PARASITES AND ECOSYSTEM ENERGY FLOW

by

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ABSTRACT OF THE DISSERTATION

Parasites and ecosystem energy flow

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In this dissertation I argue that the best way to include parasites into ecological studies is by using the direct energetic costs of parasites at the individual, population, and community levels. Thus, the objective of this dissertation was to determine the direct and indirect energetic costs of parasitism within individuals, populations, and communities of hosts to assess their functional roles. To determine the energetic effects of parasites, field surveys, bomb calorimetry, and respirometry were used to create energy budgets for all species collected from streams of the New Jersey Pinelands, including all parasites. The most common parasite was *Acanthocephalus tehlequahensis*. At the individual and population level, this parasite significantly altered the energy allocation patterns in its isopod intermediate host and extracted 6.7% of the production energy from the isopod population (infected and uninfected hosts). However, in the definitive host, the parasite had little effect on energy allocation, and there were no significant differences in the energy budgets between infected and uninfected pirate perch hosts infected with *A. tehlequahensis* and the trematode *Phyllodsitomum sp.*, and parasites within the fish population received 1.3% of the host's production energy. At the ecosystem level,

energy budgets were created within two pineland streams, one with a high-level of parasitism and one with a low level of parasitism. Parasites extracted a small amount of energy from both streams (<1%), but proportionally less energy went to parasitism in the stream with low levels of parasitism. Parasite establishment may be constrained by energy flow through the food web because little energy makes it up the food web to trophic positions that parasites inhabit. This study also demonstrated that parasite species may derive their energy from many trophic levels within a food web even if they co-occur in the same host. The results of this dissertation suggest that parasites extract a small amount of energy from their hosts at all levels of ecological organization. The effects of parasites are larger because they alter energy allocation patterns of their hosts. An overall conclusion is that energetic constraints limit parasite establishment and maintenance at the ecosystem level.

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Chapter 1

INTRODUCTION

Parasites are broadly defined as species that live in or on another species (hosts), and which have long intimate relationships with their hosts. It is generally accepted that more that 50-70% of all species are parasitic at some point in their life, and that parasitism occurs more often than any other trophic interaction (Price 1980, Toft 1991, Freeland and Boulton 1992). However, the ecological effects of parasites in their host populations, and within their host communities remains unclear. In the past, parasites were generally omitted from ecological studies because they were thought to have insignificant effects on ecosystem functions (Odum 1957, Price et al. 1986, Freeland and Boulton 1992, Macrogliese and Cone 1997). Our views have changed. Recent studies have demonstrated that the effects of parasites are often subtle, indirect, and difficult to measure, but significant (Combes 2001). It has been difficult to include new parasitological findings into ecological studies, and to compare significant patterns because there is a disjunction between parasitological and ecological studies. It is not clear why this is, but there is some anecdotal evidence that these two fields have evolved independently. Historically, ecological studies have been funded by the NSF to determine large-scale ecosystem processes, while most parasitology studies have been funded by the medically centered NIH. In addition, these fields attend separate annual scientific meetings and publish in their own journals. However, the tide is changing. Many ecologically trained parasitologists have started to merge ecology and parasitology (Kennedy 1976, Esch and Fernandez 1993, Poulin 1998, Combes 2001). Still, there seems to be difficultly incorporating parasites into ecological studies because the two

fields have different languages and methodologies. What is needed is a common metric that both parasitologists and ecologists can be comfortable with, and I believe that metric is energy. Parasites are directly linked to their hosts through the acquisition of host energy, and energy is an important factor of most ecological patterns (DeAngelis 1992, deRuiter 1995). The first step to incorporate parasites into ecological studies is to measure energetic effects of parasites at several levels of ecological organization.

Energetics

Energetics is the study of energy flow or transformation through a biological system, and it is thought to be the principle determinant of most ecological patterns (Elton 1927, Lindeman 1942, Odum 1957, Teal 1957, 1962, Engelmann 1961, DeAngelis 1992, deRuiter et al. 1995). Elton (1927) was the first to propose looking at trophic relationships to understand ecological patterns with his "food cycles" or modern food webs. He organized species into trophic levels and was the first to recognize an underlying pattern in all ecosystems. One of these patterns, the pyramid of numbers resulted because the numbers of individuals decreased as trophic levels increased. However, a pyramid of numbers is not an energetic relationship but simply a graphic representation of population size. The number of organisms at any trophic level is not an accurate measure of energy at that level because size and numbers covary, and individual species within an ecosystem are not energetically equal. Very large individuals are often less numerous but have larger energetic impacts on an ecosystem while smaller individuals are much more numerous but have very little ecosystem energetic impacts (Lindeman 1942). Thus, Lindeman (1942) and later Odum (1957) and Teal (1957) suggested that energy flow is the appropriate metric for ecosystem organization, this is

now the generally accepted norm in ecological studies (Macfayden 1948, Odum 1968, Fischer and Linken 1973, Crisp 1984, Wetzel 1995, Yee et al. 2007). Lindeman (1942) was the first to demonstrate this energetic relationship between trophic levels with his biomass pyramids. He showed that biomass decreases with increasing trophic level and over a collective ecosystem it forms a pyramidal shape. The pyramidal shape occurs because energy transfer between trophic level is inefficient, and only 5-15% of a trophic level's energy is transferred to the next level. Lindeman (1942) also measured the caloric value of each species within his lake food webs, and his data elegantly confirmed the inefficiency of energy transfer across trophic levels. Of course, there were many criticisms of Lindeman's ideas on ecosystem energetics. His harshest critics argued that the trophic paradigm was too simplistic and unrealistic, and that his data on trophic level production (energy sequestered within a species) and his measures of respiration (energy given off as heat by a species) were technically flawed (Ivlev 1945, Kozlovsky 1968). However, Lindeman had the right idea and the efficiency of energy transfer between trophic levels resulting in a pyramid shaped biomass distribution has been demonstrated in a number of ecosystems by many other ecologists (Odum 1957, Teal 1957, 1962, Richmann 1958, Slobodkin 1959, 1960, Patten 1959, Engelmann 1961, Gerking 1962). This energetic view of ecosystem organization is now the generally accepted view (DeAngelis 1992, deRuiter et al. 1995, Smith and Smith 2001, Townsend et al. 2003).

Elton's (1927) and Lindeman's (1942) ideas on trophic organization set the stage for an explosion of ecological energetics over the next 50 years (Real and Brown 1991, Smith and Smith 2001, Townsend et al. 2003). A primary goal of these early studies was to identify the mechanisms behind the inefficient transfer of energy between trophic

levels (Odum 1957, Teal 1957, 1962). The earliest studies were conducted at the ecosystem level, and designed to measure community metabolism. Community metabolism measures the amount of energy directed to production, respiration and decomposition for a group of interacting species, and tracks how energy flows through the system. Typically these studies employed bomb calorimetry, which measures the amount of heat or energy within a sample. The most complete studies of community metabolism where done by Odum (1957) and Teal (1957, 1962) in cold springs and a salt marsh, respectively, where the naturally low diversity of these systems allowed for realistically complete energetic studies (see Fig. 1). Overall patterns suggest that up to 90%, of energy absorbed by producers may be given off as heat through respiration, and this limits the number of trophic levels that can occur within an ecosystem. Only 10% of the energy from the producer level is available for the next trophic level, and each additional trophic level will lose a large proportion of their acquired energy to respiration. Thus, there are limits on the amount of energy available to each trophic level. In a similar study of soil arthropod communities and their energy source, Engelmann (1961) found that only 20% of the energy ingested by arthropods went to respiration, but 75% of the ingested energy was never assimilated and passed out as feces. Although, this study found differences in the amount of energy allocated to respiration, the same pyramidal patterns of energy flow emerged. These early studies provided strong support for Lindeman's ideas on the trophic organization of ecological studies, and it is still the dominant paradigm in ecology.

The energetic principles that regulated community and ecosystem level processes were thought to radiate downwards to the population and individual level. It was

predicted that the efficiency of energy transferred to production (new tissue growth and reproduction) should also range between 5-15%. However, it wasn't until the laboratory studies of the ecological efficiency of *Daphnia pulex* did we see solid support for this idea (Richmann 1958, Slobodkin 1959). Over a period of six years, both these scientists studied the population dynamics, energetics, and predator-prey relationships of laboratory populations of *D. pulex* and its prey *Chlamydomonas reihardi*. They created a simple energy budget by measuring all the energy inputs (consumption of *C. reihardi*), outputs (respiration) and utilization (*D. pulex* growth and reproduction) by the population. The ecological efficiency of their population was 10-12% and predators feeding exclusively on daphnia did not have an ecological efficiency of greater than 6%. Ecological efficiency is defined as the percentage of ingested energy converted to production within an individual, population, or trophic level (Lindeman 1942, Teal 1957, Lam et al. 1991, Smith and Smith 2001). These data suggested that all levels of ecological organization are constrained by energy transfer inefficiency, and this could affect all ecological processes including individual fitness, population dynamics and community structure. Slobodkin (1960) found the consistency of this 5-15% ecological efficiency of daphnia populations and community level energetics so striking that he argued that no system, at any level, would be able to convert ingested energy into new tissue production at a rate greater than 15%. He argued that biological systems are constrained by the second law of thermodynamics, which maintains that energy does not transfer efficiently from one body to another and 15% is that efficiency for biological system. These studies paved the way for many additional studies on individual and population energetics in a range of species (none included parasites), and in all cases found ecological efficiencies of about 15%

(Hargrave 1971, Dowdy 1975, Davis and Wilson 1985, Strayer and Likens 1986, Lam et al. 1991). It is now generally accepted that energetic principles are important regulator of ecological patterns.

More recent studies have tried to incorporate energetics into evolutionary and ecological theory (Brown et al. 1993, Morgan Ernest et al. 2003). Theory generally confirms that energetics is important in most ecological patterns. For example, evolutionary theorists have attempted to determine if energy allocation patterns to growth and reproduction are similar among species with very different life histories, and what effect this might play on individual fitness (Morgan Ernest et al. 2003). The allocation patterns of energy to growth and reproduction are not universal between species. Species of indeterminant growth tend to invest more energy in new biomass production and less in reproduction, while species with determinant growth allocate more energy to reproduction and less to biomass growth (Harper 1977, Peters 1983, Charnov 2001). These studies suggest that changes in energy allocation can affect fitness, which is the prime factor affecting population and community dynamics, and overall evolution of species (Morgan Ernest et al. 2003). Some authors even argued that the amount of energy allocated to reproduction is a better surrogate of fitness than the number of offspring (Brown et al. 1993). Organisms may produce offspring continuously or in discrete clutches, and in both cases it is logistically difficult to estimate lifetime fecundity, but energy allocation to reproduction could be a more rigorous estimate. This idea of an energetic value to fitness has not taken off outside the field of ecological modeling. Many theoretical ecologists have also tried to use energetics as an underlying principle in modeling population dynamics (Damuth 1981, 1987, Nee et al. 1992, Allen

et al. 2002). It is know thought that energy flow through a population does not change as a function of body-size because individual metabolic rates increase with increasing body-size, and population density decreases with increasing body-size (Damuth 1981, 1987, Nee et al. 1992, Allen et al. 2002). This is called the energetic-equivalence rule, and it proposes that a linear negative relationship exists between population density and body-size regardless of energy availability (Damuth 1981, 1987, Nee et al. 1992). Thus, energetics at the individual and population level can determine several ecosystem patterns including changes in biodiversity along latitudinal gradients (Allen et al. 2002). There is some evidence to support this view. Models that incorporate organismal metabolism suggest that population densities of plants and ectothermic animals are positively correlated with temperature, supporting an energetic component to latitudinal gradient biodiversity patterns. On the other hand, detractors feel that the energetic-equivalance rule does not hold for endothermic organisms. They propose that body-size may not be independent of population energy use and that there is a wide-range of metabolic rates among organisms. For mammalian species the highest population densities are associated with an optimal body size, below or above which population densities decrease (Marquet et al. 1995). This suggests that species of the optimum body size acquire and metabolize energy more efficiently, and this leads to larger population sizes (Brown and Maurer 1987). They also report that, at least in North American bird species, those species with the optimum body-size have the largest populations and the largest geographic range. This occurs because species that can attain high populations tend to be better able at exploiting many different energy sources and conditions enabling them to increase their range (Brown and Maurer 1987).

The energetics of a system clearly limits the available energy to any particular species within an ecosystem (Marquet et al. 1995). One important factor that has generally been left out of studies on community metabolism and energy budgets is parasitism. There have only been a few studies that have included parasites in energy budgets, they suggest that parasites co-opt a minimal proportion of their host's energy budget, and that infection has no effect on host metabolic rate or ecological efficiency (Bailey 1975, Munger and Karasov 1989, 1994). On the other hand, there have been numerous studies that clearly show a variety of energetic effects of parasites on their individual hosts including, severe pathology, increases and decreases in metabolic rates, increases and decreases in growth, and reduced fitness in hosts (Walkey and Meakins 1970, Meakins and Walkey 1975, Oetinger, 1987, Connors and Nickol 1991, Théron et al. 1992, Gérard and Théron 1997).

In this thesis, I argue that parasites are constrained by the same energetic principles as every other organism in the system. I suggest that the first step in understanding the ecological effects of parasites is to measure the patterns of energy flow through parasites and their hosts, and to determine ecological efficiency of parasites within an ecosystem.

The ecological effects of parasites

Parasites are believed to have significant effects on ecosystems at all levels of organization. These effects may include changes in behavior, resource allocation, population regulation and community dynamics of the host. Studies on parasites are complicated because parasites occur in distinct populations and different dynamics may occur at each stage of the lifecycle. Thus, the majority of parasite ecology studies have

centered around individual hosts. A general consensus is that the pathology and diseases caused by parasites can change host life history characters (survival, growth and fitness) and behavior (Moore 1984, Minchella et al. 1985, Théron et al. 1992). Several species of parasites have negative effects on host survival and fitness, but simultaneously increase host growth because of altered host energy allocation (Sorenson and Minchella 1998). Castration and gigantism are common occurrences in many trematode and acanthocephalan infections (Oetinger, 1987, Théron et al. 1992, Gérard and Théron 1997, Kakakazi et al. 2003). For example, the snail *Lemnaea elodes* serves as the intermediate host for the trematode parasite, *Echinostoma revolutum*. Infections with this parasite are associated with significant increase in the growth of snail hosts resulting in "gigantism" and this occurs at a significant cost to host survival and fitness (Sorensen and Minchella 1998). *E. revolutum* lives within the gonad of the snail host, where it undergoes asexual reproduction and feeds on the gonadal tissue effectively castrating the snail, and providing space and energy for the parasites (Sorensen and Minchella 1998). These studies demonstrate that parasites present significant energetic consequences for their hosts, and these changes in host survival, fitness, and growth could cascade upwards to have impacts on host population and community dynamics.

Parasite induced changes in host fitness and survival are thought to play a significant role is host population regulation (Anderson and May 1978, May and Anderson 1978, Dobson and Hudson 1992, Hudson et al. 1992, Hudson et al., 1998). The nematode *Trichostrongylus tenuis* is a parasite of the red grouse on the British Isles and large parasite populations can cause severe disease within these birds, increasing host mortality and reducing host fitness (Hudson et al. 1992). Increased parasite infections are

negatively correlated with host population size, so that the parasite and host populations cycle similarly to typical predator-prey cycles (Dobson and Hudson 1992, Hudson et al. 1992). Host population cycles were completely eliminated with regular administration of anti-parasitic drugs to the bird hosts suggesting that parasites are responsible for these population cycles (Hudson et al. 1998). Similar population cycles have also been seen in other host-parasite relationships. For example, the gastrointestinal nematode *Ostertagia gruehneri* causes a significant decrease in the fitness of their reindeer hosts. Infected reindeer produce one less calf per year than uninfected reindeer (Albon et al. 2002). As, these reindeer normally only produce one or two calves per year this decrease in fecundity is enough to cause significant changes in host population density. Models of two populations of reindeer indicate that parasite-reduced fecundity acts as a regulator of these populations (Albon et al. 2002).

Parasite-induced changes in individual host behavior can also have significant ecological effects on a host's interaction with its environment. Changes in host behavior due to parasite infection often occur in order to increase parasite transmission, and the greatest effects are seen in parasite infections that are trophically transmitted (Moore, 1984, Lafferty 1992, Moore 2002). These parasites require their intermediate host be ingested by the definitive host for a complete lifecycle, and the behavioral changes typically occur in the intermediate host (Moore 2002). Infected intermediate hosts often express increased activity levels, changes in phototaxis and preferred habitat, or reduced predator avoidance. These aid in making infected hosts more conspicuous to predators that serve as definitive hosts for the parasite. Definitive hosts will often acquire more energy in a system where their prey items are parasitized than in systems with no

parasitism (Lafferty 1992). For example, infected amphipods that serve as intermediate hosts for the acanthocephalan *Pomphorhychus laevis* experience increased phototaxis and spend more time in the water column than uninfected amphipods. Stickleback fish which serve as the definitive host for this parasite species feed on infected amphipods. The fish captured significantly more parasitized prey than uninfected. In this situation, the presence of the parasite facilitates the host's acquisition of energy, and the host ingests more energy than if parasitism is not present (Mazzi and Bakker, 2003). Similar predator-prey patterns due to parasite changes in host behavior are found in many other host-parasite relationships (Moore 1981, Webster et al. 1994, Lafferty and Morris 1996). These results generally support the idea that parasites can greatly affect the interactions of hosts with their environments.

Changes in host behavior due to parasite infection may cascade up to affect host communities. One study has reported community level effects of a parasites even though there has been much agreement that parasites can have significant effects on communities (Macrogliese and Cone 1997, Combes 2001, Thomas et al. 1998). In a New Zealand intertidal coast, echinostome trematodes that encyst within the muscles of cockles alter the ability of the cockle to bury itself, increasing predation by the bird definitive hosts. However, the effect of this parasite also clearly spreads beyond its intermediate host. The cockle also represents the only substrate that sea anemones and limpets colonize in this area. Normally, sea anemone are the dominant colonizers of the cockles, but when cockles are parasitized by this trematode, limpets appear to out compete the sea to become the dominant colonizers (Thomas et al. 1998). The authors suggest that the parasite is working as an ecological engineer, an organism that modifies the space or

resources available to other organisms in an ecosystem (Jones et al. 1994, 1997). This study shows that parasitism can result in changes in bottom dweller diversity along the New Zealand shore, and supports the notion that parasites can exert significant community level effects of parasites.

In recent years, parasitologists have attempted to determine the impact parasites have at the ecosystem level by examining the role of parasites in food web dynamics. The study of food webs has developed from simple feeding link diagram to theoretical studies that incorporate many statistical parameters to determine the stability of food webs. These parameters include the total number of links in a web, the average linkage density per species, the degree of omnivory, nestedness (the degree of asymmetry in a web), and connectance (ratio of actual links to the total possible links in a web). There is some debate over what these food web parameters mean for real ecosystems. For example, some ecologists feel that an increase in connectance indicates a less stable web, while others believe with increased connectance there is increased stability in a web (Paine 1988). Although, food web studies have been prevalent in ecological literature for some time, prior to the last 15 years, parasites were completely omitted from food web studies, (Marcoglise and Cone 1997, Huxham et al. 1995). Over the last few years there has been the construction of several food webs that include parasites in which these parasitologists have tried to use standard food web parameters to measure the impact of parasites (Huxham et al. 1995, Thompson et al. 2005, Hernandez 2006, Lafferty et al. 2006). Although changes in food web parameters are not universal when parasites are included, there are a few general patterns such as increased linkage number and density, increased omnivory, and decreased connectance (Huxham et al. 1995, Thompson et al.

2005, Hernandez 2006, Lafferty et al. 2006). It is intuitive that incorporation of parasites would increase the number of links, the density of links, and omnivory in the web. However, it is not clear what parasite-induced changes in connectance means for a web and it remains difficult to articulate the role parasites play in ecosystems based on changes in these food web parameters. Determination of the role of parasites in ecosystems may require measurement of the energy flow through feeding links of parasites and their hosts.

Biomass has often served as a surrogate measure of energy for many ecologists, and little is known about the accumulation of parasite biomass within an ecosystem. It has often been assumed that the contributions of parasite biomass to an ecosystem is minimal (Odum 1957, Combes 2001), but studies on parasites of stream fish communities in the New Jersey Pinelands clearly indicate that this assumption may be wrong. In these streams, biomass patterns followed the typical pyramidal shape, with parasites acquiring 15% of energy flowing to the top predators in the system (Sukhdeo and Hernandez 2005). However, there have been no studies directly measuring how much energy in an ecosystem goes to parasites, or what energetic effects parasites might have on host populations and communities.

The New Jersey Pinelands as a model ecosystem

My model system is the helminth community of fish species within New Jersey Pineland streams. The pinelands forests are positioned between the two major metropolitan regions of New York City and Philadelphia (Fig. 2) and a large proportion of these areas have been preserved by New Jersey legislation. These pineland streams are unique ecosystems that have been minimally disturbed by residential development

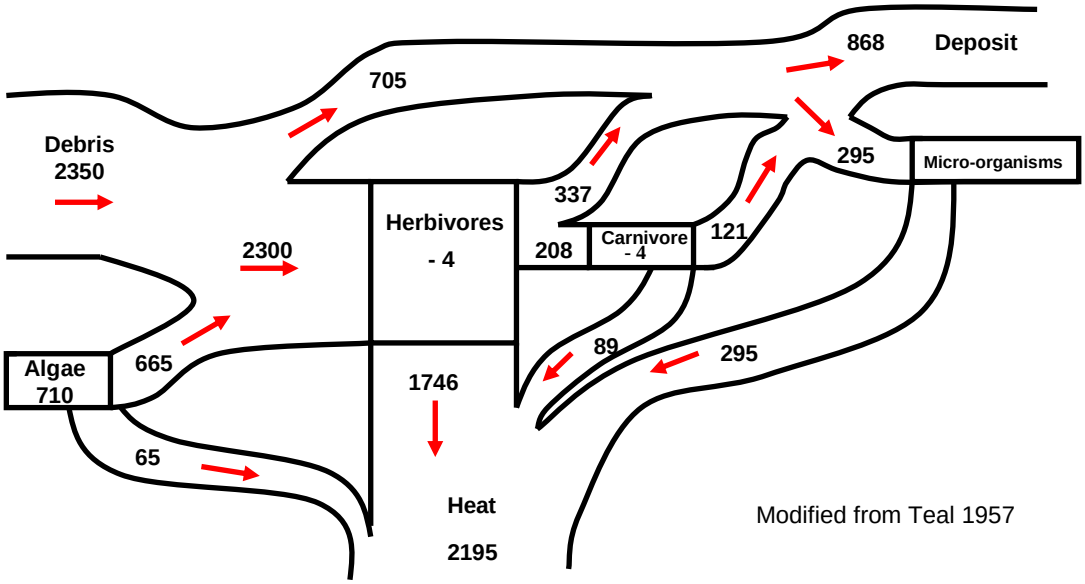
and agriculture. They are categorized as black water streams, and are naturally dark in color, have low pH (~ 4) and low concentrations of dissolved solids (Zampella and Bunnell 1998). The pinelands make a good model system for study on parasite energetics because the high acidity and low productivity leads to naturally low biodiversity (Zampella and Laidig 1997, Zampella and Bunnell 1998). These streams have low plant and algal growth, and most of the energy delivered to these systems is by way of allochthonous leaf detritus. Additionally, these streams only support 14 native fish species (Hastings 1984, Zampella and Bunnell 1998) and 4 dominant parasite species that infect fish (Sukhdeo and Hernandez 2005, Hernandez et al. 2007).

I will focus on two of the dominant parasite species in these stream ecosystems, the acanthocephalan, *Acanthocephalus tehlequahensis*, and the trematode, *Phyllodistomum sp*. In my sampling over the last two years these two parasites make up 90% of the parasite supracommunity in fish hosts, and I suspect that because of their large abundance, these parasites would have the largest energetic impact on pineland streams. Both are trophically transmitted parasites. *Acanthocephalus tehlequahensis* resides within the intestine of its fish definitive hosts, where it mates and produces eggs. Eggs are released into the stream with fish feces and then ingested by the isopod intermediate host, *Caecidotea communis*. The lifecycle is completed when the infected isopod is predated upon by the fish definitive host. The trematode, *Phyllodistomum sp*., has a three host lifecycle. Adults live within the urinary tract of the fish host, and eggs are released into the stream with host urine and feces. Eggs hatch into ciliate miracidia which swim to find and penetrate the next host, a fingernail clam (*Psidiiae sp*.) There it develops into a juvenile stage called a redia and undergoes asexual reproduction to

produce swimming cercaria, which are released into the stream. The cercaria will penetrate and encyst in either a damselfly or a caddisfly, and when ingested by a fish definitive host the lifecycle is completed.

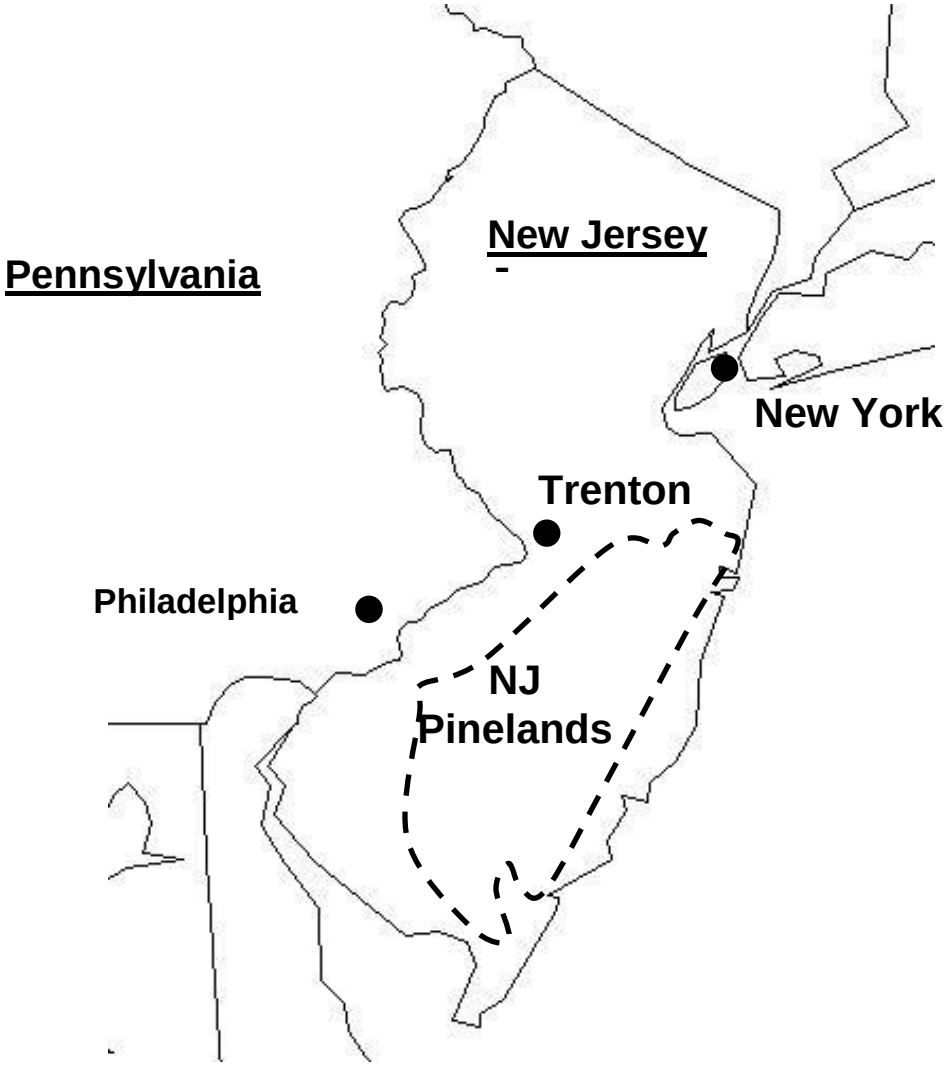
Chapter 2 of my thesis examines the energetic cost of *A. tehlequahensis* on its isopod intermediate host at the individual and population level, by creating an energy budget for infected and uninfected isopods. Chapter 3 will examine the difference in the energetic cost of 2 parasites (*A. tehlequahensis* and *Phyllodistomum sp.*) in the same definitive host, a pirate perch. Chapter 4 will estimate the energetic cost of all parasites on the entire stream community. Chapter 5 will use stable isotope analysis to determine ecosystem energy flow to *A. tehlequahensis* and *Phyllodistomum sp.* and their hosts.

Figure 1.1: Energy flow diagram of a temperate cold spring modified from Teal 1957.



Modified from Teal 1957

Figure 1.2: Map of the state of New Jersey indicating Pinelands region (dashed line).


Pennsylvania
New Jersey
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Philadelphia
NJ
Pinelands

Chapter 2

THE ENERGETIC COST OF PARASITISM IN ISOPODS.

Abstract

There is a significant and direct energetic cost (kj/m^2/yr) of parasitism by the acanthocephalan *Acanthocephalus tehlequahensis* on its freshwater isopod intermediate host *Ceacidotea communis*. Bomb calorimetry was used to measure energy allocated to host growth, reproduction, and respiration in both infected and uninfected isopods, and to parasite tissue in infected isopods. While infected isopod individuals allocated ~21% of their production energy to parasite growth, they were larger (length), consumed more leaf detritus, and allocated significantly more energy to respiration than uninfected controls ($p \leq 0.05$). In addition, infected isopods allocated proportionally less energy to tissue growth, zero energy to reproduction, and were less efficient at converting energy into isopod biomass when compared to uninfected controls ($p \leq 0.05$). In the field, isopod populations were surveyed monthly for one year. The parasite occurs in the stream at an infection prevalence of 30.19% ± 8.31 with a mean intensity of 1.12 ± 0.39 parasites/host in the population. Therefore, it is estimated that 6.7% of total production energy of the isopod population (infected and uninfected) is diverted towards this parasite. These results suggest that parasites elicit a significant energetic cost on their hosts at both the individual and population levels.

Keywords- acanthocephalans, crustacean, parasite energy allocation, energy budget, respiration

Introduction

Host-parasite interactions are always asymmetric, and while it is clear that parasites extract costs from their hosts, measuring the ecological impact of these costs has been difficult (Munger and Karasov 1989, 1994, Connors and Nickol 1991, Booth et al. 1993, Kuris et al. 2008, Lafferty et al. 2008). In this paper, we propose that the actual energetic cost of parasites to their hosts may serve as a basic currency when quantifying the ecological effects of parasites. Energy is one of the principle determinants of ecological patterns in nature (Lindeman 1942, Odum 1957, DeAngelis 1992, deRuiter et al. 1995) and all biological systems are constrained by thermodynamic laws which limit energy availability to organisms at all trophic levels. This energetic framework underlies all population, community, and ecosystem organization (Lindeman 1942, Odum 1957, Teal 1957, DeAngelis 1992, DeRuiter et al. 1995). Thus, it can be assumed that parasites are also governed by the same energetic principles that govern all ecological organization.

Traditionally, the cost of parasitism has been measured at the individual host level using either socio-economic metrics (e.g. loss of meat or wool production, number of sick days absent) or surrogate measures of host fitness (Minchella 1985, McLeod 1995, Niezen et al. 1998, Webster and Woolhouse 1999, Deressa et al. 2007). In regard to host fitness, negative effects on host fitness have been demonstrated for several parasite species where infections typically reduce the numbers of host offspring, or in some cases cause complete castration (Hamilton and Zuk 1982, Théron et al. 1992, Møller 1993, Théron and Gérard 1994, Gérard and Théron 1997, Bollache et al. 2002). For example, swallows heavily infected with the ectoparasitic mite, *Ornithonyssus bursa* experience

decreased breeding (15.6%), hatching (1.4%) and fledging (15.5%) success when compared to controls (Møller 1993). There are cases where parasites cause no measureable detrimental effects on their host's fitness, and at the other extreme, there are parasites that castrate their invertebrate hosts. For example, some trematode parasites completely castrate their snail intermediate hosts; the larvae feed on the reproductive tissue of the snail host, but not on organs that are vital for the snail's survival (Wilson and Denison 1980, Sousa 1983). Castrated snails often grow bigger and live longer, thus benefitting the parasites (Théron et al. 1992, Sorensen and Minchella 1998). There may also be indirect costs of parasitism related to host defense mechanisms. For example, hosts will allocate proportionally more energy towards immune responses when infected with parasites (Lloyd 1995, Demas et al. 1997, Penn and Potts 1998, Martin et al. 2003) affecting host fitness directly, and also indirectly through increased susceptibility to other pathogens (Sheldon and Yerhulst 1996, Cox 2001). Other indirect effects of parasites on host fitness may be related to decisions on mate choice, and the important role of parasites in sexual selection strategies has been addressed in several reviews (Hamilton and Zuk 1982, Zuk 1992, Clayton 1991, Penn and Potts 1998). Finally, the costs and benefits of particular strategies are not always clear-cut in host parasite interactions. For example, some species of trematode are known to decrease the survival of their snail host, but the increased mortality does not occur until after the parasite propagules have been released (Sorensen and Minchella 1998).

In addition to their effects on host individuals, parasites also exert significant ecological effects on their host populations (Anderson and May 1978, May and Anderson 1978, Minchella and Scott 1991, Ives and Murray 1997). For example, population cycles

in red grouse are driven by infection with the parasite *Trichostrongylus tenuis* (Hudson et al. 1992a, Hudson et al. 1992b, Hudson et al. 1998), and these oscillating population cycles are eliminated after treatment of the grouse with anti-parasitic drugs (Hudson et al. 1998). Similarly, predation on hare populations infected with the gastrointestinal nematode *Obeliscoides cuniculi* is significantly increased when compared to anthelminthic-treated controls, and the destabilizing effect of the parasite results in population cycles (Ives and Murray 1997).

While is it apparent that parasite-induced fitness costs affect host individuals and host populations, it is less clear how these costs scale in relation to higher levels of ecological organization (Marcogliese and Cone 1997, Combes 2001, Lafferty et al. 2008). Parasites were once overlooked in food web studies (Marcogliese and Cone 1997, Combes 2001), but several published food webs now include parasites, and the effect of parasites on food web topology is being widely debated (Thompson et al. 2005, Lafferty et al. 2006, Hernandez and Sukhdeo 2008a). Parasites also appear to exert a major role in the energetics of food webs. In a California salt marsh ecosystem, it is estimated that parasites extracted more biomass from the food web than all of the top predators combined in a California salt mash ecosystem (Kuris et al. 2008, Lafferty et al, 2008). In these studies where biomass is used as a surrogate of production energy, an important conclusion is that parasites, as a group, are constrained by the energy flow in the system (Kuris et al. 2008). This suggests that more accurate estimates of the ecosystem costs of parasitism can accrue if we measured the actual energetic costs in the interaction between the host and parasite.

To measure the energetic effect of parasitism, an energy budget of both infected and uninfected hosts must be constructed. Energy budgets are the accepted method to measure the amount of energy that is allocated to different physiological functions, such as reproduction, growth, and respiration (Brafield and Llywellyn 1982). There have only been a few studies that have considered the energetics of parasitism. In studies that did directly measure the energy flow to parasites using bomb calorimetry, it was reported that parasites extracted up to 1% of the host's energy budget, and that the parasite infections caused hosts to increase their metabolic rate, deplete their energy reserves and increase their ecological efficiency (Walkey and Meakins 1970, Meakins and Walkey 1975, Connors and Nickol 1991, Booth et al. 1993). However, other studies that did not directly measure energy suggest that parasites may have no effect on host metabolic rate or on the ecological efficiency of the host, and that parasites do not consume a significant fraction of the host energy budget (Bailey 1975, Munger and Karasov 1989, 1994).

Acanthocephalan parasites of crustaceans provide a good model system for measuring the energetic costs of parasites on their hosts. These parasites are trophically transmitted between their fish or bird hosts and crustacean hosts (usually an isopod or an amphipod). The crustacean ingests parasite eggs released into water with fish feces and the parasite develops into a juvenile stage (cystacanth) within the haemocoel. Acanthocephalan larvae can potentially have significant energetic costs for their crustacean hosts because they are large and take up large part of the crustacean's haemocoel (Dezfuli et al. 2002). These parasites alter resource allocation in some hosts by directing energy towards growth and away from reproduction, to provide space and energy for the parasite (Oetinger and Nickol 1982, Oetinger 1987, Kakizaki et al. 2003).

In addition, acanthocephalan parasites often castrate their crustacean intermediate hosts (Oetinger and Nickol 1981, 1982, Brattey 1983, Oetinger 1987, Zohar and Holmes 1998, Bollache et al. 2002, Kakizaki et al. 2003, Sparkes et al. 2006).

In this study, we measure the energetic cost of the fish parasite *Acanthocephalus tehlequahensis* on *Caecidotea communis* by creating an energy budget for infected and uninfected isopods. Our working hypothesis was that infection with this parasite would accrue significant energetic costs to its isopod hosts, at both the individual and population level.

Materials and Methods

Isopods were collected from a 50m stretch of a New Jersey Pineland stream, Muskingum Brook (39º49'05.10"N, 74º44'15.80"W). Isopods are the dominant macroinvertebrate in this stream (Sukhdeo and Hernandez 2005, Hernandez and Sukhdeo 2008a). To estimate the size of the isopod population in the stream and the prevalence of the acanthocephalan parasites, isopods were collected monthly for 1 year (January 2006-December 2006) from 10 random sites within the stream (area=0.093 m^2) using a dipnet. Isopods were separated from sediment, counted, sex determined, length measured, and examined for parasites with a dissecting scope. Infection status is easily detectable because the large white cystacanths (larval stage of the parasite) are clearly visible through the exoskeleton. Isopods collected from surveys were used in laboratory experiments. They were maintained alive in aerated aquaria with ad-lib leaf detritus until used.

To determine the effect of acanthocephalan parasites on isopod survival, 50 infected and 50 uninfected adult isopods (6.0mm-11.0mm) were separated into groups of 5 individual isopods and placed in plastic containers (n=10) with 250ml of aged tap water (maintained at the average stream temperature 15 C) and ad lib leaf detritus. Isopod survival was monitored every 3 days for 42 days. Dead isopods were removed and dissected to confirm infection. We used generalized linear models (GLMs) with infection status, treatment container and time as predictor variables and isopod survival as the response variable. The effect of treatment container was not significant ($p>0.20$), thus only the infection status and time were considered as potential cofactors of isopod survival. All p-values are conservatively two-tailed with a critical value of 0.05. We

performed all analyses using the software platform JMP Versions 7.0.1 (SAS Institute 2007).

To measure consumption rates of infected and uninfected isopods, 20 infected and 20 uninfected isopods were placed individually in 50 ml plastic containers (n=20) with aged tap water at 15C. A pre-weighed disc of dried Red Maple leaf detritus, which is a typical food source for this isopod species was added after rehydration in stream water, and isopods were allowed to feed for 1 week. At the end of 1 week, leaf discs, isopods, and feces were collected and dried at 60°C for 48 hours. Dried samples were weighed to the nearest 0.0001 g.

Respiration was measured by placing 30 uninfected and 30 infected isopods (6.0-7.0mm) individually in 50ml sealed containers with aged tap water at 15C. Isopods were left to acclimate to containers for 12 hours. Changes in dissolved oxygen were measured with a polarographic dissolved oxygen probe (American Marine®, Connecticut). Respiration rates for individual isopods were based on their weights during the measurement period, and were calculated from measurements taken every 12 hours over 48 hours. A conversion factor of 3.38 (cal/mg) was used to determine the amount of energy allocated to respiration (Ivlev 1934, Odum 1957, Teal 1957).

To measure the energy budgets of infected and uninfected isopods all consumption, fecal output, isopod growth, isopod reproduction, isopod respiration and parasite growth were measured by bomb calorimetry. All infected isopods (parasites removed), uninfected isopods, leaf detritus, infected isopod feces, uninfected isopods feces, isopod eggs, and cystacanth parasites (removed from infected isopods) collected throughout the year from field samples were dried at 60°C for 48hrs. Samples (n=5)

were pooled, pelleted and energy content (kilojoules) was determined with an adiabatic semi-micro bomb calorimeter (Parr Scientific, Illinois).

Energy flow through infected and uninfected isopod populations was calculated using the standard energy budget equation (modified from Brafield & Llewellyn 1982):

$$C = Pg + Pr + Pp + R + F \quad (1)$$

where C is consumption of leaf detritus, Pg is isopod growth production, Pr is isopod reproductive production, Pp is parasite production, R is respiration, and F is feces. Energy lost to nitrogenous wastes and exuvia shedding were not measured and assumed to be neglible (Lam et al. 1991). At the population level, energy budgets are collected over a specific area of the stream (per m^2/yr).

The average energy devoted to consumption, feces, production, and respiration was calculated with the following equations:

$$C = EC_l * m_l * I \quad (2)$$

$$F = EC_f * m_f * I \quad (3)$$

$$Pg = EC_g * m_g * I \quad (4)$$

$$Pr = EC_r * m_r * I \quad (5)$$

$$Pp = EC_p * m_p * I * X \quad (6)$$

$$R = DO * 3.38 * I \quad (7)$$

where *EC*= the average energy content of the samples in kj/g (l, leaf detritus; f, feces; g, isopod; r, isopod eggs; p, parasites), m= the average mass of the sample in grams, I= the mean number of isopods collected from field samples (per m^2/yr), DO= average oxygen consumption (mg) and X= average parasite intensity per isopod from field samples. The mean number of isopods/ m^2 (I) was calculated over the entire year, and the average

parasite intensity per isopod (X) was calculated using Quantitative Parasitology 3.0 (Rozsa et al. 2000)

All proportional data were arcsine transformed for all statistical analyses and Student's t-tests were performed to determine differences in mean consumption, respiration, length, width, assimilation, production, and production efficiency for infected and uninfected isopods. ANOVA was performed on monthly collection of isopods collected and acanthocephalan prevalence. Treatments were considered significantly different with a p-value of 0.05 or less.

Results

The mean number of isopods collected during the year and the prevalence of the parasite (percentage of hosts infected) during the year are shown in Figure 1. The mean density of isopods in Muskingum Brooks was 63 ± 14.49 isopods/m^2. The mean parasite prevalence throughout the year was 30.19% ± 8.31 with an infection intensity level of 1.12 ± 0.39 parasites/host (Fig. 1B).

Infection with the acanthocephalan parasite significantly decreased the survival rate of isopods ($p \leq 0.05$) (Fig. 2). Under laboratory conditions, infected isopods consumed significantly more leaf detritus ($p \leq 0.05$) (Fig. 3A), allocated more energy to respiration ($p \leq 0.05$) (Fig. 3B), and were significantly longer (mm) than uninfected isopods ($p \leq 0.05$) (Fig. 3C). However, there was no difference in the somatic dry weights (mg) (Fig. 3D) and energy content (kj/g) of infected with parasites removed and uninfected isopods. There were no significant differences between sexes for both infected and uninfected isopods.

Uninfected isopods consumed 38.07 kj/m^2/yr ± 11.82 of leaf detritus while infected isopods consumed 58.48 kj/m^2/yr ± 8.16. A large proportion of this consumed energy was not assimilated, with 33.9% (12.87 kj/m^2/yr ± 2.28) and 36.0% (21.02 kj/m^2/yr ± 3.71) lost as feces for uninfected and infected isopods, respectively (Fig. 4). The majority of the consumed energy went to respiration. Uninfected isopods allocated 67.11% (25.55 kj/m^2/yr ± 3.12) of their energy to respiration while infected isopods used 64.12% (37.50 kj/m^2/yr ± 4.53) towards respiration (Fig. 4). Host growth (i.e. biomass production) required 2.9% (1.09 kj/m^2/yr ± 0.1992) of energy consumed by uninfected isopods, but only 1.1% (0.62 kj/m^2/yr ± 0.1122) of the energy budget went to host growth

in the infected isopods (Fig. 4). Uninfected isopods used 1.8% (0.69 kj/m^2/yr ± 0.1381) of their ingested energy in reproduction (Fig. 4A), but infected isopods allocated zero energy towards reproduction (Fig. 4B) (no gravid infected females were ever recovered). Overall, these acanthocephalan parasites extracted 0.3% (0.17 kj/m^2/yr ± .009) of their infected host's energy budget (Fig. 4A). The total energy assimilated by uninfected isopods was significantly less than infected isopods (Fig. 5A), but uninfected isopod production was significantly greater than infected isopod production (Fig. 5B). Infected isopods were significantly less efficient at converting assimilated energy to production energy, or had decreased production efficiency when compared to uninfected isopods (Fig. 5C).

There were large differences in the allocation of production energy (consumption-respiration and feces), to uninfected and infected isopods. In uninfected isopods, 61.3% of the production energy went to host tissue growth, and 38.7% to host reproduction. In the infected isopod production 78.1% was directed to host growth, 0.0% to host reproduction, and 21.9% was allocated to the parasite (Fig. 6). For the entire stream population, which includes infected and uninfected isopods, it was estimated that 66.5% of the production energy was allocated to host growth, 26.8% to host reproduction, and 6.7% to growth of the parasite (Fig. 6).

Discussion

Energy budgets allow quantitative comparisons of energy consumption among several functions in infected and uninfected isopod hosts. In their natural isopod hosts the parasites alter energy allocation to host reproduction, growth, and respiration, and cause decreased survival of the host. In terms of the energetic cost of individual infected hosts, ~21% of production energy was diverted towards the parasite's growth and survival. When these effects are translated to the isopod populations in the stream, it is estimated that in this natural stream, parasites extract 6.7% of their hosts' total production energy. As a rough comparison, this value falls within the typical range of energy transfer efficiency (5-15%) between predators and prey in food web interactions (Lindeman 1942, Macfayden 1948, Odum 1957, Teal 1957).

The parasite induced significant behavioral and metabolic changes in the infected isopods who consumed more food, and allocated less energy to their own production and reproduction than uninfected isopods. Infected isopods allocated zero to reproduction, and this suggests castration, a typical phenomenon in this parasitic phylum. Most species of acanthocephalan parasites castrate their intermediate host (Brattey 1983, Oetinger 1987, Bollache et al. 2002, Kakizaki et al. 2003, Sparkes et al. 2006). Infected female isopods are rarely found in mate-guard pairings with males, and their oocysts fail to develop properly (Bollache et al. 2002, Kakizaki et al. 2003). Furthermore, acanthocephalan parasites can also behaviorally castrate their male hosts, and although reproductive structures remain intact, infected males do not perform the necessary mate-guarding behavior required for insemination, and do not attempt to mate (Poulton and Thompson 1987, Sparkes et al. 2006). Of the more than 500 isopods collected and

examined in this study, no infected gravid females were ever found, supporting the notion that this parasite is a castrator.

Consumption of red maple leaf detritus significantly increased in isopods infected with the acanthocephalan parasite. The food source may be a variable in this response since a previous study suggested that these infected isopods have decreased consumption of oak leaf detritus when compared to uninfected isopods (Hernandez and Sukhdeo 2008b). Isopods may prefer red maple leaf over oak leaf because oaks may produce chemical compounds such as tannins that make them unpalatable to many invertebrate species as a defense mechanism against herbivory (Wallace et al. 1970, Schultz and Baldwin 1982, Irons et al. 1988, Karowe 1989). Red maple makes up the bulk of the leaf detritus in Muskingum Brook.

Infection with the parasite caused isopods to grow significantly bigger than uninfected isopods. The larger size of infected isopod was the result of parasite growth only because weights of infected isopods with the parasites removed were not different from uninfected isopods. This phenomenon is not unusual and numerous parasite species, mostly trematodes and acanthocephalans, induce increased growth or gigantism (Combes 2001). The parasites may benefits from increased resources or enhanced trophic transmission (Oetinger 1987, Minchella 1985, Gérard and Théron 1997, Sorenson and Minchella 1998, Kakizaki et al. 2003, Hasu et al. 2007). Acanthocephalan parasites are trophically-transmitted, and it is likely that the larger infected isopods are more conspicuous to fish predators and aid in trophic transmission (Kakizaki et al. 2003).

Parasite infection also produced a significant increase in the amount of energy that isopods allocated to respiration. It is reported that some parasites cause no changes

in host respiration (Varó et al. 2000, Huxham et al. 2001), while other species cause significant decreases or increases in host respiration rates (Meakins and Walkey 1975, Hayworth et al. 1987, Kilgore et al. 1988, Booth et al. 1993). Our data shows that infected isopods consumed and assimilated significantly greater quantities of food than uninfected isopods, and the digestion of this food will expend more energy in respiration. In addition, acanthocephalan parasites are known to stimulate hyperactivity in their crustacean intermediate hosts (Muzzal and Rabalais 1975, Bethel and Holmes 1977, Camp and Huizinga 1979). We did not measure activity levels in the current study, but in related species, increased activity of infected intermediate hosts boosts transmission success because infected hosts are more exposed to predation by fish definitive hosts (Camp and Huizinga 1979). This would also increase respiration rates.

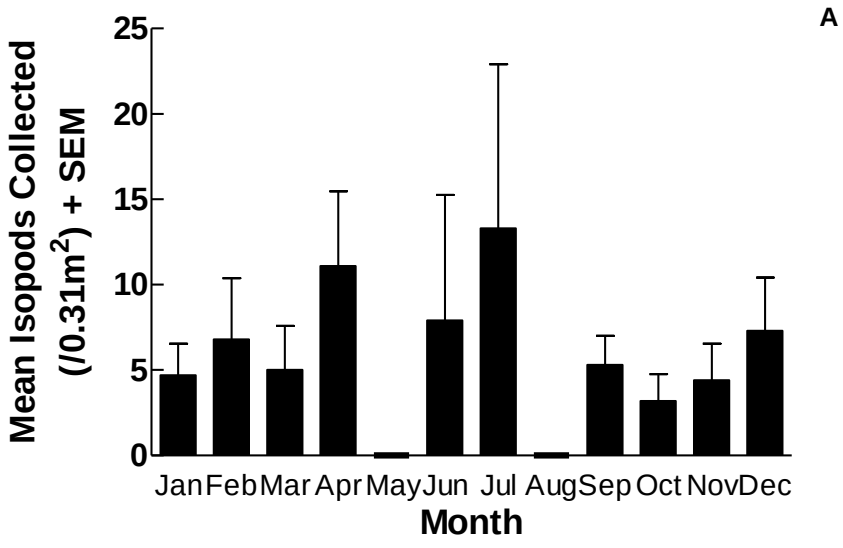
In addition, immunological functions can be energetically draining (Demas et al. 1997, Martin et al. 2003, Sandland and Minchella 2003), and the energetic costs of immune response are included in the measure of respiration (Demas et al. 1997). Many acanthocephalans seem to avoid stimulating much of an immune response in their invertebrate hosts (Loker 1994, Damian 1997, Rigaud and Moret 2003). Invertebrate immune responses against parasites included phagocytosis, encapsulation, or increases in phenoloxidase (PO)-enzyme activity (Ratcliffe et al. 1985, Rigaud and Moret 2003). We did not observe encapsulation or phagocytosis in our acanthocephalan-isopod interaction, and all larval parasites were alive after dissection from isopod hosts.

There are additional indirect costs at the individual level that could not be measured in this study but which are potentially important for full understanding of the host-parasite relationship. For example, there could be biological costs associated with

decreases in host survival and castration that are not quantified. These fitness-related costs are difficult to measure but they can be substantial. For example, when snails are infected with host-castrating trematodes, the costs to the snail's reproductive fitness was as high as 100% in infected snails (Gérard and Théron 1997). In our isopod system there may have also been some level of parasite-mediate sexual selection. Related isopod species infected with acanthocephalans do not engage in the proper mating behavior with females that insure reproduction (Sparkes et al. 2006). Thus, although measuring indirect fitness costs was beyond the scope of the present study, they should not be ignored in the overall accounting of ecological costs of parasites.

There is renewed interest in the functional roles of parasites at the community and ecosystem levels (Sukhdeo and Hernandez 2005, Byers 2009, Kuris et al. 2008, Lafferty et al. 2008). Studies on the energetic impact of trematodes on snail species in the salt marshes of Southern California suggest that energetic constraints can regulate parasite infections (Kuris et al. 2008). This study used real energy budgets to measure the direct costs of the acanthocephalan parasite *A. tehlequahensis* on its isopod intermediate hosts *C. communis*. Using energy to quantify the costs of parasitism within their hosts potentially allows ecologists to scale these effects to higher levels of organization.

Figure 2.1: (A) Mean isopods collected monthly, and (B) Mean monthly prevalence of *A. tehlequahensis* infections in isopods from January 2006-December 2006.


A
Mean Isopods Collected (/0.31m2) + SEM
25
20
15
10
5
0
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month

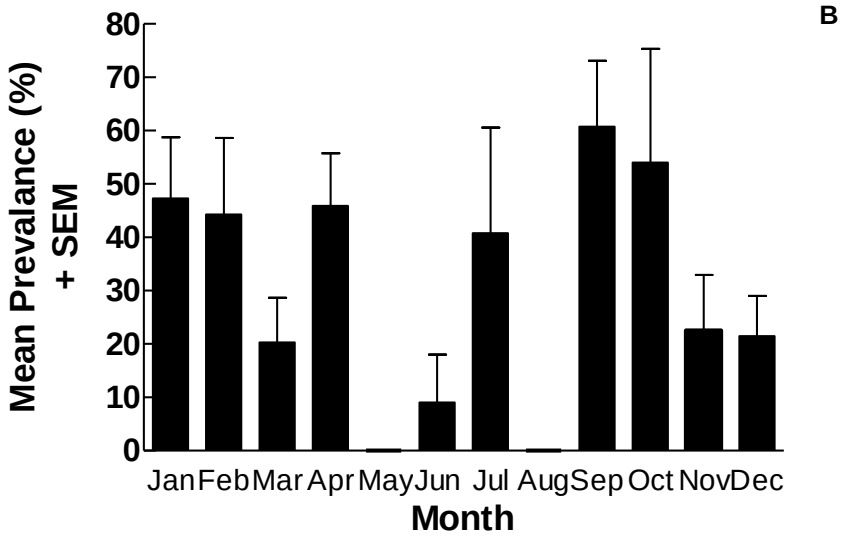

B
Mean Prevalance (%) + SEM
80
70
60
50
40
30
20
10
0
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month

Figure 2.2: Survival curves for infected (■) and uninfected (○) *C. communis*.

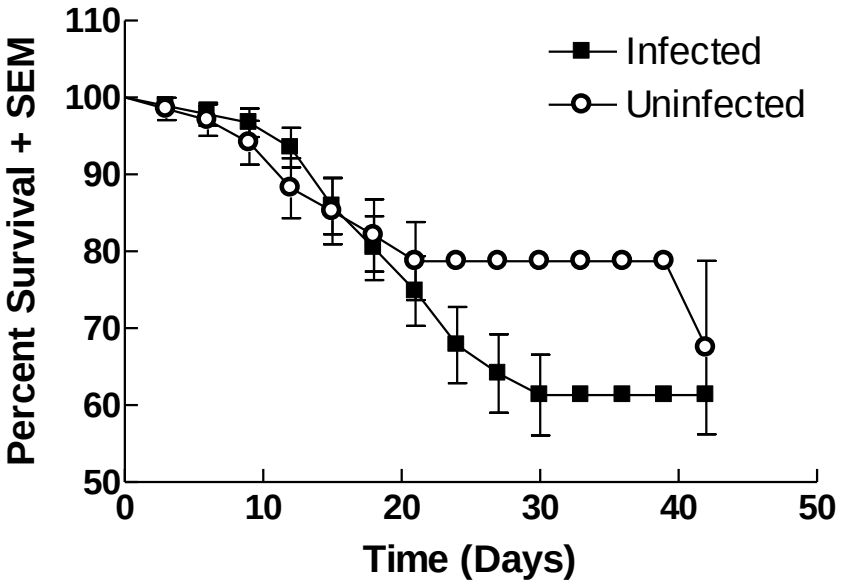

Percent Survival + SEM
110
100
90
80
70
60
50
0
10
20
30
40
50
Time (Days)
Infected
Uninfected

Figure 2.3: (A) Mean daily consumption of leaf detritus (kj/day) by infected (black) and uninfected isopods (white). (B) Mean daily respiration (kj/day) by infected (black) and uninfected isopods (white). (C) Mean length (mm) of infected (black) and uninfected isopods (white) and (D) Mean dry weight (mg) of infected isopods with parasite removed (black) and uninfected isopods (white).

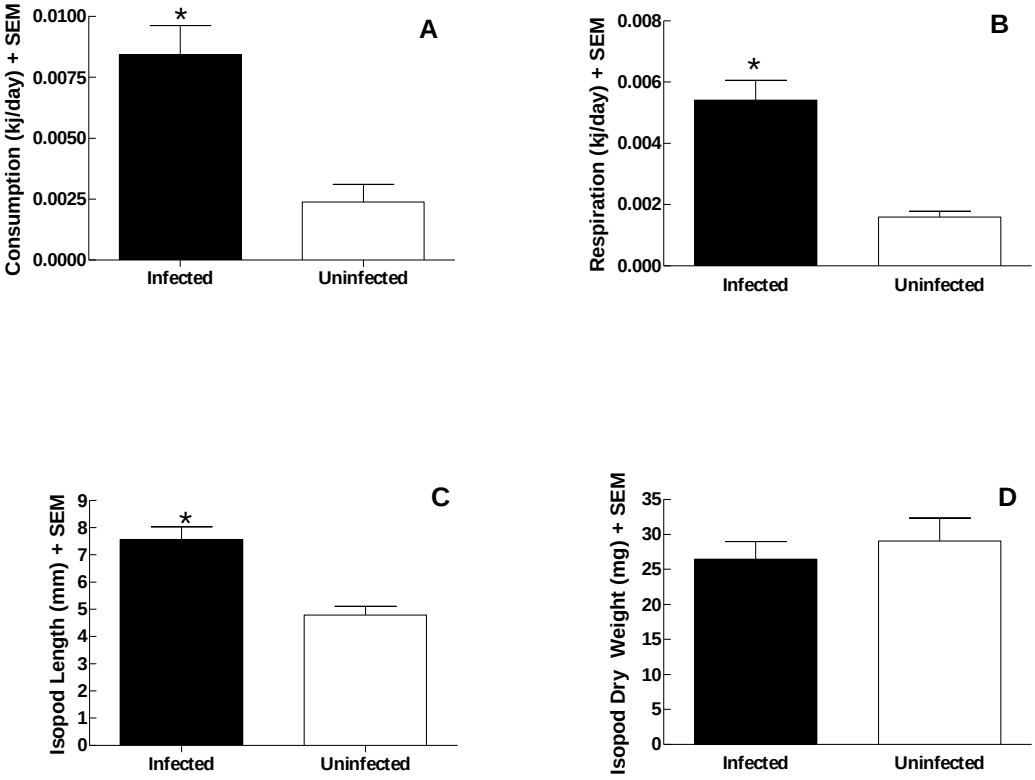
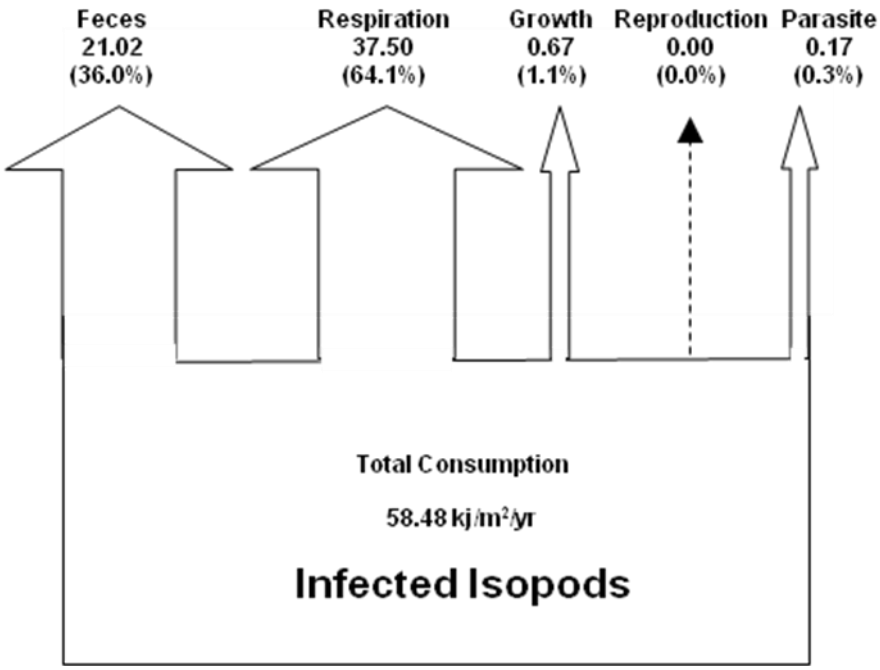
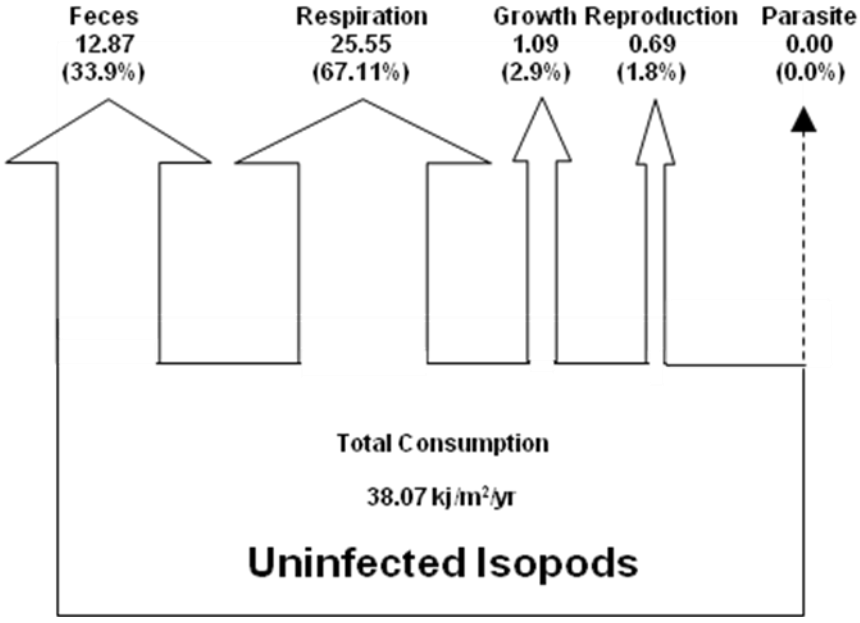

A
Consumption (kj/day) + SEM
0.0100
0.0075
0.0050
0.0025
0.0000
*
Infected
Uninfected
B
Respiration (kj/day) + SEM
0.008
0.006
0.004
0.002
0.000
*
Infected
Uninfected
C
Isopod Length (mm) + SEM
9
8
7
6
5
4
3
2
1
0
*
Infected
Uninfected
D
Isopod Dry Weight (mg) + SEM
35
30
25
20
15
10
5
0
Infected
Uninfected

Figure 2.4: (A) Energy budget (kj/m^2/yr) of infected isopods. (B) Energy budget (kj/m^2/yr) of uninfected isopods. The width of each arrow is directly proportional to the total energy consumed, unassimilated (feces), or allocated to respiration, growth, reproduction and the parasite.

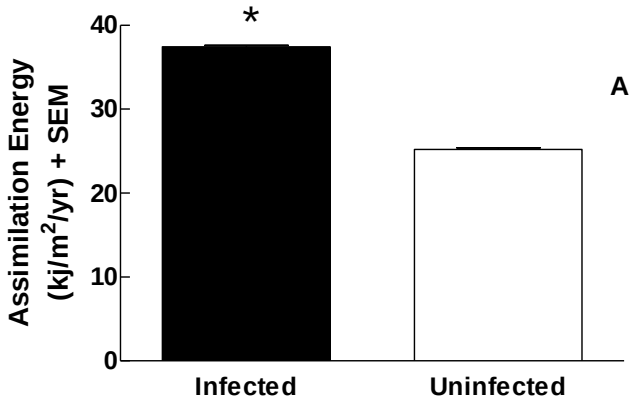

Feces
21.02
(36.0%)
Respiration
37.50
(64.1%)
Growth
0.67
(1.1%)
Reproduction
0.00
(0.0%)
Parasite
0.17
(0.3%)
Total Consumption
58.48 kj/m²/yr
Infected Isopods

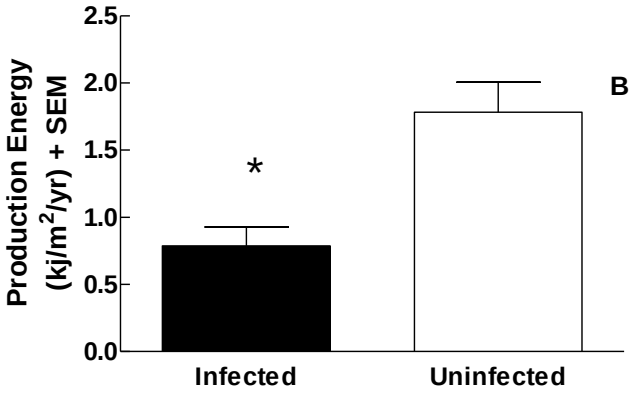
A


Feces
12.87
(33.9%)
Respiration
25.55
(67.11%)
Growth
1.09
(2.9%)
Reproduction
0.69
(1.8%)
Parasite
0.00
(0.0%)
Total Consumption
38.07 kj/m²/yr
Uninfected Isopods

B

Figure 2.5: (A) Mean assimilated (respiration, growth, reproduction, and parasite) energy ($kj/m^2/yr$); (B) Mean production (growth, reproduction, and parasite) energy ($kj/m^2/yr$), and (C) Mean production efficiency of infected (black) and uninfected (white) isopods.


Assimilation Energy
(kj/m2/yr) + SEM
40
30
20
10
0
*
A
Infected
Uninfected


Production Energy
(kj/m2/yr) + SEM
2.5
2.0
1.5
1.0
0.5
0.0
*
B
Infected
Uninfected

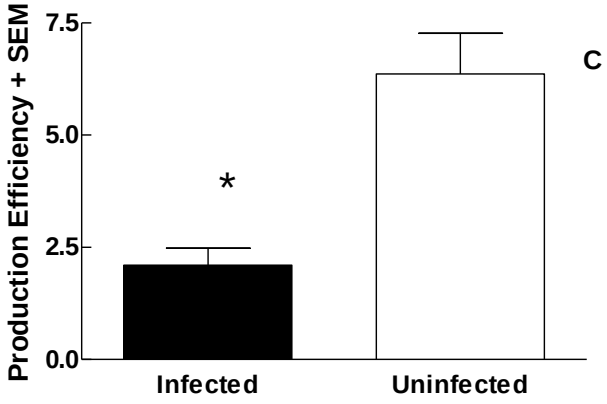
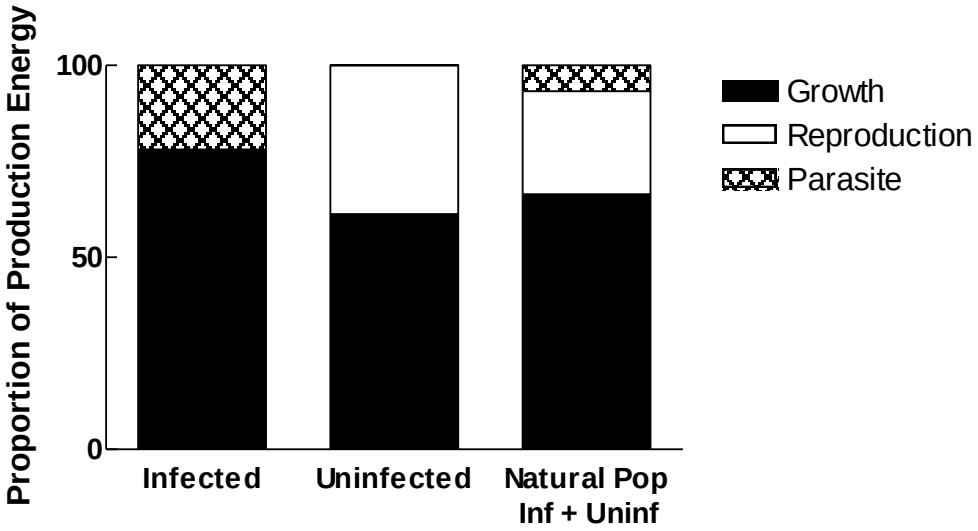

Production Efficiency + SEM
7.5
5.0
2.5
0.0
*
C
Infected
Uninfected

Figure 2.6: Proportion of total production energy allocated to growth (black), reproduction (white), and parasite (hatch) in infected and uninfected isopods.


Proportion of Production Energy
100
50
0
Infected
Uninfected
Natural Pop
Inf + Uninf
Growth
Reproduction
Parasite

Chapter 3

ENERGETIC COSTS OF PARASITISM IN A NATURAL POPULATION OF FISH DEFINITIVE HOSTS.

Abstract

This study investigated the energetic effects of two parasites, an acanthocephalan *Acanthocephalus tehlequahensis* and a trematode *Phyllodistomum spp*., their definitive host, the pirate perch, *Aphredoderus sayanus*. Pirate perch were sampled from a 50 meter stretch of stream in every season for two years, and parasite prevalence and intensities were determined. Bomb calorimetry and respirometry were used to create population energy budgets of acanthocephalan-infected, trematode-infected, dual-infected, and uninfected pirate perch. There were no significant differences in length, or weight in pirate perch as a result of parasite infection. In terms of production energy, parasitism was allocated 1.1%, 1.9%, and 3.0% for acanthocephalan infected, trematode infected, and dual infected pirate perch. At the population level (including all infected and uninfected hosts), parasites extracted even smaller proportion of the overall pirate perch energy budget (<0.1% of consumed energy, and 1.3% of production energy). However, parasitism had significant indirect effects on host allocation of energy. Pirate perch infected with either one or both parasites doubled their allocation of production energy to reproduction from 7% to 14%. It is unclear if this shift in energy allocation would affect host fitness. Overall, this study suggests that parasitism has negligible energetic effects on this pirate perch population, but it remains unclear if parasite-induced shifts in energy allocation toward fish reproduction might have any impact on host fitness.

Introduction

Parasites derive all energy from their hosts and quantifying this energetic relationship might provide a fundamental measure of the cost of parasite infection.. Energetics is considered to be the principle determinant of ecological patterns from the individual to the ecosystem (De Angelis, 1992; de Ruiter et al., 1995), and parasites are a natural part of the paradigm of ecological energetics (Sukhdeo and Hernandez, 2005; Kuris et al., 2008; Lafferty et al., 2008). Traditionally, energetic studies at the individual and population levels measure energy flow through a species by creating an energy budget to estimate the amount of energy allocated to the physiological functions of growth, reproduction and respiration (Brafield and Llywellyn, 1982). Energy budgets have been created for many species (Hargrave, 1971; Dowdy, 1975; Davis and Wilson, 1985; Strayer and Likens, 1986; Lam et al., 1991), but only a few studies have included parasites. Some reports concluded that parasites may only take a small fraction of their host's energy budget, and had very little effect on host energetics (Bailey, 1975; Munger and Karasov, 1989; 1994). However, other found that parasitism caused significant changes in host respiration, growth, and reproduction; and infection made the host more efficient at converting consumed energy into new tissue growth (Walkey and Meakins, 1970; Meakins and Walkey, 1975; Booth et al., 1993; Connors and Nickol, 1991; Gérard and Théron, 1997; Sorenson and Minchella, 1998).

The goal of this study was to determine the energetic impact of two parasites (a trematode and an acanthocephalan) on their fish definitive host by creating energy budgets for infected and uninfected fish populations in their natural habitat. The pirate perch, *Aphredoderus sayanus* (Gillum 1924) was selected as the model organism for this

study. It is native to many black water streams located in the eastern United States, and is one of the most abundant fish in streams of the New Jersey Pinelands (Hernandez et al., 2007). Pirate perch inhabit stream banks, and feed primarily on a diet of macroinvertebrates (Shepherd and Huish, 1978; Sukhdeo and Henandez 2005), and it serves as a definitive host to upwards of eight species of metazoan parasites including trematodes, acanthocephalans, cestodes and nematodes (Hernandez et al., 2007). The trematode *Phyllodistomum sp.* and the acanthocephalan, *Acanthocephalus tehlequahensis* constitute 90% of the parasite infections in this fish species (Hernandez et al., 2007). These two species were the focus of this study.

Phyllodistomum sp., and *A. tehlequahensis* are both trophically-transmitted parasites, but they have very feeding strategies and lifecycles (Hernandez et al., 2007). *Phyllodistomum sp.* lives within the ureters of the pirate perch where it feeds on host tissues and fluids. Parasite eggs are released into the environment with the feces where they hatch into miracidia that infects the next hosts, the fingernail clam. There the trematode undergoes asexual reproduction to produce cercaria that are released into the water column to infect either a damsel fly or a caddisfly which is ingested by the pirate perch to complete the life cycle. *Acanthocephalus tehlequahensis* on the other hand lives within the intestine of the pirate perch where it absorbs host digesta. Its eggs are also released into the environment with the feces where they are ingested by isopods. The lifecycle is complete when a fish ingests an infected isopod.

Habitat and feeding differences in these two categories of helminthes may cause the host to respond to infection in different ways (Kennedy, 1976), and little is known about how these differences in parasite energy acquisition might affect host energetics.

Absorption of nutrients allows acanthocephalan parasites to forego investing in sophisticated mouthparts and/or a digestive tract, and they are generally not subject to large immune responses from their hosts (Von Brand, 1979; Bogitsh and Cheng, 1998). On the other hand, these parasites are often confined to relatively poor quality habitats in the alimentary tract of their hosts (Von Brand, 1979). Trematode parasites that feed on host fluids and tissues have mouths and digestive tracts and are known to elicit strong host immune responses (Secombes and Chappell, 1996). However, they may utilize sophisticated methods to avoid the host's immune response including secretion of immunosuppressive agents, frequent turnover of parasite surface antigens, and acquisition of host antigens on the parasite's surface as camouflage (Smithers and Worm, 1976; Secombes and Chappell, 1996).

Parasite-induced immunological and physiological alteration in their hosts may significantly impact their reproduction, respiration, and energy allocation patterns (Walkey and Meakins, 1970; Meakins and Walkey, 1975; Wilson and Denison, 1980; Sousa, 1983; Connors and Nickol, 1991; Théron et al., 1992; Théron and Gérard, 1994; Gérard and Théron, 1997). In addition, parasite effects may be subtle or indirect, and infection can often alter the phenotypes of their hosts, including changes in host behavior, appearance and survival (Muzzal and Rabalais, 1975; Bethel and Holmes, 1977; Camp and Huizinga, 1979; Oetinger and Nickol, 1981; 1982; Brattey, 1983; Moore, 1984; Oetinger, 1987; Poulton and Thomas, 1987; Sorensen and Minchella, 1998; Bollache et al., 2002; Kakizaki et al., 2003; Seppälä et al., 2004; Bierbower and Sparkes, 2007). These parasite-induced alterations of host phenotype and physiology can also result in significant ecological effects including effects on host population cycles, predation rates,

and energetics (Walkey and Meakins, 1970; Connors and Nickol, 1991; Booth et al., 1993; Ives and Murray, 1997; Hudson et al., 1998).

A working hypothesis in this study is that infections with the trematode *Phyllodistomum sp.* will have a greater energetic impact on the pirate perch that have infections with *A. tehlequahensis* because the feeding behavior of the trematode damages the hosts gut and is more likely to evoke a strong immune response.

Materials and Methods

All pirate perch were collected from Muskingum Brook, a black water stream located in the southern New Jersey pinelands reserve. The New Jersey pineland is a 1.0 million acre pine-oak reserve located between the large metropolitan areas of New York City, NY and Philadelphia, PA. All streams in the pinelands are categorized as black water, and have low pH (~4-5), conductivity, dissolved solids, and biodiversity (Zampella and Bunnell, 1998).

To determine the population size of pirate perch and the prevalence and intensity of the parasites in this population, Muskingum Book was sampled using a seine net along a 50m stretch for one hour every three months over two years. All fish were immediately euthanized in ethyl 3-aminobenzoate methanesulfonate salt and frozen for later examination. Individual pirate perch were defrosted, weighed, length measured, sex determined, and necropsied for parasites. Parasites were collected stored in 70% ethanol. In addition, during dissection, all stomach contents, feces in the anus, and gonads were removed.

To measure the energy budget of infected and uninfected pirate perch bomb calorimetry was used to determine the energy allocation to consumption, feces, growth, reproduction, and parasite growth. All pirate perch and dissected parts were homogenized in 50ml of distilled water with a Waring blender. Homogenized fish tissues, stomach contents, feces, gonads and parasites (removed during necropsy) were oven-dried at 60 C for 48 hr and then weighed for biomass measurement. All dried samples were pooled if necessary, pelleted, and a Parr Adiabatic Bomb Calorimeter ® (Illinois) was used to measure the energetic content (kj/g) of samples.

To measure energy allocated to respiration, 18 individual pirate perch collected during the spring were weighed, and measured, and placed in small closed plastic containers (250 ml) to minimize movement in the field. Containers were then placed in the stream to maintain average water temperature. Fish were allowed to acclimate for 5 min, and a polarographic dissolved oxygen probe (American Marine ®, Connecticut) was used to measure oxygen consumption (mg/l) at time 0 min, 30 min, and 1 hr (Holeton and Jones 1975, Miranda and Hodges 2000). A conversion factor of 3.38 cal/mg (Ivlev,1945; Teal, 1957; 1962) was used to determine the amount of energy in calories allocated to respiration.

The energy budget of infected and uninfected pirate perch was estimated using the following equation (Brafield and Llywellyn, 1982):

$$C= P_g + P_r + P_p + R + F \quad (1)$$

where C is consumption, P_g is pirate perch growth production, P_r is pirate perch reproduction, P_p is parasite growth production, R is pirate perch respiration, and F is pirate perch feces. Energy lost to nitrogenous wastes was assumed to be negligible and not measured. These factors (consumption, fecal output, production, and respiration) were estimated using the equations below:

$$C= EC_s * m_s * A \quad (2)$$

$$F= EC_f * m_f * A \quad (3)$$

$$P_g= EC_g * m_g * A \quad (4)$$

$$P_r= EC_r * m_r * A \quad (5)$$

$$P_p= EC_p * m_p * X * A \quad (6)$$

$$R= EC_o * A \quad (7)$$

Where EC = the average energy content of the sample in kj/g (s, stomach content, f, feces, g, fish tissue, r, gonads, p, parasite, o, oxygen consumption), m= the average mass of the sample (g/m^2), A = mean number of pirate perch (per m^2/yr), and X = average parasite intensity per pirate perch.

All data were analyzed using one-way analysis of variance (ANOVA), and multiple comparisons of simple infections, dual infection and uninfected were analyzed using Tukey's intervals. All statistical measurements were conducted using GraphPad Prism 4 (2005).

Results

Pirate perch was the most abundant fish collected from Muskingum Brook (41% of fish collected). A total of 86 pirate perch were collected over the 154 m^2 stretch of Muskingum Brook from January 2006-October 2007 and the seasonal averages are shown in Figure 1A. *Acanthocephalus tehlequahensis* and *Phyllodisotmum sp.* were found in single and dual infections. The prevalence (proportion of infected hosts) of *A. tehlequahensis* was 26.7% and the mean infection intensity of 4.09 ± 0.94 per infected fish (Fig. 1B). *Phyllodistomum sp*. infection occurred at a prevalence of 54.7% and with a mean infection intensity of 4.31 ± 0.43 per infected fish (Fig 1C). Dual infections of both parasites were significantly less common, and only 17.4% of the pirate population was concurrently infected (Fig 1D).

Infection with either parasite or dual infections had no significant effect on individual pirate perch weight, length or weight to length ratio (Fig 2). Infection with these parasites also did not result in any significant changes in the yearly energy consumption (Fig. 3A) and yearly respiration rate (Fig. 3B) of the pirate perch population.

At the host population level, uninfected pirate perch consumed 7.22 ± 2.7 kj/m^2/yr, while pirate perch infected with the acanthocephalan, the trematode, or had dual infections ingested 2.29 ± 0.77 kj/m^2/yr, 9.15 ± 3.1 kj/m^2/yr and 4.30 ± 1.4 kj/m^2/yr, respectively. The energy that was not assimilated and went out as feces was 3.62 ± 0.85 kj/m^2/yr for uninfected fish, 0.93 ± 0.22 kj/m^2/yr for acanthocephalan infected, 3.74 ± 0.89 kj/m2/yr for trematode infection, and 1.76 ± 0.42 kj/m^2/yr for fish infect with both parasites (Fig. 4). The parasite used very little of the assimilated energy in all cases with

acanthocephalans taking 0.00076 ± 0.0002 kj/m^2/yr, trematodes taking 0.00479 ± 0.00093 kj/m^2/yr, and dual infections taking 0.00368 ± 0.0002kj/m^2/yr from the fish population (Fig. 4). This accounts for 0.033%, 0.052%, and 0.085% of the host's ingested energy in acanthocephalan, trematode and dual infections respectively. Energy allocation to respiration, growth and reproduction also differed between infected and uninfected pirate perch. Uninfected pirate perch allocated 61.1% (4.41 ± 2.30 kj/m^2/yr) to respiration. Acanthocephalans allocated 41.0% (0.94 ± 0.54 kj/m^2/yr), trematodes allocated 48.2% (4.41 ± 0.85 kj/m^2/yr), and dual infection allocated 32.0% (1.38 ± 0.71 kj/m^2/yr) to respiration (Fig. 4). The largest proportion of consumed energy directed towards new tissue growth was in uninfected pirate perch with 3.1% (0.23 ± 0.019 kj/m^2/yr). Fish infected with acanthocephalans, trematodes, and both parasites directed 2.5 % (0.06 ± 0.003 kj/m^2/yr), 2.4 % (0.22 ± 0.013 kj/m^2/yr) and 2.4 % (0.10 ± 0.006 kj/m^2/yr) to new tissue growth respectively (Fig. 4). A small proportion of energy was allocated to reproduction in all cases, but infected pirate perch allocated more energy to host reproduction than uninfected hosts. Acanthocephalan infected hosts allocated 0.4% (0.009 ± 0.002 kj/m^2/yr), trematode infected hosts allocated 0.4 % (0.036 ± 0.008 kj/m^2/yr), and dual infected hosts allocated 0.4% (0.017 ± 0.004 kj/m^2/yr) while uninfected hosts allocation 0.2% (0.017 ± 0.005 kj/m^2/yr) to reproduction (Fig. 4).

The amount of energy that pirate perch allocated to overall production (fish growth, fish reproduction, and parasite growth) was also significantly different between the types of parasite infection. Uninfected pirate perch and trematode infected pirate perch populations directed significantly more energy toward production than infected pirated perch (Fig 5A). However, when the production efficiency (production

energy/assimilated energy *100) of pirate perch that were dually infected was significantly more efficient than single parasite infections (Fig 5B). There were also differences in how this production energy was allocated. Uninfected pirate perch allocated a larger proportion of their production energy to growth (93.0%) when compared to infected pirate perch (~84%) (Fig. 5C). Infected fish in all cases allocated a larger proportion of their production energy to reproduction (~14%) when compared to uninfected fish (~7.0%) (Fig. 5C). The parasites siphoned off a small proportion of production energy with 1.1%, 1.9%, and 3.0% going toward acanthocephalan infections, trematode infections, and dual infections respectively (Fig. 5C). In addition, when the cost of production energy is extrapolated to the entire natural pirate perch population (uninfected and infected), only 1.3% of the production energy was allocated to the parasites (Fig 5C).

Discussion

This study examined the energetic impact of two parasites, the acanthocephalan *A. tehlequahensis*, and the trematode *Phyllodistomum sp*. on their definitive hosts, the pirate perch (*A. sayanus*) in a natural population. It was expected that due to difference in feeding strategies, habitat locations, and potential immune response stimulation between the two parasites, that infections with *Phyllodistomum sp*. would present greater energetic costs than *A. tehlequahensis*. This was not the case. At the individual host level, there were no significant differences in the length, weight, or length to weight ratio of pirate perch infected with either or both parasites concurrently. In addition, the proportion of production energy allocated to physiological functions did not change with type of parasite and there were no significant differences in the allocation patterns of infected pirate perch. However, the allocation patterns of infected pirate perch differed from uninfected fish, and significantly more production energy was proportioned towards reproduction in the infected fish. At the population level, although there were no significant differences in the energy allocation patterns of infected and uninfected, the majority of the energy within the pirate perch population was distributed among uninfected pirate perch and trematode infected pirate perch, these two groups constituted ~73% of the population. This caused uninfected and trematode infected pirate perch to allocate significantly more energy to overall production (host growth, reproduction, and the parasite), but pirate perch with dual infections were the most efficient at converting assimilated energy to production.

The lack of a significant direct energetic cost at the population level may be due to the natural aggregation of parasites within host populations (Crofton, 1971; Anderson

and Gordon, 1982; Rohde, 1984; Shaw et al., 1998). Both parasite species were over dispersed in the pirate perch population with variance/mean ratios of 7.83 for the acanthocephalan and 3.97 for the trematode. *Acanthocephalus tehlequahensis* and *Phyllodistomum sp.* had mean parasite intensities of only 4.09 and 4.31 parasites/host, respectively, but one individual pirate perch harbored as many as 21 acanthocephalans and another had 14 trematodes. Thus, these data cannot be directly compared with previous studies on the energetic costs of parasitism that were conducted in definitive hosts using manipulated parasite loads. In these studies, host with large parasites loads had lowered digestibility, increased respiration, and increased thermoregulation compared to uninfected hosts (Munger and Karasov, 1989; Booth et al., 1993; Connors and Nickol, 1991). The natural occurrence and intensities of the parasites in these populations were not measured (Munger and Karasov, 1989; Booth et al., 1993; Connors and Nickol, 1991), but it is likely that these high parasite loads are not typical of infections in a natural population.

It has been demonstrated in several systems that parasites can have significant fitness costs on their hosts with effects on sexual selection, decreasing reproductive output, and causing complete castration (Hamilton and Zuk, 1982; Kennedy et al., 1987; Théron et al., 1992; Møller, 1993; Théron and Gérard, 1994; Gérard and Théron, 1997; Marzal et al., 2005). Changes in host fitness induced by the parasite can have profound effects on host populations and have been shown to regulate population cycles (Hudson et al., 1992; 1997; Ives and Murray, 1997). Although parasite infections did not significantly affect the energy going toward infected pirate perch, in these fish the proportion of production energy allocated to reproduction was doubled when compared

to uninfected hosts. This suggests that parasites may actually be increasing host fitness within this population. This type of effect of parasitism has not been previously reported, probably because there have been so few studies on host-parasite energetics. However, a shift the proportion of energy allocated to reproduction may not necessarily equate to increased production of offspring or increased host fitness. It could also be the case that a greater proportion of production energy is required for infected hosts to maintain the same level of fitness. Studies on host fitness in pirate perch might be logistically difficult. It was originally thought that the pirate perch was a bronchial breeder (Brill, 1977; Boltz and Stauffer, 1986). However, the pirate perch is an elusive fish that does not take to captivity well; and laboratory studies were unable to confirm this (Fontonet and Rutherford, 1999; Katula, 1987; 1992). It took an elaborate field study that employed underwater cameras to find that the pirate perch is a nest breeder with the female doing much of the parental care, and not a bronchial breeder (Fletcher et al., 2004).

In many host-parasite interactions, the immune response is thought to be a major energetic drain on the host (Damian et al., 1997; Martin et al., 2003; Sandland and Minchella, 2003). Our data did not support this idea because the effects of trematode infections on the energetics of the hosts did not differ significantly from the acanthocephalan infection or the uninfected hosts. Although the immune response was not quantified in this study, these results may suggest that neither parasite is eliciting large energetically costly immune responses. However, the energetic cost of the immune response may be present in individual hosts with abnormally large infections. While it would seem that these individual energetic effects would not have much of an effect on

the host population as a whole, it is possible that if host fitness is significantly alter, host population ecology may be affected (Booth et al., 1993; Hudson et al., 1992; 1998).

In conclusion, the energy allocation patterns of pirate perch were not significantly different in fish infected with *A. tehlequahensis*, *Phyllodistomum sp.*, or dual infections. These two parasites species do not present a significant energetic cost to their pirate perch host populations. However, the allocation of energy to reproductive effort was much higher in infected fish than uninfected fish, and this might have important consequences on host fitness.

Figure 3.1: (A) The mean number of pirate perch collected, (B) mean prevalence of single acanthocephalan infection, (C) mean prevalence of single trematode infections, and (D) mean prevalence of dual infections (acanth and trem) in pirate perch per season.

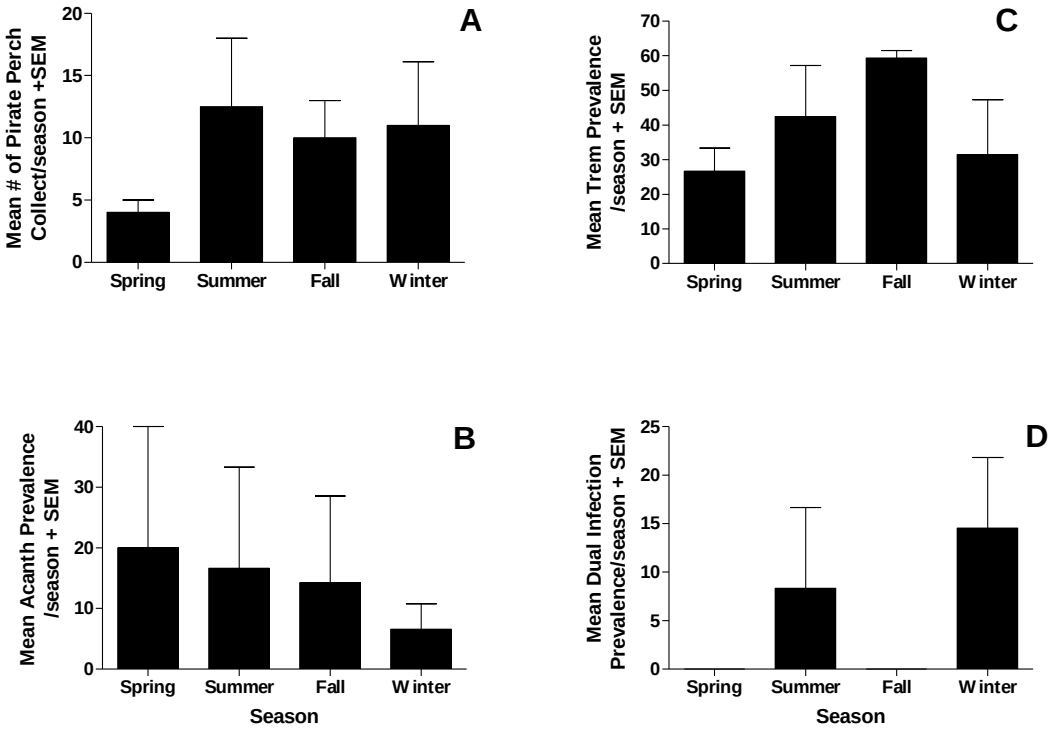

A
Mean # of Pirate Perch Collect/season +SEM
20
15
10
5
0
Spring
Summer
Fall
Winter
C
Mean Trem Prevalence /season + SEM
70
60
50
40
30
20
10
0
Spring
Summer
Fall
Winter
B
Mean Acanth Prevalence /season + SEM
40
30
20
10
0
Spring
Summer
Fall
Winter
Season
D
Mean Dual Infection Prevalence/season + SEM
25
20
15
10
5
0
Spring
Summer
Fall
Winter
Season

Figure 3.2: The mean (A) length, (B) weight, and (C) length/weight ratio of acanthocephalan (black), trematode (gray), dual infected (white) or uninfected (hatched) pirate perch.

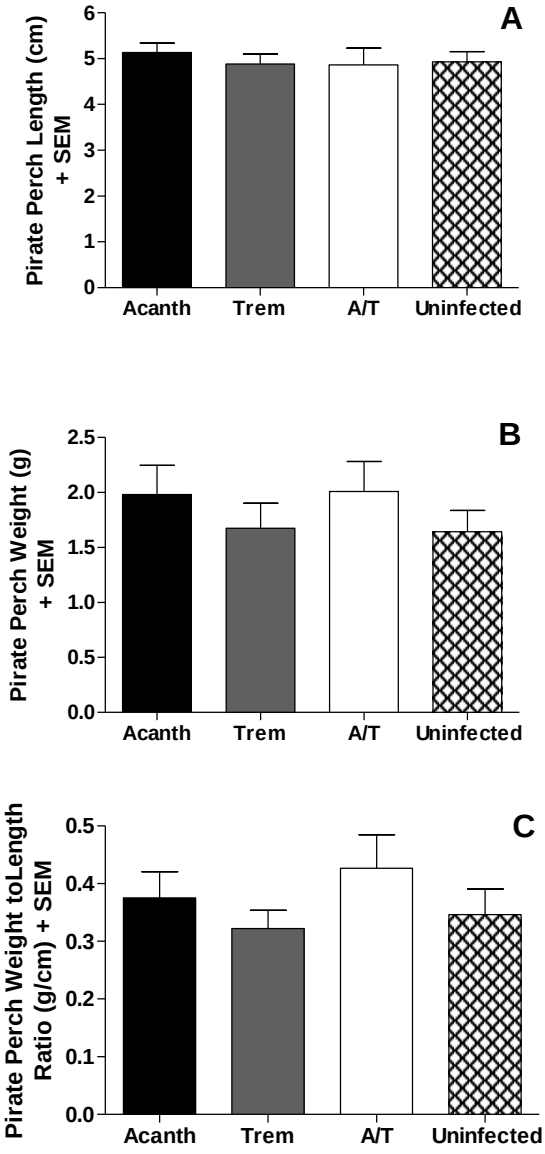

A
Pirate Perch Length (cm) + SEM
6
5
4
3
2
1
0
Acanth
Trem
A/T
Uninfected
B
Pirate Perch Weight (g) + SEM
2.5
2.0
1.5
1.0
0.5
0.0
Acanth
Trem
A/T
Uninfected
C
Pirate Perch Weight toLength Ratio (g/cm) + SEM
0.5
0.4
0.3
0.2
0.1
0.0
Acanth
Trem
A/T
Uninfected

Figure 3.3: The mean consumption (A) and respiration (B) (kj/m^2/day) of acanthocephalan (black), trematode (gray), dual infected (white), and uninfected (hatched) pirate perch.

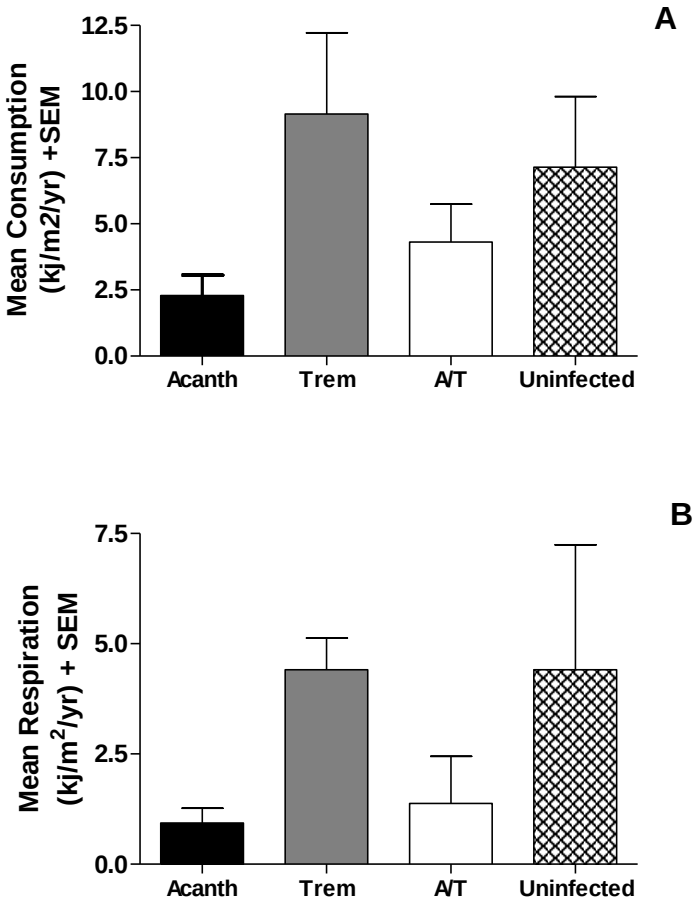

A
Mean Consumption (kj/m2/yr) +SEM
12.5
10.0
7.5
5.0
2.5
0.0
Acanth
Trem
A/T
Uninfected
B
Mean Respiration (kj/m2/yr) + SEM
7.5
5.0
2.5
0.0
Acanth
Trem
A/T
Uninfected

Figure 3.4: Energy budgets for (A) uninfected, (B) acanthocephalan, (C) trematode, and (D) dual infected pirate, showing energy allocated to feces, respiration, host growth, host reproduction, and the parasites (kj/m^2).

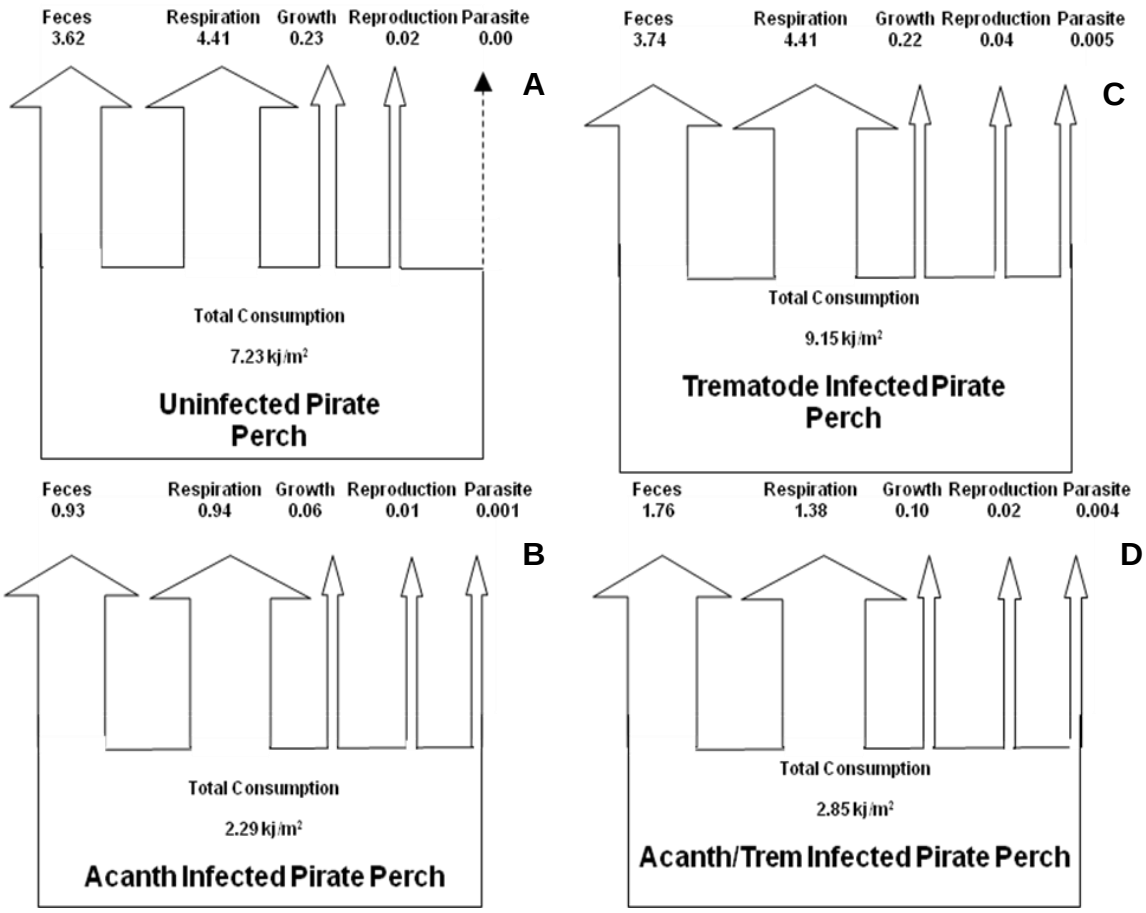
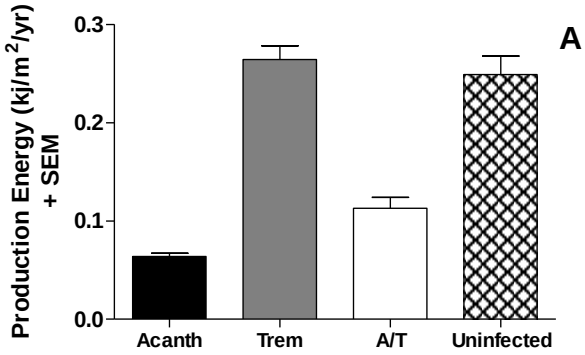
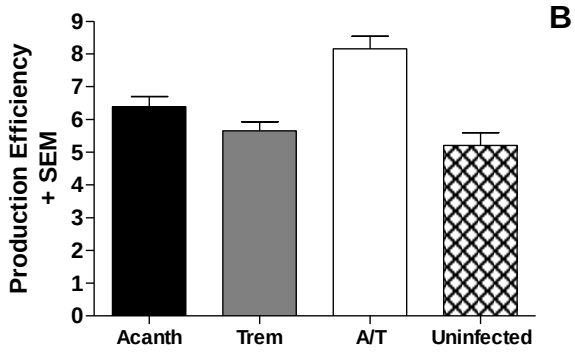
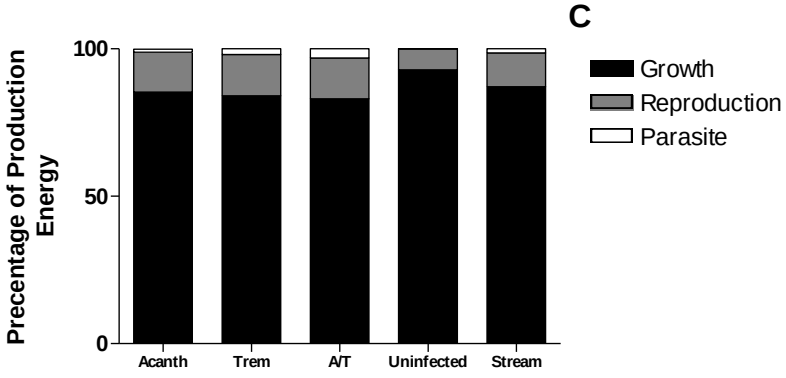

Feces
3.62
Respiration
4.41
Growth
0.23
Reproduction
0.02
Parasite
0.00
A
Total Consumption
7.23 kj/m²
Uninfected Pirate Perch
Feces
3.74
Respiration
4.41
Growth
0.22
Reproduction
0.04
Parasite
0.005
C
Total Consumption
9.15 kj/m²
Trematode Infected Pirate Perch
Feces
0.93
Respiration
0.94
Growth
0.06
Reproduction
0.01
Parasite
0.001
B
Total Consumption
2.29 kj/m²
Acanth Infected Pirate Perch
Feces
1.76
Respiration
1.38
Growth
0.10
Reproduction
0.02
Parasite
0.004
D
Total Consumption
2.85 kj/m²
Acanth/Trem Infected Pirate Perch

Figure 3.5: Energy allocation to production (A) and production efficiency (B) of acanthocephalan (black), trematode (gray), dual infected (white), and uninfected (hatched) pirate perch. (C) The percentage of production energy allocated to growth (black), reproduction (gray), and parasite (white) of infected pirate perch, uninfected pirate perch, and the natural stream population (infected and uninfected pirate perch).


A
Production Energy (kj/m2/yr) + SEM
0.3
0.2
0.1
0.0
Acanth
Trem
A/T
Uninfected


B
Production Efficiency + SEM
9
8
7
6
5
4
3
2
1
0
Acanth
Trem
A/T
Uninfected


C
Precentage of Production Energy
100
50
0
Acanth
Trem
A/T
Uninfected
Stream
Growth
Reproduction
Parasite

Chapter 4

THE COSTS OF PARASITISM ON ENERGY FLOW IN A STREAM ECOSYSTEM.

Abstract

This study measured the energetic costs of parasitism within two New Jersey Pineland streams, one with high levels of parasitism and one with low levels of parasitism. Annual ecosystem energy budgets, including all metazoan parasites, were created for each stream. Each stream was sampled monthly over a 50 m^2 transect for two years, and all inputs (leaf detritus), macroinvertebrates, fish, and metazoan parasites were collected, identified to genus, counted, and biomass measured. In the laboratory, bomb calorimetry and respirometry were used to determine the contribution of all energetic inputs and outputs within the streams. Within both streams, parasites extracted only a fraction of the energy from the total ecosystem budget (0.16% and 0.00003%). In the stream with high levels of parasitism, trophic energy flow patterns were similar to that of healthy ecosystems with 56.5% of the energy going to respiration, 22.3% to biomass production and 21.2% of the energy undergoing decomposition. The stream with low levels of parasitism was much less productive and there was little energy flowing from the detritus to upper trophic levels. In these streams 9.2% of the energy going went to respiration, 3.6% was allocated to biomass production, and the majority of the energy in the system (87.2%) went to decomposition. These data suggest that although parasitism extracts relatively small costs on total ecosystem energetics, some unproductive ecosystems may not be able to support even the small energetic demands of parasites.

Keywords- Ecosystem, Energy Budget, Energy Flow, Parasite, Bomb Calorimetry

Introduction

Parasitism occurs more often than any other feeding strategy on the planet, with at least 50% of all organisms being parasitic during some part of their life (Price 1980, Toft 1991, Freeland and Boulton 1992). By their very nature parasite-host interactions are asymmetrical, and parasitism can often result in significant costs to the host. Theory and experiments have assessed these costs on their hosts at both the individual and host population levels (Anderson and May 1978, May and Anderson 1978, Hamilton and Zuk 1982, Moore 1984, Minchella and Scott 1991). For example, at the individual host level, parasites may cause complete castration in their intermediate hosts and significantly reduce survival and reproductive fitness in definitive hosts (Wilson and Denison 1980, Hamilton and Zuk 1982, Sousa 1983, Théron et al 1992, Sorensen and Minchella 1998, Marzal et al. 2005, Sparkes et al. 2006). Fitness costs of parasites on individual hosts also translate to the population level. For example, long-term studies of population cycles in red grouse (*Lagopus lagopus scotica*) have demonstrated that these cycles are caused by the nematode *Trichostrongylus tenuis*, which decreases grouse survival and fitness in infected birds (Hudson et al. 1992, Dobson and Hudson 1992). When the parasites are removed from the grouse populations with anti-parasitic drugs, the host population cycles abate (Hudson et al. 1998).

Recent studies in diverse systems also support the idea that parasites may have profound effects on the structure of host communities (Poulin 1999, Hernandez and Sukhdeo 2007, Lafferty et al. 2008). In some systems, parasites may even serve a similar function as keystone predators. For example, the cockle (*Austrovenus stutchburyi*) inhabits the shores of New Zealand and serves as substrate for both sea anemones and

limpets. Sea anemones out-colonize limpets for the cockle substrate, but when the cockles are infected with the parasitic trematode (*Curtuteria austeralis*), they are unable to bury themselves very deeply into the mud, and this favors limpet colonization. Thus, under high levels of parasitism, limpets tend to be the dominant colonizers, displacing the sea anemones (Poulin 1999). In addition, when all species colonizing these cockles were examined, colonizer species richness and diversity was higher in the parasitized cockles (Mouritsen and Poulin 2005). The key mechanism driving this effect is that the parasites infect the foot of the cockle and this inhibits their ability to bury themselves.

While it is clear that parasites can induce costs at the individual, population and community levels, it is not apparent what the costs of parasites might be at the ecosystem level, or how to measure these costs (Macrogliese and Cone 1997, Combes 2001, Lafferty et al. 2008). We suggest that energy flow within ecosystems can provide an estimate of the costs of parasitism. Energy is the foundation of all ecosystems, and energy flow dynamics underlie diverse ecological phenomena, including resource allocation, population regulation, food web structure and community dynamics (Lindeman 1942, Odum 1957, Teal 1957, 1962, Slobodkin 1959, Odum 1968, Brown et al. 1993).

When parasites are included in food webs, several web parameters are increased, including the number of feeding links, linkage density, omnivory, and the stability of food webs (Huxham et al. 1995, Thompson et al. 2005, Lafferty et al. 2006, Hernandez and Sukhdeo 2008a). In addition, trophic biomass patterns suggest that energetics may constrain parasitism (Kuris et al. 2008, Lafferty et al. 2008). For example, in salt marsh ecosystems, parasites may constitute a significant proportion of the biomass within the

system, and more biomass accumulates into parasite tissue than into the top predators (piscivorous birds) (Kuris et al. 2008). Similarly results have been reported from studies on food webs in black water streams of the northeast US. In these streams where top predators like piscivorous birds are almost completely absent, total parasite biomass in the system may constitute up to 10-15% of total predator (fish) biomass (Sukhdeo and Hernandez 2005). These data strongly support the idea that parasites extract significant amounts of energy from their ecosystems.

The best representations of ecosystem energy flow are annual ecosystem energy budgets (MacFayden 1948, Teal 1957). Transfer of biomass between trophic levels is not always equal to the flow of energy because biomass is recycled through the system while energy is not (MacFayden 1948, Teal 1957). In addition, biomass patterns do not address energy allocation to either respiration or decomposition, but most of the energy ingested by organisms goes to respiration and decomposition (Odum 1957, Teal 1957, 1962, Engelmann 1961). Examples of energy budgets exist for many different ecosystems including lakes, marine estuaries, cold springs, and old fields (Juday 1940, Lindeman 1942, Odum 1957, Teal 1957, 1962, Engelmann 1961), but parasites were not included in these classic ecosystem studies.

There have only been a few investigations into the effects of parasites on the energy budgets of their hosts, and mostly at the individual host or population levels. Some studies concluded that parasites had no significant effect on individual host energy budgets (Bailey 1975, Munger and Karsov 1989, 1994) while other studies suggested that while parasites had a small direct effect (extracting only small amounts of energy from their hosts), they had significant indirect energetic costs by negatively affecting energy

allocation to host production, reproduction and respiration (Walkey and Meakins 1970, Meakins and Walkey 1975, Connors and Nickols 1991).

In this study, annual energy budgets were created for two New Jersey Pineland streams, one with high levels of parasitism and one with low levels of parasitism to determine how much energy goes to parasites within these stream communities. Each stream was rigorously samples for all inputs and outputs over the course of two years, and bomb calorimetry and respirometry was used to determine the energy allocated to respiration, production, and decomposition by all members of each trophic level and by all metazoan parasites in the system.

Materials and Methods

Study sites

This study was done in two New Jersey Pineland streams, Skit Branch (39°49'08.10"N, 74°40'39.20"W) and Muskingum Brook (39°49'05.10N, 74°44'15.80"W). Skit Branch (Low Parasitism – LP) is a historically undisturbed stream surrounded mostly by pine-oak forest and has low pH (~4), dissolved solids and exotic species (Zampella and Bunnell 1998). It has a low diversity and number of parasites (Hernandez et al. 2007). Muskingum Brook (High Parasitism– HP) is considered a disturbed Pinelands streams and is completely surrounded by residential development and agricultural lands. Agricultural run-off from the surrounding area has resulted in increased pH (~6) and dissolved solids, as well as in species diversity (Zampella and Bunnell 1998). These conditions support many crustaceans and mollusks that serve as intermediate hosts of parasites in these stream systems. This stream has higher diversity and numbers of parasites than Skit Branch LP (Hernandez et al. 2007).

Collection & protocols

The two streams communities were sampled over 50m transects every season for two years using methods from Teal (1957) and Odum (1957). All fish, the predators in these streams, were sampled by seine for the entire 50m stretch for one hour in each stream. Macroinvertebrates were sampled by dipnet from 10 randomly selected 1 ft^2 quadrants. Zooplankton were collected by dragging a plankton net through the streams water column for ten minutes at 5 random sites. Algal accumulation was measured on 5-3in^2 ceramic tiles placed in the stream. Leaf detritus was collected in 5 screen-lined milk crates (1 ft^2) placed randomly in the stream. All samples were brought back the lab; fish

were frozen, and all macroinvertebrates and zooplankton were placed in 70% ethanol. All species were identified to the nearest taxonomic level, and following necropsy all parasites were recovered and preserved in 70% ethanol for identification. Each specimen was then dried for 48hrs at 60°C and biomass was weighed to the nearest 0.0001g.

Ecosystem Energy Budgets

Production energy of all species collected from the streams including parasites was determined using bomb calorimetry. All dried samples were pelleted, weighed, and incinerated in a adiabatic bomb calorimeter (Parr Instrument Company, Illinois), standardized with benzoic acid. Three to five samples of each species were used to determine the mean energy content (kj/g).

A polargraphic dissolved oxygen probe (American Marine, Conneticut) was used to measure changes in dissolved oxygen concentration of live organisms places in sealed containers (Holeston and Jones 1975, Miranda and Hodges 2000). For fish species, changes in dissolved oxygen were all conducted in the field to minimize stress, and to maintain natural stream temperature. All fish collected were weighed and placed in small plastic containers (250ml) to minimize movement. Fish were allowed to acclimate for 5 mins and then dissolved oxygen concentrations in the containers were measured at time 0, 30, 60, and 90 mins. A similar procedure at a smaller scale was used to measure respiration in macroinvertebrates. Macroinvertebrates of mixed species were placed in 50ml containers with aged tap water and allowed to acclimate for 5 minutes (n=10) (Teal 1957). Dissolved oxygen was measured at time 0, 6, 12, and 24 hrs. Immediately following respiration measurements all macroinvertebrates in the sampled were dried for 48 hours at 60°C and weighed to the nearest 0.0001g. To estimate the amount of energy

(calories) to respiration, the amount of oxygen consumed (mg) per gram of dried weight was calculated for each trophic level, and using a conversion factor of 3.38 cal/mg of oxygen (Ivlev 1945, Teal 1957, 1962).

Decomposition energy was comprised of leaf detritus that was not ingested, fecal output, molting casts, and death not due to predation. The efficiencies of energy allocated to ecosystem respiration, decomposition, and production were calculated as proportions of total energy entering the ecosystem. Lindeman's production efficiencies for each trophic level were also calculated using the following:

$$\text{Production efficiency} = \Lambda_{x+1}/\Lambda_x * 100$$

where Λ, total production energy in a given trophic level

Statistical Analysis

One- way ANOVA and Tukey's post- hoc test were used to analyze the seasonal differences in macroinvertebrates, fish and parasite seasonal abundances, biomass and production energy. Two-way ANOVA was used to compare seasonal differences in abundances, biomass, and production energy between the 2 streams. Significance was set at $p \leq 0.05$.

Results

Overall organismal diversity (macroinvertebrates, fish and parasites) was greater Muskingum Brook HP than Skit Branch LP. A total of 45 macroinvertebrate species from 13 families were recovered from Muskingum Brook HP (Table 1), and 23 macroinvertebrates species from 10 families were found in Skit Branch LP (Table 2). Muskingum Brook HP contained 11 species of fish (Table 3) while in Skit Branch LP only 7 species were caught (Table 4). Seven parasites species were recovered from Muskingum Brook HP (Table 5), and 5 species of parasites were found in Skit Branch LP (Table 6).

Zooplankton and algae did not colonize the ceramic plates, and were not found in plankton net samples. These functional groups are not prominent parts of both the Muskingum Brook and Skit Branch food webs (Hernandez and Sukhdeo 2008).

Leaf detritus is the predominant basal energy source in both streams. However, in Muskingum Brook HP most of the leaf detritus enters the stream in the fall (Figure 1), while most leaf detritus enters Skit Branch LP in the winter (Figure 1). No leaf detritus entered the streams in the summer and a very small amount in the spring (Figure 1).

The seasonal abundance, biomass, and production energy of macroinvertebrates were significantly different in the 2 streams ($p<0.05$). Muskingum Brook HP had significantly larger macroinvertebrate abundances, biomass and production energy in spring and winter when compared to summer and fall ($p<0.05$) (Figure2), but there were no significant seasonal differences in macroinvertebrates from Skit Branch LP (Figure 2). Although, biomass and production energy were significantly greater only in the spring

and the winter ($p<0.05$). There were always more macroinvertebrates in Muskingum Brook HP at all times of the year ($p<0.05$) when compared to Skit Branch LP.

Fish species showed no significant difference in seasonal abundance, biomass, or production energy in Muskingum Brook HP (Figure 2). On the other hand there were seasonal differences in Skit Branch LP, which had significantly higher abundances, biomass and energy content in the summer and fall ($p<0.05$) (Figure 2). Muskingum Brook HP was more productive and I addition to there being more fish in this stream, these fish greater individual biomass and energy content than fish collected from Skit Branch LP ($p<0.05$).

There were significant differences in the abundance, biomass and production energy of parasites between the 2 streams. In Muskingum Brook HP, parasites were more abundant, and had greater biomass and energy content in all seasons when compared to Skit Branch LP ($p<0.05$). Parasites only infected fish hosts in the summer within Skit Branch LP (Figure 2), while parasites were found in all seasons within Muskingum Brook HP (Figure 2). There were no significant seasonal differences in parasite abundances, biomass, or energy content in Muskingum Brook HP (Figure 2).

Ecosystem energy flow diagrams ($kj/m^2/yr$) were constructed for Muskingum Brook HP and Skit Branch LP using the graphical methods of Teal (1957) (Figures 3). Muskingum Brook HP had 191 $kj/m^2/yr$ of leaf detritus entering the stream. Of this energy, 185 $kj/m^2/yr$ were processed by macroinvertebrates with the majority going to respiration (134 $kj/m^2/yr$) and 51 $kj/m^2/yr$ allocated to invertebrate production. Fish within this stream ingested 8 $kj/m^2/yr$ and allocated 3 $kj/m^2/yr$ to fish production. Parasites within this stream took 0.31 $kj/m^2/yr$ from macroinvertebrates and fish (Figure

5A). The flow of energy through Skit Branch LP displayed a very different pattern from that of Muskingum Brook HP (Figure 5). More energy in the form of leaf detritus enters Skit Branch LP (326 kj/m^2/yr), but the majority of the leaf detritus is not processed by macroinvertebrates and is left to decompose 293 kj/m^2/yr. In Skit Branch LP, macroinvertebrates ingested 33 kj/m^2/yr and allocated 22 kj/m^2/yr to respiration and 11 kj/m^2/yr to production. Fish processed most of the production energy contained within the macroinvertebrates by ingesting 10 kj/m^2/yr and allocating most of that to respiration (9 kj/m^2/yr). Parasite production energy from fish hosts was minimal (.0001 kj/m^2/yr) and took no energy from macroinvertebrates (Figure 3B).

In Muskingum Brook HP 56.5% of total energy was given off as heat through respiration, 22.3% ended up in biomass (i.e. production), and 21.2% went to decomposition. Skit Branch LP on the other hand had the largest proportion of its energy going towards decomposition (87.2%). Only 9.2% of the ecosystems energy was lost as heat through respiration and 3.6% ended up as biomass. Additionally, there were also differences in the Lindeman efficiencies (Lindeman 1942) for each trophic level within the ecosystems between the 2 streams. In Muskingum Brook HP invertebrates were the most efficient at converting energy into production (26.9%). Fish were next with an efficiency of 6.0%, and parasites were the least efficient (0.6%) (Figure 4). However, in Skit Branch LP fish (12.3%) were more efficient than either invertebrates (3.4%) or parasites (.0008%) (Figure 4).

Discussion

Ecosystem energy budgets have been critical to our current understanding of ecological dynamics and food web structure (Juday 1940, Lindeman 1942, MacFayden 1948, Odum 1957, Teal 1957, 1962, Engelmann 1961, Morin 1999, Dunne 2006). However, the use of ecosystem energy budgets has gone out of fashion in recent years, partly because their construction is very labor intensive, and other methods have evolved to answer questions on energy flow and food web theory including theoretical models, biomass measurement, and stable isotope analysis (Pimm and Lawton 1977, DeAngelis 1992, Loreau 1995, Finlay 2001, Kuris et al. 2008). The current study was feasible because the streams are highly acidic (pH~4) black water streams, which have naturally low biodiversity (Zampella and Bunnell 1998). These naturally depauperate systems make it easy to quantify the entire food web (Hernandez and Sukhdeo 2008) and allow an ecosystem study of energy flow.

There were clear differences in the flow of energy between Muskingum Brook HP and Skit Branch LP. Muskingum Brook HP showed an energy flow pattern that is characteristic to other productive ecosystems with the efficiencies of energy transfer between trophic levels ranging from 6%-26% (Juday 1940, Lindeman 1942, Macfayden 1948, Odum 1957, Teal 1957, 1962, Engelmann 1961). For example, in arctic ecosystems, these values ranged from 3-7% but in marine systems in the Atlantic, efficiencies range from 12-27% (Glazier 1991, Pomeroy 2001, Jennings et al. 2002). Muskingum Brook HP has the flow patterns of healthy systems because it is considered a disturbed stream in this region. It is surrounded on all sides by agricultural and residential lands, and the runoff from these lands has increased the pH (~6) and the

calcium inputs into this stream (Zampella and Bunnell 1998). This increase in pH has made this stream more suitable for the establishment of invertebrate species that are not normally found in these streams including isopods, amphipods, fingernail clams, snails, and mayflies. The low pH (~4) and low calcium inputs in the natural streams prevents shell and exoskeleton formation of many of these detritivore groups (Sutcliffe and Carrick 1973, Mackay and Kersey 1985, Sutcliffe and Hildrew 1989, Zampella and Bunnell 1998). In the more natural stream, Skit Branch LP, the energy flow patterns would be considered atypical or unproductive. There were fewer macroinvertebrates and their abundances, biomass, and energy content were significantly lower in Skit Branch LP was not processed by macroinvertebrates.

Ecologists have generally assumed that energy flow to parasitism within an ecosystem is small (Odum 1957, Loreau et al. 2005), and this appears to be right. The direct energetic costs of parasites within both streams were quite small 0.31 kj/m^2/yr in Muskingum Brook HP and 0.0001 kj/m^2/yr in Skit Branch. In addition the Lindeman efficiencies (Koslovsky 1968, Brafield and Llywellyn 1982, Hairston and Hairston 1993) for parasites (i.e. parasite energy as a percentage of total macroinvertebrate or fish energy in the system) within Muskingum Brook HP were 0.6% and 0.3% for energy derived from macroinvertebrates and fish respectively. These efficiencies were even lower in Skit Branch LP, with only 0.008% of going to parasites from fish (Figure 4). Nevertheless, similar values were reported from studies of biomass transfer between parasites and their hosts in California salt marshes (Kuris et al. 2008). Within these highly productive marsh systems where biomass is used as a surrogate for energy, Lindeman efficiencies for parasites ranged from ~3.0% of biomass from crab hosts to as

little as 0.1% from the top carnivores. Overall, parasite biomass accounted for only 0.2-1.3% of all animal biomass within these marsh systems (Kuris et al. 2008). These data support the idea that parasites may be constrained by some energetic dynamics that limit their access to energy.

There was proportionally less energy going to parasites in Skit Branch LP than in Muskingum Brook HP. The difference in energy allocation to parasites within the two streams may be due to availability of hosts. Many of the parasites that infect fish within the streams of the New Jersey Pinelands are trophically-transmitted parasites that require invertebrate detritivores as intermediate hosts (Hernandez et al. 2007). In Muskingum Brook HP, the two most abundant parasites species are *Acanthocephalus tehlequahensis* and *Phyllodistomum sp*., which use and isopod and a fingernail clam as intermediate hosts respectively. These two parasite are completely absent from Skit Branch, and their intermediate hosts were either absent or in very low numbers. These data support the positive relationship that has been reported between parasite diversity and host diversity (Combes 2001, Holt et al. 2003, Keesing et al. 2006, Hechinger and Lafferty 2005, Poulin 2007), but suggests that energetic dynamics impose greater constraints on parasite diversity than the effects of host diversity.

Table 4.1: Macroinvertebrate species collected from Muskingum Brook HP.

Species	Family	% of Community
Synurella sp.	Amphipoda	3.46
Pisididiiae sp.	Bivalvia	13.06
Carabidae sp	Coleptera	0.13
Corixdae sp.	Coleptera	0.13
Laccophilus sp.	Coleptera	0.13
Oulimnius sp.	Coleptera	0.38
Unknown Coleptera sp.	Coleptera	0.51
Unknown Collembola sp.	Collembola	0.26
Ablabsemyia sp.	Diptera	28.81
Chyrsops sp.	Diptera	0.26
Leptoconops sp.	Diptera	0.13
Probezzia sp.	Diptera	0.38
Simulinium sp.	Diptera	0.51
Twinnia sp.	Diptera	0.26
Unknown Chrominae sp.	Diptera	0.13
Gyraulus sp.	Gastropoda	0.13
Helisoma sp.	Gastropoda	0.26
Physa sp.	Gastropoda	9.86
Moorebdella sp.	Hirudinia	0.38
Ceacidotea communis	Isopoda	5.76
Chauloides sp.	Megaloptera	0.13
Sialis sp.	Megaloptera	0.51
Argia sp.	Odonata	0.13
Boyeria sp	Odonata	0.13
Calopteryx sp.	Odonata	11.78
Gomphus sp.	Odonata	0.26
Neurocordulia sp.	Odonata	0.13
Unknown Oligocheate sp.	Oligocheate	0.51
Brachycentropus sp.	Trichoptera	0.77
Cheumatopschye sp.	Trichoptera	1.79
Helicopsyche sp.	Trichoptera	0.13
Molanna sp.	Trichoptera	0.13
Neutrichia sp.	Trichoptera	0.13
Oectis sp.	Trichoptera	0.13
Platycentropus sp.	Trichoptera	1.79
Phagocata sp.	Turbelleria	0.26
Leptophlebia sp.	Ephemeroptera	4.99
Leuctra sp.	Plecoptera	0.13
Neoephemera sp.	Ephemeroptera	11.14
Parasleptophelbia sp.	Ephemeroptera	0.13

Table 4.2: Macroinvertebrate species collected from Skit Branch LP.

Species	Family	% of Community
Pisididiiae sp.	Bivalvia	1.08
Unknown Coleptera sp.	Coleptera	1.08
Ablabesmyia sp.	Diptera	61.29
Chrysops sp.	Diptera	2.15
Probezzia sp.	Diptera	3.23
Nigronia sp.	Megaloptera	2.15
Cordulagaster sp.	Odonata	1.08
Gomphus sp.	Odonata	6.45
Calopteryx sp.	Odonata	1.08
Unknown Oligocheate sp.	Oligocheate	2.15
Leuctra sp.	Plecoptera	13.98
Parapsyche sp.	Trichoptera	3.23
Neotrichia sp.	Trichoptera	1.08

Table 4.3: Fish species collected from Muskingum Brook HP.

Common Name	Species Name	% of Community
Yellow Bullhead	*Ameiurus natalis*	0.53
Banded Sunfish	*Enneacanthus obesus*	18.95
Blue Gill Sunfish	*Lepomis macrochirus*	11.05
Blue Spotted Sunfish	*Enneacanthus gloriosus*	7.37
Creek Chubsucker	*Erimyzon oblongus*	3.16
Eastern Mudminnow	*Umbra pygmaea*	1.58
Redfin Pickerol	*Esox americanus*	0.53
Chain Pickerol	*Esox niger*	1.05
Pirate Perch	*Aphredoderua sayanus*	40.53
Pumpkin Seed	*Lepomis gibbosus*	9.47
Swamp Darter	*Etheostoma fusiforme*	5.79

Table 4.4: Fish species collected from Skit Branch LP.

Common Name	Species Name	% of Community
Banded Sunfish	*Enneacanthus obesus*	52.38
Blue Gill Sunfish	*Lepomis macrochirus*	9.52
Blue Spotted Sunfish	*Enneacanthus gloriosus*	9.52
Creek Chubsucker	*Erimyzon oblongus*	4.76
Eastern Mudminnow	*Umbra pygmaea*	4.76
Chain Pickerol	*Esox niger*	4.76
Pirate Perch	*Aphredoderua sayanus*	14.28

Table 4.5: Parasite species dissected from fish in Muskingum Brook HP.

Class	Species	% of Community
Acanthocephala	*Acanthocephlus tehlaquehensis*	22.26
	Neoechinorhychus sp.	5.38
Trematoda	*Phyllodistomum sp.*	30.06
	Crepidostomum sp.	1.30
	Posthodilostomum sp.	25.23
Nematoda	*Nematode sp. 1*	5.19
	Nematode sp. 2	10.58

Table 4.6: Parasite species dissected from fish in Skit Branch LP.

Class	Species	% of Community
Acanthocephala	*Neoechinorhynchus sp.*	12.50
Trematoda	*Posthodilostomum sp.*	12.50
Nematoda	*Nematode sp. 1*	25.00
	Nematode sp. 2	37.50
	Nematode sp. 3	12.50

Figure 4.1: The amount of biomass (g/m^2) in the form of leaf detritus entering Muskingum Brook HP (black) and Skit Branch LP (white) seasonally.

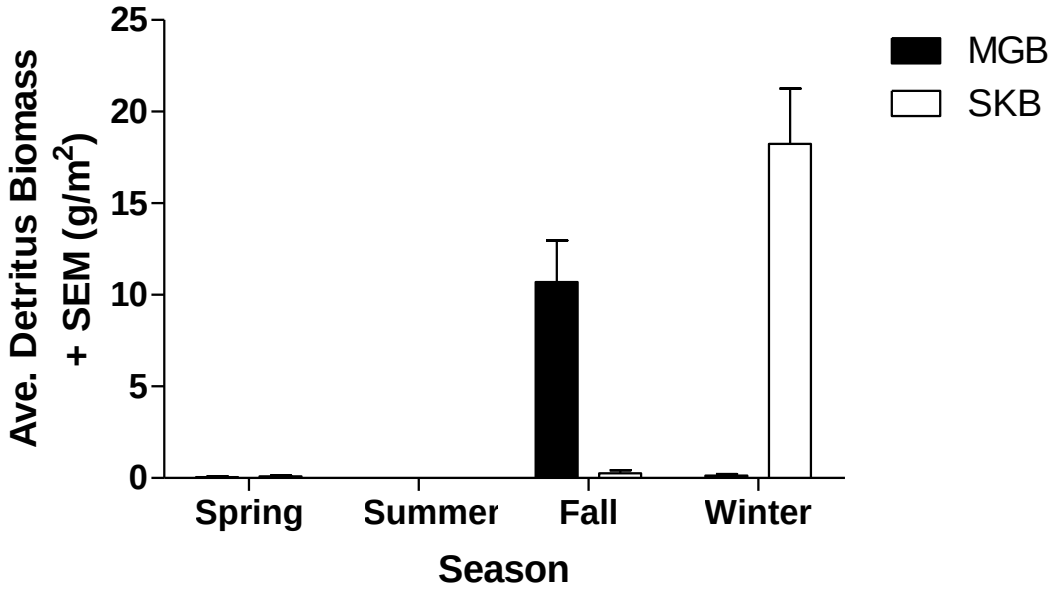

Ave. Detritus Biomass
+ SEM (g/m2)
25
20
15
10
5
0
Spring
Summer
Fall
Winter
Season
MGB
SKB

Figure 4.2: Seasonal abundance (A), biomass ($g/m^2/yr$) (B), and production energy ($kj/m^2/yr$) (C) of macroinvertebrates, fish and parasites collected from Muskingum Brook HP (black) and Skit Branch LP (white).

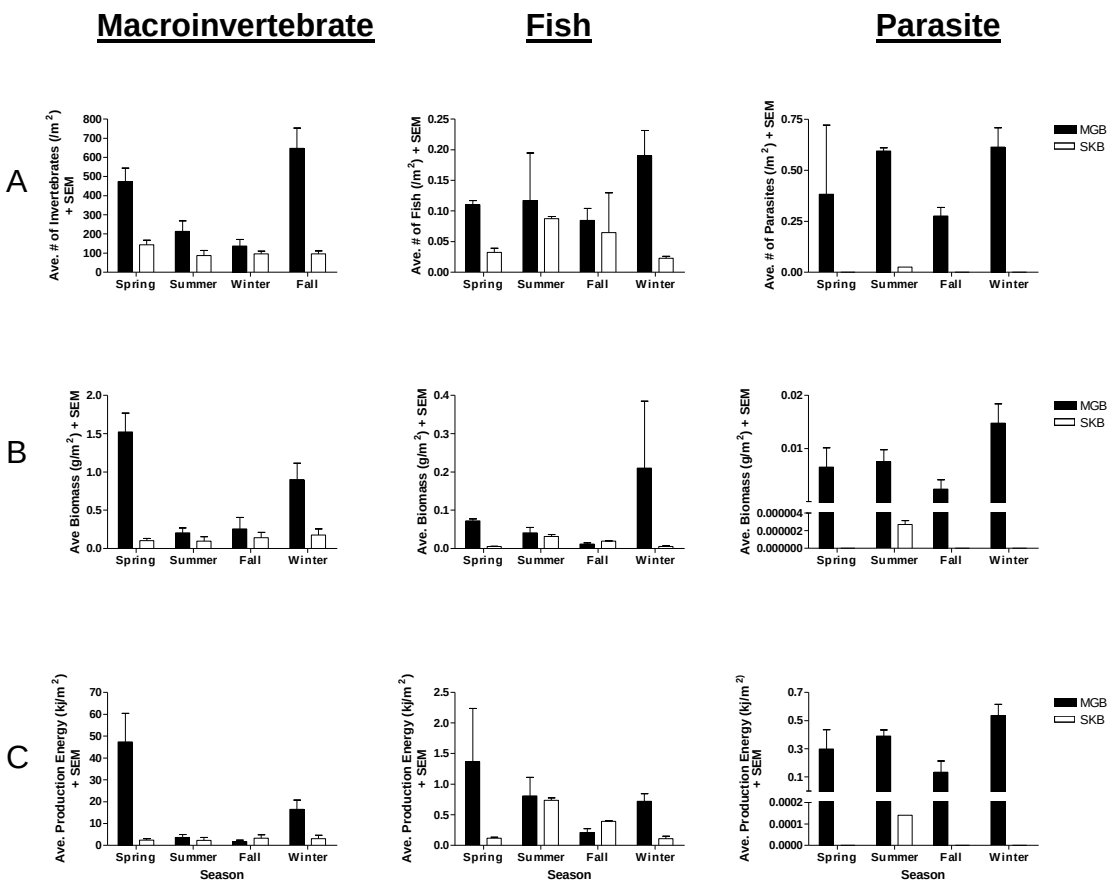
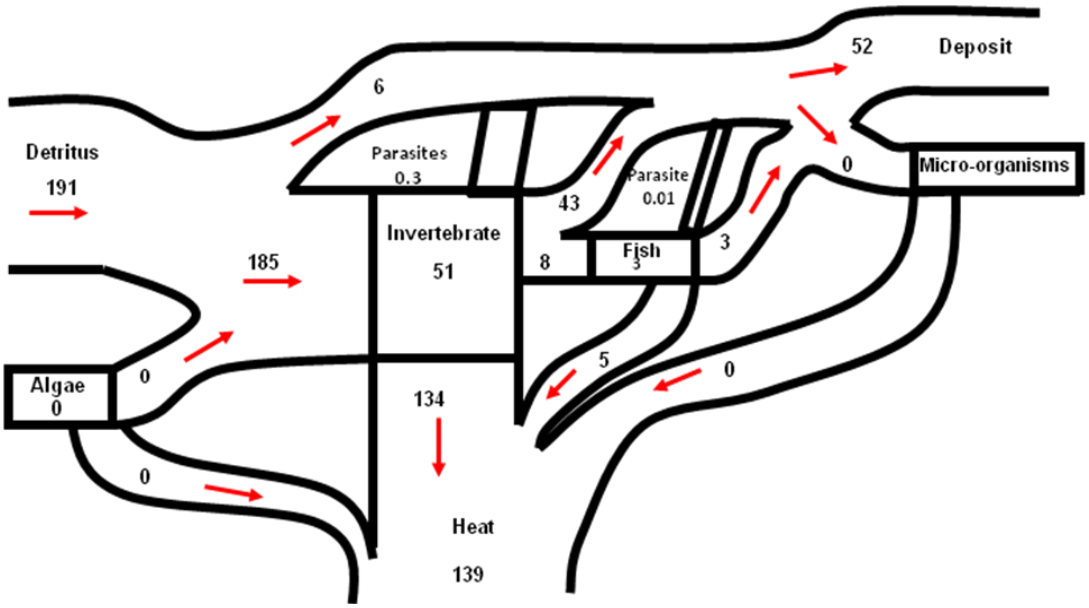

Macroinvertebrate
Fish
Parasite
A
B
C
Ave. # of Invertebrates (/m 2) + SEM
Ave. # of Fish (/m 2) + SEM
Ave. # of Parasites (/m 2) + SEM
Ave Biomass (g/m 2) + SEM
Ave. Biomass (g/m 2) + SEM
Ave. Production Energy (kj/m 2) + SEM
Spring
Summer
Fall
Winter
Season
MGB
SKB

Figure 4.3: Muskingum Brook HP (A) and Skit Branch LP (B) ecosystem energy flow diagram (kj/m^2/yr) (not shown to scale).

A


Detritus
191
Parasites
0.3
6
52
Deposit
Micro-organisms
0
Parasite
0.01
43
Invertebrate
51
8
Fish
3
3
185
5
0
Algae
0
0
134
0
Heat
139

B

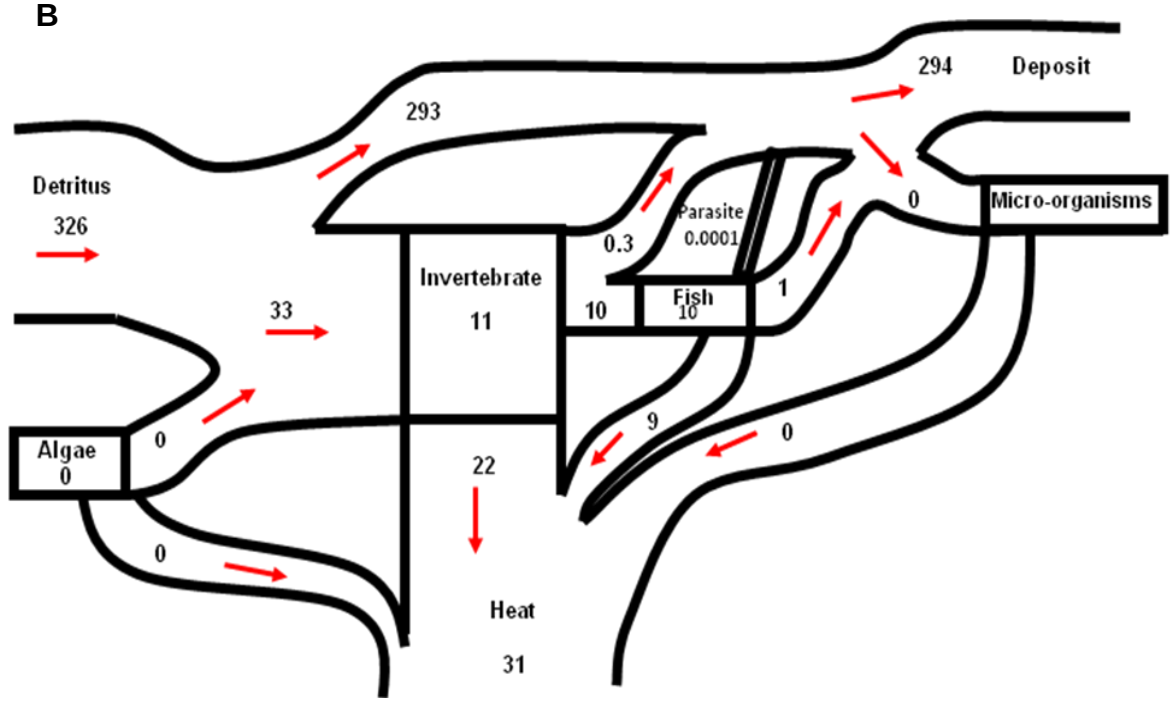
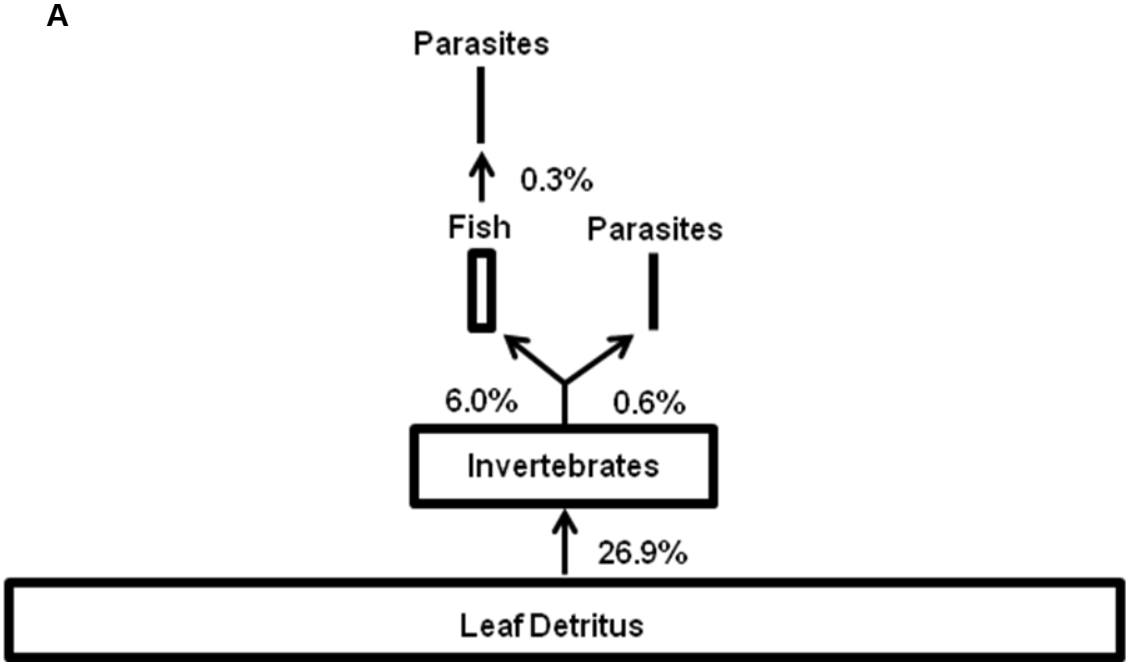
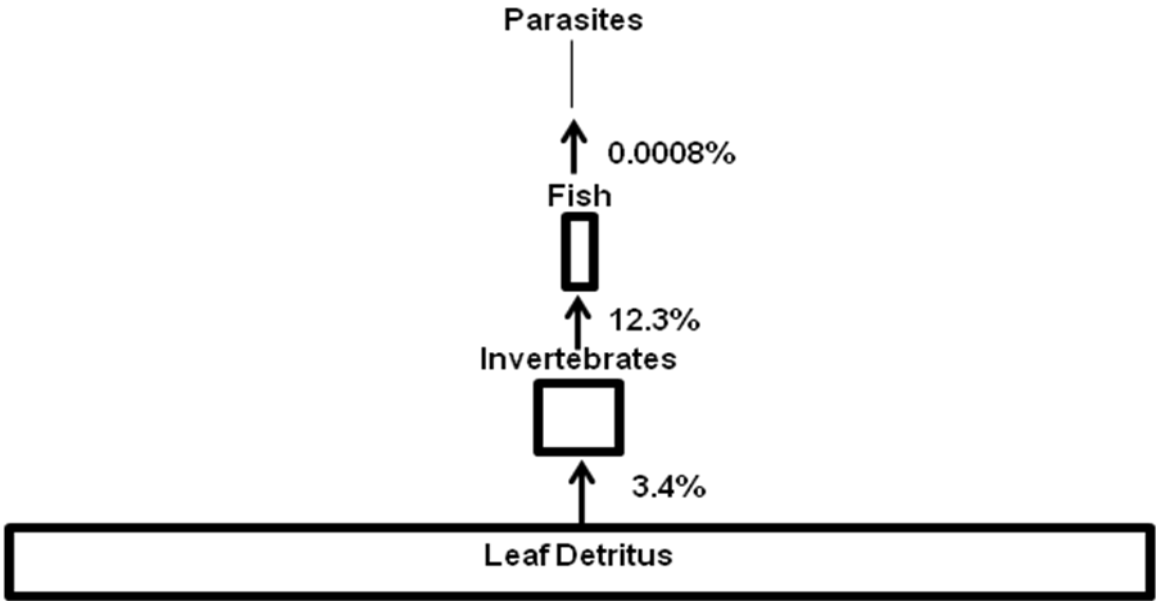

Detritus
326
293
294
Deposit
Micro-organisms
0
Parasite
0.0001
0.3
Invertebrate
11
10
Fish
10
1
33
9
0
Algae
0
0
22
0
Heat
31

Figure 4.4: Lindemanm (1942) efficiencies for each trophic level within Muskingum Brook HP (A) and Skit Branch LP (B).

A


Parasites
0.3%
Fish
Parasites
6.0%
0.6%
Invertebrates
26.9%
Leaf Detritus

B


Parasites
0.0008%
Fish
12.3%
Invertebrates
3.4%
Leaf Detritus

Chapter 5

FEEDING STRATEGY DETERMINE PARASITE'S TROPHIC LEVEL.

Abstract

In food web studies, it is not clear to which trophic level parasites be assigned. The current study used stable isotope analysis (δ 13C and δ15 N) to determine the trophic position of two internal parasites infecting the same fish hosts using different feeding strategies. The acanthocephalan parasite lives in the intestine and feeds on host food. The trematode parasite lives in the ureters and feeds on host tissue. Changes in carbon and nitrogen isotopic signatures indicate that these parasites can occupy different trophic levels. There was no significant differences between the isotopic signatures of adult acanthocephalan parasites and their pirate perch host. Whereas the trematode tissues were significantly enriched in δ15N compared to its fish hosts, suggesting that it occupies a trophic level above fish.

Introduction

Recently the roles of parasites in food webs has received considerable attention, and there is rigorous debate on how best to include parasitism into present food web theory (Macrogliese and Cone 1997, Lafferty et al. 2008). A major problem when trying to incorporate parasites in food webs is determining their trophic positions. Parasites often feed at multiple trophic levels during their lifecycle. To get around this, some analyses treat them as trophospecies that can occur at multiple trophic levels within a food web (Huxham et al. 1996). Normally, because the host is considered sole energy source, most practitioners place parasites one trophic level above their hosts (Lafferty et al. 2006, Hernandez and Sukhdeo 2008). However, internal parasites can have distinct feeding strategies, and they obtain their energy from a host is many ways. For example, parasites of the intestine can obtain their energy from host blood, host tissue, host food or bacteria lining the gut of the rabbit (Neilson et al. 2005). Parasite feeding strategies can be divided into two broad categories. The first is tissue and/or blood feeding and this is usually associated with parasite species that possess mouths such as nematodes and trematodes. The second category is food absorption, and these are always gut parasites that lack mouths (e.g. tapeworms and acanthocephalans), and the food they absorb is the host's food. These differences in parasite feeding strategies have the potential to greatly affect their trophic position within a system.

Stable isotope analysis (SIA) has often been used to help determine energy flow through food webs ($\delta^{13}C$ enriched 0-1‰ from energy sources) and trophic position of the species in the web ($\delta^{15}N$ enriched 0-5‰ between trophic positions) (Fry and Sherr 1984, Peterson and Fry 1987, Fry 1991). In host-parasite relationships, parasites are

significantly depleted in carbon and nitrogen isotopes when compared to their hosts suggesting they are at a lower trophic level than their host (Boag et al. 1998 Iken et al. 2001, Pinnegar et al. 2001, Deudero et al. 2002, Neilson et al. 2005, Persson et al. 2007). Other studies found no significant difference in the isotopic signatures between parasites and hosts (O'Grady and Dearing 2006), while some have found parasites have significantly enriched ^{13}C and ^{15}N values when compared to hosts (Boag et al. 1998, Doucett et al. 1999, Iken et al. 2001, Deudero et al. 2002, Neilson et al. 2005, Persson et al. 2007). In the present study we use SIA to determine the trophic position of two adult parasites species, a trematode and an acanthocephalan that share the same fish definitive host.

The trophic positions were determined for all organisms within a natural food web in a black water stream in the New Jersey Pinelands. All leaf detritus (input), macroinvertebrates, fish, and parasites were sampled every three months for two years. These streams have low biodiversity and relatively simple food webs (Hernandez and Sukhdeo 2008). Energy flow is primarily driven by leaf detritus which falls into the stream each year (Zampella and Bunnell 1998, Hernandez and Sukhdeo 2008). During the course of this study nine species of fish, 46 species of macroinvertebrate, and 7 species of parasite were collected. Only the most abundant species were used for SIA (Table 1). In these streams, 90% of all adult parasite infections came from two trophically-transmitted parasites, the acanthocephalan *Acanthocephalus tehlaquehensis* and the trematode *Phyllodistomum sp* The larval acanthocephalan *A. tehlaquehensis* infects an isopod intermediate host, *Ceacidotea communis*, which is eaten by the definitive host the pirate perch, *Aphredoderus sayanus*, where the adult lives in the gut .

The larval *Phyllodistomum sp.* infects its first intermediate hosts, the fingernail clam (*Pisidiiae sp.*) and then it infects a second intermediate host, either a damselfly or caddisfly, which are consumed by the pirate perch. The adults live in the swim bladder of the pirate perch feeding on blood and tissue. Although, both parasites occasionally infected other fish species, 80% of these parasites in the stream were in pirate perch.

Trophic position of all species was determined based on a ~3.4‰ change in the nitrogen signature (Minagawa and Wada 1984, Owens 1987, Peterson and Fry 1987, Cabana and Rasmussen 1994). The primary energy sources of mix leaf detritus had a $\delta^{13}C$ value of -29.66 (Table 1 & Fig. 1). All fish species carbon signatures were enriched by 0-2‰ compared to leaf detritus (Table 1 & Fig. 1). However, macroinvertebrates were both significantly enriched and depleted in ^{13}C compared to leaf detritus with *Platycentropus sp.* (a caddisfly and known detritivore) being the most depleted (~5‰) and the algavore *Pisidiiae sp.* (a fingernail clam) the most enriched (~5‰) (Table 1 & Fig. 1). The large differences in the $\delta^{13}C$ among the macroinvertebrates clearly represent differences in feeding strategies. Species may selectively feed on different species of leaf detritus (Wallace et al. 1970, Irons et al. 1988), and may ingest some algal species. Parasites were not significantly enriched compared to leaf detritus ($p<0.05$, Fig. 1). These data suggest that although there may be some species-specific differences in energy sources for the macroinvertebrates of this stream, leaf detritus is the primary energy source of energy flow to the top of the food web.

All species were significantly enriched in nitrogen compared to leaf detritus (Fig.1). Difference in $\delta^{15}N$ (~3.4 ‰) and known feeding relationships were used to determine the trophic position of species within the food web. Several species of

macroinvertebrate were placed in the second trophic level (*Physa sp.*, *Ceacidotea communis*, and *Calopteryx sp.*). *Calopteryx sp.* is a known invertebrate predator. However both *Physa sp.* and *Ceacidotea communis* are known detritivores. These species also feed on fungi and bacteria that grow on the leaf detritus (Arsuffi and Suberkropp 1989, Graça et al. 1993, Hernandez and Sukhdeo 2008). Isopods in particular are known to selectively feed on the fungi growing on detritus, and this may greatly affect nitrogen enrichment in these species. Fish species were enriched 1-5‰ from their macroinvertebrate prey, and were placed at two trophic levels within the food web (Table 1 & Fig. 1).

The relationship between stable isotope signature and trophic level of host-parasite interactions within this stream are show in Figure 2. In terms of energy flow, juvenile acanthocephalans (cystacanths) were significantly depleted in $\delta^{13}C$ compared to their isopod host (Table 1 & Fig. 2A). This is most likely due to selective absorption particular of carbohydrate and lipid molecules (Neilson et al. 2005). There were no significant differences in the carbon signature of adult acanthocephalan parasites and their pirate perch host (Table 1 & Fig. 2A). Pirate perch nitrogen signatures were not significantly different from isopods or juvenile acanthocephalans ($p>0.05$), but were significantly more enriched than adult acanthocephalans ($p<0.05$) (Fig. 2B). Juvenile acanthocephalan had significantly greater $\delta^{15}N$ values than isopods and adult acanthocephalans ($p<0.05$), Thus, adult acanthocephalans were placed half a trophic level lower than juvenile acanthocephalans (Fig. 2B).

There were no significant differences in the $\delta^{13}C$ of pirate perch, and *Phyllodistomum sp.* (Table 1, Fig 2A), suggesting they derive their energy from the same

carbon source, leaf detritus. However, the fingernail clam, which serves as this trematode's intermediate host was significantly enriched in carbon compared leaf detritus, pirate perch, and *Phyllodistomum sp.* This may be due to the fact that it feeds on algae (Hernandez and Sukhdeo 2008). No juvenile trematodes (redia, sporocysts, or cercaria) were recovered from the fingernail clams, and therefore no comparison of $\delta^{13}C$ of these stages was possible. However, it is clear that adult *Phyllodistomum sp.* feeds directly on its fish definitive host because they are significantly enriched in $\delta^{15}N$ compared to their fish host (Fig. 2B). These data suggest that *Phyllodistomum sp.* occupies the top trophic level within this stream food web.

A parasite species can obtain its energy from multiple trophic levels within a food web, and the SIA of the parasites and hosts from a stream in the New Jersey Pinelands suggests that feeding relationships between a single parasite species and it hosts can be complicated. Based on the lifecycle of the acanthocephalan parasite, it could be assumed that juvenile and adult parasites feed at the same trophic level. However, SIA indicates that these two life stages feed at different trophic levels. Specifically, *A. tehlaquehensis* juveniles feed higher up in the food web than adult parasites. This is probably because isopods are omnivorous and feed on both leaf detritus and fungal decomposers living on the leaf detritus (Graça et al. 1993). On the other hand, adult acanthocephalan obtain energy from absorption of pirate digesta within the intestine, and pirate perch feed on many species of strict detritivore (Hernandez and Sukhdeo 2008), most likely resulting in fish ingesta being less ^{15}N enriched than isopods.

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Ultimately, parasite-host feeding relationships are not easy to determine, and can be more complex than simply considering parasites as feeding on the host. Our results clearly indicate that life stages of the same parasite species or different parasite species within the same hosts can occupy different trophic positions. Additionally, these results clearly suggest that parasites can occupy multiple trophic levels within a food web, and the top trophic position within this stream was occupied by a parasite.

Table 5.1: $\delta^{13}C$ and $\delta^{15}N$ values of species collected from a New Jersey Pineland stream.

Species	Common Name	δ13C	Error	δ15N	Error
Leaf Detritus		-29.66	0.0536	3.62	0.5909
Macroinvertebrates					
Platycentropus sp.	Caddisfly	-34.86	2.7540	8.75	0.6089
Ceacidotea communis	Isopod	-24.88	0.5000	12.05	0.3258
Calopteryx sp.	Damselfly	-32.35	0.0901	12.28	0.1201
Physa sp.	Snail	-23.76	0.7254	10.10	0.1936
Pisidiiae sp.	Fingernail Clam	-19.98	0.5267	9.20	0.5908
Fish					
Aphredoderus sayanus	Pirate Perch	-28.86	0.1635	13.04	0.2822
Lepomis gibbosus	Pumpkinseed	-29.05	0.6164	13.07	0.1686
Enneacanthus obesus	Banded Sunfish	-28.14	0.2663	11.47	0.6152
Enneacanthus gloriosus	Blue Spotted Sunfish	-27.05	0.4320	14.08	0.1535
Lepomis macrochirus	Bluegill	-27.51	0.4363	13.23	0.0945
Esox niger	Juvenile Chain Pickerel	-29.18	0.4062	10.85	0.5379
Parasites					
Acanthocephalus tehlaquehensis	Juvenile Acanthocephalan	-31.90	0.4135	14.01	0.2595
	Adult Acanthocephalan	-29.57	0.0703	11.52	0.1351
Phyllodistomum sp.	Adult Trematode	-29.70	N/A	18.43	N/A

Figure 5.1: The relationship between $\delta^{15}N$ and $\delta^{13}C$ of all species including fish (solid squares), macroinvertebrates (solid triangles), parasites (open circles), leaf detritus (solid diamond), and standards (solid circles).

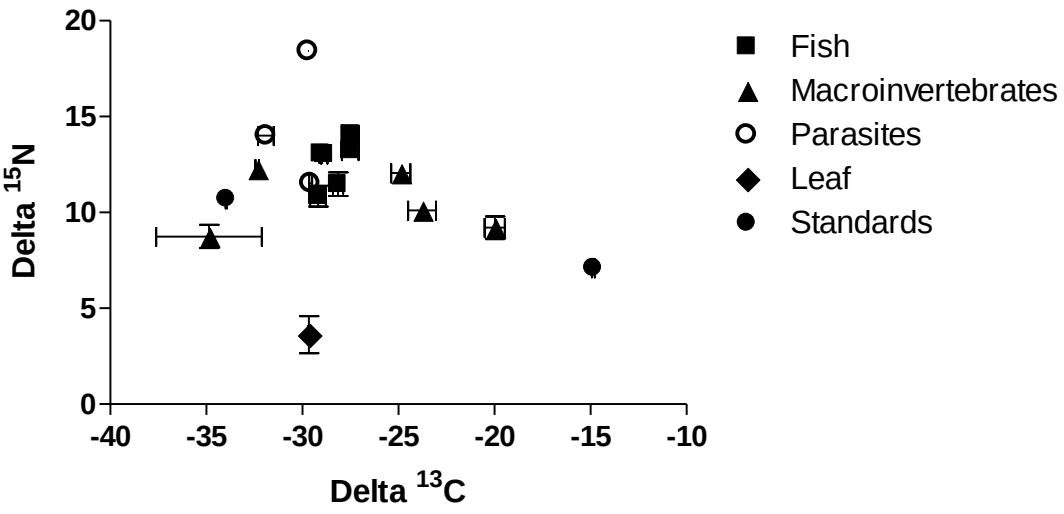
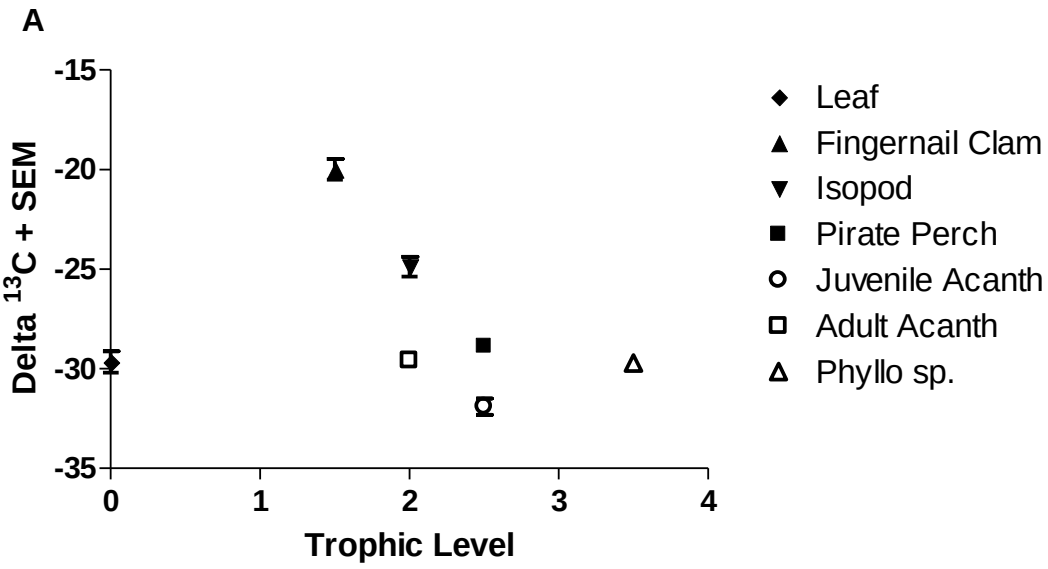
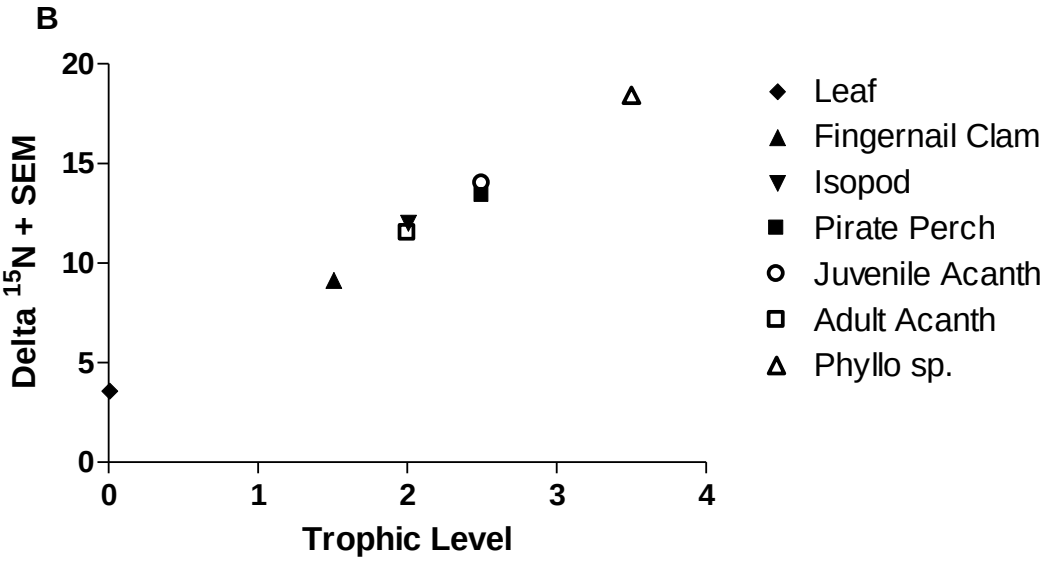

20
15
10
5
0
-40
-35
-30
-25
-20
-15
-10
Delta 15N
Delta 13C
Fish
Macroinvertebrates
Parasites
Leaf
Standards

Figure 5.2: The relationship between $\delta^{15}N$ (A) and $\delta^{13}C$ (B) and trophic level of the host-parasite food chain with leaf detritus (solid diamond), fingernail clam (solid triangle), isopod (inverted solid triangle), pirate perch (solid square), juvenile acanthocephalan (open circle), adult acanthocephalan (open square) and adult trematode (open triangle). Standard error bars are sometimes smaller than the symbols.


A
-15
-20
-25
-30
-35
Delta 13C + SEM
0
1
2
3
4
Trophic Level
Leaf
Fingernail Clam
Isopod
Pirate Perch
Juvenile Acanth
Adult Acanth
Phyllo sp.


B
20
15
10
5
0
Delta 15N + SEM
0
1
2
3
4
Trophic Level
Leaf
Fingernail Clam
Isopod
Pirate Perch
Juvenile Acanth
Adult Acanth
Phyllo sp.

Chapter 6

GENERAL DISCUSSION

The goal of this dissertation was to determine if measures of energy could be used to assess the ecological impact of parasitism at multiple levels of organization within the streams of the New Jersey Pinelands. To this end, bomb calorimetry field surveys of all host species and all metazoan parasites were used to create energy budgets at the individual, population, and ecosystem levels. The results of these investigations suggest that at all levels of organization, parasites require only minimal amounts of energy from their host's energy budget (<1.0%) in order to persist. Despite this small energetic drain, the effects of parasitism could still be significant. For example, at the individual level, the acanthocephalan parasite *Acanthocephalus tehlequahensis* caused decreased survival, increased consumption and respiration, and complete castration of its isopod intermediate hosts. In addition, this parasite took 6.7% of the entire production energy budget of the isopod population (infected and uninfected). On the other hand there were few energetic costs were of parasitism in the fish definitive host (1.3 % of the pirate perch population's production energy) even when two parasites, an acanthocephalan and a trematode co-infected the host. In fact, infected pirate perch allocated more production energy to reproduction than uninfected hosts, conferring benefit to the host in direct fitness. At the ecosystem level, the energetic costs of parasites species within two streams was relatively small, but the stream with high secondary production supported higher levels of parasitism.

Intermediate hosts tend to experience more negative from parasite infection when compared to definitive hosts (Moore 1984, Combes 2001). Juvenile parasites can alter

host growth, survival, reproduction, and behavior, and these alterations often aid the parasites growth or transmission (Moore 1984, Lafferty and Morris 1996, Combes 2001). The acanthocephalan parasite *A. tehlaquehensis* had significantly different energetic effects when infecting its isopod intermediate host and fish definitive host. Specifically, this parasite altered energy allocation patterns of isopods at the individual and population level, and 21% of the isopod production energy went to parasite growth in infected hosts. At the host population level, 6.7% of the production energy was allocated to this parasite species. There were few energetic costs during *A. tehlaquehensis* infection of its definitive host, the pirate perch. There were no significant differences between the energy budgets of infected and uninfected isopods, and at the population level only 1.3% of the production energy went to parasites (including the trematode *Phyllodistomum sp*.). These differences in costs of this parasite may reflect the relaxation of evolutionary pressure not to kill the intermediate host that has been demonstrated in some definitive hosts (Smith 1934, Simon 1960, Ewald 1983, Ebert and Weisser 1997). In many trophically-transmitted parasites, infection affects the physiological functions of the intermediate host (i.e. host growth, activity, reproduction, and respiration) (Walkey and Meakins 1970, Meakins and Walkey 1975, Camp and Huizinga 1979, Sousa 1983, Moore 1984, Oetinger 1987, Sorensen and Minchella 1998, Kakizaki et al 2003), and these changes often make the intermediate host more conspicuous to the predatory definitive host (Camp and Huizinga 1979, Moore 1984, Combes 2001). In fact, definitive hosts are more likely to ingest infected hosts than uninfected hosts (Moore 1984, Lafferty 1992, Perrot-Minnot et al. 2007). In addition, there is little selection on the definitive host to

avoid ingested intermediate hosts because the cost of the parasite infection is significant less compared to the benefit of increased prey to consume (Lafferty 1992).

There are significant direct energetic costs associated with parasite infection at the individual and population level, but the overall amount of energy allocated to parasitism (in terms of energy consumed by the host) was minimal (<1%). The direct energetic drain of parasites on their hosts may be small due to the very nature of parasite infections. Parasites tend to have aggregated distributions within their hosts, with only a few members of the host population maintaining large infection intensities and most of the population having low levels of infection (Crofton 1971, Anderson and Gordon 1982, Combes 2001, 2005). Aggregation of parasites is seen at all scales from the individual host to the ecosystem. Within the individual host aggregation occurs because only specific organs are parasitized. In host populations parasites are aggregated so that few hosts harbor most of the parasites, and within communities parasites are aggregated within particular species (Combes 2005). Aggregated distributions have been documented for most parasites species (Combes 2001, 2005, Poulin 2007), and the nature of parasite distributions is often measured by the variance to mean ratio of the parasite within a population of hosts. If the ratio is close one it suggests that the parasite population is randomly distributed; less than one suggests that it is uniformly distributed, and greater than one suggests aggregated distributions (Combes 2001). The distribution of the two most abundant parasites within Muskingum Brook, *A. tehlaquehensis* and *Phyllodistomum sp.* were aggregated with variance to mean ratios of 7.828 and 3.973 respectively within their pirate perch host, indicating that these parasites species are aggregated. Additionally, pirate perch was the most heavily infected fish hosts (~80% of

the parasite infection). These data suggest that parasites within this system are drawing their energy from a select group of individuals. Although many hypotheses have been proposed to explain these aggregations in nature invoking mechanisms of spatial heterogeneity of free-living infective stages and host species, and differences in the ability of hosts to fight infection; it remains unclear what evolutionary pressures on the host-parasitic relationship help to maintain these distributions. Nevertheless, these distributions may constrain the overall energetic cost of parasites to their hosts.

The aggregated nature of parasite infections within their hosts will also have an impact on the amount and efficiency of energy flow to parasites at the ecosystem level. Within an ecosystem the flow of energy is often measured as the amount of energy that passes from one trophic level to the next and is frequently reported as an efficiency of energy transfer (Lindeman 1942, Odum 1957, Slobodkin 1959, Teal 1957, 1962, Engelmann 1961, Kozlovsky 1968, Hairston and Hairston 1993). Energy efficiencies have been reported for many different ecosystems, and flow of energy between trophic levels tends to be between 5-15% (Lindeman 1942, Odum 1957, Slobodkin 1959, Teal 1957, 1962). In streams of the New Jersey Pineland, the efficiency of energy flow to parasites ranged from 0.0008% in Skit Branch-LP (low levels of parasitism) and 0.6% in Muskingum Brook-HP (high levels of parasitism), which are significantly less than 5-15% efficiency reported for free-living organisms. However, the proportions of energy flowing to parasites in these streams is similar to that for parasites in a California salt marsh (Kuris et al. 2008). These results suggest that parasites do not follow the typical energy transfer efficiencies seen in free-living organisms within other ecosystems, and aggregated distributions of parasites may explain why these patterns of energy flow differ

with parasites. Although almost every species of fish was infected in this study, parasite populations were present in significant levels in only two species, the pirate perch and the pumpkinseed, accounting for 75% of the parasites recovered. Therefore, it is not surprising that energy flow to parasites was less than that of free-living organisms, and the fact that parasites require little energy to persist may explain how parasites are able to establish in most habitats.

Although parasites require only a small proportion of the energy budgets from individual hosts to ecosystems, parasites may be regulated by ecosystem energy flow. I measured the energy flow to parasitism in two Pineland streams, one with a high level of parasitism (Muskingum Brook-HP) and one with a low level of parasitism (Skit Branch-LP), and there were distinct differences is the amount of energy going to parasites in the streams. Muskingum Brook-HP had significantly more energy allocated to parasites when compared to Skit Branch-LP. Moreover, the energy flow patterns between these two streams were very different. Skit Branch-LP showed an altered energy flow regime with much of the energy remaining as leaf detritus and not making its way up the food web. In addition, there was very little secondary production in this stream with an absence of many of the important detritivores present in large abundances in Muskingum Brook-HP. However, many of the abundant parasite species in Muskingum Brook-HP require detritivore intermediate hosts which are absent in Skit Branch-LP, and this may also limit parasitism. It has been demonstrated in other system that host species density can significantly alter parasite community structure (Morand and Poulin 1998, Arneberg 2002), but it is unclear how this would affect energy allocation to parasites. Therefore,

the lack of parasite establishment within Skit Branch-LP may be due to either the absence of intermediate hosts, altered energy flow through the food web, or both.

Limited energy flow to parasites at all level of ecological organization does not mean that parasites are insignificant players at the ecosystem level. Parasites play an important role in food web structure and topology, but the optimal method for incorporation of parasites into food web studies is still debatable (Huxham et al. 1996, Marcogliese and Cone 1997, Thompson et al. 2005, Sukhdeo and Hernandez 2005, Lafferty et al. 2006, Hernandez and Sukhdeo 2008, Kuris et al. 2008, Lafferty et al. 2008). This occurs because many are trophically-transmitted, and different life stages may derive their energy from multiple trophic levels within the ecosystem (Lafferty et al. 2008). Parasites are included within food webs either as top predators or as trophospecies where life stages are used to indicating energy flow to parasites (Sukhdeo and Hernandez 2005, Lafferty et al. 2008). A stable isotope analysis (SIA) of the most abundant species within Muskingum Brook including both *A. tehlaquehensis* and *Phyllodistomum sp.* indicated that parasites have very complex energetic relationships with their hosts. Specifically, juvenile stage parasites can sometimes feed higher up in the food webs than their adult counterparts, and different parasites species within the same hosts can occupy two different trophic positions. In addition, although parasites occupy the uppermost trophic level of food webs, during their complete lifecycle, they derive energy from a several trophic levels within a web. Therefore, classifying parasites into any single trophic level can misrepresent the flow of energy to parasites and under estimate the potential impact of parasites on energy flow.

This dissertation explored the practicality of using energy as a metric for quantifying the ecological role of parasites within individuals, population, and ecosystems. Energy proved to be a metric that provided estimates of the cost of parasitism across all levels of ecological organization. It is clear that parasites can alter host energy allocation patterns and present significant costs to their hosts, and this study confirms that these effects have the potential to cascade up to higher levels of ecological organization via their effects on important host functions such as host growth, activity, and fitness. However, these costs are not universal to all hosts, and parasites may be more benign in terms of their energetic effects within definitive hosts. The direct energetic cost of parasitism at the ecosystem level was insignificant, but parasites can obtain their energy from multiple trophic levels. There may also have been significant indirect costs from parasitism. Specifically, the greatest energetic impact of parasites may be seen at lower levels of the food web because parasite can alter intermediate host reproductive output and predation rates. These indirect costs within the ecosystem level have the potential to significantly alter energy flow. However, although, parasites can alter energy flow, their establishment and maintenance may be dependent on ecosystem energy flow because they often require energy from at least two trophic levels for completion their lifecycles. Without typical energy flow to the trophic levels that parasites occupy, the direct energetic costs of parasitism should be small. In conclusion, the energetic relationships between their parasites and their hosts are more complex than typical predator-prey relationships, and have the potential to both directly and indirectly affect energy flow at all levels of ecological organization. To incorporate parasite into ecosystem level studies required a new way to quantify the impact of parasites on the

system, and measuring the energetic relationships between parasites and free-living organisms provides a way to account for both the direct and indirect effects of parasites.

APPENDIX

RH: LETTINI AND SUKHDEO-ANHYDROBIOSIS IN TRICHOSTRONGYLES

ANHYDROBIOSIS INCREASES SURVIVAL OF TRICHOSTRONGYLE NEMATODES

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ABSTRACT: This study demonstrates that infective stage larvae of 2 trichostrongyle ruminant gastrointestinal nematodes, *Haemonchus contortus* and *Trichostrongylus colubriformis*, can enter into anhydrobiotic states when completely desiccated. Larvae of control trichostrongyle species, *Heligmosomoides polygyrus* and *Nippostrongylus brasiliensis,* that infect mice, were unable to survive desiccation or to enter into anhydrobiosis. Ruminant larvae were able to survive up to 7 desiccation/rehydration cycles and during anhydrobiosis, metabolic activity was decreased and survival of the larvae was prolonged both in the laboratory and in the field. Relative humidity had no effect on ruminant larval survival following anhydrobiosis when compared to controls. Temperature had a significant effect, 85.8 ± 2.3% of larvae in anhydrobiosis could survive low temperatures (0 C) that killed all control larvae. Metabolic activity, measured by changes in lipid content and CO_2 respiration, was significantly lower in larvae that entered anhydrobiosis when compared to controls ($P<0.05$). In field experiments using open-meshed chambers under ambient environmental conditions, larvae in anhydrobiosis had significantly higher survival rates in the field when compared

to controls ($P<0.05$) during summer and winter trials. These data suggest that anhydrobiosis in ruminant larvae promotes survival at freezing temperatures, decreases metabolic activity, and prolongs survival under natural field conditions.

Ecological and epidemiological studies of ruminant gastrointestinal nematode survival on pasture have generally concluded that temperature and humidity are critical to the development and survival of the infective stage (L_3 larva) of gastrointestinal nematodes in domestic animals (Shorb, 1942; Crofton, 1952; Gibson and Everett, 1967; Donald and Leslie, 1969; Levine, 1980; Krecek, et al., 1992; Stromberg, 1997). Control strategies focused on predicting peak populations on pasture, and were designed to minimize larval survival on pasture (Bairden et al., 1995; Barger, 1997, 1999; Stromberg and Averback, 1999). Nevertheless, these strategies have largely been proven ineffective, primarily because ruminant larvae can survive extreme variation in weather, and are often able to over-winter successfully (Bairden et al., 1995; Barger, 1997, 1999; Behm, 1997; Stromberg and Averback, 1999). It is not clear how environmental factors impinge on the activities and behaviors of individual larvae to increase their survival during drastic environmental fluctuations.

Many aquatic invertebrates including rotifers, tardigrades, and nematodes (free-living and parasitic) can survive temperature and moisture extremes for prolonged periods (Keilin, 1959; Crowe, 1971; Crowe and Madin, 1975, Campbell and Gaugler, 1991; Behm, 1997). These organisms enter a state of anhydrobiosis when completely desiccated (Pigon and Weglarska, 1953, 1955; Keilin, 1959; Crowe 1971, Crowe and Madin, 1975; Campbell and Gaugler, 1991; Behm, 1997), and metabolic activity completely ceases (Pigon and Weglarska, 1953, 1955; Hinton, 1968). Studies on

anhydrobiosis in plant-parasitic, free-living, and entomopathogenic nematodes, suggest that 5 predictable physiological or behavioral modifications accompany anhydrobiosis in these organisms. First, is the nematode's ability to survive complete or partial desiccation for some period of time. Entomopathogenic nematodes undergo partial desiccation, but rarely survive complete desiccation (Solomon et al., 1999; Grewal, 2000; Grewal and Jagdale, 2002), whereas some plant parasitic and free-living nematodes can survive in an anhydrobiotic state for mo to yr (Wharton et al., 1988; Aroian et al., 1993; Ricci and Pagan, 1997; Treonis et al., 2000). Second, successful anhydrobiosis is increased by a slow rate of desiccation (Womersley and Ching, 1989, Aroian et al., 1993; Solomon et al., 1999). A slow rate of desiccation is thought to allow for the accumulation of trehalose, and/or glycerol, which are protectants that maintain the structure of the nematode cell membranes (Behm, 1997, Qiu and Bedding, 2002). Third, individual nematodes will coil, or groups of nematodes will aggregate in clumps to decrease cuticle surface area and this aids in slow desiccation (Koenning and Schmitt, 1986; Womersley and Ching, 1989; Solomon et al., 1999; Treonis et al., 2000; O'Leary et al., 2001). Fourth, nematodes in anhydrobiosis cease all metabolic activity; they do not take up oxygen and they do not significantly alter their energy reserves (Pigon and Weglarska, 1953; Bhatt and Rhode, 1970; Grewal, 2000). Finally, anhydrobiosis is revived by the addition of water, and the worms quickly rehydrate and return to normal locomotion in about 2 hr.

Gastrointestinal nematodes are reported to have some ability to survive desiccation on pasture, but the mechanisms are unclear (Anderson and Levine, 1968; Ellenby, 1968; Allan and Wharton, 1990). The present study examines anhydrobiosis in

2 gastrointestinal nematodes of sheep, *Haemonchus contortus* and *Trichostrongylus colubriformis,* to identify conditions that trigger the process, and determine its effect on larval survival in the laboratory and in the field.

MATERIAL AND METHODS

Infective (L_3) larvae of *H. contortus* and *T. colubriformis* were obtained from single infections in sheep maintained by Eli Lilly and Company (Chicago, Illinois). Infective (L_3) larvae of *Heligmosomoides polygyrus* and *Nippostrongylus brasiliensis* (controls) were obtained from stock infections in mice at Rutgers University. All larvae were kept in distilled water at 4 C until use.

Preliminary experiments with ruminant parasites established that slow drying at 25 C for 24 hr consistently elicited the anhydrobiotic response, and larvae recovered from this treatment successfully regardless of length of time in anhydrobiosis, ranging from a few hr to 18 mo. This treatment was used as a baseline standard to elicit anhydrobiosis. Individual larvae of *H. contortus, T. colubriformis, H. polygyrus,* and *N. brasiliensis* were placed 96-well plates containing 80-µl of distilled water, per well. Larvae were kept at 25 C and left to slowly dry for 24 hr during which time, all water evaporated. At weekly intervals, 80-µl of distilled water was added to all cells to rehydrate the larvae and recover them from anhydrobiosis, and (n=12), larvae were sampled for viability assays. The larvae were subjected to 8 cycles of dehydration/rehydration. Viability of larvae was determined by larval motility and exclusion of methylene blue, a vital stain. Larvae were considered dead if they were immobile and remained stiff after prodding with a needle. Methylene blue uptake was used to confirm that immobile larvae that did not respond to prodding were dead.

To determine the effect of humidity on survival of larvae in anhydrobiosis, *Haemonchus contortus* larvae were transferred to filter papers in batches of 500, and placed in airtight chambers with no liquid water that were maintained at 97%, 90%, 75%, 50%, and 25% relative humidity. Relative humidity in the chambers was maintained with glycerol water solutions according to Kung et al. (1991). Control larvae were kept moist at 100% relative humidity to prevent anhydrobiosis. Batches (n=5) were randomly sampled weekly for 4 wk, and rehydrated in 2-ml of distilled water for 24 hr. The numbers of living and dead larvae were used to determine survival rate.

To determine the effect of temperature on the ability of larvae in anhydrobiosis to survive, batches of 500 *H. contortus* and *T. colubriformis* larvae were transferred to filter paper inside 2-ml wells. In half of the batches anhydrobiosis was induced by slow drying for 24 hrs, and half (controls) were kept moist at 25 C for 24 hr to prevent anhydrobiosis. Larvae in anhydrobiosis and control larvae were incubated at 25 C or 0 C, and at intervals of 1 wk, 1 mo, 3 mo, and 6 mo, (n=6) batches of each species were rehydrated with 2 ml of distilled water for 24 hr, and survival rate was determined by counting the living and dead larvae.

To measure the changes in energy reserves that occur during anhydrobiosis, batches of 500 *H. contortus* and *T. colubriformis* larvae were transferred to filter paper inside 2-ml wells. Half were induced into anhydrobiosis by slow drying at 25 C for 24 hr while control larvae were kept moist at 25 C for the same time to prevent them from entering anhydrobiosis. At intervals from 1 wk to 6 mo (n=6) batches for each species were rehydrated in 2-ml of distilled water for 24 hr, fixed, and stained for lipids with Oil Red O, using a method modified from Stamps and Linit (1995). Briefly, larvae were

fixed with a formal-acetic fixative for 24 hours, the fixative was removed and replaced with saturated Oil Red O, and incubated at 60 C for 25 min. Oil Red O was removed by washing twice with 70% ethanol for 10 min, and 2-ml of Seinhorst I solution (glycerol:ethanol:water, 1:20:79) was added. Following incubation at 40 C for 24 hr, Sienhorst II (glycerol:ethanol, 10:90) solution was added, and the larvae were incubated at 40 C until all the ethanol had evaporated. Stained larvae were mounted on glass slides using the wax ring method. A circle of wax 1 mm high was applied to slides with a metal applicator, larvae in glycerol were placed in center of ring, and the cover slip was placed on top and sealed by carefully melting the wax ring on a hot plate. Digital photographs of individual larvae were taken at 400x, and mean lipid content was estimated using ImageJ (Rasband, 2005) to measure the area of 10 lipid globules of n=10 nematodes per sample interval.

Carbon dioxide respiration was used to compare the metabolic activities between larvae in anhydrobiosis and control larvae. Large numbers of larvae were slowly dried at 25 C to initiate anhydrobiosis. For each measurement, batches of 20,000 larvae in anhydrobiosis were place in 500-ml glass jars with 50-ml of distilled water and carbon dioxide respiration of each batch was recorded (n=5). Carbon dioxide respiration was recorded similarly in control larvae that were not stimulated to enter anhydrobiosis. Carbon dioxide levels were measured with a Micro-Oxymax respirometer (Columbus Instruments) at 1 hr intervals for 5 hr. Prior to the first reading, larvae were kept for 1 hr in the chambers for calibration.

To determine larval survival under ambient conditions on pasture, infective larvae of both species were placed in the field in experimental chambers that allowed free

exchange of moisture and air. Chambers were constructed from plastic test tubes (15 ml) cut to 6.5 cm long and the open bottoms sealed with 0.2 μm porous filters. Autoclaved soil (2 g) was added to each tube (3 cm in height), and 1,000 *H. contortus* or *T. colubriformis* larvae were carefully pipetted onto the soil surface. The tube was capped with cheesecloth (Fig. 1). Half the chambers were dried for 1 wk at 25 C to induce anhydrobiosis, and half (controls) were maintained moist at 25 C to prevent anhydrobiosis. On 15 June, and 15 December 2004, all tubes were buried to the same depth (3 cm) in the pasture so that the soil surface in the chambers was level with soil surface of the surrounding soil. Every 3 days for 30 days, (n=3) tubes for each treatment were collected, and living and dead larvae recovered by a Baermann technique. In this method, soil was broken up into a double layer of cheese cloth, placed in glass funnels (with sealed bottoms), distilled water was added to the funnels, and soil was agitated periodically over 12 hours as larvae settled to the bottom of the funnels. Experimental fields were located on Cook College Farms, Rutgers University, and local meteorological data was collected by the Rutgers University Weather Center (New Brunswick, New Jersey).

All statistical analysis were conducted using SigmaStat 2.30 1997. Percentage data were arcsine root transformed prior to statistical analysis. Data were analyzed using one-way analysis of variance (ANOVA) for repeated desiccation survival, humidity, and lipid concentration experiments. Two-way ANOVA was used for laboratory anhydrobiosis survival, respiration and outdoor anhydrobiosis survival experiments. Tukey's post-hoc test was used to make multiple comparisons of treatments. Differences were considered significant at $P \leq 0.05$.

RESULTS

Slow drying for 24 hr at 25 C was a consistent trigger of anhydrobiosis in the infective L_3 larvae of ruminants. Larvae in anhydrobiosis were significantly shrunken within the cuticle when compared to pretreatment controls (Fig. 2). These larvae tended to assume a coiled posture, became immobile, and did not respond to prodding with a needle although they excluded methylene blue. When water was added, the larvae swelled to normal size within 2 hr and resumed typical sinusoidal motility. Species that entered anhydrobiosis once, could survive through several weekly desiccation events (Fig. 3). The mouse-parasitic species, *H. polygyrus* and *N. brasiliensis* (control larvae), did not respond with the morphological and behavioral responses typical of anhydrobiosis, and did not survive even a single desiccating event. In ruminant larvae, complete desiccation i.e., the absence of liquid water, was required to trigger anhydrobiosis. When in the anhydrobiotic state, larval survival was unaffected by relative humidity, and their survival upon rehydration was no different from that of control larvae that did not enter anhydrobiosis. There were no significant differences in the long term survival rate between larvae in anhydrobiosis and control larvae at 25 C, but at 0 C, larvae in anhydrobiosis survived significantly longer than control larvae ($P<0.05$), which died in the 0 C treatment (Fig. 4).

When prevented from entering anhydrobiosis, control larvae of both ruminant species showed significant decreases in lipids when kept at 25 C for 6 mo when compared to their counterparts in anhydrobiosis ($P<0.05$) (Figs. 5, 6). *Trichostrongylus colubriformis* had significantly greater initial lipid reserves (0.0219 ± 0.0021 mm^2) than *H. contortus* (0.0041 ± 0.0015 mm^2; $P<0.05$). Control larvae were quickly killed by the

low temperatures, and lipid content in control larvae at 0 C did not decrease during the experiment (Figs. 4, 5, 6). Larvae emerging out of anhydrobiosis had significantly lower levels of CO_2 production when compared to controls ($P<0.05$) (Fig. 7).

Larvae stimulated to enter anhydrobiosis had increased survival of larvae under field conditions (Figs. 8, 9). Average daily temperature and total precipitation varied daily over 30 days, and there were prolonged periods of no rainfall both in summer and winter trails (Figs. 8A, 9A). During summer trails, control larval survival was significantly decreased after day 15 on pasture, when compared to the survival of larvae in anhydrobiosis, which remained high over 30 days (Figs. 8B, C, $P<0.05$). In winter trials, control larvae of both species, showed significant decreases in survival by day 9 when compared to larvae in anhydrobiosis (Figs. 9B, C, $P<0.05$).

DISCUSSION

The four species of nematodes used in this study were trichostrongylids with similar lifecycles. Infective larvae of ruminant hosts, *H. contortus* and *T. colubriformis* can repeatedly enter anhydrobiosis and recover without significant losses in survival, but in contrast, infective larvae of the mouse parasites, *H. polygyrus* and *N. brasiliensis*, could not survive any desiccation, and did not demonstrate any of the morphological or behavioral changes that are typical of anhydrobiosis. The mouse strains have been in culture for many generations and may have lost the ability to undergo anhydrobiosis, but it is more likely that the evolutionary history of these species reflects physiological constraints that make them unable to survive desiccation. The development of mechanisms for anhydrobiosis may be related to the parasite's specific mode of transmission. Ruminant parasites utilize a sit and wait transmission strategy (Rogers and

Somerville, 1963), and anhydrobiosis may be an important survival adaptation for these species that spend a significant amount of time in harsh desiccating conditions on vegetation. In contrast, *H. polygyrus* is ingested via allogrooming (Hernandez and Sukhdeo, 1995), and *N. brasiliensis* infects through penetration of the skin during infection (Rogers and Somerville, 1963). These mouse parasites presumably must remain close to their host, and may not spend significant periods of time in desiccating environments.

In nematodes, humidity greatly affects the anhydrobiotic this process (Crowe and Madin, 1975; Womersley and Ching, 1989; O'Leary et al., 2001). Many nematodes that enter anhydrobiosis under desiccating conditions at low relative humidity survive more poorly than nematodes that enter anhydrobiosis at higher relative humidity, and several species require a narrow and specific range of relative humidity to survive desiccation through anhydrobiosis (Crowe and Madin, 1975; Womersley and Ching, 1989; O'Leary et al., 2001). Our data show that in two ruminant larvae, low relative humidity had no effect on anhydrobiosis or desiccation survival, and larvae could enter anhydrobiosis and survive desiccation under a wide range of relative humidities (97%-25%). Regardless of relative humidity, the absence of liquid water is the primary trigger in the initiation of anhydrobiosis in ruminant larvae.

Anhydrobiotic mechanisms can provide protection from extreme temperatures (hot and cold) under desiccating conditions in several nematode species (Keilen, 1959; Hinton, 1968; Crowe, 1971; Crowe and Crowe, 1992; Treonis et al., 2000; Grewal and Jagdale, 2002). Our data shows that at 25 C, *H. contortus* and *T. colubriformis* survival in larvae that entered anhydrobiosis did not differ from control larvae. However, at 0 C,

only larvae in anhydrobiosis survived, while all control larvae not in anhydrobiosis died. This is clear evidence for the benefits of anhydrobiosis during low temperatures, and suggests that anhydrobiosis may be a mechanism that allows ruminant larvae to over-winter on pasture. Previous studies reporting that *H. contortus* and *T. colubriformis* did not survive winter conditions (Rogers and Somerville, 1963; Gibson and Everett, 1967) did not induce the larvae into anhydrobiosis prior to testing.

Lipid metabolism plays an important role in the survival of non-feeding infective larvae with their limited energy stores (Rogers, 1939; Rogers and Somerville, 1963; Clark, 1969; Stamps and Linit, 1995; Medica and Sukhdeo, 1997; Hass et al., 2002). Lipids are an efficient form of energy because they provide twice as many calories as carbohydrates and protein by weight; moreover, lipid catabolism provides water that is essential for larval metabolism (Medica and Sukhdeo, 1997). Lipids used for energy are stored in the form of globules within the body cavity (Clark, 1969; Stamps and Linit, 1995; Fitters et al., 1997). In the present study, lipid content did not change after 6 mo in larvae that had entered anhydrobiosis, while lipid content in control larvae that were prevented from entering anhydrobiosis decreased significantly within 7 days. These data support the idea that *H. contortus* and *T. colubriformis* larvae are metabolically inactive during anhydrobiosis, and this is further validated by measurement of CO_2 respiration of larvae in anhydrobiosis compared to control larvae. Carbon dioxide is a by-product of lipid metabolism (Rogers and Somerville, 1963), and CO_2 production was significantly lower in larvae recovering from in anhydrobiosis. Carbon dioxide respiration in larvae that are in complete anhydrobiosis was not possible because the method requires hydration of the samples. After rehydration and resumption of motility in larvae in

anhydrobiosis, metabolic activity remained lower than controls for up to 6 hours, suggesting that revival of enzymatic metabolic pathways lag behind somatic rehydration during recovery from anhydrobiosis.

The effect of anhydrobiosis on larval survival under natural field conditions was tested during summer and winter. During both seasons, survival of the larvae in anhydrobiosis was significantly higher than in control larvae. The summer decrease in survival of control larvae may reflect the normal depletion of their lipid stores when compared to larvae in anhydrobiosis. On the other hand, high winter survival of larvae in anhydrobiosis compared to control larvae is more likely due to the inability of control larvae to survive freezing, than from depletion of their lipid reserves.

In this study, we report that the ruminant parasites, *H. contortus* and *T. colubriformis*, can enter anhydrobiosis under desiccating conditions. For larvae that must sit and wait in fluctuating environmental conditions to infect a host, anhydrobiosis provides protection from desiccation and freezing, thus prolonging their survival. In addition, during anhydrobiosis, larvae are metabolically inactive and survival is further increased by conserving lipid energy reserves.

ACKNOWLEDGMENTS

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FIGURE LEGENDS

FIGURE 1. Diagram of soil chambers used in outdoor experiments.

FIGURE 2. (**A**) Infective L_3 larva of *Haemonchus contortus* before anhydrobiosis. (**B**) Infective L_3 larva in anhydrobiosis. Arrow indicates empty space within the cuticle where larva has significantly shrunken during anhydrobiosis (Scale bars= 100 μm).

FIGURE 3. Survival of L_3 larvae through sequential desiccation and rehydration events. (**A**) *Haemonchus contortus*. (**B**) *Trichostrongylus colubriformis.* (**C**) *Heligmosomoides polygyrus*. (**D**) *Nippostrongylus brasiliensis.*

FIGURE 4. The effect of temperature on the survival of (**A**) *Haemonchus contortus,* and (**B**) *Trichostrongylus colubriformis* larvae during anhydrobiosis. (▽) 25 C in anhydrobiosis; An-25°, (○) 25 C control; Con-25, (▼) 0 C in anhydrobiosis; An-0, and (●) 0 C control; Con-0. Letters denote statistical significance between groups ($p \leq 0.05$).

FIGURE 5. The effects of temperature on the lipid content (μm^2) of *Haemonchus contortus* (25 C in anhydrobiosis, 25 C control, 0 C in anhydrobiosis, and 0 C control) from 1 wk to 6 mo. Lipids remained high in the 0 C control group because they were dead. Letters denote statistical significance between groups ($p \leq 0.05$).

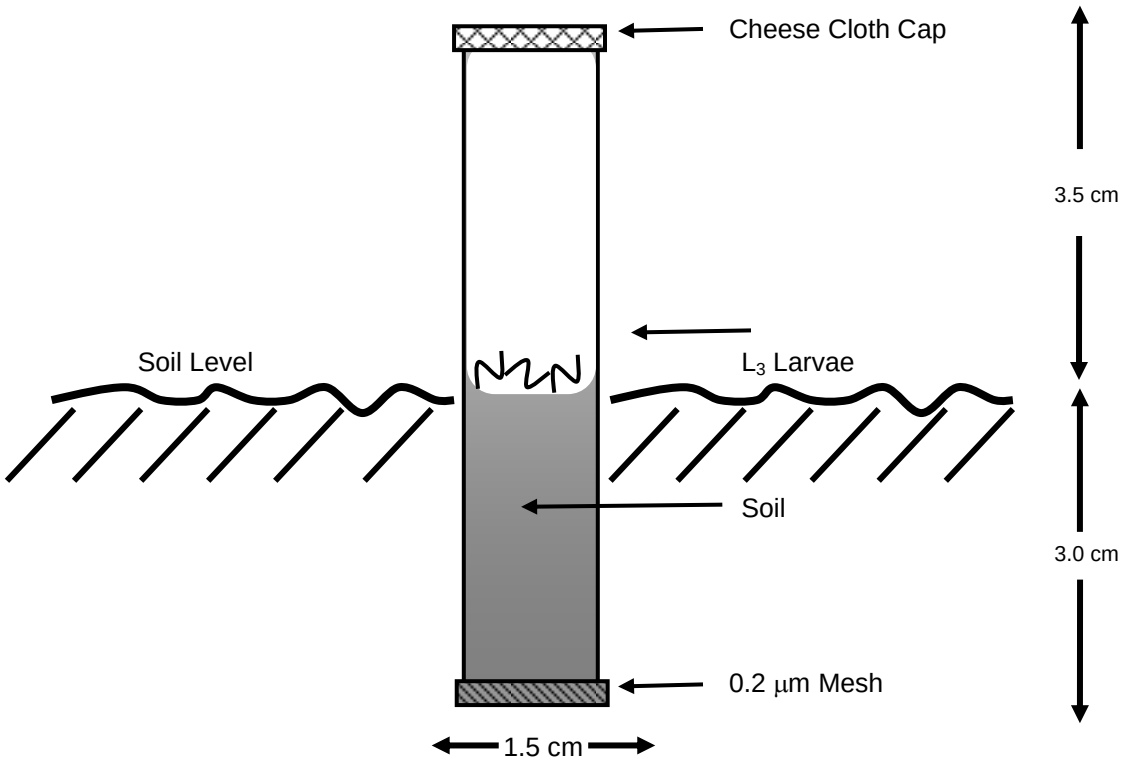
FIGURE 6. The effects of temperature on the lipid content (μm^2) of *Trichostrongylus colubriformis* (25 C in anhydrobiosis, 25 C control, 0 C in anhydrobiosis, and 0 C control) from 1 wk to 6 mo. Lipids remained high in the 0 C control group because they were dead. Letters denote statistical significance between groups ($p \leq 0.05$).

FIGURE 7. CO_2 production (μg/min) in 20,000 ruminant larvae. (**A**) *Haemonchus contortus,* and (**B**) *Trichostrongylus colubriformis.* (●) larvae in anhydrobiosis and (○) control larvae.

FIGURE 8. (**A**) Summer average daily temperature (dotted line) and total precipitation (solid line) in the experimental field. The effect of season (summer) on the survival of ruminant larvae. (**B**) *Haemonchus contortus,* and (**C**) *Trichostrongylus colubriformis,* (●) in anhydrobiosis; (○) control on pasture for 30 days.

Figure 9. (**A**) Winter average daily temperature (dotted line) and total precipitation (solid line) in the experimental field. The effect of season (winter) on the survival of ruminant larvae. (**B**) *Haemonchus contortus,* and (**C**) *Trichostrongylus colubriformis,* (●) in anhydrobiosis; (○) control on pasture for 30 days.

Figure 1. Lettini and Sukhdeo



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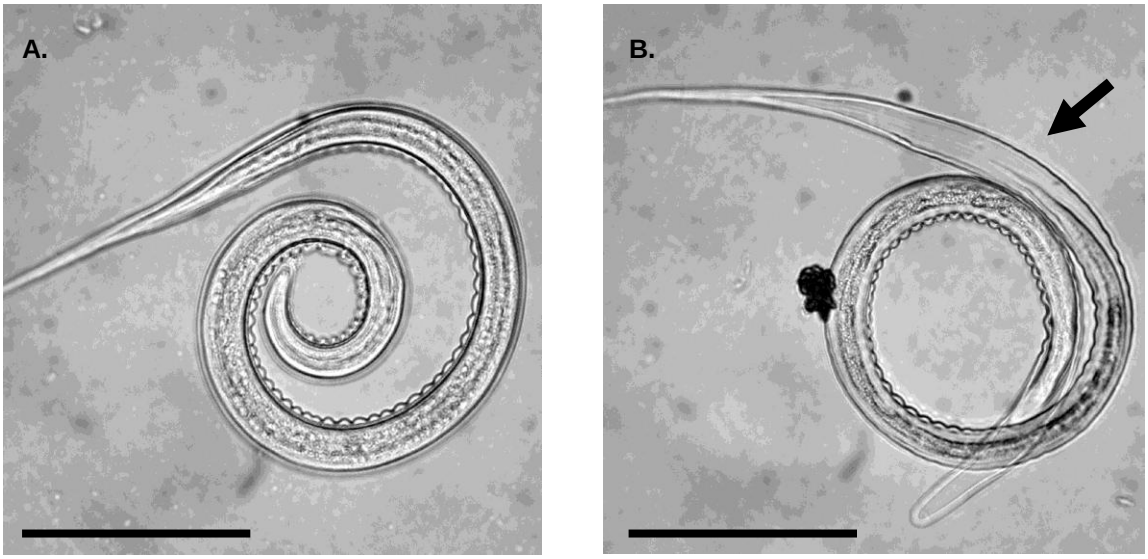

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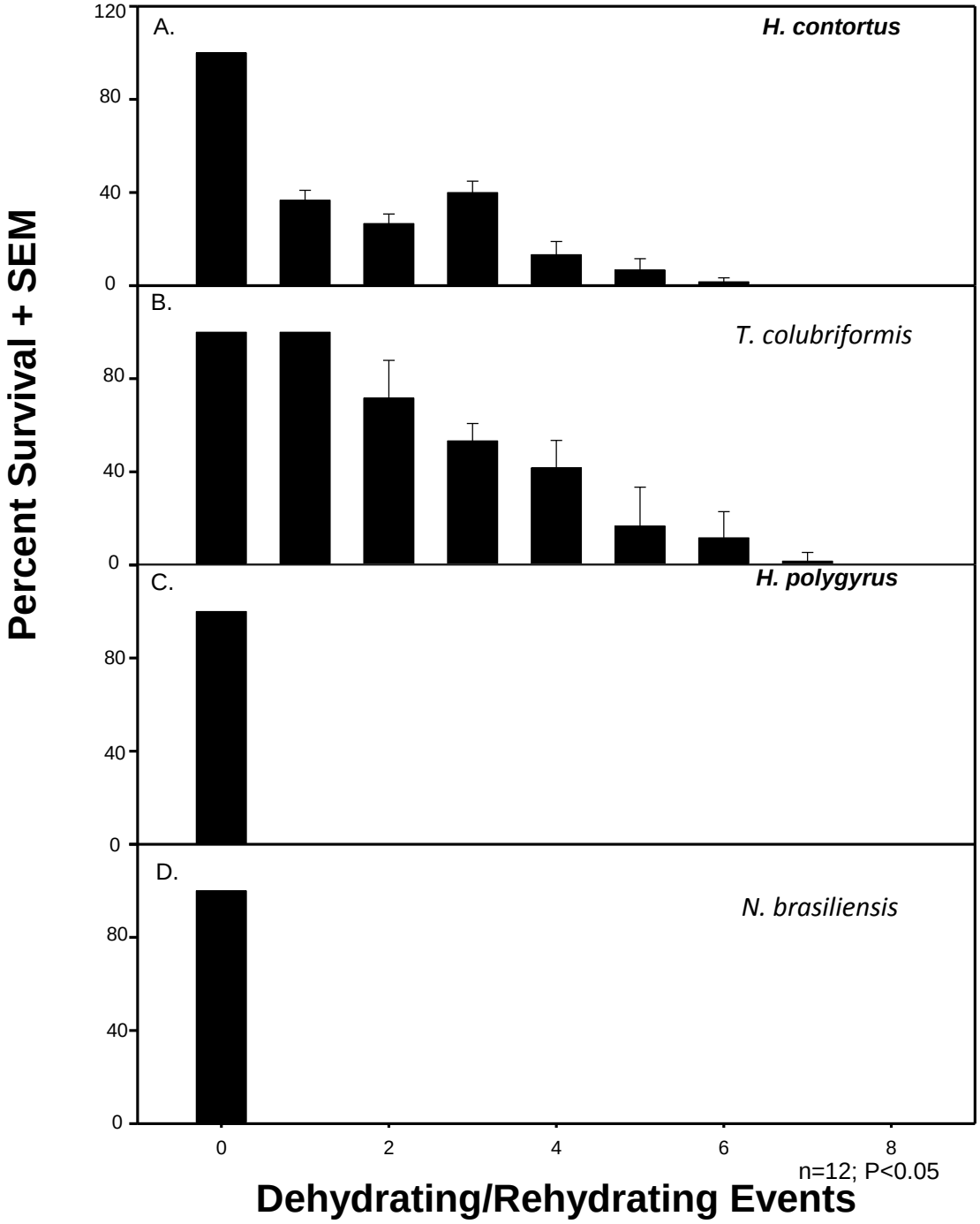

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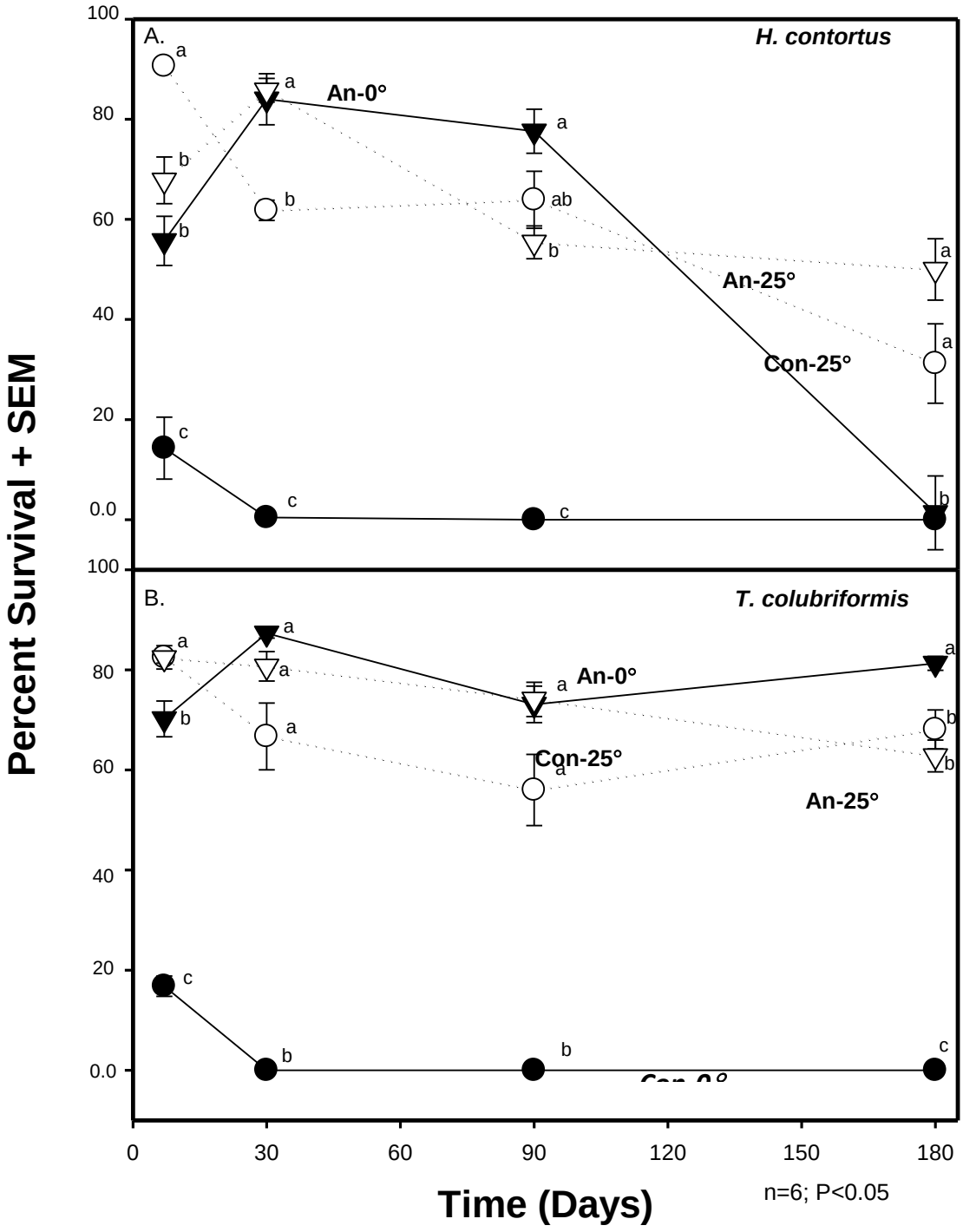

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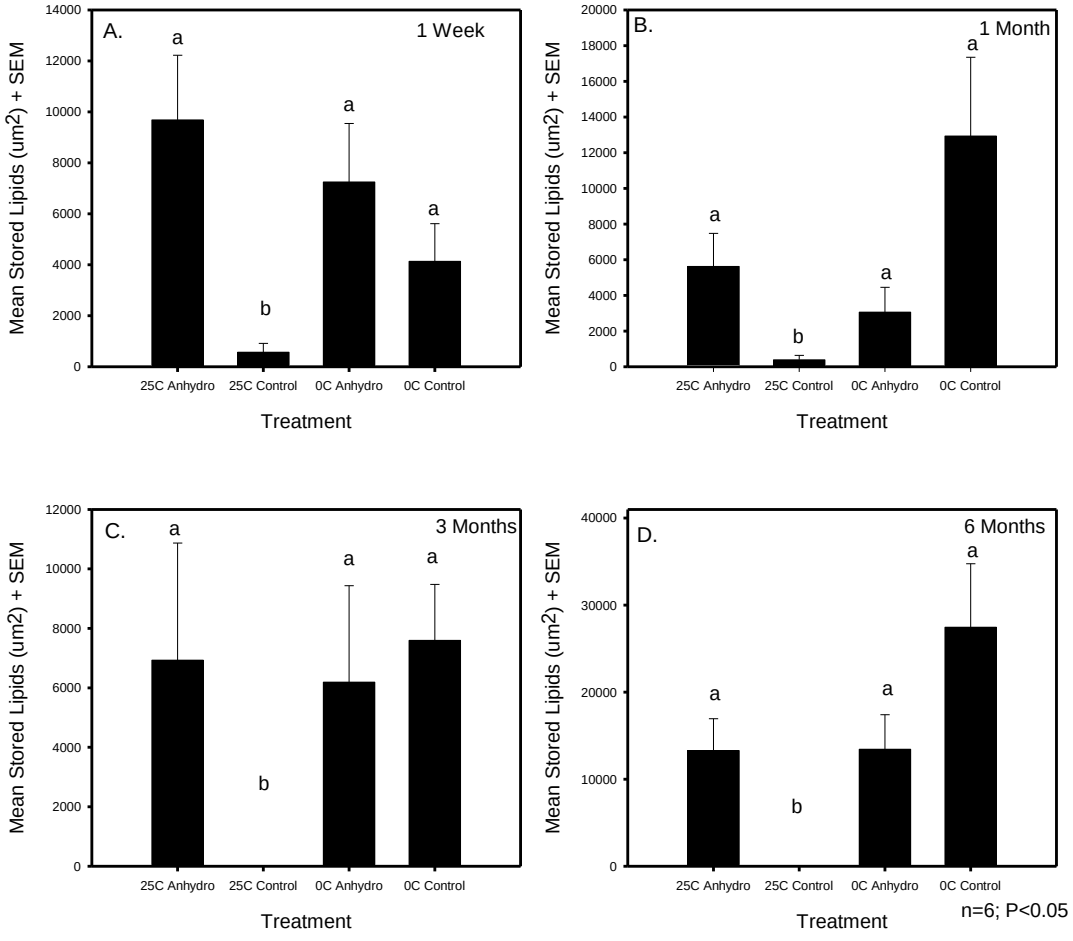

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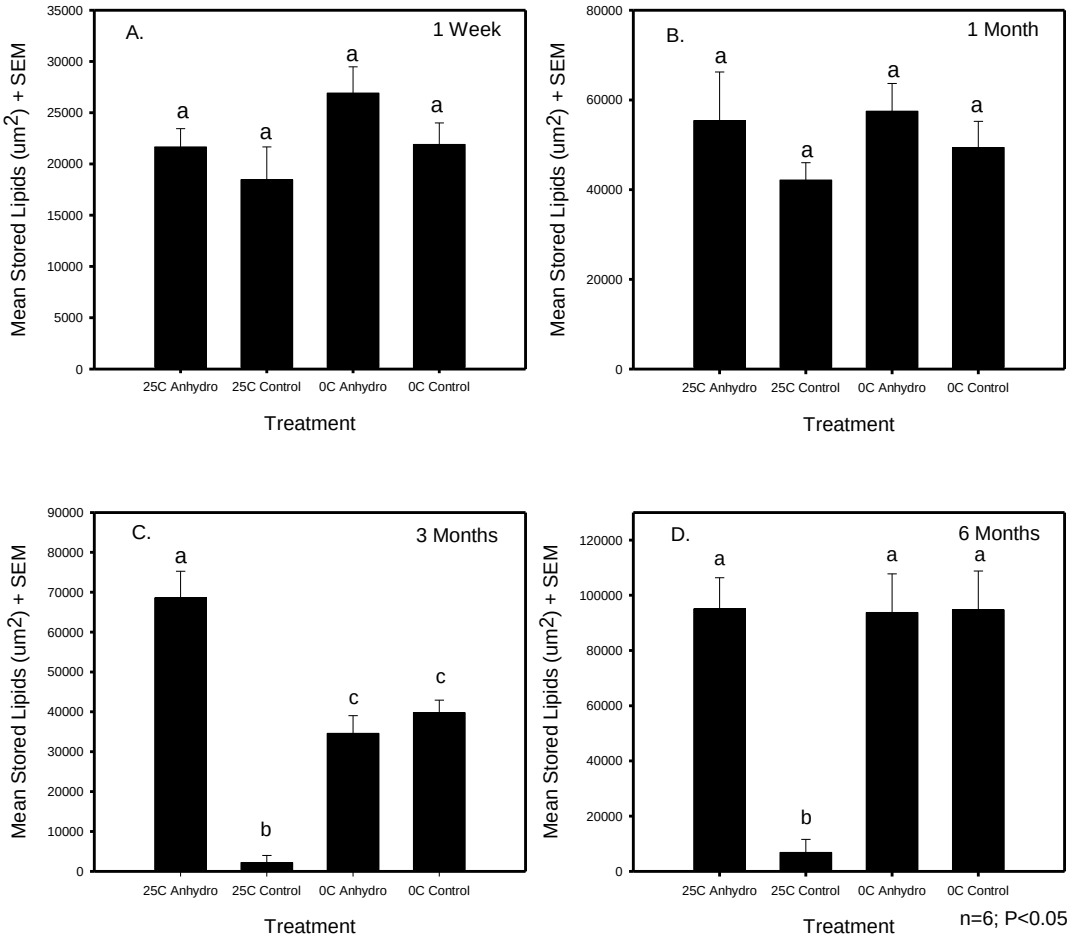

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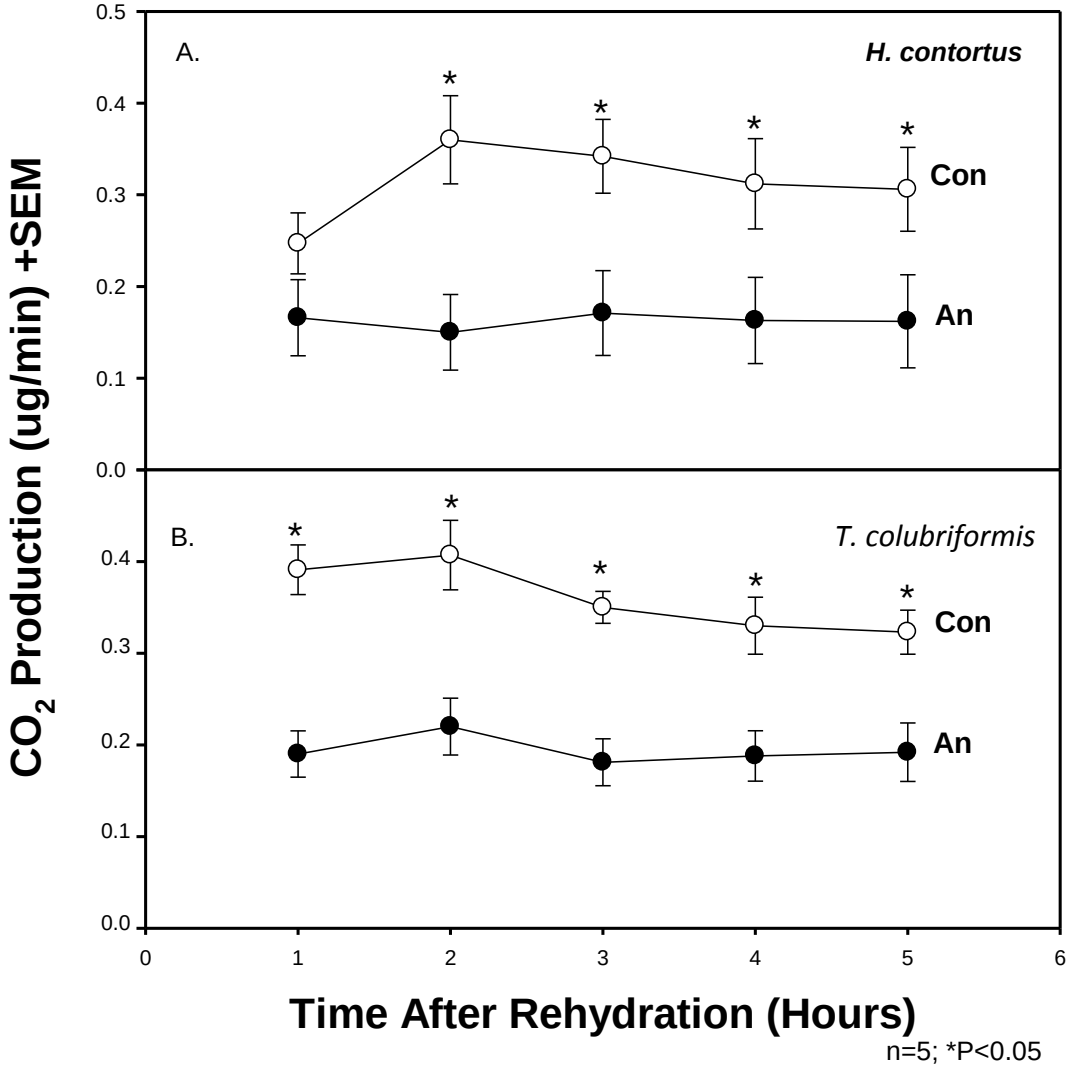

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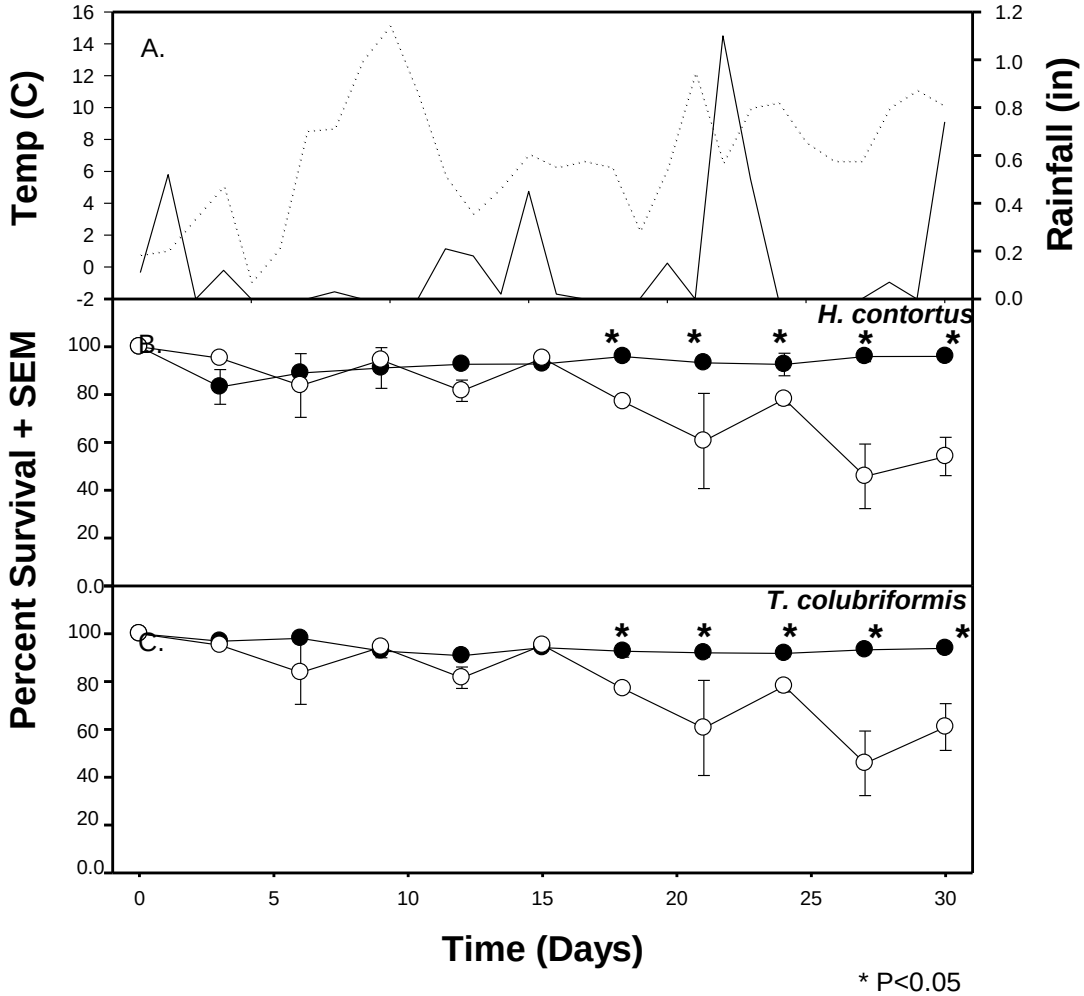

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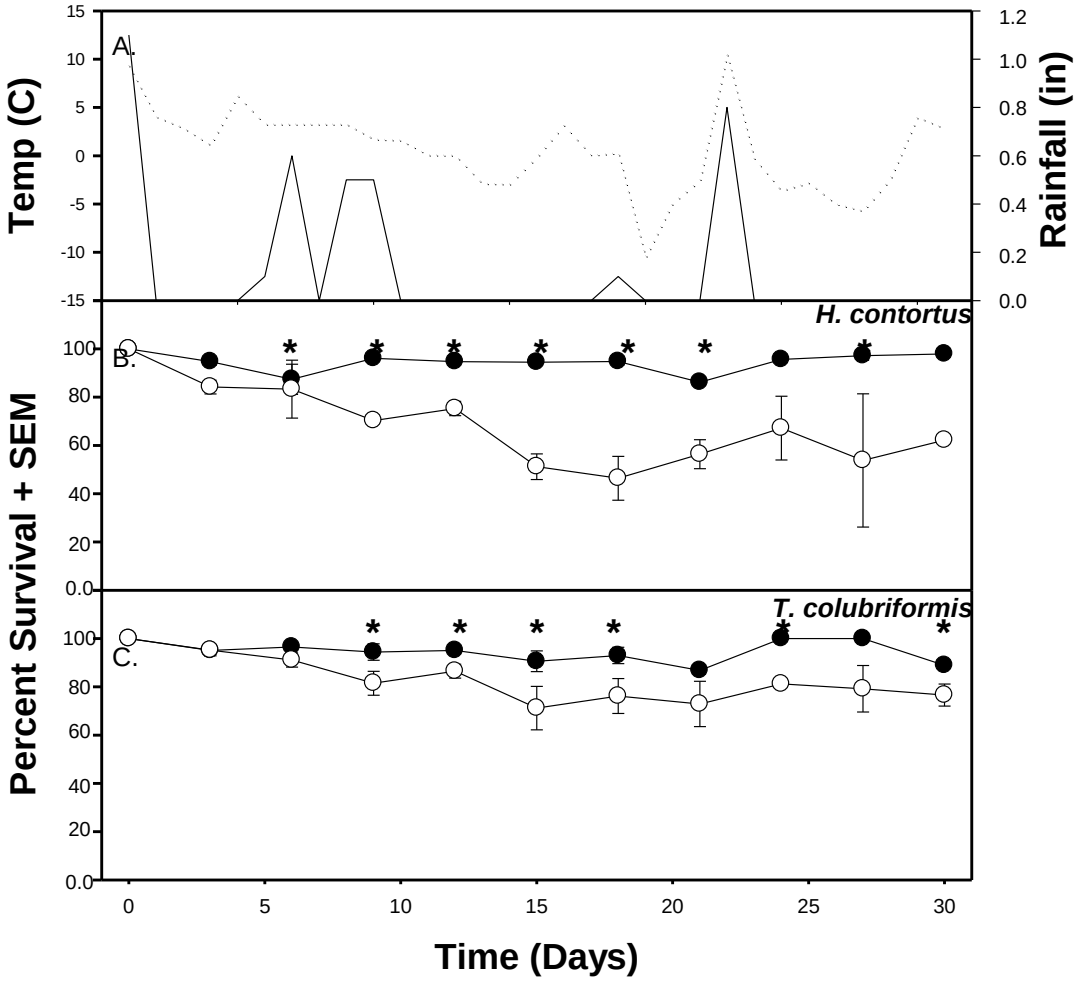

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