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ORIGINAL PAPER

Encystment patterns and metacercarial size of an opecoelid trematode in two polychaete hosts

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Abstract The growth, and thus to some extent the fitness, of parasites may vary among individuals based on a range of factors including which host species they infect or how many other parasites share the host with them. Here, we investigate the determinants of the size attained by metacercariae of an opecoelid trematode within two species of intertidal polychaetes serving as second intermediate host, *Heteromastus filiformis* and *Abarenicola affinis*. Metacercarial cysts were found predominantly in the anterior region of *H. filiformis*, particularly in segments 3–6, whereas no apparent pattern of encystment was observed in *A. affinis*. No relationship was found between the volume achieved by an individual metacercarial cyst and either host body volume, total number of cysts per host, the segment number in which a cyst was located, or the number of other cysts sharing a distinct segment; this was true for parasites in either polychaete species. However, cysts were found to be significantly larger, on average, within *A. affinis* than within *H. filiformis*. It remains to be determined whether the polychaete species in which larger sizes are achieved is also the one in which transmission to fish hosts is maximized.

Introduction

For many trematode species with low degrees of host specificity, organisms belonging to the same taxonomic group or ecological niche often serve interchangeably as potential second intermediate hosts (Galaktionov and Dobrovolskij 2003; Leung et al. 2009). As long as they are physiologically and immunologically compatible with the host organism, many trophically transmitted trematodes can utilize any species considered prey by the definitive host in order to complete their life cycles (Koehler and Poulin 2010). However, not all potential intermediate hosts are equally suitable; trematodes may achieve higher growth or larger final sizes in certain host species than in others.

Availability of resources is one of the most important variables determining the growth and reproductive success of any organism. For endoparasitic trematodes, the host represents not only the surrounding environment but also the sum of the available potential resources at their disposal. Given the relative size between an average trematode and its host, the host generally provides ample resources for trematode development, although the supply may become stretched close to its limits when several trematodes share the same host (Poulin 2007). It is not uncommon for trematode metacercariae within the second intermediate host to exhibit not only a range of sizes but also substantial growth while encysted (Shaw 1933; McCurdy 2001; Poulin and Latham 2003). Indeed, for endoparasitic trematode communities within second intermediate hosts, the number of conspecific and/or heterospecific individuals requiring similar resources often has the potential to limit the final size of any given metacercarial cyst (Brown et al. 2003; Fredensborg and Poulin 2005; Saldanha et al. 2009), indirectly affecting its overall fitness (Fredensborg and Poulin 2005; Poulin and Latham 2003).

Here, we investigate the determinants of the size achieved by trematode metacercariae (family Opecoelidae) in their intermediate hosts. Metacercariae of an opecoelid trematode related to, but genetically distinct from, the only

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other opecoelids known from the area (see Donald et al. 2004, 2007) are found encysted within the body musculature of the polychaetes *Heteromastus filiformis* (Capitellidae) and *Abarenicola affinis* (Arenicolidae; Leung et al. 2009). Tentatively termed opecoelid E (see Leung et al. 2009), encysted metacercariae of this trematode within *H. filiformis* and *A. affinis* show variations in size, potentially indicating metacercarial growth occurring post-encystment within these two host polychaetes. Additionally, the sites of encystment for invading cercariae that develop into metacercariae also vary, with some host segments harboring many metacercariae and others harboring only one, an uneven distribution that may favor variability in final cyst sizes because competition for resources should be more intense between metacercariae sharing the same host segment than among those from different segments.

Polychaete growth occurs with the addition of new segments in the germinal region just in front of the pygidium. Thus, in adult polychaetes the oldest segments are those closest to the head of the animal (Barnes et al. 1988). In younger polychaetes, the rate of segment proliferation is usually higher than in older ones. However, the rate of segment production typically slows with age, as older segments become enlarged because of gamete production (Barnes et al. 1988). Infective cercariae may follow one of two encystment patterns upon penetration of a second intermediate host: (a) they migrate to a physiologically ideal location before encysting in a targeted manner, or (b) they encyst almost immediately upon penetration in a generalized manner (Taylor and Hall 1993; Schotthoefer et al. 2003; Matisz et al. 2010). If a correlation exists between polychaete length and total number of segments, depending on the polychaete's life history, the location of encysted metacercariae within its body could indicate the time of infection relative to the polychaete's life. Alternatively, some segments of a polychaete may be more exposed to infection than others, leading to uneven and crowded infections in a subset of segments. The distribution of metacercariae among host segments therefore determines the intensity of competition for resources, which should be highest when parasites are concentrated in a few shared segments. The first aim of the current study will be to describe the distribution of encysted metacercariae along the body of both *H. filiformis* and *A. affinis*.

A polychaete represents a shared habitat for the encysted metacercariae it harbors. As with free living organisms, both interspecific and intraspecific competition for resources within the host can influence aspects of individual parasite fitness (Fredensborg and Poulin 2005; Gower and Webster 2005). We hypothesize that the size of metacercariae should be greatest (a) in older hosts or older/anterior segments, which may harbor older parasites, (b) in larger hosts, which should provide more resources, and (c) in segments with single metacercariae, where resource competition among parasites should be weak. The second aim of this study will be to determine what factors affect the size of an individual metacercaria. Relationships between metacercarial size and host age (measured as the total number of host segments), host body volume, the segment number in which a particular cyst is located, and the number of other cysts (if any) sharing that segment will be examined.

Materials and methods

Polychaetes were collected in Lower Portobello Bay, within Otago Harbor, Dunedin, New Zealand (45°50′S, 170°40′E) from June to October 2009 (austral winter) as part of a survey of the local polychaete fauna. Roughly equal numbers of both target species were collected on each occasions. Specimens of the polychaete *H. filiformis* were collected using a 0.5 mm sieve. *A. affinis* were collected using targeted sampling, as visible castings on the surface sediment indicated their location. Active collection ceased once >40 individuals of both *H. filiformis* and *A. affinis* were obtained.

Live polychaetes were brought back to the laboratory and placed in 3 l containers lined with 3 cm of surface sediment and filled with seawater. Polychaetes were kept at 16°C for up to 1 week following collection, at which time they were measured and examined for parasites. Measurements of total body length, maximum thoracic width (following Young and Young 1978; Micaletto et al. 2002), and number of segments were recorded under an Olympus CX-41 compound microscope for *H. filiformis* and under an Olympus SZ-ST dissection microscope for *A. affinis*. Polychaetes were pressed between two glass plates in order to observe and count encysted metacercariae present within the polychaete tissue and coelom (following Vanoverschelde and Vaes 1980; McCurdy 2001). Due to their large sizes and wide girths, specimens of *A. affinis* were cut into manageable pieces, which were then sliced along the anterior–posterior axis, in order to open up the body cavity, before being pressed between glass slides. Length and width measurements were made for each encysted metacercaria, and the segment in which each parasite was located was also recorded, with segments numbered starting from the anterior end of the polychaete. Trematode cysts were identified by sequencing about 700 base pairs of the cytochrome oxidase 1 gene as part of another study (for details of genetic analyses see Leung et al. 2009), and all sequenced specimens consisted of the as yet undescribed opecoelid E (GenBank accession numbers, FJ765504–FJ765508).

Polychaete volume was calculated using the formula for the volume of a cylinder:

$$V = \pi r^2 h$$

where r is half the maximum thoracic width, and h is body length. Metacercarial cyst volume was calculated by using the formula for the volume of a scalene ellipsoid:

$$V = \left(\frac{4}{3}\right)\pi\left(\frac{a}{2}\right)\left(\frac{b}{2}\right)\left(\frac{c}{2}\right)$$

where a is cyst length and b is cyst width, and c, the height, was approximated as half of the width, or b/2.

Upon collection, a certain portion the *H. filiformis* specimens gathered during the survey were found to fragment (i.e., break into two or more pieces). For the purposes of the current study, all relatively long anterior-end fragments and complete specimens of *H. filiformis* were considered either separately or together, depending on the analysis.

Generalized linear models (GLMs) were used to determine what factors affect cyst volume; a separate GLM was performed separately for each host species. Cyst volumes were log-transformed to achieve normality (Kolmogorov–Smirnov–Lilliefors test, $P>0.05$), thus all models had normal error structure and identity link function, as these provided the best match for the data. The four explanatory variables considered were: total number of segments in the host, host body volume, the segment number in which the cyst was located, and the number of other cysts sharing that segment. After starting with a full model, significance levels were based on the deviance explained by each factor, based on χ^2 statistics, following backward stepwise elimination of nonsignificant ($P>0.05$) terms. Subsequently, a two-sample t-test was used to determine if there were any differences in cyst volume between cysts from *H. filiformis* and cysts from *A. affinis*. The statistical analysis program JMP 7.0 was used to perform all tests.

Results

Data on the total number of specimens collected, average polychaete size, and parasite prevalence are reported in Table 1. A linear relationship was found to exist between the number of segments and overall polychaete length in *H. filiformis* ($r^2=0.795$, $P<0.001$), but not in *A. affinis* ($P=0.74$). Most encysted metacercariae were found predominantly in the anterior region, particularly in segments 3–6 within *H. filiformis* (Fig. 1). In contrast, encysted metacercariae showed no obvious generalized pattern of encystment and were more evenly distributed across all segments within *A. affinis* (Fig. 2).

In both host species, the frequency distributions of cyst volumes were skewed, so that most cysts had volumes closer to the lower end of the observed range (Figs. 3 and 4). Based on theGLMs, none of the four explanatory variables (total number of host segments, host body volume, the segment number in which the cyst was located, and the number of other cysts sharing that segment) had any effect on cyst volume, in either polychaete species (all $P>0.05$ for the full models or any subsets of factors). For *H. filiformis*, this was true whether all individuals were included, either complete or anterior fragments, or whether only complete polychaetes were included in the analysis. However, the two-sample t-test performed on log-transformed cyst volumes revealed that cysts were significantly larger in *A. affinis* when compared to cysts from *H. filiformis* ($t=5.89$, $df=207$, $P<0.0001$; Fig. 5).

Discussion

Patterns of growth and parasite accumulation differed between *H. filiformis* and *A. affinis*. In *H. filiformis*, a linear relationship was found between the total number of segments and polychaete length, indicating that *H. filiformis* grows directly as a result of the addition of new segments. In contrast, there was no such relationship in *A. affinis*, suggesting that two individuals with the same number of segments can have very different body lengths. Metacercarial encystment patterns of opecoelid E also differed between the two polychaete species. Within *H. filiformis* encysted metacercariae were found predominantly in the anterior portion of the polychaete, particularly in segments 3–6. Within *A. affinis* encysted metacercariae showed no overall tendency to occur in any particular segment/group of segments.

A. affinis lives in a mucous-lined U-shaped tunnel with its posterior end ascending up one side of its burrow. Through peristaltic action of longitudinal muscles it maintains a near-constant water flow that helps to oxygenate its burrow as well as aid in the delivery of food particles from the water column (Barrow and Wells 1982). A space between the polychaete and the walls of its burrow encourages water flow. Cercariae of opecoelid E most likely infect *A. affinis* through the water current created by the feeding polychaete. Cercariae in the polychaete's feeding current would be passed along the polychaete's body, eventually attaching to the polychaete, penetrating and encysting within it. Some cercariae appear to penetrate polychaetes through the gills (Rangel and Santos 2009); in *A. affinis*, well-developed gills occur along most of the body. The semiregular distribution of encysted metacercariae observed along the body of *A. affinis* might be due to

Table 1 Measurements of polychaete hosts, prevalence of opecoelid E, and total number of opecoelid E metacercariae from two species of polychaetes

Polychaete species	Number examined	Mean length±SE (mm)	Mean no. segments±SE	Number of infected hosts (prevalence,%)	Total no. metacercariae (mean no. per infected host)
Heteromastus filiformis	71	23.6±2.1	69.9±8.0	44 (61.9%)	128 (2.9)
Abarenicola affinis	46	80.9±5.2	148.1±3.4	22 (47.8%)	82 (3.7)

cercariae of opecoelid E encysting near their respective sites of penetration.

Compared to *A. affinis*, the tunnels of *H. filiformis* are exceptionally narrow, and leave very little, if any, leeway between the polychaete and the walls of its burrow. *H. filiformis* lives by positioning itself in an indiscriminately shaped burrow perpendicular to the surface sediment, its anterior end facing downwards. In this position, it feeds on microbial biota and decaying matter at the aerobic/anaerobic interface (Rouse and Pleijel 2001). This position leaves its anterior segments relatively sheltered from the water column (i.e., potential cercarial infection) when compared to its posterior segments. This suggests that infection must occur early in the life of the polychaete, when it consists of a few segments only. All polychaetes begin their lives as planktonic trochophore larvae before metamorphosing into vermiform shapes and settling into the benthic environment (Qian 1999; Huang and Hadfield 2003). Metamorphosed polychaetes continue to grow by adding posterior segments (Barnes et al. 1988). If the cercariae of opecoelid E encyst within *H. filiformis* near their respective sites of penetration, the segments in which they are located should indicate at what point infection occurred during the growth of the host polychaete. The predominance of encysted metacercariae in segments 3–6 suggests a high rate of infection at the time during which these segments were being added. This conclusion assumes that cercariae of opecoelid E do not migrate upon penetration to targeted locations within the body of *H. filiformis*, but instead encyst within the segment initially penetrated.

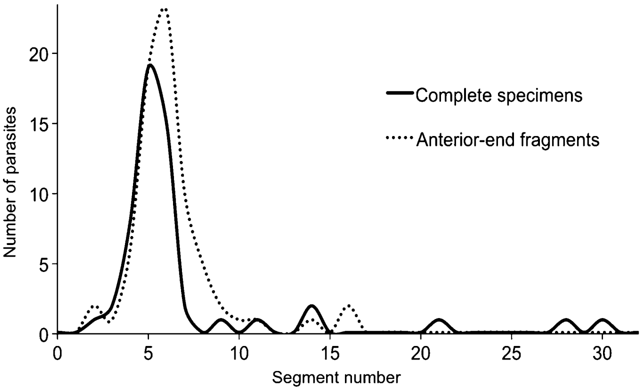

Fig. 1 Distribution of encysted metacercariae collected from all infected *Heteromastus filiformis* (anterior-end fragments and complete specimens), in relation to segment of encystment

There are several reports of parasites encysting within the anterior regions of host polychaetes, particularly in those species of polychaete that spend a good portion of their lives burrowed and/or protected by a tube (Brown and Prezant 1986; Rangel and Santos 2009). In these species, the anterior end is the only portion of the polychaete's body exposed to the water column. The behavior of the host polychaete combined with the observed infection patterns indicate that invading cercariae simply encyst near the site of initial contact with the host (i.e., the only exposed region). Thus in *H. filiformis*, with opecoelid E found almost exclusively in the anterior segments, the polychaete must only be susceptible to infection when at an early age while it is only a few segments long.

An alternate explanation for the encystment patterns observed in *H. filiformis* involves the location of primary host resources within the polychaete. The pharynx and blood vessels capable of peristaltic action are located in the first ten segments and may represent areas of high-energy supply within the host polychaete. Thus an alternative explanation could be that invading cercariae migrate to this region prior to encystment in order to access more readily available nutrients. Although the distribution of cysts in *A. affinis* is more evenly spread out across the entire body of the polychaete, encysted metacercariae were consistently found near major blood vessels, further suggesting a correlation between the location of encystment and areas

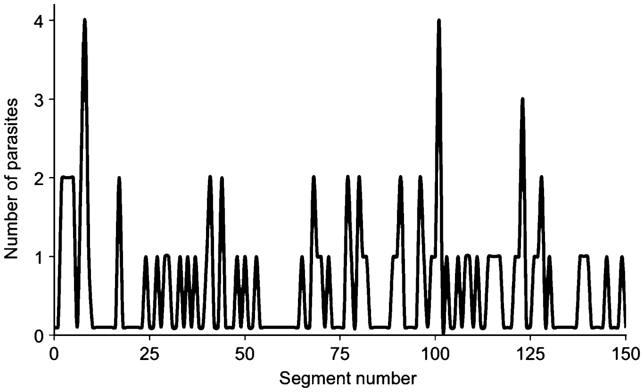

Fig. 2 Distribution of encysted metacercariae collected from all infected *Abarenicola affinis* in relation to segment of encystment

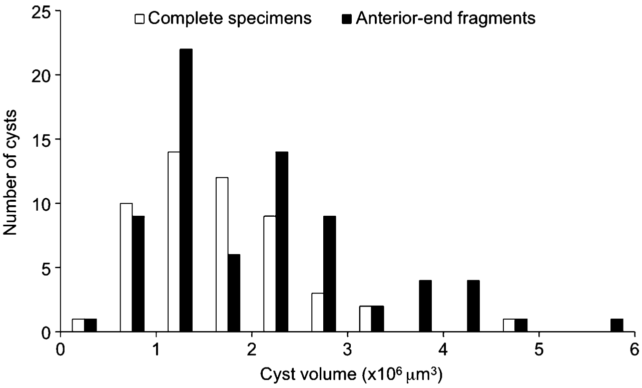

Fig. 3 Frequency distribution of metacercarial cyst volumes from *Heteromastus filiformis* (anterior-end fragments and complete specimens)

within the host polychaete associated with high energy supply. Previous authors have made this suggestion regarding other trematode species encysting near major blood vessels within polychaete hosts (Shaw 1933; Vandergon et al. 1988; Vanoverschelde and Vaes 1980). Further information regarding cercarial behavior is needed before encystment patterns of opecoelid E can be fully understood.

No relationship was found between the volume achieved by an individual metacercarial cyst, and either host body volume, the total number of cysts per host, the segment number in which the particular cyst was located, or the number of other cysts sharing a distinct segment. This was true for parasites in either polychaete species. There was also no difference in cyst volume between cysts from anterior-end fragments and cysts from complete specimens of *H. filiformis*. These findings do not support the hypothesis that competition over host resources exists among metacercariae of opecoelid E, as there are more metacercarial cysts, on average, in the anterior segments of *H. filiformis*. There was also no evidence that metacercariae achieve larger sizes in larger hosts within a given polychaete species, or within distinct areas of a particular host. However, it was clear that metacercariae within one polychaete species achieve a larger size, on average. *A. affinis* is a much larger polychaete and provides, to a parasite, a greater amount of potential host resources and weaker space constraints when compared to *H. filiformis*. Accordingly, metacercariae within *A. affinis* were found to be significantly larger, by about 50%, on average, than metacercariae within *H. filiformis*, indicating that host size is relevant to parasite size and that growth of metacercariae may occur post-encystment. Similar findings have been reported by Rangel and Santos (2009) regarding the trematode *Gymnophallus choledochus* encysting within several polychaete species of varying sizes. In our system, the implication could be that *A. affinis* is a higher-quality host than *H. filiformis* since metacercarial size is a key determinant of later adult size and fecundity in trematodes (Fredensborg and Poulin 2005; Poulin and Latham 2003).

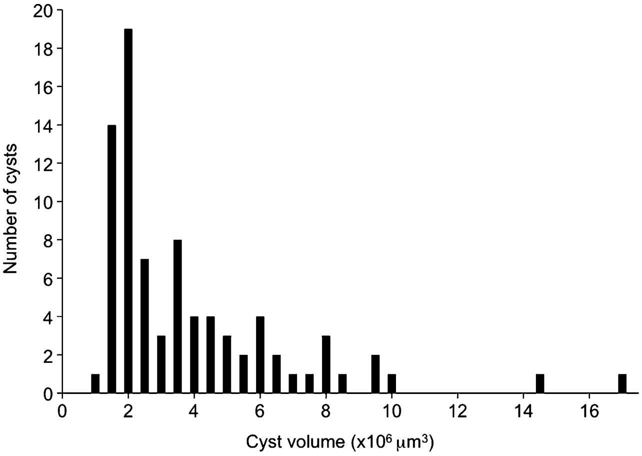

Fig. 4 Frequency distribution of metacercarial cyst volumes from *Abarenicola affinis*

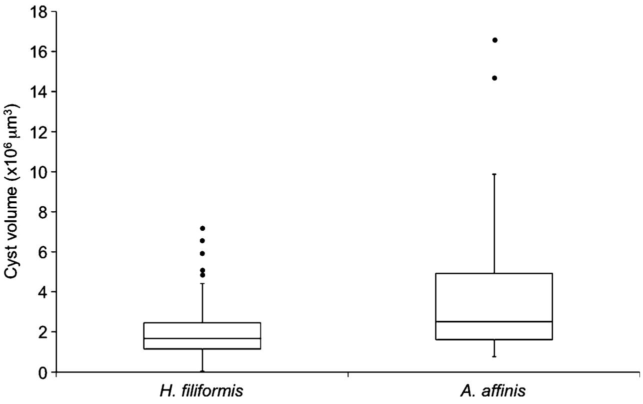

Fig. 5 Cyst volumes (µm^3) of encysted metacercariae of opecoelid E collected from parasitized individuals of both *Heteromastus filiformis* (anterior-end fragments and complete specimens, total=128 cysts) and *Abarenicola affinis* (n=82 cysts), showing median, interquartile ranges and outliers

Opecoelids develop as adults within the digestive tracts of fish belonging to numerous different families (Cribb 2005; Durio and Manter 1968; Bray and Justine 2007). While the definitive host of opecoelid E is most undoubtedly a fish, the identity of the gastropod serving as its first intermediate host remains entirely unknown. What little is known about this trematode is based solely on observations of metacercariae encysted within polychaete second intermediate hosts. Our study revealed that within-host factors play no significant role in determining metacercarial sizes; in contrast, the identity of the host species has substantial implications for metacercarial growth. It remains to be determined whether the polychaete species in which growth is highest, i.e., *A. affinis*, is also the one in which transmission to fish hosts is maximized. Future studies aiming to uncover the full life history of opecoelid E, as well as shedding further light on the relationships between the larval stages of this trematode and its second intermediate polychaete hosts, are needed to evaluate the parasite's role in the benthic ecosystem.

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