**Reversing the negative spiral: a Dynamic Energy Budget approach**

Gastrointestinal (GI) helminths infect over a quarter of the human population[1] and the costs of helminth infection are similar to HIV-AIDS, in terms of years lost to disability or premature death[2]. Helminth infection can also affect susceptibility to and severity of other major human pathogens (e.g., malaria, HIV, tuberculosis), as well as the reduce the efficacy of vaccination and treatment options[3,4]. Yet, comparably few resources are devoted to understanding the basic biology and treatment of helminths[3]. Frequent mass-administration of anthelmintics raises concerns regarding development of drug resistance, particularly given the small number of available anthelmintic drugs and widespread emergence of resistance in helminths of livestock[5]. New management solutions that maximize health while reducing reliance on anthelmintics could prolong the lifespan of these valuable drugs. **Nutrition is a key axis of health with the potential to promote better management of helminth infections, but for which greater understanding is needed.**

Helminth infection and malnutrition follow a similar geographic distribution and frequently co-occur within the same individuals. This overlap is not coincidental. Both processes can work synergistically, creating **a "negative spiral" in which helminth infection exacerbates malnutrition, and vice versa,** to the detriment of host health[6]. Furthermore, some GI helminths actively immunosuppress their hosts[7], enhancing the vicious cycle of susceptibility and malnutrition. **The consequences of the negative spiral of helminth infection and malnutrition are profound** and include immunosuppression, severe anemia, diarrhea, growth stunting, irreparable cognitive impairment, and risk of mortality[2,8–11]. **The persistence and widespread prevalence of helminth infections in human populations[1] attests to the difficulty of both preventing the onset of pathological synergy and breaking out of an established negative spiral[3].** While both improved nutrition and anthelmintic treatment offer potential benefits to infected individuals, they are often insufficient for breaking this cycle[3]. Biomedical scientists, livestock physiologists, and disease ecologists have approached the negative spiral from different perspectives based upon their differing priorities (e.g. detailed descriptions of immune mechanisms, maximizing production efficiency, and understanding population-level disease dynamics, respectively). Interplay between these disciplines, though rare, can reveal new biological insights relevant for human health.

Reinfection with GI helminths is common and anthelmintic drug treatments typically provide only temporary parasite clearance and short-term gains in host growth[6]. Nutritional supplementation offers the potential to enable individuals to fight off current and future infections, but may not be sufficient to improve health outcomes in helminth-infected individuals[12]. Providing additional resources often enable livestock to tolerate helminth infections without declines in health, but also without corresponding decreases in helminth intensity[13]. **Indeed, in my own work on a mouse model of GI helminth infection, I found evidence for tolerance of infection (i.e. lower anti-helminth immune defenses, higher parasite loads) in high resource conditions compared to moderate protein restriction**[14]. Such a pattern, if borne out in human or other populations under food supplementation, could be problematic for reducing parasite transmission. While combined approaches of nutritional supplementation and anthelminthic treatment have proven effective[15], they remain rare and the type, duration, and magnitude of supplementation necessary to maximize health gains is unclear.

The interrelationships among resources, immune responses and parasites are complex. The distribution of resources to immune function versus those stolen by parasites will impact individual health by influencing infection severity and duration. Thus, **to understand the effects of resources on GI helminth load and host health, it is critical to understand the prioritization and competition for resources within hosts.** From the parasite's perspective, well-nourished individuals may provide superior habitats for replication, but at the same time represent better-defended territories. In addition to directly stealing resources, GI helminths often alter nutrient absorption and reduce appetite[6,16]. Frequently, resource-limited individuals are less able to fight parasites and are more susceptible to new infections[6,17] because immune responses depend upon resource availability[18,19]. For example, vaccine-induced antibody responses increase metabolic rate by 15-30% in humans and mice[16,20–22]. Furthermore, as the total pool of available resources decreases, tradeoffs among competing physiological demands will grow stronger. But, because they occur within the confines of a living host, interactions among resources, immunity, and parasites are exceedingly difficult to observe. **Fortunately, various ecological modeling approaches have provided insights into within-host interactions and improved understanding and treatment of human diseases**, such as the predicting the outcome of co-infections[23,24].

Infection with multiple helminth species is not only widespread in human populations, but often leads to greater health consequences than single-species infections[25–29]. For example, in a study of children in the Philippines, 78% of children were co-infected with at least two helminth types, and the odds of having anemia

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**Comment [1]:** The "greater understanding" of this clause technically only applies to nutrition (from the first clause), not the interaction between nutrition and helminth infection.

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**Comment [2]:** I'm not sure I follow the logic here: the first part of the sentence says that helminth infections are widespread, but how does it then follow from that fact that *pathological* synergy between malnutrition and infection is hard to prevent? Does the causality run the other way, e.g., "The potential for synergy between malnutrition and infection provides an explanation for the persistence and widespread prevalence of helminth infections."

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**Comment [3]:** These sentences seem a bit out of place, as the first sentences of the next paragraph seem to follow from the sentence preceding these sentences.

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**Comment [4]:** These sentences don't cohere. First you say that nutritional supplementation helps fight current and future infections; then you say that it doesn't necessarily improve health outcomes (why not?); but then you say it often does improve health outcomes by increasing tolerance.

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**Comment [5]:** Maybe this was your intent, but these sentences read like a list of possible interactions, but I kind of want them to lay out a story (e.g., I want each sentence to follow from the preceding one, laying out a complex story).

despite a low parasite load were nearly 5-fold higher amongst children co-infected with hookworms and *Trichuris trichura*[26,29]. These costs of co-infection typically manifest as anemia and wasting[25–29], suggesting that the compound effects of multiple infections may be due to depletion of host resources. The cause of the exacerbated consequences of helminth co-infection for individual energy balance are largely undescribed, but a survey of human infections showed that parasites interact most frequently via shared resources[30]. Similarly lacking are data on the effects of helminth co-infection on worm loads and transmission potential. Additionally, my dissertation research demonstrated that helminth species may respond in opposing manners to protein limitation[14]. **Given the substantial effects of helminth co-infection on human morbidity, the tight, yet complex, links between nutrition and helminth co-infection warrant further investigation**.

**I propose that a quantitative, mechanistic understanding of the interplay between an individual's energetics, immune responses, and parasite infection could be used to predict the consequences of malnutrition and to maximize success of nutritional supplementation and deworming programs**. Individual pieces of the complex negative spiral dynamics have been studied (i.e. the effects of protein malnutrition on immune function), but a theoretical framework is needed to integrate them. First applied to parasite infection in the 1980s, the classic energy budget approach is based on subtracting energy lost in feces, urine, and respiration from those ingested to estimate energy available for production (e.g. growth, reproduction; Fig 1A). By comparing budgets of infected and non-infected hosts, the net effects on energy available for production can be determined. However, the classic energy budget framework does not incorporate immunity, parasite load, or feedback among compartments in the model. Accordingly, it fails to capture the complex effects of nutrition on immune function and of immune responses on parasite infection. My training as an integrative ecologist has given me the tools to unite and build upon previous multidisciplinary advances by taking a new dynamic energy budget (DEB) approach (Fig 1B).

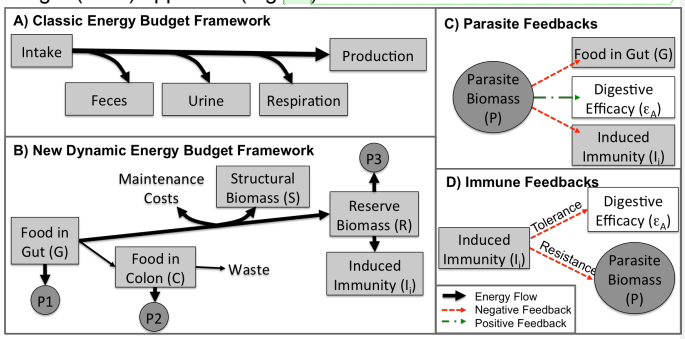


**Figure 1.** A) The classic energy budget framework posits that energy intake goes to feces, urine, respiration, or production. B) The proposed new dynamic energy budget (DEB) framework that showing three places where different types of parasites (P1, P2, P3) can steal host resources. The DEB also includes positive (green) and negative (red) feedbacks due to C) parasite load and D) resistance and tolerance induced immune responses.

### Specific Approach

With this project, I will develop and test DEB models of the helminth-malnutrition negative spiral. **I propose to pair mechanistic DEB models with an empirical resource manipulation experiment to track the distribution of within-host resources to parasites versus host tissues in a human disease model system.** I will focus on three critical questions:

**Q1. Where do hosts allocate nutrients and how/why does that distribution change with malnutrition and parasite infection?**

**Q2. What is the optimal treatment strategy to reverse the negative spiral and maximize health gains for hosts on a given nutritional plane?**

**Q3. How do optimal treatment strategies change in a multi-parasite context?**

First, I will use current knowledge of pairwise interactions among nutrition, immunity, and parasite infection to develop a plausible mechanistic energy budget framework. Next, I will use this framework to predict how changing aspects of the energy budget (e.g., ingestion rate, investment in induced immune defenses) will affect host health, immune responses, and parasite load. To test these predictions, I will use a resource manipulation experiment in a mouse model to quantify the outcomes of host resource allocation, but few of the within-host resources distributions themselves can be traced directly. However, by matching experimental data to the mathematical predictions, I will identify the 'best fit' mechanistic framework that describes the complex dynamics occurring within infected hosts.

It is easy to state that a model can inform treatment options, but too rarely are they experimentally validated. Using the 'best fit' model from Q1, I will predict the most effective treatment strategies (e.g. timing and amount of supplementation) to reverse the negative spiral and conduct a second experiment to test those hypotheses (Q2). Finally, I will use insights learned from Q1 and Q2 to predict and then test how to mitigate the

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**Comment [6]:** Why no P4 parasite? You discuss it later, so it probably should be here as well.

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**Comment [7]:** Awesome.

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**Comment [8]:** Not sure what you mean here.

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**Comment [9]:** At the beginning of this paragraph, you talk about developing a single mechanistic energy budget framework; now you are talking about evaluating among competing frameworks. Based on what you have written below for this objective, I think what you mean is that you will attempt to parameterize the general model for the specific system using the data generated by the experiment. That is not the same as comparing different models (which you would be doing if, e.g., you parameterized the models implied by Fig. 1A and Fig. 1B and then compared the fits of each model to the data). Of course, it might be the case that we will need to modify the model structure in order to capture the observed patterns in the experimental data. However, that is not really being discussed in the proposal (you only mention a single model), and would add significantly to the complexity of the model description to talk about plausible alternative model structures.

consequences of helminth co-infection (Q3). **This novel approach will unite experimental, theoretical, and practical methodologies to advance understanding of how to reverse synergistic effects of helminth infection and malnutrition.** Moreover, this approach can easily be adapted by other scientists to understand similar parasitic (co-)infections and to predict the ability of disease control strategies to improve human health.

**Q1. Where do hosts allocate nutrients and how/why does that distribution change with malnutrition and parasite infection?**

*Q1A. DEB model of helminth infection*

Dynamic energy budget (DEB) theory uses simple, quantitative mechanistic rules and allometric scaling (i.e. body size relationships) for the intake and use of energy by living organisms[31]. Over thirty years of theoretical and empirical studies demonstrate the accuracy, utility, and flexibility of this modeling approach[31–34]. In particular, DEBs provide a framework to quantify the complex interactions among resources, hosts, and parasites[35–38], but to date host-parasite DEBs have been limited to invertebrate hosts and simple representations of immune function. For example, a set of DEB models were developed by Cressler et al. to predict microbial abundance and immune responses of an invertebrate host under conditions of differing resource overlap and prioritization[35]. Their model shows that if parasite energy needs take precedence, parasite load will increase with intake rate (Fig 2A), but if resources preferentially go to immune responses, parasite load will peak at low intake rates (Fig 2B)[35]. The implications of these different outcomes are great; high resource conditions will reduce infection under immune priority, but increase infection under pathogen priority. **DEB models offer a robust way to determine the outcomes of complex biological interactions and, in particular, offer enormous potential for improving understanding within-host dynamics of vertebrate hosts. I will build a novel DEB model characterizing helminth infection in a vertebrate host to explore the implications of interactions among host nutrition, immune function, and parasite load for individual health.**

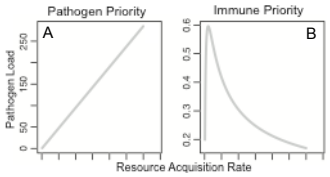


**Figure 2.** Resource prioritization to different components can strongly influence pathogen load (Adapted from [35])

Dr. Cressler is now a formal collaborator on the project and together we have recently parameterized a working DEB model that fits the biology of a mouse in program R. Our model includes aspects of mouse physiology (e.g. asymptotic growth, demand-driven feeding), parasite-load dependent feedback on food intake and digestive efficiency (Fig 1C), innate and adaptive immune defenses, and both tolerance and resistance feedbacks (Fig 1D). As observed in our preliminary study (Fig. 3), the model allows mice to increase feeding in response to infection, up to a physiological limit (also informed by our preliminary ingestion data). Resistance involves energetically expensive defenses (e.g. lymphocyte proliferation, antibody production) to reduce parasite burden. Tolerance involves maintaining health despite a given parasite burden, but also requires energy (e.g., repairing GI tract damage)[39,40]. These processes are not mutually exclusive; resource supplementation often enhances both resistance and tolerance of hosts[13]. **Distinguishing between resistance and tolerance responses is critical because of their potentially opposing effects on host health, resource dynamics and parasite loads.** Our DEB model includes this crucial dichotomy and allows us to determine the optimal defense strategy for particular parasites and nutritional conditions. **[insert example graph (Fig 4) showing protein vs host condition with three lines for no immune, resistance, and tolerance].**

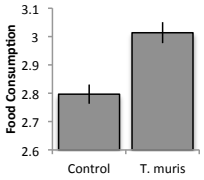


**Figure 3.** Feeding (g/day) was higher *T. muris* infected C57BL/6J mice than controls ($p = 0.004$).

We depict resource flow through this DEB model using a set of differential equations describing the connections among within-host components (Box 1). Different parasite species and life-stages are able to steal host resources at different places along the digestion pathway (P1: before host nutrient absorption, P2: nutrients not absorbed by the host, P3: after host have transformed resources into tissues, P4: host energy stores). This flexibility allows us to tailor the model to specific parasite species such at *Trichuris* species nematodes, which feed on ingesta in the colon (P2), and hookworms, which feed on cells of the intestinal lining or host blood (P3)[41]. To date, we have developed this novel DEB model for a mouse, parameterized it with data from our trial experiment and other published studies, and validated that it gives reasonable outputs for food consumption, growth, and mortality of an uninfected mouse. Currently we are beginning to add parasite infection, to modify allocation to resistance and tolerance feedbacks, and to test different resource priority frameworks. By modifying coefficients to reflect interaction strengths and the preferential distribution of resources to parasites, **I will predict the consequences for stored reserves, helminth biomass, and immune responses under different resource distribution scenarios.** Lastly, **I will modify resource intake**

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**Comment [10]:** Technically, it is energy, not biomass, which is the currency.

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**Comment [11]:** I'm not sure why my model would be considered a model of an invertebrate host.

**and predict how resource limitation affects host health (growth, survival) and parasite loads under different resource apportionment scenarios.**

**Box 1:** Dynamic energy budget model for a parasite-infected mouse. Space limitations precluded definition of all terms, so as an example, the formulation of the equations describing the dynamics of an induced immune response are described.

**Dynamics of:**

Food biomass in the gut
$$\frac{dG}{dt} = \frac{\iota_{\max} S^{2/3}}{1+\exp\left(\eta(R/S-\theta)\right)} - \rho G - \frac{\sigma_G P_1 G}{H_G + G},$$

Food biomass in the colon
$$\frac{dC}{dt} = \rho(1-\epsilon_A)G - \rho C - \frac{\sigma_C P_2 C}{H_C + C},$$

Structural biomass
$$\frac{dS}{dt} = 3\gamma(S)\left(\alpha^{1/3} L_\infty S^{2/3} - S\right) - \frac{\sigma_S P_3 S}{H_S + S},$$

Reserve biomass
$$\frac{dR}{dt} = \epsilon_R\left(\rho\epsilon_A G - M(S,R,I_i) - C_G(S)\right) - bR(P_1+P_2+P_3+P_4) - \frac{\sigma_R P_4 R}{H_R + R},$$

Induced immunity
$$\frac{dI_i}{dt} = \epsilon_i bRP - \mu_I I_i,$$

Mortality risk
$$\frac{d\mu}{dt} = \mu_{\min} + \mu_{accel}\max\left(\theta\frac{S}{R} - 1, 0\right),$$

Parasite biomass
$$\begin{aligned}
\frac{dP_1}{dt} &= \epsilon_P \frac{\sigma_G P_1 G}{H_G + G} - \mu_P P_1 - \mu_c I_c P_1 - \mu_i I_i P_1,\\
\frac{dP_2}{dt} &= \epsilon_P \frac{\sigma_C P_2 C}{H_C + C} - \mu_P P_2 - \mu_c I_c P_2 - \mu_i I_i P_2,\\
\frac{dP_3}{dt} &= \epsilon_P \frac{\sigma_S P_3 S}{H_S + S} - \mu_P P_3 - \mu_c I_c P_3 - \mu_i I_i P_3,\\
\frac{dP_4}{dt} &= \epsilon_P \frac{\sigma_R P_4 R}{H_R + R} - \mu_P P_4 - \mu_c I_c P_4 - \mu_i I_i P_4.
\end{aligned}$$

**Dynamics of an induced immune response ($I_i$)**

$$\frac{dI_i}{dt} = \epsilon_i bRP - \mu_I I_i.$$

In our model, $b$ is the rate of reserve biomass ($R$) that is allocated to the immune system per unit of parasite biomass ($P$), $\epsilon_i$ is the cost of converting reserve biomass into *effective* induced immune biomass, and $\mu_i$ is the background rate that immune cells are lost.

**Additional functions:**

Mass
$$M = m(S+R) + m_c I_c + m_i I_i,$$

Constitutive immunity
$$I_c = k(S+R).$$

Cost of growth of structural biomass
$$C_G(t) = \epsilon_G 3\gamma(R)\left(\alpha^{1/3} L_\infty S(t)^{2/3} - S(t)\right),$$

Growth rate in structure
$$\gamma(S) = \gamma_{\min} + \gamma_{\text{catchup}}\left(\frac{S_{\text{target}}(t)}{S(t)} - 1\right),$$

Target structural mass
$$S_{\text{target}}(t) = \alpha\left((L_b - L_\infty)e^{-\gamma t} + L_\infty\right)^3.$$

*Q1B. Empirical testing of DEB model predictions*

DEB models of infection are rarely coupled with empirical data on resource availability (but see [37]). Moreover, biomedical data on the negative spiral have never been compared to DEB model predictions. Yet, empirical data are necessary for testing the ability of the model to accurately capture and predict biological interactions. I will experimentally manipulate resource availability and concurrently monitor intake, body mass, reserve biomass, digestive efficiency, parasite biomass, and aspects of constitutive and induced immunity (Table 1) in a mouse model of helminth infection. The empirical parameters will be measured in biomass directly (e.g. food consumption, mouse, helminth, or spleen weight) or converted to biomass using standard conversions[31,42].

I will use **a factorial experimental design where helminth-infected and uninfected laboratory mice are fed one of five diets with varying protein content** (n = 9 mice per treatment, 90 mice total, of dose-dependent susceptible strain C57BL/6J). While recognizing that energy, protein, and micronutrient deficiencies can individually and jointly influence disease susceptibility and severity (see future directions), I chose to initially focus on protein because of its strong effects on host immune defenses[6,14,43–45]. The diets will have equivalent energy content. To reduce the significant effects of compensatory feeding (i.e. eating greater quantities of low quality diet to obtain desired protein levels), all mice will be kept on 80% *ad lib* food restriction. Young adult (6 wk old) female C57BL/6J mice will be randomly assigned to each of the five resource treatments (4-24% protein), acclimated to that diet for 2 wks, then half of the mice on each diet will be infected with a high dose of the GI nematode *Trichuris muris*. *T. muris* has long been a model system for studying human GI helminth infections and host immune responses to *T. muris* have been particularly well characterized (reviewed in [46]). A close relative of this worm, *Trichuris trichiura*, infects over one billion people, including up to 95% of children in many locations where malnutrition is also prevalent[47]. After ingestion, *T. muris* eggs take approximately 30 days to develop into reproductive adults and eggs are passed with host feces. In this mouse strain, moderate doses of *T. muris* (200+ eggs) generally lead to worm expulsion and protective antibody responses in well-fed mice[48–50], creating the possibility for decreased or delayed immunological protection in malnourished individuals.

A unique combination of established methods will be used to sufficiently and regularly monitor each component of the model (Fig 1B) for 60 days post-infection (Table 1). In addition to measurements listed below (Table 1), I will freeze feces for future investigation of interactions among resources, GI helminths, and the gut microbiome (see future directions). Finally, I will compare the experimental data to theoretical predictions from DEB model developed in Q1A. This will allow me to estimate the values for all of the parameters the DEB model, including those that cannot be directly measured. This fully parameterized model will be a powerful tool for studying Q2 and Q3. In particular, **the model will indicate the degree of resource overlap between immune responses and parasites and reveal which was able to preferentially capture limited resources.**

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**Comment [12]:** Do you *want* to limit compensatory feeding? If that is a significant tolerance mechanism….

Sarah Budischak 7/15/15 12:39 PM
**Comment [13]:** A – will IACUC easily approve this? If not, I think we can just take this feeding issue out. It will be something we continue to think about how to control/compensate for, but not something that will probably raise a red flag with the reviewers.

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**Table 1.** Empirical methods for assessing components of DEB model.

| Component | Measurement | Method | Schedule |
|---|---|---|---|
| **Intake ($\iota$)** | Food consumption | Food weight[51] | weekly |
| **Mass (M)** | Body weight | Balance | 48 hrs |
| **Reserves Biomass (R)** | Body fat content | Carcass fat[52] , serum leptin ELISA | at cull, weekly |
| **Digestive Efficiency ($\varepsilon_A$)** | Undigested protein | Fecal nitrogen[53] | weekly |
| **Parasite Biomass (P)** | # and biomass of adult helminths | Necropsy | at cull |
| **Constitutive Immunity ($I_c$)** | WBC count (uninfected) | Blood smear | weekly |
| | Spleen mass (uninfected) | Necropsy | at cull |
| **Induced Immunity ($I_i$)** | Elevation in WBC count (infected – uninfected) | Blood smear | weekly |
| | Elevation in spleen mass (infected – uninfected) | Necropsy | at cull |
| | *T. muris* specific antibodies | IgG ELISA[56] | weekly |

ELISA: Enzyme linked immunosorbent assay, WBC: white blood cell, IgG: Immunoglobulin G.

**Q2. What is the optimal treatment strategy to reverse the negative spiral and maximize health gains for hosts on a given nutritional plane?**

**Surprisingly, a combined approach including both supplementary feeding and anthelmintic treatment has rarely been applied in laboratory or human studies**, despite evidence that both anthelmintic treatment and supplementary feeding may be necessary to achieve positive health outcomes [15,57]. The empirical data and best-fit dynamic DEB model (Q1) can provide informed answers to critical questions for treating GI helminth infection. **For example, the relationship between resources and resistance responses (from plots such as Fig 2B) can be used to identify 'tipping points' where modifying resource quality may be most effective for reducing disease susceptibility.** Critically, under some mechanistic resource distribution scenarios, parasite replication is highest at intermediate or high resource levels [35]. Under those circumstances, I predict increasing host resources will, counterproductively, lead to higher parasite reproduction and disease transmission. **To maximize the benefits of resource supplementation, we first need to understand the conditions under which resources can enhance or supplant anthelmintic treatment by enhancing host immunity. My work will fill that gap.**

This factorial experiment will also test the effectiveness of resource supplementation prior and post-helminth infection to mimic potential intervention strategies in human populations. Resource supplementation prior to infection will likely increase host growth and stored resources, and the DEB model will predict whether those stored resources will be preferentially allocated to immune defenses (to the benefit of host health) or to fuel parasite replication (to the detriment of host health). Resource supplementation post-infection will not significantly improve individual health if the parasite has preferential access or reduces digestive efficiency, but will improve outcomes if those resources can be allocated to resistance or tolerance responses. **Accordingly, manipulating whether individuals receive high quality resources before and/or after infection will allow me to test whether parasites or induced immune responses have preferential access to stored reserves, ingested resources, or both.** For *T. muris* (hypothesized to be a parasite of ingested resources), the DEB model predicts… [insert graph(s) (Fig 5) showing predictions for pre/post feeding on host condition and/or parasite biomass].

Specifically, I will use the DEB model to predict (1) changes resource levels that will maximize gains in individual health and (2) how hosts and parasites will respond to resource supplementation under four conditions: no supplementation, and supplementation before, after, or throughout infection. These four resource treatments will be crossed with three helminth treatments: uninfected controls, *T. muris* infection, or *T. muris* infection followed by anthelmintic treatment (ivermectin). The infective dose will be selected to approximate *Trichuris trichura* burdens and chronicity observed in human populations. Nine mice per treatment combination (108 mice total) will be fed each diet for one month prior to infection and monitored for two months post infection. As above, mice will be assessed for food intake, digestive efficiency, stored reserves, immune responses, and parasite biomass (Table 1). Additionally, fecal egg counts will be used to monitor infection intensity every other day once the worms mature (30 days post infection). I will test for the individual and interactive effects of protein nutritional plane and helminth infection treatment on each component in the model using general linear models and compare these empirical results with DEB model predictions. **The results of this study will inform the efficacy of resource supplementation for enhancing individual health as well as the predictive power of our DEB model.**

**Q3. How do optimal treatment strategies change in a multi-parasite context?**

Sarah Budischak 7/15/15 12:36 PM
**Comment [14]:** I'll make sure this is all on the same page once the text is set.

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**Comment [15]:** "allocated" is probably not the right word here, as the host allocates resources to immune defenses, but not to fuel parasite replication.

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**Comment [16]:** Even if the parasite reduces digestive efficiency, resource supplementation can improve host health (by offering the possibility for compensatory feeding).

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**Comment [17]:** How is this different from the goal stated in the final sentence on page 4?

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**Comment [18]:** One thing that seems like it might be missing here is how you plan to include anthelminthic treatment in the DEB model. In particular, it might be interesting to use the DEB model to predict the optimal combination of nutrition and anthelminthic treatment to maximize health (or minimize transmission, or any other goal). We can discuss this in more detail if you want.

After empirical validation and refinement after Q1 and Q2, I will build in additional levels of complexity and realism to the DEB model, starting with multiple-species helminth infections. Co-infection with multiple helminth species predominate in human populations and impose greater health costs than single infections[25–29], even at low intensities[29]. The effects of helminth co-infection on individual health are typically manifest as wasting and anemia[25–29], suggesting that resources may play a key role the synergistic costs of poly-parasitic infections. *Trichuris*-hookworm co-infection is particularly detrimental to the health of children[29] and, as such, will be the focus of my first multi-parasite investigations.

To incorporate helminth co-infection in the DEB model, I will depict *Trichuris* as a parasite of ingested food (P2), whereas hookworms steal host structural biomass and will be represented as P3 parasites. The parasites will interact with each other via host resources and induced immune responses. Hookworm-infected hosts will be forced to repair tissue damage, leaving behind fewer resources for *Trichuris*. Although hookworms are 'upstream' of *Trichuris* in the intestinal tract, immune responses to *Trichuris* are energetically costly and can reduce digestive efficiency, both of which will reduce the amount of host structural biomass available to hookworms. Some resistance and tolerance immune responses will be effective against both worms (e.g. eosinophil up-regulation, intestinal tissue repair), while others are species-specific (e.g. antibody responses). Modifications to the species-specific induced-immunity killing rates in the DEB model will allow me to test outcomes for host condition, survival, and parasite load across a range immune cross-protection levels. These modifications to the DEB model will allow me to make predictions for the effects of infection order, cross-immunity and nutrition on individual health and parasite loads.

After identifying the conditions under which helminth co-infection may have the greatest impacts on individual health, I will again test those hypotheses in a mouse model. *Heligmosomoides bakeri* is the most commonly used murine proxy of human chronic hookworm infection[58], and a species I have experience working with[14]. The interactions among *T. muris*, *H. bakeri*, resources, and the host's immune system are too complex to predict *a prori*, illustrating the need for the DEB model predictions to identify the combinations that are most realistic, interesting, and relevant to improving health outcomes. Accordingly, the final experiential design will depend upon the DEB model identifying the most interesting combinations of infection order, resource levels, and supplementary resource timing to test experimentally.

**Conclusions and future directions:**

By building a DEB framework of parasite infection and validating it experimentally, I will be able to identify the most likely mechanistic pathways shaping outcomes of parasite infection and the effectiveness of treatment strategies. Not only will I use the validated DEB model to predict responses to intervention strategies during single and co-infections, but I will also experimentally test the health and disease transmission consequences of those treatments. **This novel approach will unite experimental, theoretical, and practical methodologies to advance understanding of how to break the negative spiral of helminth infection and malnutrition.** Critically, over a billion people suffer from helminth infection and the risk of anthelmintic resistance will remain a global threat until we better understand how to reverse the negative spiral[1,3].

Furthermore, the proposed project will serve as a concrete launching point for my future independent research career. First, this fellowship training will provide the empirical and mathematical tools to explore the effects of nutrition and helminth infection on additional co-infections, including with bacterial, viral, and protozoan parasites. In particular, the combination of my doctoral and post-doctoral training will leave me well positioned to investigate helminth-*Mycobacterium* co-infection in future studies. Second, throughout all proposed experiments, I will collect and store fecal samples for subsequent microbiome analysis. The importance of gut microbiome for human health and well-being is increasingly recognized [59]. Microbiota are influenced by diet, and can, reciprocally, influence nutritional status [60,61]. In addition, gut microbiota may interact with GI helminths directly or via the host's immune system [45]. In the future, I will test for interactions among nutrition, helminth infection and the gut microbiome. **In combination, I will be able to start a faculty position with my own area of conceptual expertise that I will have investigated in multiple host-parasite systems, plus valuable, informative samples and innovative experimental designs to quickly launch my independent career.**

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**Comment [19]:** The current model would not predict this, because the amount of resources available for growth+maintenance and storage is influenced by ingestion and assimilation. That is, we don't currently have any way for the host to e.g. increase assimilation efficiency in order to repair damage done to structure. Ingestion rate depends on a structure to reserve ratio (which would be affected in a weird way by parasites of structure), but assimilation efficiency is only impacted by the abundance of P2 parasites.

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**Comment [20]:** This is also not correct for the current model structure: right now, structure receives a fixed allocation that is independent of ingestion, assimilation, or immune defense.

As currently constructed, P2 and P3 parasites would interact thru resources in the following way: P2 parasites would reduce digestive efficiency, thereby reducing the amount of assimilate available for maintenance, structural growth, and reserves (and induced immune response); P3 parasites reduce the amount of structure, and since the host has a genetically fixed growth schedule this means that more resources are used for structure (essentially to both repair the damage caused by the parasite and to build the requisite amount of new structure); both of these effects will reduce the amount of resource that is available to be stored as reserves, thereby weakening the induced immune response.

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