Intensity-dependent host mortality: what can it tell us about larval growth strategies in complex life cycle helminths?

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SUMMARY

Complex life cycle helminths use their intermediate hosts as both a source of nutrients and as transportation. There is an assumed trade-off between these functions in that parasite growth may reduce host survival and thus transmission. The virulence of larval helminths can be assessed by experimentally increasing infection intensities and recording how parasite biomass and host mortality scale with intensity. I summarize the literature on these relationships in larval helminths and I provide an empirical example using the nematode *Camallanus lacustris* in its copepod first host. In all species studied thus far, including *C. lacustris*, overall parasite volume increases with intensity. Although a few studies observed host survival to decrease predictably with intensity, several studies found no intensity-dependent mortality or elevated mortality only at extreme intensities. For instance, no intensity-dependent mortality was observed in male copepods infected with *C. lacustris*, whereas female survival was reduced only at high intensities (> 3) and only after worms were fully developed. These observations suggest that at low, natural intensity levels parasites do not exploit intermediate hosts as much as they presumably could and that increased growth would not obviously entail survival costs.

Key words: crowding effect, growth cost, life history strategy, *Macrocyclops albidus*, Nematoda, resource constraint, virulence.

INTRODUCTION

There has been much recent interest into how complex life cycle parasites optimize their larval growth (Choisy *et al*. 2003; Parker *et al*. 2003*a*,*b*; Duclos *et al*. 2006; Iwasa and Wada, 2006; Michaud *et al*. 2006; Ball *et al*. 2008; Shostak *et al*. 2008; Parker *et al*. 2009*a*,*b*; Benesh, 2010*a*,*b*; Chubb *et al*. 2010). On the one hand, a fast larval growth rate and a large size at infectivity are expected to increase fitness. Rapid growth reduces the time until developmental thresholds are reached, thus lowering the chances of dying before being capable of transmission to the next host (Day and Rowe, 2002), whereas a large transitional size may increase infection success (Steinauer and Nickol, 2003) or adult fecundity (Fredensborg and Poulin, 2005). On the other hand, rapid growth entails an elevated consumption of host resources, and may thus decrease host survival and parasite transmission. This trade-off between growth and transmission is thought to shape larval life history (Parker *et al*. 2003*b*).

In the case of larval helminths, normal infection levels are very low (frequently a single worm per host) (Poulin, 2007), and infection does not always lower host survival (Poulin *et al*. 1992; Hurd *et al*. 2001; Guinnee and Moore, 2004; Benesh, 2010*b*). This suggests that some parasites may not normally exploit their hosts at levels where mortality costs are measurable. In such systems, mortality costs may only be detected at unnaturally high levels of parasite growth. The rate and amount of parasite growth can be manipulated by experimentally producing a range of infection intensities. The total biomass accumulated by multiple worms should exceed that of a single worm, unless single parasites are maximally exploiting the available host resources (Parker *et al*. 2003*b*). Thus, if there is a trade-off between total worm growth and host viability, then we expect some pattern of intensity-dependent mortality (IDM).

Older studies of IDM in larval helminths were stimulated by population models (Anderson and May, 1978), and focused on whether parasites regulated intermediate host populations (Keymer, 1980; Uznanski and Nickol, 1980; Skorping, 1984, 1985). Here, I emphasize the insights into larval growth strategies that may be gleaned from IDM studies. In particular, the relationship between total parasite biomass and intensity indicates the infection levels at which host resources limit larval growth, whereas patterns of IDM indicate when and if 'host mortality' costs are manifested. I start by outlining a simple conceptual framework in which to categorize patterns of IDM. Using this framework, the patterns of intensity-dependent helminth growth and intermediate host survival found in the literature are summarized. An example study of IDM using the nematode

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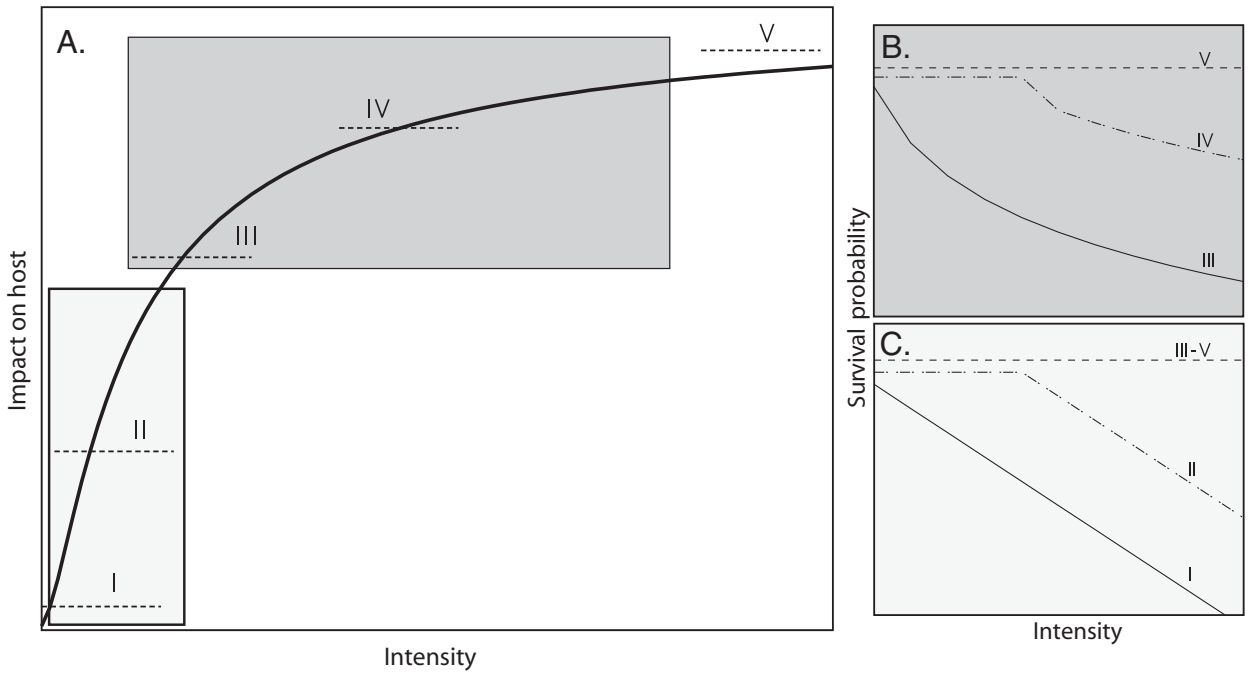

Fig. 1. (A) Generalized relationship between intensity of infection (arbitrary units) and impact on the host, which can presumably be indirectly assessed by quantifying parasite growth. Hypothetical threshold burdens, beyond which host survival decreases, are indicated by dashed lines labelled I to V. (B and C) The predicted patterns of intensity-dependent mortality are shown for a parasite exhibiting a decelerating intensity-burden relationship (dark grey box) as well for the case in which burden increases linearly with intensity (light grey box). The survival patterns are shown for the particular 'danger' thresholds I–V.

Camallanus lacustris and its copepod intermediate host is also provided. The aims in this paper are to (1) evaluate the typical relationship between larval parasite growth/size and infection intensity, (2) assess the patterns of IDM associated with parasite growth, and (3) establish whether normal levels of intermediate host exploitation entail risks for parasites.

MATERIALS AND METHODS

Conceptual background for categorizing the literature

Complex life cycle parasites use resources from their intermediate hosts to fuel their larval growth. Therefore, by quantifying overall parasite growth (how fast and how much), we can estimate the impact that parasites have on the host. However, if worms vary considerably in their biochemical makeup, i.e. some are of higher quality than others, then worm size need not be well correlated with the actual drain on host resources. Indeed, the effect of helminth infection on the intermediate host can depend on the availability of particular macronutrients (Ponton *et al*. 2011). Nonetheless, overall parasite biomass typically varies several-fold with intensity, and this extensive variation in parasite size should be at least roughly related to the consumption of host nutrients.

The generalized relationship between intensity and burden imposed on the host is likely to be an asymptotic function (Fig. 1A) (Parker *et al*. 2003*b*). Detriment to the host initially increases as more parasites sap host nutrients, but this should eventually level off as parasites approach the maximum possible level of host exploitation. By estimating the shape of this function over a natural and unnatural range of intensities, we can assess how extensively a parasite species normally exploits its intermediate host. Consider 2 extremes. When the burden on the host increases proportionally over a large range of intensities, i.e. many individuals can infect a host without approaching a maximum level of exploitation, this suggests the parasite is a rather mild-exploiter of the host. An example of such parasites might be acanthocephalans in crabs (Poulin *et al*. 2003). On the other end of the spectrum, the resources consumed and growth attained by a parasite may already be maximized in single-worm infections. A potential example of such aggressive parasites might be some tapeworms infecting copepods (Parker *et al*. 2003*b*).

Two areas of the function in Fig. 1A are highlighted, the initial, near-linear trajectory (light grey box) and the non-linear, decelerating advance to the maximum (dark grey box). Assume that we observe these two intensity-burden relationships. What are the expected patterns of IDM? This depends on the parasite burden that hosts can tolerate. The dashed lines in Fig. 1A indicate hypothetical burdens beyond which IDM occurs. In the parlance of recent theoretical models, these thresholds represent the total

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parasite mass at which the noise mortality rate for the parasite becomes size-dependent (Parker *et al*. 2003*b*; Ball *et al*. 2008; Parker *et al*. 2009*a*). Different thresholds produce different qualitative patterns of IDM (Fig. 1B and C). If hosts are sensitive to even low parasite burdens, then each increase in intensity should decrease host survival, either proportionally (threshold I, Fig. 1C) or with a slowing rate (threshold III, Fig. 1B). Alternatively, hosts may be able to harbour low numbers of parasites with no ill effect, but once a threshold is passed, survival decreases, resulting in a discontinuous pattern of IDM (threshold II in Fig. 1C and threshold IV in Fig. 1B). When parasites are far from maximally exploiting the host, even a steep impact-intensity relationship could be tolerable, so no IDM is expected (thresholds III to V, Fig. 1C). At the exploitation maximum, increases in intensity do not add much burden to the already strained host. If hosts are able to tolerate this extreme, then IDM is never expected (threshold V, Fig. 1B).

Survey and categorization of the literature

What is the typical relationship between intensity and growth? And is mortality related to growth? To address these questions, I searched for studies on intensity-dependent growth and mortality in the larval stages of trophically transmitted helminths. I focused on species that do not asexually reproduce in their intermediate host. Studies were found during a more comprehensive survey of growth and development in larval helminths (Benesh *et al*. 2011), in which literature databases (Web of Science, PubMed, Google Scholar) were queried with genus names and the terms 'life cycle', 'growth', or 'development'.

Two growth characteristics may affect host well-being, rate and final size attained. Typically, investigators have recorded how average parasite size, but not growth rate, changes with intensity. These two parameters are likely correlated, but it should be kept in mind that they do not necessarily produce the same intensity-burden relationships. I was interested in how overall parasite growth affects host viability, so I examined how the total parasite burden (size) scaled with intensity. Species were categorized into 1 of 3 intensity-burden relationships. When each additional worm increased the total by about the same amount, then burden was considered proportional to intensity (the light grey area in Fig. 1A). Note that a proportional increase in total worm size with intensity does not imply the absence of crowding effects. When the slope of the intensity-burden relationship is less than 1 the average worm size decreases with intensity. If the total increased by a smaller and smaller amount with each worm, then the burden-intensity relationship was categorized as decreasing (the dark grey area in Fig. 1A). Finally, if the maximum burden has been reached already at the lowest observed intensities, then the total worm biomass may not increase at all with intensity.

For intensity-mortality relationships, the hypothetical scenarios of IDM depicted in Fig. 1B and 1C were used to categorize observed patterns. Potential relationships between survival probability and intensity were: proportional (case I), flat and then a linear decrease (case II), decreasing with a slowing rate (III), flat followed by a non-linear decrease (IV), or completely flat (V). Unfortunately, the ability to discriminate between case II and IV is low, because in most studies, there has been little replication within intensity levels and the range of observed intensities may not be large enough to differentiate a non-linear from a linear decrease. Thus, cases in which host survival decreased past a certain intensity level were simply considered to have a discontinuous pattern of IDM.

An example: the effect of intensity on growth and mortality in Camallanus lacustris

The nematode *Camallanus lacustris* has a 2-host life cycle. Free, first-stage larvae are eaten by freshwater copepods where they invade the body cavity. The worms undergo 2 moults in copepods before reaching the infective L3 stage after about 12 days at room temperature (Moravec, 1969). Several species of fish are potential final hosts, but perch (*Perca fluviatilis*) is probably the most frequent. As nematodes must reach the L3 stage before being infective (Anderson, 2000), the final size attained by larvae was expected to be relatively constant, at least in comparison to cestodes or acanthocephalans. Consequently, unless growth rates varied considerably, the total burden on the host was predicted to increase proportionally with intensity.

Experimental infection and maintenance of copepods

Gravid female worms were collected from the guts of perch caught in the Grosser Plöner See, Germany (54°09′N 10°25′E). L1 larvae were dissected from 10 female worms, pooled together, and then kept at 4 °C overnight before being used for infection the following day. Copepods (*Macrocyclops albidus*) were taken from a laboratory culture (see van der Veen and Kurtz (2002)). Two groups of copepods were used for infection; adult males and adult females. Female copepods are larger than males, and likely provide more nutrients and/or space to developing larvae. Copepods were isolated individually in 24-well microtitre plates ($n=930$). To create a range of infection intensities, copepods were exposed to 2, 4, or 6 *C. lacustris* larvae 2 days after isolation. Some copepods served as unexposed controls ($n=111$). Copepods were maintained at 18 °C with a 18:6 h light:dark cycle, and they were fed every second day with 3 freshly hatched *Artemia salina* nauplii.

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Copepod dissection and larval parasite growth

Copepods were checked daily and dead individuals were frozen at −20 °C. Dead copepods were later thawed and dissected to determine the intensity of infection. Copepods that died within the first 2 days post-exposure were not processed ($n=28$), because the L1 larvae can actively leave the dead host, leading to under-estimates of intensity. Additionally, to quantify worm growth rates, random subsamples of live copepods were taken 5 and 11 days post-exposure ($n=164$ and $n=160$, respectively). On these days, worms are typically undergoing the L1 to L2 moult and the L2 to L3 moult (Moravec, 1969). After 75 days, the experiment was terminated and all remaining copepods were frozen.

For most of the infected copepods, all worms were measured. Worms were placed on a microscope slide in 10 μl of water under a cover-slip. Each worm was photographed with a digital camera, and length and width were measured using the freeware Image J 1·38× (Rasband, W.S., NIH, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997–2009). Body width was recorded where the worm's pharynx ended. Worms were considered cylindrical in shape, so their volume (mm^3) was estimated with the equation $(\pi lw^2)/4$ where l is worm length and w is worm width. The weight of the cover-slip compressed worms slightly. This was necessary, because L3 worms are often coiled, making their length otherwise difficult to measure. However, because of this flattening the worm volumes presented here may be somewhat distorted in comparison with other studies (Moravec, 1969).

Relationship between intensity and burden on the host

To assess how growth rate and final size change with intensity, growth curves were fitted to the data for each intensity level. The following asymptotic function was used: $V(t)=2{\cdot}06\times10^{-4}+A\times(e^{B/t})$ where V is the total worm volume harboured by a copepod, A is the asymptotic volume, and B is the relative rate at which the asymptote is approached (see Shostak *et al*. (1985) and Michaud *et al*. (2006) for similar approaches). The function's intercept was set at $2{\cdot}06\times10^{-4}$ mm^3 because this was the average volume of free-living infective L1 larvae ($n=23$). The parameters for this non-linear function, as well as their standard errors, were estimated via an iterative procedure executed with SPSS version 18.0 (SPSS Inc., Chicago, Illinois, USA). Parasite growth was analysed separately in male and female copepods.

Relationship between intensity and host survival

The effect of intensity on copepod survival was assessed with Cox regression, a method commonly used for survival analyses (Andersen, 1991). Male and female copepods had clearly different life spans (average survival of males was 19·7 days versus 54·9 days for females), so they were analysed separately. Uninfected copepods were either unexposed controls or exposed, but uninfected. These 2 groups did not differ significantly in a preliminary Cox regression, so for simplicity all uninfected copepods were pooled for the analysis. Intensity was entered into the regression model as a categorical covariate. An assumption of Cox regression models is that the ratio of the hazard function for any 2 individuals is dependent on their covariate values and the baseline hazard function, but not time (proportional hazards assumption). However, I was interested in whether intensity-dependent mortality occurs at particular times, i.e. during or after the major growth phase, so I checked the validity of the proportional hazards assumption. Two predictor terms were entered into a preliminary model: intensity alone as well as the interaction between intensity and a time-dependent covariate. If the intensity by time interaction explained more variation (had a higher Wald χ^2 value), then the effect of intensity was considered to be time dependent and intensity alone was removed from the model.

Checking dose effects

Copepods harbouring the same number of worms may not be entirely comparable, because they were exposed to different doses. For example, a copepod with 1 worm, depending on the dose it received, may have been infected by 50%, 25% or 12·5% of the larvae to which it was exposed. If susceptibility is correlated with host quality, then within each intensity level dose may affect parasite growth or host survival. To check this, the data were split by copepod sex and by intensity level, so that within groups only dose varied. For each group, a Cox regression was performed with dose as the only predictor. ANOVAs were also conducted using these data subsets to assess whether total worm volume depended on dose. As worms were measured at different time-points, the values for worm size were 'corrected' for time by taking the residuals of the fitted growth curves (e.g. see Fig. 2). These residuals were then used as dependent variables in the ANOVAs.

RESULTS

Intensity-dependent growth and mortality in larval Camallanus lacustris

Dose effects. Sample sizes were large enough to confidently assess dose effects for only intensities of 1, 2, and 3. For both male and female copepods, dose did not significantly affect copepod survival at any infection level (Cox regressions, all $P>0{\cdot}089$). Likewise, there were no clear effects of dose on parasite growth (ANOVAs, all $F<2{\cdot}154$, $P>0{\cdot}101$).

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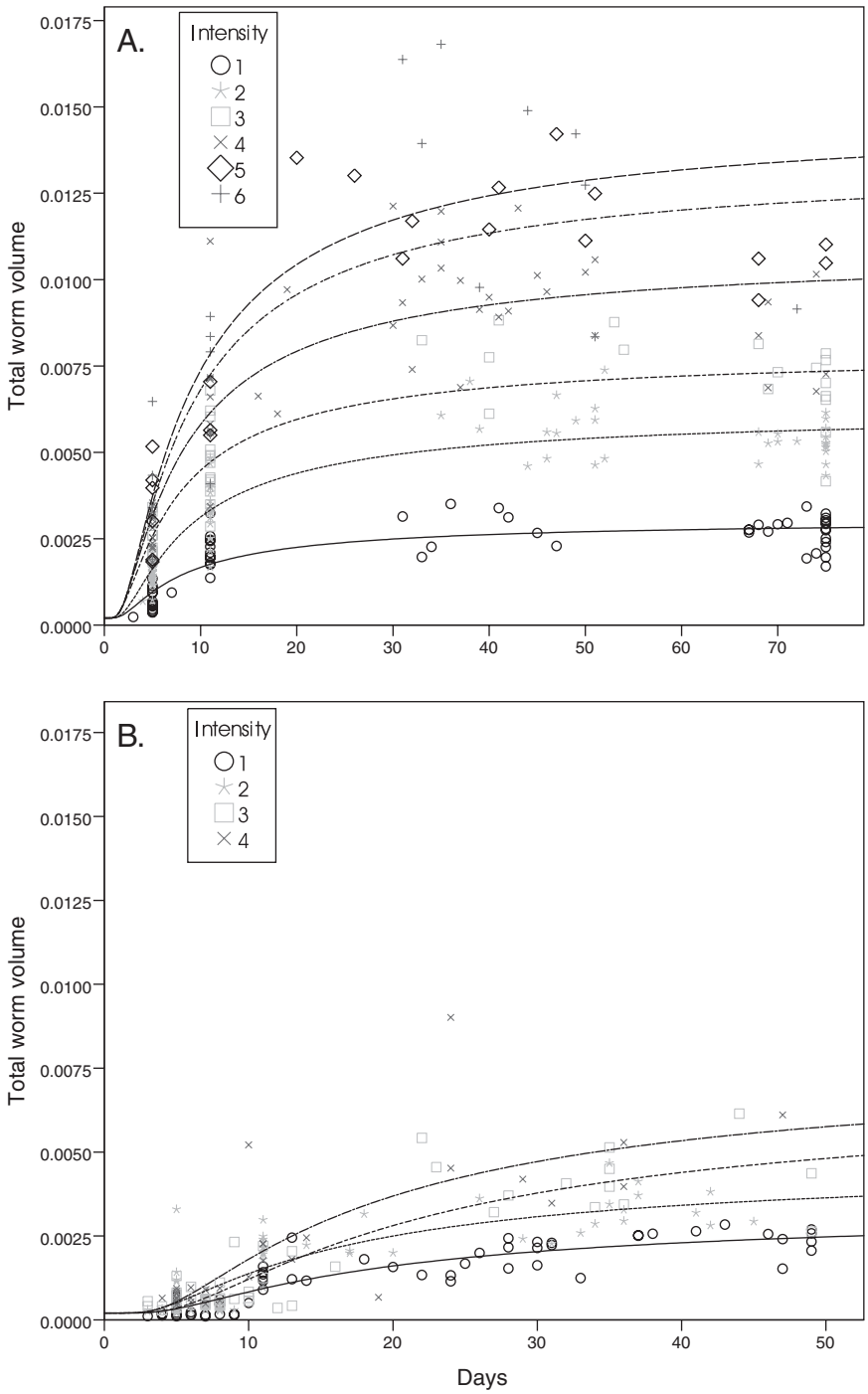

Fig. 2. The total volume (mm^3) of larval *Camallanus lacustris* at different infection intensities in (A) female copepods and (B) male copepods.

Consequently, in the following analyses intensity was considered without regard to dose.

Larval growth at different intensities. There were not many male copepods infected with 5 or 6 larvae ($n=10$ and 14, respectively), so growth curves were only fitted for the intensity levels 1–4. In general, the growth curves fit the data well, usually explaining over 70% of the variation in total worm volume (Table 1, Fig. 2). Parasite growth differed between the host sexes; the estimates for asymptotic total worm volume and relative growth rate tended to be lower in male copepods (Table 1). The asymptotic size increased with intensity, particularly in female copepods (Fig. 3), but within each copepod sex, growth rate estimates were rather consistent, indicating that the asymptotic volume for each intensity level is approached at about the same *relative* rate. Additionally, there was a weak crowding effect. In single infections, the asymptotic volume was about 0·003 mm^3, but the total worm volume did not increase by 0·003 mm^3 with each additional worm

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Table 1. Parameter estimates for the fitted growth curves

(The following asymptotic function was fitted at each intensity level: $V(t) = 2{\cdot}06 \times 10^{-4} + A \times (e^{B/t})$ where t is days post-exposure, V is the total worm volume harboured by a copepod, A is the asymptotic volume, and B is the relative rate at which the asymptote is approached. The curves were fitted separately for adult female and male copepods.)

	N	A – Asymptotic volume in mm^3 (S.E.)	B – Relative growth rate (S.E.)	R^2
Females				
1	61	$0{\cdot}00285\ (1{\cdot}17 \times 10^{-4})$	−6·67 (0·74)	0·748
2	74	$0{\cdot}00600\ (1{\cdot}62 \times 10^{-4})$	−7·18 (0·46)	0·864
3	41	$0{\cdot}00773\ (3{\cdot}28 \times 10^{-4})$	−5·93 (0·65)	0·770
4	51	$0{\cdot}01064\ (4{\cdot}45 \times 10^{-4})$	−6·41 (0·73)	0·758
5	22	$0{\cdot}01326\ (7{\cdot}40 \times 10^{-4})$	−6·97 (1·09)	0·819
6	18	$0{\cdot}01460\ (1{\cdot}47 \times 10^{-3})$	−7·12 (1·83)	0·636
Males				
1	67	$0{\cdot}00312\ (2{\cdot}14 \times 10^{-4})$	−16·02 (1·71)	0·827
2	70	$0{\cdot}00449\ (3{\cdot}54 \times 10^{-4})$	−13·43 (1·46)	0·738
3	49	$0{\cdot}00673\ (6{\cdot}61 \times 10^{-4})$	−19·00 (2·37)	0·796
4	32	$0{\cdot}00757\ (1{\cdot}29 \times 10^{-3})$	−15·52 (3·27)	0·627

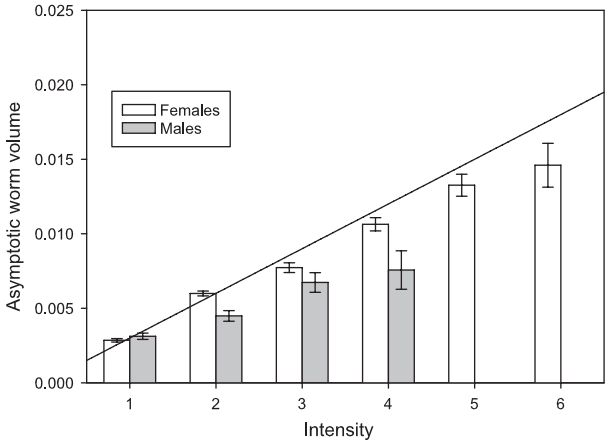

Fig. 3. Estimated asymptotic total volume (mm^3) of *Camallanus lacustris* larvae reached at different infection intensities in female and male copepods. The diagonal line depicts the expected increase in worm volume without crowding effects, i.e. when worms from multiple infections are the same size as those from single infections. Bars represent S.E.

(Fig. 3). In summary, total volume increases proportionally with intensity, albeit at a faster rate in female copepods. In male copepods, worm volume seems to level off above an intensity of 3 worms (Fig. 3), suggesting that the slope of the intensity-burden relationship might be decreasing past this intensity.

Host survival

For female copepods, but not males, the intensity by time interaction had a higher Wald χ^2 score than intensity alone in a preliminary Cox regression analysis. Thus, for males just intensity was a predictor in the Cox regression, whereas for females the intensity by time interaction was checked. Intensity did not affect the survival of male copepods (Wald $\chi^2 = 5{\cdot}941$, D.F. = 4, $P = 0{\cdot}204$). For female copepods, however, the intensity by time interaction was significant, with copepods harbouring 4 or more worms having slightly lower survival (Table 2). Pronounced mortality in female copepods was only observed after about 20 days, with individuals harbouring 4–6 worms dying at an elevated rate after this time (Fig. 4).

Summarizing the literature

Studies fell into 3 categories: (1) only intensity-dependent growth recorded, (2) only intensity-dependent mortality described, or (3) both. Studies of the second type are difficult to interpret, because mortality cannot be correlated with growth, so they are not discussed further here (Kisielewska, 1959; Huizinga, 1967; Rosen *et al*. 1970; Moravec, 1978; Courtney and Christensen, 1987; Okaka, 1989; Solomon *et al*. 1996; Lopez *et al*. 1998; Meissner and Bick, 1999; Ferreira *et al*. 2005; Hansen and Poulin, 2005; Kokkotis and McLaughlin, 2006). Moreover, a number of studies could not be categorized into the outlined framework. For instance, a few studies compared just 2 intensity levels (high *vs* low), making it impossible to evaluate whether the relationship between intensity and growth/mortality is linear or non-linear (Measures, 1988; Robert and Gabrion, 1991; Sandland and Goater, 2000; Dezfuli *et al*. 2001). Also, some authors wrote that larval growth was intensity-dependent, but did not support these observations with data (Calentine, 1965; Awachie, 1966; Denny, 1969; Wootten, 1974; Korting, 1975).

My main goal was to evaluate the connection between total parasite growth and intermediate host mortality, and I found 10 species in which intensity, growth, and host mortality were quantified (Table 3). Nine of these cases involved helminths in their first

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Table 2. Results of Cox regression survival analysis assessing intensity-dependent mortality in female copepods ($N = 288$)

(A preliminary test suggested the effect of intensity varied over time, so the interaction between intensity and time was used as predictor variable. The estimated survival probabilities at each intensity level were compared to that of uninfected copepods to give the odds ratio (= increase in mortality probability relative to uninfected group). *P*-values in bold indicate groups that differed significantly from uninfected copepods.)

	β	Wald	D.F.	*P*	Odds ratio
Intensity by time		36·43	6	< 0·0001	
Intensity = 1	− 0·003	0·48	1	0·489	0·997
Intensity = 2	0·004	0·94	1	0·332	1·004
Intensity = 3	0·003	0·54	1	0·461	1·003
Intensity = 4	0·018	19·14	1	**< 0·0001**	1·018
Intensity = 5	0·013	5·69	1	**0·017**	1·013
Intensity = 6	0·023	14·47	1	**< 0·0001**	1·023

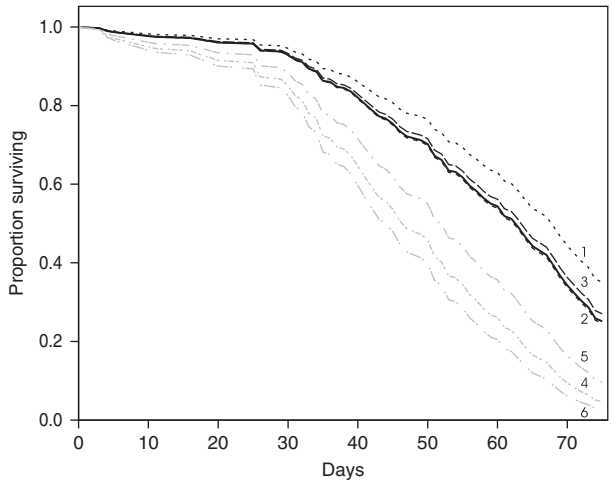

Fig. 4. Survival of female copepods infected with 1 to 6 *Camallanus lacustris* larvae. The black solid line represents the survival of uninfected copepods (both unexposed controls and exposed, but uninfected individuals).

host, while only 1 focused on a trematode exploiting its second intermediate host (Fredensborg *et al*. 2004). I found an additional 23 cases in which only intensity-dependent growth was recorded (Table 4). Most studies involved experimental infections (85%) that produced intensities far exceeding those typically observed in nature. In every study, parasite impact on the host, usually gauged by the total worm size, increased with intensity (Tables 3 and 4). However, in 21% of cases the increase had a decreasing slope, suggesting that the burden on the host often approaches a maximum level at higher intensities.

Occasionally, patterns of IDM predictably follow patterns of intensity-dependent parasite growth. For example, the total larval growth rate of the nematode *Elaphostrongylus rangiferi* increases linearly with intensity, and accordingly snail survival rate decreases linearly with intensity (Skorping, 1984, 1985). However, there are several studies in which IDM is discontinuous and thus less predictable (Table 3). Three of the 5 cases of discontinuous IDM occurred when the total burden on the host appeared to approach a maximum, suggesting that host exploitation only decreases host survival at very high levels (Rosen and Dick, 1983; Duclos *et al*. 2006; Michaud *et al*. 2006).

The absence of IDM has been observed under very different conditions. On the one hand, Sakanari and Moser (1985) observed lowered survival in infected copepods, but this did not depend on the number of tapeworm larvae they harboured. At the very high intensities in their study, the exploitation of the host was maximized, suggesting that extreme host exploitation entails risks but that it does not matter how many individual parasites are actually exploiting the host. On the other hand, Uznanski and Nickol (1980) found no IDM in amphipods infected with the acanthocephalan *Leptorhynchoides thecatus*, although the total parasite volume increases proportionally with intensity, at least in the studied intensity range (see Steinauer and Nickol, 2003 for the intensity-parasite size relationship). This suggests that these worms are far from maximally exploiting their hosts and that amphipods can tolerate intensities above the normal range.

Finally, 1 study does not fit into any of the scenarios presented in Fig. 1. Working with the tapeworm *Hymenolepis diminuta*, Keymer (1980) observed a linear decrease in host survival with intensity, even though total worm size increased with a decelerating slope with intensity. This suggests that parasite growth is not the cause of mortality. In this case, as well as in the lone trematode example (Fredensborg *et al*. 2004), other mechanisms that are proportional to intensity are presumably responsible for host death, such as the damage done to the host during invasion of the body cavity.

DISCUSSION

Helminths that aggressively drain host resources to fuel their own growth may increase the chances of host death and thus failed transmission. This

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Table 3. Studies on complex life cycle helminths that have examined the relationship between intensity, larval parasite growth and intermediate host mortality

(For each parasite species, some aspect of larval growth, typically the final size, was used to estimate the impact on the host. The relationship between this growth character and intensity was then categorized as increasing proportionally (light grey box in Fig. 1A), increasing but with a decreasing slope (dark grey box in Fig. 1A), or not increasing at all. Patterns of intensity-dependent mortality (IDM) were considered to be either proportional to intensity, a discontinuous function of intensity, or absent, i.e. no IDM.)

Species*	Intermediate host	Typical natural intensity	Experimental intensity	Growth character used to gauge burden on host	Shape of intensity-burden relationship	Pattern of IDM	Comment/critique	Reference
Schistocephalus solidus (C)	copepod	1	1 to 3	asymptotic size and relative growth rate	decreasing slope	discontinuous, decrease from intensity = 3		Michaud *et al*. (2006)
Triaenophorus crassus (C)	copepod	1	1 to 4	size of 21 to 28 day old worms	decreasing slope	discontinuous, decrease from intensity = 3		Rosen and Dick (1983)
Bothriocephalus claviceps (C)	copepod	1	up to an average of 14	mean size of 10 day old worms	proportional	proportional	at higher intensities total worm size might level off	Nie and Kennedy (1993)
Lacistorhynchus tenuis (C)	copepod	1	1 to more than 50	size of 21 day old worms	decreasing slope	none		Sakanari and Moser (1985)
Hymenolepis diminuta (C)	beetle	tens	an average over 60 with highest dose	size of worms older than 2 weeks	decreasing slope	proportional		Keymer (1980)
Acanthocephalus lucii (A)	isopod	1	average of 14	total worm size relative to time, early-growth, late-final size	early infection – proportional, later infection – flat	none		Benesh and Valtonen (2007)
Corynosoma constrictum (A)	amphipod	1	up to an average of 6	—	probably decreasing slope in the intensity range observed	discontinuous, decrease from intensity = 6	Intensity-burden relationship based on that in related Polymorphus species (Petrochenko, 1971)	Duclos *et al*. (2006)
Leptorhynchoides thecatus (A)	amphipod	? probably low	up to an average of 5	size of worms older than 32 days	proportional	none		Uznanski and Nickol (1980); Steinauer and Nickol (2003)
Elaphostrongylus rangiferi (N)	terrestrial snail (juveniles)	less than 5	up to more than 50	growth rate and size at 28 days post infection	proportional	proportional	a high intensity did not affect the survival of adult snails	Skorping, (1984, 1985)
Camallanus lacustris (N)	male copepods	1	1 to 4	asymptotic size and relative growth rate	proportional	none	at higher intensities total worm size might level off	this study
C. lacustris (N)	female copepods	1	1 to 6	asymptotic size and relative growth rate	proportional	discontinuous, decrease from intensity = 3		this study
Maritrema novizealandiae (T)	amphipod	up to 24	up to 60	metacercaria size after 10 weeks	proportional	discontinous, decrease at cercarial doses $\geqslant 25$	mortality attributed to penetration rather than growth	Fredensborg *et al*. (2004); Fredensborg and Poulin (2005)

* (C), cestode; (A), acanthocephalan; (N), nematode; (T), trematode.

Table 4. Studies on complex life cycle helminths that reported the relationship between intensity and some aspect of larval growth, typically the final size

(The relationship between this growth character and intensity was then categorized as increasing proportionally (light grey box in Fig. 1A), increasing but with a decreasing slope (dark grey box in Fig. 1A), or not increasing at all.)

Species*	Intermediate host	Typical natural intensity	Experimental intensity	Growth character used to gauge burden on host	Shape of intensity-burden relationship	Comment/critique	Reference
Anoplocephala perfoliata (C)	mite	probably 1	1 to 9	cysticercoid volume	proportional	at highest intensities total volume may level off	Trowe (1997)
Paranoplocephala mamillia (C)	mite	probably 1	1 to 3	cysticercoid volume	proportional	"	Trowe (1997)
Moniezia benedeni (C)	mite	probably 1	1 to 5	cysticercoid volume	proportional	"	Trowe (1997)
Mo. expansa (C)	mite	probably 1	1 to 4	cysticercoid volume	proportional	"	Trowe (1997)
Monoecocestus sp. (C)	mite	probably 1	1 to 6	cysticercoid diameter	proportional		Freeman (1952)
Diorchis inflata (C)	copepod	up to 4	1 to 4	cysticercoid diameter	proportional	For the species in this Table, Valkounova stated that size was unaffected by intensity, but that size decreased with intensity in three other species. No data were given, however, so the shape of the decrease is unknown.	Valkounova (1980)
D. ransomi (C)	copepod	up to 4	1 to 4	cysticercoid diameter	proportional		Valkounova (1980)
Microsomacanthus compressa (C)	copepod	up to 4	1 to 4	cysticercoid diameter	proportional		Valkounova (1980)
Mi. paracompressa (C)	copepod	up to 4	1 to 4	cysticercoid diameter	proportional		Valkounova (1980)
Mi. paramicrosoma (C)	copepod	up to 4	1 to 4	cysticercoid diameter	proportional		Valkounova (1980)
Sobolevicanthus gracilis (C)	copepod	up to 4	1 to 4	cysticercoid diameter	proportional		Valkounova (1980)
So. krabbeella (C)	copepod	up to 4	1 to 4	cysticercoid diameter	proportional		Valkounova (1980)
So. octacantha (C)	copepod	up to 4	1 to 4	cysticercoid diameter	proportional		Valkounova (1980)
Schistocephalus solidus (C)	fish	up to 10	—	plerocercoid weight	decreasing slope	naturally-infected fish	Heins *et al*. (2002)
Acanthocephalus anguillae (A)	isopod	1	1 to 14	cystacanth volume	decreasing slope	total worm size increases proportionally until intensity of ~5	Pilecka-Rapacz (1986)
Ac. ranae (A)	isopod	1	1 to 6	cystacanth volume	proportional		Pilecka-Rapacz (1986)
Echinorhynchus borealis (A)	amphipod	up to 9	—	cystacanth length and width	proportional	natural infections	Benesh, unpublished data
Pomphorhynchus laevis (A)	amphipod	1	1 to 4	cystacanth volume	proportional		Cornet (2011)
Profilicollis sp. (A)	crab	tens to more than 100	—	cystacanth volume	proportional	natural infections	Poulin *et al*. (2003)
Physaloptera maxillaris (N)	cricket	?	up to 200	L3 length	proportional		Cawthorn and Anderson (1976)
Coitocaecum parvum (T)	amphipod	1 to 3	—	metacercaria volume	proportional	natural infections, crowding was only observed in progenetic larvae	Lagrue and Poulin (2008)
Maritrema novizealandiae (T)	isopod	up to 170	—	metacercaria volume	proportional	natural infections	Saldanha *et al*. (2009)
Ma. novizealandiae (T)	crab	up to 300	—		proportional	natural infections	Fredensborg and Poulin (2005)

* (C), cestode; (A), acanthocephalan; (N), nematode; (T), trematode.

trade-off between using intermediate hosts for nutrition and transportation is thought to shape parasite life-history strategies (Choisy *et al*. 2003; Parker *et al*. 2003*b*; Iwasa and Wada, 2006; Ball *et al*. 2008; Parker *et al*. 2009*a*; Chubb *et al*. 2010). Experimental manipulation of the burden on the host by using a range of infection intensities is one way to evaluate this trade-off, and I have summarized the results from such studies. Only 15% of the cases in Tables 3 and 4 involve the second intermediate host; most studies to date focus on the first host. Helminths usually enter the first intermediate host as eggs or small larvae before rapidly increasing in size, so growth costs and IDM are intuitively expected. Size at establishment in the second intermediate host is much larger, which likely favours reduced growth or paratenesis (Poulin and Latham, 2003; Parker *et al*. 2009*a*; Chubb *et al*. 2010). Perhaps researchers have assumed that the relatively low levels of growth in second intermediate hosts are unlikely to affect host viability, explaining the fewer studies at this life-cycle stage.

What is the typical growth-intensity relationship and what does it imply?

In all studies to date, the overall parasite mass increased with intensity, but in some species, this increase decelerated at high intensities. These observations indicate that (1) at low, natural intensities individual parasites do not exploit the host at a maximum level, and (2) resource ceilings are detectable and some species may approach them sooner than others. When resource ceilings have been observed, it has been at either extreme intensities (Pilecka-Rapacz, 1986; Heins *et al*. 2002; Benesh and Valtonen, 2007) or in small hosts (Rosen and Dick, 1983; Sakanari and Moser, 1985; Michaud *et al*. 2006). Indeed, in *C. lacustris* there were hints of a resource ceiling in male copepods but not in larger female copepods. Even here, though, indications of a ceiling only appear at an intensity of 3, which is probably 3 times higher than the normal infection level. Thus, at low intensities larval growth is not obviously constrained by resources.

Why don't larval worms take advantage of all resources available? One possibility is that the benefits associated with larger size are marginal. For instance, if worms are able to grow faster with lower mortality in the next host, then there may be little incentive to spend a long time extensively exploiting the first host (Werner and Gilliam, 1984; Abrams *et al*. 1996; Choisy *et al*. 2003; Parker *et al*. 2003*a*; Gandon, 2004; Iwasa and Wada, 2006; Ball *et al*. 2008; Parker *et al*. 2009*a*). Additionally, the exact relationship between a large larval size and its potential advantages, like increased infectivity (Rosen and Dick, 1983; Steinauer and Nickol, 2003), is not well explored. Perhaps extremely large worms reap few additional fitness benefits. The benefits associated with rapid growth seem less disputable; the faster worms reach a minimum size for infectivity, the less likely they will die before transmission (Day and Rowe, 2002). Although overall parasite biomass tends to accumulate faster with increasing intensity, this does not necessarily imply that individual parasites could grow faster. Individual growth might be limited by some intrinsic factor, like the speed of nutrient uptake, rather than overall resource availability. However, under normal circumstances most organisms do not grow at a maximum rate (Gotthard, 2001; Metcalfe and Monaghan, 2001), and the substantial phenotypic plasticity and considerable variation across species hints that larval helminth growth may be rather labile (Shostak *et al*. 2008; Benesh, 2010*a*). Despite these alternatives, the most frequently cited limitation on parasite growth is virulence costs.

Does growth determine IDM? And are natural levels of intermediate host exploitation risky?

As total parasite growth usually increases with intensity, we expect host mortality to be intensity dependent, assuming there is a trade-off between growth and mortality. In 2 cases, host mortality has indeed been observed to increase proportionally with intensity (Skorping, 1984, 1985; Nie and Kennedy, 1993). Surprisingly, though, a number of studies found discontinuous IDM, suggesting only extreme parasite burdens reduce host survival. And some studies have failed to find any IDM, such as the case study presented here. Presumably, all *C. lacustris* larvae must reach a threshold size to moult to the L3 stage. As all worms need to achieve some minimum amount of growth, virulence-reducing plastic responses to intensity are perhaps less likely, such as smaller final sizes (Parker *et al*. 2003*b*). Although final size appears somewhat flexible, given the different sizes attained in male and female hosts, the burden on the host increased proportionally with intensity. However, no IDM was observed in male copepods and in females IDM was only observed at the highest intensities. Thus, copepods were able to tolerate all but the most extreme parasite burdens. This result is not unique; the literature summary indicates that intermediate hosts can often survive exceptional infection levels. This implies that at low, natural intensities, larval growth is often not at a level that induces any host mortality (Uznanski and Nickol, 1980; Poulin *et al*. 1992; Hurd *et al*. 2001; Guinnee and Moore, 2004; Benesh, 2010*b*).

In female copepods infected with *C. lacustris*, IDM was time dependent. Decreased survival in hosts with 4 or more worms occurred mainly after 20 days post-exposure (dpe), when worm growth had nearly slowed to a stop. This is surprising, as host exploitation presumably slows with the arrest of worm growth, and it suggests that the mortality

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associated with excessive growth may be delayed. The vast majority of worms reached the L3 stage by 11 dpe (data not shown, but in accordance with Moravec (1969)), so most appear to be capable of transmission before any increase in mortality. Thus, growth costs could be completely avoided if transmission occurs quickly enough. Day (2003) argued that if virulence costs (e.g. a decrease in transmission due to host mortality) usually occur after virulence benefits have been accrued (e.g. a large larval size), then selection should favour increased virulence, because there is only a low probability that its costs will be paid. Parker *et al*. (2009*a*) assumed that larval growth arrest serves to reduce host mortality. If mortality actually increases after arrest, as observed here, then there is no clear reason for parasites to stop growing, and a highly aggressive growth strategy may be optimal (Parker *et al*. 2009*a*). The delayed mortality of copepods heavily infected with *C. lacustris* further suggests that individual parasites are much less aggressive that they could be and perhaps less than what is theoretically favourable. Future studies should also note the temporal pattern of IDM, given the important implications for virulence evolution (Chubb *et al*. 2010).

To conclude, overall parasite growth/size generally increases with intensity, but frequently this does not result in a proportional increase in host mortality. Indeed, IDM is often absent or only observed at extreme levels. This indicates that (1) at natural intensity levels parasites do not exploit the host as much as they presumably could and that (2) increased growth would not entail obvious mortality costs. It is important to note that all these studies have been conducted in rather benign laboratory conditions, i.e. *ad libitum* food, absence of predation and competition. Under less favourable conditions, larval parasite growth may have a much larger impact on host survival. A number of field surveys have uncovered patterns indicative of IDM in larval helminths (Crofton, 1971; Amin *et al*. 1980; Brattey, 1986; Thomas *et al*. 1995; Brown *et al*. 2001; Latham and Poulin, 2002; Brown *et al*. 2003; Outreman *et al*. 2007; Bates *et al*. 2010; Heins *et al*. 2010), but the source of host mortality cannot usually be unambiguously inferred (e.g. transmission to the next host or just a generally higher death rate?), so the impact on the costs and benefits of larval parasite growth is unclear. Undoubtedly, additional experiments incorporating natural causes of mortality, such as food limitation or predation, are needed before concluding that mortality is not an important constraint on larval helminth growth.

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