

Helminth parasite infracommunities and dietary analysis of the  
rough-skinned newt, *Taricha granulosa* from south-eastern  
Vancouver Island, B.C.

An undergraduate research project

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## ABSTRACT

The rough-skinned newt, *Taricha granulosa*, is a common amphibian in the Pacific Northwest, but very little is known about its helminth parasites. This species typically overwinters on land and returns to breeding ponds in the spring and summer. A primary objective of this study was to determine the nature of parasite infracommunities of the rough-skinned newt. A second was to relate patterns of parasite prevalence and intensity to aspects of rough-skinned newt ecology, including diet. A total of 107 road-killed newts collected in the spring and fall from Doumont Marsh in Lantzville, BC were examined in this study. Necropsies and stomach content analysis were performed on newts in good condition, and snout-vent-length and sex were determined for all newts. Three different parasite species, the trematode, *Glyphelmins sp.*, the acanthocephalan, *Neoechinorynchus sp.*, and the nematode, *Cosmocercoides sp.* were found in the intestine of newts. The first two were rare, with prevalences of 8 and 4% respectively. *Glyphelmins sp.* is a new record in this host. *Cosmocercoides sp.* were found in most newts with a prevalence of 88% and a mean intensity of 3.2 +/- 2.0 worms/host. Prevalence and mean intensity were not influenced by gender, season of collection, or body size. The diets of newts consisted of a wide variety of terrestrial invertebrates, with springtails, snails and mites representing 65% of total prey items observed. This terrestrial diet indicates that newts have limited exposure to parasites transmitted via predator-prey interactions in aquatic habitats. This factor accounts for the low helminth species diversity observed in these newts and the high prevalence and abundance of *Cosmocercoides sp.*, a parasite transmitted by a direct life cycle in terrestrial habitats.

## INTRODUCTION

Community ecology is the study of the interactions between groups of organisms with each other and the common environment they share. This can include predator-prey relationships, competitive interactions, or how certain groups of organisms might alter the environment to indirectly affect their neighbours. One of the major difficulties in the study of community ecology is the clear definition of what constitutes a habitat (Poulin, 1997). As one habitat or ecosystem fades into another, it is difficult to know where to draw the line between, for example, riparian zone and cedar hemlock forest. As the scope of community ecology is already so broad, this blurriness adds yet another confounding factor which makes it difficult to tease apart the biotic and abiotic factors that interact to determine community structure and function.

In such an ambitious field of study, it is helpful to start by examining simple models to understand the basics before looking at a very complex system. Looking at the ecology of parasites within a living host can provide a small-scale model within which to study community interactions and also a habitat – the host – with clearly defined boundaries (Poulin, 1997). Another advantage of studying parasite communities of a certain host is the ability to compare parasite assemblages between individuals to test predictions or hypotheses about the nature of those communities. As individuals of the same species represent virtual replicates of the parasite community's habitat, this allows the application of rigorous statistical analyses (Holmes and Price, 1986; Poulin, 1997).

Margolis *et al.* (1982) remarked on the importance of clearly defining ecological terms used in parasitology, especially parasite population and community ecology. In keeping with this recommendation, terms used later in this paper will be defined here

first. At the population level, there are several important terms to define. An infrapopulation of parasites includes all the individuals of one parasite species within a single host (Margolis *et al.*, 1982), whereas a component population refers to all individuals of a parasite species, comprising all life stages, in a defined location. Increasing in scale, there are some corresponding terms that describe parasite communities. An infracommunity refers to all the species of parasites within an individual host. A component community is defined as all the species of parasites within a host species population (Bush *et al.*, 1997).

Studies of parasite communities tend to focus on intestinal helminths. Helminths encompass a huge diversity of parasites and include those from the phyla Nematoda, Platyhelminthes and Acanthocephala (Roberts and Janovy, 2005). Holmes and Price (1986) have identified helminth parasite communities as either isolationist or interactive. Isolationist communities are formed of members whose probability of infecting a host is low. These communities are composed of small populations that seldom interact with one another – if at all – so interspecific competition plays no role in community structure. This low level of colonization leaves many niches within the host unoccupied.

Conversely, interspecific competition plays a potentially significant role in interactive helminth parasite communities, which are composed of large populations of a greater diversity of species with a high probability of colonizing a host. Because there are several aspects of host biology that influence the nature of their parasite infracommunities, Goater *et al.* (1987) suggested that these infracommunities could be classified along a scale with isolationist and interactive communities being the extremes at either end of a continuum.

Many parasites have evolved to exploit predator-prey pathways as a way to get into their host. In other words, larval or immature life stages infect a prey item, or an intermediate host, and are transmitted to the definitive host when it consumes the prey item. A definitive host is the habitat in which the parasite reaches sexual maturity and reproduces. In many cases, the parasite may actually alter the behaviour or even the morphology of the intermediate host to enhance the likelihood of transmission (Roberts and Janovy, 2005). The ability of parasites to exploit food-web relationships in order to gain access to their definitive host makes all aspects of host foraging ecology hugely influential in determining where along the isolationist-interactive continuum their parasite infracommunities lie. Generalist diets can expose hosts to a greater diversity of helminths, but so can specialized diets if they focus on a prey item that acts as a common intermediate host for different helminth species (Kennedy *et al.*, 1986).

Typically, endotherms such as birds and mammals have interactive parasite communities, due to the relatively greater food intake requirements associated with maintaining their own body heat. Consuming more prey individuals increases the probability of consuming both a greater diversity of parasites and increasing recruitment for parasite populations already established within a host. Alternately, ectotherms such as fish, reptiles and amphibians, have lower energy costs than endotherms and therefore eat less food relative to body size. This reduces the opportunity for the transmission of parasites that exploit predator-prey pathways (Kennedy *et al.*, 1986).

Several other factors that relate to host feeding ecology also influence the nature of parasite infracommunities. One of these is host vagility, or the ability to disperse. Hosts with larger ranges can exploit a wider variety of habitats, which allows them to

exploit a wider variety of prey populations and/or species, or to be exposed to direct life cycle parasites from different habitats (Kennedy *et al.*; 1986, Aho, 1990).

Another factor is host body size. Holmes and Price (1986) and Esch *et al.* (1990) have applied MacArthur and Wilson's theory of island biogeography to parasite communities. In their theory, they state that larger islands offer more habitat than smaller islands, and therefore will have a greater diversity of species. In the case of parasitism, where the host is the island, larger hosts offer more space for parasites to colonize. Host size may also influence the size of the gape, which sets a limit on the size of the prey that may be consumed. The larger the gape, the greater the variety of prey that can be eaten that, in turn, may act as intermediate hosts for helminth parasites.

Complexity of the enteric system may also affect the nature of parasite infracommunities. As more complex enteric systems offer a larger area for colonization and a greater diversity of microhabitats, they tend to harbour more diverse interactive parasite infracommunities (Kennedy *et al.*, 1986).

In a review of 1222 studies on 582 host species, Bush *et al.* (1990) observed the general trend that aquatic species hosted more intestinal helminths than terrestrial species. A major reason they identified for this is that a majority of cestodes and trematodes require an aquatic environment for at least some stage in their complex life cycles. On the contrary, the only major helminth group that flourishes on land are the nematodes, many of which have direct life cycles. This relates to another factor identified by Kennedy *et al.* (1986) as contributing to interactive helminth communities, namely host factors that result in increased exposure to direct life-cycle helminths that enter by penetration.

It must be noted that all the factors of a host's biology discussed above do not act in isolation. Rather, they interact to determine the position of the host's parasite infracommunity along the isolationist-interactive continuum (Goater *et al.*, 1987).

Since those pioneering studies in parasite community ecology, amphibian helminth parasite communities have figured prominently in the literature (e.g. Aho, 1990; Muzzall, 1991; McAlpine, 1998; Goldberg *et al.*, 1998; Goater and Goater, 2001; Bolek and Coggins, 2003), for several reasons. Global trends in amphibian population declines have been the cause of increasing concern in recent years. Many different factors, biotic and abiotic, have been implicated as causes – including parasites (Blaustein and Kiesecker, 2002). Also, amphibians are found in a wide variety of habitats and exhibit a diversity of life history strategies, enabling the use of the comparative method in studying their parasite communities. Finally, in the case of secretive animals such as amphibians, the presence of parasites can provide information about ecology and food-web relationships that would be difficult to glean through direct observation (Goater and Goater, 2001).

In a review of 98 studies of intestinal helminth infracommunities of amphibians, Bush *et al.* (1990) found that the mean number of parasite species was  $1.8 \pm 1.3$ . This clearly illustrates the depauperate, isolationist nature of amphibian intestinal helminth infracommunities.

As many amphibians are opportunistic feeders, their helminth infracommunities are typically unpredictable, and highly variable (Aho, 1990). Infracommunities also tend to be dominated by generalists that exploit a wide variety of hosts, and so may be common parasites of other sympatric amphibian species. Several studies of frog and salamander helminth communities found that most of the helminths found had direct life

cycles and did not exploit predator-prey pathways to get into their hosts (Goater *et al.*, 1987; Goldberg *et al.*, 1998; Bolek and Coggins, 2003). Conversely, in a study of red-spotted newts, *Notophthalmus viridescens* in Michigan, it was found that the newt infracommunities were dominated by helminths with complex life cycles (Muzzall, 1991).

In a study of sympatric frogs and salamanders, Bolek and Coggins (2003) noted that blue spotted salamander infracommunities were less diverse than those of the northern leopard frog and the eastern American toad. They attributed the differences in parasite community structure to aspects of host ecology, notably dietary, life history and habitat differences between the hosts. Goater *et al.* (1987) also noted that ecological and behavioural factors led to differences in parasite community structure in their study of sympatric salamander species.

Several studies have found that amphibian helminth community structure can vary seasonally (Muzzall, 1990; Muzzall, 1991; Joy and Pennington, 1998). Abiotic factors are intimately linked with the ecology of the host, its foraging behaviour and any free-living stages of parasites that may exist within the ecosystem (Roberts and Janovy, 2005). In his study of *N. viridescens*, Muzzall (1991) found marked monthly differences in the composition of parasite infracommunities. Parasite diversity was relatively low in the spring, peaked in June and remained relatively high until September. Muzzall (1991) connected increasing diversity with increasing size of the newts as the spring progressed. In another study looking specifically at the trematode *Megalodiscus temperatus* in *N. viridescens*, Joy and Pennington (1998) also found that prevalence increased from February to October and then dropped in December, although they found no correlation between prevalence of this parasite and host size. Instead, they postulated that increasing



prevalence in the summer and fall was linked with increasing production of cercaria by snail intermediate hosts throughout the summer, and the tendency of adult newts to cannibalize their larvae in the late summer (thus acquiring their parasite loads).

This study will focus on the helminth infracommunities of the rough-skinned newt, *Taricha granulosa*. As the host organism of a parasite acts as its environment, knowledge of host natural history offers important insights into the structure of parasite populations and communities. In both a large-scale review of vertebrate helminths (Bush *et al.*, 1990) and a review of amphibian helminths (Aho, 1990), the authors have identified host ecology as a major factor in determining the structure of parasite communities. Consequently, aspects of the rough-skinned newt's biology such as habitat selection, seasonal migrations and position in the food web with relation to prey and predators need to be stressed.

Rough-skinned newts are found in western North America, from southern Alaska to Northern California (Oliver and McCurdy, 1974). They become sexually mature at 4-5 years of age, and although their life expectancy is unknown, the average age of rough-skinned newts captured in Marion Lake, B.C. was 12 years (Efford and Mathias, 1969). Adult newts reach total body lengths of 12 to 18 cm (Green and Campbell, 1984). They are quite abundant across their range and are not considered to be in decline or of conservation concern (Marks and Doyle, 2005).

In their survey of wetlands in the Willamette Valley, Oregon, Pearl *et al.* (2005) examined several different habitat attributes to determine which had the greatest impact on the abundances of several local species. Overall, rough-skinned newts were well dispersed, inhabiting 70% of 85 wetlands surveyed, showing no preference for either permanent or temporary ponds. Newt abundance was positively correlated with forest

cover, which was the best predictor for newt abundance. Road density and the presence of exotic fish species were the next best predictors, both having a negative correlation with newt abundance. Interestingly, the authors found no noticeable differences in abundance of newts from ponds with and without breeding bullfrog populations. The bullfrog, *Rana catesbiana*, is an introduced species spreading throughout western North America. Its large size, voracious appetite and generalist feeding behaviour have been implicated in the declining numbers of some native amphibians (Orchard, 1991).

Rough-skinned newts divide their lives between aquatic and terrestrial environments. Gilled larvae are aquatic until metamorphosis. Typically, adults are aquatic throughout the breeding season and spend the winter in a terrestrial environment. However, the length and timing of the breeding season varies with latitude and altitude, and may also be influenced by the permanence of the aquatic habitat (Marks and Doyle, 2005). They can be found in temporary and permanent lakes, ponds, marshes and slow moving streams. When terrestrial they prefer open woodlands with woody debris for cover, although they avoid cedar forests (Green and Campbell, 1984). In a survey of red alder stands in Oregon, McComb *et al.* (1993) found positive correlations between newt capture and amount of evergreen shrub cover, the number of stumps and the presence of logs more than 19 cm in diameter. They found newt capture was negatively correlated with deciduous tree basal area.

Pimentel (1960) studied the migratory habits of *T. granulosa* in Oregon and California. He found that newts were inactive at air and water temperature below 5 °C. Only reproductively mature individuals migrated to ponds in the summer time, while metamorphosed juveniles and non-breeding adults remained on land in a state of dormancy. Newts occasionally emerged from dormancy to undertake seemingly random

movements believed to be associated with foraging for up to 8 weeks. Pimentel (1960) also observed what he termed wandering movements, where reproductive adults left their breeding ponds to wander short distances on land for varying periods of time. Although both sexes were found to wander, males wandered more frequently and for longer periods of time. All newt movements occurred during or following rainfall.

The timing and nature of breeding migrations in the rough-skinned newt have been found to vary geographically between populations. In some areas, the entire population migrates (Efford and Mathias, 1969), while in one study only females overwintered on land, while males remained aquatic year-round (Oliver and McCurdy, 1974).

Looking at the stomach contents of more than 200 *T. granulosa* from four lakes in the Cascade mountains in Oregon, Taylor (1984) found it to be a true generalist and opportunistic predator. He found that stomach contents differed markedly between lakes, and also between the same lake in different years. Rough-skinned newts from Marion Lake, B.C. were also found to exploit diverse prey, and diet varied in response to seasonal availability of prey (Efford and Tsumura, 1973). In other localities *T. granulosa* has been found to eat the eggs and larvae of many sympatric amphibians, including the red-legged frog, Pacific tree frog, long toed salamander and northwestern salamander (Marks and Doyle, 2005). They have also been known to eat slugs and snails (Green and Campbell, 1984).

The skin of *T. granulosa* contains tetrodotoxin, a powerful neurotoxin that makes it deadly to almost every possible predator. It advertises its toxicity by means of a bright orange underbelly and when threatened will arch backwards to make this warning coloration even more obvious (Green and Campbell, 1984). In their study at Marion

Lake, Efford and Mathias (1969) found that although rainbow trout in the lake regularly ate the sympatric north-western salamander *Ambystoma gracile*, no rough-skinned newts were ever found in the stomachs of these fish. The garter snake, *Thamnophis sirtalis* has evolved a resistance to tetrodotoxin, and is the only natural predator of *T. granulosa* (Green and Campbell, 1984). Interestingly, rough-skinned newts from Vancouver Island populations have almost completely lost their toxicity, and *T. sirtalis* has accordingly lost its resistance (Brodie and Brodie, 1991). Aho (1990) suggested that reduced predation pressure on toxic red-spotted newts, which allows them to forage for longer than their non-toxic counterparts, might lead to more diverse parasite infracommunities.

Few studies have looked at the parasites of *T. granulosa*, even fewer in British Columbia, and none have adopted a community analysis. Studies conducted in the Nanaimo, Sooke and Campbell River areas on Vancouver Island have been very small in scale with sample sizes ranging from 3 to 20 individuals (Richardson and Adamson, 1988; Zamparo and Brooks, 2005; Jennifer Hoare, 2006, personal communication). The only studies to use large sample sizes were conducted in Oregon and on the lower mainland of British Columbia (Efford and Tsumura, 1969; Taylor, 1984). The trematode *Megalodiscus microphagus* and the nematode *Megalobatrachonema moravecii* were found in a majority of component communities, with several other intestinal helminth species found in single studies. Species richness of the component communities was two or less, except in Moravec's 1984 study, in which five species were found. Prevalences ranged from 10 to 65% and intensities ranged from 1 to 9. The details of these studies are summarized in Appendix I.

In conclusion, the parasite infracommunities of *T. granulosa* have, to date, garnered little attention from biologists. In 1984, Moravec remarked on the lack of

studies of western amphibian parasites, and it seems that, for the rough-skinned newt at least, little has changed. According to Aho (1990), helminth communities of rough-skinned newt are only 20-50% similar among populations, so their species composition is highly unpredictable. Aho (1990) also noted that rare helminth species make up a significant component of the parasite infracommunities of the rough-skinned newt. Both of these observations confirm what the few studies available on *T. granulosa* parasite communities imply: that further research is required to determine this newt's parasite fauna and its community structure.

In the current global atmosphere of massive biodiversity loss, including a trend in amphibian decline (Blaustein and Kiesecker, 2002), it is important to study healthy populations of species before they become vulnerable. An amphibian species with such large populations and well-known ecology affords an excellent opportunity to examine the structure of parasite communities.

Considering the general trends in amphibian parasite communities and previous examinations of *T. granulosa* parasites discussed above, it was predicted that rough-skinned newts would harbour isolationist helminth communities. To confirm this, a study was undertaken that examined parasite communities of rough-skinned newts of varying sizes and ages collected both during spring and fall migrations to and from Doumont Marsh in Lantzville, Vancouver Island, British Columbia. Stomach contents of the newts were examined to assess local diet and seasonal variation in diet between the spring and fall, and any relationship these findings may have to parasite species found within the host in both seasons. This study had three major objectives. The first was to determine the prevalence, mean intensity, and species richness of helminth parasites in *T.*

*granulosa*. Prevalence is the percentage of hosts infected with a given parasite, mean

intensity is the average number of parasites of a species found per host and species richness is the number of parasite species found in the host (Bush *et al.*, 1997). The second objective was to relate these parameters to aspects of newt ecology, particularly diet and migratory behaviour. The final objective was to determine if these parasite infracommunities varied seasonally. As there have been no studies yet looking at seasonal variation in rough-skinned newt parasite communities, this study was first of its kind.

## MATERIALS AND METHODS

Doumont Marsh is located in Lantzville on Vancouver Island, British Columbia. It is roughly several hundred meters in length by 50 m wide, and is relatively shallow. It is moderately eutrophic with considerable emergent vegetation, particularly bullrushes. The surrounding area consists mainly of forested foothills. Being the major wetland in the area, Doumont Marsh hosts many different amphibian species, including red-legged frogs, *Rana aurora*, Pacific tree frogs, *Pseudacris regilla*, long-toed salamanders, *Ambystoma macrodactylum*, red-backed salamanders, *Plethodon vehiculum*, western toads, *Bufo boreas*, rough-skinned newts and American bullfrogs, *Rana catesbeiana*. Bullfrogs, an invasive species on Vancouver Island, were introduced to the marsh in 2000 (Tim Goater, personal communication). In recent years, a residential access road (Sywash Road) was built that curves around the marsh and runs parallel along its length, so that it runs between the marsh and the forested foothills.

Many of the amphibians that use the marsh as a breeding site suffer mortalities as they cross Sywash road to disperse. The most abundant species is *T. granulosa* (Tim Goater, personal communication). Road-killed animals can provide valuable information

regarding dispersal habits and diet. As many of these newt corpses are salvageable for parasite examination purposes, they were utilized for this study.

Newts were collected in the spring (January - March) and the fall (September-November). In the morning following rainfall, the road was surveyed for corpses, as this was when road-kills were observed in high numbers (but before they were run over multiple times by multiple vehicles). Of the 107 newts examined, 49 were sampled from the fall (September-November) and 58 from the spring (January-April). Of these, 10 from the spring and 16 from the fall had uncompromised alimentary canals and were therefore suitable for necropsy. These twenty-six individuals were selected as suitable for necropsy because they had intact alimentary canals, which were not severed or pierced at any point from the stomach to the colon. Exceptions were made only if the alimentary canal was entirely contained within the body of the newt, in which case it was deemed that any parasites that had escaped the alimentary canal through piercings would still be contained within the body cavity of the newt.

Although only a small subsample of 107 newts were in acceptable condition to collect parasitological and diet data, ecological data such as body size (SVL), sex and ventral colour was recorded for 106, 102 and 87 newts respectively. Gender was determined by inspection of the gonads during dissection. In many specimens the vent was ripped open, so measurements of body size are probably accurate within +/- 1 mm. Colour was quantified using the Munsell Book of Colour. The book is full of swatches assigned a value and chroma and grouped by page under hue headings. In this study, differences between swatches was deemed to be too subjective, so newt colour differences were analyzed based on more general colour grouping of hue. Due to damage

resulting from freezer burn or colour leaching, colour could only be recorded for 87 specimens.

The body cavity, lungs, liver, spleen, kidneys, stomach and intestinal tract were examined for parasites, with particular focus on the latter. Standard necropsy procedures were used, as described in Goater and Goater (2001). In brief, this entailed examining and teasing apart organs under a dissecting microscope. All nematodes were preserved in 70% alcohol while platyhelminths and acanthocephalans were preserved in AFA. Parasites were tentatively identified, counted and stored for subsequent positive identification to genus with reference to published taxonomic descriptions. The presence of larvae was noted to calculate prevalence, but as these are extremely small and easily missed they were not counted and could not be used in calculations of intensity or mean intensity. Stomach, intestine and colon contents of newts were examined for identifiable prey items, which were counted and identified to taxonomic Order. The prevalence and mean abundance of each prey taxa in 28 newts was determined. Many prey items, including soft-bodied prey, were found intact and in some cases in almost perfect condition in the intestines and even the colon, so no distinction was made between prey found in the stomach, intestine and colon.

Prevalence, range and mean intensity of all parasite species were calculated as described in Bush *et al.* (1997). Further analysis was conducted for *Cosmocercoides sp.* only, due to the rarity of *Glyphelmins sp.* and *Neochinorhynchus sp.* The variance to mean ratio was calculated to determine whether or not *Cosmocercoides sp.* exhibited the negative binomial distribution typical of many macroparasites (Roberts and Janovy, 2005). To identify trends in the data, Pearson's Product Moment Correlation Coefficient was used to determine the relationship of mean intensity with snout-vent length. Kruskal-



Wallis ANOVA and R x C contingency tables were used to determine if mean intensity and prevalence respectively varied significantly between sexes or seasons.

## RESULTS

### Helminth Parasite Infracommunities

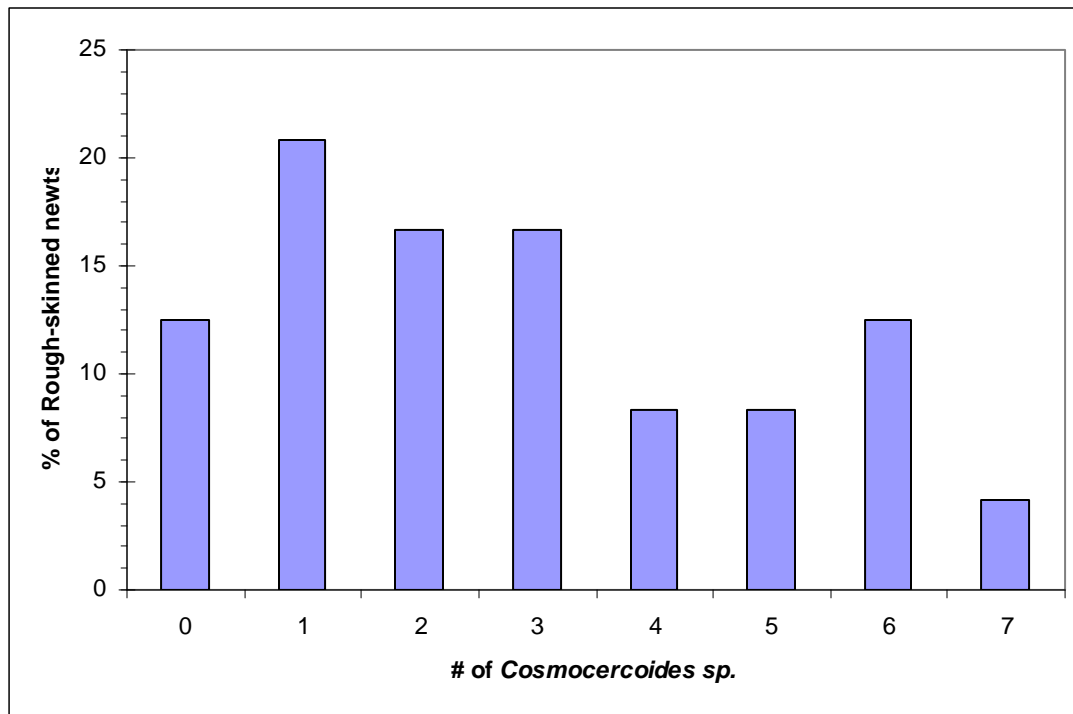
Helminth parasite infracommunities were depauperate and dominated by the nematode *Cosmocercoides sp.*, occurring in 88% of newts sampled. The trematode, *Glythelmins sp.*, and the acanthocephalan, *Neoechinorhynchus sp.* were both extremely rare (Table I). *Cosmocercoides sp.* were found in the stomach, intestine, colon and body cavity. The number of individuals per newt ranged from zero to seven. The mean intensity was 3.2 +/- 1.9 worms/host (Table I). Body size and mean intensity of *Cosmocercoides sp.* had a weak positive correlation ( $r = 0.25$ ) that was not significant ( $P > 0.05$ ). Although the prevalence and mean intensity of the nematode were both higher in the spring than in the fall (Table I), these differences were not found to be significant ( $P > 0.05$ ). Prevalence and mean intensity of *Cosmocercoides sp.* were also not found to differ significantly between males and females ( $P > 0.05$ ).

The variance to mean ratio of *Cosmocercoides sp.* for both seasons was 1.59, indicating that there was a degree of clumping of this parasite in the rough-skinned newt population sampled, with 23% of hosts harbouring 52% of the parasites (Figure 1).

*Glythelmins sp.* and *Neoechinorhynchus sp.* were rarely found in the intestines of newts. Both were only found in the spring sample. *Glythelmins sp.* was found in only two hosts – one newt harboured three individuals and the second harboured one individual. Only one specimen of *Neoechinorhynchus sp.* was found in a single newt (Table I; Appendix II).

**Table I. Prevalence, range and mean intensity of intestinal helminth parasites of *Taricha granulosa* (n=24).**

Parasite species	Prevalence %	Range	Mean intensity	Standard deviation
<b>Total</b>				
<i>Cosmocercoides sp.</i>	88	0-7	3.2	1.9
<i>Glypthelmins sp.</i>	8.0	0-3	2.0	
<i>Neoechinorhynchus sp.</i>	4.0	0-1	1.0	
<b>Spring</b>				
<i>Cosmocercoides sp.</i>	100	1-7	4.0	1.9
<b>Fall</b>				
<i>Cosmocercoides sp.</i>	81	0-6	2.5	1.8



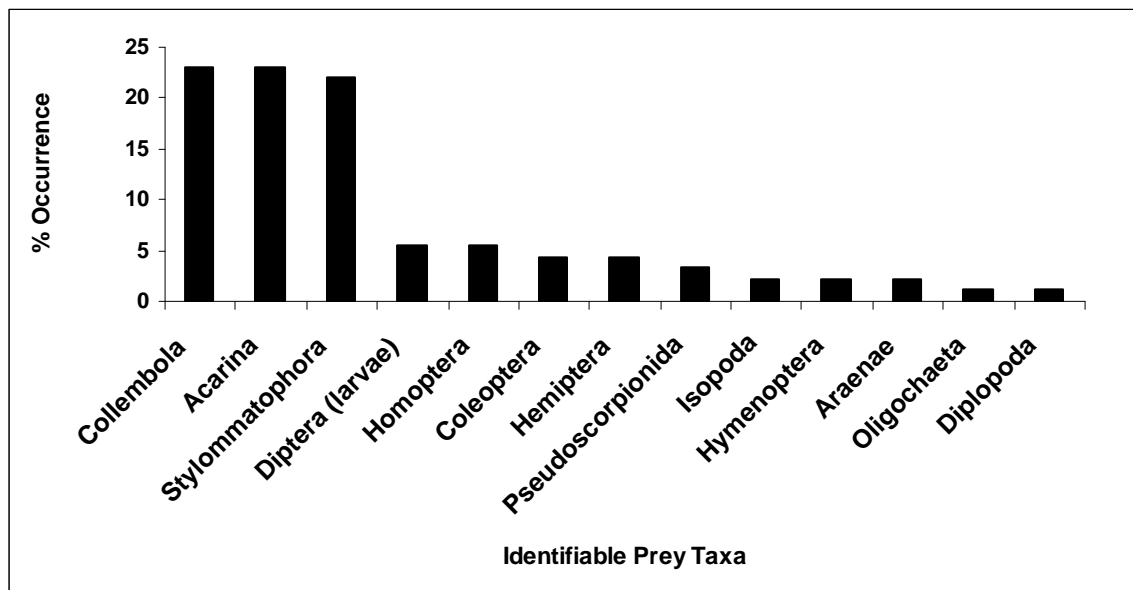
**Figure 1. Frequency distribution of *Cosmocercoides sp.* in a population of rough-skinned newts (n = 24).**

## Dietary Analysis

Prey items representing at least 13 different Orders were identified in 28 different newts. All recovered prey items were terrestrial arthropods except for the terrestrial gastropods and annelids. Springtails (*Collembola*), mites (*Acarina*) and terrestrial snails (*Stylommatophora*) were the three most common prey items, each representing 22-23% of the total prey items identified (Figure 2). All other taxa made up only 1-5.5% of the total prey recovered from newts (Figure 2). Three different species of terrestrial snails and at least five different species of mites were represented in the prey items.

Homopterans were represented entirely by ensign coccids (Family *Ortheziidae*).

Although springtails were the most abundant prey item, mites were the most prevalent prey taxa recovered, being found in 57% of newts, and had a mean abundance of 0.75 +/- 1.27 mites per newt (Table II). Springtails and snails were the second most prevalent prey taxa, both were found in 36% of newts, and had mean abundances of 0.75 +/- 0.89 and 0.71 +/- 1.26 individuals per newt respectively (Table II). Appendix III provides the raw data for the prey recovered from the 28 newts sampled.



**Figure 2.** Proportion of prey items found in a population of rough-skinned newts (n=28).

**Table II. Prevalence and mean abundance of prey taxa in rough-skinned newts (n=28).**

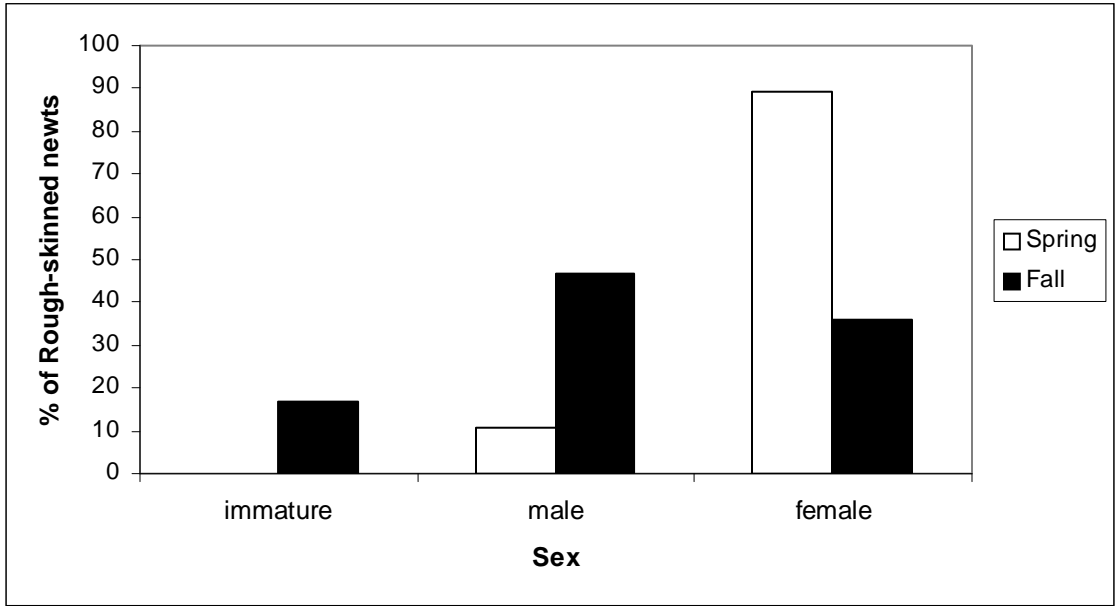
<b>Prey Taxa</b>	<b>Prevalence (%)</b>	<b>Mean abundance (+/- SD)</b>
Acarina	57.1	0.75 +/- 1.27
Collembola	35.7	0.75 +/- 0.89
Stylommatophora	35.7	0.71 +/- 1.26
Diptera (larvae)	14.3	0.18 +/- 0.48
Homoptera	14.3	0.18 +/- 0.48
Coleoptera	14.3	0.14 +/- 0.36
Hemiptera	14.3	0.14 +/- 0.36
Pseudoscorpionida	10.7	0.11 +/- 0.31
Isopoda	7.1	0.07 +/- 0.26
Hymenoptera	7.1	0.07 +/- 0.26
Araenae	7.1	0.07 +/- 0.26
Oligochaeta	3.6	0.04 +/- 0.19
Diplopoda	3.6	0.04 +/- 0.19

### **Rough-skinned newt ecology**

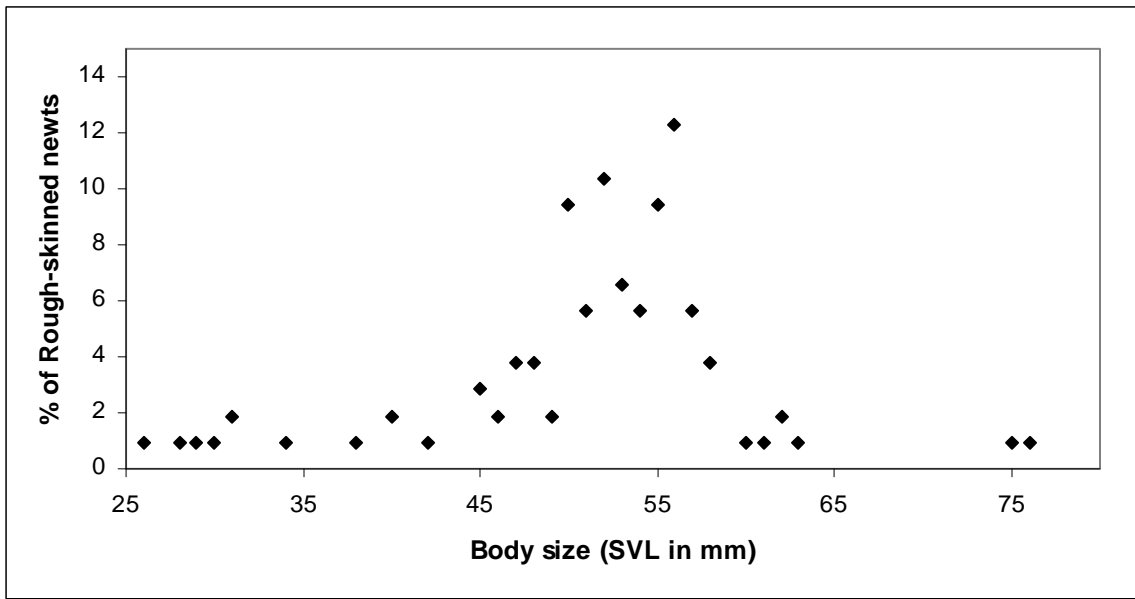
In addition to parasitological and dietary data, other ecological data including gender, body size and ventral colouration of newts collected in the spring and fall were also recorded. Eight immature newts were found in the fall sample, and none in the spring sample. Seasonal differences in sex ratio between newts were marked; a total of 89% of newts collected in the spring were females, compared to 36% in the fall (Figure 3).

Snout vent length for 106 newts ranged from a 26 mm immature individual to a 76 mm mature female (Figure 4; Appendix IV). The average snout vent length of adults was 53 +/- 5.5 mm. Newts in the spring sample were significantly larger ( $P < 0.05$ ). If the immatures are not included in the fall sample, there is no statistically significant difference in snout vent length between the seasons ( $P > 0.05$ ).

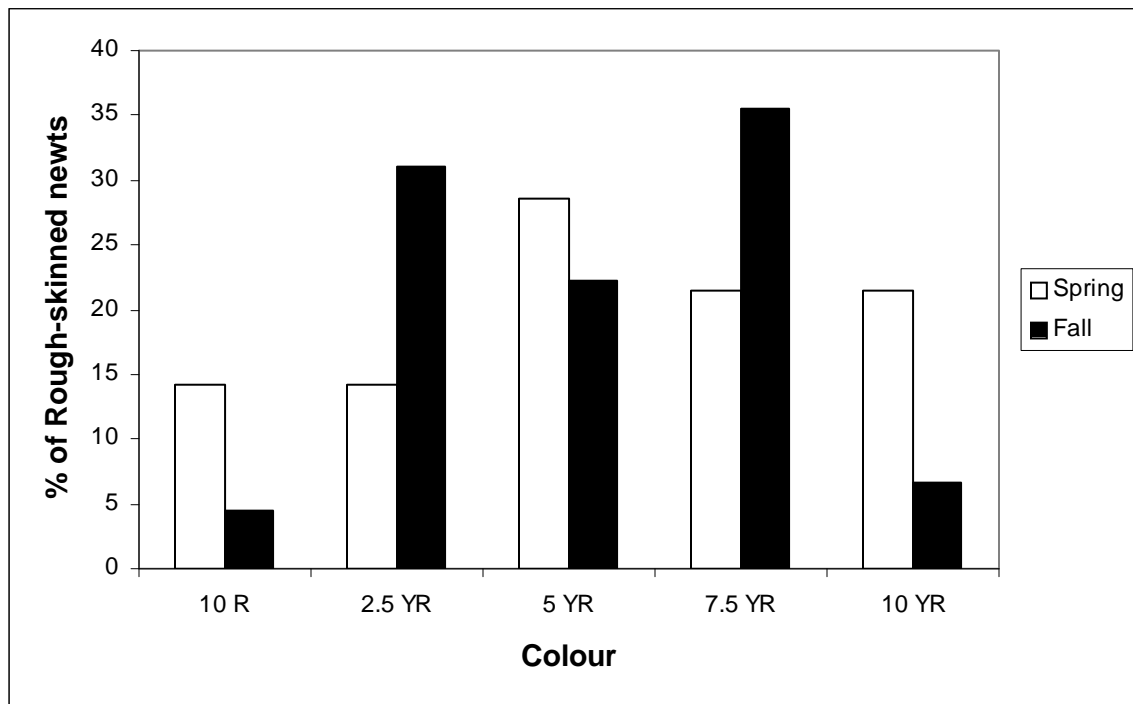
A total of 87 newts, 42 from the spring and 45 from the fall, were also examined for their ventral colour variation. Colours ranged from Red (10 R) to Yellow-Red (10 YR). The range was the same for both seasons, and in the total sample, the extremes were less common with 9% of the sample at 10 R and 13% at 10 YR (Figure 5; Appendix IV). The colour distribution differed markedly between the spring and fall samples. In the spring the colour distribution was much more uniform, with a slight peak at 5 YR. In the fall, the extremes were much less well represented, with both making up less than 7% of the sample, with peaks occurring at 2.5 YR and 7.5 YR.



**Figure 3. Comparison of sex ratio of rough-skinned newts collected in the spring and fall (n=102).**



**Figure 4. Body size distribution of newts collected in the spring and fall,  $r = 0.25$  (n=106).**



**Figure 5. Comparison of ventral colour frequency of newts collected in the spring and fall (n=87). Ventral colour was estimated using the Munsell Book of Colour (see methods).**

## DISCUSSION

### Helminth Parasite Infracommunities

Parasite infracommunities of the rough-skinned newts from Doumont Marsh in Lantzville were extremely depauperate and are clearly isolationist in nature (*sensu* Holmes and Price, 1986). Of the three parasite species found, one was extremely common and the other two were extremely rare.

Parasite infracommunities of rough-skinned newts were dominated by a generalist, direct life-cycle parasite that does not exploit predator pathways to get into their host, a common finding in previous studies of amphibian helminth parasite infracommunities (Goater *et al.*, 1987; Goldberg *et al.*; 1998, Bolek and Coggins, 2003).

There are several reasons why the parasite infracommunities of the rough-skinned newt were extremely depauperate. First of all, newts were relatively small in size, with the mean body length of adults collected being 53 +/- 5.5 mm. As a result they offer relatively few niches and little area for parasite colonization (Holmes and Price, 1986). Body size also influences the length and complexity of the alimentary canal, which again, in newts is relatively short and uniform. However, even the most heavily infected host, with seven adult nematodes, had a meager parasite load. Seemingly, there was still abundant unused host tissue available for colonization. As gape-limited predators, their small size also limits the prey they can exploit, thus reducing potential parasite transmission via intermediate hosts.

Another factor is host vagility. Rough-skinned newts in Oregon and California were found not to travel farther than 400 m on land during breeding migrations (Pimentel, 1960). Although no previous studies could be found detailing the vagility of newts in aquatic environments, this population must at least be restricted to Doumont



Marsh, as there are no other suitable breeding sites in the immediate vicinity (Tim Goater, personal communication).

In addition, amphibians are ectothermic, and their lower metabolic requirements relative to endothermic animals result in a relatively lower food intake. These four factors – small body size, simple alimentary canal, low vagility, and ectothermy - are common explanations for the depauperate nature of amphibian helminth parasite infracommunities (Aho, 1990). In addition, they all have some influence on what are often considered the most important factors contributing to the depauperate nature of amphibian helminth parasite infracommunities: diet and habitat.

Diet is important because many parasites utilize intermediate hosts or paratenic hosts as a transmission pathway to their definitive hosts (Goater and Goater, 2001). Amphibian helminth parasite infracommunities tend to be dominated either by trematodes or nematodes (Perez Ponce de Leon *et al.*, 2002), as there are few acanthocephalans (McAlpine, 1996) or cestodes that parasitize amphibians. Most trematodes have complex life cycles that use aquatic snails as first intermediate hosts and use aquatic intermediate hosts for transmission to the definitive host. As this group is often tied by its life cycle to the aquatic environment, trematodes tend to dominate aquatic amphibian helminth parasite infracommunities (Brooks, 1984). On the other hand, the parasite helminth infracommunities of terrestrial amphibians tend to be dominated by direct life cycle nematodes and are typically less speciose (Rankin, 1937; Goater *et al.*, 1987; McAlpine and Burt, 1998; Bolek and Coggins, 2003).

The specimens of *Cosmocercoides sp.* found were probably either *C. dukae* or *C. variabilis*. *Cosmocercoides dukae* has been reported from a wide variety of amphibian species from all over North America (Dyer and Brandon, 1973). This nematode utilizes

terrestrial gastropods, such as slugs and snails as definitive hosts and is thought to be incidental in amphibians, ingested when they prey on terrestrial gastropods (Vanderburgh and Anderson, 1986). Anderson (1960) concluded that the ability of *C. dukae* to pass through the gut of amphibians unharmed could be an important factor in the dispersal of this species. Lehmann (1954) and Moravec (1984) provide the only previous records of *C. dukae* in the rough-skinned newt (Appendix I). The former examined newts captured from terrestrial and aquatic environments in Oregon and California and found all six terrestrial newts were infected, and 93% of aquatic newts were infected. The latter found a prevalence of 50%, although he does not state the season of collection. In his study of the blue-spotted salamander, *Ambystoma laterale*, Bolek (1998) noted that the prevalence of *C. dukae* varied seasonally, with a relatively low prevalence of 6-14% in the spring and summer, peaking at 50% in September. He found that peaks in prevalence of the parasite corresponded with a greater dependence on terrestrial snail prey.

In this study, prevalence and mean intensity of *Cosmocercoides sp.* were slightly higher in the spring than the fall sample, although this was not statistically significant, possibly due to low seasonal sample sizes. Given the consistently high prevalence of this nematode in both seasons, it seems there is little potential for significant seasonal variation in the Doumont Marsh newt population. The overall prevalence of snails as a dietary item for newts was 36%, which is quite low in comparison to the overall presence of *Cosmocercoides sp.*, which was 88%. However, due to the small sample size, no attempt was made to determine the relationship, if any, between the presence of *Cosmocercoides sp.* in newts and the ingestion of snails.

*Cosmocercoides variabilis* utilizes toads as definitive hosts. It may utilize other amphibians as definitive hosts, although it does not seem to infect ranid frogs

(Vanderburgh and Anderson, 1987). Transmission occurs through direct penetration of the skin. Larvae then migrate to the lungs, and then to the colon as fourth stage larvae or adults (Vanderburgh and Anderson, 1987). Although the high prevalence of *Cosmocercoides sp.* found in the rough-skinned newt in this study relative to previous studies (Appendix I) indicates that it may be *C. variabilis*, no larvae were found in the lungs of the newts examined. This was not surprising, as the lungs of the rough-skinned newt are very small and thin-walled, and do not offer much space for colonization. There is no previous record of this species in the rough-skinned newt.

The rough-skinned newt is a new host record for *Glythelmins sp.* (see Appendix I). Specimens of *Glythelmins sp.* in this study were gravid, with a conspicuous egg-filled uterus. Specimens found are probably either *G. californiensis* or *G. quieta*. *Glythelmins californiensis* has been found in the intestines of red-legged frogs on Vancouver Island (Moravec, 1984; Zamparo and Brooks, 2005). *Glythelmins quieta* has also been found in bullfrogs on Vancouver Island (Sturhan, 2000). Both of these frog species are found in Doumont Marsh. There are no previous records of *G. quieta* infecting a caudate host, although it does infect several species of anurans, notably species of *Rana* and *Bufo*, from across North America (Brooks, 1976). *Glythelmins sp.* do not utilize a predator-prey pathway for transmission; instead, the cercaria penetrate the skin of adult or larval anurans and encyst as metacercaria, these are ingested by the host when it sheds and consumes its molted skin (Prudhoe and Bray, 1982).

In his review of Acanthocephala records from North American amphibians, McAlpine (1996) found no records for *T. granulosa*. He also notes that North American amphibians have few acanthocephalan parasites, although the majority of them are found in aquatic salamanders. He found only two records of *Neoechinorhynchus spp.* in

amphibians. *Neoechinorhynchus rutili* was found with a very low prevalence in the ileum of bullfrogs, the authors dismiss it as an accidental infection, as this is primarily a parasite of fish (McAlpine and Burt, 1998). However, in an ecological study of the rough-skinned newt not included in McAlpine's (1996) review, Taylor (1984) reports finding *Neoechinorhynchus sp.*, although he does not state the site they were found or the prevalence. Due to the rarity of *Neoechinorhynchus sp.* in this sample and also the lack of previous records for this parasite in the amphibian, this probably represents an accidental infection.

Interestingly, there was no overlap in parasite species found in this study and a similar study that looked at twenty road-killed newts collected from the same location in the spring of 2006 (Jennifer Hoare, personal communication). Hoare found *Megalodiscus sp.*, which has been found in the same host by other authors in Nanaimo and other Southern Vancouver Island locations (Moravec, 1984; Zamparo and Brooks, 2005). She did not find *Glythelmins sp.* or *Neoechinorhynchus sp.*, which is not surprising considering the low prevalence of these parasites in the current sample. However, it is very surprising that no *Cosmocercoides sp.* were found, considering a prevalence of 100% in the current spring sample from the same location at the same time of year. The best explanation for this is that *Cosmocercoides sp.* were lost from her specimens, which had punctured or severed alimentary canals on the outside of the body, through which parasites could escape (Jennifer Hoare, personal communication). It is also possible that *Cosmocercoides sp.* found in her study were misidentified as *Megalobachtrachonema moraveci*. In addition, small sample sizes in both studies may have likely influenced the results.

Future studies of rough-skinned newt helminth parasite infracommunities should use larger sample sizes, particularly for seasonal comparisons of helminth species richness. Although utilizing road-killed specimens provides valuable information and avoids euthanizing newts and acquiring collection permits, this method yielded small and unpredictable overall and seasonal sample sizes. It is predicted that newts collected throughout both aquatic and terrestrial phases of their life cycle would have more speciose helminth parasite infracommunities.

### **Ecology of *Taricha granulosa*: Dietary Analysis**

The diets of the newts collected in this study consisted exclusively of terrestrial invertebrates. Of the 91 prey items found in the stomach and intestines of 28 newts, none were aquatic. In previous studies, rough-skinned newts collected in aquatic environments had diets dominated by aquatic prey, such as amphipods, cladocerans, aquatic insect larvae and amphibian eggs and larvae (Efford and Tsumura, 1973; Taylor, 1984).

Terrestrial insects also accounted for a significant portion of the prey items from newts in Marion Lake, B.C. (Efford and Tsumura, 1973), although the authors were unsure if these were ingested during forays onto land or if they were eaten after falling into the water.

Considering their exclusively terrestrial diet, in combination with the parasitological evidence (dominance of helminth infracommunities by a terrestrial, direct life-cycle nematode, and lack of aquatically transmitted parasites) it is clear that newts were leading a terrestrial lifestyle before collection. This is not surprising for the spring sample, as it was assumed that these newts were heading to Doumont Marsh to breed, after overwintering on land in nearby forested areas across the road. Previous studies have found that newts overwintering on land undertake migrations to aquatic breeding

sites in the spring (Pimentel, 1960; Efford and Matthias, 1969; Oliver and McMurdy, 1974).

On the other hand, it was not expected that newts from the fall sample would have been leading a terrestrial lifestyle prior to collection. Previous studies have shown that newts leave aquatic breeding sites in the fall on a second migration to return to a suitable, terrestrial overwintering site (Pimentel, 1960; Efford and Matthias, 1969; Oliver and McMurdy, 1974). Therefore, it was assumed that newts from the fall sample had recently been leading an aquatic lifestyle, and were hit after emerging from the marsh, on their way to a terrestrial overwintering site. However, if these newts had recently emerged from Doumont Marsh, either some of their prey and/or their parasites should be aquatic in origin.

There are three possible explanations for this. The first explanation is that the individuals collected were wandering, or undertaking temporary forays onto land from the breeding site (Pimentel, 1960). This latter explanation seems unlikely for several reasons. The predominance of prey taxa associated with leaf litter found in a forested habitat indicates that newts must have crossed Sywash Road, to forage in the only forested area nearby. If this was the case, it would be expected that wandering newts would be hit both when leaving and returning to Doumont Marsh. Those just emerging from the marsh would most likely have eaten at least some aquatic prey items or harboured parasites to indicate their recent aquatic lifestyle.

The second possible reason for the lack of aquatic prey taxa and parasites in newts from the fall sample, is that these newts were actually heading to the marsh to overwinter in preparation for the breeding season the following spring. As immature and non-breeding newts remain terrestrial (Pimentel, 1960), these could be individuals that had

just matured and would be breeding for the first time the following spring, or individuals that were already mature but had not bred the previous year.

If newts in this population breed every other year, as Pimentel (1960) found in Oregon and California, then some newts that had bred the previous season and were leaving the marsh should have been collected in either the fall or the spring. Also, there is no obvious reason why newts would undertake two separate mass migrations towards the marsh in the spring and fall.

The third explanation is that some newts were leading an aquatic lifestyle prior to collection, but for some reason their prey taxa and parasites provided no evidence of this. The indication for this comes from the immature newts that were found in the fall sample. These must have been the product of the previous breeding season, leaving the marsh for the first time after metamorphosing (Corkran and Thoms, 2006). Of the eight immature newts collected, half of these had recognizable stomach contents and were suitable for necropsy. All prey items were terrestrial, and three out of four immature newts were infected with *Cosmocercoides* sp. (see Appendix I). Thus, the evidence indicates that these immatures were also terrestrial prior to collection, but that cannot be the case. Ultimately, the small seasonal and overall sample sizes make it difficult to draw confident conclusions about the lifestyles of newts prior to collection.

To truly resolve this issue of whether the rough-skinned newts were migrating to or from Doumont Marsh in the spring and fall, the marsh must be sampled for newts in the winter. Past studies have revealed the overwintering habits of the rough-skinned newt to be quite variable between different populations. Efford and Matthias (1969) found that both sexes migrated from breeding habitat in Marion Lake, B.C. to overwinter on land. In Victoria, B.C. only females overwintered on land while males remained aquatic year

round (Oliver and McCurdy, 1974). Oliver and McCurdy (1974) found that male newts buried themselves under the mud in the winter in mildly to moderately eutrophic ponds in Victoria. If either or both genders are overwintering in Doumont Marsh, they would most likely be found at the bottom of the marsh, under the mud. As Oliver and McCurdy (1974) provide the only previous record of rough-skinned newts remaining aquatic throughout the year, the finding of similar behaviour in Doumont Marsh would be a valuable contribution to the understanding of rough-skinned newt ecology.

### **Ecology of *Taricha granulosa*: body size, sex ratio and ventral colour variation**

Although newts in the spring sample were significantly larger than those from the fall sample, these differences in body size were due to the presence of eight immature newts in the fall sample (the product of the previous breeding season). No juvenile newts were observed in the spring sample, as only sexually mature individuals return to the water to breed (Pimentel, 1960). Subsequent comparison of adults from both samples indicated no significant seasonal difference in body size among newts.

Differences in proportions of females represented in each season was surprising. Results were expected to be similar to Oliver and McCurdy (1974), who found that only females participated in breeding migrations over land, as their study site in Victoria, B.C., was relatively close to Doumont Marsh. In this study, while females accounted for 89% of the spring sample, they only comprised 36% of the fall sample. When immatures (17%) are not included in the fall sample, it is roughly half male and half female. One likely explanation for this disparity is that females bloated with eggs in the spring are more susceptible to being hit by cars on roads than males (Elke Wind, personal



communication). Either this, or some other aspect of their breeding physiology or behaviour could make mature females more likely to be hit than males in the spring.

There have been no previous studies looking at variation in ventral colour in the rough-skinned newt. This is surprising, given the use of the bright ventral colour to advertise toxicity in defensive displays. Although several studies have found variation in tetrodotoxin levels in rough-skinned newts both between and within populations (Brodie and Brodie, 1991; Hanifin *et al.*, 1999), none have looked for a relationship between ventral colour and toxicity.

In this study, colour was quantified using the criteria provided by the Munsell Book of Colour. Although this method is subjective, and the exact Munsell colour could not always be found to match newt colour, it nevertheless provided a general idea of the range and distribution of colours represented in this population. It should be noted that although colour was sorted and analyzed according to the hue groupings in the Munsell Book of Colour, swatches from different hue groupings can appear very similar, and so this method is somewhat arbitrary.

Most newts were a brownish orange colour with a minority being a bright red (10R) or a more yellow colour (10YR). Interestingly, the spring sample and fall samples differed quite markedly, with a more even distribution in the spring sample. The fall sample had two peaks at 2.5 and 7.5YR, with less than 7% represented by either extreme. With such a tentative system of measurement, it seems premature to attempt to draw any conclusions from this, other than the fact that ventral colouration variation exists within populations of rough-skinned newts. In future studies, it would be interesting to investigate if this observed ventral colour variation in these newts is correlated with

varying levels of toxicity or influences behaviour and/or ecology (ie more brightly coloured newts might forage more).

In summary, rough-skinned newt helminth infracommunities were extremely depauperate, and dominated by a direct life cycle, generalist species of nematode, *Cosmocercoides sp.* The isolationist nature of the newt infracommunities was the result of the terrestrial lifestyle of the newts collected in this study, as shown by their exclusively terrestrial diet, and the lack of aquatic parasites transmitted by predator-prey interactions. Future ecological parasitological studies should utilize larger sample sizes by selectively collecting rough-skinned newts from both terrestrial and aquatic habitats. Sampling Doumont Marsh for newts in the winter would also resolve the uncertainty surrounding the migratory behaviour of this population.

In closing, it should be noted that the 107 rough-skinned newts collected for this study were the individuals deemed as potentially salvageable for ecological and/or parasitological data. Therefore they represent only a sub-sample of the total fatalities suffered on Sywash Road adjacent to Doumont Marsh. Although in much smaller numbers, other species also suffered mortality. These included Pacific tree frogs American bullfrogs, long-toed salamanders, Western red-backed salamanders, and red-legged frogs, the latter of which is a species of special concern in Canada (Anonymous, 2006). This study highlights the huge volume of amphibian road-kill that can be generated by a single, residential road, and the need to consider amphibians when planning roads and infrastructure. It is hoped that this pilot study will prompt local community groups in the Lantzville area to undertake amphibian monitoring initiatives on Sywash Road and other roads near critical amphibian breeding habitat. This would help raise public awareness about the severity of this problem, and hopefully stimulate

public interest in the general biology and conservation of amphibians. In addition, information on amphibian mortality resulting from road kill gathered from these monitoring efforts could be used to lobby for funding from public and/or private sources to construct drift fences and underpasses to mitigate amphibian mortality resulting from road kill on Sywash Road and other poorly planned pre-existing roads.

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## APPENDIX I.

**Comparison of helminth parasite infracommunity species richness in the rough-skinned newt from past studies with the results of this study. Prevalence and intensity of parasites was provided where possible, blank spaces indicate this data was not available from the original studies. Intensity was recorded either as a range or as mean intensity, depending on the data available.**

Citation	Location	n	Parasite species	Site	Prevalence (%)	Intensity
Chandler (1918)			<i>Hedruris spp.</i>	Stomach		
Lehmann (1954)	Oregon/ California	6	<i>Brachycoelium salamandrae</i>	Intestine	17	
		28	<i>Megalodiscus americanus</i>	Rectum	21	
		34	<i>Cosmocercoides dukae</i>	rectum, intestine	94	
		28	<i>Hedruris siredonis</i>	Stomach	29	
Taylor (1984)	Cascade Mtns., Oregon		<i>Cosmocerca sp.</i> <i>Neoechinorynchus sp.</i>			
Efford and Tsumura (1969)	Marion Lake, B.C.	>100	<i>Megalodiscus microphagus</i>	Gut		
			<i>Cephalouterina dicamptodonti</i>	Unknown		
Moravec (1984)	Nanaimo/Sooke	10	<i>M. microphagus</i>	Intestine	30	1 to 8
			<i>Brachycoelium salamandrae</i>	Intestine	30	3 to 5
			<i>Cosmocercoides dukae</i>	rectum, intestine	50	2 to 6
			<i>Hedruris androphora</i>	stomach, intestine	40	2 to 3
			<i>Megalobatrachonema sp.</i>	Intestine	10	1
Richardson and Adamson (1988)	Nanaimo/Campbell River	12	<i>Megalobatrachonema moravecii</i>	Intestine	50	5.8/host
Zamparo and Brooks (2005)	Sooke	3	<i>M. microphagus</i>	esophagus, intestine	66.6	1 to 9
Jennifer Hoare 2006 (personal communication)	Lantzville	20	<i>Megalodiscus temperatus</i>	Colon	65	2.92/host
			<i>Megalobatrachonema moravecii</i>	Colon	10	1
Parkinson (2007)	Lantzville	26	<i>Cosmocercoides sp.</i>	colon, body cavity, intestine, stomach	88.5	3.19/host
			<i>Glypthelmins sp.</i>	Intestine	8	2.0/host
			<i>Neoechinorynchus sp.</i>	Intestine	4	1.0/host

**APPENDIX II.**

**Helminth parasites recovered from rough-skinned newts collected in the spring and fall from Doumont Marsh.**

<b>Specimen</b>	<b>Date</b>	<b>SVL</b>	<b>Sex</b>	<b><i>Cosmocercoides sp.</i></b>	<b><i>Glypthelmins sp.</i></b>	<b><i>Neoechinorhynchus sp.</i></b>
1	March '06	75	?	1	0	0
2	March '06	61	?	3	0	0
3	Nov '06	38	imm	larvae	0	0
5	Nov '06	56	M	1	0	0
6	Nov '06	56	M	6	0	0
7	Nov '06	34	imm	1	0	0
8	Nov '06	54	M	2	0	0
9	Nov '06	50	M	1	0	0
11	Nov '06	56	M	2	0	0
13	Nov '06	53	F	5	0	0
15	Nov '06	31	imm	2	0	0
19	Nov '06	55	M	5	0	0
20	Nov '06	56	M	4	0	0
21	Nov '06	52	F	1	0	0
29	Nov '06	55	F	0	0	0
34	Nov '06	53	F	0	0	0
46	March '06	49	F	6	0	0
49	March '06	56	F	larvae	0	0
50	March '06	58	M	7	0	0
60	March '06	60	F	6	0	0
76	March '06	76	F	3	0	0
80	March '06	56	F	4	0	0
81	April '06	45	F	3	3	0
90	Jan '06	57	M	3	1	1
106	Sept '06	50	F	2	0	0
110	Sept '06	29	imm	0	0	0

APPENDIX III.

Prey taxa recovered from rough-skinned newts collected in the spring and fall from Doumont Marsh

Specimen	Date	Collembola	Acarina	Stylom	Diptera	Homoptera	Coleoptera	Hemiptera	Pseudo	Hymen	Isopoda	Diplopoda	Oligo	Araenae
2	March '06	1	1	1	0	0	0	0	0	0	0	0	0	0
3	Nov '06	2	4	0	0	2	0	0	0	0	0	0	0	0
4	Nov '06	0	1	0	0	0	0	0	0	0	0	0	0	0
7	Nov '06	3	1	4	1	0	0	0	0	0	1	0	0	0
9	Nov '06	0	2	0	0	0	1	0	0	1	0	0	0	1
11	Nov '06	1	0	0	0	0	0	0	0	0	0	0	0	0
12	Nov '06	0	1	0	0	0	1	0	0	0	0	0	0	0
13	Nov '06	0	0	0	0	1	0	0	0	0	0	0	0	0
15	Nov '06	0	0	0	1	0	0	0	0	0	0	0	0	0
16	Nov '06	2	0	0	0	0	0	0	0	0	0	0	0	0
19	Nov '06	3	1	0	0	0	1	0	1	0	0	0	0	0
21	Nov '06	0	0	3	2	0	0	1	0	0	0	0	0	0
24	Nov '06	0	1	0	0	1	0	1	0	0	0	0	0	0
26	Nov '06	0	0	1	0	0	0	0	1	1	0	0	0	0
29	Nov '06	5	1	1	0	0	0	0	0	0	0	0	0	0
30	Nov '06	0	1	1	0	0	0	0	0	0	0	0	0	0
34	Nov '06	0	0	1	0	0	0	0	0	0	0	0	0	0
46	March '06	1	1	3	0	0	0	0	0	0	0	0	0	1
58	March '06	1	1	0	1	0	0	0	0	0	0	0	0	0
60	March '06	0	2	1	0	0	0	0	0	0	0	0	0	0
76	March '06	0	0	0	0	0	0	0	0	0	1	0	0	0
80	March '06	0	0	0	0	0	0	0	0	0	0	1	0	0
81	April '06	0	1	0	0	0	0	0	0	0	0	0	0	0
90	Jan '06	0	0	0	0	0	0	0	1	0	0	0	0	0
96	April '06	0	0	2	0	0	0	0	0	0	0	0	0	0
99	Oct '06	0	0	0	0	0	0	1	0	0	0	0	0	0
106	Sept '06	0	1	0	0	1	1	1	0	0	0	0	1	0
110	Sept '06	2	1	2	0	0	0	0	0	0	0	0	0	0

#### APPENDIX IV.

Sex, body size and ventral colouration of rough-skinned newts collected in the spring and fall from Doumont Marsh.

Specimen	Date	Body size (SVL)	Sex	Colour
1	March '06	75	?	
2	March '06	61	?	
3	Nov '06	38	imm	
4	Nov '06	30	imm	
5	Nov '06	56	M	2.5 YR
6	Nov '06	56	M	2.5 YR
7	Nov '06	34	imm	2.5 YR
8	Nov '06	54	M	2.5 YR
9	Nov '06	50	M	7.5 YR
10	Nov '06	51	M	7.5 YR
11	Nov '06	56	M	2.5 YR
12	Nov '06	57	?	2.5 YR
13	Nov '06	53	F	5 YR
14	Nov '06	50	?	10 YR
15	Nov '06	31	imm	5 YR
16	Nov '06	57	?	5 YR
17	Nov '06	52	M	5 YR
18	Nov '06	53	M	10 R
19	Nov '06	55	M	10 YR
20	Nov '06	56	M	2.5 YR
21	Nov '06	52	F	5 YR
22	Nov '06	28	imm	7.5 YR
23	Nov '06	63	F	7.5 YR
24	Nov '06	55	M	10 R
25	Nov '06	62	M	7.5 YR
26	Nov '06	54	M	2.5 YR
27	Nov '06	56	M	5 YR
28	Nov '06	46	F	5 YR
29	Nov '06	55	F	7.5 YR
30	Nov '06	54	M	2.5 YR
31	Nov '06	55	M	5 YR
32	Nov '06	56	M	2.5 YR
33	Nov '06	54	M	5 YR
34	Nov '06	53	F	5 YR
35	Nov '06	57	F	7.5 YR
36	Nov '06	42	F	7.5 YR
38	Nov '06	50	F	7.5 YR
39	Nov '06	52	M	7.5 YR
41	Nov '06	31	imm	7.5 YR
42	April '06	52	F	10 YR
43	April '06	52	F	5 YR

44	March '06	56	F	10 YR
45	March '06	51	F	7.5 YR
46	March '06	49	F	5 YR
47	March '06	50	F	7.5 YR
49	March '06	56	F	7.5 YR
50	March '06	58	M	5 YR
51	March '06	?	F	2.5 YR
52	March '06	53	F	
53	March '06	55	F	
54	March '06	55	F	5 YR
55	March '06	62	F	10 YR
56	March '06	56	F	5 YR
57	March '06	58	F	10 YR
58	March '06	53	F	5 YR
59	March '06	52	F	10 R
60	March '06	60	F	5 YR
61	April '06	56	F	7.5 YR
62	March '06	52	F	
63	March '06	57	F	7.5 YR
64	March '06	47	F	
65	March '06	54	F	5 YR
66	March '06	50	F	10 YR
67	March '06	52	M	
69	March '06	47	F	
70	March '06	48	M	
71	March '06	40	F	10 R
72	March '06	55	F	10 YR
73	March '06	49	F	2.5 YR
74	March '06	51	F	
75	April '06	58	F	7.5 YR
76	March '06	76	F	2.5 YR
77	March '06	50	F	10 YR
78	March '06	47	F	5 YR
79	March '06	55	F	
80	March '06	56	F	
81	April '06	45	F	5 YR
82	March '06	55	F	
83	March '06	58	F	
84	March '06	55	F	10 R
85	March '06	46	F	
86	April '06	52	F	10 R
87	April '06	48	F	7.5 YR
88	April '06	50	M	2.5 YR
89	April '06	48	F	5 YR
90	Jan '06	57	M	2.5 YR
91	March '06	57	M	5 YR
92	April '06	45	M	7.5 YR
93	April '06	51	F	10 YR

94	April '06	47	F	10 R
95	April '06	40	F	2.5 YR
96	April '06	52	F	10 YR
97	April '06	48	F	2.5 YR
98	April '06	53	F	7.5 YR
99	Oct '06	51	M	10 YR
100	Oct '06	56	F	7.5 YR
101	March '06	50	F	10 R
102	Sept '06	50	F	7.5 YR
103	Sept '06	54	M	
104	Sept '06	56	F	
105	Sept '06	51	F	2.5 YR
106	Sept '06	50	F	2.5 YR
107	Sept '06	52	F	2.5 YR
108	Sept '06	26	imm	7.5 YR
109	Sept '06	45	F	
110	Sept '06	29	imm	7.5 YR



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