Research Article

Full Title: High mortality associated with tapeworm parasitism in geladas (*Theropithecus gelada*) in the Simien Mountains National Park, Ethiopia[1].

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High fitness effects of parasitism in geladas

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Highlights

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- Parasitism is associated with high mortality and low reproductive success in wild geladas.
- Infection with the larvae of the tapeworm *Taenia serialis* significantly decreases survival in females.
- Offspring of infected females have lower survival due to maternal death.

ABSTRACT

Despite increasing appreciation for parasitism as an important component of primate ecology and evolution, surprisingly few studies have demonstrated the costs of helminth parasitism in primates. Detecting parasite-related costs in primates is particularly difficult because it requires detailed, long-term data on individual host reproductive success, survival, and parasitism. The identification of the larval tapeworm *Taenia serialis* in geladas under intensive long-term study in the Ethiopian Highlands [Schneider-Crease *et al.* 2013, Nguyen *et al.* 2015] provides an opportunity to examine how an endemic parasite impacts host reproductive success and survival. We used survival analyses to assess the mortality risk associated with protuberant larval cysts characteristic of *T. serialis* using a decade of data from a gelada population in the Simien Mountains National Park (SMNP), Ethiopia. We demonstrated strikingly high mortality associated with *T. serialis* cysts in adult females, particularly for younger adults. The estimated effect of cysts on male mortality was similar, although the effect was not statistically significant, likely owing to the smaller sample size. Additionally, the offspring of mothers with cysts experienced increased mortality, which was driven almost entirely by maternal death. Mothers with cysts had such high mortality that they rarely completed an interbirth interval. Comparison with a study of this parasite in another gelada population on the Guassa Plateau [Nguyen *et al.* 2015] revealed lower cyst prevalence in the SMNP and similar cyst-associated mortality. However, many more females with cysts completed interbirth intervals at Guassa than in the SMNP, suggesting that *T. serialis* cysts may kill hosts more rapidly in the SMNP. Our results point toward the underlying causes of individual and population-level heterogeneity in *T. serialis*-associated mortality as important areas for future research.

KEYWORDS

Cestodes, coenurosis, infectious disease, primates, parasite ecology

INTRODUCTION

Parasites are increasingly appreciated as drivers of wildlife ecology and evolution [Grenfell & Dobson 1995, Nunn & Altizer 2006]. Parasites may influence the reproductive success of their hosts [Anderson & May 1978, Tompkins *et al.* 2011] and the persistence of endangered species and populations [McCallum & Dobson 1995, Smith *et al.* 2006]. However, few studies have demonstrated fitness consequences of endemic parasites on wild primate hosts [Milton 1996, Keele *et al.* 2009]. This dearth can largely be attributed to the scarcity of detailed, long-term field data on primate life history and parasitism that would permit the detection of fitness costs [Kappeler & Watts 2012]. Additionally, the long evolutionary relationships between many parasites and hosts may result in selection against extreme fitness costs, both because parasites that rely on host survival and reproduction are under selection to temper their costs [Ewald 1983] and because hosts are under selection to mitigate such costs [Boots *et al.* 2009, Roy & Kirchner 2000, Wenk & Renz 2012].

For decades, geladas (*Theropithecus gelada*) inhabiting the Ethiopian Highlands have been observed with protuberant cysts characteristic of infection with the tapeworm *Taenia serialis* (Fig. 1), a parasite that exploits predator-prey relationships. *Taenia serialis* requires separate adult and larval stages that infect predator ("definitive") and prey ("intermediate") hosts to complete a single life cycle [Nagaty & Ezzat 1946, Meyer 1955]. Canid definitive hosts harbor adult tapeworms and deposit infective eggs in feces, while herbivorous intermediate hosts harbor intrasomatic asexually budding larvae (coenuri; sing., coenurus) that develop when herbivores ingest parasite eggs [Nagaty & Ezzat 1946, Meyer 1955]. Upon canid predation or

scavenging of infected herbivores, the larvae develop into adult tapeworms in the canid gastrointestinal tract, and the cycle is perpetuated [Nagaty & Ezzat 1946, Meyer 1955] (Fig. 2). Wild-caught captive geladas have been described with protuberant cysts characteristic of the larval stage of *T. serialis* beginning in the early 20th century [Scott 1926, Schwartz 1926, Schwartz 1927, Urbain & Bullier 1935, Elek & Finkelstein 1939, Rodhain & Wanson 1954, Bertolino 1957, Clark 1969], and these cysts have been described in wild gelada populations for decades [Ohsawa 1978, Dunbar 1980]. The recent application of molecular techniques confirmed the diagnosis of *T. serialis* as the etiological agent behind the cysts [Schneider-Crease *et al.* 2013, Nguyen *et al.* 2015]. Herbivorous geladas are currently the only primate species known to be parasitized by the larval stage of *T. serialis*.

Figure 1. Female gelada exhibiting a protuberant cyst characteristic of *Taenia serialis* on the right pectoral region.

Figure 2. Hypothesized *Taenia serialis* life cycle in the gelada-canid system.

Taenia serialis can cause severe damage in its intermediate hosts, leading both to increased mortality and decreased reproductive success. Mortality associated with *T. serialis* cysts can result directly from physical damage to somatic tissue, with larvae causing muscle atrophy, spastic limb paralysis, and organ failure in captive geladas [Scott 1926, Urbain & Bullier 1935, Elek & Finkelstein 1939]. These pathologies can also indirectly increase mortality by enhancing the vulnerability of infected individuals to predation by definitive hosts (candidate definitive hosts include jackals (*Canis mesomelas*, *Canis aureus*), spotted hyenas (*Crocuta crocuta*), and Ethiopian wolves (*Canis simensis*)). Taeniid-induced reductions in reproductive

success can result from mechanical damage to the reproductive tract or from neuroendocrinological modulation leading to irregularities in or suppression of sexual behavior, estrous cycling, and placental maintenance [Esch 1967, Lin *et al.* 1990, Sciutto *et al.* 1991, Terrazas *et al.* 1994, Larralde *et al.* 1995, Morales-Montor *et al.* 2002, 2004, Gourbal & Gabrion 2004, Morales-Montor & Larralde 2005, Arteaga-Silva *et al.* 2009]. In males, taeniid infection is associated with drastic decreases in testosterone, sexual and aggressive behaviors, and, accordingly, dominance tenure length [Morales-Montor *et al.* 1999, Gourbal *et al.* 2002]. In females, taeniid infection is associated with the cessation of sexual behavior and damage to the reproductive system [Arteaga-Silva *et al.* 2009], both of which are likely to inhibit conception and thus lead to lengthened interval intervals. Perhaps most directly, *T. serialis* can impede offspring growth and survival through parasite-induced maternal death; infants are exceedingly vulnerable, and rarely survive without their mothers in the wild [Nowak *et al.* 2005].

In the first study of the consequences of *T. serialis* cysts in wild geladas, Nguyen *et al.* [2015] found that *T. serialis* cysts were associated with increased mortality and decreased reproductive success. The authors used likelihood ratio tests to compare mortality rates between individuals with and without cysts across 6 years in a population inhabiting the Guassa Plateau, Ethiopia, and found that a greater proportion of individuals with cysts died. The authors also used survival models to demonstrate that offspring of mothers with cysts had higher mortality than offspring of mothers without cysts. This result persisted even after the authors excluded infants that died along with their mothers, suggesting an indirect effect of maternal cysts on offspring survival.

In the present study, we investigated the prevalence and impact of *T. serialis* cysts on adult mortality and offspring survival in geladas inhabiting a distinct region of the Ethiopian

Highlands, the Simien Mountains National Park (SMNP). First, we report the prevalence of cysts among demographic categories (age and sex) in our study group. Second, we assess how *T. serialis* cysts impact adult survival, extending the approach used by Nguyen *et al.* [2015] by implementing survival analyses using estimated and known dates of birth for all adults. This allows us to more precisely quantify the relative risk associated with *T. serialis* cysts across the gelada lifespan. Third, we examine the consequences of cysts on reproductive success by comparing offspring survival and interbirth interval length between females with and without cysts. Following Nguyen *et al.* [2015], we compare the impact of maternal *T. serialis* cysts on offspring whose mothers perished and on offspring whose mothers survived. Fourth, we compare the prevalence of *T. serialis* cysts and patterns of associated mortality between the SMNP geladas to those reported by Nguyen *et al.* (2015) for geladas inhabiting the Guassa Plateau.

METHODS

Subjects and Study Site

We studied geladas in the Sankaber area of the SMNP (13.1833'N, 38.0667'E). All subjects derived from three bands [Snyder-Mackler *et al.* 2012] totaling 351 females and 336 males of all ages (387 adults and 300 immatures) in ~40 reproductive units observed over 10 years. The exact number of reproductive units in the study was variable due to the fusion, fission, or disappearance of units (exact sample sizes are indicated with each statistical test below).

Longitudinal Data Collection

Near-daily behavioral and demographic data were collected across 10 years (Jan 2006 - Dec 2015) by the Simien Mountains Gelada Research Project (SMGRP). Individuals born within

the study period have known birthdates (n = 383), while those born prior to the study or outside of study groups were assigned estimated birth dates according to maturational milestones and morphological traits [Barale *et al.* 2015, Roberts *et al.* 2017]. We use 4 years of age as the cutoff for "maturity", a broad age category that facilitates the analysis of the impact of *T. serialis* cysts in adult geladas while minimizing background rates of mortality related to infancy and juvenility. Study animals were recognizable based on corporeal idiosyncrasies such as injuries or skin and hair patterns, and are identified by researchers with near-perfect accuracy ensured by intensive trainings and frequent interobserver reliability evaluations.

Because geladas are female-philopatric [Dunbar 1984, le Roux *et al.* 2011], females that disappeared from their natal units were assumed to have died and assigned a date of death at the time of disappearance. By contrast, because males move between units and bands throughout their lives [Dunbar 1984, Pappano 2013], male deaths were more difficult to assign with certainty. We adopted the conservative approach to assigning male deaths taken by Nguyen *et al.* [2015]. In brief, male disappearances were only classified as deaths if the disappearance was accompanied by a major injury or an obvious decline in health, or if the individual was an infant (and thus dependent on his mother for nutrition).

Census data, including unit composition and health, were collected weekly. Changes in leader males, infant presence/absence, body condition, and emergence of *T. serialis* cysts were recorded on a near-daily basis. The close proximity of researchers to habituated study geladas (~1.5 m), along with minimum monthly requirements for data collection on individuals, ensured that UMGRP researchers were able to observe cysts within ~1 month of emergence. All analyses performed here use the presence of visible cysts characteristic of *T. serialis* infection, which may underestimate actual prevalence by ignoring infections that do not present externally.

Ethics Statement

All research was approved by the University Committee on the Use and Care of Animals at the University of Michigan (UCUCA #09554), the Duke University Institutional Animal Care and Use Committee (IACUC #A218-13-08), followed all laws and guidelines in Ethiopia, and adhered to the American Society of Primatologists' *Principles for the Ethical Treatment of Non Human Primates*.

Adult Mortality

We employed survival models to evaluate the impact of *T. serialis* cysts on adult gelada mortality because these models account for the timing of cyst appearance and death and are therefore sensitive to how long individuals survive after cysts appear. Additionally, survival analysis can incorporate data on censored individuals that only contribute to estimates of model parameters during the portion of the study for which they were observed. Our data are well-suited to survival analysis since we have fairly accurate ages at birth, first cyst appearance, and death for all study subjects, and many of our subjects were right-censored (i.e., individuals that were alive at the conclusion of the study or that disappeared before the end of the study and are not confirmed to be dead or alive). Survival analyses were performed in R [R Core Team 2015] with the package "survival" [Therneau 2015].

We used Cox proportional hazards models, which are flexible, robust, and do not require specification of a probability distribution for survival times. These models estimate the relative risk of death associated with a covariate (in this case, the presence of a *T. serialis* cyst) across the gelada lifespan. Because cysts can emerge at any age, individuals can contribute to the estimate of risk as both "non-cyst individuals" (pre-cyst emergence) and "cyst individuals" (post-cyst

emergence). Our Cox models took the form $h(t|X) = h_0(t) \exp(\beta_1 X_1(t))$, where $h(t|X)$ is the instantaneous hazard rate at time t; $h_0(t)$ is the baseline hazard rate; β_1 is the log hazard ratio associated with cysts; and $X_1(t)$ is 0 if $t < t_c$, where t_c is the time of first cyst appearance, and 1 if $t \geq t_c$.

Because male and female geladas are subject to different demographic processes that likely result in a biased sample of adult males being observed in a given study group (i.e., we are more likely to observe males that successfully immigrate into the study group and less likely to observe male deaths), we fit separate Cox models for adult males (n = 170, 92.4% right-censored) and females (n = 216, 72.2% right-censored), including the presence of one or more cysts (cyst = 1) as a time-dependent covariate [Cox 1972]. Because cysts rarely appeared in immature individuals, data were left-truncated at 4 years of age, excluding from the analysis any individuals that did not survive to at least 4 years of age. Thus, our models evaluate the impact of *T. serialis* cysts on the risk of death as a function of age for adults only. In addition, we right-truncated data at 24 years of age because beyond this age, small sample sizes and high age-related mortality resulted in poor model fit.

Proportional hazards models assume that model covariates have a constant, proportional (i.e., multiplicative) effect on the baseline risk of death. However, the proportional hazards assumption is violated when the effect of a covariate on the hazard rate changes over time. Using a scaled Schoenfeld residual test, we found that the proportional hazards assumption was violated in our model, and fitted extended Cox models of the form $h(t|X) = h_0(t) \exp(f_1(t)X_1(t))$. This differs from the Cox model in that the coefficient β_1 is replaced by a linear function of time, $f_1(t)$, allowing the effect of the covariate to change linearly through time.

Infant Mortality

To assess the impact of maternal cysts on offspring survival, we compared infant survivorship to 3 years of age among mothers with and without cysts using Kaplan-Meier survival curves and Cox proportional hazard models. We analyzed the full dataset (36 infants born to 27 cyst mothers, 339 born to 154 non-cyst mothers), as well as a subset of the data that excluded infants that died along with their mothers (leaving 25 infants born to 17 cyst mothers, and 325 born to 149 non-cyst mothers) to assess whether maternal mortality was the primary driver of infant death. Our Cox models took the form $h(t|X) = h_0(t) \exp(\beta_1 X_1)$, where $h(t|X)$ is the instantaneous hazard rate at time t; $h_0(t)$ is the baseline hazard rate; X_1 is 0 if the infant's mother had a cyst at the time of birth and 1 if not; and β_1 is the hazard ratio associated with maternal cysts. Scaled Schoenfeld residual tests revealed no violation of the proportional hazards assumption. To account for potential non-independence of infants born to the same mother, we fit Cox models that included a frailty term (i.e., a random effects term for mother ID) [Therneau, Grambsch & Pankratz 2003]. The frailty term has a multiplicative effect on the baseline hazard, and allows the offspring of different mothers to have different baseline hazard rates.

Interbirth Interval

Due to a surprisingly low sample size of 7 complete interbirth intervals (IBIs) observed for only 4 females with cysts over 10 years, we were unable to statistically analyze the impact of cysts on IBI. The majority of cyst females died before completing a single interbirth interval. Four of the 7 recorded IBIs came from a single female, and the remaining 3 IBIs came from 3 different females.

Comparison with Guassa

We compared the prevalence of *T. serialis* cysts and mortality rates of individuals with cysts between geladas in the SMNP and those studied by Nguyen *et al.* [2015] on the Guassa Plateau. We limited our data to the most recent 6.5 years to match the duration of the study of Nguyen *et al.* [2015] and performed G-tests for independent proportions for both mortality and prevalence analyses. We compared (1) cyst prevalence and (2) the proportion of deaths among cyst adults between the SMNP and Guassa populations. Our prevalence dataset contained 351 adult individuals (161 males, 190 females), and our mortality dataset contained 320 individuals (130 males, 190 females) after excluding individuals that could not be confidently coded as dead or alive at the end of the study.

RESULTS

Cyst Prevalence

Our dataset included 386 adult individuals: 170 males and 216 females. Over 10 years, 54 adults across 3 study bands exhibited cysts characteristic of *T. serialis*, producing a 14% period prevalence. Thirty-eight of 216 females exhibited cysts during the study period (17.6%), while 16 of 170 males exhibited cysts during the study period (9.4%). Only 2 of 407 immature individuals ever exhibited cysts (0.05%).

Adult Mortality

Survival analyses revealed reduced survival for adult males and females with cysts, although the effect was only significant for females, and demonstrated that younger adults with cysts have a higher risk of death than older adults with cysts (Table 1). The Cox proportional hazards model showed a positive effect of cysts on the hazard ratio for males and females (Table

1). Scaled Schoenfeld residuals tests revealed a significant deviation from the proportional hazard assumption for females but not males (Table 1), and plots of scaled Schoenfeld residuals against time indicated a clear negative relationship between time and the coefficient for cysts in males and females (Fig. 3). We therefore fit extended Cox models with time-varying coefficients for the cyst variable, specifying a linear relationship between time and the effect of cysts on the log hazard ratio. For females, this yielded a significant positive effect of cysts on the hazard ratio (Table 1, Fig. 4B) and a significant negative relationship between the effect of cysts and time (Table 1, Fig. 4B), indicating that cysts increase the risk of death for females and that the risk is the greatest for younger adults. A similar effect of cysts was estimated for males (Table 1, Fig. 4A) but was not statistically significant.

Figure 3: Scaled Schoenfeld residuals plotted against age for the cyst variable in Cox proportional hazards models for males (A) and females (B). Dashed lines represent confidence intervals. Deviations from a line with slope 0 indicate violations of the proportional hazard assumption.

Figure 4: Log hazard ratios for cyst presence over age, estimated from the extended Cox model with time-varying coefficients for males (A) and females (B). The thick solid turquoise lines represent the log hazard ratio, thin solid turquoise lines represent confidence intervals, light gray dotted lines represent 0 (i.e., no effect of cysts), and black dashed lines represent the constant log hazard ratio estimated from a standard Cox proportional hazard model. For females, the line representing the log hazard ratio has an intercept of 4.8, and a slope of -0.19 (reflecting the change in the log hazard per year as individuals age); for males, the log hazard ratio line has an

intercept of 3.85 and a slope of -0.27.

Table 1: Results of Cox models and scaled Schoenfeld residual tests. Coefficients and confidence intervals (CI) for the effects of cysts on hazard ratios for males and females in the standard and extended Cox proportional hazard models.

Infant Mortality and Interbirth Interval

Infants born to mothers with cysts experienced higher mortality than those born to mothers without cysts (Fig. 5A). The frailty term for mother ID was not significant ($p = 0.76$), indicating that maternal identity did not affect infant survival in this analysis; thus, we report results for a Cox proportional hazards model without a frailty term. Females varied in number of births during the study period (maximum: 7, median: 2). The model revealed that infants born to cyst mothers experience a ~2.5-fold increase in the hazard rate (hazard ratio = 2.49, CI = 1.47-4.23, $p < 0.001$). To assess whether maternal mortality drove patterns of infant mortality among offspring of mothers with cysts, we fit a Cox model to infant data that excluded offspring of mothers that died with their infants (4% of non-cyst mothers, 30.6% of cyst mothers). We found that maternal cysts no longer had a significant effect on infant survival (hazard ratio = 1.37, CI = 0.59-3.17, $p = 0.467$; Fig. 5B).

Low sample size precluded quantitative analysis of interbirth interval data. Four of the 7 IBIs to females with cysts came from a single female that possessed a small cyst on her chest for most of her adult life. Of the five infants born to this female while she had a cyst, only one survived to sexual maturity, three died, and one was still dependent at the end of the study. The other 3 complete IBIs were to 3 different females; one lost both infants at the start and end of the IBI, another died along with her second infant, and a third successfully weaned both offspring.

Figure 5: Kaplan-Meier survival curves for infants born to mothers with (dark blue) and without cysts (light blue). The estimated curves in plot (A) are for all infants, while the curves in plot (B) were estimated for data excluding infants that died along with their mothers. Hatch marks indicate right-censoring, and dashed lines represent confidence intervals.

Comparison with Guassa

We found lower prevalence of cysts in SMNP geladas compared to that reported for Guassa geladas by Nguyen *et al*. [2015]. Cyst prevalence among SMNP adults was 13% (45 of 351) compared to 30% at Guassa (50 of 167), while prevalence among SMNP immatures was 0.5% (2 of 407) compared to 3.8% at Guassa (7 of 181). Cyst prevalence in the SMNP was significantly lower among adult males (df = 1, $G = 13.16$, $p < 0.001$), adult females (df = 1, $G = 8.38$, $p = 0.004$), and immatures (df = 1, $G = 8.58$, $p = 0.003$). Adult mortality rates (proportion of individuals with cysts that died) were not significantly different between Guassa and the SMNP for males (df = 1, $G = 1.42$, $p = 0.233$) or females (df = 1, $G = 1.1$, $p = 0.294$).

DISCUSSION

Cysts characteristic of the tapeworm *Taenia serialis* were associated with increased mortality in female geladas in the Simien Mountains National Park (SMNP), Ethiopia. While this effect was statistically significant only for females, the similarity between the magnitude of the estimated effect and the age-dependent pattern in both sexes suggests that similar processes are occurring in both males and females. Offspring of mothers with cysts had significantly higher mortality, which was driven by high maternal mortality. In fact, maternal mortality was so high that females rarely survived the duration of an interbirth interval, precluding statistical analysis

of interbirth intervals. Comparisons of cyst prevalence and cyst-associated mortality in the gelada populations of the SMNP and Guassa revealed lower prevalence in the SMNP, but no parallel difference in mortality; however, the strength of mortality in females indicated by the lack of IBIs among cyst mothers suggests that SMNP females may succumb to infection more rapidly than Guassa females despite the lower prevalence. These results contribute to the sparse literature demonstrating explicit fitness costs of an endemic helminth parasite infection in a wild primate and expand the nascent body of research on *T. serialis* in wildlife.

Demographic Variation in Prevalence

Among adults, cyst prevalence over 10 years was 14%, with 18% prevalence in females and 9.4% prevalence in males. We refrained from statistically comparing prevalence rates between the sexes because males and females are subject to different demographic processes, which would likely result in an underestimation of prevalence among males. Natal males disperse at sexual maturation to roving all-male groups (AMGs), from which they challenge dominant males ("leaders")—generally in non-natal bands—for control of reproductive units [Dunbar 1984, Pappano 2013]. Following takeovers, deposed leaders can become subordinate "followers" in their units, rejoin AMGs, or join other bands [Dunbar 1984, Pappano 2013]. Thus, we only observe natal males until their dispersal and adult males during their limited tenures in study bands. Furthermore, as suggested by Nguyen *et al.* [2015], *T. serialis* infection likely precludes the ascension of infected males to dominance and increases the likelihood of disappearance, which would result in further underestimation of prevalence among males.

Over the same period, 14% of adults and ~0.5% of immatures exhibited *T. serialis* cysts. A similarly strong age bias was reported by Nguyen *et al*. [2015] for the Guassa geladas, and is consistent with evidence from other taeniid species that age is associated with higher infection

risk, that infection persists throughout the host's lifetime, and that hosts do not develop immunity with prolonged exposure [Gemmell *et al.* 1987, Torgerson *et al.* 1998].

Impact of Cysts on Mortality

The direct, extreme parasite-induced mortality demonstrated by our survival analyses is rarely observed in wild primates or other long-lived mammalian hosts [see Milton 1996, Keele *et al.* 2009, Nguyen *et al.* 2015 for notable exceptions]. The magnitude of the effect of cysts on mortality was similar for both males and females, although the effect was only statistically significant for females. Because of the similar effect size estimated for both sexes and the relatively small sample size for males, we interpret the lack of significance in the male analysis to reflect our small male sample size and not a true sex difference. We hypothesize that the high degree of host mortality in this system results from a parasitic life cycle that requires the demise of the intermediate host for its survival and reproduction. As in the intermediate stages of many tapeworm species, *T. serialis* must be ingested by the carnivorous definitive host to reach sexual maturity [Parker *et al.* 2015]. Thus, *T. serialis* and related tapeworms should be under selection to increase the probability that their intermediate hosts are consumed by predators or scavengers [Lafferty 1999, Parker *et al.* 2015]. This phenomenon, "parasite increased trophic transmission", may select for *T. serialis* to harm its intermediate host, promoting parasite survival through increased infection of predators and scavengers [Poulin 1994, Lafferty 1999, Lafferty & Kuris 2002, Choisy *et al.* 2003].

Indeed, geladas in our study population were observed with cysts that appeared to impede breathing, movement, and mating, and often became infected after bursting. Thus, *T. serialis* larvae appear to act similarly to those of other taeniid species, causing mechanical injury to muscular, visceral, neural, and connective tissue, physical impingement of nerves, and

obstruction of arterial and venous flow [Sharma & Chauhan 2006, Godara *et al.* 2011, Oryan *et al.* 2014]. Beyond directly precipitating death, these pathologies can thwart the predation-protection ostensibly offered by large social groups [van Schaik 1983, Janson & Goldsmith 1994] by hampering the ability of infected geladas to keep pace with their groups. Thus, the damage caused by *T. serialis* likely facilitates the transmission of larvae to the definitive host, either through scavenging of infected corpses or predation of lagging individuals.

Our survival analyses revealed an unexpected time-dependent relationship between age and mortality risk in adults, with the impact of cysts on mortality declining with age across adulthood. In other words, younger adult geladas with cysts faced higher mortality risk than older adult geladas with cysts. This effect, again only significant in females but with a similar trend observed in males, could be due to a time-dependent effect of cysts on mortality, in which earlier stages of infection are more virulent than later stages. This effect could alternatively be an artifact of unmodeled individual-level heterogeneity, in which individuals differ in their innate response to infection [Raberg, Graham & Read 2009], in immunocompetence as a function of age, or in the severity of their infections. If severity is related to cyst location, for example, individuals with cysts in more benign locations (e.g., subcutaneous connective tissue) will survive longer than individuals with cysts in more harmful locations (e.g., viscera). Unfortunately, our dataset is too small to explore these potential explanations, and future work should explore individual variation in *T. serialis*-associated mortality with fine-grained descriptive data on cysts and immunological measures.

Impact of Cysts on Reproductive Success

Consistent with evidence from experimental work showing taeniid-induced reductions in host reproductive success, offspring of female geladas with *T. serialis* cysts incurred a ~2.5-fold

increase in mortality. Many parasite infections demand energetic resources and immune activation at the cost of reproductive effort [Forbes 1993, Ilmonen *et al.* 2000, Hurd 2001], or incite immunological or endocrinological changes that interfere with implantation and pregnancy [Lin *et al.* 1990, Krishnan *et al.* 1999, Gourbal & Gabrion 2004, Arteaga-Silva *et al.* 2009]. In this system, however, cyst-associated declines in reproductive success take the shape of heightened offspring mortality for mothers with cysts, and this effect appears to be driven primarily by cyst-associated maternal death. When infants whose mothers perished were excluded from the analysis, no effect of maternal cysts was detected. Remarkably, mortality of cyst mothers in the SMNP was so high that we were unable to analyze the impact of cysts on interbirth interval (IBI). Over 10 years of data collected on this population, only 7 IBIs were observed for 31 mothers with cysts.

Comparison with Guassa

Prevalence of cysts in SMNP geladas was markedly lower than in Guassa geladas. Because the *T. serialis* life cycle involves multiple host species, it is expected to be sensitive to ecological shifts; indeed, the SMNP has historically experienced high levels of anthropogenic impact, while the Guassa Plateau has remained largely pristine and protected. The Guassa Plateau is named for its abundant Guassa grasses (*Festuca* spp.) that are home to large rodent and lagomorph populations [Ashenafi, Leader-Williams & Coulson 2012], which support large populations of candidate definitive host species (e.g., jackals, Ethiopian wolves). In contrast, land cultivation in the SMNP has eliminated Guassa grasses, thereby reducing rodent density [Ashenafi, Leader-Williams & Coulson 2012, Yihune & Bekele 2014]. Smaller rodent populations should lead to smaller predator populations, resulting in low definitive and

intermediate host prevalence in the SMNP. With fewer infected intermediate hosts, the infection rate in definitive hosts will be lower and fewer eggs will be shed into the environment. Thus, the impact of anthropogenic disturbance may explain the lower prevalence of *T. serialis* cysts in SMNP geladas compared to Guassa geladas.

Offspring mortality related to maternal *T. serialis* cysts was similar at the two sites, although the mechanisms driving this relationship appear to differ. Whereas Nguyen *et al.* [2015] found elevated mortality among offspring of cyst mothers even after excluding offspring that died with their mothers, we found no evidence for such indirect fitness costs despite observing a similar number of offspring of mothers with cysts (n = 27 at Guassa, n = 25 at the SMNP). Thus, high maternal mortality is the primary driver of reduced survival in the offspring of SMNP cyst mothers, while indirect effects of maternal cysts on offspring survival appear to play a more important role for Guassa geladas. One explanation for the divergence between the two sites is that cyst-associated mortality may be more rapid in the SMNP than at Guassa. Thus, the effect and frequency of maternal mortality in the SMNP geladas may be too strong to detect alternate mechanisms of offspring mortality that might be at play. Future work should look to parasite-related decreases in milk quality and lactation persistence as candidate mechanisms driving infant mortality not related to maternal mortality in the Guassa population [Lopes *et al.* 2016].

G-tests did not reveal a significant difference in cyst-associated mortality rates, measured as proportions of individuals with cysts that died, between SMNP and Guassa geladas. However, this limited comparison does not incorporate information on the timing of death relative to cyst emergence, nor does it include data from individuals that could not be confidently coded as dead or alive at the conclusion of the study, a common occurrence in studies of wild primates. Thus, this comparison should be interpreted with caution, and future research should use survival

analyses with data from both sites to obtain a more nuanced and accurate comparison of cyst-associated mortality. Moreover, based on the vast difference in the number of IBIs observed among females with cysts between the two sites, we suspect that geladas with cysts in the SMNP die more quickly than their counterparts on the Guassa Plateau. While only 7 IBIs were recorded among 31 females with cysts in the SMNP over 10 years, with most females dying before the completion of a single IBI, 27 IBIs were recorded among 31 females with cysts over 6.5 years in the Guassa population. This suggests that females with cysts in the SMNP may die, on average, faster than females with cysts in Guassa. This pattern could arise through a number of mechanisms, including the evolution of infection tolerance [Roy & Kirchner 2000, Raberg, Graham & Read 2009, Rohr, Raffel & Hall 2010, Hayward *et al.* 2014] in the Guassa geladas, hormonally-modulated immunosuppression induced by increased anthropogenic stressors [Lafferty & Holt 2003] in the SMNP, comorbidity with other parasites [Pullan & Brooker 2008], or the presence of a more lethal strain of *T. serialis* in the SMNP. However, additional analyses must be conducted in order to directly compare mortality rates between the two populations.

Future Directions

The presence of *T. serialis* cysts in geladas under intensive study at two sites in the Ethiopian Highlands with drastically different degrees of anthropogenic habitat change provides an exciting opportunity to evaluate the impact of habitat disturbance on an ecologically complex parasite. Further work should quantify anthropogenic habitat change at each site, describe intermediate and definitive host identity and infection prevalence, and assess environmental *T. serialis* risk. Furthermore, external cysts may not be the only manifestation of *T. serialis* infection, and research is underway to non-invasively diagnose infections that do not present externally and thus to facilitate a more complete study of *T. serialis* prevalence and associated

mortality.

DATA AVAILABILITY

Data and R source code are available on GitHub (https://github.com/rgriff23/Gelada_parasites).

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Table 1

Cox model	Cyst coefficient	95% CI	Time-transform coefficient	95% CI	Schoenfeld residual test *p*-value
Standard- Males	0.6	-0.81, 2	NA	NA	$p = 0.18$
Standard- Females	1.75 ***	1.8, 2.33	NA	NA	$p < 0.001$ ***
Extended- Males	3.85	-0.18, 7.87	-0.27	-0.63, 0.07	NA
Extended- Females	4.8 ***	3.24, 6.34	-0.19 ***	-0.28, -0.1	NA

Figure 1

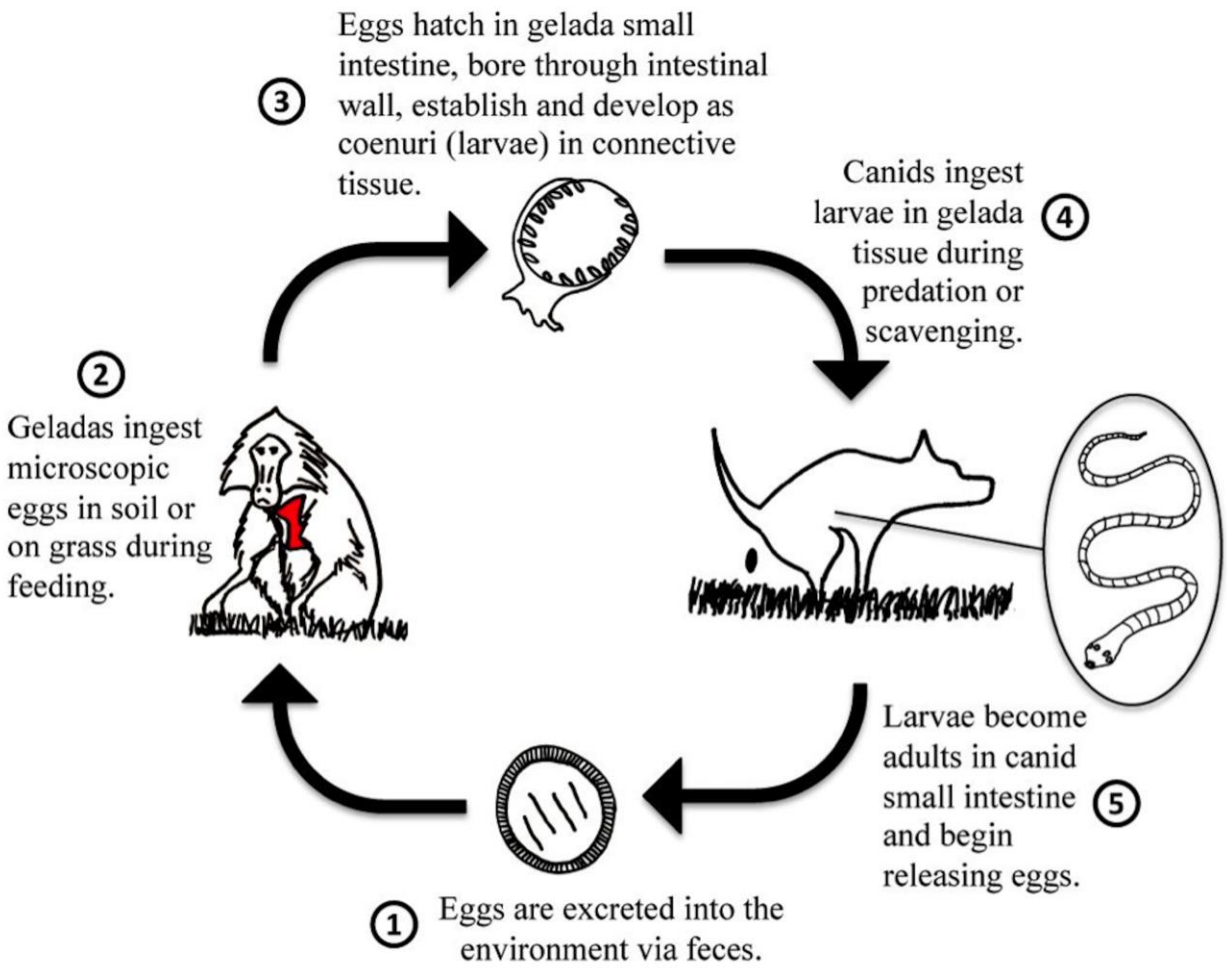

Figure 2

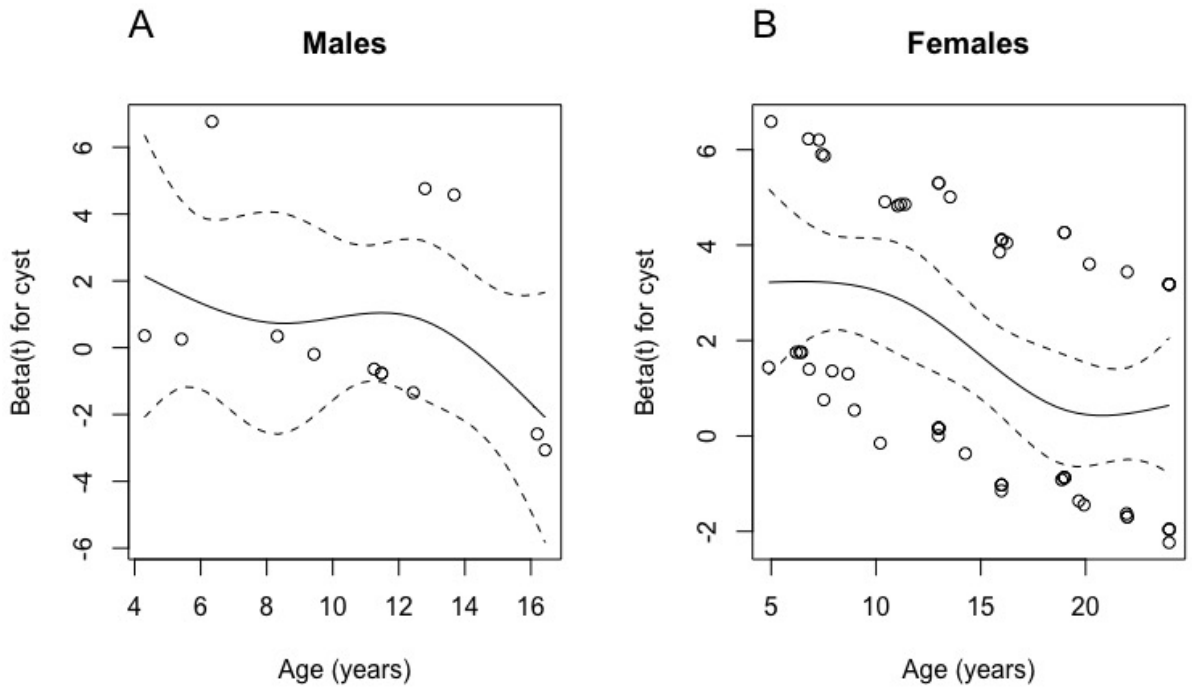

Figure 3

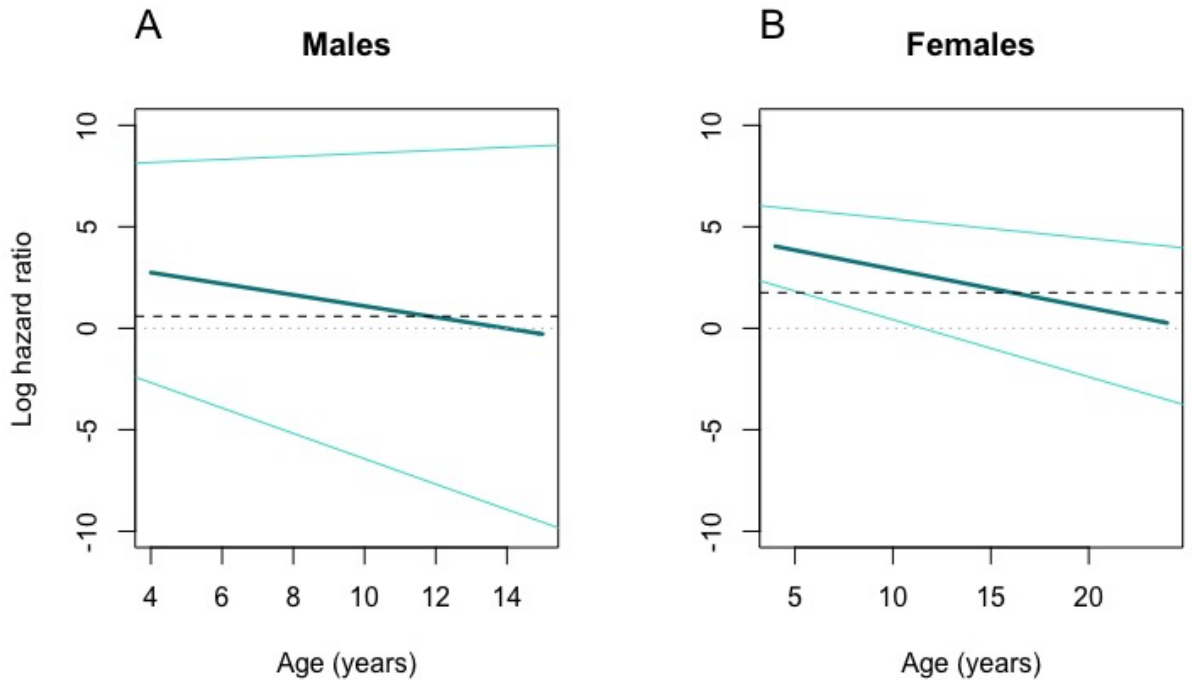

Figure 4

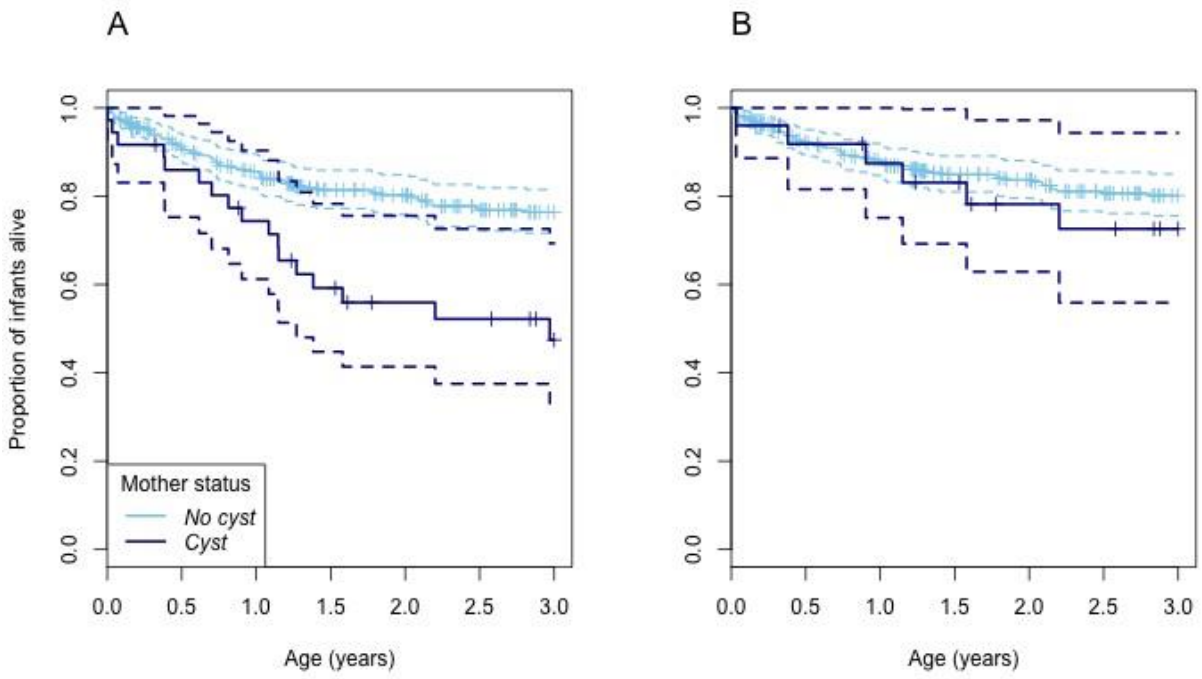

Figure 5

Graphical abstract