Caracal aurata, Crocuta crocuta, or Leptailurus serval*
Mountain Gorilla	Dung Heard Tracks Nest Sites Sighting	To the date, if possible, or >5d	*Gorilla beringei beringei*

recorded. Note that some species and sign types that were included in 2003 or 2010 surveys were not incorporated into the protocol of this study.

Table 2 describes the human activities and types of observations recorded. Note again that some types of human activities that were included in 2003 or 2010 surveys were not incorporated in 2015-2016 protocols. Teams entered all data into rugged, handheld

electronic devices (Toughpad FZX1, Panasonic™) equipped with Cybertracker software that was customized for this survey (Photo 4). In addition, teams plotted their location on paper maps at 'control points' every 250 m to track their progress and survey coverage for coordination with other teams. These control points were also logged in the electronic devices.

Reconnaissance Routes "Recces"

To survey a sector, field teams moved through the vegetation on foot, typically following an initial pre-determined bearing until they came within 200 m of either a sector or National Park boundary. Adjacent reconnaissance routes, termed recces, were spaced c. 500 m apart. Recces departed from

the bearing, becoming irregular, when teams circumnavigated obstacles such as ravines or peaks, and when teams detected fresh gorilla trails. For direct and indirect observations of mountain gorillas and other select mammals, as well as illegal activities, teams recorded the age, species, and type of sign (e.g. track, dung, heard, seen). Table 1 describes the complete set of species and types of mammal observations

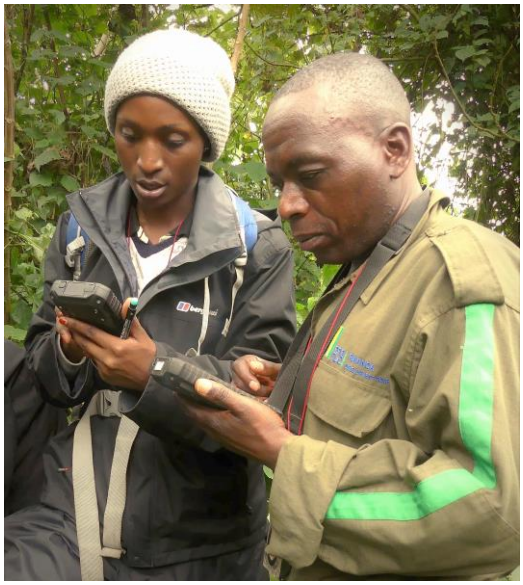

Photo 4. Esther Kakuze and Jean Damascene Hakizimana enter data into rugged handheld electronic devices

Vegetation Typing

At every location where mammal signs were recorded, teams also recorded the dominant vegetation type within a circle of 10-m radius around the observation. Dominant vegetation was categorized into the following vegetation types, roughly following the classification of Plumptre (1991), Watts (1983), and Grueter *et al* (2013): bamboo; mixed bamboo; *Hagenia* forest; *Hypericum* woodland; herbaceous; meadow; *Mimulopsis*; high-low-open-closed mixed forest; *Neobutonia* forest; subalpine; and alpine. A mix of *Poa*, *Carex*, *Dendrosenecio*, and *Lobelia* species tended to characterize subalpine; and a combination of rock, shrub and grasses typically characterized alpine. Geo-referenced locations of vegetation types collected during this survey were used as ground-truth data in a supervised landcover classification of remotely sensed imagery (WWF-Germany and IGCP 2017). That separate effort will eventually contribute to future studies relating species occurrences to vegetation and land-use change, as well as larger planning efforts.

Table 2. Human activities, types of observations, and manner of aging signs

Human Activity	Observation Type	Age of Sign (days)
Poaching	Snare Poacher Hunt Camp	NA
	Animal in Snare Poached Carcass	Fresh (0-1d) Recent (2-4d) Old (>4d)
Wood cutting	Tree Cut	Fresh (0-1d) Recent (2-4d) Old (4-30d)

Photo 5. Field crew collects fecal ample for genetic analysis to individual

Sample Collection

When teams encountered a gorilla trail estimated (based on field experience) to be recent (≤5 days old), they left the bearing of the recce to follow the trail in search of a gorilla nest site. Once at a nest site, teams assigned the gorilla group a unique identity (either an alpha-numeric code or, if it was a known habituated group, then the group's name), searched for each nest (ground and arboreal), and collected fecal samples from every nest that contained ≥1 dung. If dung diameters were markedly different within a nest, each dung was sampled separately. Each sample collected was associated with data regarding the sector, group ID, nest site ID, nest ID, individual's estimated sex and age class, date of collection, estimated age of the sample, and GPS coordinates. Rarely, nests were so high in the vegetation as to be inaccessible. In those cases, nests were recorded as having no dung in them, because none could be collected.

All genetic samples were collected following the two-step procedure (Nsubuga *et al* 2004). In the field, approximately 4-g of feces (about the size of a teaspoon) were collected in a tube containing 96% ethanol such that the entire sample was submerged (Photo 5). After 24-30 h, the ethanol was removed, and samples were transferred into tubes filled with silica beads to complete desiccation. Silica tubes were then stored at room temperature until export to Germany. Once in Leipzig, samples were stored at room temperature until extraction, then at +4°C for long-term storage.

During the first sweep, fecal samples were also collected from each nest site for viral pathogen and parasite surveillance. An approximately 3-g piece of fresh feces per gorilla dung was placed in each of two plastic specimen tubes containing (1) 10% formalin and (2) RNALater™ for laboratory analysis of parasites and viruses, respectively. Specimens in RNALater™ were transferred to a subzero freezer as soon as they were transported from the field to the laboratory.

During the second sweep, a single sample was collected per gorilla dung, preserved in 96% ethanol, and transferred to silica (for genetic analyses only). Based on field evidence for each detected group, teams followed the gorilla trails and aimed to sample all nests from 3 consecutive nest sites per group – ideally including one fresh nest site from the previous night. Genetic analyses would later verify or correct the identification of which gorilla group constructed each nest site.

Laboratory Methods

DNA Extraction

We extracted a selection of 1,123 samples from the 2,233 collected using the Stool DNA Kit (ROBOKLON) following the manufacturer's instructions, except for one modification: we added 20-60 mg of desiccated stool sample (instead of 200 mg of wet stool sample) to the bead tube. After cell lysis, we incubated samples in the bead tube for 12-48 h before continuing the extraction procedure.

DNA Amplification

We attempted to amplify each extract at one sex-specific microsatellite locus (Amelogenin) and 13 autosomal microsatellite loci: vWF, D16s2624, D7s2204, D10s1432, D14s306, D5s1457, D5s1470, D4s1627, D2s1326, D1s550, D8s1106, D6s1056, D7s817.

We amplified each extract in 2-6 replicates using a two-step multiplexing PCR approach (Arandjelovic *et al* 2009). During the first step, we amplified all loci simultaneously in a single 20 µL PCR reaction containing: 10 µL of Type-it MultiplexPCR MasterMix® (QIAGEN), 0.03 µL of each forward and reverse primer (100-µM concentration), 3.95 µL of water and 5 µL of DNA extract. DNA amplification was performed in PTC-200 Thermocycler (MJ Research) and Thermocyclers T100 (BIO-RAD) following the protocol: initial denaturation at 95°C for 5 min, then 30 cycles of 20 s at 94°C, 90 s at 57°C and 30 s at 72°C, completed by a 30-min elongation step at 72°C.

In order to allow further genotyping of extracts at other microsatellite loci commonly used in gorilla research, we included three additional primer pairs in the Multiplex: D1s2130, D3s2459, D6s474.

In a second step, we used 1.5:100 diluted Multiplex product as a DNA template for four smaller Multiplexes including 3-4 loci: GroupA (D10s1432, D5s1457, D14s306), GroupC (D5s1470, D4s1627, D2s1326), GroupD (Amelogenin, vWF, D7s2204, D16s2624) and GroupE (D8s1106, D1s550, D6s1056,

D7s817). Each 10-µL reaction contained 5 µL of Type-it MultiplexPCR MasterMix®, 0.03-0.4 µL of forward (FAM-, HEX-, NED- or VIC-labelled) and reverse primers (10-µM concentration), 0.3-2 µL of water and 2.5 µL of diluted DNA. We amplified each group with the same Thermocycler protocol, but with 30 s of group-specific annealing temperature (55-60°C).

After the second amplification step, we electrophoresed each PCR product on an ABI PRISM 3130 Genetic Analyser and analyzed results with GeneMapper Software version 3.7 (Applied Biosystems).

Genotype Comparison

To confirm monitored group identities versus those samples that likely originated from unmonitored groups, we compared the extracted genotypes to one another and to genotypes from independent reference samples from known, habituated individuals. To that end and as part of a separate long-term effort, genetic samples were collected from as many known habituated individuals as possible in order to create a genetic database as a cross-reference for confirming individual and group identities. For the genetic database, sampling occurred moments after observed defecations to ensure that the reference sample indeed originated from the known individual. Of note, the genetic database in the Virunga Massif was incomplete at the time of this study, with three habituated groups in ViNP and several individuals in VNP and MGNP yet to be sampled.

Pathogen Analysis

Samples were screened by consensus polymerase chain reaction (PCR) for select viral families according to the USAID Emerging Pandemic Threats PREDICT project methodology. This strategic approach combines high sensitivity with broad reactivity (i.e. detects viruses at low levels while casting a wide net) and allows the detection of both known and novel viruses in a wide range of samples and host species (Goldstein *et al* 2013; Anthony *et al* 2013 a,b). Consensus PCR (cPCR) was used to screen gorilla fecal samples for coronaviruses, filoviruses, flaviviruses, paramyxoviruses, and influenzaviruses.

RNA was extracted from 155 fecal samples collected from gorilla nests in Volcanoes National Park (Rwanda) between 6-October and 21-November-2015, at the One Health Institute Research & Diagnostic Laboratory at the University of California, Davis (UCD OHI; samples received 12-July-2017). Following cDNA synthesis, samples were tested for housekeeping genes [Cytochrome B (CytB) and ß-actin] to assess RNA quality. All samples were then tested using conventional PCR assays for coronaviruses, influenza viruses, filoviruses and paramyxoviruses.

As well, to assess specific parasite burdens in individual gorillas and in the population, 370 fecal samples were examined for roundworms (strongylid) and tapeworms using standard fecal sedimentation methods to count eggs per gram feces (Modry *et al* 2018).

Analytical Methods

Spatial Analyses

To estimate survey effort (km-walked), we used the control points and basic observations recorded by each team in each 2-week phase of work and converted those points to lines, linking points consecutively in time. We then merged all lines for a sweep into a single shapefile to determine the

total km-walked per sweep. For each sweep's km-walked layer, we conducted a neighborhood analysis with windows of radius 1-km to produce a raster of km-walked per window that depicts survey effort spatially across the Virunga Massif. Areas >600-m from recces were considered outside the survey effort (e.g. mountain tops).

For each of the mammal and illegal activities recorded, we converted point observations to raster, then conducted a neighborhood analysis, again using a 1-km-radius moving window to count the number of observations per window. To determine the encounter rate in raster calculator, the number of observations was then divided by km-walked in that same window. Because tracks and dung of dogs could not be reliably distinguished from the tracks and dung of other carnivores, and because dogs likely function as carnivores (e.g. predators) in the ecosystem, we combined all carnivore and dog observations per sweep for this spatial analysis.

To eliminate potentially redundant records, where identical sign types (e.g. old elephant dung) were recorded within 30-m of each other, one of two observations was deleted for tabular reporting. However, if two records within 30-m of each were aged differently (e.g. fresh v recent elephant dung), then both records were retained. Encounter rates reflect observations after this data-cleaning step.

Estimate of Gorilla Abundance

The total number and age-sex composition of *habituated* groups is known independently of the Virunga 2015-2016 survey described herein because habituated gorilla groups are typically monitored daily, with all births, deaths, and dispersal events recorded (e.g. Robbins *et al* 2011; Gray *et al* 2013). Gorillas could exit the monitored subset via death or emigration, although uncertainty exists around some of these designations. For example, monitoring teams listed a gorilla as dead based on either finding its corpse and/or the individual exhibiting ill health prior to disappearing. Whereas, monitoring teams classified gorillas as emigrating to the unmonitored subset if they: disappeared within the typical age range that gorillas disperse from their natal group, were seen again in an unmonitored group, were seen again as solitary, or were later detected in an unmonitored group through genotyping. However, there remains an unquantifiable uncertainty around the recorded deaths and emigrations that were not confirmed subsequently, which may influence the estimations of growth rates.

Photo 6. Monitored gorilla in Susa Group

For a count of the total abundance of all gorillas throughout the Massif, we needed to estimate the number of unmonitored gorillas in the study area and then add that to the known number of monitored gorillas. The number of unique genotypes of unmonitored gorillas was termed the "minimum count" of individuals in the unmonitored subset. We then added this minimum count of unmonitored gorillas to the known count of monitored gorillas (as of 1-June-2016) to obtain a total minimum count of the entire Virunga subpopulation.

Minimum counts do not reflect the true abundance of unmonitored gorillas, because some individuals remain undetected in the densely forested steep mountains of the Virunga Massif. To improve on a minimum count and generate a confidence interval (CI), we will employ a non-invasive genetic-mark-recapture approach on detected genotypes to estimate the abundance of the unmonitored subset of gorillas in the Virunga Massif for a forthcoming scientific publication. Here, we present the minimum count based on the sum of the known monitored individuals plus the number of detected unique genotypes from unmonitored gorillas to compare the minimum count from 2015-2016 to the 2010 minimum count.

Although past abundance estimates of mountain gorillas included an infant-correction factor, we do not do so here. Typically, infant feces are very difficult to find, therefore the correction factor was an effort by researchers to estimate the number of unmonitored infants whose feces, and therefore whose genetic identities, were undetected (Guschanski *et al* 2009, Gray *et al* 2013b). That factor was based on an assumption that the unmonitored gorillas had the same ratio of infants to adult females as the monitored gorillas (Guschanski *et al* 2009). However, there is a growing understanding that monitored and unmonitored gorillas may be exposed to different types and intensities of threats, such that the assumption of equal ratios of infants to adult females may be unfounded. A more conservative and comparable estimate (for use in generating population trends) would be to use abundance estimates generated by capture-mark recapture techniques that account for detection probability and avoid the use of potentially inaccurate correction factors that may add noise to the estimate. Such mark-recapture approaches will be used and described in a forthcoming scientific publication.

Calculation of Growth Rates

For both monitored and unmonitored subsets, we used a time-series calculation that accounts for movements between the two subsets since the previous census (Robbins *et al*., 2011; Gray *et al* 2013). The growth rate was determined by starting with an initial number of gorillas and using Eq. (1) to calculate the number of gorillas in each subsequent month:

$$N_i = [N_{i-1} * (1 + r_m)] + A_i \quad (1)$$

In Eq. (1), N_i represents the number of gorillas in month i, N_{i-1} is the number of gorillas in the previous month, and r_m is the monthly growth rate. The adjustment factor A_i equals the number of gorillas that immigrated from the other subset during each month, minus the number of gorillas that emigrated. Note that we did not use this equation for the entire Virunga population because it is geographically closed due to isolation from human settlement, and migrations can only happen between the monitored and unmonitored subsets.

We report the average values from two sets of calculations for A_i: one set assumes that unexplained disappearances of monitored gorillas were deaths, and the other set assumes that those disappearances were due to dispersal to unmonitored groups. We used iterative calculations with the bisection method to find the value of r_m that enabled us to match the observed size of the monitored subset at the end of the study period. The monthly growth rate was converted into an annual growth rate (r_a) using Eq. (2) to account for monthly compounding:

$$(1 + r_a) = (1 + r_m)^{12}. \quad (2)$$

Finally, we estimated the growth (Eq. 3) of the entire Virunga population since the previous census using the sum of the count of monitored gorillas plus the minimum count of unmonitored gorillas for 2010 and 2016:

$$\left(\frac{P_x}{P_0}\right)^{\left(\frac{1}{x}\right)} - 1 \quad (3)$$

where P_0 is the sum of those counts from the 2010 survey, P_x is sum of those counts during this 2015-2016 survey, and x is the number of years between censuses (Kalpers *et al* 2003).

Results

Reconnaissance Routes "Recces"

Effort (km-walked) in this 2015-2016 survey totaled 2,132 km; broken down by sweep, effort was 1,069 km in 2015 and 1,063 km in 2016. As expected, this 2-sweep approach resulted in approximately twice the effort as previous surveys. Specifically, survey effort was 2.63-times and

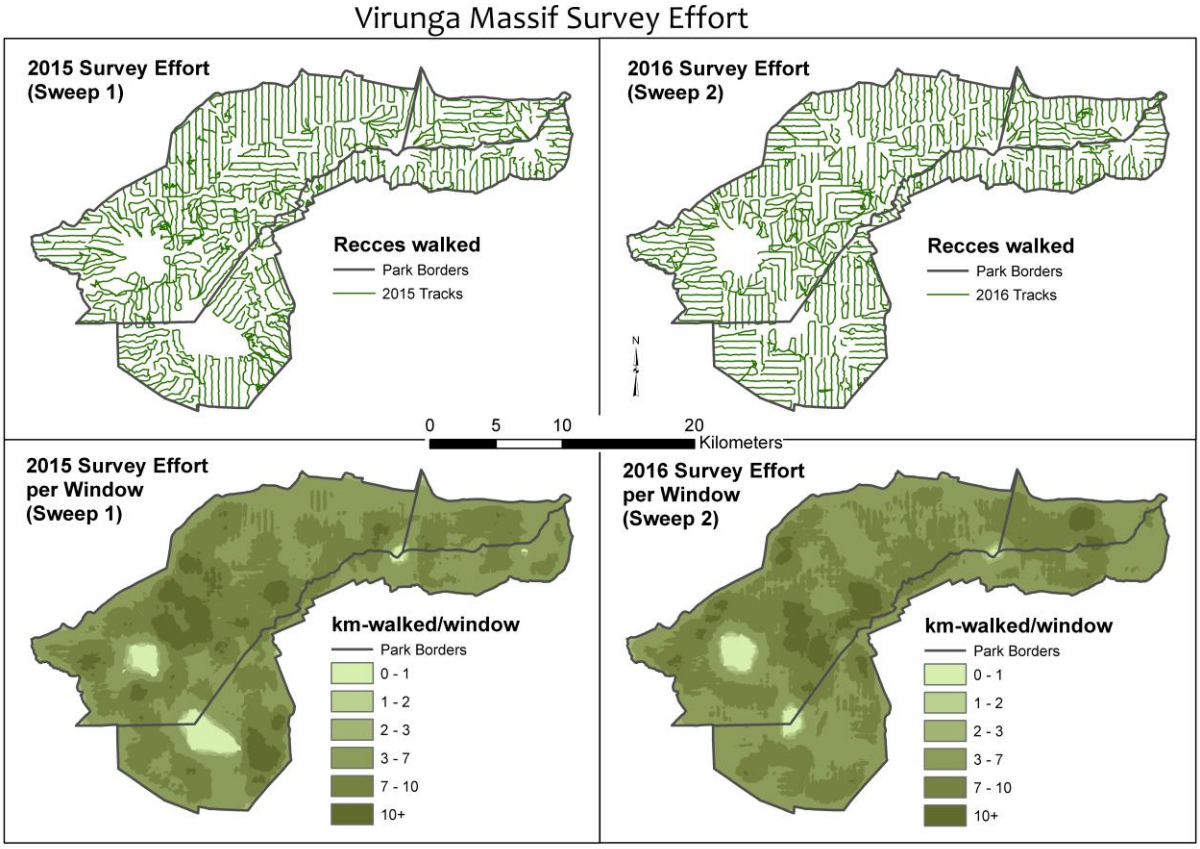

Figure 2. Survey effort in km-walked per 1-km-radius moving window in sweeps 1 & 2 of the Virunga 2015-2016 Mountain Gorilla Population Survey

1.87-times more than the 2003 and 2010 surveys, respectively (Gray *et al* 2005, Gray *et al* 2013a). Maps of the spatial distribution of survey effort for 2015 and 2016 demonstrate the thorough coverage with only high-elevation mountaintops and the steepest of ravines excluded from the survey effort (Figure 2).

Mountain Gorillas

Genetic Data Analysis

A total of 2,233 fecal samples were collected during the two sweeps: 1,102 in 2015 and 1,131 in 2016. Of these, 740 and 1,493 samples were from putative unhabituated and putative habituated gorillas, respectively. At the time of collection, field samples were described with the terms 'habituated' and 'unhabituated' before any genetic confirmation of group status, therefore we refer to them as 'putatively habituated' or 'putatively unhabituated'.

In fact, the genetic analysis revealed that some putative unhabituated groups were actually habituated as determined by comparison with genotypes from reference samples (see below for more details). Habituated gorillas sometimes disperse to unhabituated groups where they are not monitored; therefore, we refer to the confirmed habituated groups (after genetic analysis) as 'monitored' and the confirmed unhabituated groups as 'unmonitored'.

We attempted genotyping 739 samples from putative unhabituated gorillas (1 arrived at the lab too late to be genotyped) and a subset of 384 samples from putative habituated gorillas (2-3 per putative habituated nest site). We collected raw genotyping data for 13 autosomal loci and 1 sexing locus on these 1,123 samples. Analyses showed high PCR success rates (averaging 83% and ranging from 64% for D7s2204 to 93% for Amelogenin) and low allelic dropout varying from 0.1 to 3%, which allows 99% homozygote certainty with two replicates only.

As references to verify the identity of the monitored groups during the census, we also analyzed 464 samples collected from known individuals from the groups habituated for tourism in Rwanda and obtained 149 genotypes from unique individuals. These samples were collected independently from the population-survey work and the 149 genotypes represent 87% of the tourist gorillas accessible in Rwanda at the time (Karisimbi group was not available for sampling, as it crossed into a remote portion of DRC). We also used genotypes of the gorillas from the groups habituated for research by the Dian Fossey Gorilla Fund (DFGF).

The allele frequency analysis using these data indicated that even if two genotypes could be compared only at the eight *least* informative loci, the probability that two identical genotypes might represent two siblings, rather than one and the same gorilla, was very low ($PID_{sib} = 0.0072$). From the 1,123 extracts we attempted to genotype, 305 could not be confidently assigned to unique individuals, (177 [46%] from monitored and 128 [17.3%] from unmonitored groups). These failure rates are comparable to previous rates from mountain gorilla surveys and in the lower range for genetic analyses of non-invasively collected samples.

After adjusting for the 305 failed samples, the remaining 818 extracts (549 from unmonitored and 269 from monitored groups) were on average 80% complete (range: 6-13 loci), and we could determine the sex for 96% of them. We sorted these 818 genotypes into unique individuals ('consensus genotypes') with a $PID_{sib} < 0.01$ (Table 3). When sorting individuals into consensus

genotypes we found 5 pairs of genotypes that mismatched at only one locus. These pairs were re-examined to confirm the mismatch thereby indicating that all were unique individuals, and similar genotypes typically indicated parent-offspring pairs. These cases all concerned monitored gorillas.

Table 3. Summary of fecal samples collected and analyzed from the 2015-2016 field survey.

	Monitored	Unmonitored	Total
# Samples collected (putative status)	1493	740	2233
# Attempted extracts (putative status)	384	739	1123
# Samples collected (confirmed status)[a]	1555	678	2233
# Attempted extracts (confirmed status)[a]	446	677	1123
# Failed extracts	177	128	305
# Complete genotypes	269	549	818
# Unique individuals found	**168[b]**	**2[c]+184**	**354**
# Groups	27[d]	13	41[d]
# Solitary males		2[c]+12	14

[a]In the field, samples were labeled as either habituated or unhabituated. The genetic analysis demonstrated that some of the putative unhabituated samples were actually from habituated groups. Therefore, we confirmed the status of samples with the genetic analysis and updated their classification.

[b]This number reflects the number of unique individuals from monitored groups found genetically during the surveys, and not the total number of monitored gorillas.

[c]One unmonitored solitary male (Ra1) was sampled and not genotyped but is added here. A second unmonitored solitary male (Mukunda who was previously monitored) was physically seen and a fecal sample was collected, but no genotype was obtained because the sample did not amplify.

[d]There were 28 monitored groups at the time of the surveys, but one group, Musilikale (n=13), was never detected during either sweep.

Unmonitored Gorillas

The 549 genotypes from unmonitored individuals corresponded to 184 unique gorillas. Each individual was typed from 1-8 fecal samples. All consensus genotypes were based on comparisons with $PID_{sib} < 0.01$. The 184 consensus genotypes were on average 95% complete (range: 7-13 loci), and we determined the sex for 98% of them. One unmonitored solitary male (Ra1) was sampled and not genotyped (sample received in Leipzig too late for analysis), but is added here as an unique individual, because it was found in an area (Sector R) where no other samples were collected within a 3-km radius in either sweep. Additionally, one previously monitored male, Mukunda, is no longer monitored, yet was sighted during sweep 2 and is added here as well.

Therefore, as of June 2016, the minimum count of uniquely identified unmonitored gorillas was 186 individuals. Of those 186 unmonitored gorillas, we detected 130 and 134 individuals in 2015 and 2016, respectively. Specifically, 78 unmonitored individuals were found in both sweeps, 52 were found only in 2015 and 56 were found only in 2016. The 186 unmonitored gorillas were comprised of 172 individuals in 13 groups of average size 13 and 14 unmonitored solitary males (Table 4). Six of those solitary males (Himbara, Irakoze, Kubona**,** Mukunda, Shirimpumu, Urugwiro) were previously monitored gorillas that dispersed. Whereas fourteen of the unmonitored females were previously monitored (Bishushwe, Faida, Gufasha, Gusura, Haguruka, Igisubizo, Kanama, Kubaka, Kunga,

Makuba, Mawingu, Sabato, Umutekano and Umutuzo) that dispersed and, accordingly, were found in unmonitored groups.

Table 4. Unmonitored groups, solitary individuals, group sizes, country where detected, and number of times each group was found during 2015-2016 Virunga surveys. Total reflects the number of unique individuals based on genotype data.

Group	Times found	Number individuals found			Country Where Found
		2015	2016	Total	
A2-A1-A2	3	22	27	30	DRC
B1-Ga2	2	14	17	17	DRC
D2-D1	2	8	13	13	DRC
Ga1-D3b	2	10	11	12	DRC
K1-K2	2	9	18	19	DRC
K4-K3-K4a	3	8	10	10	DRC
K6-K4b	2	15	9	15	DRC
Na2-Na1	2	13	1[a]	13	DRC
D3a-D5	2	16	Not found	16	DRC
V2	1	2	Not found	2	Rwanda
V4	1	4	Not found	4	Rwanda
D2b	1	Not found	13	13	DRC
Ja1	1	Not found	8	8	DRC
Irakoze	3	1	1	1	Rwanda
Kubona	4	1	1	1	Rwanda
A1a	1	1	Not found	1	DRC
E2	1	1	Not found	1	DRC
Gb5	1	1	Not found	1	Rwanda
J1	1	1	Not found	1	DRC
J2	1	1	Not found	1	DRC
Ra1	1	1	Not found	1	Uganda
Shirimpumu	1	1	Not found	1	Rwanda
Himbara	2	Not found	1	1	Rwanda
K1b	1	Not found	1	1	DRC
Mukunda	1	Not found	1[b]	1	DRC
Na2b	1	Not found	1	1	DRC
Urugwiro	1	Not found	1	1	Rwanda
Total		**130**	**134**	**186**	

[a]One female found in Na2 in 2015 was detected via sampling at a lone nest 2016, whereas the other members of the group were not detected.

[b]Mukunda was previously monitored and was physically identified in one sweep. The fecal sample collected from him did not amplify so no genotype was obtained.

Less than 4 months passed between the two sweeps and therefore the time interval was short enough that, given the slow life history of mountain gorillas, there were likely very few births,

deaths, or dispersal events during this period and the subpopulation was assumed closed. This assumption is further supported by an estimated birth rate of 0.255 births per adult-female year and an estimated mortality rate of 0.037 deaths per gorilla year (Robbins *et al* 2011). The detections of inter-group transfers were cross-referenced to the extent possible.

Monitored Gorillas

Rather than genetic analyses, routine long-term monitoring of habituated gorillas (Table 5) provided direct counts of monitored groups. As of June 2016, there were 418 known monitored individual gorillas in the Virunga Massif. These monitored gorillas were distributed among 28 groups with mean group size of approximately 15 individuals.

Table 5. Number of gorillas in each monitored group in Virunga Massif as of 1-June-2016.

Country Where Managed	Group	Number of Gorillas
DRC	Bageni	24
DRC	Humba	9
DRC	Kabirizi	19
DRC	Lulengo	10
DRC	Mapuwa	22
DRC	Munyaga	9
DRC	Nyakamwe	11
DRC	Rugendo	9
Rwanda	Giraneza	6
Rwanda	Isabukuru	19
Rwanda	Iyambere	5
Rwanda[a]	Kuryama	10
Rwanda	Mafunzo	11
Rwanda	Musilikale	13
Rwanda	Ntambara	8
Rwanda	Pablo	33
Rwanda	Titus	7
Rwanda	Agashya	19
Rwanda[a]	Amahoro	19
Rwanda	Hirwa	19
Rwanda	Isimbi	15
Rwanda[a]	Karisimbi	12
Rwanda	Kwitonda	28
Rwanda	Sabyinyo	16
Rwanda	Susa - Igisha	26
Rwanda	Susa - Kurira	17
Rwanda	Umubano	12
Uganda	Nyakagezi	10
		418

[a]During the survey was found in both DRC and Rwanda

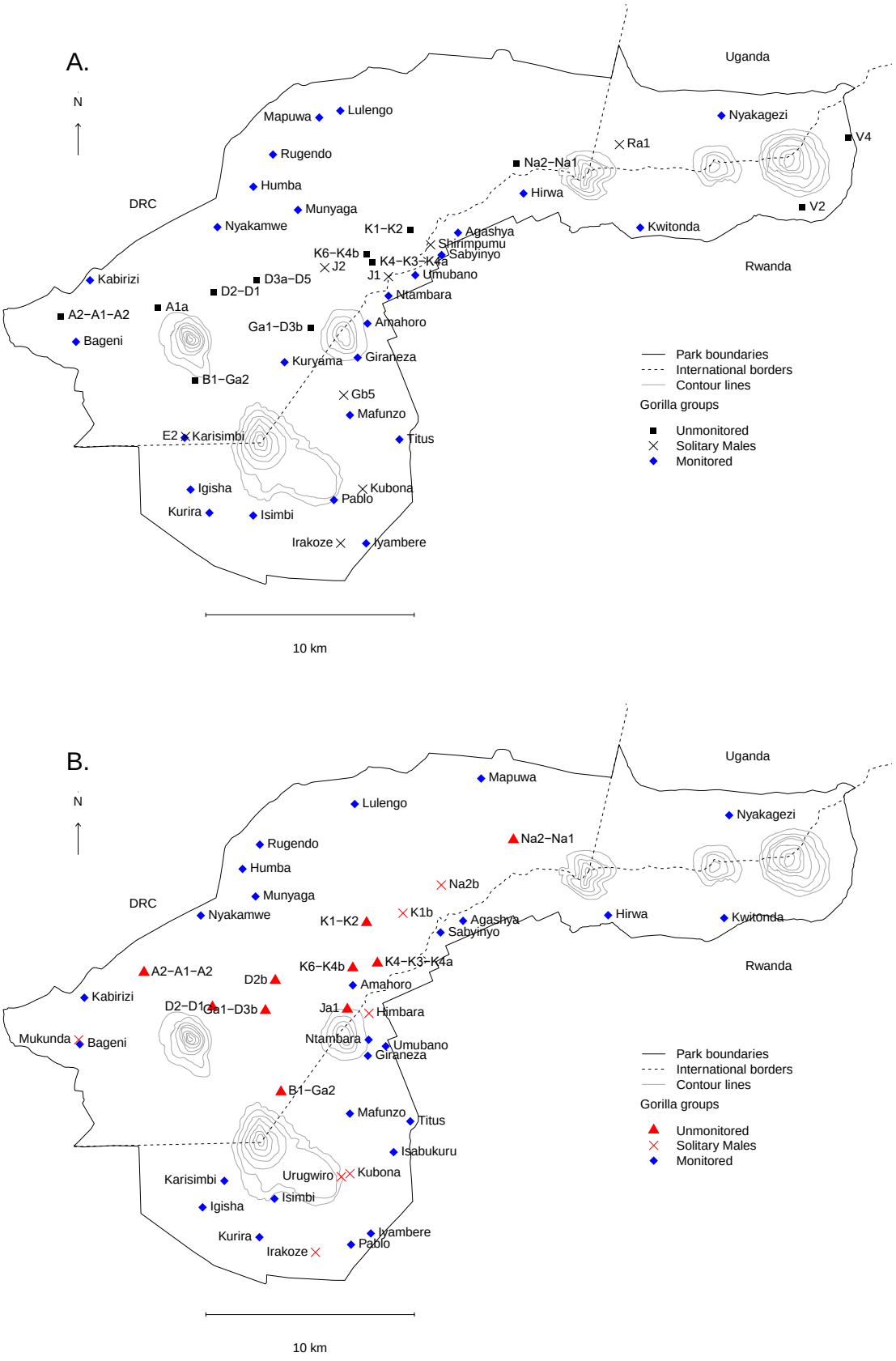

Figure 3. Location of groups and solitary males during the sweep 1 (A) and sweep 2 (B). The locations are based on average longitude and latitude for each group during a given sweep, and adjusted for a few groups to allow readability of the names. The ⍈ symbol denotes solitary males, with the same color code as the groups.

Unlike previous census reports, we no longer distinguish monitored groups in Rwanda by their original purpose of habituation (research or tourism) because these distinct management practices no longer apply and, for a variety of reasons, some previously monitored gorillas are no longer monitored, yet are still habituated (i.e. accustomed to, and typically tolerant of, human presence).There were 188 births, 83 deaths, 39 emigrations, 2 immigrations, and 2 unexplained disappearances in the monitored groups between 2010 and 2016. Of the those that emigrated out of monitored, some merely are no longer monitored, as opposed to an actual movement of the gorillas out of a particular group. Figure 3 displays the approximate locations of gorilla groups at time of detection during each sweep, which may be compared to detected group locations in the 2010 surveys (Figure 4).

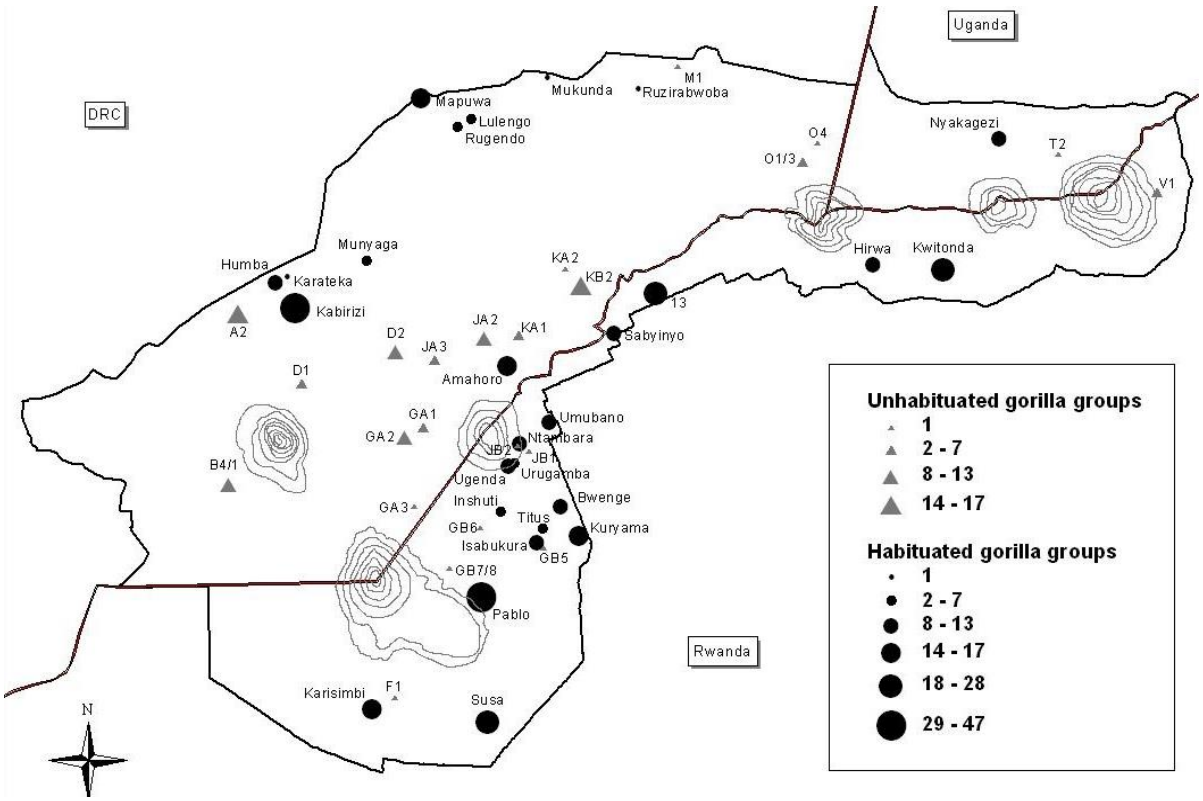

Figure 4. Locations of gorilla groups and solitary individuals detected during the 2010 survey of the Virunga Massif

Abundances and Growth Rates

Adding the minimum count of 186 unmonitored gorillas to the 418 monitored gorillas, we obtain a minimum count of 604 gorillas for the entire subpopulation in the Virunga Massif as of 1-June-2016 (Table 6). A total of 41 groups (average group size 14.1) and 14 solitary males were detected in 2015-2016 compared to 36 groups (average size 12.5) and 14 solitary males in 2010. Between 2010 and 2016, we designated 39 monitored gorillas as dispersed thereby becoming unmonitored and 2 unmonitored gorillas dispersed to monitored groups. Therefore, we estimated that a net of 37 monitored gorillas dispersed to the unmonitored subset between 2010 and 2016.

After adjusting for those exchanges between the subsets, based on Equation (2), the unmonitored subset increased approximately 5.1% annually. Importantly, this annual rate of increase for the

Table 6. Summary of the minimum count of mountain gorillas in the Virunga Massif as assessed in 2010 versus 2016.

	Monitored		Unmonitored Genotyped		Unmonitored Not Genotyped		Total	
	2010	2016	2010	2016	2010	2016	2010	2016
Number of Gorillas	352	418	106	184	6	2[a]	464	604
Number of Groups	24	28	12	13			36	41
Solitary Individuals	3	0	11	14			14	14

[a]These individuals were considered detected because the previously monitored individual named Mukunda was seen and the unmonitored individual, Ra1, was found in an area (Sector R) where no other samples were collected within a 3-km radius in either sweep.

unmonitored gorillas does not control for increased detection, with nearly double the search effort in 2015-2016 as in 2010, and it therefore overestimates the intrinsic growth. Hence, as an alternative perspective, we also estimated the growth rate between the single sweep of 2010 and either one or the other sweep of 2015 or 2016, separately (using only the detections from one sweep at a time). In that case, the unmonitored subset appeared to decline (or exhibit a negative growth rate) by either -2.0% or -1.4% annually depending on whether we compared 2010 abundance to that of 2015 or 2016, respectively. These alternate perspectives (comparing the 2010 abundance estimate to the 2015/2016 estimates based on either one or two sweeps of data) demonstrate the range of the uncertainty in annual rate of change (e.g. -2.0% to 5.1%) when detection probability changes between survey efforts. For monitored gorillas, which are known, the analysis is much simpler; the estimated annual growth rate was 4.4%. If any of the gorillas designated as emigrating were actually deaths, then the growth rate of the monitored subset would have been lower, and that of the unmonitored subset would have been higher than reported here. However, there was conclusive evidence that 25 of the designated emigrants did, in fact, emigrate because they were either seen again as unmonitored and/or detected via genotyping during these surveys. The other 14 potential emigrants were last seen in the monitored subset at a typical age of dispersal.

For consistency across surveys, we needed to compare estimates calculated the same way. Therefore from Table 6, we summed the minimum count of unmonitored genotypes (n=106) and known habituated gorillas (n=352) for a total minimum count of 458 individuals in the Virunga Massif in 2010 (Gray *et al* 2013). The increase in minimum counts from 458 to 604 gorillas, in 2010 versus 2016 respectively, represents a mean annual rate of growth of 4.7% for the entire Virunga subpopulation. If we had incorporated the correction factors from the 2010 abundance estimate, including 16 potentially undetected infants and 6 samples that could not be genotyped, then the rate of increase would be lower, yet we would be comparing estimates calculated in different ways and with different assumptions, therefore we refrain from such a comparison.

We discourage literal interpretations of changes in the abundance estimates of the subpopulation over the decades (Table 7), because methods changed and effort increased from survey to survey. Rather, we offer the following summary of abundance estimates because they represent the best information available at the time of each survey, and they provide evidence for a generally increasing trend over the last 45 years.

Table 7. Population parameters for Virunga Massif Mountain Gorilla Subpopulation from 1971- 2016, NA = data not available for calculating this variable (adapted from Gray *et al* 2013b).

Census Year	Total Gorillas Counted	Estimated Sub-population Size	# of Social Groups	Mean Group Size (SD)	Median Group Size	# of Solitary Males	% Multimale Groups	% Immature	% of Social Groups with >20 individuals
1971-73[a]	261	274	31	7.9 (NA)	NA	15	42	39.8	NA
1976-78[b]	252	268	28	8.8 (4.4)	7	6	39	35.8	3.5
1981[c]	242	254	28	8.5 (NA)	NA	5	40	39.7	NA
1986[d]	279	293	29	9.2 (5.5)	8	11	14	48.1	7
1989[e]	309	324	32	9.2 (7.1)	7	6	28	45.5	9
2000[f]	359	359-395	32	10.9 (9.7)	8	10	53[*]	44.7[*]	15.6
2003[g]	360	380	32	11.4 (11.2)	7.5	11	36[†]	41.0[†]	15.6
2010[h]	464[**]	480	36	12.5 (9.1)	10.5	14	61[*]	45.2[*]	11.1
2015-16[i]	604	604[††]	41	14.4 (7.0)	13.0	14	75[*]	42.8[*]	14.6

a. Harcourt & Groom (1972), Groom (1973); b. Weber & Vedder (1983); c. Aveling & Harcourt (1981); d. Vedder & Aveling (1986); e. Sholley (1991); f. Kalpers *et al* (2003); g. Gray *et al* (2009); h. Gray *et al* (2013b); i. This survey

[*]For 2000, 2010, and 2016, the percent multimale groups and percent immature are calculated from the monitored groups only

[**]For 2010, the minimum count of unmonitored genotypes (n=106) plus known monitored gorillas (n=352) equated to a minimum of 458 individuals; the value here incorporates correction factors that add 16 potentially undetected infants and 6 samples that could not be genotyped

[†]does not include the four groups found only by Ranger Based Monitoring, for which only the number of nests was observed

[††]minimum count

Pathogens

Of the 155 specimens preserved in RNA-Later, 21 samples were determined to have good quality RNA based on CytB or ß-actin PCR. All 155 fecal samples were tested for coronaviruses, influenza viruses, filoviruses and paramyxoviruses and none were positive by PCR. Additional viral screenings of fecal samples collected in DR Congo and Uganda have not yet been conducted.

Results of eggs per gram feces counts for strongylids and tapeworms are pending.

Select Mammals

We mapped the spatial distribution of encounter rates (observations/km-walked) for the following types of select mammal signs: sightings of buffalo; sightings, fresh or recent dung, and tracks of elephants; sightings or calls of golden or blue monkeys; and all carnivore signs (Figures 5 & 6). Distributions of species were similar between sweeps 1 and 2. For comparison to past surveys, we summarized those types of mammal observations that were also collected in 2003 and 2010 (Table 8). We included the chimpanzee (*Pan troglodytes*) dung detected in the current study because we believe that if such a sign had been detected in previous studies, the researchers would have reported it. This single dung was found below chimpanzee nests observed in the trees and could be reliably distinguished from gorilla dung. However, it should be noted that some of the participants in the current and previous censuses had never worked in locations containing chimpanzees, so they may not have been able to reliably identify chimpanzee feces. The 2010 spatial distributions of select mammals may be compared to those recorded solely in the second (2016) sweep, as these survey efforts were both conducted between March and May. However, the 2010 and 2016 distributions were summarized at different spatial scales, such that the 2016 maps are more spatially explicit than

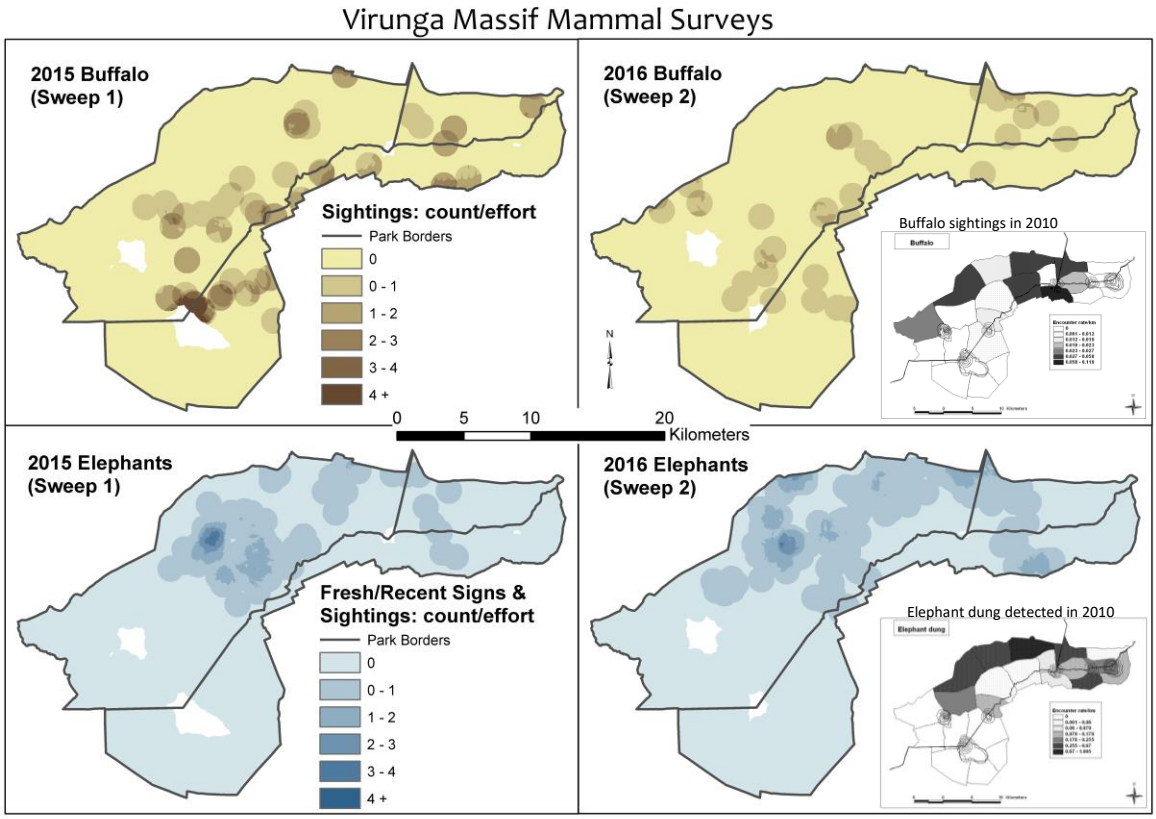

Figure 5. Distribution of buffalo and elephants in Sweeps 1 & 2 of the Virunga 2015-2016 mammal surveys
**Inlay map of elephant dung in 2010 reproduced from Gray et al 2013a*

the 2010 inlays. Yet a visual comparison demonstrates that the saddle between Mts. Karisimbi and Visoke continues to harbor buffalo, as does MGNP, whereas fewer buffalo were observed in the northern portion of Mikeno Sector than in 2010. Notably, no buffalo signs were found south of Mts. Karisimbi or Mikeno in either 2010 or 2016.

Importantly, elephant distributions from 2010 and 2016 are not truly comparable because the 2016 (Figure 5, Sweep 2) map included tracks and sightings in addition to fresh and recent dung, whereas the 2010 map (Figure 5, inlay) portrayed only dung, yet included all ages (including old dung). Nonetheless, the elephant distribution appears relatively stable between 2010 and 2016, and data from the current and past surveys suggest that elephants do not use the southwestern region of the Massif. Consistent with past surveys, elephants were rarely actually seen; however, much more elephant dung was recorded in this survey than in previous efforts even after condensing identical observations within 30-m of each other to single observations. Otherwise, mammal encounter rates were similar between the 2015-2016 survey and previous surveys (Table 8). In order to focus the survey effort on gorilla detection and help teams move more efficiently through the forest, the 2015-2016 protocols did not record sightings of duikers, bushbucks, or bushpigs nor observations of buffalo dung and several types of illegal activities (e.g. human tracks/paths and collection of honey, water, or bamboo). By reducing the reporting burden (number and type of signs to record), observers may have more thoroughly documented separate elephant-dung observations.

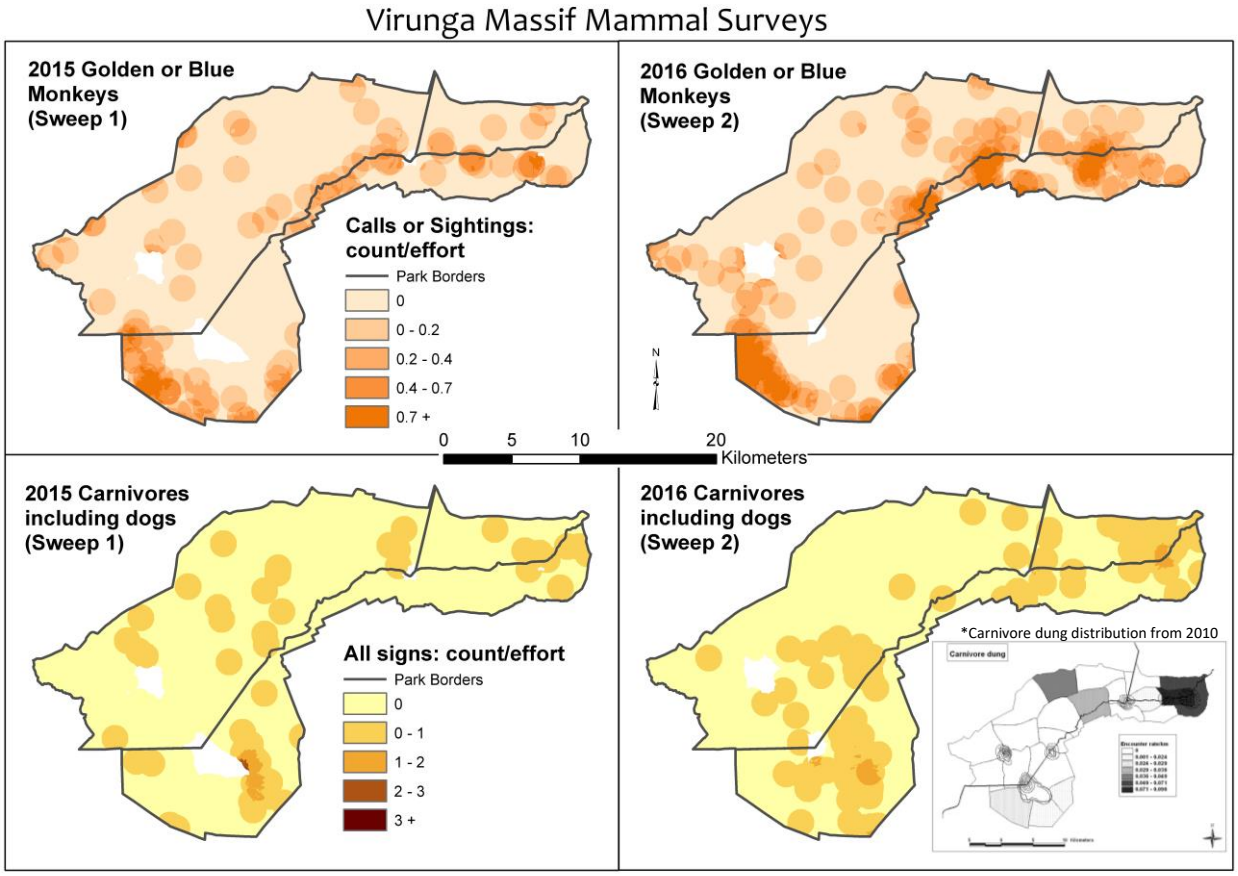

Figure 6. Distribution of monkeys and carnivores in Sweeps 1 & 2 of the Virunga 2015-2016 mammal surveys
**inlay map of carnivore dung in 2010 reproduced from Gray et al 2013a*

Table 8. Total number of encounters and encounter rates (observation/km-walked) of select mammals in 2003, 2010, and 2015-2016 surveys. Note that 2015-2016 was the first Virunga survey to incorporate two full sweeps of the study area, one each year, therefore the encounter rate indicated for each year equals number of encounters divided by km-walked for the respective sweep. Encounter rates do not reflect total counts of individuals seen, but rather, 1 or more occurrence per observation.

	2003		2010		2015 (Sweep 1)		2016 (Sweep 2)	
Large mammal observation / sign	**Total number of encounters**	**Encounter rate per km walked**	**Total number of encounters**	**Encounter rate per km walked**	**Total number of encounters**	**Encounter rate per km walked**	**Total number of encounters**	**Encounter rate per km walked**
Elephant dung	181	0.22	224	0.20	741	0.69	623	0.59
Carnivore dung	58	0.07	17	0.02	59	0.06	94	0.09
Golden or Blue monkey	46	0.06	28	0.02	55	0.05	87	0.08
Buffalo	27	0.03	21	0.02	45	0.04	32	0.03
Elephant	1	0.00	0	0.00	3	0.00	2	0.00

Carnivore signs were common in MGNP for both 2010 and 2016, however more carnivore signs were observed in the higher elevations around Mt. Mikeno in 2016 and fewer were recorded in the lowlands of Mikeno Sector than in 2010. No map of monkey detections was included in 2010, so we cannot compare their distribution to previous Virunga survey.

Illegal Activities

The map of all detected illegal activities (Figure 7) demonstrates that poaching activity continues in the Virunga Massif. Illegal activities were most prevalent in ViNP, followed by VNP, with very little illegal activity detected in MGNP. All signs deduced as feral dogs in the field were located in the southern-most portion of the Virunga Massif, near Karisimbi in VNP. A dead gorilla was found in a snare within an area of high density snares shown in Mikeno Sector, DRC (Figure 7).

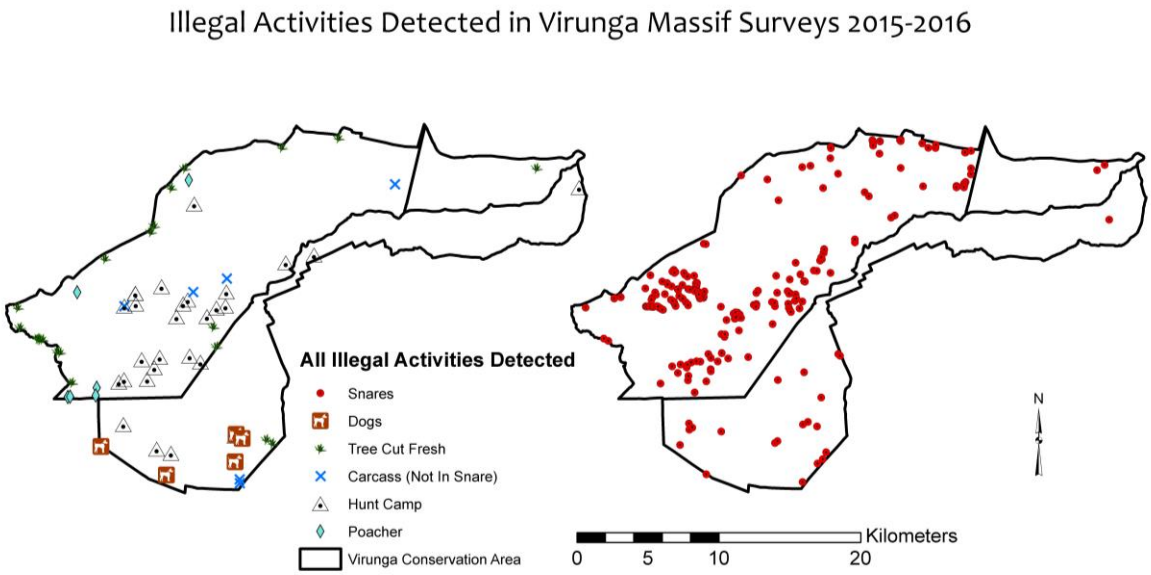

Figure 7. Distribution of all illegal activities, with snares shown on right, detected in both Sweeps of the Virunga 2015-2016

For comparison to past surveys, we summarized those types of illegal activities that were also collected in 2003 and 2010 (Table 9). We mapped observations of all poaching signs combined (poachers, snares, animals in snares, poached carcasses, hunting camps) as well as a count of snares (including snares with animals still caught in them) (Figure 8). The distribution of snares and all poaching activity were similar between sweeps 1 and 2, although intensity of poaching and snaring activity may have declined negligibly between sweep 1 and sweep 2 (from late 2015 to early 2016, respectively, Table 9). As with mammal distributions, the 2010 spatial distribution of illegal activities may be compared to those recorded solely in the second (2016) sweep, as they were both conducted between March and May. Such a comparison demonstrates that the vicinity of Mts. Mikeno, Karisimbi, and Visoke continue to be problem areas for poaching. In contrast, fewer poaching activities were recorded in the far eastern portion of the Massif. Additionally, more poaching activities were documented in the northern portion of Mikeno Sector in 2015-2016 than in 2010. However, since animals move and poaching activity is also spatially and temporally dynamic, surveys such as these – which pass through any given area very quickly – can only provide approximate distributions because they are essentially snapshots in time rather than comprehensive accounts of occurrence of illegal activities in a given year.

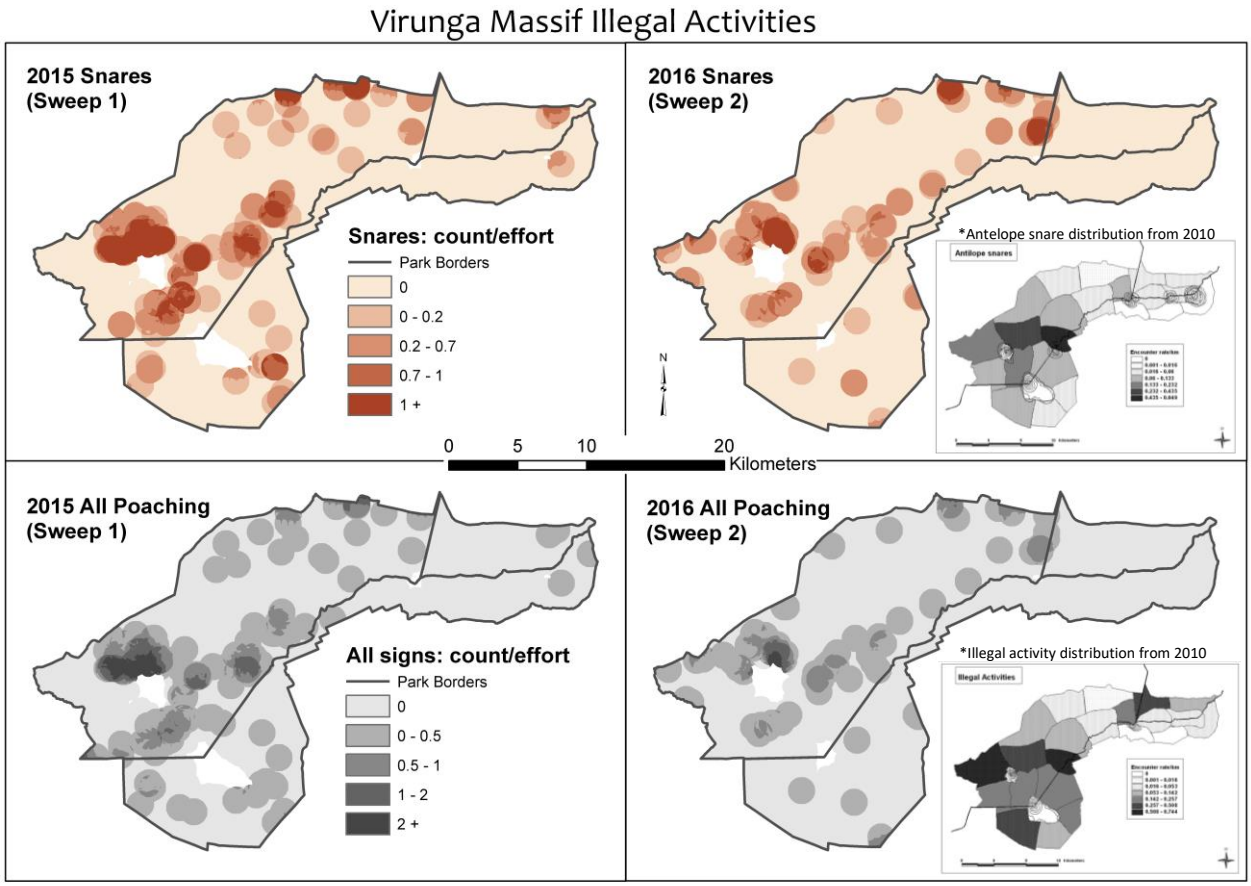

Figure 8. Distribution of snares and all poaching activity in Sweeps 1 & 2 of the Virunga 2015-2016 Surveys
**inlay maps of antelope snares and illegal activities in 2010 reproduced from Gray et al 2013a*

Table 9. Total number of encounters and encounter rates (observation/km-walked) of illegal activities in 2003, 2010, and 2015-2016 surveys. Note that 2015-2016 was the first Virunga survey to incorporate two full sweeps of the study area, therefore the encounter rate indicated for each year equals number of encounters divided by km-walked for the respective sweep. Encounter rates do not reflect total counts of signs seen, but rather, 1 or more occurrence of that sign type per observation.

	2003		2010		2015 (Sweep 1)		2016 (Sweep 2)	
Human sign	Total number of encounters	Encounter rate per km walked	Total number of encounters	Encounter rate per km walked	Total number of encounters	Encounter rate per km walked	Total number of encounters	Encounter rate per km walked
Snares	167	0.21	172	0.15	165	0.15	92	0.09
Wood cutting	39	0.05	35	0.03	14	0.01	7	0.01
Camps	20	0.03	12	0.01	23	0.02	5	0.00
Poachers	4	0.01	8	0.01	4	0.00	2	0.00
Dogs/carnivores	4	0.01	1	0.00	3	0.00	2	0.00

Discussion

Mountain Gorillas

The 2015-2016 survey of this subpopulation detected the largest number of gorillas ever recorded in the Virunga Massif. Importantly, any comparison of the abundances between 2010 and 2015-2016 should be done cautiously, because search effort in 2015-2016 was nearly double that of the 2010 surveys and increased effort conveys a concomitant increased opportunity to find more gorillas. That said, the entire subpopulation in the Virunga Massif comprised a minimum of 604 gorillas as of June 2016, which is a notable increase from the minimum count – without correction factors – of 458 gorillas documented in 2010 (Gray *et al* 2013). The greater number of groups found in 2015-2016 (n = 41) compared to 2010 (n = 36) is likely due to a combination of group fissions, solitary males acquiring females, and the detection of 1 additional unmonitored group. Despite methodological differences between the 2010 and 2015-2016, we can make some comparisons of the growth rate estimates by considering the unmonitored and monitored subsets separately.

Considering only the unmonitored gorillas, the increased number of detected individuals (from 106 in 2010 to 186 in 2015-2016) was likely caused by a combination of three factors. First, the increase may reflect intrinsic growth in abundance for this subset of gorillas. Second, it reflects more dispersal by gorillas from monitored to unmonitored groups (39 transfers from monitored to unmonitored versus 2 transfers from unmonitored to monitored). Third, the increase may reflect better detection because we conducted two sweeps instead of one. The estimated annual rate of change ranging from -2.0% to 5.1% in the number of unmonitored gorillas widely encompasses the estimated mean annual increase of 0.9% between the 2003 and 2010 surveys (Gray *et al* 2013) and represents the uncertain combination of increased detection, net movement from monitored groups, and intrinsic growth, which we will investigate in a forthcoming publication (Granjon *et al* in prep). If we compare 2010 results to only those of one or the other sweep of 2015 or 2016, respectively, then the estimated annual rate of change was -2.0% or -1.4%, respectively. Only when we compare the number of unmonitored gorillas detected in the single sweep of 2010 to the total minimum count of unmonitored gorillas detected in both sweeps of 2015 and 2016 (with approximately double the detection of one sweep alone) does the estimated growth rate appear to

be 5.1% annually. Taken together, these disparate values demonstrate the range of uncertainty around the annual rate of change (e.g. -2.0% to +5.1%) when detection probability changes between survey efforts and suggest that most of the apparent increase was due to increased detection, rather than births far exceeding deaths.

The results of both sweeps combined show that, as in the BINP census in 2011 (Roy *et al* 2014), some unmonitored gorilla groups were detected in only one sweep. That said, the sampling effort in the field was similar for any single sweep such that we can assume that a similar proportion of groups and individuals were detected in the single sweep of 2010 as in each individual sweep of 2015 and 2016, and all three sweeps can thus be compared to each other. We found 130 and 134 unique unmonitored gorillas in the 2015 and 2016 sweeps, respectively, whereas 106 were found in 2010, although much of this increase seems to be attributed to the net movement out of monitored groups. Interestingly, the number of unmonitored groups seems to have remained relatively stable, with 12, 11 and 9 groups found in 2010, 2015 and 2016, respectively, and 13 groups found in 2015-2016 combined. The detected groups were larger in 2015-2016 than in 2010, which explains the increased number of gorillas found in a similar number of groups.

It is important to track the potential differences in mean annual growth rate, and at the same time recognize the potential limitations of this comparative analysis. Although we cannot conclusively state that the unmonitored subset is experiencing intrinsic growth (versus simply an increased detection of unmonitored gorillas), we are able to accurately calculate the growth rate of the monitored subset from the known number of monitored gorillas. Taken as a whole, the monitored subset continued increasing over the past six years, from 352 to 418 habituated gorillas, reflecting a mean annual growth rate of 4.4%. This is slightly less than the 4.7% mean annual growth rate of the monitored groups between the 2003 and 2010 population surveys. Figures from recent surveys suggest high variance in growth rates among monitored gorillas, and investigations into the nuances of various demographic rates are an area of active inquiry (e.g. Caillaud *et al* 2014).

Continued efforts should be made to accurately record all births, deaths, and dispersal events into and out of the monitored groups, as the database on these gorillas provides a relatively easy method for evaluating population dynamics of a large percentage (approximately 69%) of the Virunga mountain gorilla subpopulation. Moreover, such growth estimates could be calculated more frequently than the interval between population surveys of the entire Virunga Massif.

In addition to estimating the abundance of the entire subpopulation, the periodic Massif-wide surveys enable us to assess the spatial distribution of gorillas over time. For example, throughout the 1980-1990s no groups were detected in the area south of Mt. Karisimbi or west of Mt. Mikeno, and in 2003 only one group was found there (Gray *et al* 2013b). Whereas in 2010, a few groups were found in that same area and in 2015-2016 even more gorilla groups were detected there, thereby suggesting an expansion of the gorilla distribution into available habitat. Very few gorilla groups were detected east of Mt. Sabyinyo in 2010 (n=4) or in 2015-2016 (n=5), suggesting that either the area may provide lower quality habitat for gorillas than other parts of the Massif or that historically heavy poaching in that location had a lasting impact on current abundance. As the subpopulation continues to increase, and gorillas continue to occupy previously unused areas, future research should involve assessing changes in food availability for the gorillas (e.g. Grueter *et al* 2013) to ascertain whether the subpopulation is approaching carrying capacity. McNeilage (1995) estimated the carrying capacity of the Virunga Massif as at least 600 mountain gorillas. Interestingly, the

present study's total minimum count of 604 gorillas has now reached that projection. Recognizing that estimating carrying capacity is complex, we recommend a re-evaluation of this estimate with current land-cover data, including the forthcoming land-cover classification that will be a by-product of this survey effort. Certain areas, such as the vicinity around Mt. Visoke, already show increased densities of gorillas and number of intergroup encounters (Caillaud *et al* 2014).

Overall, the Virunga Massif subpopulation of mountain gorillas appears to have increased over the last six years and, taken as a whole, the entire subpopulation (monitored plus the detected number of unmonitored individuals) increased at an annual rate of 4.7% during that same time period partly reflecting increased survey effort through two sweeps in 2015 and 2016. These survey results further demonstrate the efficacy of conservation measures taken in the last 40 years to protect mountain gorillas. Such measures include: regular monitoring of health, status, and number of habituated gorillas; *in situ* veterinary treatment of snared gorillas; coordinated patrols throughout the mountain gorilla range by park staff; and removal of snares found during said patrols and during full Massif-wide surveys such as that described in this report. Even in remote areas of the Massif that are less accessible for "extreme" conservation actions, the unmonitored gorillas appear to be benefiting, to some extent, from the combined conservation measures occurring in all three countries.

It is important to recognize that habituation of great apes for tourism and/or research purposes comes with potential risks that should be assessed, mitigated, and monitored to the fullest extent. The process of habituation is, itself, stressful to mountain gorillas (Butynski and Kalina 1998) and, once habituated, individual gorillas are both more vulnerable to human attacks (Williamson and Fawcett 2008) and more likely to encroach cultivated fields. Furthermore, due to genetic relatedness, gorillas are susceptible to human-borne diseases and human pathogens have caused disease outbreaks in mountain gorillas, including fatal infections caused by human respiratory viruses (Palacios *et al* 2011) and a measles virus outbreak (Hastings *et al* 1991). Logically, the risk of mountain gorillas contracting human-borne diseases increases with increasing exposure to humans. Importantly, in Rwanda and Uganda, tourists and researchers now visit the same gorilla groups, whereas in the past, groups were visited in any given day by either tourists or researchers, but not both. The Section on Great Apes (SGA) of the IUCN SSC Primate Specialist Group has documented best practice guidelines on great ape tourism and health monitoring (Gilardi *et al* 2015, Macfie and Williamson 2010) related to potential impacts and risks, that continue to serve as a reference documents.

Pathogen Analysis

As would be expected of feces presumably collected from healthy individuals, the fecal samples were not positive for select viruses. These findings were not surprising, given that we were screening for relatively rare viruses and given PREDICT's overall results in testing thousands of wildlife specimens from around the world, which have generally resulted in PCR-positive results for a very small fraction of samples (<1%) (PREDICT 2014). It is important to note that negative viral findings in no way imply that gorillas were free of bacterial, parasitic or fungal pathogens.

However, the majority of samples may have been of insufficient quality to detect RNA viruses. This may have been due to the challenges of maintaining the cold chain in the field (viral nucleic acid is fragile and does not remain intact during long periods at above-freezing temperatures) and/or low sample quality may have been due to over-dilution of fecal samples in RNALater™ (feces upon

receipt at UCD OHI were in 15 ml of RNALater™, instead of 1 ml) which may have inhibited PCR assays. These potential issues are among the considerations influencing plans to conduct further screening of Uganda or DRC samples.

Of note, the quality of the fecal samples for RNA viral family screening likely did not affect the utility and quality of the samples for use for host identification using DNA extracted from these samples, as DNA is more stable and host DNA should be abundant in these samples.

Select Mammals

A chief motivation for monitoring species over the long-term is to ascertain their relative status; that is, are they still present and are signs of their presence dwindling, steady, or increasing? However, as several previous studies have highlighted (Gibbs 2000, Anderson 2001), numbers of indirect signs such as tracks or dung are not reliable measures of abundance, particularly without reliable estimates of dung-production and dung-decay rates (Barnes 2001, Laing *et al* 2003) or when effort varies between surveys. In addition to potential bias introduced via inconsistent survey effort, observer ability, or performance, can also introduce bias since some observers may be able to detect more signs than other observers (Fitzpatrick *et al* 2009). Therefore, we interpret the mammal survey results reported here as an indication of species occurrence and do not try to glean trends by comparing encounter rates observed here to past surveys.

Presence-absence interpretations suggest that elephants and buffalos have been absent from the southwestern portion of the Virunga Massif since at least 2003. As mentioned previously, we caution that the protocols in the present survey involved a lower reporting burden than the 2003 and 2010 surveys, therefore the encounter rate of signs such as elephant dung may appear increased simply due to changed protocols. However, encounter rates of the other large mammals showed remarkably little difference since 2010, at least hinting that the increase in elephant dung encounter rate may represent an increase in the number of elephants in the Virunga Massif. Such speculation would have to be verified with a separate non-invasive mark-recapture study.

In fact, if population abundance estimates are desired for species other than mountain gorillas, then future work will need to focus on either mark-recapture (of genotypes, unique markings, or actual tagged animals; Seber 1982, Barnes 2001) or distance approaches (Plumptre 2000; Buckland *et al* 2005), depending on the species. All these approaches take considerably more time in the field than recording dung observations and would slow the process of the primary objective – to detect mountain gorilla nest sites and collect fecal samples with a sufficiently short time interval between sampling occasions (sweeps) to consider the subpopulation closed (negligible births or deaths). As such, we recommend independent projects to ascertain, for example, elephant-dung production and decay rates, or to conduct double sampling to calibrate dung counts into reliable estimates of elephant abundance in the Virunga Massif (Anderson 2001, Laing *et al* 2003). Furthermore, since duikers are the target species for poachers, it would be useful to devise methods to assess their population dynamics without impeding other survey work.

Two species of interest that we did not anticipate observing were detected and should be added to future survey protocols: chimpanzees and blue monkeys.

Illegal Activities

As in previous similar efforts, the thorough sweep approach of these surveys provided benefits beyond finding gorilla nest sites, and in fact allowed us to detect illegal activities in remote areas of the Virunga Massif that are rarely patrolled by law enforcement. Whereas monitoring of illegal activities through daily ranger-based-monitoring is opportunistic, the Massif-wide survey provides a more systematic and comprehensive snapshot of the occurrence of illegal activities in the transboundary area.

The very few detections of illegal activities in the far eastern portion of the Massif are noteworthy. Most of that area is under the jurisdiction of MGNP, which is the smallest park in Uganda. MGNP law enforcement staff are therefore comparatively higher density there than in any other park in the country, and likely higher density than in other parts of the Massif. According to the management plan at the time of the 2015-2016 surveys, MGNP had 46 law enforcement staff, making every ranger responsible for patrolling only 0.7 sq. km. Additionally, the presence of many tourism-related activities well dispersed throughout the Park – mountain hiking, nature walks, the Batwa trail, golden monkey and gorilla trekking – may further deter illegal activities within MGNP. Moreover, Zone 2, which covers one-third of MGNP and stretches the length of the northern boundary of MGNP, was formerly under human habitation and intensively cultivated until 1992. That zone has not fully regenerated, and its relatively open vegetation provides an additional deterrent to people who might otherwise engage in illegal activities, as they can be easily spotted by park staff from several vantage points within the zone. Finally, ample investment in rain-water harvesting technologies in the villages adjacent to MGNP, resulted in piped water reaching second- and, in some cases, third-tier parishes from MGNP. Therefore, there may be less motivation for the local communities to enter MGNP on the pretext of collecting water, except during the long dry period. Nevertheless, we suggest caution when interpreting the apparent low number of illegal activities in the far eastern portion of the Massif, as some teams were rushed there due to circumstances outside their control, as that region marked the end of each sweep of fieldwork. Particularly during sweep 2, both observer fatigue and time pressure from survey organizers factored into what was recorded. We recommend that future surveys avoid applying undue time pressure to field teams and avoid the use of “mobile teams” which covered ground very quickly compared to the pace of the km-walked in the remainder of the Massif.

Across the entire Massif, snare encounter rates did not differ notably from 2010 to 2015-2016, indicating little or no reduction in poaching activity despite impressive conservation efforts during that period. The presence of snares and other illegal activities documented throughout the Massif, most particularly in the southern and western portions, demonstrates that increased law enforcement and new techniques to detect illegal activities should be explored to further reduce both poaching within the parks and the risk of gorillas being snared. The status quo is insufficient to stop bushmeat hunting and other illegal activities in the Virunga Massif. Further, future socio-economic surveys of bushmeat consumption among communities neighboring the Virunga Massif could help elucidate some of the causal relationships behind patterns of illegal activities documented in this report.

The conservation community would do well to increase incentives designed to further reduce the dependence of local peoples on park resources. We witnessed numerous community members hiking into the Parks to fetch water with apparently no intent to extract any other resources. While most of these water collectors intend no harm (e.g. to wildlife or vegetation), any human entry into

the mountain gorilla range increases the potential risk of zoonotic disease transfer – a hazard to both humans and gorillas (Gategeko *et al* 2017) – not to mention the risk of human injury should they encounter dangerous wildlife, such as buffalo. Moreover, the presence of water collectors exacerbates challenges faced by law enforcement, as park staff rarely, if ever, penalize a water collector for entering the park, thereby leading some community members to enter the parks under the auspices of water collection and then set snares or commit other illegal acts.

Future Works

Ultimately, several end products will arise from this single collaborative effort. Vegetation-type data have already informed an initial broad-scale land-cover classification (WWF-Germany and IGCP 2017). As those land-cover classification products become available, they will inform numerous future studies related to habitat for local species, land-cover change, landscape planning, and population viability analyses. Likewise, location data of the select mammal species included in these surveys, in combination with the new land-cover data, will allow the development of species distribution models (SDMs), as well as explorations of ecological relationships among various plants, animals, and abiotic factors such as precipitation, soil type, slope, and aspect, to name a few. IGCP plans to be forthcoming with such products (e.g. niche models) in 2019.

Regarding research on viral diseases potentially affecting mountain gorillas in the Virunga Massif, previous studies of fecal samples collected from this subpopulation focused on viruses with a double stranded DNA (dsDNA) genome (in particular polyomaviruses and herpesviruses), with the aim to understand (i) the evolution of these viruses in the hominine lineage and (ii) their potential involvement in mountain gorilla diseases. Notably, Gilardi *et al* (2014) isolated human herpes simplex virus 1 (HHSV-1) DNA from oral lesions from a small number of captive orphaned mountain and Grauer's gorillas. Subsequent population-wide surveys of wild mountain gorillas for DNA evidence for herpesvirus infections using saliva extracted from dropped forage revealed that the population was HHSV-1 free, but that other endemic herpesviruses (e.g. gorilla lymphocryptovirus) were common (Evans *et al* 2016). Future analyses of fecal samples from the 2015-2016 census will allow us to investigate viruses infecting Virunga mountain gorillas and to refine the overall understanding of the biology of these viruses, including their persistence and potential spread within and among gorilla groups.

To further determine how much of the apparent increased abundance of unmonitored gorillas reflects intrinsic growth of the subpopulation versus improved detection, we propose to re-analyze results from recent Virunga population surveys (e.g. 2003 and 2010) by adjusting for imperfect detection through a population viability assessment process. Such adjustments will allow us to re-assess historic abundance estimates with those from 2015-2016 in order to evaluate growth rates holistically with a more consistent set of assumptions across surveys. Because the 2010 surveys included genotyping of fecal samples (with 106 unmonitored gorillas identified through genotypes), we are in a unique position to track individual unmonitored gorillas from one survey to the next (Granjon *et al* in prep).

Furthermore, for those genotypes detected in both 2010 and 2015-2016, we plan to determine group membership changes and – to some extent – the fate of groups found in 2010 (e.g. group formations, fissions, and disintegrations). For example, if all or most members of a 2010 group were distributed across several groups in 2015-2016, it would indicate dissolution of a group. Conversely, if we find individuals from separate 2010 groups combined in a single 2015-2016 group, it would

indicate a group formation. Likewise, group fission and group stability can be determined in a similar manner, as well as transfers of single individuals (e.g. adult females) from one group to another. Evaluating group dynamics in this way will thus allow a fine-scale investigation of the population dynamics and growth of the unmonitored subset.

Finally, we plan to apply a non-invasive genetic capture-recapture approach to the data from this study to produce a peer-reviewed scientific publication with an overall abundance estimate for the Virunga subpopulation of mountain gorillas that accounts for imperfect detection.

Conclusions

The 2015-2016 survey detected the largest number of mountain gorillas ever recorded in the Virunga Massif – 604. Combined with the estimated abundance, including correction factors for undetected infants, of 400 gorillas in BINP in 2011 (Roy *et al* 2014), an estimated 1,004 mountain gorillas existed in the wild as of June 2016.

The results, even taking into account the increased effort for this most recent survey, represent a remarkable conservation achievement in light of the fact that the estimate was substantially lower only decades ago and other subspecies of great apes have recently experienced rapid declines (Campbell *et al* 2008, Walsh *et al* 2003, Plumptre *et al* 2016). While exercising caution due to the limitations of the study, there were no indications of population declines since 2010 for the select mammals surveyed, including elephants.

Nonetheless, protected area authorities and conservation groups cannot be complacent as the global mountain gorilla population is still vulnerable to a potential rapid decline due to factors such as its small size, limited available habitat, climate change, human dependency on park resources, other human-wildlife conflicts, and the risk of disease due to frequent contact with humans. Additionally, it appears that the density of snares in the Virunga Massif has not declined since 2010, suggesting that additional efforts need to be made to reduce poaching, because snares remain a notable threat to gorillas. Further efforts should also be made to address the possibility that this mountain gorilla subpopulation is approaching carrying capacity, with an assessment of both biophysical- and social- carrying capacities (Daily and Ehrlich 1992). The apparent increase in mountain gorillas inhabiting the Virunga Massif is a testament to the effectiveness of conservation policies and strategies in the region, notably veterinary interventions, daily protection, and regulated tourism for the monitored gorillas, as well as intensive law enforcement, community conservation projects, and transboundary collaboration that benefit all gorillas.

Recommendations

Based on the results and conclusions from this survey, the following recommendations are offered:

1) A population viability assessment (PVA) should be completed, discussed, and disseminated.
2) Socio-economic surveys of bushmeat hunting and consumption among communities neighboring the Virunga Massif should be conducted.
3) Transboundary law-enforcement monitoring and anti-poaching efforts, including regional meetings as well as joint and coordinated patrols, should be re-established in the Virunga Massif.
4) Increased efforts to provide better protection to the unmonitored gorillas should be made, in particular patrols in the areas inhabited by unmonitored gorillas.

5) Continued investments – data collection, data management, and data sharing – in the gorilla demography database for monitored gorillas should be made by the three Range States.
6) Routine collection of fecal samples for DNA analysis in areas used by unmonitored gorillas is recommended in order to achieve more regular tracking of population dynamics.
7) Population dynamics and the growth rate of the monitored mountain gorillas should be evaluated across the transboundary Virunga Massif at more regular intervals (suggested every 3 years).
8) Given the high percentage of mountain gorillas currently habituated, caution should be used – through careful assessment and collective decision-making – related to habituation of new groups or individuals.

Acknowledgements

The Virunga Massif 2015-2016 surveys of mountain gorillas, select mammals, and illegal activities were conducted by the protected area authorities in the Democratic Republic of Congo, Rwanda, and Uganda: L'Institut Congolais pour la Conservation de la Nature, the Rwanda Development Board, and the Uganda Wildlife Authority, under the transboundary framework of the Greater Virunga Transboundary Collaboration (GVTC). The surveys were supported by the Executive Secretariat of GVTC, the International Gorilla Conservation Programme (a coalition of Fauna & Flora International and WWF), the Max Planck Institute for Evolutionary Anthropology, The Dian Fossey Gorilla Fund, the Institute of Tropical Forest Conservation, Gorilla Doctors, and the North Carolina Zoo. The population survey was funded by Fauna & Flora International, WWF, and Partners in Conservation at the Columbus Zoo & Aquarium. Additional financial support to the census science committee was provided by Berggorilla & Regenwald Direkthilfe.

We would like to thank the immigration, customs, and security agencies in the three countries for their cooperation and assistance. Special thanks also to Prosper Uwingeli, Emmanuel de Merode, Innocent Mburanumwe, Pontious Ezuma, Christopher Masaba, Fidele Ruzigandekwe, Richard Bergl, Badru Mugerwa, Mike Cranfield, Méthode Bahizi, James Byamukama, Robert Bitariho, Dennis Babaasa, Anna Behm Masozera, Benjamin Mugabukomeye, Altor Musema, Stephen Asuma, Eustrate Uzabaho, Rosy Kabeya, Emmanuel Ntivuguruzwa, Jerome Baguma, Jean Diogene Komezusenge, Jean Jacques Ngenzi, Wilbur Kaiire, and André Nzasebera for their cooperation and assistance.

We would also like to thank Gorilla Doctors for use of laboratory facilities in Rwanda and Uganda, Charles Kayijamahe, Gaspard Nzayisenga, and Méthode Bahizi for organizing all the fecal samples, and anyone else who participated in any way in this survey effort.

We thank Andrew Robbins for his assistance with organizing the databases for the monitored gorillas and calculating the growth rate for the monitored subset.

This effort would not have been possible without the arduous work and dedication of all the participants, through many long, wet days in steep, rugged terrain. We are extremely grateful to all the team leaders and team members: Akantorana Moses, Arinaitwe Joseph, Bakebwa Ismael, Bakeka Felicien, Bigirimana Boniface, Bikezi Jean Pierre, Bimenyimana Theoneste, Bitegetsimana Chimanga Augustin, Bizagwira Emmanuel, Busenga Nzabonimpa Vianney, Byaruganga Alfred, Byaruhanga Charles, Byaruhanga Christopher, Faida Emmanuel, Hakiza Serafin, Hakizimana Emmanuel, Hakizimana Emmanuel, Hakizimana J Damascene, Hakizimana JMV, Hakizimana Simpeze, Harerimana Marc, Hatangimana Ignace, Ildephonse Gatete, Irakoze Emmanuel, Iyamuremye Sekabende, Izabayo Birusha Justin, Kakuze Esther, Kasereka Kivuya David, Katsala Paluku, Katutu Jacques, Kawaida Ntagene Innocent, Kazerezi Barigomwa Martin, Kendero Raymond Nestor, Kimbembe Samuel, Kwiraviwe Paluku, Magoma Richard, Majoro Emmanuel, Maniragaba Bosco,

Manzi Olivier Jean Leonce, Matabaro Anselme*, Maurice Ngiramahoro, Mazambi Mutimanwa Victor, Mbabazi Simon, Mbusa Kahavo Julien, Modeste Twongere, Mucunguzi Geoffrey, Mugiraneza Leonard, Muhindo Safari François, Mukiza John, Mumbere Kisokolo Augsutin, Munguiko Kashamba Dieu est Là, Munyaruyenzi Patrice, Munyemanzi Felicien, Musekura Habimana Theo, Mutabazi Emmanuel, Mwanaki Tsongo Diddy, Nahabwe Job, Ndabereye Jerome, Ndaganje Faustin, Ndagijimana Byabuze Augustin, Ndarifite Iyamuremye John, Ndarubyariye Semivumbi, Ndayisaba Pierre, Ndayisenga Jean De Dieu, Ndikubwimana Guheka, Ndikubwimana Jean Damascene, Nibishaka Faustin, Niyikiza Biranda Isaac, Nizeyimana Fidele, Nkurunziza Jean Damascene, Nsababera James, Nshizirungu Baudin, Nyirandayambaje Gudula, Nzayisenga Gaspard, Nzingo Jonas, Okiria Alex, Okwir Okello Ricky, Ouma Owiny Amos, Phocas Nkunzingoma, Rubasha Daniel, Rwagasore Sebushari, Safari Gabriel, Seruhara Serutoke, Sindayigaya Nyagahinga Jean De Dieu, Tafazali Kaola Jérémie, Tindimwebwa Vastine, Tumugabirwe Lawrence, Tumwesigye Bernard, Turyananuka Beda, Tushabomwe Elias, Tuyisunge Jean Nepomuscene, Uketwengu Penjonga Pierre, Uzabaho Eustrate, Uzabakiriho Bernard and all other camp keepers.

**We express a solemn dedication to Anselme Matabaro of the ICCN – a cherished friend and colleague – who was fatally wounded in the line of duty, protecting wildlife against poachers, after this survey was completed.*

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