GOALS

The task here is to attempt to fit the growth, reproduction, and spore production data from Cat Searle's experiments with *D. dentifera* and *P. ramosa*. Her experiment used a sacrifice series to quantify spore production at different infection ages, but she also measured size, cumulative reproduction, and, most importantly, feeding rate for the animals sacrificed at each age. The clearance rate data is especially valuable, as it should allow us to forego needing to fit feeding parameters separately. Based on my previous efforts and conversations with other people, the feeding model is both the most difficult to fit, and the values of those parameters will critically affect the best-fit values of other parameters.

The key question is what the model for parasite growth should look like. My Proc B paper suggested that parasites get energy from the allocation to growth. Simple carbon accounting in the Proc B paper suggested that the total carbon in spores+tissue was about equal to the amount of carbon liberated by castration (the carbon that should have ended up in eggs). Moreover, the paper suggested that about 45% of the liberated carbon ended up in spores. These observations suggest some potential models for parasite growth, but it would be good to test those explicitly here, using fitting.

The challenge will be figuring out how to model parasite growth/energy theft. A number of possibilities spring to mind:

- Parasites receive a constant fraction of energy allocated to growth, regardless of their population size. This is the model directly suggested by my results.
- Parasites have a Type I functional response on energy allocated to growth.
- Parasites have a Type II functional response on energy allocated to growth.
- Parasites growth rate is independent of energy, but the carrying capacity is determined by host size or allocation to growth.

There are also a lot of other issues to consider, such as

- Does the parasite suffer mortality within the host (maybe, but probably best left ignored for the moment)?
- Does the parasite actually get its energy from growth, or is Spencer's suggestion that the energy comes from reserves the better model?
- Do we need to consider stage structure for the parasite? The parasite is clearly stage-structured, and there is some limited evidence that the early stage might be the replicating stage, whereas the other stages are merely developing. The immediate suggestion from that observation is that replication should be more expensive than development. However, that is hard to reconcile with the dynamics of host growth and reproduction - the timing of things suggests that the early stage of parasite growth comes before castration. What's the point of castrating the host *after* the most energetically expensive stage of life? Moreover, the total carbon ending up in tissue and spores is equal to the total carbon freed over the host's lifetime. If the primary energy demand for parasite growth came very early in infection, that should reveal itself as a significant reduction in host growth and reproduction during the energetically expensive replication phase. It is my belief that replication is actually rather "cheap" for Pasteuria, but development is expensive, possibly because of the cost of building the endospore. For now, I think we are safe to treat the parasite as homogeneous.
- On the other hand, what about a *size*-structured model? That is, imagine that there is some initial proliferating stage, and then after that, the parasite transitions between size classes with no change in parasite numbers. This appears to be what the parasite is actually doing. But what sets the "carrying capacity" of the parasite? I might need to do some reanalysis of my experimental

data to see if you can predict total spore load by some aspect of host growth/reproduction from early in infection - clearly the total carbon in spores/tissue equals the total freed by castration, but can I go further?
- How do we model the dynamics of food? I have the feeding rate calculation, but I do not yet know what the feeding protocol is. Can I safely assume (even if it is not quite correct) that feeding was frequent enough that the host can be treated as having lived in a chemostat rather than batch culture?

My (current) hypothesis for infection development in the host:

- Infection occurs when ingestion spores successfully attach to the esophagus of the *Daphnia*.
- These spores migrate into the hemolymph and develop (without replication) into the "cauliflower" stage; this process takes several days.
- At some point in development, the cauliflower stages begin a process of budding off new spores (replication).
- This requires a large investment of energy, so the parasite triggers increased allocation to growth to fuel this replicative process.
- Each cauliflower cell goes through several rounds of replication, producing "grape-seed" stage spores.
- These rounds of replication are not separated (temporally) very much, which is why you don't see the coexistence of many different developmental stages simultaneously.
- After producing some number of grape-seed spores, the cauliflower stages become "dormant" - they remain visible in the hemolymph, but are no longer doing anything.
- The grape-seed cells go through a process of development whereby they eventually become transmission stages.

It would probably be very good to cross-reference this developmental timeline against anything known for *P. penetrans*, which has seen some more (limited) success in culturing outside the host.

Note that this hypothesis suggests that the total parasite burden depends very little on what is happening late in infection (that will affect how many viable transmission stages are produced, but not how many pre-transmission stages are produced). Differences among hosts in total parasite burden can be explained by differences in host allocation to growth, ingestion, or maintenance that are caused (maybe) by demographic heterogeneity. This demographic heterogeneity among hosts will be the primary challenge throughout this fitting exercise, as it was before.

The model that is implied by that hypothesis would have to involve stage structure in the parasite. For example, a potential model would be

- Spores are ingested on the day of exposure;
- The total infectious dose depends on the feeding rate and on a probability of infection p_I that is estimated on the basis of the data;
- The spore population that is ingested is "dormant" following ingestion; replication starts n days later (where n is an estimated parameter of the fit model);
- Castration also happens n days post-infection;
- Parasites replicate for m datas (where m is also estimated from the data), using energy harvested from the host to fuel replication;
- Afterwards, spores transition to a new developmental stage with some probability p_M that is estimated on the basis of data;

- These spores develop into transmission stages, using energy harvested from the host to fuel replication;
- Spore transition to transmission stage with some probability p_T.

The three probabilities allow for repeated expore to the same spore without infection (p_I), and for the persistence of both "cauliflower" stages (through p_M) and pre-transmission stages (p_T), while imposing an underlying deterministic developmental structure.

One question is whether we seek here to develop a model that works well for just this set of data, or whether we seek to develop a model that will work well for any dataset. We must take advantage of the fact that ingestion is measured, so the feeding model will not be general. A better example is that, in this dataset, castration happens prior to the onset of sexual maturity so we could, for example, assume that castration happens upon infection, ignoring the actual delay that occurs between spore ingestion and castration. There is also the reality that, in many cases, the exposure period is long enough to consider exposure happening over a period of multiple days, such that a spore might be ingestion and not attach the first time, but then reingested later. This would create asynchrony in developmental stages. Again, in Cat's data, we don't really have to worry as much about that because the exposure period was relatively short compared to some of the exposure periods I have used in my experiments. I think, for now, I will attempt to develop a model that works well with Cat's data, and then decide how such a model needs to be extended for more general use.

DEB MODEL

Let's begin by laying out the standard dynamic energy budget model, and then discuss ways to include parasitism into that model. The model begins with the dynamics of "reserves", a pool of metabolizable energy that is in temporary storage. The dynamics of reserves are simple to state, and complicated to derive: the dynamics are just the difference between assimilation rate p_A and mobilization rate p_C (the 'C' is for 'catabolization').

$$\frac{dE}{dt} = p_A - p_C. \tag{1}$$

The dynamics of assimilation depend on the feeding model. For the surface-area-dependent Type II functional response common to DEB models,

$$p_A = \rho I_{max} \frac{F}{f_h + F} L^2. \tag{2}$$

Here ρ is the fraction of ingested food which is assimilated, I_{max} is the surface-area-specific ingestion rate, F is the density of food, f_h is the half-saturation constant, and L^2 is surface area related to observed length. We can safely assume that the scaling of surface area from length is incorporated into the I_{max} term.

Cat has calculated the clearance rate as $\log(F_0/F_1) * V/T$, where F_0 is the initial number of algae cells, F_1 is the final number of algae cells, V is the volume of the tube, and T is the duration of the feeding trial (3 hours). This model is assuming an exponential decrease in the number of algae cells, with the rate of decrease determined by the ingestion rate of the daphnid; essentially, the daphnid has a linear functional response. Cat also calculates a size-corrected clearance rate as the observed clearance rate divided by the square of the length, that is, assuming that clearance rate is dependent on the surface area. **It will probably be desirable to fit a feeding model to all of this data to estimate its parameters, and also to compare alternative feeding models, although I believe that Spencer has done this to some extent already.** In the work I have seen, Spencer fits some models that allow feeding rate to depend on spore load/infection status. Spencer's results suggest that feeding rate should

depend on the number of spores. One thing to consider: if all the parasite's replication happens early, then the change in feeding rate through infection time has more to do with the *size* of the parasite spores than their numbers (see discussion above). *A key for this study is the ability to treat the feeding model parameters as fixed, rather than needing to estimate them*; even if feeding rate changes as the abundance of spores changes dynamically.

A slightly-modified version of the standard DEB model. There are lots of ways to write down the standard DEB model. I have a version that I have used that simplifies the standard DEB model in some ways that I think are useful. This version assumes that energy reserves $E(t$ increase due to ingestion, and are mobilized $p_C(E, V, t)$ to fuel growth in volume $V(t)$ and egg production $R(t)$. The simplification I make to the standard DEB model is in assuming that there are no "maturity maintenance" costs; this prevents the possibility of a sexually mature animal "reverting" to sexual immaturity. It also is beneficial for working with *Daphnia*, since, to a good approximation, sexual maturity can be measured by length (that is, it is possible to define a "size at maturity" that applies to all individuals).

$$\frac{dE}{dt} = \rho I_{max} \frac{F}{f_h + F} V^{2/3} - p_C, \tag{3}$$

$$\frac{dV}{dt} = \frac{\kappa\, p_C - k_M\, E_G\, V}{E_G}, \tag{4}$$

$$\frac{dR}{dt} = \frac{(1-\kappa)\, p_C}{E_R}, \tag{5}$$

$$p_C = E \left(\frac{\frac{v}{V^{2/3}} + k_m}{1 + \frac{\kappa E}{E_G V}} \right). \tag{6}$$

This is the description of the growth dynamic under "good" food conditions. In the experiments, although not technically in chemostats, I believe that food was held as close to constant as possible (daily food transfers, saturating food). Clearance rates were also directly measured, as were size and spore load. Because of this, we can safely remove most of the terms relating to feeding from the model. In particular, I think we can begin by using the raw measurement of size-dependent clearance rate in place of $I_{max}\ F/(f_h + F)$. Then we can focus only on estimating the assimilation efficiency ρ. We can simplify further by using the assimilation efficiency estimated for *D. pulex*, e.g. in the the work by McCauley, Nisbet et al., as the parameter ρ.

Including parasitism. Here is where things get a bit dicier, because we are faced with many possible ways of adding parasitism, as noted above. To constrain ourselves a bit, let's assume that the parasite shuts off reproduction by causing all energy allocated to reproduction to instead be allocated to growth, and let's assume that the parasite, in way way or another, uses this energy as a resource. The three issues we need to deal with are the following:

(1) *When* does the parasite shut of energy allocation to reprodution?
(2) *How* is the parasite population structured?
(3) *Where* does the parasite get its energy from growth?

The *when* question is a bit easier. Castration is not instantaneous, as many individuals are able to have clutches (including clutches that are larger than normal) after exposure to the parasite. However, if infection occurs early enough in life (that is, early enough before sexual maturity is reached), then castration happens prior to sexual maturity. In the data for Cat's experiments, castration precedes sexual

maturity for all individuals, so we could make the assumption that castration is instantaneous upon infection. Or we could follow the model structure above and assume that castration happens n days post-infection, letting n be estimated on the basis of the data. That is the structure I will assume now.

The *how* question is also not too bad. If the parasite population is unstructured, then we are explicitly treating the parasite population as homogeneous (which we know it is not), so all spores are transmission spores, and all spores use the same amount of energy. I think we cannot, in good faith, use this model. I think we must assume some simple structure (like that suggested above). In this case, we can consider three parasite subpopulations: "cauliflower stages" that produce pre-transmission stages, which requires some amount of energy from the host; "pre-transmission stage" that develop into transmission stages, which requires some amount of energy from the host; "transmission stages" that are inert, simply taking up space inside the host. To be fully general, this suggests a delay-differential equation approach, because individual infecting spores infect the host at different times and each "cauliflower stage" produce pre-transmission spores time; this means that pre-transmission stages (and, subsequently, transmission stages) have a distribution of ages within the host.

However, a simpler alternative is a continuous-time stage-structured population model. We just want to make the rates of transitioning between stages (i.e., the probabilities of transitions) dependent on ingestion rates, food densities, etc. A simple model to begin is the following:

$$\frac{dN_C}{dt} = p_I \times (clearancerate) \times N_{T,env} - \mu N_C, \tag{7}$$

$$\frac{dN_P}{dt} = b(E,V)N_C - p_T(E,V)N_P, \tag{8}$$

$$\frac{dN_T}{dt} = p_T(E,V)N_P. \tag{9}$$

In this model, the total number of cauliflower stages N_C depends upon clearance rates (feeding, not immune!), the probability of infection, and the density of transmission-stage spores in the environment. Cauliflower stage spores "deactivate" at a rate μ. Note that although this enters the model as a death rate, it isn't biologically a death rate - it is just a way of ensuring that cauliflower stages don't produce pre-transmission spores indefinitely. In experiments, new cauliflower stages would only be coming in while the host was in the exposure phase. Cauliflower stages produce pre-transmission stages N_P at a rate b that depends (potentially) on energy reserves and host size. These pre-transmission stage spores transition to becoming transmission stages N_T at a rate p_T that may depend on reserves and host size. Transmission stage spores persist within the host forever. This seems like a pretty good model to begin with - it is quite simple (structurally) but roughly captures what we know about the biology of the host. What remains is to specify functional forms for $b(E,V)$ and $p_T(E,V)$, so that they depend upon the energy being allocated to growth.

Where exactly that energy is coming from is trickier, as I noted above. I am working from the assumption that the energy used by the parasite comes from growth (in some capacity), rather than reserves are reproduction. The total energy allocated to growth is $\kappa\ E_G\ p_C$ in DEB model (or just p_C once castration happens). Maintenance costs are subtracted from this total, and the remainder is used for growth, with a conversion cost of E_G. The growth model (without parasitism) is just:

$$\frac{dV}{dt} = \frac{\kappa\, p_C - k_m\, E_G\, V}{E_G}. \tag{10}$$

There are several ways to incorporate parasite energy theft into growth. The simplest is to assume that the parasite steals some fraction of the energy allocated to growth. Focusing on the point after all energy is allocated towards growth (i.e., $\kappa = 1$), we assume that the parasite steals some fraction

$\sigma(N, E, V)$ of mobilized energy p_C. The remainder goes to fuel host growth. Then the model for growth and parasite population size (ignoring structure for simplicity) is something like:

$$\frac{dV}{dt} = \frac{(\kappa - \sigma(N, E, V))\, p_C - k_m\, E_G\, V}{E_G}, \tag{11}$$

$$\frac{dN}{dt} = \sigma(N, E, V)\, p_C. \tag{12}$$

The fraction of energy stolen by the parasite could potentially depend on the number of parasites, the size of the host, the size of the host's energy reserves, or none of these things. My results suggest that it might just be a constant fraction, regardless of the host's size or energetic state, and that the total number of parasites would depend on the host and environmental factors that affect the mobilization flux p_C.

The next simplest model is to assume that the parasite actually utilizes "structure" in some way, shape, or form, as a resource. In this case, we let $\sigma(N, V)$ be the parasite's rate of "structure conversion to parasite biomass" or some other such thing, and the model for host and parasite growth becomes

$$\frac{dV}{dt} = \frac{\kappa\, p_C - k_m\, E_G\, V}{E_G} - \sigma(N, V), \tag{13}$$

$$\frac{dN}{dt} = \sigma(N, V). \tag{14}$$

More complicated are models where the parasite is utilizing energy that is allocated to growth in a dynamic way. The reason this is slightly more complicated is that $\kappa\, p_C$ is an energy flux; it has units of energy per time. If we imagine the the parasite is acting as a "predator" on host energy, we would write down its "functional response." For example, in Hall et al. 2009, Spencer had the parasite using energy reserves E as a resource, and wrote down the parasite's per-capita growth rate as

$$a_N \frac{E}{h_N + E} - m_N. \tag{15}$$

It is less obvious how to do that when the equivalent of E in Hall et al. 2009 is p_C. The simplest way is to simply treat p_C as they did E. That's fine (mathematically), but it is worth pointing out that the terms of this "functional response" don't have the same biological interpretation (and cannot be derived on the basis of a physical argument). However, we will go with it for now, and say that $\sigma(N, p_C)$ is the functional response of the parasite. There are two ways that the parasite might access energy.

$$\frac{dV}{dt} = \frac{\kappa\, (p_C - \sigma(N, p_C)) - k_m\, E_G\, V}{E_G}, \tag{16}$$

$$\frac{dN}{dt} = \sigma(N, p_C). \tag{17}$$

In this model, the parasite reduces the amount of mobilized energy available for growth. The second model is

$$\frac{dV}{dt} = \frac{\kappa\, p_C - k_m\, E_G\, V - \sigma(N, \kappa\, p_C)}{E_G}, \tag{18}$$

$$\frac{dN}{dt} = \sigma(N, p_C). \tag{19}$$

In this model, the parasite only has access to the energy that has been allocated to growth. Of course, practically speaking, these two models will be identical because $\kappa = 1$ once castration occurs. So we can profitably reduce the number of models down to three by assuming that most (essentially, all) of the replication happens after castration has occurred.

Of course, there are mathematical details that have not been made explicit yet. In particular, we need to specify functional forms for the parasite's energy use or growth. Let's take each model in turn, considering the dynamics after castration has occurred (which we are going to assume coincides with the start of replication). I am also going to make a fairly big assumption that is not obvious here, which is that the mobilization rate rules do not change upon infection. That is, the functional form of p_C in infected hosts will be identical to that of uninfected hosts. This is problematic is because the (admittedly obscure) derivation of the form of the mobilization rate equation relies on assumptions that are not met in infected hosts. In particular, the DEB assumption of "weak homeostasis" is almost certainly violated. This assumption states that, under constant food, there is a "reserve density" (defined by the quantity E/V) which remains constant. Given that the parasite is developing inside the host, causing a rechanneling of energy and siphoning off some of that energy for itself, it seems very unlikely to imagine that the ratio of reserves to structure will remain constant. However, previous work using DEB models to study infection (Hall et al. 2007, 2009 and Flye-Sainte-Marie et al. 2009) have not worried about this assumption violation, and those have been co-authored by DEB pioneers like Nisbet and Kooijman. Thus, I will assume that this is not too big of a problem.

Model 1:

$$p_C = E\left(\frac{\frac{v}{V^{2/3}} + k_m}{1 + \frac{\kappa E}{E_G V}}\right).\frac{dE}{dt} \qquad = \rho I_{max}\frac{F}{f_h + F}V^{2/3} - p_C, \tag{20}$$

$$\frac{dV}{dt} = \frac{(\kappa - \sigma(N,E,V))\,p_C - k_m\,E_G\,V}{E_G}, \tag{21}$$

$$\frac{dN}{dt} = \sigma(N,E,V)\,p_C. \tag{22}$$

SUGGESTED FUTURE EXPERIMENTS

Infection experiment with varying initiation of reduced food. If I am correct that most (all) of the replication happens early in infection, then the total (transmission vs. pre-transmission) number of spores should be relatively unaffected by reductions in food late in infection. However, the total number of transmission stages would be affected, because reducing the food late in infection reduces the developmental rate of the pre-transmission stages. It also might increase density-dependent competition for resources.

NOTES TO SELF

Derivation of the clearance model. The clearance rate was calculated as $\log(F_0/F_T)(V/T)$, where F_T is the final number of cells, F_0 is the initial number of cells, V is the volume of the container, and T is the amount of time the feeding trial lasted. This comes from the following model of feeding (where $F(t)$ is the amount of food at any time t:

$$\frac{dF}{dt} = -a/V F(t) \tag{23}$$

Implicit in this equation are the units of the parameters: a is the clearance rate and has units of L/time; V has units of L, and $F(t)$ has units of algal cells. Solving and manipulating this equation:

$$\frac{1}{F(t)}dF = -a/V dt \tag{24}$$

$$\log(F(t)) = -at/V + C \tag{25}$$

$$F(t) = F_0\, e^(- at/V) \tag{26}$$

$$F(T) = F_0\, e^(- aT/V) \tag{27}$$

$$e^(aT/V) = \frac{F_0}{F_T} \tag{28}$$

$$\frac{aT}{V} = \log\left(\frac{F_0}{F_T}\right) \tag{29}$$

$$a = \log\left(\frac{F_0}{F_T}\right)\left(\frac{V}{T}\right) \tag{30}$$