**Changing prevalence of *Fasciola hepatica* in response to damming**

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**Abstract**

Fascioliasis [liver flukes] is an infectious disease caused by the trematode parasite *Fasciola hepatica* that causes significant economic losses in livestock worldwide (Toet *et al.* 2014) and is increasingly prevalent in humans (Ashrafi *et al.* 2014). Current forms of treatment have proven ineffective, and no vaccine exists (Toet *et al.* 2014). Previous studies have found trematode cercariae are susceptible to damage at higher water velocities (Jewsbury 1985) and increased prevalence of schistosomiasis, the infection brought on by *F. hepatica*'s close relation *Schistosoma*, occurs as a result of damming (Steinmann *et al.* 2006). Based on these findings, I am proposing a study that will test dammed and undammed sites on a global scale to determine if fascioliasis prevalence increases in response to damming. This study will also determine if higher water velocities prevent *F. hepatica* cercariae from infecting their definitive, and if so will quantify a threshold upper water velocity above which *F. hepatica* are unable to effect their definitive hosts. The data from this study can then be utilized to reform management of aquatic resources in regions where fascioliasis is endemic.

## Introduction

*Fasciola hepatica*, a trematode platyhelminth, is found on every continent save Antarctica (Mas-Coma *et al.* 2005). Over the course of its life history, *F. hepatica* occupies two hosts – an intermediate lymnaeid snail host, in which it asexually reproduces, and a definitive mammal host, which disperses the parasite's eggs in feces [see **Fig. 1**]. This second host is typically livestock, such as a cow or sheep, but *F. hepatica* will also take up residence in humans (Saba *et al.* 2005). Fascioliasis is the disease brought on by *F. hepatica* within its definitive host, which can result in liver damage, internal hemorrhaging, and eventual mortality if left untreated (Moazeni and Ahmadi 2016). Livestock typically ingest infective *F. hepatica* metacercariae by eating freshwater plants or drinking contaminated water. Humans can ingest metacercariae through these same pathways, but may also be infected by consuming plants cultivated in freshwater [e.g. watercress], washing objects with contaminated water, or consuming raw liver containing infected metacercariae. Though uncommonly found in humans in the developed world, fascioliasis persists in the developing world due to shared water resources between humans and livestock, poor sanitation, and minimal hygienic practices (Ashrafi *et al.* 2014).

Historically, research on fascioliasis has focused on livestock (Mas-Coma *et al.* 1999), understandably, as the annual costs of livestock losses to fascioliasis are estimated to exceed US $3 billion (Toet *et al.* 2014). Since the 1990s, however, reports of human infection have steadily increased in developing nations, and isolated incidents have been reported in developed nations due to import of contaminated produce from endemic regions (Ashrafi *et al.* 2014). Calculations in 2010 by the World Health Organization found that food-borne trematodiases, of which fascioliasis is a member, were the cause of 169,017 DALYs [a unit that adds years of life lost with years of disability due to disease] that year (Wood *et al.* press).

*Schistosoma*, the trematode behind another infectious disease known as schistosomiasis, is a close relative of *F. hepatica*. While some parallels may be drawn between *F. hepatica* and *Schistosoma*, there are key differences in their life histories. *F. hepatica* reproduces asexually in its intermediate snail host to produce cercariae, which then disperse and encyst on aquatic vegetation as metacercariae. These metacercariae are then ingested by the definitive host, at which point the *F. hepatica* flukes penetrate and encyst in the liver (Nithiuthai *et al.* 2004). *Schistosoma* also reproduces asexually in its intermediate snail host to produce cercariae, but these cercariae then directly penetrate the definitive host, often a human bathing in contaminated water (Gordon and Griffiths 1951). *F. hepatica* and *Schistosoma* cercariae have similar morphology – a definitive body and tail [see **Fig. 1**] – though they ultimately serve different functions within their respective life cycles (Nithiuthai *et al*. 2004).

Numerous studies over the past few decades have found that damming downstream increases the prevalence of schistosomiasis in communities upstream (Hassan *et al.* 2014), (N'Goran *et al.* 1997), (Pretorius *et al.* 1989), (Oladejo and Ofoezie 2006). In one study conducted in Ghana's Upper Region, the introduction of clay-core dams increased schistosomiasis prevalence from 17% of the population to 51% (Hunter 2003). Schistosoma cercariae are susceptible to damage of their body-tail junction at higher water speeds, and once cercariae are damaged they are unable to infect hosts (Jewsbury 1985). As damming slows water velocity, the institution of dams may enable more *Schistosoma* cercariae to survive long enough to reach their hosts, resulting in higher prevalence of schistosomiasis. Since *Fasciola* cercariae have similar morphology to *Schistosoma* cercariae, I believe damming will also increase the prevalence of fascioliasis in a region, and increased water velocities will render *Fasciola* cercariae incapable of encysting on aquatic vegetation and infecting their definitive hosts.

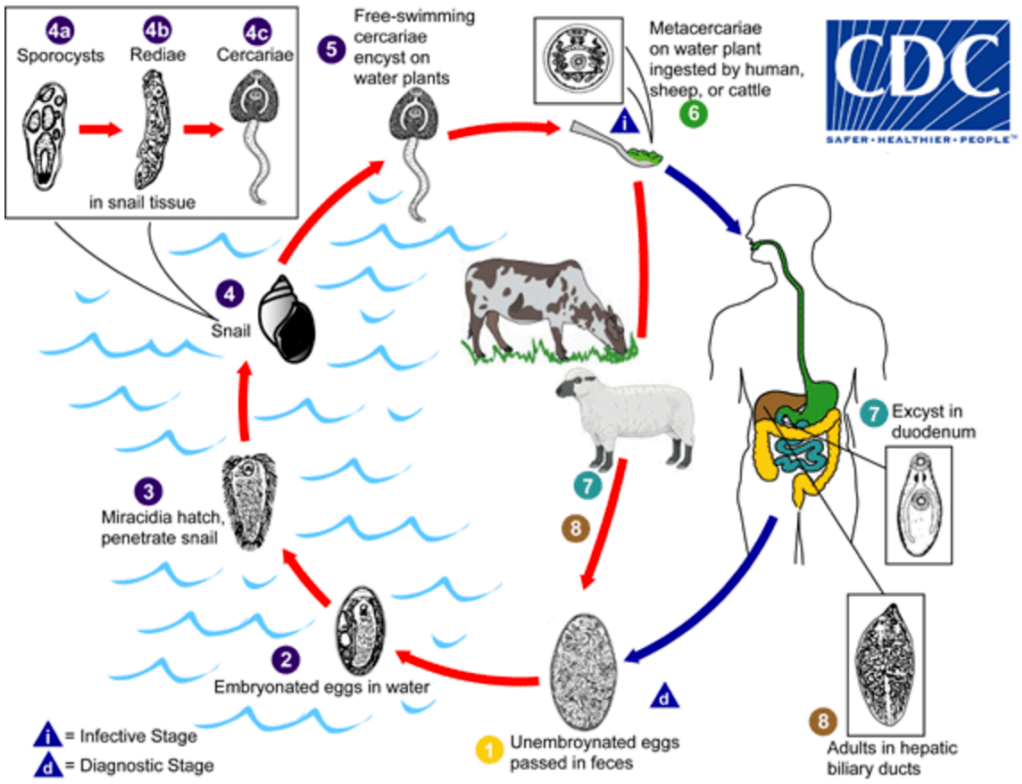


**Figure 1:** Life cycle of *F. hepatica*. (Source: Center for Disease Control)

**Motivation for Research**

Though extensive research has been conducted to indicate a direct link between schistosomiasis and damming, no experiments have tested for higher prevalence of fascioliasis in response to damming on a global scale. As previously referenced, *F. hepatica*'s devastating economic impacts and increasing prevalence in humans worldwide signify its need to be further understood and addressed. In addition, while fascioliasis can be treated with triclabendazole we are seeing increasing numbers of drug-resistant strains of *F. hepatica,* and the drug is often ineffective once fascioliasis is endemic in a region, as those treated will simply contract it again through their environment. Though vaccines have and are being researched, there is no indication that a vaccine for livestock or humans will be widely available anytime soon (Toet *et*

*al.* 2014). *F. hepatica* also demonstrates high genetic diversity and gene flow, and thus the species is more likely to evolve into drug-resistant strains, potentially rendering vaccines ineffective (Beesley *et al.* 2017).

**Research Questions**

The research I am proposing will aim to answer the following questions: (1) Does *F. hepatica* infect a greater proportion of livestock [cattle and sheep] in watersheds that have undergone anthropogenic manipulation to slow water velocity (e.g., dams), compared to regions that remain unaltered? (2) Will increased water velocity damage the body-tail junction of *F.* hepatica cercariae, rendering them incapable of encysting as metacercariae and infecting their terminal hosts? If so, (3) What is the minimum water velocity required for *F. hepatica* cercariae to sustain sufficient injuries preventing them from encysting on aquatic vegetation and infecting their terminal hosts?

***Experiment #1***

**$Q_1$:** Does *F. hepatica* infect a greater proportion of livestock [cattle and sheep] in watersheds that have undergone anthropogenic manipulation to slow water velocity compared to regions that remain unaltered?

***Experiment #2***

**$Q_2$:** Will increased water velocity damage the body-tail junction of *F. hepatica* cercariae, rendering them incapable of encysting as metacercariae and infecting their terminal hosts?

**$Q_3$:** What is the minimum water velocity required for *F. hepatica* cercariae to sustain sufficient injuries preventing them from encysting on aquatic vegetation and infecting their terminal hosts?

**Hypotheses**

***Experiment #1***

**$H_{1.0}$:** *F. hepatica*'s ability to infect its terminal host is unaltered by anthropogenic decrease of water velocity.

**$P_{1.0}$:** The proportion of cattle and sheep infected with *F. hepatica* in both dammed and undammed regions will be roughly equivalent.

**$H_{1.1}$:** Anthropogenic decrease of water velocity improves *F. hepatica*'s ability to infect its terminal host.

**$P_{1.1}$:** The proportion of cattle and sheep infected by *F. hepatica* will be significantly higher in dammed regions compared to undammed regions.

**$H_{1.2}$:** Anthropogenic decrease of water velocity impedes *F. hepatica*'s ability to infect its terminal host.

**$P_{1.2}$:** The proportion of cattle and sheep infected by *F. hepatica* will be significantly higher in undammed regions compared to dammed regions.

***Experiment #2***

**$H_{2.0}$:** Increased water velocity does not render *F. hepatica* cercariae incapable of infecting their terminal hosts.

**$P_{2.0}$:** Increased water velocity will not damage the body-tail junction of *F. hepatica* cercariae.

**$H_{2.1}$:** *F. hepatica* cercariae are rendered incapable of infecting their terminal hosts by increased water velocity.

**$P_{2.1}$:** Increased water velocity will damage the body-tail junction of *F. hepatica* cercariae.

**$H_{2.2}$:** *F. hepatica* are rendered incapable of infecting their terminal hosts at increased water velocities, but not as a result of body-tail junction damage.

**$P_{2.2}$:** *F. hepatica* will sustain body damage other than partial or full tearing of the body-tail junction.

As no previous studies have established a water velocity threshold for incapacitating *F. hepatica* cercariae, there is little evidence on which to base a projected threshold water velocity value. Jewsbury found that peak infection rates from *S. mansoni* occurred at 30–40 cm $s^{-1}$, and infection decreased drastically above 60 cm $s^{-1}$ (Jewsbury 1985).

## Methods

### *Experiment #1*

Recent studies examining the correlation between *Schistosoma* prevalence and damming practices have utilized literature searches and syntheses (Steinmann et al. 2006). However, this method is currently an ineffective tool for examining the correlation between *F. hepatica* prevalence and damming practices due to a lack of peer-reviewed literature on the subject. While some site-specific studies have been conducted (Esteban et al. 2002), the sites which have been

examined are not globally distributed or of similar topography (Mas-Coma 1999). Therefore, I will test this hypothesis through field observation.

Thirty pairs of one dammed and one undammed site in regions of similar topography, and within *F. hepatica*'s range, will be selected; ten pairs in Northern Africa, ten in South America, and ten in Southeast Asia, making sixty sites total. These are regions where fascioliasis is presently endemic (Mas-Coma *et al.* 2009). Similar topography will be defined as areas of comparable [within 300 ft.] elevation, sufficient riparian zones, and equal adjacent land use [e.g., agricultural]. Dammed regions will be classified as sites at which a dam has slowed the flow of water to less than 50 cm $s^{-1}$, while undammed regions will have a water flow of greater than 60 cm $s^{-1}$ [these thresholds are based on findings relayed by Jewsbury (1985)] and no dam within proximity to impact water velocity. Over a period of five years, the number of cases of fascioliasis occurring at each site will be tracked by testing fecal samples from cattle and sheep populations on a bi-annual basis. At each site, samples from forty-five cattle and forty-five sheep will be tested. For each animal, we will examine three successive fecal deposits for the presence of *F. hepatica* eggs, as eggs are not released continuously but rather at random (Rana et al. 2014). Following data collection, results will be analyzed in their respective dammed/undammed pairs [see **Fig. 2.0 – 2.2**]. This will also provide limited data on how *F. hepatica*'s prevalence varies between the observed global regions.

***Experiment #2***

*F. hepatica* cercariae will be placed in ten separate loop tanks [see **Fig. 2**] pumping water at each of the following velocities: 35 cm $s^{-1}$, 40 cm $s^{-1}$, 45 cm $s^{-1}$, 50 cm $s^{-1}$, 55 cm $s^{-1}$, 60 cm $s^{-1}$, 65 cm $s^{-1}$, 70 cm $s^{-1}$, 75 cm $s^{-1}$, and 80 cm $s^{-1}$. Loop tanks will be used so as to prevent cercariae

damage from crashing into the end of a straight-track tank. One-quarter of the tank's outer rim will be lined with aquatic vegetation. Thirty cercariae will be placed in each tank and rotated at their respective velocity for 48 hours. After this period, the vegetation will be pulled from the tank and we will record the number of metacercariae encysted on it. In addition, any cercariae remaining in the water will be removed with a fine sieve [~20μm] and examined under microscope for exterior damage, specifically at the body-tail junction.

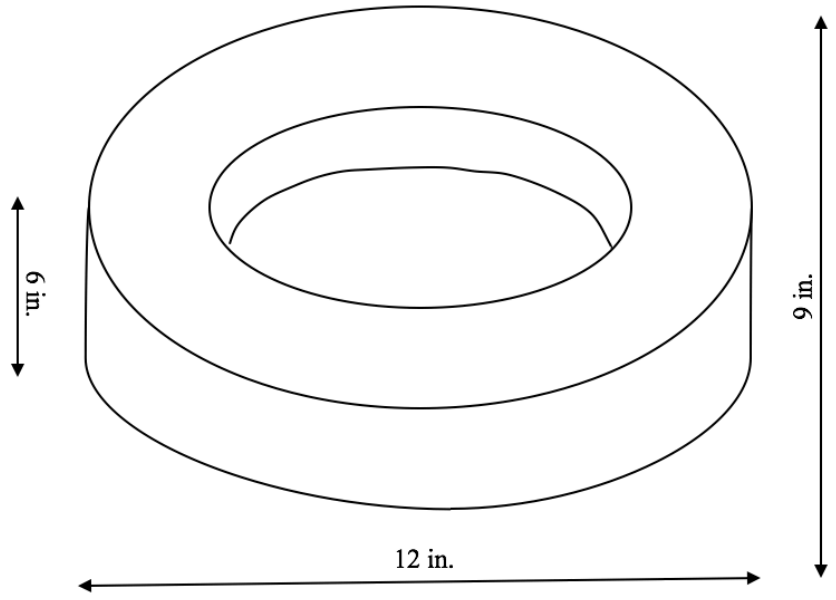


**Figure 2:** 12" x 9" x 6" loop tank.

**Anticipated Results**

***Experiment #1***

**$H_{1.0}$:** Should damming prove to have little to no impact on the prevalence of fascioliasis, we would expect to see similar proportions of positive tests at both the dammed and undammed sites within a pair [see **Fig. 3.0**].

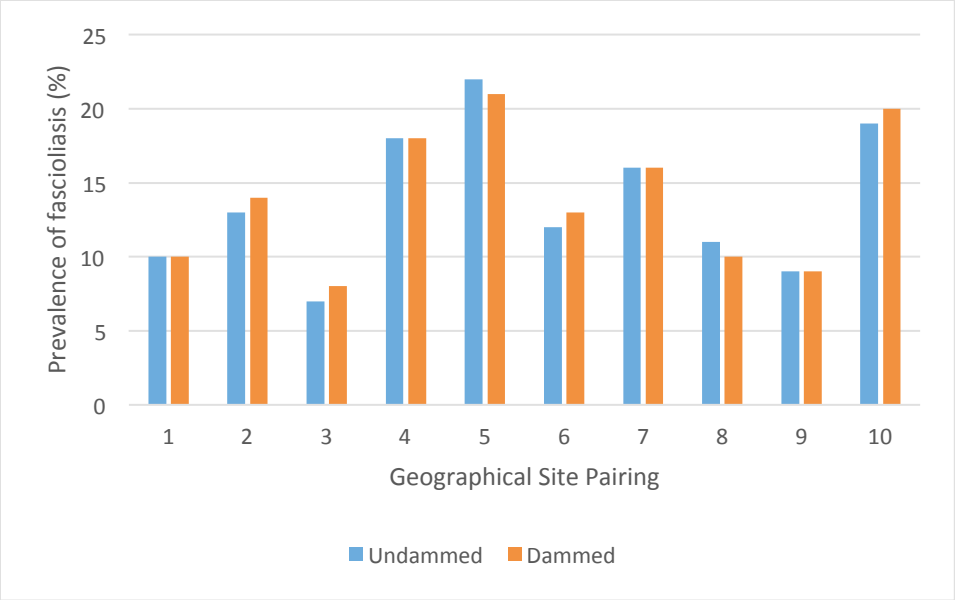


**Figure 3.0:** Projected results if damming has no impact on the prevalence of fascioliasis.

**$H_{1.1}$:** Should damming prove to increase the prevalence of fascioliasis, we would expect to see significantly higher proportions of positive tests at the dammed sites in comparison to their paired undammed sites [see **Fig. 3.1**].

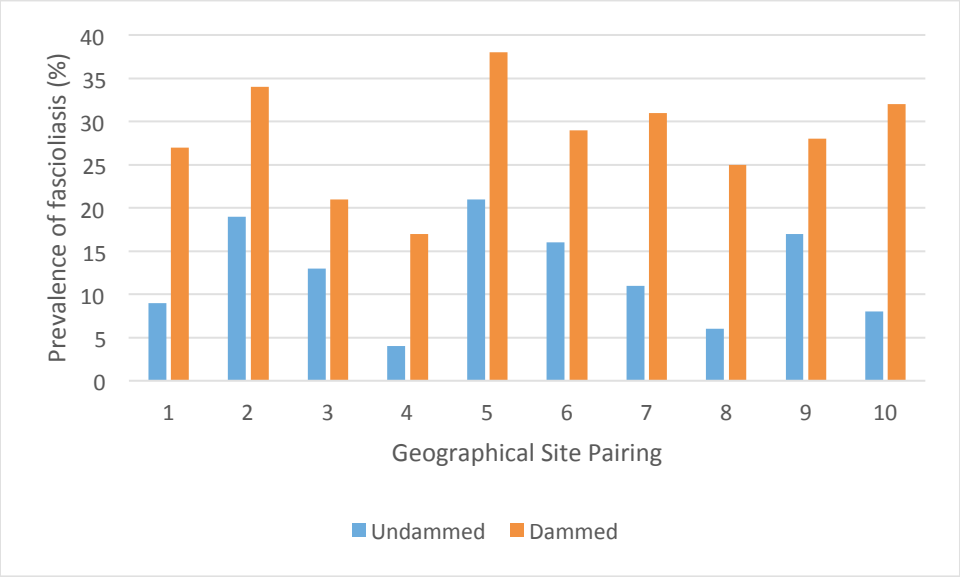


**Figure 3.1:** Projected results if damming increases prevalence of fascioliasis.

**$H_{1.2}$:** Should damming prove to decrease the rate of fascioliasis, we would expect to see significantly higher proportions of positive tests at the undammed sites in comparison to their paired dammed sites [see **Fig. 3.2**].

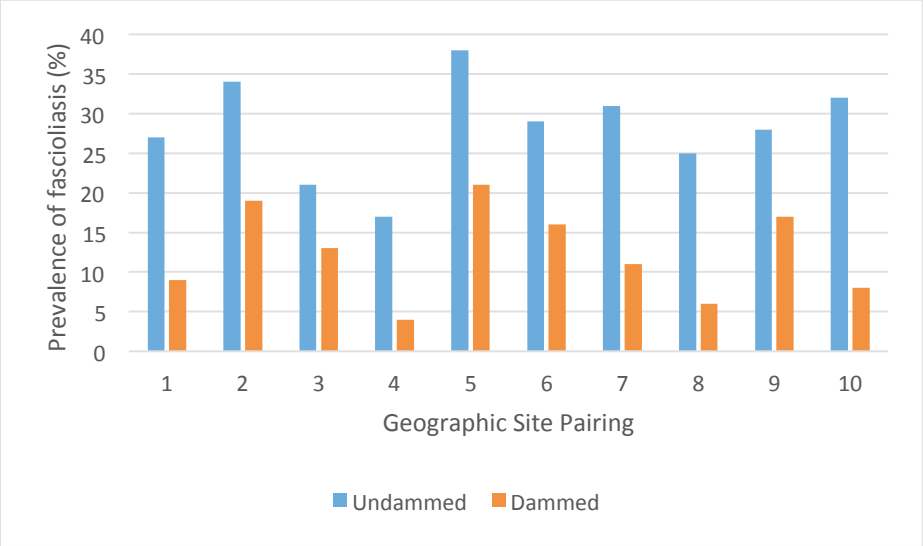


**Figure 3.2:** Projected results if damming decreases prevalence of fascioliasis.

***Experiment #2***

**$H_{2.0}$:** Should water velocity prove to have no impact on the ability of *F. hepatica* cercariae to encyst on aquatic vegetation, nearly all of the cercariae will form metacercariae in every trial [see **Fig. 4.0**] and we will see no external damage to any cercariae that fail to encyst.

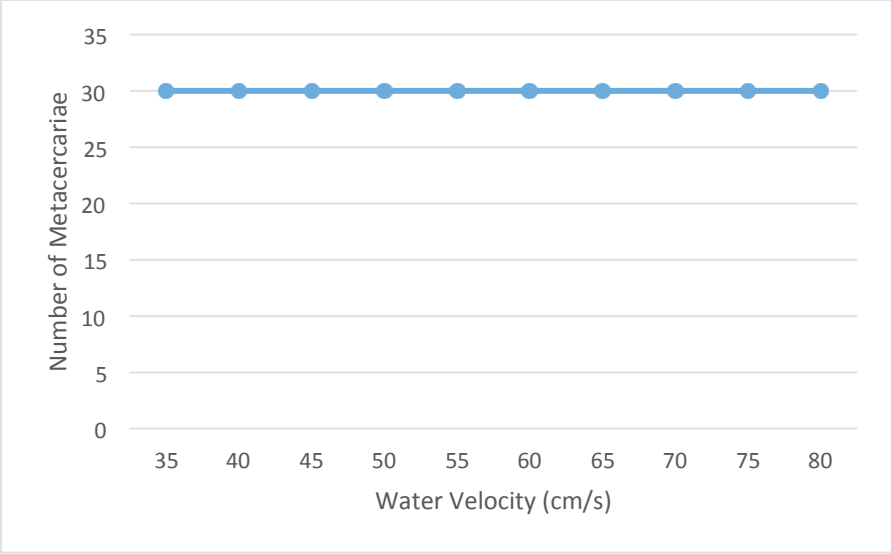


**Figure 4.0:** Projected results if increased water velocity does not impact cercariae's ability to encyst

**$H_{2.1}$:** Should damage to the body-tail junction prevent *F. hepatica* cercariae from encysting on aquatic vegetation, we would expect to see the number of metacercariae counted decrease as water velocity increases [see **Fig. 4.1**]. Additionally, we would anticipate observing external damage to the body-tail junction.

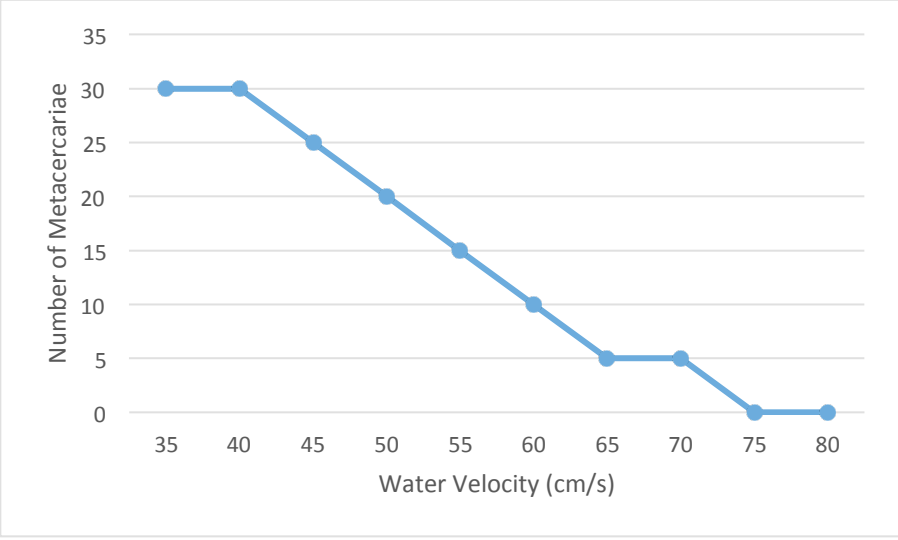


**Figure 4.1:** Projected results if increased water velocity prevents *Fasciola* from encysting on plants.

**$H_{2.2}$:** Should a cause other than damage at the body-tail junction prevent *F. hepatica* cercariae from encysting on aquatic vegetation, we will see the same results as expected in Hypothesis 2.1 [see **Fig. 4.1**], but no external signs of damage to the body-tail junction will be evident.

**Discussion**

Were my hypothesis to be refuted by our findings, in which case prevalence of fascioliasis would be shown to decrease or remain unaltered in dammed regions, little change would be expected in the management of water source infrastructure. On the other hand, if my hypothesis that dams lead to a rise in prevalence of fascioliasis were to be proven true, management entities should take such data under consideration when proposing new dam sites. While it is unlikely that governments, particularly those of developing nations, will favor the removal of dams used for irrigation, transportation, and power, the impact of newly proposed hydroelectric projects on human and livestock health must be factored into cost-benefit analyses.

Furthermore, communities already existing above dams in regions where fascioliasis is endemic should be prioritized for installation of separate water sources for livestock and humans to prevent cross-transmission of fascioliasis. Discouragement of consuming aquatic plants and funding for alternate sources of nutrition that do no promote fascioliasis may also aid these communities by removing the environmental origins of *F. hepatica* from human and livestock food systems.

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