Geothermal habitats as sites for year-round transmission of Fasciola hepatica
by Robert Stanley Potts, Jr

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Veterinary Molecular Biology
Montana State University

Abstract:
Transmission of Fasciola hepatica in western Montana usually occurs during the fall months. However, a reported infection of F. hepatica in a bison herd winter pastured near a thermal site suggested that geothermally influenced habitats may serve as year-round foci of parasite transmission. To investigate this hypothesis, snails were collected and climatological conditions were monitored at four geothermal habitats on a monthly basis from July 1993 to August 1994. Lymnaeid snails were identified and their seasonal population cycles determined. Climatological data were analyzed to determine suitability of thermal habitats as year-round sources of F. hepatica miracidia and metacercaria. Laboratory- reared generations of field-collected lymnaeid snails, originating from thermal and non-thermal habitats, were exposed to F. hepatica miracidia to determine infection rates. Lymnaeid snails were collected from three of four thermal habitats and four species were identified. Lymnaeid snails were recovered at least ten months out of the year at each thermal habitat, with population cycles peaking in March-April and July-September. Review of climatological profiles indicated that temperature and moisture regimes were suitable for F. hepatica development and transmission year-round. Snail species of thermal and non-thermal origins demonstrated significantly different F. hepatica infection rates on a site specific basis.

GEOTHERMAL HABITATS AS SITES FOR YEAR-ROUND TRANSMISSION OF FASCIOLA HEPATICA

by

Robert Stanley Potts Jr.

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Robert Stanley Potts Jr.

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Date 5 June 1995

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ABSTRACT

Transmission of Fasciola hepatica in western Montana usually occurs during the fall months. However, a reported infection of F. hepatica in a bison herd winter pastured near a thermal site suggested that geothermally influenced habitats may serve as year-round foci of parasite transmission. To investigate this hypothesis, snails were collected and climatological conditions were monitored at four geothermal habitats on a monthly basis from July 1993 to August 1994. Lymnaeid snails were identified and their seasonal population cycles determined. Climatological data were analyzed to determine suitability of thermal habitats as year-round sources of F. hepatica miracidia and metacercaria. Laboratory- reared generations of field-collected lymnaeid snails, originating from thermal and non-thermal habitats, were exposed to F. hepatica miracidia to determine infection rates. Lymnaeid snails were collected from three of four thermal habitats and four species were identified. Lymnaeid snails were recovered at least ten months out of the year at each thermal habitat, with population cycles peaking in March-April and July-September. Review of climatological profiles indicated that temperature and moisture regimes were suitable for F. hepatica development and transmission year-round. Snail species of thermal and non-thermal origins demonstrated significantly different F. hepatica infection rates on a site specific basis.

INTRODUCTION

Three factors determine the development of liver fluke disease caused by members of the Fasciolidae. These include characteristics of the definitive host, the intermediate host and the parasite (Malone and Zukowski, 1992). The definitive host is usually a wild or domestic ungulate but may include other herbivorous animals as well as humans. The snail intermediate hosts for liver flukes belong to the family Lymnaeidae. The principle species of liver fluke of concern to the livestock industry in Montana is Fasciola hepatica although the North American deer fluke, Fascioloides magna, may also be present (Knapp et al., 1992). Parasite transmission is highly complex as each of the components of the Fasciola-Lymnaea system may affect the others and is influenced by the environment. Whereas each component is essential and their biological characteristics affect the incidence of disease, environmental conditions are rate limiting with respect to snail and parasite development in the northwestern United States. The snail intermediate hosts and parasite are sensitive to temperature and moisture and as a result development and transmission of the parasite is usually seasonal in Montana. Additionally, transmission tends to be focal in nature, often occurring in specific microhabitats. Thermal habitats are abundant and widely distributed throughout the state and exhibit many characteristics of microhabitats that favor the occurrence of fasciolosis. The relationship of thermal habitats to the development of liver flukes and the possibility of such areas presenting a year-round microhabitat for transmission is the topic of this study.

Fasciola hepatica

Fasciola hepatica, the common liver fluke, is believed to have been first described in 1379 by Jehan de Brie in France, making it one of the oldest known parasites (Ollerenshaw, 1959). The life history was worked out in 1882 when Lymnaea truncatula was determined to be the intermediate host in Germany (Leuckart, 1882) and England (Thomas, 1883). In North America, Lymnaea (= Galba) bulimoides was the first intermediate host identified for Fasciola hepatica (Shaw and Simms, 1929).

Life Cycle

Fasciola hepatica is a digenetic trematode. The intermediate host is a mollusc and the final host is a vertebrate. The life cycle consists of an alternation of developmental and transport phases, each of which typically result in a variable level of mortality (Ollerenshaw, 1959). Adult flukes occur in the liver and bile duct of the final host, where they have been reported to maintain an infection for as long as 11 years (Price, 1956). Flukes are hermaphroditic and produce thousands of eggs which are deposited in the bile duct and pass to the intestine by way of the common bile duct. Further development to the hatching stage is dependent upon liberation from the feces as well as temperatures of at least 10 C and soil moisture conditions at or exceeding soil water holding capacity (Ollerenshaw, 1959). The time required for the egg to develop to the miracidial stage varies with environmental conditions between two and six weeks. The ciliated miracidia have an active period measured in hours during which time contact and penetration of an intermediate host must occur. Once penetration has occurred the miracidia change into sporocysts. Each sporocyst gives rise to many redia which in turn give rise to daughter

redia. Ultimately, daughter redia give rise to cercaria which leave the snail through the mantle. The cercaria are free swimming and eventually encyst on vegetation, losing their tail in the process and becoming metacercaria. Metacercaria are infective to animals which ingest them while grazing. Upon ingestion, the metacercaria excyst in the stomach and bore through the wall of the gut into the abdominal cavity and eventually reach the surface of the liver. The immature flukes then bore into the liver parenchyma, tunneling through and feeding until they reach the bile ducts. Upon arrival, this new generation of adult flukes deposit eggs and the cycle is complete.

Fasciola hepatica in Montana

Until recently, (Knapp et al., 1992) fasciolosis was not considered to be an important disease of domestic livestock in Montana as the cold dry climate was considered to limit the disease to a few of the western counties. Jacobson and Worley (1969) reported detecting fluke ova in 2.4% of 791 samples and noted that infected cattle were primarily distributed in the western region of the state. In 1973, 5% of the livers from Montana slaughter cattle were reported to be condemned because of F. hepatica (Foreyt and Todd, 1976). Recently, however, a study of United States Department of Agriculture (USDA) inspected slaughter plants in Montana reported that liver flukes were present in 17.24% of the 6,032 cattle processed during a 12 month period and that the parasite is widely distributed (Knapp et al., 1992). Subsequently, a survey of beef cow processing facilities in the western United States reported the prevalence rate of F. hepatica to be 19.2 ± 1.2% (Briskey et al., 1994). The parasite has been reported in 26 of 56 counties in Montana. Infections appear to be heaviest in the Bitterroot Valley area, where 90% of the

livers from slaughter cattle were reported to be condemned because of liver fluke (Knapp et al., 1992). Based on studies using tracer sheep, transmission is thought to occur primarily in the autumn as metacercaria are not able to overwinter, rendering pasture essentially parasite free in the spring (Hoover et al., 1984; Knapp and Abrahamsen, 1994).

Lymnaeid Snails

The Lymnaeidae are amphibious snails that belong to the subclass Pulmonata. The family contains over one thousand species (Cruz-Reyes, 1982) and is world-wide in distribution.

Life Cycle

Lymnaeid snails are hermaphroditic, although they may cross fertilize. Eggs are laid in gelatinous capsules and are attached to surrounding substrate. The capsules contain a varying number of eggs ranging from 4 to 180 (Hyman, 1967). The eggs develop into veliger larva within the egg capsule and emerge as young snails anatomically complete except for the reproductive system. Length of development within the egg capsule is temperature dependent. Snails do not develop at temperatures below 9 C or above 37 C. At 9 C the snails hatch from their eggs in about 30 days, at 17-19 C in 17-22 days, and at 25 C in only 8-12 days (Roberts, 1950). Snails typically undergo logarithmic growth with rapid growth in length or diameter at first, then slowing down to a level with little or no growth. Growth commonly ceases after attainment of sexual maturity. Most pulmonates live about one year and die after one spawning (Hyman, 1967).

Ecology

Members of the family Lymnaeidae can be found in many habitat types, including lakes, ponds, ditches, rivers, swamps, and irrigated and non-irrigated pastures. Lymnaeids occur in a wide range of conditions, from sea level to 10,000 feet elevation, from ice waters to hot springs, and in lakes from shallow waters to depths of 250 m (Hyman, 1967). Temperature and moisture have been reported to be the most important factors regarding the development of lymnaeid snails (Ollerenshaw, 1959), but other environmental factors are considered to be important as well. Lymnaeids are generally not associated with rapidly flowing water, however, a moderate flow rate is beneficial (Boray, 1964). A preference of soil type has been reported by several investigators. Ökland (1935), Peters (1938) and Wetzel (1953) each concluded that lymnaeids prefer clay soil. Schmid (1934) suggested that an important factor of soil may be the level of impermeability to water. Several authors have suggested the importance of both soil and water pH. The range of soil pH favored is given variously as 6.2 - 7.2 (Mehl, 1932; Bryant, 1935) and 6 - 9 (Schadin, 1937). Hyman (1967) reported that lymnaeids live in slightly alkaline waters to a maximum of pH 8.5. The alkalinity of the water was due to the presence of calcium carbonate, of which a minimum of 20 mg/l is essential for the well-being of the snails (Boycott, 1936).

Lymnaeids in Montana

The history of lymnaeids in Montana dates back to 1860 when several species were collected from the Missouri River and Hell Gate River (Cooper, 1868). Currently, 18 species have been identified (Table 1) and specimens have been found in 29 of 56

counties. Of the 18 species of lymnaeid snails found in the state, 6 have been reported as natural vectors and 5 have been reported as experimental vectors of F. hepatica in various places in the United States (Tables 2 and 3).

Thermal Habitats

There are 96 thermal habitats in the state of Montana with estimated reservoir temperatures ranging from 20 C to 136 C (Sonderegger et al., 1981). The various springs and wells are located in 29 of 56 counties. Thermal waters in Montana are classified according to water temperature as hot, warm or tepid and they represent the largest and most varied group of hot springs in the world (Brues, 1932). Climatic differences relating to temperature and humidity limit the distribution of animals, resulting in regionally distinct fauna (Brues, 1932), but thermal waters present notable exceptions. In Montana, thermal habitats provide opportunities for faunal growth year-round that would not otherwise occur.

Lymnaeids and Thermal Habitats

Many molluscs, including lymnaeid snails, inhabit hot springs in Europe, Iceland and America (Brues, 1932). These snails have been found in waters ranging in temperature up to 45 C. In Montana, Oswald (1979) reported finding lymnaeid snails from the Ringling thermal well in Meagher county and Dunkel et al. (1995) collected several species from thermal habitats throughout the state.

Rationale for the Research

First, Fasciola hepatica is a parasite of considerable economic importance in Montana and throughout the world (Knapp et al., 1992; Boray, 1994). Liver flukes negatively affect the condition of livestock in numerous ways and result in decreased food production and significant economic losses (Chick, 1979; Dargie, 1987). Second, agriculture is the top grossing industry in Montana, resulting in 2.2 billion dollars in cash receipts per year, of which livestock sales accounted for 52.7% in 1993 (Sands and Lund, 1994). Third, the few studies that have been completed suggested that F. hepatica transmission in the northern Rocky Mountain region was seasonal, occurring primarily during the autumn (Hoover et al., 1984; Knapp and Abrahamsen, 1994). However, these studies did not consider the possible effects of microclimates, which Smith and Wilson (1980) and Malone and Zukowski (1992) consider to be important factors in the transmission of F. hepatica. Observation of an outbreak of fasciolosis in a herd of American bison (Bison bison) that had been winter pastured near a thermal habitat (Knapp, personal communication), and a report of clinical fasciolosis in a goat herd in Hot Springs Montana in which parasite transmission occurred in November (Leathers et al., 1982) suggested that transmission could occur year-round in association with thermal habitats. Fourth, thermal habitats are abundant in Montana, occurring in 35 of 56 counties, and their spatial distribution is positively correlated ($p < 0.01$) to the occurrence of liver flukes. Finally, environmental conditions related to temperature and moisture are the most important factors influencing the development of lymnaeid snails and F. hepatica.

Statement of Problem

The purpose of this study was to explore the effects of geothermal habitats on lymnaeid snail populations and liver fluke transmission in Montana. The proposed research was designed to determine the following:

1. the species of lymnaeid snails present at several thermal habitats;

2. the seasonal population dynamics of lymnaeid snails at thermal habitats (population dynamics refers to the number of snails present and the age structure of the population each month over the course of the year);

3. the macro- and microclimatological conditions of several thermal habitats throughout the year and, thereby, indirectly determine their suitability as a year-round source of F. hepatica miracidia and metacercaria; and

4. if snails from thermal and non-thermal habitats differ in susceptibility to F. hepatica.

Snail Species	Collection Site, Date	Reference
Lymnaea bulimoides (Lea)	Missouri river, 1860	Cooper, 1868
L. disidiosa (Say)	Missouri river, 1860	Cooper, 1868
L. humilis (Say)	Missouri river, 1860	Cooper, 1868
L. palustris (Linn)	Hell Gate river, 1860	Cooper, 1868
L. montanensis	Hayes Creek, 1912	Baker, 1913
L. caperata	Winnecook Lake, 1914	Berry, 1916
L. obrussa (Say)	Elk Creek, 1914	Berry, 1916
L. parva	Winnecook Lake, 1914	Berry, 1916
L. binneyi	Madison River, 1924	Taylor, 1952
L. hinkleyi (Say)	Yellowstone Lake, 1935	Taylor, 1952
L. elrodiana (Baker)	St. Mary's Lake, 1960	Russell and Brunson, 1967b
L. stagnalis (Say)	Lake McDonald, 1966	Russell, 1967a
L. dalli	Beaverhead Co, 1989	Dunkel et al., 1995
L. elodes	Gallatin Co., 1989,	Dunkel et al., 1995
L. modicella	Beaverhead Co., 1989	Dunkel et al., 1995
L. techella (Haldeman)	Missouri River, 1990	Knapp, pers. comm., 1993
L. auricularia	Rattlesnake Lake, 1993	Knapp, pers. comm., 1993
L. catascopium	Flathead river, 1993	Knapp, pers. comm., 1993

Table 1. List of lymnaeid snails found in Montana.

Snail Species	Collection Site	Reference
Lymnaea bulimoides	Eastern Washington	Lang, 1977
L. caperata	Sanders Co., Montana	Knapp, pers. comm., 1993
L. modicella	Eastern Washington	Lang, 1977
L. obrussa	Beaverhead Co., Montana	Knapp, pers. comm., 1993
L. palustris	Eastern Washington	Lang, 1977
L. stagnalis	Eastern Washington	Lang, 1977

Table 2. Species of lymnaeid snails collected in Montana which have previously been found to harbor natural infections of F. hepatica.

Snail Species	Reference
Lymnaea modicella	Krull, 1933
L. montanensis	Rowan et al., 1966
L. palustris	Lang, 1977
L. stagnalis	Lang, 1977
L. bulimoides	Foreyt and Todd, 1978

Table 3. Species of lymnaeid snails collected in Montana which have been experimentally infected with F. hepatica.

STUDY SITES

The four thermal habitats represented three geographically distinct regions of the state and were selected based on existing knowledge of the distribution of liver flukes, lymnaeid snails (Knapp et al., 1992) and thermal habitats (Sonderegger and Bergantino, 1981) (Fig.1). Each thermal habitat had previously been visited as part of a state-wide survey for lymnaeid snails (Dunkel et al., 1995).

Green Springs (Hot)

Green Springs (Fig. 2) effluent emanates from a Precambrian Piegan rock formation and Alluvium sediments (Sonderegger et al., 1981) in Sanders Co., Latitude 47D 26M 35S, Longitude 114 D 40M 7S. An analysis of water chemistry is presented in Table 4. The thermal habitat was a marshy depression in the middle of a pasture which was continuously filled by the spring. The area covered about 0.2 ha and was 0.6 meters deep near the center. Cattle were present much of the year and created a mud flat region around the perimeter of the spring. The elevation was 850 meters. Regional land cover was designated as strongly sloping range (8-15%) and the soil had a water holding capacity of 10.7 cm and an average pH of 7.0 (Caprio et al., 1990). The climax vegetation was Subalpine Fir climax forest, with a typical overstory of 50% Subalpine Pine, 35% Douglas Fir, 10% Ponderosa Pine and 5% Engelmann Spruce (Caprio et al., 1990).

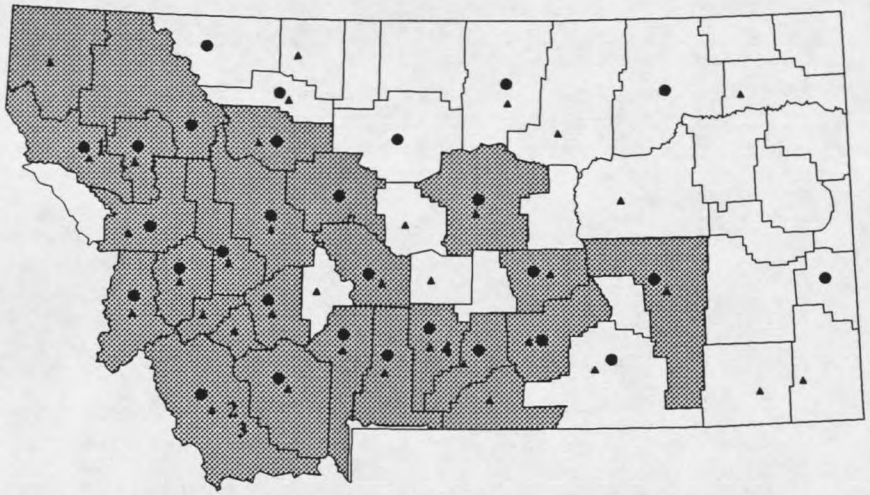

Figure 1. Location of the 4 thermal habitats studied. Distribution of liver flukes (shaded counties), lymnaeid snails (●) and thermal habitats (▲) in Montana by county. (1), Green Springs; (2), New Biltmore Hot Spring; (3), Beaverhead Warm Springs; (4), McLeod Hot Spring.

Fig. 2. Green Springs, Sanders Co.

New Biltmore Hot Spring

New Biltmore Hot Spring (Fig. 3) effluent emanates from the Madison aquifer in Beaverhead Co., Latitude 45D 27M 58S, Longitude 112D 29M 1S. The estimated reservoir temperature was 71 C and an analysis of water chemistry is presented in Table 4 (Sonderegger et al., 1981). The spring continuously flowed from a pipe into a ditch that was approximately 1.5 meters across and had high vertical sides. The water column was less than 0.3 meters deep. The elevation was 1,460 meters. Regional land cover was designated as gently sloping range (2-4%) and the soil had a water holding capacity of 12.4 cm and an average pH of 7.9 (Caprio et al., 1990). The climax vegetation was Saline Lowland Range, characterized by various grass communities (Caprio et al., 1990).

Beaverhead Warm Springs

Beaverhead Warm Springs (Fig. 4) effluent emanates from tertiary sediments over the Madison aquifer (Sonderegger et al., 1981) in Beaverhead Co., Latitude 45D 22M 47S, Longitude 112D 26M 33S. The spring percolated through the sediments of a stream bed. The channel of the stream was 3.0 - 4.5 meters wide and the banks ranged from vertical to gently sloping. The average depth of the water column was 0.76 meters at the center of the channel. The study site was at an elevation of 1,480 meters. Regional land cover was designated as gently sloping range (2-4%) and the soil had a water holding capacity of 13.0 and an average pH of 7.9 (Caprio et al., 1990). The climax vegetation was Silty Range, characterized by various grass communities (Caprio et al., 1990).

Fig. 3. New Biltmore Hot Spring, Beaverhead Co.

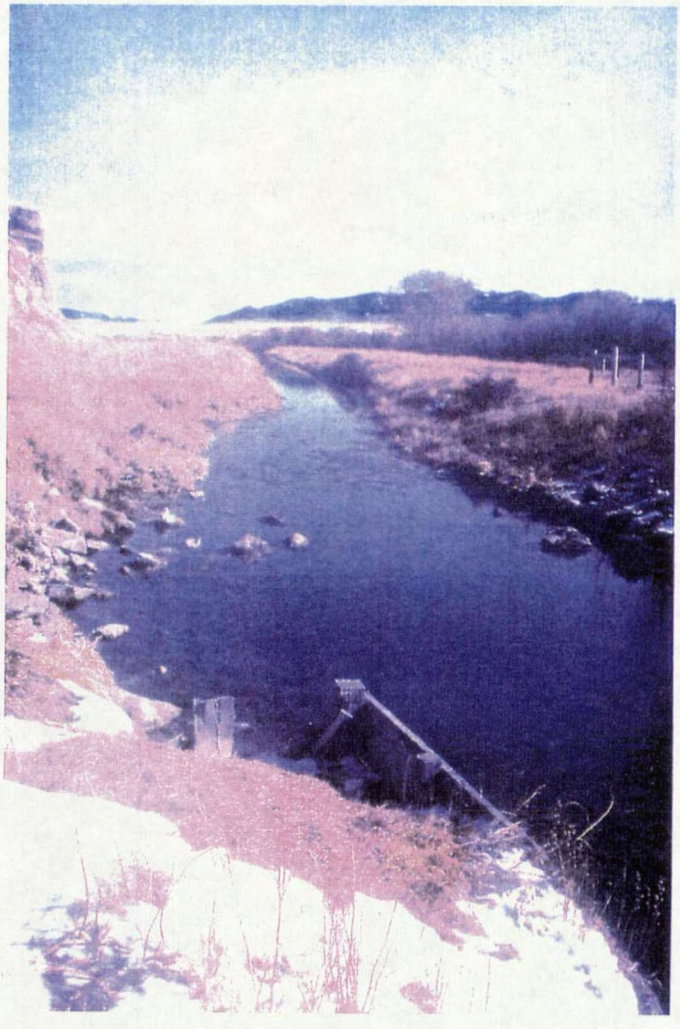

Fig. 4. Beaverhead Warm Springs, Beaverhead Co.

McLeod Hot Spring

McLeod Hot Spring (Fig. 5) effluent emanates from a Kibbey rock in Sweet Grass Co., Latitude 45D 40M 8S, Longitude 110D 5M 41S. The estimated reservoir temperature was 50 C and an analysis of water chemistry is presented in Table 4 (Sonderegger et al., 1981). The spring continuously flowed through a trench and into a holding tank. The sampling sites were located along a marshy region created by water that spilled over the edges of the trench. The elevation was 1460 meters. Regional land cover was designated as strongly sloping range (8-15%) The soil had a water holding capacity of 12.2 cm and an average pH of 7.1 (Caprio et al., 1990). The climax vegetation was Hardwood climax forest with a typical overstory comprised of 80% Cottonwoods (Caprio et al., 1990).

Fig. 5. McLeod Hot Spring, Sweet Grass Co.

Table 4. Analysis of water chemistry at the Green Springs, New Biltmore Hot Spring and McLeod Hot Spring (Sonderegger et al., 1981). Measurements given in mg/L. (ND), not detected/detection limit unknown. Data not available for the Beaverhead Warm Springs.

Site	Ca	HCO_3	CO_3	Cl	SO_4	K	Fe	NO_3
Green Springs	ND	101.0	12.0	5.0	18.0	61.0	0.1	ND
New Biltmore Hot Springs	290.0	230.0	<1.0	46.0	1100.0	ND	0.10	ND
McLeod Hot Spring	473.0	122.0	0.0	3.0	1350.0	ND	0.40	0.0

MATERIALS AND METHODS

Snail collections and observations of climatological conditions were made from July 1993 through July 1994. Sampling was done at four sampling sites along a 75 meter transect at each of the thermal habitats. The transects originated at the source of thermal activity and proceeded along the descending thermal gradient. The sampling sites (1-4) were spaced at 25 meter intervals, covering an area not exceeding $1m^2$, and were situated at the interface of the water and bank so as to allow for sampling of aquatic, transitional and terrestrial environments. Sampling was done according to a repeated measures design, although other methods were considered. This method was selected because the origin of thermal activity was a point source, creating a temperature gradient along the length of the habitat. Therefore, regions differing in distance from the source represented unique microhabitats, each of which were likely to be more or less suitable for lymnaeid snails. The repeated measures design works well in this situation because it is non-random and the same microhabitats are sampled during each effort. This is important because it allows the population dynamics for each microhabitat to be determined. Also, the microhabitat which is best suited for lymnaeid snails could be determined. If a random design were used, inferences about population dynamics and habitat suitability would be limited to the system as a whole.

Snail Collections

A 10 minute visual search of the soil and vegetation was made within each of the $1m^2$ study sites along the transect. Forceps were used to recover snails from the terrestrial and transitional sections of the study site and an aquatic net was used to sample the aquatic section. Snails were transported to the laboratory in sealed plastic containers filled with water collected on site. Containers were marked to indicate the thermal habitat and sampling site (1-4) where the snails originated. Visual searches were used as opposed to the water extraction of snails from soil cores (Ross and O'Hagan, 1968; Malone et al., 1984) because the objective was to identify active lymnaeid populations. A distinction was made between active and non-active or aestivating snails because of the underlying objective to understand F. hepatica transmission. According to Lynch (1965) lymnaeid snails burrow prior to aestivation 85% of the time. Therefore, while active snails would be susceptible to acquiring new F. hepatica infections, aestivating snails generally would not.

Climatological Characterization

Climatological conditions of the macro- and microhabitats associated with the thermal habitats were monitored each month over the course of the year. The region immediately surrounding a thermal habitat was considered to be representative of the macrohabitat and the sampling sites (1-4) were each considered to be microhabitats. Climatological conditions, such as precipitation, have been reported to be useful as indicators of the probability of F. hepatica transmission (Ross, 1970; Malone et al., 1984), however, Smith and Wilson (1980) stated that such factors are useful only to the extent to

which they affect microclimates. The purpose of this experiment was to compare the suitability of the macro- and microhabitats for lymnaeid snails and F. hepatica over the course of a year. Temperature and moisture were the principle climatological conditions measured because they are the rate limiting factors for growth and development of lymnaeid snails and F. hepatica (Ollerenshaw, 1959; Boray, 1969; Malone et al., 1984).

Macroclimatological Characterization. Characterization of the macroclimate was achieved by monitoring air temperature and precipitation. Air temperature was measured at 1.2 meters above the ground, to the nearest 0.1 C, with a Tegam 871A digital thermometer using a 20 cm air/gas thermocouple probe. Average monthly precipitation was retrieved from the Montana Agricultural Potential System Atlas, Version 5.0, Geographic Information System (Caprio et al., 1990).

Microclimatological Characterization. Characterization of microclimates included monitoring water temperature and electrical conductivity and soil temperature. Water temperature was measured to the nearest 0.1 C and electrical conductivity to the nearest 0.1 mS with an Extech (Model 650) digital conductivity meter/thermometer. Measurements were made by suspending the probe within the aquatic section of the sampling site. Soil temperature was measured, to the nearest 0.1 C, with a Tegam 871A digital thermometer using a 10 cm surface thermocouple probe. Measurements were made by fully inserting the probe within the terrestrial section of the sampling site.

Specimen Preservation

Snails were relaxed prior to preservation using menthol crystals. Specimens were placed in a container of water and powdered menthol was sprinkled over the water surface

(50 mg/ml). The container was sealed and maintained at 10 C for 10-24 hours. Specimens were relaxed until their bodies were extended from the shell and insensitive to touch. Snails were placed in hot water (60 C) for 2 seconds and preserved in 70% ethanol. Specimens were stored as wet collections (70% ethanol) in 25 ml screw top vials. Each collection was assigned an accession number which was indicated on both the collecting data label and the identification label, which were placed in the vials. A duplicate set of each accession was made and submitted to the mollusc collection at the Museum of Natural History at the University of Colorado, Boulder.

Identification

Snails were identified by Dr. Shi-Kuei Wu at the University of Colorado, Boulder. Shell characters were relied upon for most identifications. Dissection of specimens and microscopic examination of soft body parts were performed when necessary to place snails into the proper species group.

Snail Culture Technique

Field collected snails were cultured to produce generations free of F. hepatica for experimental use. Cultures were prepared as described by Taylor and Mozley (1948) with a few modifications (Fig. 6). Twenty-five centimeter unglazed clay pots were soaked in distilled water for 2 days and mud collected from the sampling sites was sterilized by autoclave to remove any potential for parasite transmission. Sterile aquaria gravel was placed in the bottom of the clay pot and mud was formed into a slope (m = 0.1) extending from near the lip of the pot on one side to the bottom of the pot on the other. Distilled water was added to the pots to form a pool at the base of the slope creating an

approximate 2:1 ratio of exposed soil to submerged soil. Pots were placed in plastic spill trays filled with distilled water. The cultures were misted with distilled water daily to compensate for evaporative losses. Cultures were placed in a controlled environment and allowed to equilibrate for 2 days, at which time 5-10 snails were introduced. Temperature was maintained at 22-27 C, and lighting, which was controlled by a timer, was set to 12 hour light:dark cycles. Cultures were placed in racks that had wide spectrum (40 W) plant and aquarium fluorescent lighting mounted at a height of 45 cm overhead. Snails were fed red leaf lettuce to supplement the algal growth which occurred in the cultures. Lettuce was prepared by soaking in distilled water for 24 hours and then rinsing in distilled water. Chalk was added ad libitum as a source of calcium.

Determination of F. hepatica
Experimental Infection Rates

Generations of snails reared in laboratory cultures were exposed to F. hepatica miracidia to determine infection rates of the various species . Exposures were designed to compare infection characteristics among species of lymnaeids and also within species of lymnaeids which originated from thermal and non-thermal habitats (Table 5). Snails ranging in size from 2-6 mm were individually exposed to 3-8 miracidia (Montana bovine origin) for 6 hours and then maintained in cultures. Snails were crushed and examined microscopically for redia, sporocysts and cercaria at 50 days post-exposure to determine infection rates. Lymnaea columella was used as a positive control during experiments as it has been consistently demonstrated to be susceptible to infection at a rate of 60- 80% in the laboratory (Knapp, pers. comm., 1993). Differences in infection rates of specimens

from thermal and non-thermal habitats were tested for statistical significance; the comparison of infection rates was based on the difference of the sample proportions of the two populations and used the z statistic (Moore and McCabe, 1989). Lymnaea obrussa and L. modicella were used in this experiment because of their availability from thermal and non-thermal origins. Laboratory reared snails were used to ensure that the snails being exposed were free of F. hepatica. Field collected specimens could not be used because it is impossible to detect the parasite without crushing the snail. First generation snails were used whenever possible to ensure that they were not appreciably different than the parental generation. It is generally accepted that lymnaeid snails evolve from their wild type when maintained in culture. Boray (1966) reported that lymnaeids acclimate to laboratory conditions within 2-3 generations. Therefore, while first generation snails would still be likely to respond to parasite exposure in a similar manner as the thermal and non-thermal field collected snails, second generation and beyond might not.

Fig. 6. Culture for lymnaeid snails.

Table 5. Lymnaeid snails from thermal and non-thermal habitats exposed to F. hepatica. (a), based on identification of snails used to start cultures. (b), average water temperature of the sampling site from which snails originated.

Snail[a]	Origin	Temperature[b]
L. obrussa	Beaverhead Warm Springs	18.6 C ± 3.3
L. obrussa	Beaverhead Warm Springs	14.4 C ± 5.2
L. modicella	New Biltmore Hot Spring	23.5 C ± 4.0
L. modicella	Beaverhead River	12.9 C ± 8.6

RESULTS

Species of Snails Collected

In this study 3,500 snails representing 2 subclasses and 5 genera were collected, including 4 species of lymnaeids (Table 6). Lymnaeid snails were collected from the Green Springs, New Biltmore Hot Spring and Beaverhead Warm Springs, but were not found at the McLeod Hot Spring. Snails demonstrated some variability in habitat type selection, however, the majority of snails was recovered from the transitional segment of the study sites on gently sloping mud flats at the interface of the water and bank.

Green Springs

Lymnaeid snails comprised 93% of the 498 snails collected from July 1993 to July 1994 (Fig 7). Lymnaea caperata and L. montanensis were found at sampling sites 1, 2 and 4; the majority was collected at site 1 (Fig. 8). The snails were always found in the transitional section of the sampling sites, usually in the tracks of livestock that grazed near the springs perimeter. Oxyloma spp. was the only other snail collected at the Green Springs and it always appeared on the terrestrial segment of the sampling sites.

Table 6. List of snail species collected from each thermal habitat.

Subclass	**Genus/Species**	**Collection Site**
Pulmonata	Lymnaea caperata	Green Springs
	L. obrussa	Beaverhead Warm Springs
	L. modicella	New Biltmore Hot Spring
	L. montanensis	Green Springs
	Physa skinneri	New Biltmore Hot Spring, Beaverhead Warm Springs, McLeod Hot Spring
	Gyraulus parvus	New Biltmore Hot Spring Beaverhead Warm Springs McLeod Hot Spring
	Oxyloma spp.	Green Springs New Biltmore Hot Spring Beaverhead Warm Springs
Prosobranchia	Juga spp.	Beaverhead Warm Springs

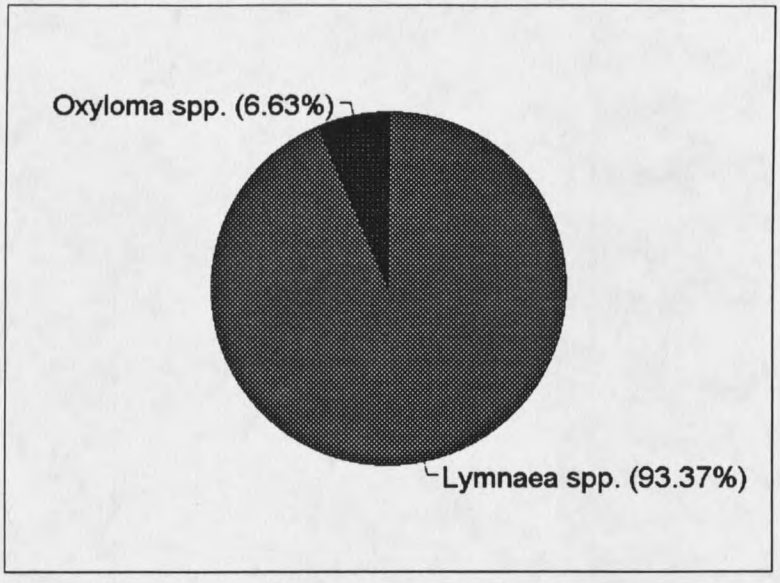

Fig 7. Percentage of each snail species collected at the Green Springs from July 1993 to June 1994.

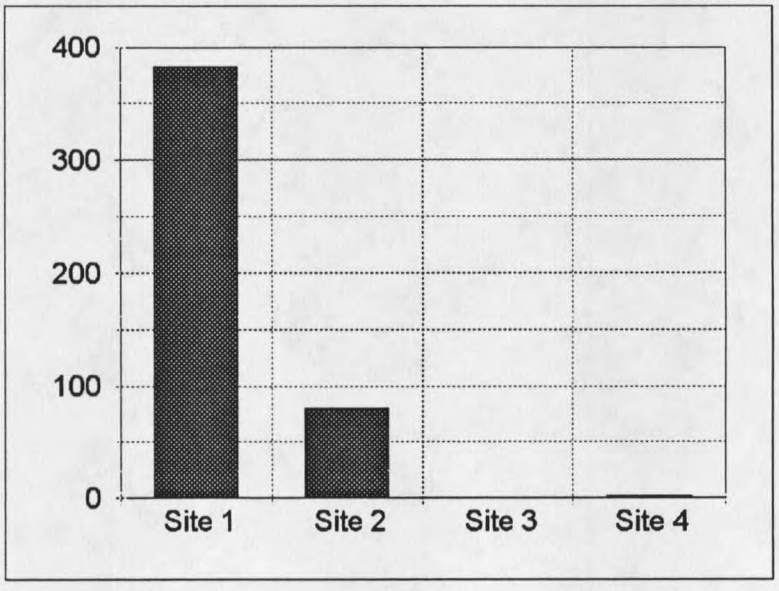

Fig 8. Number of lymnaeid snails collected at each sampling site (1-4) from July 1993 to July 1994 at the Green Springs.

New Biltmore Hot Spring

Lymnaea modicella, the only lymnaeid snail found at the New Biltmore Hot Spring, comprised 30% of the 1,090 snails collected from July 1993 to July 1994 (Fig 9). Lymnaea modicella were found only at sampling sites 3 and 4 (Fig. 10). The snails were always found in the transitional segment of site 3, but were found in both the transitional and aquatic segments of sampling site 4. Physa skinneri, Gyraulus parvus and *Oxyloma* spp. snails were also collected. Physa skinneri was found in all 4 sampling sites and was always in the aquatic segment of the sites, usually on the surface of rocks or woody debris. Gyraulus parvus and Oxyloma spp. were collected at site 4 and were always found in the aquatic and terrestrial segments of the site, respectively.

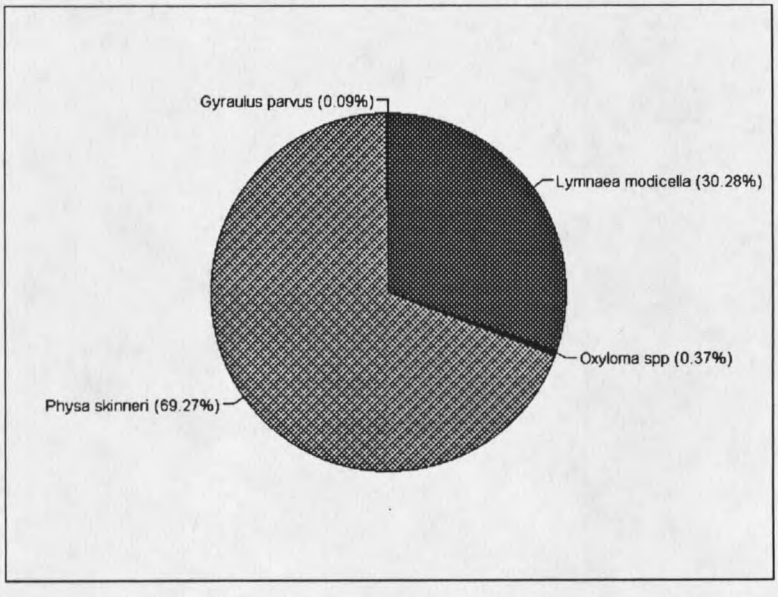

Fig 9. Percentage of each snail species collected at the New Biltmore Hot Spring from July 1993 to July 1994.

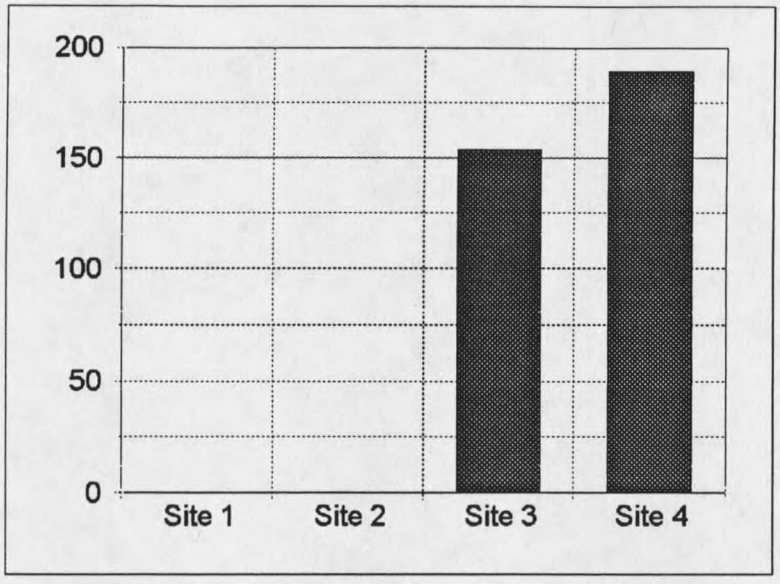

Fig 10. Number of Lymnaea modicella collected at each sampling site (1-4) from July 1993 to July 1994 at the New Biltmore Hot Spring.

Beaverhead Warm Springs

Lymnaea obrussa, the only lymnaeid found at the Beaverhead Warm Springs, comprised 67% of the 1,600 snails collected from July 1993 to July 1994 (Fig 11). Lymnaea obrussa was collected from all 4 sampling sites, but most were found in the muddy portion of site 3 (Fig 12). Physa skinneri and Juga spp. were collected from all 4 sampling sites and Gyraulus parvus and Oxyloma spp. were collected from sites 1, 2 and 4. Physa skinneri, Juga spp and Gyraulus parvus were always found in the water and Oxyloma spp. was always found in the terrestrial section of the sampling sites.

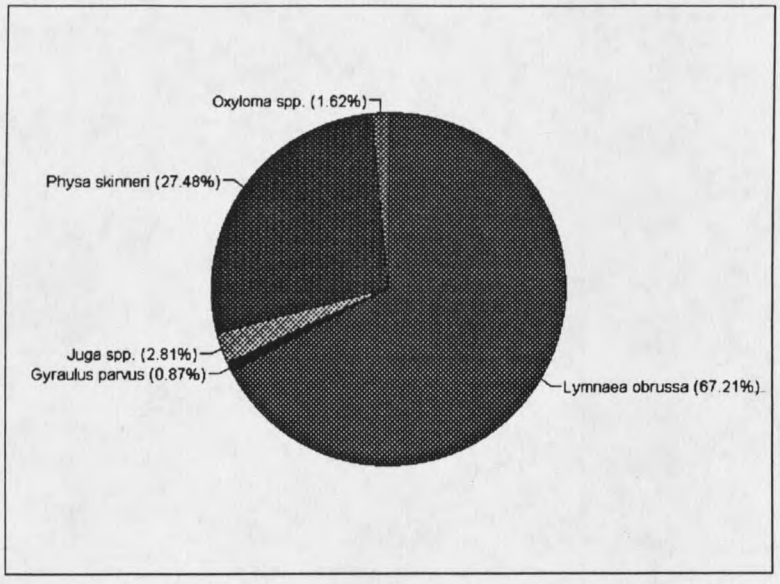

Fig 11. Percentage of each snail species collected at the Beaverhead Warm Springs from July 1993 to July 1994.

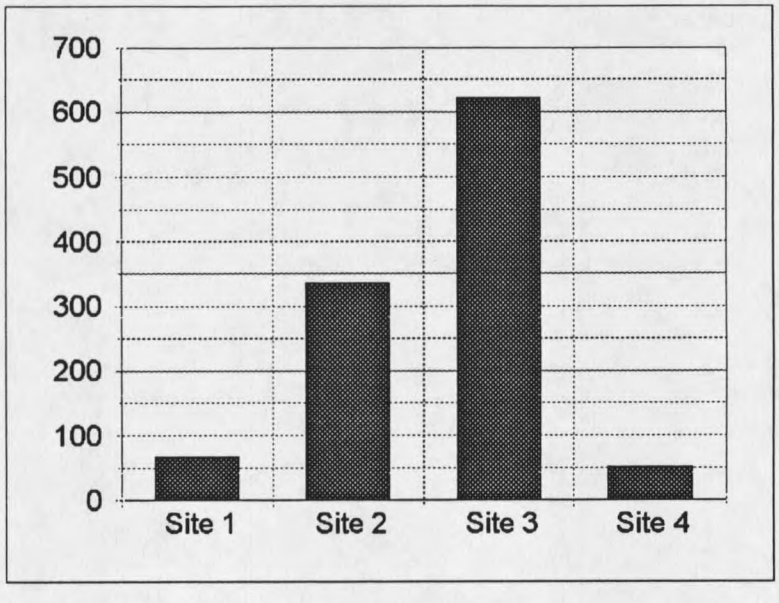

Fig 12. Number of Lymnaea obrussa collected at each sampling site (1-4) from July 1993 to June 1994 at the Beaverhead Warm Springs.

Lymnaeid Population Dynamics

Green Springs

Lymnaeid snails were found during 10 months of the year. The number of snails collected per month ranged from a low of 2 in June to a high of 116 in September. The population cycled seasonally with peaks in September and April (Fig 13). Two generations of snails were produced during the year, as indicated by the presence of juvenile snails in September and June (Fig 14).

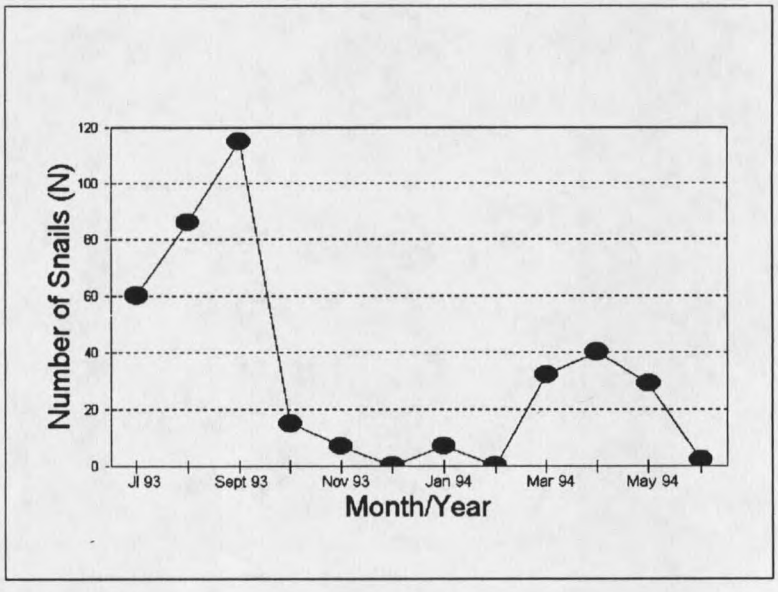

Fig 13. Number of lymnaeid snails collected each month at the Green Springs from July 1993 to June 1994.

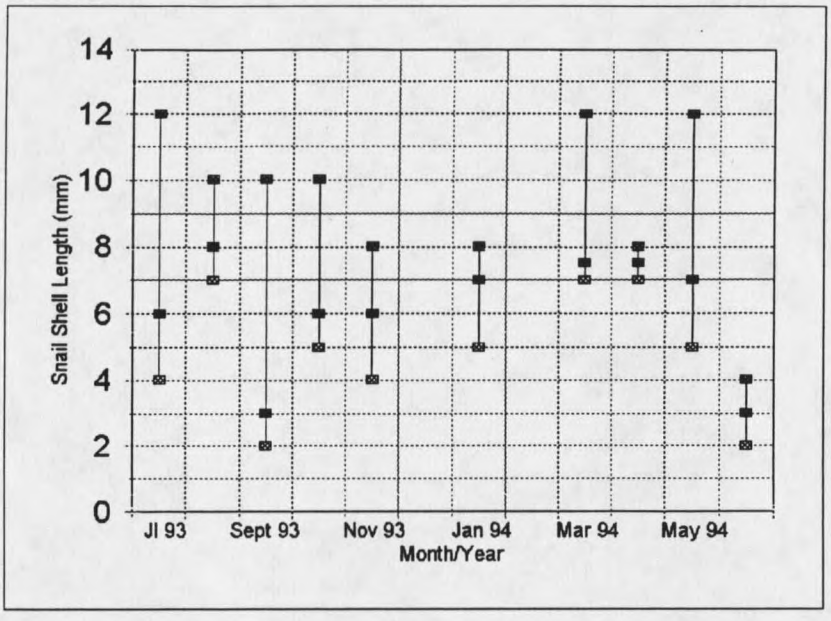

Fig 14. Maximum, minimum and average shell length of lymnaeid snails at the Green Springs from July 1993 to June 1994.

New Biltmore Hot Spring

Lymnaea modicella was found throughout the year. The number of snails collected per month ranged from a low of 2 in February to a high of 65 in March. In general, the population cycled seasonally with peaks in March and October followed by a gradual decline in the size of the population, however, the number of snails collected in April did not follow this trend (Fig 15). Two generations of snails were produced during the year, as indicated by the presence of juvenile snails in October and April (Fig 16).

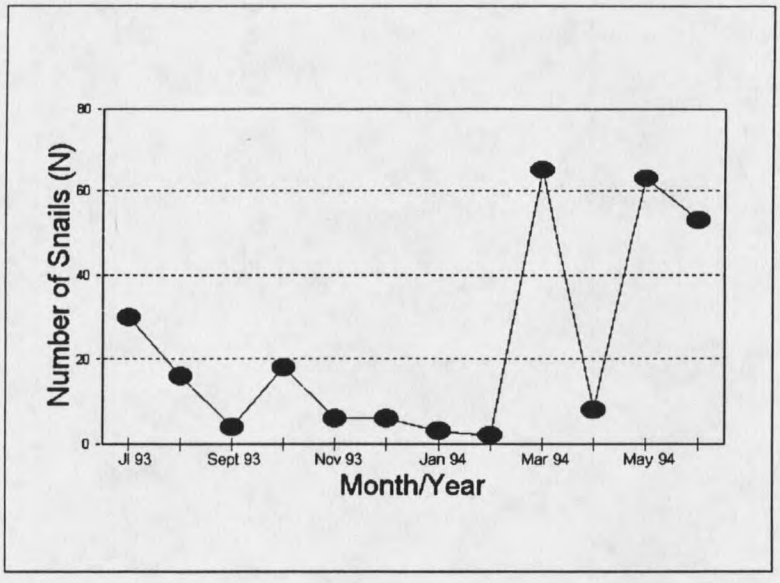

Fig 15. Number of Lymnaea modicella collected each month at the New Biltmore Hot Spring from July 1993 to June 1994.

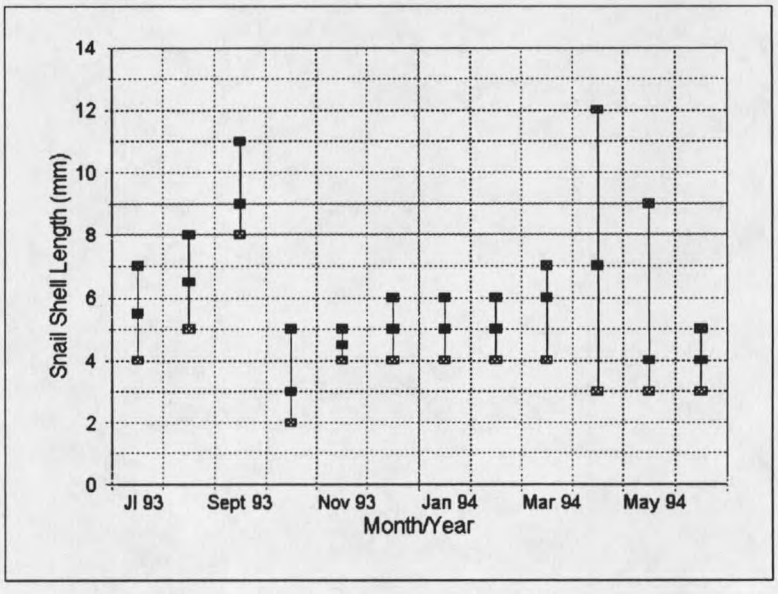

Fig 16. Maximum, minimum and average shell length of Lymnaea modicella collected at the New Biltmore Hot Spring from July 1993 to June 1994.

Beaverhead Warm Springs

Lymnaea obrussa was found during 10 months of the year. The number of snails collected per month ranged from a low of 3 in January to a high of 473 in July. The population size cycled 3 times during the year, reaching peaks in April, July and October (Fig 17). At least 2 generations of snails were produced during the year, as indicated by the presence of juvenile snails in January, March, April and May and July (Fig 18).

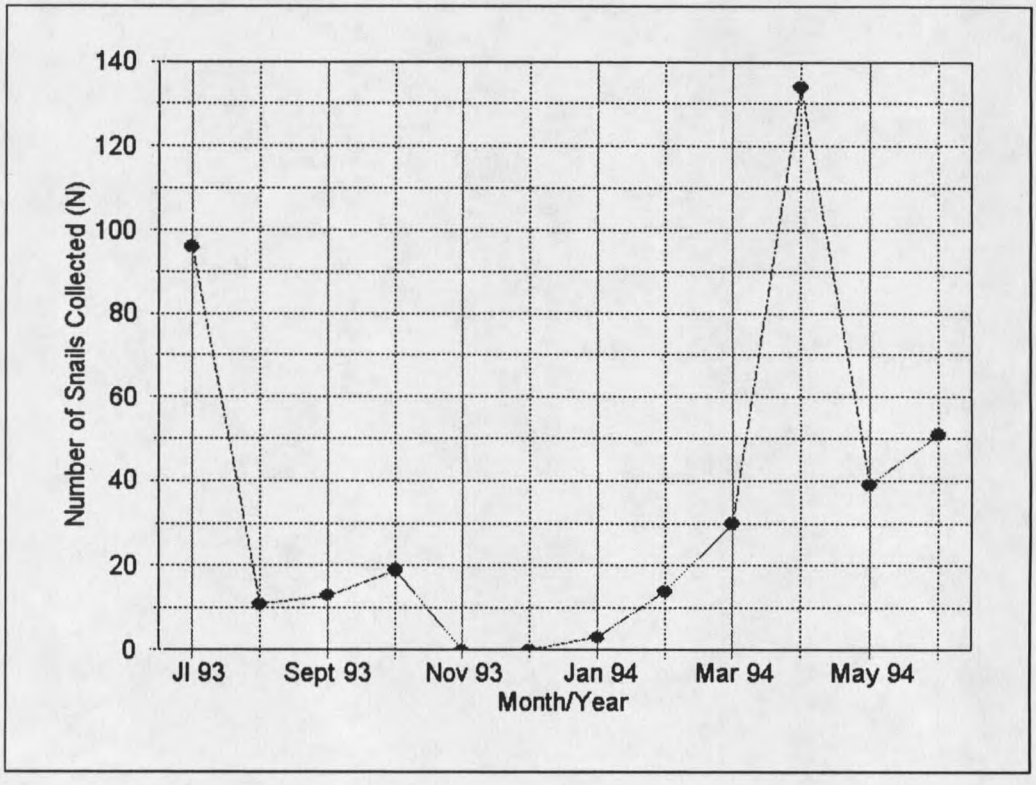

Fig 17. Number of Lymnaea obrussa collected each month from July 1993 to June 1994 at the Beaverhead Warm Springs.

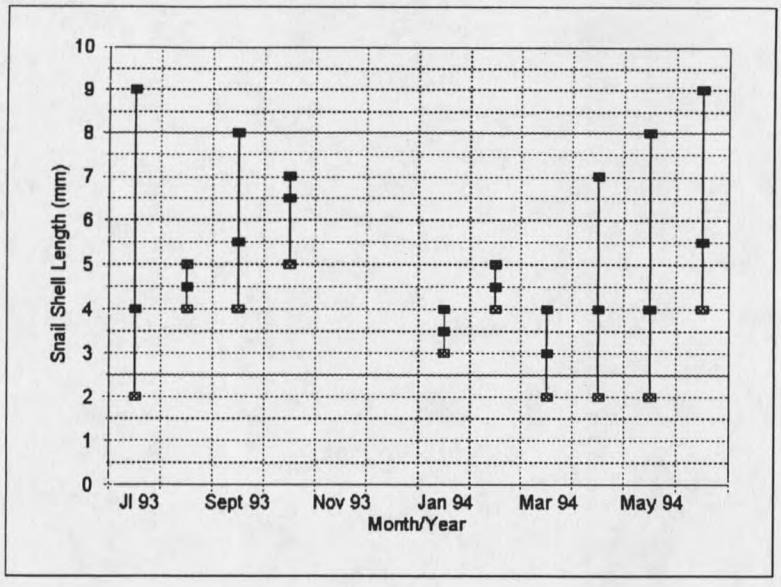

Fig 18. Maximum, minimum and average shell length of Lymnaea obrussa collected at the Beaverhead Warm Springs from July 1993 to June 1994.

Characterization of Thermal Habitats

Green Springs

Macroclimatological Conditions. Air temperature cycled seasonally reaching a maximum of 16.7 C in July and a minimum of -5.0 C in January. The mean annual precipitation was 43.2 cm (Caprio et al., 1990). Precipitation reached a maximum of 5.8 cm in June and a minimum of 2.5 cm in April and October. Air temperature and precipitation values for each month from July 1993 to June 1994 are presented in Figure 19.

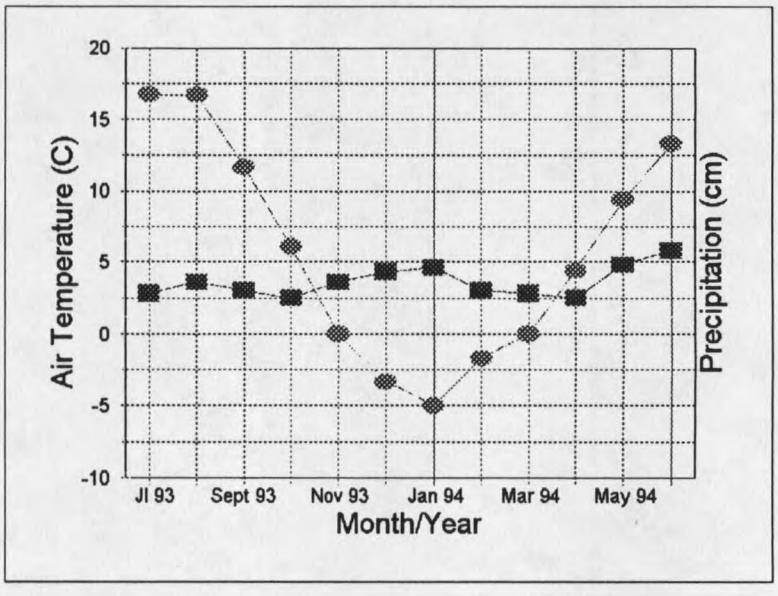

Fig 19. Air temperature and precipitation values reported for each month at the Green Springs from July 1993 to June 1994. (●), air temperature. (■), precipitation.

Microclimatological Conditions. The average water temperature of study sites 1-4 ranged from 27.1 C (±4.2) to 15.4 C (±8.3) (Fig 20). The water temperature cycled seasonally reaching a maximum of 31.8 C at site 1 in August and a minimum of 5.2 C at site 4 in November (Fig 21). The average electrical conductivity of the water ranged from 0.18 mS (±0.1) at sites 1-3 to 0.19 mS (±0.1) at site 4. Soil temperature cycled seasonally, similar to the water temperature, reaching a maximum of 16.6 C at site 1 in May and a minimum of -3.4 C at site 2 in February (Fig 22).

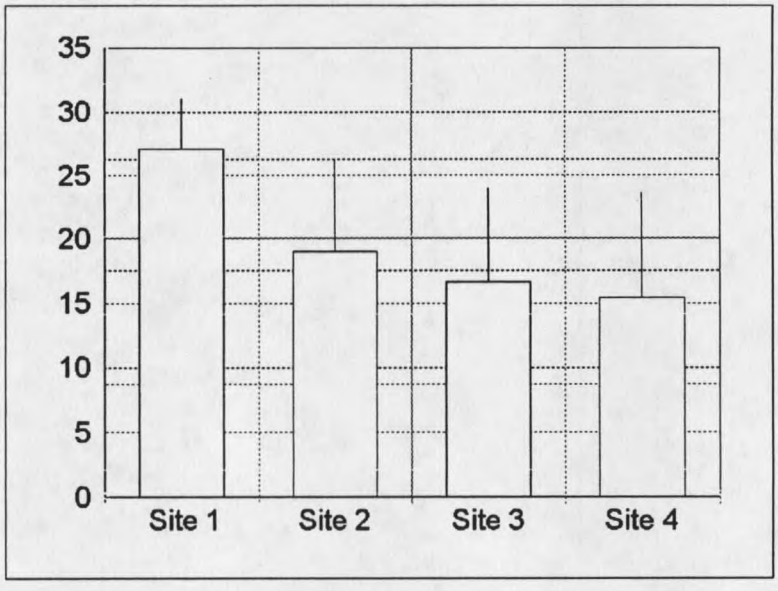

Fig 20. Average water temperature (± standard deviation) at sampling sites 1-4 from July 1993 to June 1994 at the Green Springs.

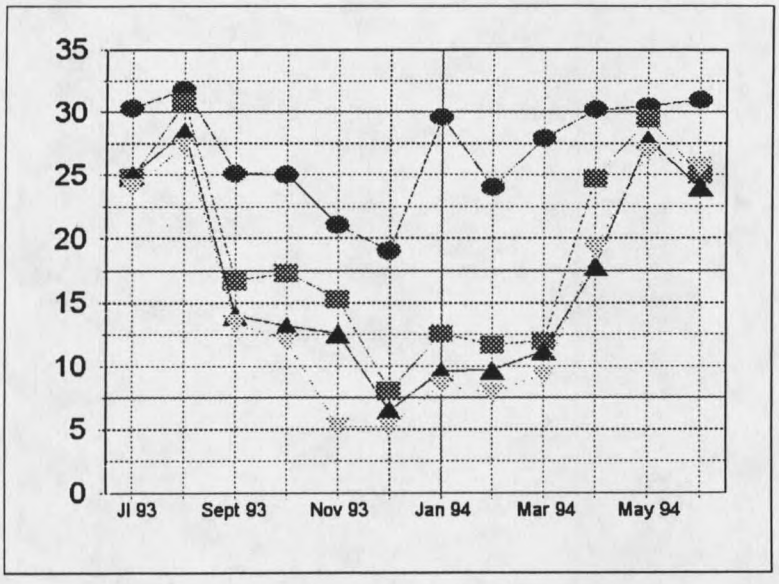

Fig 21. Water temperature at sampling sites 1-4 from July 1993 to June 1994 at the Green Springs. (●), site 1; (■), site 2; (▲), site 3; (▼), site 4.

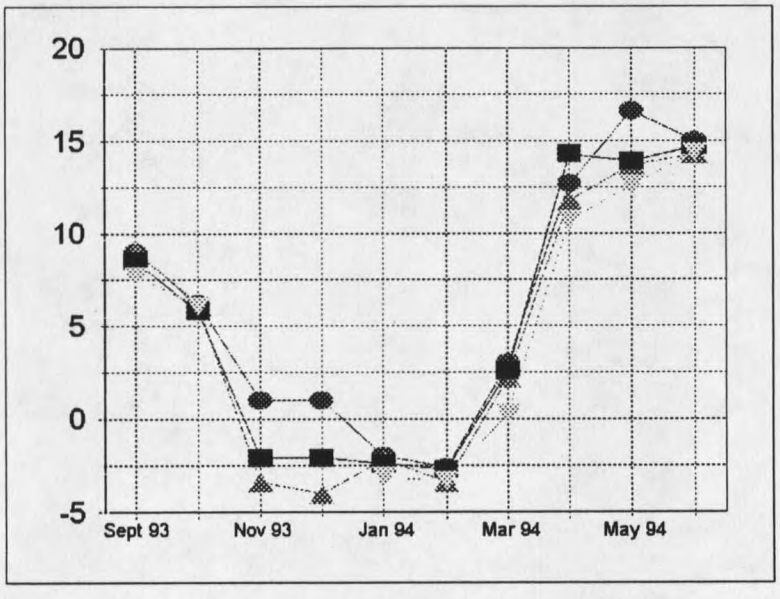

Fig 22. Soil temperature at sampling sites (1-4) from September 1993 to June 1994 at the Green Springs. (●), site 1. (▲), site 2; (■), site 3; (▼), site 4.