Age Determination of Individual Garter Snakes (Thamnophis spp.) Using Skeletochronology

by

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ABSTRACT

Individual and population–specific patterns of growth, and variations in these patterns, can be determined if age structure is known; this allows the dynamics of populations to be more accurately modelled and projected into the future. Besides contributing to our understanding of the fundamental ecological issue of limitation of distribution and abundance, such knowledge is critical to management or conservation plans for many species. Skeletochronology, the reading of growth rings in bony structures, has been used extensively to determine the ages of individual fish and, to a lesser extent, reptiles and amphibians. This study evaluates the use of skeletochronology to determine the ages of garter snakes (Thamnophis spp.) with emphasis on the development of techniques that allow the sampling of bone structures from live animals. Rings were observed in the caudal vertebrae of three species of garter snakes and were consistent in number within individual snakes. The validity of growth rings as indicators of age was established using snakes raised in the laboratory under differing hibernation regimes, and through recapture and resampling of snakes caught the previous year. The age structure of this population of garter snakes is discussed, and a preliminary life table based on age was constructed. Skeletochronology has the potential to be an important and useful technique for the study of age in snakes, but is very labour-intensive and best used as part of a large, long-term sampling project.

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INTRODUCTION

Determination of the age of individual animals is an important component of the study of populations, because demographic processes (e.g. fecundity and survivorship) are often age–specific. Knowledge of age structure can therefore yield essential data on the dynamics of the population and life history of the species as a whole (Parker and Plummer 1987). Individual and population–specific patterns of growth and maturation, and individual and year–specific variations in these patterns, can also be determined if age structure is known; this allows the dynamics of populations to be more accurately modelled and projected into the future. Besides contributing to our understanding of the fundamental ecological issue of limitation of distribution and abundance, such knowledge is critical to management or conservation plans for many species.

Accurate data on age have rarely been collected for populations of amphibians and reptiles, especially snakes. This has limited significantly our understanding of population dynamics and life histories of snakes, which may have unique ecological properties correlated with their unique morphology. The need to study population dynamics of snakes is made more urgent by the fact that 180 species or populations of snakes worldwide are considered rare, declining, or in need of assessment and management (Dodd 1993). For many species, most of the information available is anecdotal, and the largest problem facing conservation efforts is the lack of basic knowledge.

Reptiles generally show rapid growth to the time of first breeding, and from then on growth is slower. In those species that have been studied carefully, much variance is seen in the body size of individuals that have reached age of first breeding, and this variance is presumably maintained through the later age classes (Parker and Plummer 1987). Body size and fecundity usually are positively correlated, at least in females, so it is important to know if body size is a function of age or of early growth. Size–age relationships may not be constant in a species and can vary among populations (Halliday and Verrell 1988). The purpose of this study is to investigate the relationship between age and size in garter snakes, by using growth rings as a direct measure of age.

Age Determination:

In most animals, the age of individuals is difficult to establish with certainty. Indicators of age may vary with the age of the individual, and older age classes are usually more difficult to define. Therefore, techniques of age determination must be tested for validity for every age class in the population for each species examined, and possibly for each population or stock. The precision of sets of readings can be examined by comparing separate readings by an individual or by different readers. Accuracy of the age determination technique can be determined when a method is proved valid for all age–groups in the population, or an approximation of accuracy can be made by comparing several techniques; if all readings are similar then there is more confidence in the techniques, but if they are variable then the individual techniques must be validated (Chilton and Beamish 1982).

Halliday and Verrell (1988) list available methods for estimating the age of amphibians and reptiles. All methods have inherent problems and none are currently wholly reliable except mark–recapture; ideally, therefore, more than one method usually should be used in concert.

1) The recapture of marked individuals is potentially the most reliable method, especially if animals are marked when newly–hatched or at a known age. Advantages of this method include the production of precise data on age-specific growth over a specific time period and an accurate measure of variance in growth rates within age–classes. This method is disadvantageous because it is labour–intensive; a long time is required to obtain results and many animals need to be marked. It also might be virtually impossible in some cases to recapture animals while they are growing to adulthood.

2) The extrapolation of age from size–frequency data assumes that all size classes have an equal probability of capture and that all measurements are made at an instantaneous point in time or over a short time period. The age classes are assigned to a size–frequency distribution statistically or through visual inspection, and it is often not clear what age to assign to the smallest class. This method also assumes that age and size are related; this

may be the case for many reptiles but usually with very large variance.

3) The method of skeletochronology is based on the assumption that periodic growth (in the form of rings or lines) is recorded in the bones. The counting of annuli can be difficult because of factors such as variable numbers of lines due to variation among and within particular bones and/or among readings by workers. Annuli can be split, fused, or false, but researchers rarely explain how the final count was reached in a particular publication. Studies that lack known–age material cannot claim that the annuli change with age in a predictable manner. Lack of statistical analysis of the technique in a large number of papers has made it difficult to determine the accuracy of the regression equation obtained (Dapson 1980).

Skeletochronology:

Zug and Rand (1987) state that there are three major assumptions in the use of skeletochronology for aging any vertebrate: 1) regular cyclic growth occurs in the skeleton or elements (i.e. each cycle consists of an active growth period and a period of no or very reduced growth that leaves visible permanent markers in the bone); 2) growth markers represent a specific period of time in the life of the individual, so the total number of markers is a direct measure of age; 3) where markers are lost through bone remodelling (e.g. expansion of the marrow cavity can erase the first few rings in older individuals), the number lost can be estimated from the number and size of markers remaining. Griffiths (1962) pointed out that rings are found in tropical species, so could be questionable indicators of interrupted growth (although wet and dry seasons could affect growth). He also emphasized that lamellar counts can be different between different elements of one individual and at different levels of the same element, and bone elements are always undergoing reconstruction. However, as detailed below, the annular hypothesis has been tested and confirmed in some temperate zone frogs, lizards, turtles, and snakes.

Early students tended to assume automatically that the rings seen in the bones were annual in nature. Mattox (1936) studied transverse sections of femurs, mandibles and humeri from the turtle Chrysemys marginata and suggested that the observed rings are due to "temporary cessation of growth during hibernation causing heavy ossification of the bone tissue on the outside of the bone" and that the age of the individual can be read directly from the number of rings. Similarly, Bryuzgin (1939) found a series of parallel lines at right angles to the long axis of the maxillary end of the ectopterygoid in snakes. He also felt that these lines were due to alternating active and hibernating seasons and that one line equalled one year. However, he used length measurements of the snakes to standardize the counting of rings and did not differentiate between sexes. A few studies have concluded that growth rings are not useful for determining the age of reptiles. Griffiths (1962), using known–age snakes of Boiga dendrophila and Natrix natrix, concluded that the number of ectopterygoidal bands is not in simple relationship to the annual growth cycle, but the number and extent of each ring is a function of individual external and internal environments.

Hailey and Davies (1987) collected the ectopterygoids of the snake Natrix maura, and concluded that most of the rings seen were produced by periods of faster growth, not slow growth. They determined the relationship between the number of rings and length of the snakes and arrived at separate equations for males and females that predict annual growth rates and hatchling lengths. They did not verify that the rings were growth rings and seemed to base that conclusion on the paper by Peabody (1958), who assumed that the rings seen on Pituophis catenifer bones were annular.

There are many cases in which skeletochronology has been verified as a technique to measure age in amphibians and reptiles. Hemelaar and van Gelder (1980) demonstrated that a layer of bone was deposited each year in two populations of Bufo bufo by marking individuals and taking samples in successive years. Schroeder and Baskett (1968) validated the skeletochronology technique for Rana catesbeiana. Male Bufo bufo have been shown to have minimal resorption in the toes (Hemelaar 1981), which allows the age to be read directly from the rings. Breeding frogs show a reduction in growth rate compared to nonbreeding frogs of the same size (Ryser 1989); thus, age of first breeding can be determined by the closeness of the growth rings. Rana temporaria has also been shown to form only one growth ring and one resting line each year in the phalanges (Hemelaar 1981).

Turtles also have been studied for growth rings. The long bones of Testudo hermanni and T. graeca were examined by Castanet and Cheylan (1979) and the annual growth pattern was confirmed through known–age specimens and correlation between numbers of bone rings and rings on the shell. A calculation was developed to account for any resorption of juvenile rings. The annular nature of the bone rings has also been suggested, but not fully verified, for Chelydra serpentina (Hammer 1969).

Fewer species of lizards and snakes have been examined for growth rings than frogs and turtles. However, growth rings have been confirmed in a few cases. A population of the lizard Lacerta vivipara was studied by Pilorge and Castanet (1981) to establish a relationship between size and age. They validated the skeletochronological method using mark–recapture. Another study using known–age Lacerta lepida concluded that the rings in lacertilians are laid annually (Castanet 1978).

Minakami (1979) examined two species of Trimeresurus vipers and did in vivo staining of the bone rings on the vertebral centrum by lead–acetate injected into the dorsal muscle at certain times of the year. He determined that a ring on the centrum is laid down once a year between June and September and that the age of the snake can be determined by adding one year to the number of rings (as the first ring is laid down in the second year of life). He concluded that there is a relationship between the number of bone rings and the total body length (Minakami 1979). Study of Vipera aspis using known–age animals and fluorochrome labelling also demonstrated the relationship between skeletal growth marks and age (Castanet and Naulleau 1974).

Skeletochronology has been verified in several amphibian species in which toes can be removed in subsequent years without sacrifice of the animals. In the few studies in which this technique has been verified in snakes, the study animals were all sacrificed to obtain vertebrae or ectopterygoids. The establishment of a sampling technique that enables researchers to determine the age of a snake without killing it is sorely needed, both for ethical reasons and to allow us to study population dynamics simultaneously. For snakes, the only structure that can be removed without sacrificing the animal is part of the tail. Jayne and Bennett (1989) found that 33% of the tail of garter snakes can be removed without affecting the speed of the snakes, making this a feasible way of obtaining bone for determining ages of living snakes.

The specific objectives of this study are three-fold:

1) To establish presence of growth rings and to correlate their numbers among different bones in a preserved collection of the common garter snake (Thamnophis sirtalis) from Manitoba. In turn, this will allow a preliminary assessment of the age structure of this population.

2) To test, by laboratory experiment and by mark-recapture in the field, that growth rings are annual. The effect of other potentially influential factors on growth ring deposition also is tested.

3) To use skeletochronology to determine age-structure of a population of western terrestrial garter snakes (Thamnophis elegans) and to examine relationships between life-history parameters and age and size in this population.

METHODS

I used a sample of preserved specimens of Thamnophis sirtalis from the Interlake region of Manitoba to determine if growth rings could be seen in the bones of garter snakes. Snakes in this population spend at least six months in hibernation each year (Gregory 1977), so I thought they would be an ideal population in which to look for growth rings, and the sample of dead snakes, taken in the summer of 1993 at and around the den sites, was already available in the laboratory. These snakes were fixed in 10% formalin, and later rinsed with water and kept in 70% ethanol. A vertebra was taken from one of the snakes and prepared using typical histological techniques, outlined in detail below. Rings were observed in the bone, so each snake was measured and the sex determined, and a section of tail, a rib from the mid-body region, and an ectopterygoid (Fig. 1) was taken from each. This was done to compare the bones within each individual snake for uniformity in number of rings. The ectopterygoid was chosen as the skull element as it has been shown to provide the best sample for rings in previous studies (e.g. Hailey and Davies 1987); the tail vertebra was taken as the bone of choice in this study (for reasons outlined above), and the rib to provide a third point of comparison. Each snake was given an individual number that was used to label the bone structures and the slides, so it was impossible to tell from the number on the slide either the length or sex of the snake. The three structures were kept in one vial labelled with the identification number of the snake, and were processed together until they were embedded in paraffin in separate blocks. The second ectopterygoid was taken from one snake and cleaned of tissue, cleared in xylene, and examined through the microscope with both transmitted and reflected light. This was to determine if the growth rings could be observed using the technique described in older studies (e.g. Peabody 1961).

Study Site and Capture Methods:

The study site was at the Canadian Wildlife Service wildlife reserve at the mouth of the Little Qualicum River, just north of Qualicum, B.C (49°24' N, 124°37'W) (Fig. 2). The site consisted of several small fields, some overrun with rose bushes, series of hedgerows

Figure 1. Generalized colubrid upper skull showing location of ectopterygoid (after Cundall and Gans 1979).

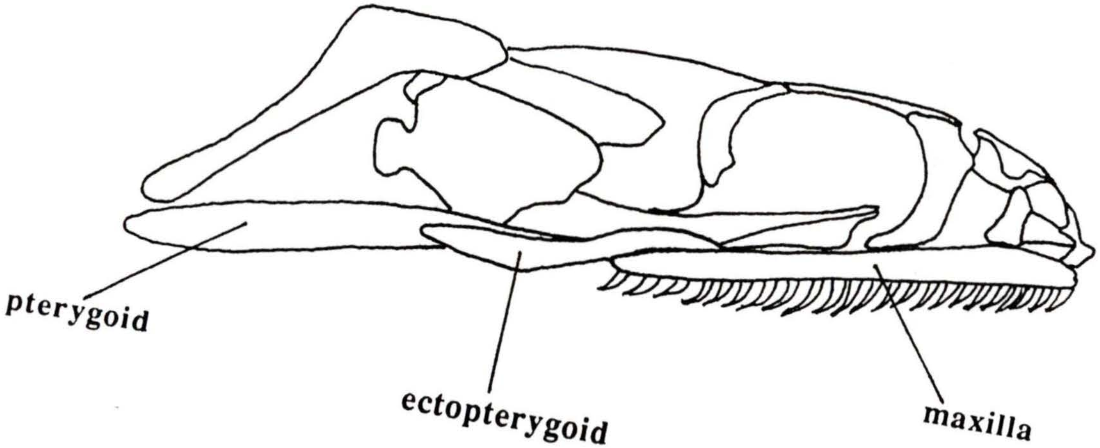
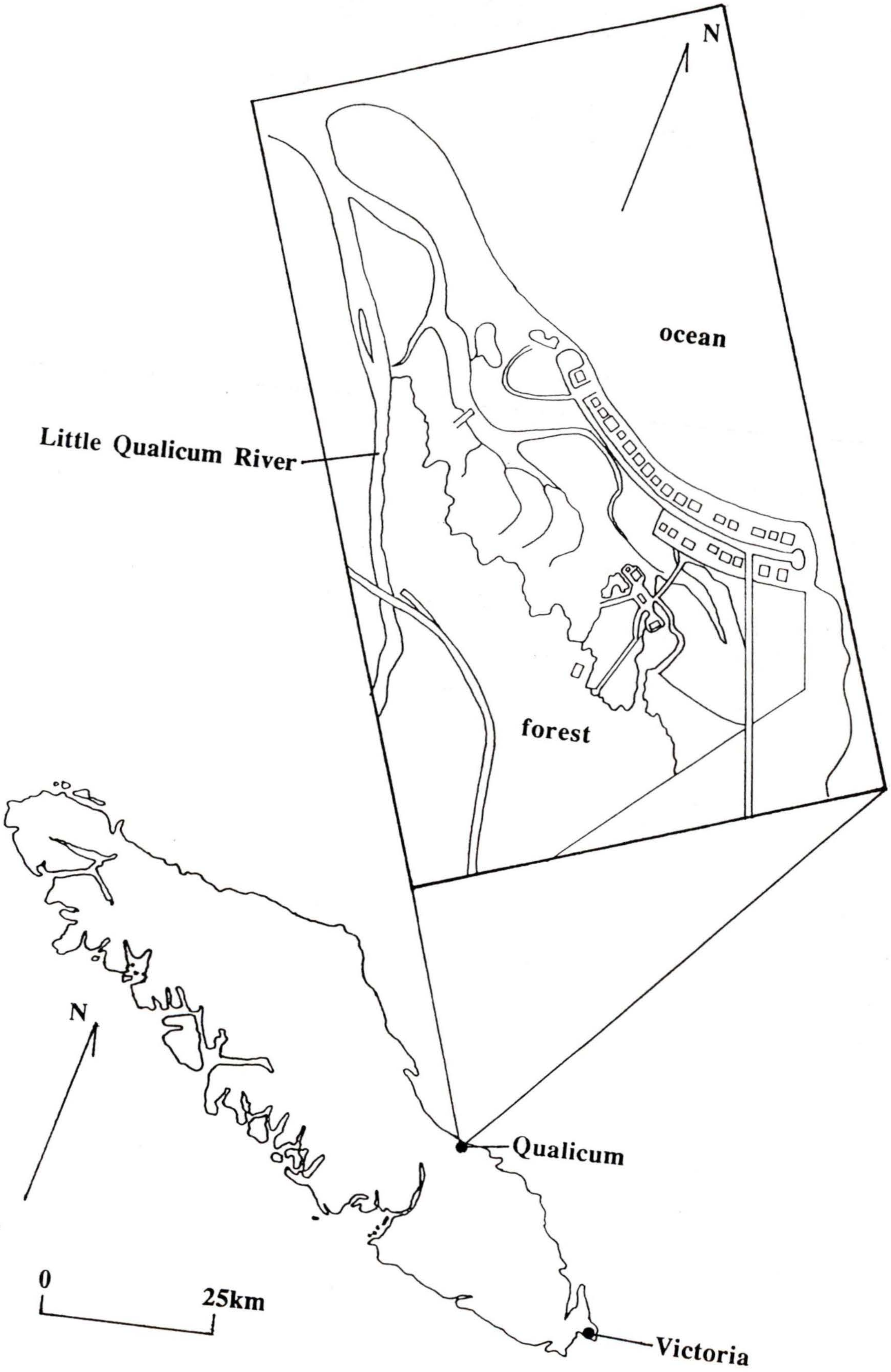

pterygoid
ectopterygoid
maxilla

Figure 2. Vancouver Island, British Columbia, showing location of study site. Inset is a map of the Little Qualicum River estuary Canadian Wildlife Service wildlife reserve (squares indicate houses, Highway 19 cuts across the lower left of the inset map).


N
ocean
Little Qualicum River
forest
N
Qualicum
0
25km
Victoria

separating the fields, a forested area running along Highway 19 to the south, a tidally-influenced marshy estuarine area, the mud and gravel banks of the river, and a stretch of sandy ocean beach with many logs and grasses above the tide line. Several old farm buildings still stand on the site; many snakes were observed emerging from and disappearing under them and basking along the bases of the walls. A gravel road runs through part of the field, leading past a caretaker's house to the office, a converted farmhouse. The area is fenced along the highway and McFeely Road to the east and along the beach boundary. Along the north edge of the reserve is a row of houses that back onto the fields and run from McFeely Road along Shoreline Road to a public beach access.

Thamnophis elegans and the northwestern garter snake, T. ordinoides, are known to hibernate in several areas around the estuary (N. Dawe, pers. comm.), including an old road bed beside the bridge over Little Qualicum River, an old road bed in the middle of the estuary, under the old farm buildings, and in a small pile of rocks beside the driveway to the last house, which is located on the reserve. In March 1995, I observed snakes apparently emerging from hibernation from the two old road beds, and from under the old building nearest to the office.

The first field season started at the end of March, 1994. I caught snakes by hand while walking around the site, paying particular attention to areas where snakes had been caught in numbers before. Only T. elegans and T. ordinoides were found; T. sirtalis is not common on this site, and none were found in the course of this study, although several have been seen in the past ten years (Neil Dawe, pers. comm.). Each snake was given an individual mark by clipping a unique combination of subcaudal scales. The snout-vent length (SVL) and total length were measured to the nearest 5 mm, the snake was weighed to the nearest gram with a Pesola$^{(R)}$ scale, and the sex, colouring, markings, scars, and general condition of the snake was noted. Finally, the distal portion of the tail was cut off, using scissors cleaned in 70% ethanol. The length of the clipped portion varied, depending on the snake's length and whether the original tail was whole or not. Not more than $^1/3$ of the tail was taken, and generally much less than that; on an adult snake, about 1.5 cm was removed from the tip of the tail. Several snakes were not sampled at all, as too much of the tail was already missing, but they were marked, weighed and measured. About 0.5 cm of the cut end of the piece of tail was cut off and placed in 10% formalin in a labelled glass vial, and the rest was placed in a cryovial and stored in liquid nitrogen for DNA analysis in another project. During the second field season, any snakes that had been marked in 1994 and recaptured in 1995 were resampled, to determine if one more ring had been added. Any dead snakes found on the site were kept and the ectopterygoid, rib, and tail vertebrae sampled to compare the number of rings among bones within individuals. The sex of some of the snakes caught at the start of the 1994 season was determined incorrectly through improper visual sexing techniques. These snakes were placed in the "unknown" category until they were recaptured and the sex verified using a probe.

Histological Techniques:

Samples were allowed to sit in 10% formalin for at least 24 hours, then soaked in water for several hours and placed in 70% ethanol until further processing. To prepare the bones for sectioning, I removed the skin from the pieces of tail, and decalcified the bone in 5% nitric acid for between 5 and 8 hours. A test series was done to determine the optimal time for decalcification; samples left for 3, 7, and 10 hours in nitric acid showed little difference, although the 7- and 10-hour samples were slightly better than the 3-hour sample (the rings stained somewhat darker). I rinsed the bones in tap water and placed them in 70% ethanol for 24 hours, with at least one change of solution. They were then moved through the following series, each sample in its own labelled vial:

-95% ethanol for 24 hours (at least one change)
-100% alcohol for 30 hours (at least one change)
-xylene for 12 hours (at least one change)
-paraffin for 3 hours (one change)

I embedded each bone in fresh paraffin in a labelled paper boat that was then floated in cool water until the paraffin was set. I gave the samples at least a day to harden before they were mounted on blocks and sectioned with a AO Spencer "820" microtome. Sections of

10μ thickness were made and placed on slides coated with albumin. I made two slides for each sample, each filled with as many sections as possible (about 20 to 50 sections per slide), but I stained only one slide (the other was kept in case the first staining did not work). The initial staining procedure followed this protocol:

-3 minutes each in 2 changes of xylene
-3 mins in 100% alcohol
-3 mins in 95% ethanol
-3 mins in 70% ethanol
-5 mins in running tap water
-3 mins in Delafield's hematoxylin
-3 mins in running tap water
-1 min in eosin
-quick dips in 70% and 95% ethanol
-3 mins each in 2 changes of 100% alcohol
-3 mins in 100%:xylene (in 1:1 solution)
-3 mins each in 2 changes of xylene
-covered with Permount (R) mounting medium and slidecover applied.

The slides were allowed to dry in the fume hood for about two days, then examined with a light microscope. I switched to an alternative staining technique about half-way through the samples, which went as follows:

-4 mins in xylene
-3 mins in xylene
-3 mins each in 2 changes of 100% alcohol
-3 mins 95% ethanol
-30 secs in 1% toludine blue in 1% sodium borate
-quick dip in distilled water
-quick dip in 95% ethanol
-quick dip in 100% alcohol
-1 min 100% alcohol

-2 mins each in 2 changes xylene

-mounted with Permount $^{(R)}$

This technique produced much clearer rings in the bone, although the stain intensity often varied within each individual slide.

Hibernation Experiment:

Gravid female T. elegans and T. ordinoides were collected in July 1994 and brought back to the laboratory (10 T. ordinoides and 16 T. elegans). After giving birth in the laboratory, they were released in early September 1994; the neonates were kept for the hibernation experiment. The 53 T. ordinoides neonates were released October 10, 1994 as they did not start to feed and it was feared that all would die over the winter. The remaining T. elegans neonates were divided into four categories:

1) In hibernation the entire winter (November 25, 1994 to April 11, 1995)
2) Out of hibernation and fed *ad libitum*
3) Out of hibernation and fed 2 small guppies/feeding
4) In hibernation November 25, 1994 to January 25, 1995, taken out of hibernation and fed *ad libitum* until February 28, 1995, back in hibernation until April 25, 1995.

These categories were established to determine if one ring is laid down for each hibernation period, and whether low amounts of food will also produce a ring (i.e. due to reduced growth). Out of 104 T. elegans neonates born in the laboratory, 32 survived to the start of the experiment, and 22 of those survived until August 2, 1995, when they were released.

One method used to validate age determination techniques is the application of oxytetracycline (OTC) to the animal to mark the bone. The date at which that part of the bone was laid down is then clearly marked and the number of "growth rings" compared to the amount of time that has passed. This technique has been used on many species of fish to verify growth rings in the bone, fin rays, and otoliths (Chilton and Beamish 1982). Oxytetracycline is an antibiotic used to treat conditions such as bacterial septicaemia and

Salmonella in reptiles (Cooper 1981). Tetracyclines are generally thought to be safe for reptiles. The suggested dose is 6–10 mg/kg daily intramuscularly or intravenously, or 6–12 mg/kg daily orally (Holt 1981); however, OTC has been given orally to turtles and tortoises at 50–100 mg/kg daily for 1 week to treat Vibrio (Jacobson 1988). Injections are thought to be more effective than oral dosages for reptiles as they tend to not eat or drink regularly, and absorption from the gastrointestinal tract can be erratic. Therefore, 50 mg/kg OTC injected intramuscularly just before hibernation was the treatment used in this study. These snakes were injected on November 15, 1994, with Liquamycin* - LP$^{(R)}$, an oxytetracycline hydrochloride injectable solution (100 mg/mL oxytetracycline hydrochloride in an aqueous solution for intravenous or intramuscular administration). The solution was injected into the tail about halfway down its length, and no mortalities occurred directly after injection. Known-age adult T. elegans (n=10) in the collection were similarly injected with OTC to look at results in older snakes. The first bone sample was taken November 21, 1994, and the snakes placed into hibernation soon after. The neonates in the hibernation experiment were resampled (by removing the tip of the tail, as in field-caught snakes) on May 11 and 21, and the adults were taken out of hibernation on May 30 and resampled on June 28. Two samples were taken from each snake both before and after hibernation; one for the staining protocol, and the other to observe the OTC.

To observe the OTC label, I air dried samples of bone in open vials, cut them into smaller pieces (about 1 or 2 vertebra(e) long) and attached them to a glass slide with Krazy Glue$^{(R)}$. Each slide was labelled with the identifying number of the snake. The number of pieces per snake varied depending on the length of the original sample and how easily each piece could be mounted in the correct orientation. Once the glue had dried, I ground down the bones using a grinding stone attached to a Craftsman $^{(R)}$ rotary tool to create a smooth flat surface exposing a cross-section of the middle of the vertebra. These sections were then viewed under ultraviolet light (360 nm wavelength) using a Zeiss UV filter set 02 and IIIRS epifluorescence condenser mounted on a compound microscope; the OTC glowed bright yellow (about 550 nm wavelength) against the faint blue background of the natural fluorescence of bone.

Counting the Rings:

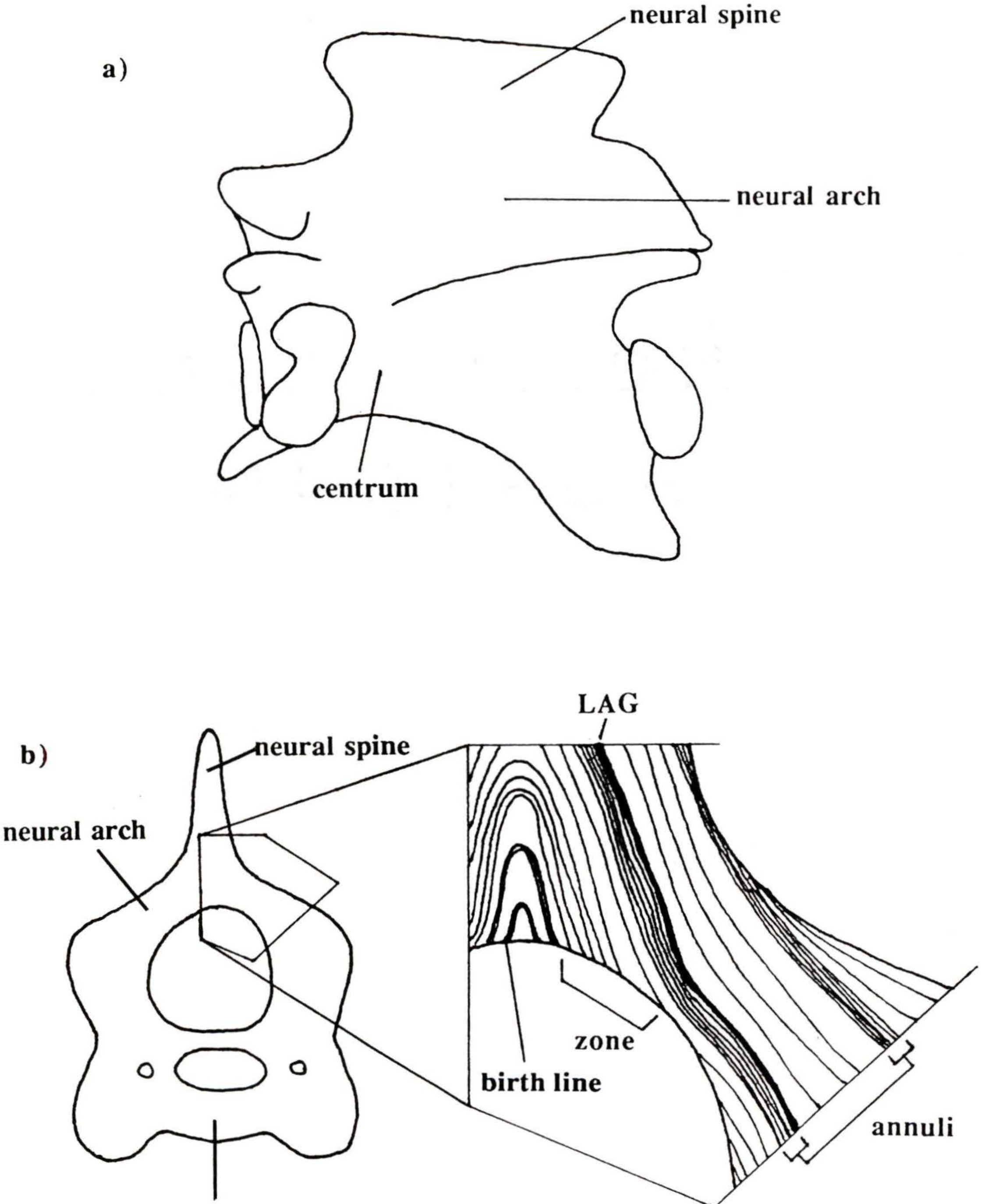
Castanet et al. (1993) define three kinds of growth marks: opaque "zones", translucent "annuli", and lines of arrested growth (LAG). The zones correspond to periods of active bone growth and consist of woven-fibered bone in juveniles and parallel-fibered bone matrix in slower-growing adults. Annuli mark periods of slow bone growth, and so are narrower than zones. They are composed of lamellar bone matrix. LAG are also known as "rest lines" and are very thin and stain very dark. They generally appear within annuli or bordering them. Both the annuli and the LAG stain dark blue with the toludine blue stain, but the LAG show up as dark, sharp lines and are usually quite distinct from typical annuli.

I viewed each stained slide with a compound microscope under 100X power. Each section on a slide was scanned and the number of annuli counted in several areas of the bone sections. The dark bands, representing hibernation events, were counted at the areas where they were most distinct, usually along the neural arch or the centrum (Fig. 3). The outer edge of the bone was counted as a ring if the snake was sampled in early spring, as was the case with the T. sirtalis from Manitoba. I tried to find an inner edge along the centrum of the vertebra where the first ring could be seen; in many cases the rest line from birth (Castanet and Smirina 1990) could be identified as a dark ring, similar to a LAG, at the inner edge along the central cavity and projecting into the neural spine.

I recorded the "ages" as the number of annuli counted. As the snakes are born in the late summer, they do not have a full growing season before the first annulus is formed; therefore, a snake with 1 annulus could be between 6 and 16 months old, depending on what time of year the sample was taken. According to Castanet et al. (1993), a zone plus an annulus indicates a complete growth cycle; in this case, I counted each annulus plus the zone immediately preceding it (counting from the inside to the edge) as one growth cycle.

I read 25 samples without knowing the month of capture of the snake, then reread the

Figure 3. a) Left lateral view of a generalized colubrid vertebra; b) Cross-section through the centre of a vertebra; inset is a diagram of a zone, annuli, and a LAG.


a)
neural spine
neural arch
centrum
b)
neural spine
neural arch
centrum
LAG
zone
birth line
annuli

slides with that information. The second count varied from the first only for snakes sampled at the start or end of the season, when the edge could contain a ring or not. I felt that knowing the month that the snake was sampled increased the accuracy of the reading, so subsequent readings were done with that information in front of me.

The number of rings counted in the vertebrae, ectopterygoids, and ribs of the T. sirtalis and the dead T. elegans and T. ordinoides were compared within individuals to establish whether the number of rings was constant throughout an individual. I later discarded the counts from the ribs, as they generally gave very poor results, if any rings could be observed at all. The samples from recaptured snakes were compared to the 1994 samples of the same snakes and the samples from the snakes in the hibernation experiment were compared before and after in an effort to validate the rings. The growth rate of the age classes of the recaptured snakes was compared, combining T. elegans and T. ordinoides males and females to increase the sample size. The 1994 age was assumed to be correct, and the second age obtained by adding 1 to the first age for this comparison, as it is generally found that younger age classes are easier to age than older ones (Beamish and McFarlane 1983).

I selected 20 samples at random and gave them to a second reader to determine reader bias. The second reader had no experience reading growth rings in reptiles, and was given slides of known-age individuals to establish criteria with. This subsample of slides was then re-read by me to test repeatability of readings.

The estimated age of each T. elegans was plotted against SVL to examine the relationship, if any, between age and size, and the age structure of males and females of the population were compared with the size structures. The SVL used for individuals recaptured within the same year was the initial length measurement. The correlation coefficient, r, of each regression was compared to the critical value for level of significance (Zar 1984).

Life tables based on age classes were constructed for females. The 1995 ages were used as calculated to compare to SVL, but each was reduced by 1 and added to the 1994 ages to construct the age-frequency histograms and life tables. For the life table, I calculated the average litter size for the T. elegans that gave birth in the laboratory (as there was no relationship between their age and litter size) and multiplied that by the number of females that were gravid when caught in 1994 to give the number of age 0 animals. This number was divided by 2 (for an assumed 1:1 sex ratio) and used as the 0 age class for the smoothing of the age frequencies. The frequencies in each age class were smoothed using log-polynomials (Caughley 1977). I felt that this was a valid procedure for constructing the life tables, as the actual number of 0 age class individuals caught can not possibly reflect the actual number of neonates in the field in the late summer; neonate snakes are usually underrepresented in population studies (Parker and Plummer 1987) and this appears to be the case with these data. The average litter size was also used to calculate fecundity (b_x) and the projected rate of change in population size under stable conditions (R_o), but was only applied to age classes 3 to 5. Survivorship (l_x) was calculated as a proportion from the difference between the number of individuals in one age class and the number in the next age class, d_x was the number dying during the interval, and the finite rate of mortality (q_x) was calculated as the number dying within an age interval divided by the number alive at the start of that interval; the equation for the finite rate of survival (p_x) was $1-q_x$ for each age interval (Caughley 1977).

The T. ordinoides samples were put aside, as there were not enough collected to construct life tables for this species. The snakes of unknown sex could not be assigned to either the male or female sample, so they were left out of the analyses, but included in the age frequency graph to show that this group is not made up of a narrow group of individuals (e.g. all neonates or all very large snakes).

Statistical Analysis:

The population size of T. elegans and T. ordinoides was estimated using the Petersen index (Caughley 1977). The "first sample was all snakes caught in 1994, and the "recapture" sample was all snakes caught in 1995. The assumptions that must be met for this estimate are:

1) Equal catchability for all individuals. The assumption of equal susceptibility to capture was definitely not met; I found that certain snakes (e.g. large gravid females) were much easier to find and catch than others (e.g. neonates).

2) No birth or immigration into the study area between marking and recapture. There was probably minimal immigration into the study site, as the area is bounded by houses and roads, reducing the chance of a snake from another population wandering into the site. However, the dates that I sampled included the 1994 and 1995 birth season (late July to mid-August).

3) Marked and unmarked individuals have the same mortality or emigration rate. Marking snakes by clipping subcaudal scales is not known to cause differential mortality in a population.

4) Marks are not lost. I found that the marks on recaptured snakes were generally clear and easy to read.

Although the main assumptions for the Petersen estimate were not met, I used this calculation because it accommodated the type of data that I collected the best. Other estimates are more constrained than Petersen's or require a specific number or timing of marking and recapturing occasions (Caughley 1977), while I marked and recaptured constantly throughout the season.

The difference between the T. sirtalis tail and ectopterygoid "ages" within individuals was tested for significant variation from 0 using a two-tailed paired sample t-test (α=0.05). Age-length regressions were calculated for males and females using both tail and ectopterygoid values. The length-mass regressions for male and female T. elegans were compared using Student's t-test (α=0.05). The number of rings in the recaptured T. elegans and T. ordinoides were compared between years using a two-tailed paired sample t-test (α=0.05); the difference between the number of rings counted for each individual in

1995 and 1994 was examined for significant difference from 1 (Zar 1984).

Many of the papers that discuss ways to calculate between-reader bias and precision in age determination are concerned with comparing readings between structures or between two or more independent readers. They typically calculate an index, which is then compared to the index obtained by another reader or structure (e.g. Beamish and Fournier 1981, Chang 1982). As I did not have an index to which to compare my results, and I was less concerned about which reader was more precise than whether two readers arrived at the same counts, I applied the paired-sample two-tailed t-test (α=0.05) to the number of annuli counted by the two readers, and to the samples that I re-read (Zar 1984).

RESULTS

Validation Within Individuals:

I examined a total of 41 T. sirtalis (9 females and 32 males) for constancy in growth rings within individual snakes. Tail vertebrae were read for each snake, but only 37 ectopterygoids were read, as the others were either lost in processing or did not have any readable rings. Rings were observed in the flattened end of the ectopterygoid that was only cleared with xylene, but this method did not seem easily applied to vertebrae, so the sectioning and staining technique was followed for the rest of the samples.

The annuli appeared to be consistent between structures within any given individual; even if the exact number of rings was difficult to determine, dark rings or patterns of rings could be followed throughout the sections of each structure and between the structures (Figs. 4 and 5). The difference between the number of rings in the ectopterygoids and in the corresponding tail vertebrae did differ significantly from 0 ($0.05>P(t_{0.05(2)36}\geq 2.3909)>0.02$). The majority of individuals showed no difference in the number of rings between structures (Fig. 6), and the differences that did occur ranged from -2 (the ectopterygoid had 2 more annuli than the vertebra) to 3 (the tail had 3 more annuli than the corresponding ectopterygoid). Overall, when there was a difference in number of annuli, the vertebrae tended to show more rings than the ectopterygoid. The majority of the males had the same number of annuli in the vertebra and the ectopterygoid, while half of the female snakes had more vertebral annuli.

I compared the SVL of each snake to the number of rings in its vertebra and ectopterygoid. The simple linear regressions of the relationship between number of annuli and size for males and females all had very low correlation coefficients, indicating the straight-line relationship is not strong (Fig. 7) (male tail annuli vs. length $P(r_{0.05(2)28}\geq 0.037)>0.5$; female tail annuli vs. length $0.2>P(r_{0.05(2)7}\geq 0.562)>0.1$; male ectopterygoid annuli vs. length $0.1>P(r_{0.05(2)28}>0.317)>0.05$; female ectopterygoid annuli vs. length

Figure 4. Cross-section of T. sirtalis vertebra, stained with hematoxylin and eosin (200x). Arrows mark the annuli.

Figure 5. Cross-section of T. sirtalis ectopterygoid, from the same snake shown in Figure 4, stained with hematoxylin and eosin (400x). Annuli are marked with arrows.

Figure 6. The frequency of differences between the number of annuli counted in tail vertebrae and in ectopterygoids within individual Manitoba T. sirtalis (number of annuli in vertebra minus number in ectopterygoid).

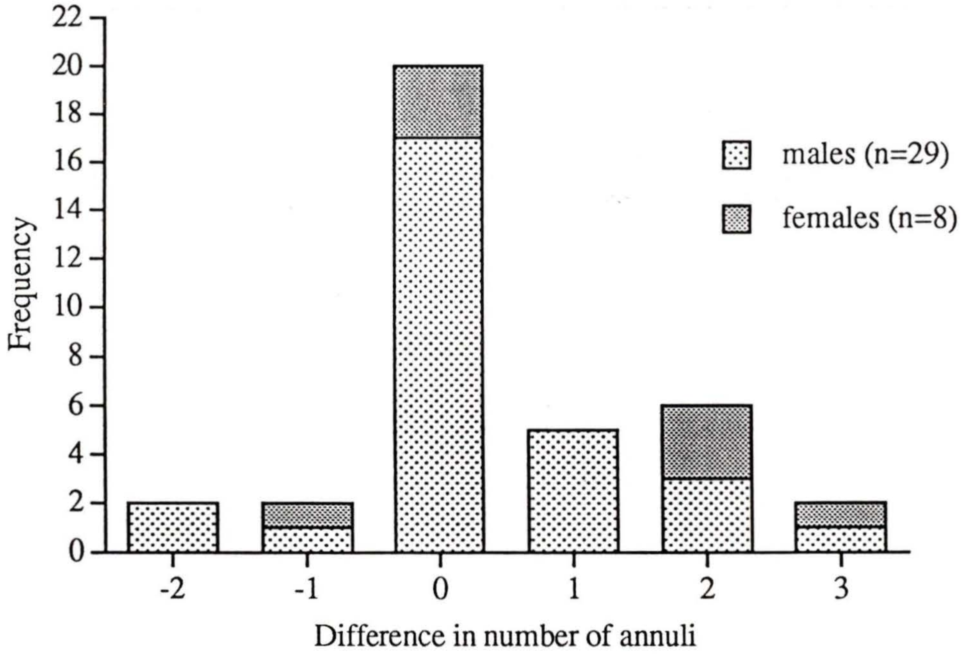
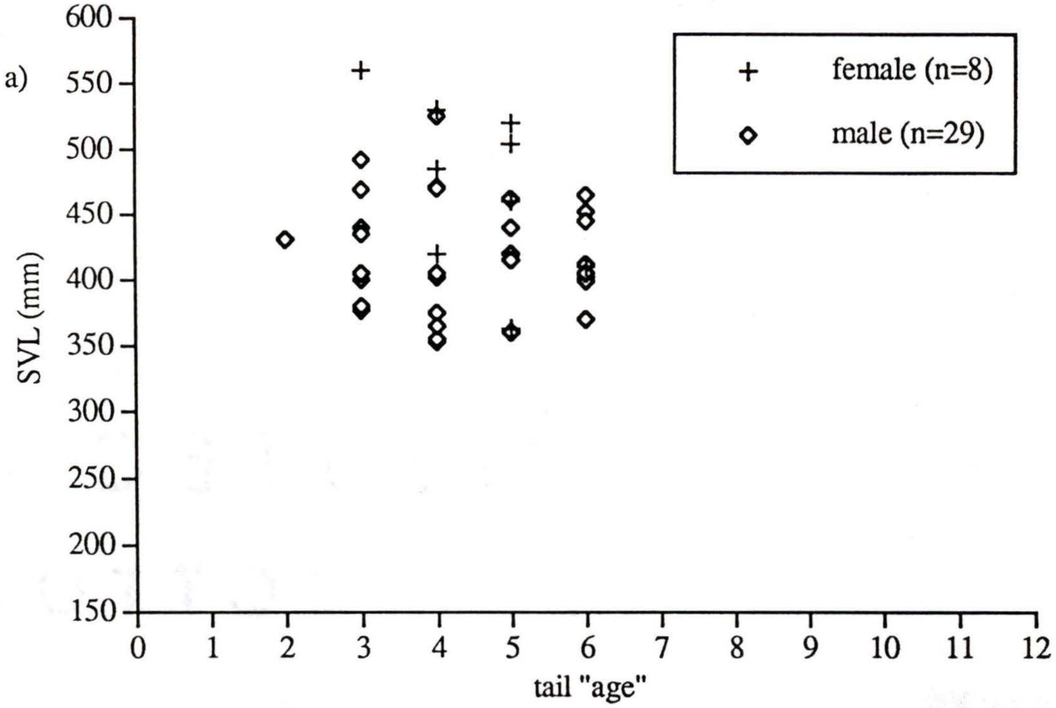
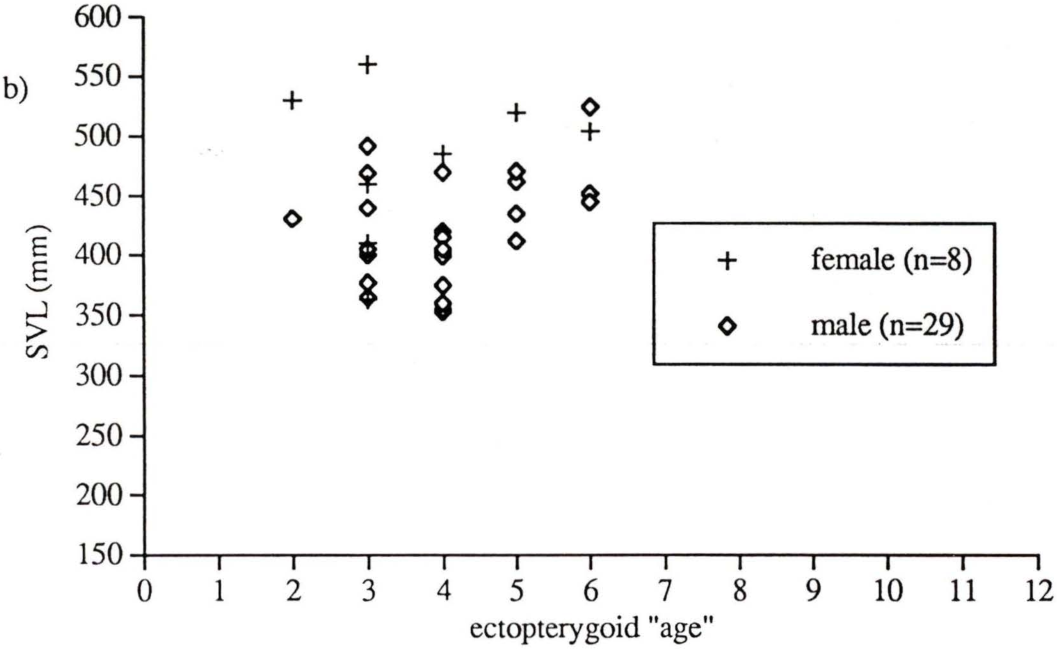

22
20
18
16
14
12
10
8
6
4
2
0
Frequency
-2
-1
0
1
2
3
Difference in number of annuli
males (n=29)
females (n=8)

Figure 7. T. sirtalis age and snout-vent length relationships a) snout-vent length compared to number of annuli in the vertebra; b) snout-vent length compare to number of annuli in the ectopterygoid.


a)
SVL (mm)
600
550
500
450
400
350
300
250
200
150
0
1
2
3
4
5
6
7
8
9
10
11
12
tail "age"
female (n=8)
male (n=29)


b)
SVL (mm)
600
550
500
450
400
350
300
250
200
150
0
1
2
3
4
5
6
7
8
9
10
11
12
ectopterygoid "age"
female (n=8)
male (n=29)

$P(r_{0.05(2)7} \geq 0.187) > 0.5$). The average SVL for the T. sirtalis age groups decreases with increasing age, but the 95% confidence limits show much overlap between males and females (Fig. 8).

Field Samples:

I collected a total of 210 T. elegans and 119 T. ordinoides from April 29 to October 10, 1994 and March 25 to September 24, 1995 (Table 1). Tail samples were not taken from 3 female T. elegans, as most of or all of the tail was already missing. More T. elegans were missing part of the tail upon capture than T. ordinoides (Table 1), and T. elegans females had the highest rate of tail loss overall. At the end of the 1995 season, 17 T. elegans and 18 T. ordinoides remained in the "unknown" category, and could not be added to the analyses of male and female SVL and age.

Only 1 female T. elegans, 3 female and 1 male T. ordinoides were found dead, but the annuli appeared to be consistent between the structures examined for each. I noticed that the outermost annulus progressed away from the edge of the vertebrae as the samples were taken later in the season (i.e. samples taken in early spring had the last annulus along the edge of the vertebra, whereas those taken in late summer had space between the last annulus and the edge of the vertebra).

I recaptured 18 snakes in 1995 that were originally sampled in 1994 (Table 1). The difference between the two ages for each snake was not significantly different from 1 ($0.2 < P(t_{0.05(2)17} \geq 0.8997) < 0.5$). Again, the patterns of the annuli could be followed from the 1994 sample to the 1995 sample, even when the actual number of annuli was difficult to count (Fig. 9). Three of the 1995 samples had a dark LAG just inside or along the last annulus, and a similar phenomenon was noted for 3 of the adult T. elegans in the hibernation experiment below, possibly a result of the trauma of the first sampling of the tail. The difference in SVL between the recaptures, comparing the SVL at first capture in 1994 with that at first capture in 1995, show that growth rate within the age classes

Figure 8. T. sirtalis average snout-vent length for each age class, with 95% confidence limits.

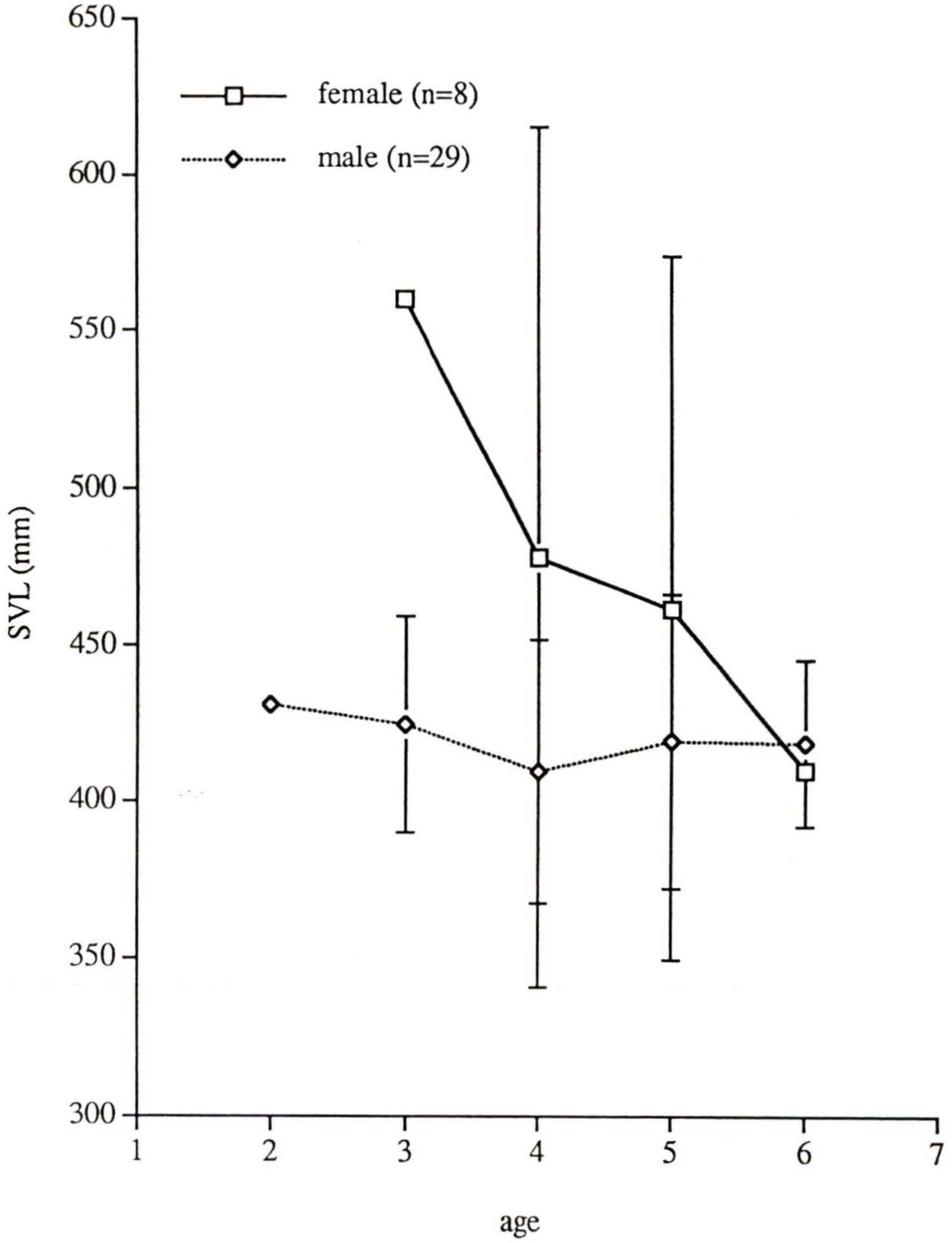
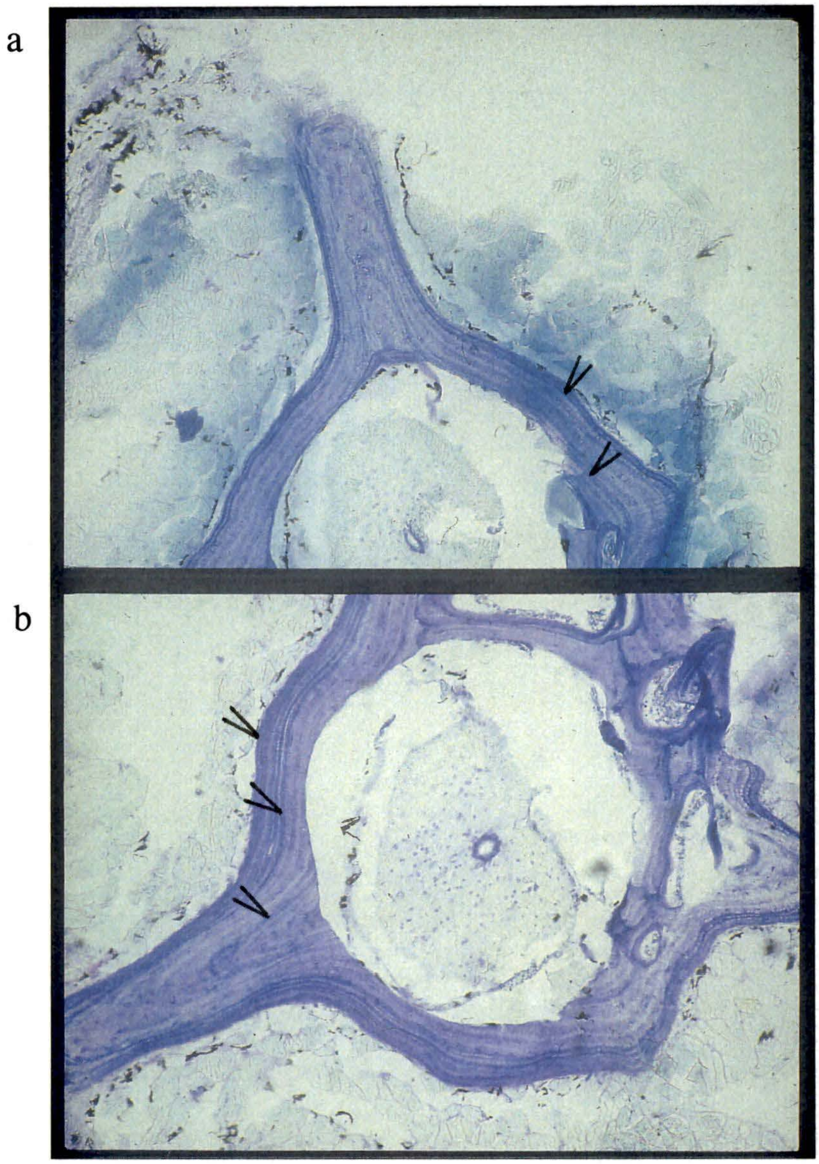

650
600
550
500
450
400
350
300
SVL (mm)
female (n=8)
male (n=29)
1
2
3
4
5
6
7
age

Table 1. Summary statistics for T. elegans and T. ordinoides caught at Qualicum, B.C. in 1994 and 1995. Tail loss was recorded before initial tail sample was taken, and the population size was estimated using the total number of inidividuals in each species.

	Thamnophis elegans				Thamnophis ordinoides			
	female	male	unknown	total	female	male	unknown	total
number caught	111	82	17	210	56	45	18	119
sex ratio (f/m)				1.35				1.24
mean SVL (mm)	510	421	419		373	325	300	
st. dev.	117	75	102		106	71	111	
tail loss (% of total captures)	12.61%	9.76%	5.88%	10.95%	5.36%	8.89%	0	5.88%
recaptures (1994 to 1995)	8	4		12	4	2		6
est. population size				902.31				457.71
SE				221.95				145.24

Figure 9. Cross-sections of vertebrae taken from a recaptured female T. elegans; a) sample taken in 1994; b) sample taken in 1995. Stained with toludine blue (75x). Annuli are marked with arrows; outermost arrow in b) shows the annulus formed over the winter of 1994-1995.


a
b

generally decreases with increasing age (Fig. 10). The T. ordinoides samples appear to have more growth between captures than the T. elegans, although there are two samples (one of each species) that are the same size at the same age (5 to 6, both at about 400 mm SVL at age 5) that have the same difference in size. Some of the variability seen in this figure is due to the time of year of capture (e.g. the snake that did not grow at all between age 3 and 4 was sampled in September 1994 and resampled in April 1995). The main difference is between the age 4 to 5 snakes and the age 5 to 6 snakes.

Very few snakes less than 300 mm SVL were caught; the majority of males were in the 325 - 500 mm SVL range (Fig. 11) and most of the females were between 400 - 700 mm SVL (Fig. 12). More snakes were caught at the beginning of spring to early summer than later in the season; in 1995 most of the males were caught in March and April near the den sites. The connections between the recapture data points shows the amount of growth that occurred between captures. Some of the steeper lines in 1994 are probably due to changes in measuring technique; as I gained experience I tended to stretch the snakes more when I measured them. Similarly, recapture lengths that were smaller than the original lengths were discarded as being obviously incorrect. Overall, there is very little growth in the larger snakes (over 400 mm SVL), although much variation can be seen between individuals; the few small snakes that were recaptured showed a comparatively high rate of growth.

I combined the 1994 and 1995 length measurements to produce the size-frequency histograms in Fig. 13 because the rate of growth between the start of one season and the end of the same season would be more than that between the end of the first season and the start of the second. Females attain greater lengths than males; many of the larger females were over 600 mm SVL whereas none of the males reached 600 mm. The two small clumps of individuals at 170 mm and 230 mm could indicate neonates and 1-year olds (one hibernation), but any older putative age classes are impossible to define from this data. The smallest gravid female was 470 mm SVL, and there were gravid females in every size class above that.

Figure 10. Changes in snout-vent length between captures according to age group for T. elegans and T. ordinoides. The length measurements used were those taken at first capture in 1994 and first recapture in 1995.

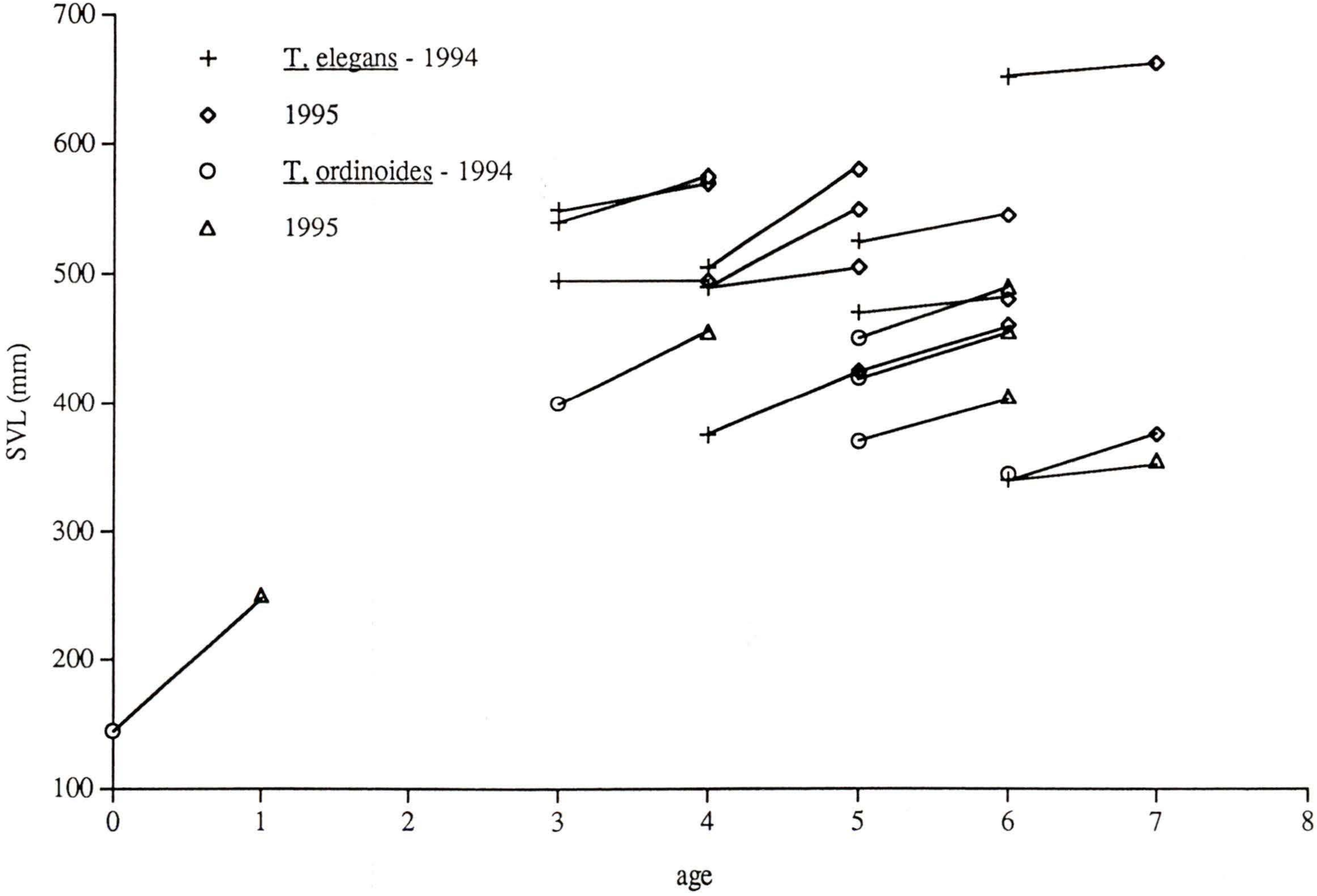
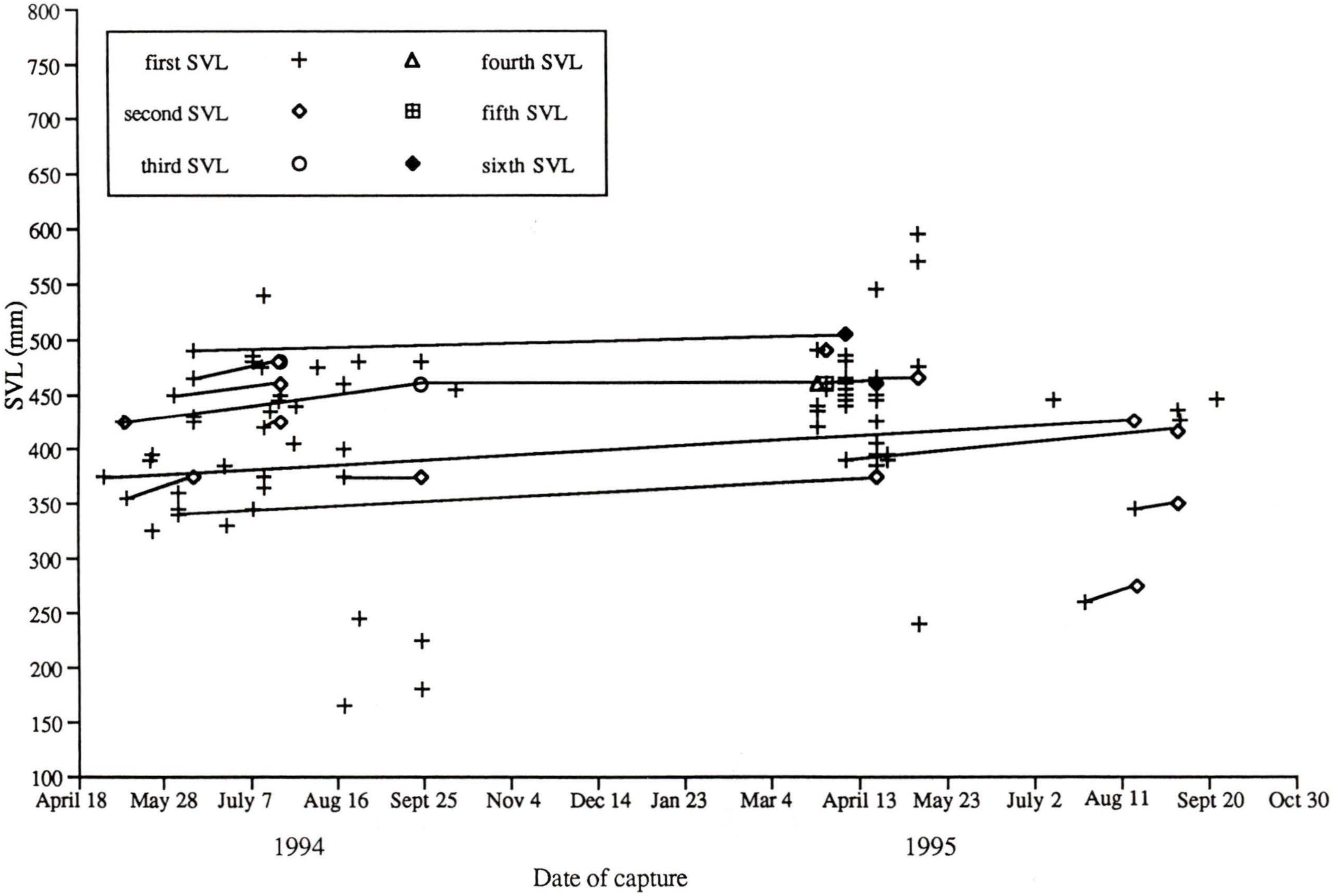

700
600
500
400
300
200
100
SVL (mm)
0
1
2
3
4
5
6
7
8
age
+ T. elegans - 1994
◇ 1995
○ T. ordinoides - 1994
△ 1995

Figure 11. Length of male T. elegans caught during 1994 and 1995 at Qualicum, B.C., showing recaptures connected by lines.



800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
SVL (mm)
first SVL +
second SVL ◇
third SVL ○
fourth SVL △
fifth SVL ⊞
sixth SVL ◆
April 18
May 28
July 7
Aug 16
Sept 25
Nov 4
Dec 14
Jan 23
Mar 4
April 13
May 23
July 2
Aug 11
Sept 20
Oct 30
1994
1995
Date of capture

Figure 12. Length of female T. elegans caught during 1994 and 1995 at Qualicum, B.C., showing recaptures connected by lines.

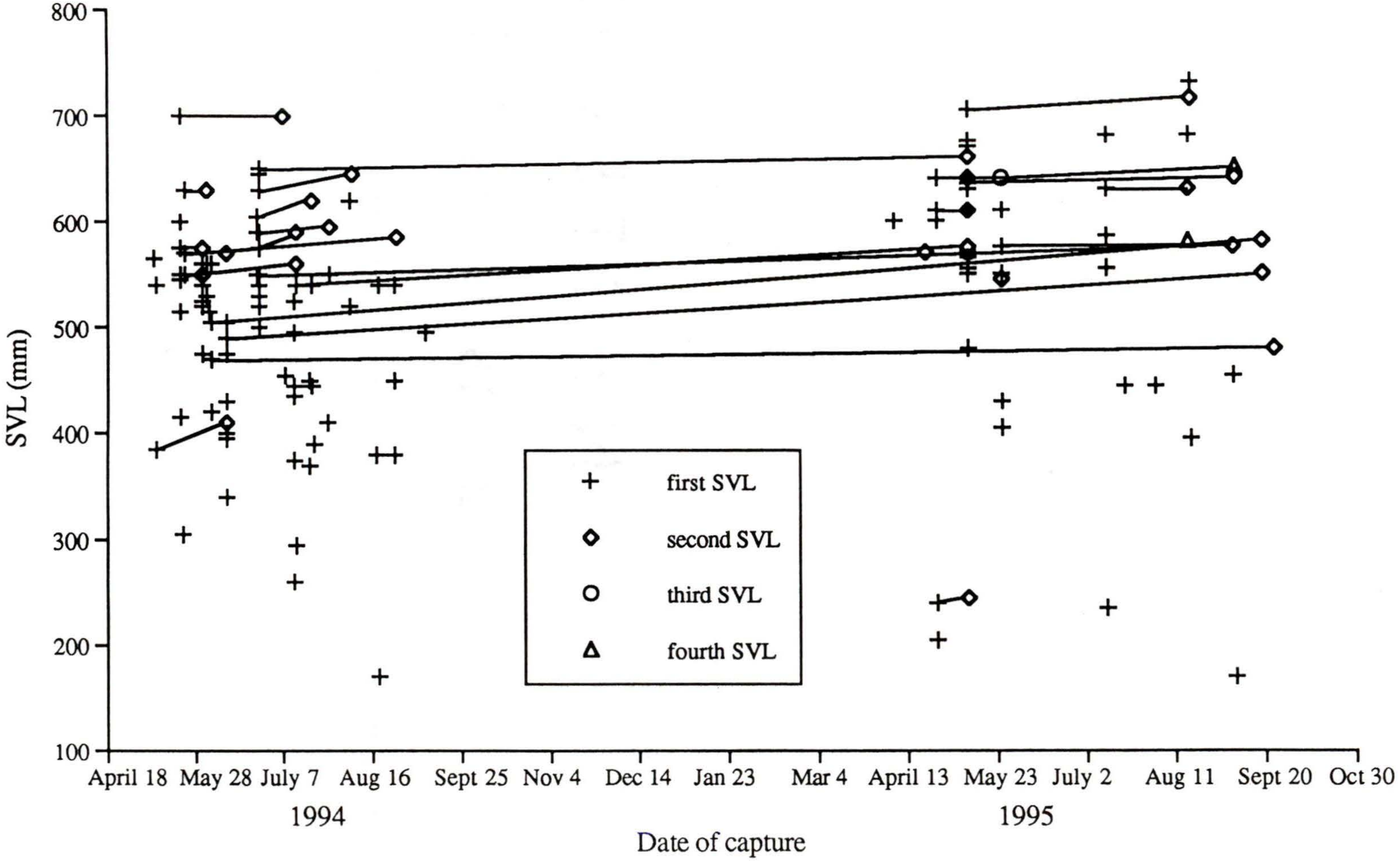
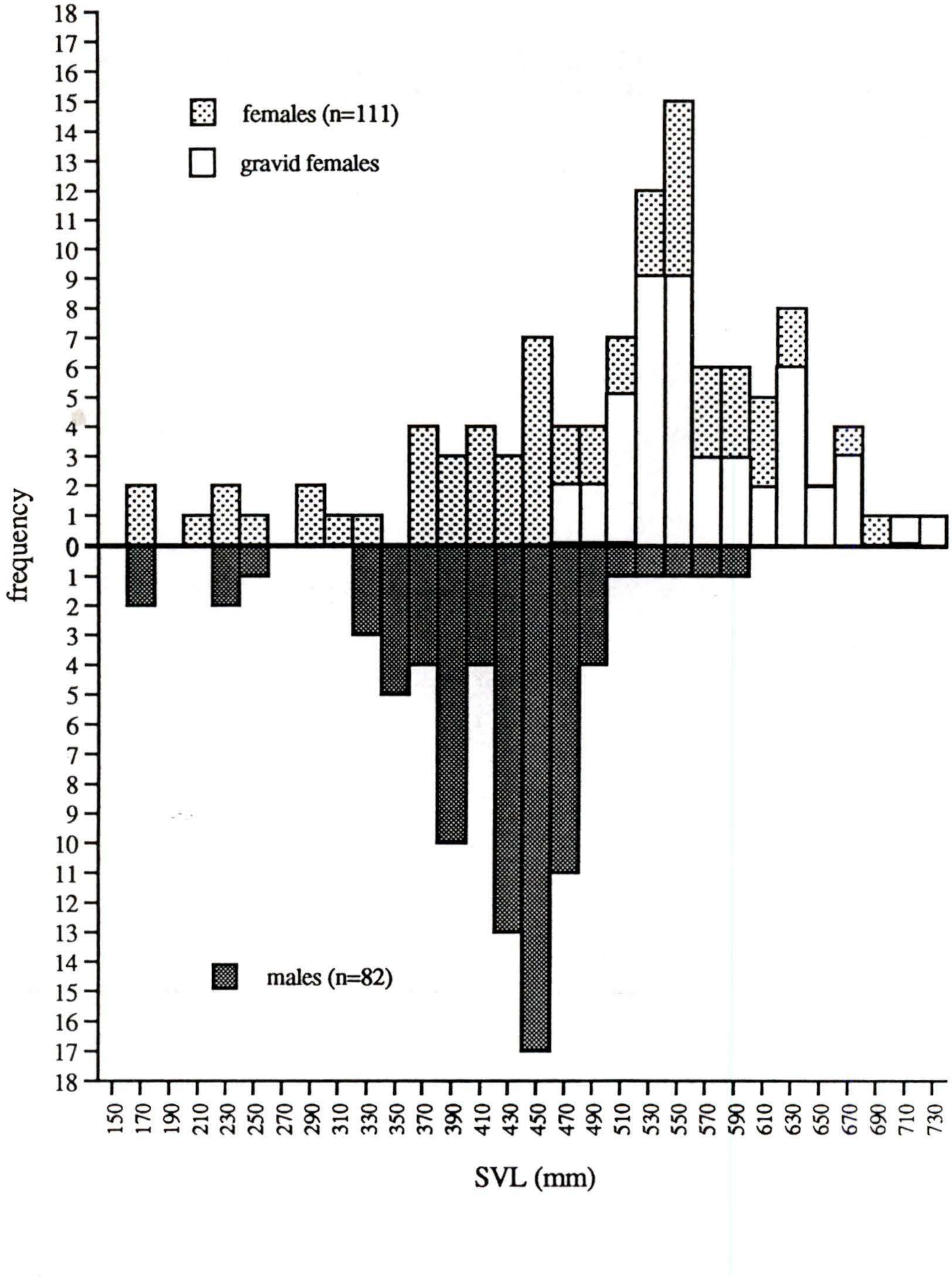

800
700
600
500
400
300
200
100
SVL (mm)
April 18
May 28
July 7
Aug 16
Sept 25
Nov 4
Dec 14
Jan 23
Mar 4
April 13
May 23
July 2
Aug 11
Sept 20
Oct 30
1994
1995
Date of capture
+ first SVL
◇ second SVL
○ third SVL
△ fourth SVL

Figure 13. Size frequency distribution of male and female T. elegans at Qualicum (1994 and 1995 lengths combined, not including recaptures).


females (n=111)
gravid females
males (n=82)
frequency
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
150
170
190
210
230
250
270
290
310
330
350
370
390
410
430
450
470
490
510
530
550
570
590
610
630
650
670
690
710
730
SVL (mm)

The relationships between SVL and mass for the male and female T. elegans were very similar (Fig. 14) but when the slopes of the regressions of the log transformed data were compared (Fig. 15), they were shown to be significantly different ($P(t_{0.05(2)186} \geq 6.4634) < 0.001$). The correlation coefficients of these lines were very high ($P(r_{0.05(2)110} \geq 0.981) < 0.001$ for females, $P(r_{0.05(2)81} \geq 0.976) < 0.001$ for males) indicating a close relationship between length and mass; the lines do not intercept the axis at the origin, indicating allometric growth. The SVL and the mass of the snakes compared to the number of annuli showed a generally positive exponential relationship with a large amount of scatter (Fig. 16). The correlation coefficent was significant for all regressions ($P(r_{0.05(2)110} \geq 0.567) < 0.001$ for female and $P(r_{0.05(2)81} \geq 0.404) < 0.001$ for male number of annuli vs. SVL; $P(r_{0.05(2)110} \geq 0.592) < 0.001$ for female and $P(r_{0.05(2)81} \geq 0.422) < 0.001$ for male number of annuli vs. mass). As the snakes get older and larger, the data points spread out more, possibly indicating much variability in individual growth rates. The average SVL in each age class increases in both males and females to "age" 4, then does not show much relationship to age. The average male SVL is greater than the average female SVL for age class 2, whereas female averages are larger than male averages for all other age classes (Fig.17). The 95% confidence limits overlap between male and female average SVL in most of the age groups, especially those with few individuals.

The age-frequency distributions for 1994 (the 1995 ages were reduced by 1 and added to the 1994 ages) show some features that correspond to the size-frequency distributions (Fig. 18). There are few individuals in both the smaller size classes and the lower age classes, although the numbers do not match perfectly. Most of the male and female snakes have undergone between 2 and 6 hibernation periods, with most of the males showing 2, 3, or 4 annuli and most of the females showing 3, 4, or 5 annuli; the youngest gravid female had 3 annuli. The ratio of mature to immature snakes (immature individuals were counted as those in age classes 0 and 1 for males and 0, 1, and 2 for females) was 6.8 to 1.

The life table for female T. elegans in 1994 show that the highest mortality rate occurs

Figure 14. Relationship between length and mass for female, male, and unknown sex T. elegans at Qualicum, 1994 and 1995.

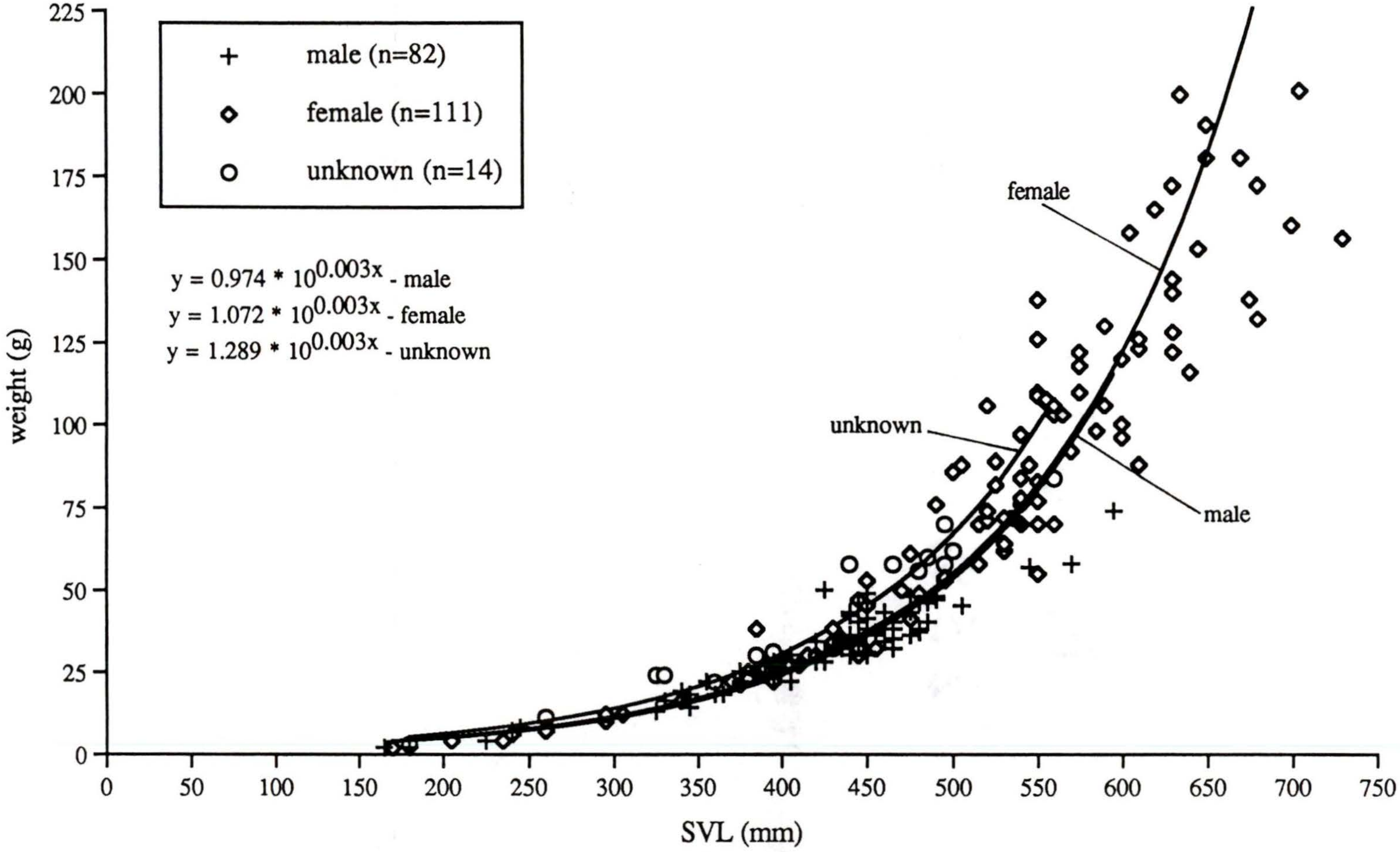

male (n=82)
female (n=111)
unknown (n=14)
y = 0.974 * 10^0.003x - male
y = 1.072 * 10^0.003x - female
y = 1.289 * 10^0.003x - unknown
female
unknown
male
weight (g)
0
25
50
75
100
125
150
175
200
225
SVL (mm)
0
50
100
150
200
250
300
350
400
450
500
550
600
650
700
750

Figure 15. Log mass and length relationship for female and male T. elegans at Qualicum, 1994 and 1995.

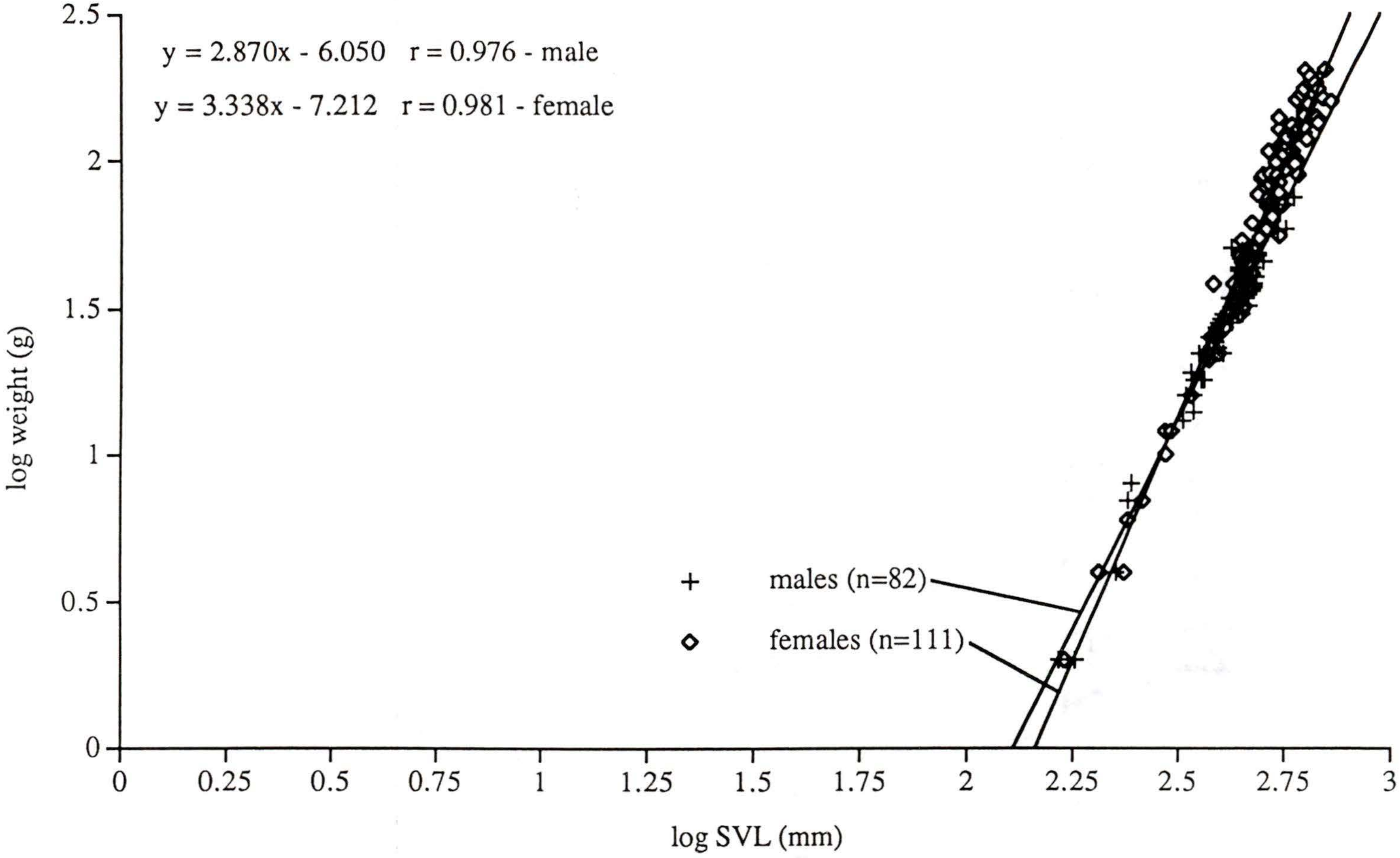
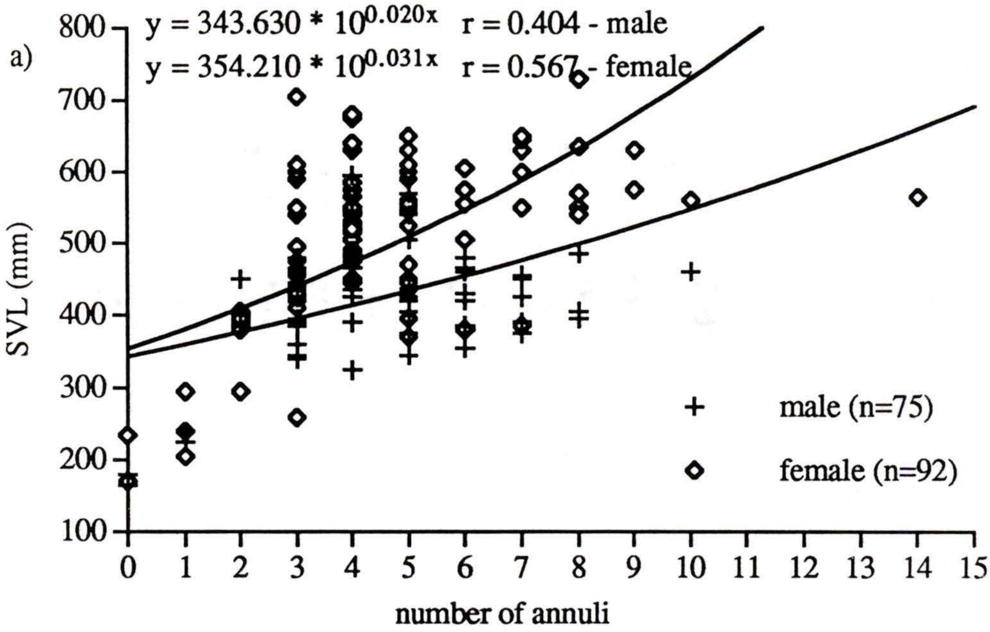

y = 2.870x - 6.050 r = 0.976 - male
y = 3.338x - 7.212 r = 0.981 - female
+ males (n=82)
◇ females (n=111)
log weight (g)
2.5
2
1.5
1
0.5
0
0
0.25
0.5
0.75
1
1.25
1.5
1.75
2
2.25
2.5
2.75
3
log SVL (mm)

Figure 16. Relationship between a) age and length, and b) age and mass for male and female T. elegans at Qualicum, 1994 and 1995.

a)


y = 343.630 * 10^0.020x r = 0.404 - male
y = 354.210 * 10^0.031x r = 0.567 - female
SVL (mm)
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
number of annuli
+ male (n=75)
◇ female (n=92)

b)

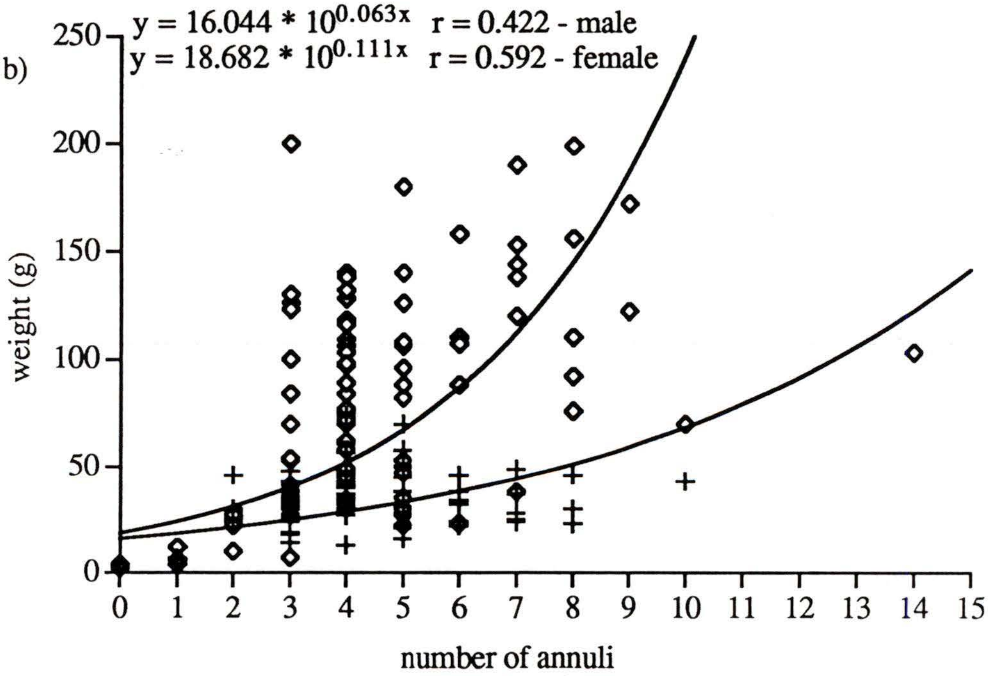

y = 16.044 * 10^0.063x r = 0.422 - male
y = 18.682 * 10^0.111x r = 0.592 - female
weight (g)
250
200
150
100
50
0
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
number of annuli

Figure 17. Average snout-vent length for each age class, and 95% confidence limits, for female and male T. elegans at Qualicum, 1994 and 1995.

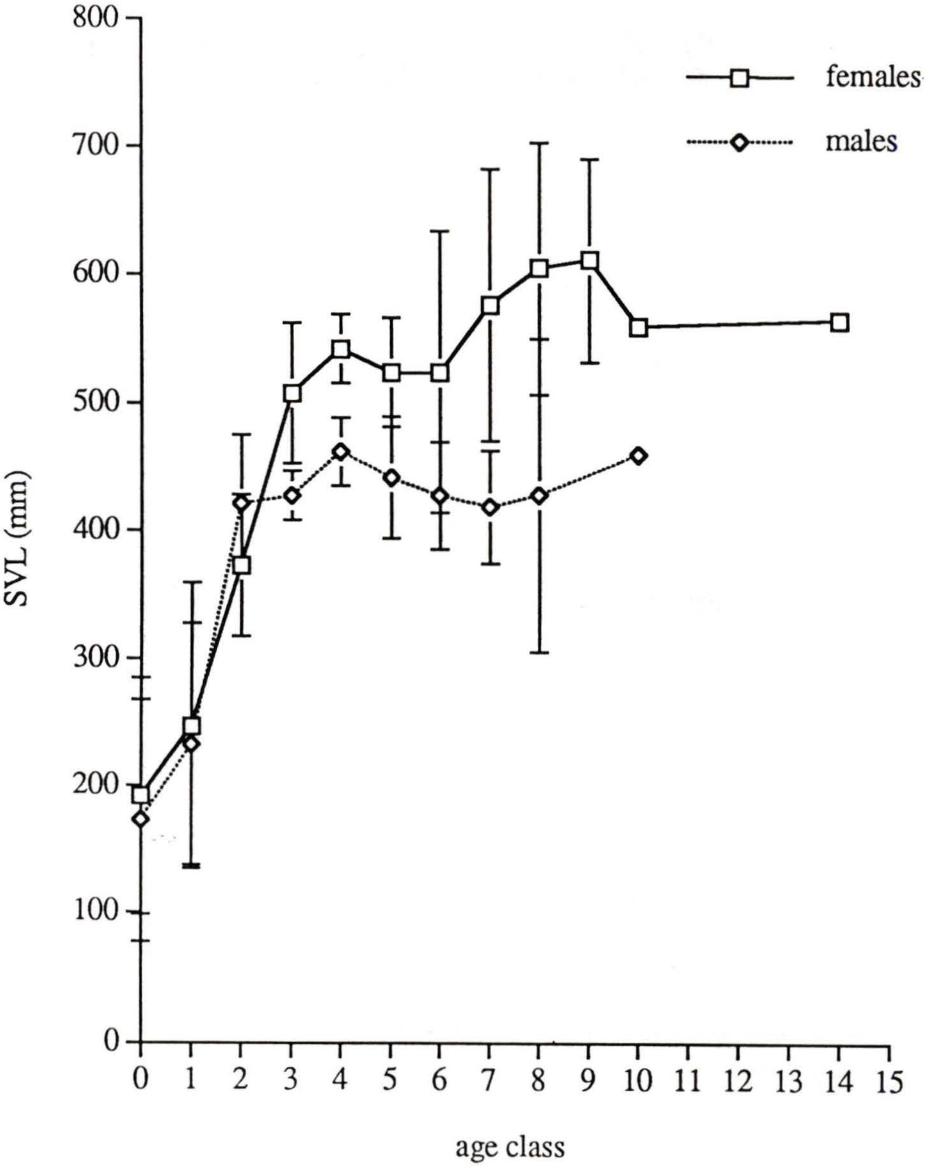
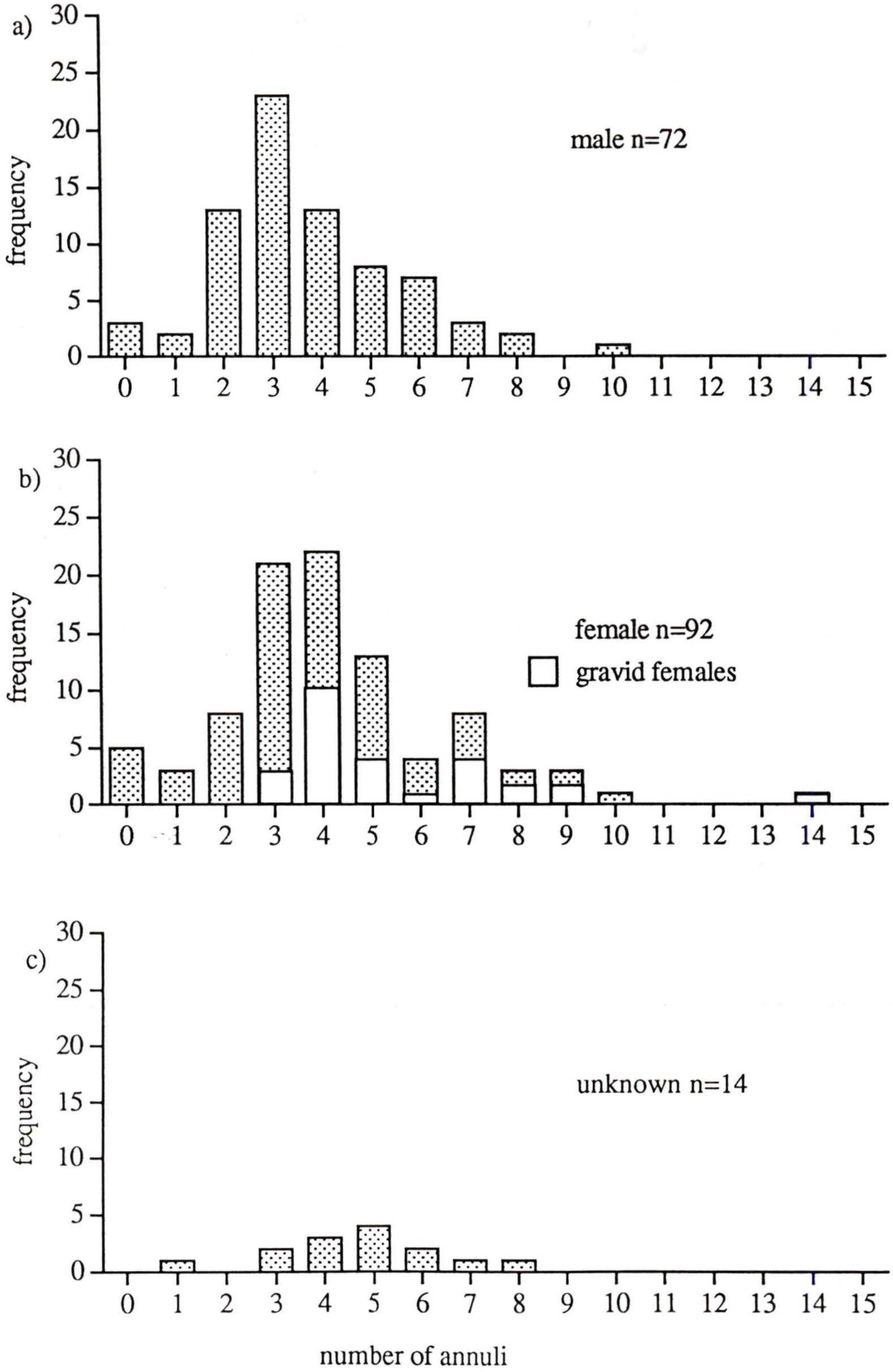

800
700
600
500
400
300
200
100
0
SVL (mm)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
age class
females
males

Figure 18. Age frequencies for a) males, b) females, and c) unknown sex T. elegans from Qualicum, 1994. Ages for 1995 samples were added to this figure by subtracting 1 to obtain the 1994 age. Only females that were known to be gravid in 1994 were included in the gravid female sample; females that were gravid in 1995 were not included.


a)
frequency
30
25
20
15
10
5
0
male n=72
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
b)
frequency
30
25
20
15
10
5
0
female n=92
☐ gravid females
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
c)
frequency
30
25
20
15
10
5
0
unknown n=14
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
number of annuli

between the first and second age classes (age 0 and 1), and the mortality rate steadily decreases with increasing age class (Table 2). The projected rate of increase of the population (R_o) is 5.2751.

The difference in ages between the samples that I re-read did not differ significantly from 0 ($P(t_{0.05(2)19} \geq 0.2521)$). The largest difference in ages for any individual was 2 annuli, and the percentage agreement within 1 year was 90% (i.e. 90% of the samples had differences between the first and second readings of 0 or 1 year). The difference in ages between the samples that I re-read and the ages obtained by a second reader for the same samples was also not significantly different from 0 ($0.10 > P(t_{0.05(2)19} \geq 1.8136) > 0.05$). The largest difference was 3 annuli, and the percentage agreement within 1 year was 75%. The mean difference in the first comparison was 0.05, while in the second it was 0.55, and the variance in the sample was much higher in the comparison with the second reader than in the re-reading comparison (1.8395 compared to 0.7868).

Hibernation Experiment:

The results of the neonate hibernation experiment are as follows:

1) the snakes that were in hibernation the entire time all had one definite dark band between the birth line and the edge of the vertebra (Fig. 19) and generally had the least growth (Fig 20);

2) the snakes that were removed from hibernation, fed, then placed back into hibernation generally showed two fainter rings between the birth line and the edge (Fig. 21);

3) the snakes that were fed *ad libitum* over the winter had many wide, faint bands (Fig. 22) that only roughly correspond to ecdysis episodes ($P(r_{0.05(2)5} \geq 0.26) > 0.5$) and showed the greatest growth (Fig 20);

4) those that were fed a minimal amount did not grow as much as those in group 3, but also had faint wide bands (Fig. 23).

It appears that the formation of annuli occurs over hibernation periods and not just over periods of low feeding.

Table 2. Life table for female T. elegans from Qualicum, B.C. 1994. Ages for 1995 were added to the sample by subtracting 1 to obtain the age in 1994.

age (x)	No. alive at start of age interval, n(x)	Proportion surviving at start of age interval, l(x)	No. dying within age interval x to x+1, d(x)	Finite rate of mortality q(x)	Finite rate of survival p(x)	Fecundity per female b(x)	l(x)b(x)
0	1000	1	216	0.22	0.78	0.00	0.00
1	784	0.7838	167	0.21	0.79	0.00	0.00
2	617	0.6169	129	0.21	0.79	0.00	0.00
3	488	0.4875	101	0.21	0.79	4.46	2.17
4	387	0.3869	79	0.20	0.80	4.46	1.73
5	308	0.3083	62	0.20	0.80	4.46	1.38
6	247	0.2467	48	0.20	0.80	0.00	0.00
7	198	0.1982	38	0.19	0.81	0.00	0.00
8	160	0.1600	30	0.19	0.81	0.00	0.00
9	130	0.1296	24	0.19	0.81	0.00	0.00
10	105	0.1054	19	0.18	0.82	0.00	0.00
11	86	0.0861	15	0.18	0.82	0.00	0.00
12	71	0.0707	12	0.18	0.82	0.00	0.00
13	58	0.0582	10	0.17	0.83	0.00	0.00
14	48	0.0482	8	0.17	0.83	0.00	0.00
15	40	0.0400	40	1.00	0.00	0.00	0.00
16	0	0	-	-		0.00	
						Ro=	5.28

Figure 19. Cross-section of vertebra from a T. elegans neonate placed in hibernation from November 25, 1994 to April 11, 1995 as part of the laboratory experiment; a) vertebra from November sample, b) vertebra from April sample. Stained with toludine blue (300x). Arrow marks annulus formed over the winter of 1994-1995.

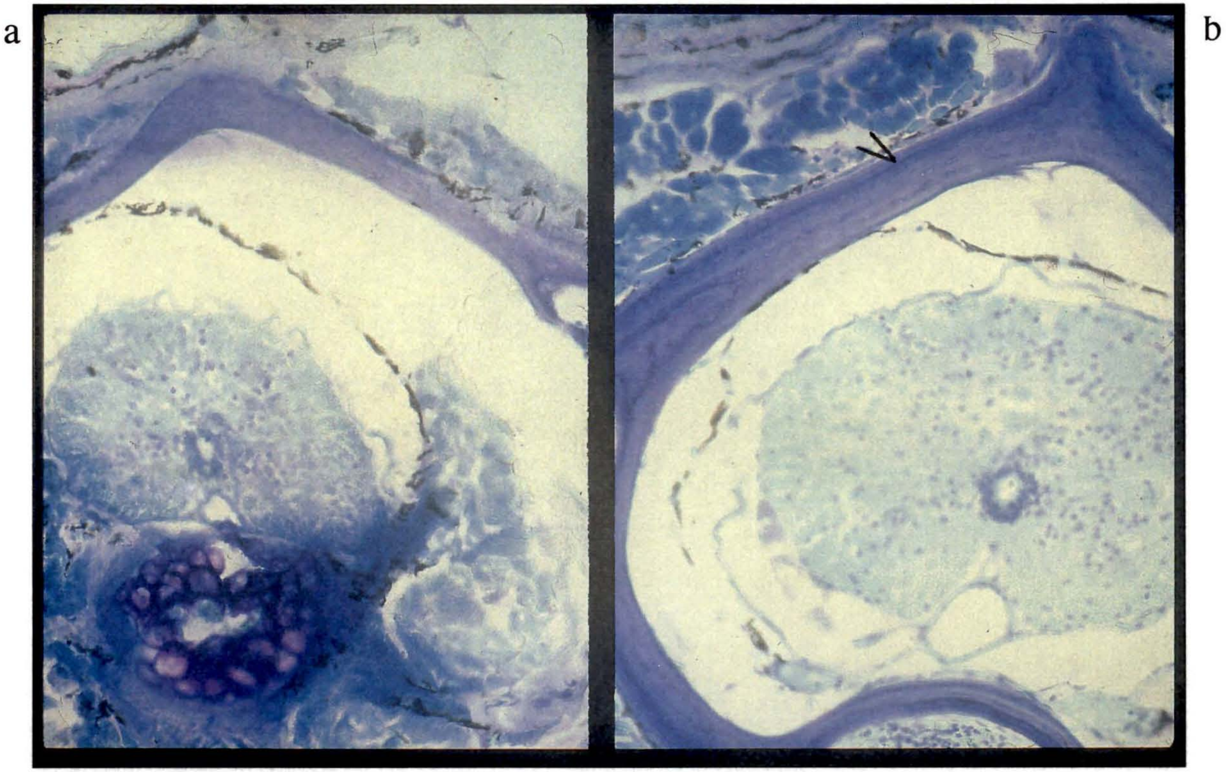

a
b

Figure 20. Measurements of snout-vent length of neonate T. elegans in the hibernation experiment, grouped by protocol.

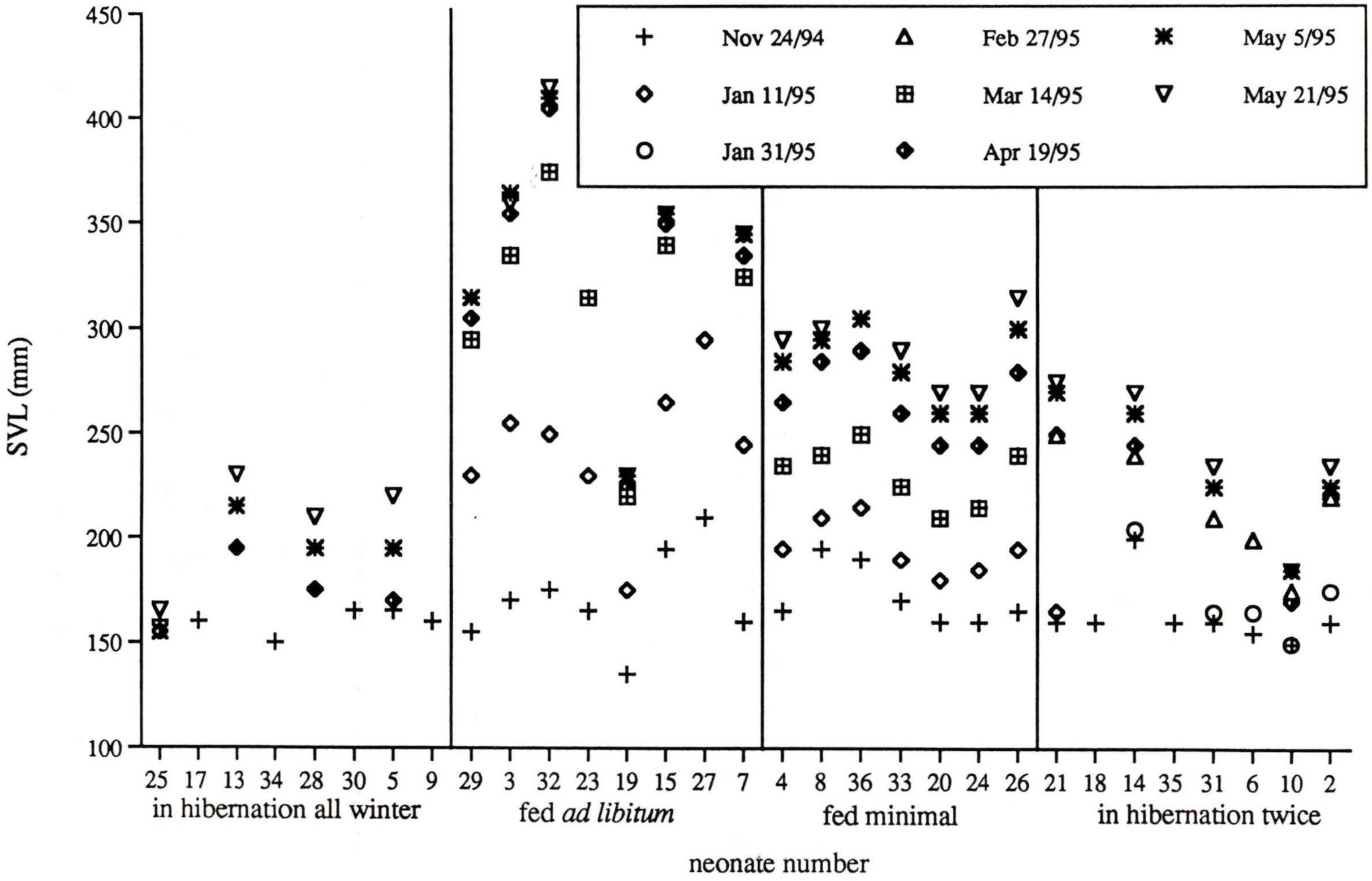

SVL (mm)
450
400
350
300
250
200
150
100
Nov 24/94
Jan 11/95
Jan 31/95
Feb 27/95
Mar 14/95
Apr 19/95
May 5/95
May 21/95
25 17 13 34 28 30 5 9
in hibernation all winter
29 3 32 23 19 15 27 7
fed *ad libitum*
4 8 36 33 20 24 26
fed minimal
21 18 14 35 31 6 10 2
in hibernation twice
neonate number

Figure 21. Cross-section of vertebra from a T. elegans neonate placed in hibernation from November 25, 1994 to January 25, 1995 and from February 28, 1995 to April 25, 1995 as part of the laboratory experiment. Stained with toludine blue (350x). Arrow marks rings formed over the winter of 1994-1995.

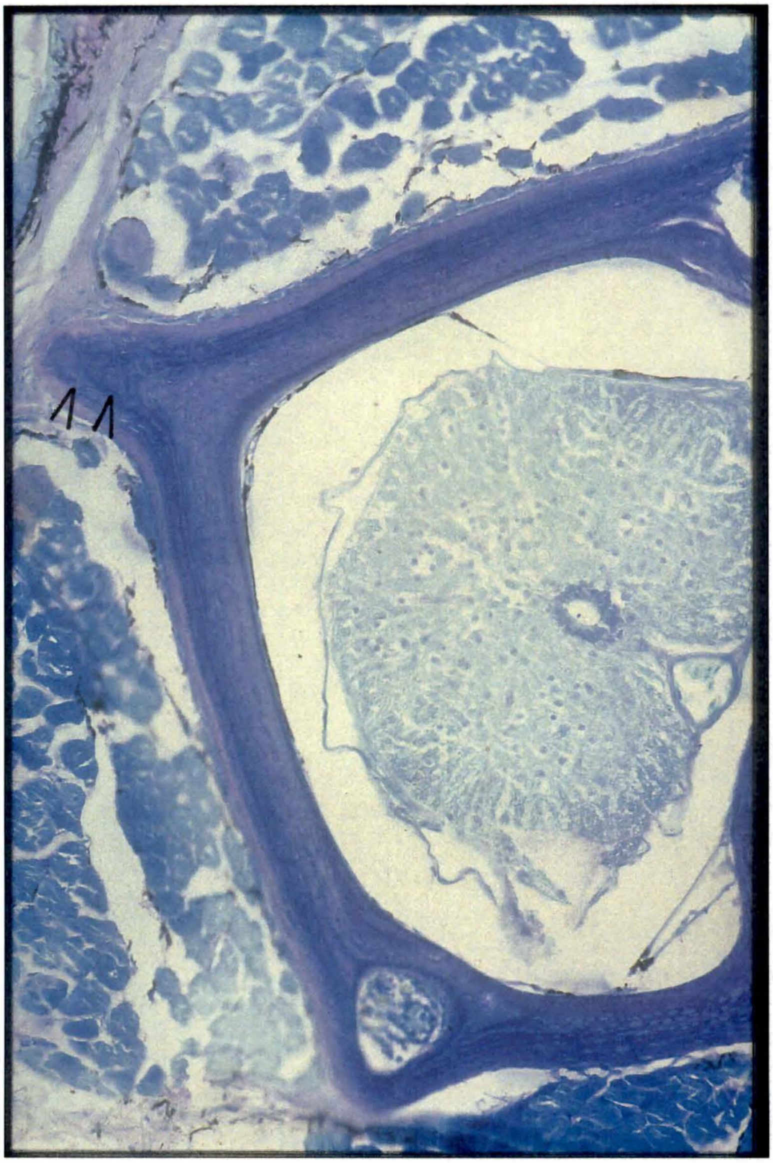


Figure 22. Cross-section of vertebra from a T. elegans neonate kept out of hibernation and fed *ad libitum* as part of the laboratory experiment; a) vertebra from November sample, b) vertebra from April sample. Stained with toludine blue (350x). Many faint bands that roughly correspond to episodes of ecdysis can be seen.

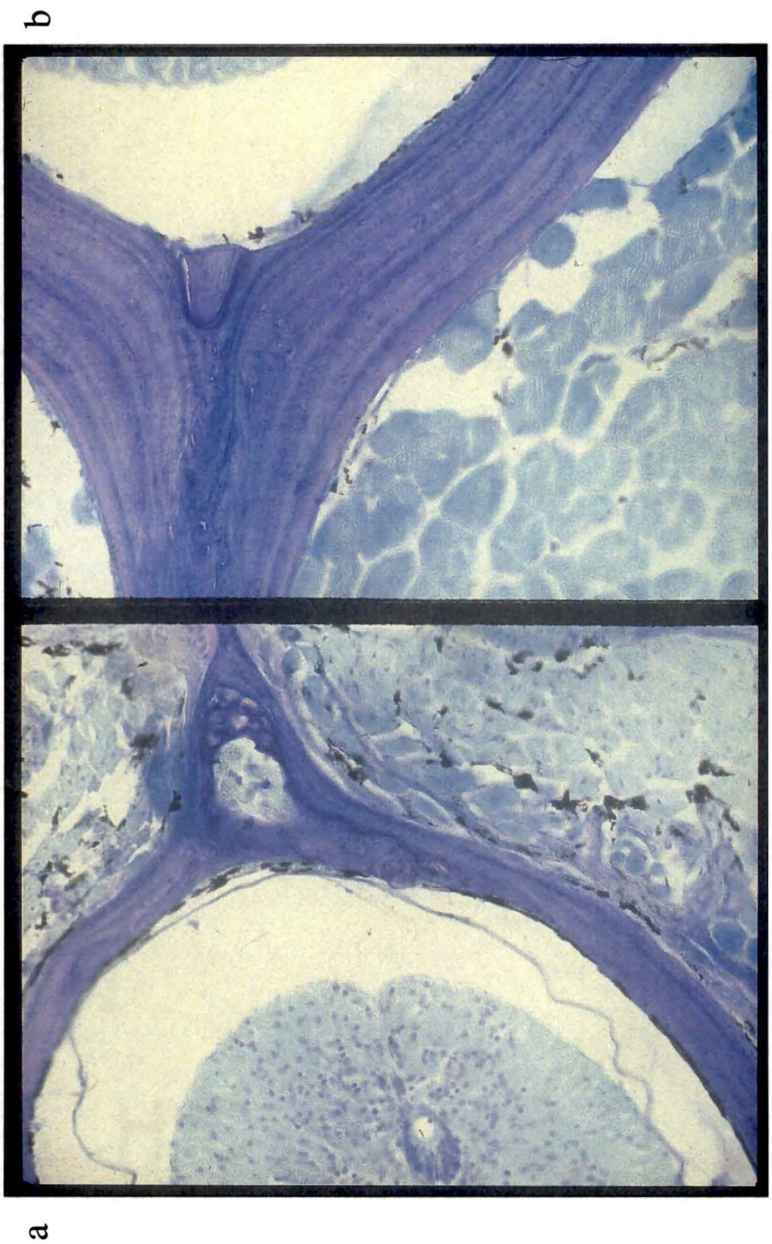
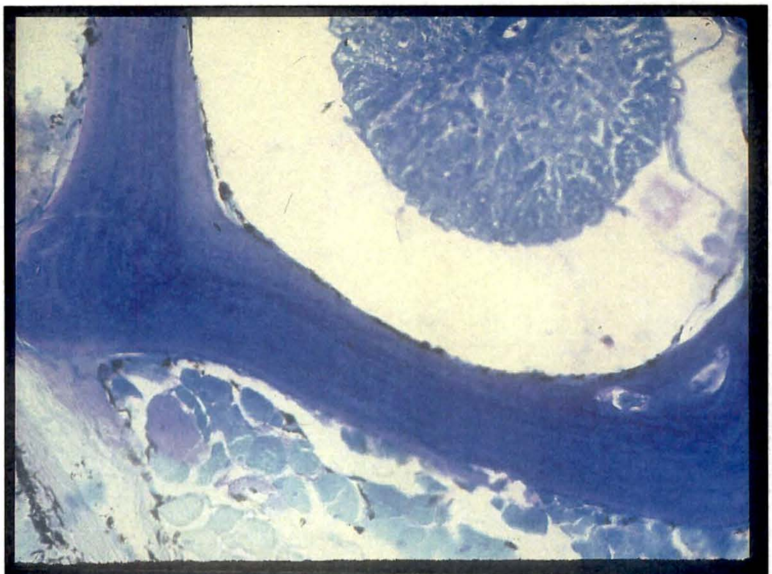
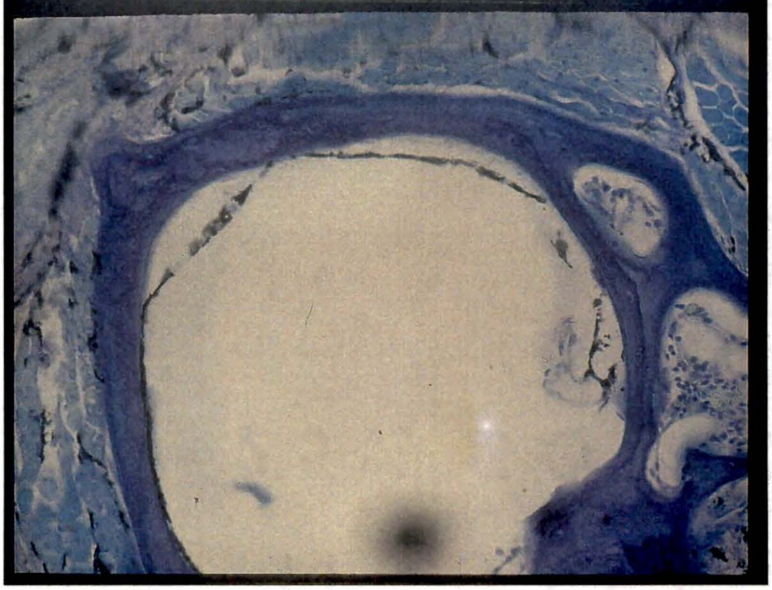

b
a

Figure 23. Cross-section of vertebra from a T. elegans neonate kept out of hibernation and fed a minimal amount of food as part of the laboratory experiment; a) vertebra from November sample, b) vertebra from April sample. Stained with toludine blue (350x).

b



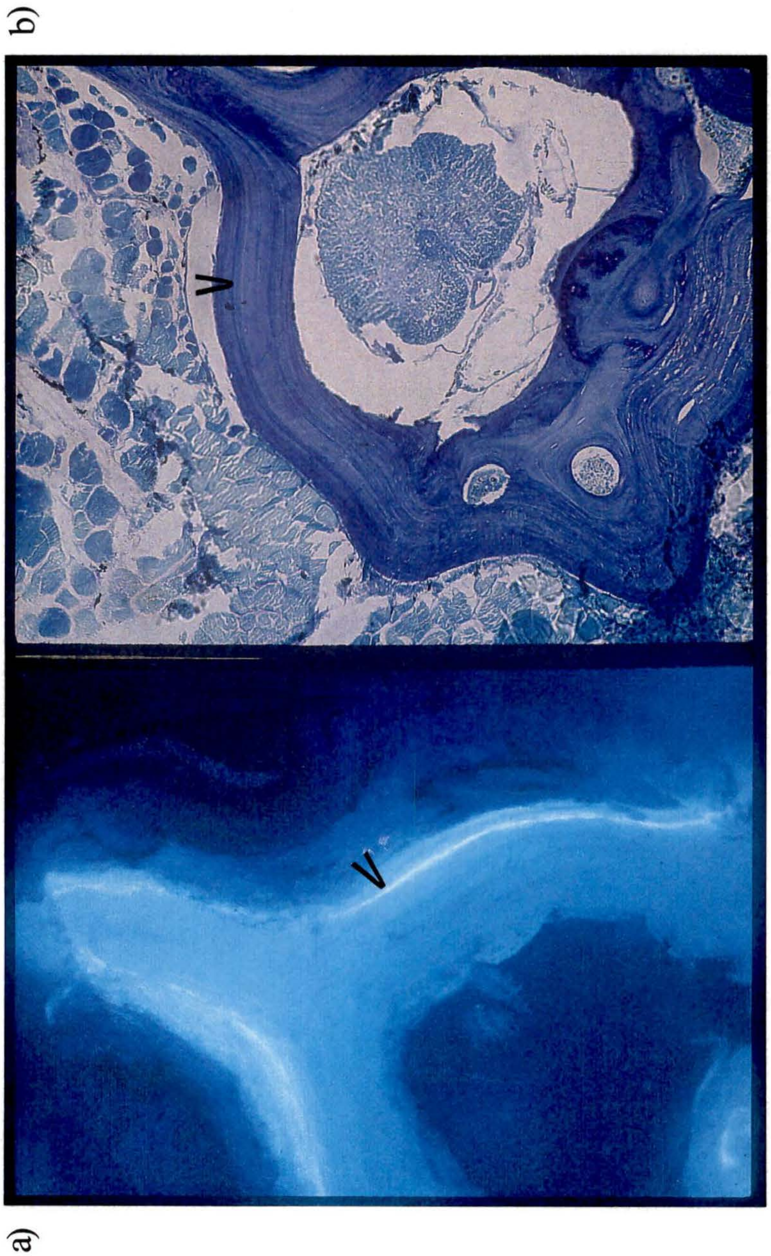


a

The adult snakes that I included in this experiment were all of known age; each had been in hibernation twice previously, and each had spent one or two winters out of hibernation. The difference between the number of annuli in the samples taken before hibernation and those taken after are consistent with the theory that one annulus is formed every hibernation. I did not feel that I could determine the age of these samples objectively, knowing that each had been in hibernation twice before, so I simply compared the samples taken in November 1994 with those taken in May 1995. Most of the samples showed two annuli between the birth line and the edge in November (corresponding to the previous two hibernation periods) and three annuli in May, although some of the samples did have annuli consistent with the winters in which they did not hibernate.

The oxytetracycline component of this experiment was not entirely successful; only 22 of 33 samples examined (both before hibernation and after) showed any part of a fluorescent ring, and of these only 3 had a complete ring. In those samples that had the OTC mark, it was consistent with the inner edge of the annulus formed over that hibernation period (seen in the corresponding decalcified stained sections) (Fig. 24).

Figure 24. Example of oxytetracycline fluorescence as a label to indicate growth rings. a) oxytetracycline ring along the neural arch and neural spine of a vertebra from a laboratory adult T. elegans, b) cross-section of vertebra from the same snake, taken from the snake at the same time. Arrows indicate OTC band in a) and annulus formed after the OTC injection in b), indicating the annulus formed during the winter of 1994-1995 (85x).


b)
a)

DISCUSSION

The size structure of a population may depend on various factors, but the age structure is affected by age-specific population parameters, including age at first reproduction, fecundity, and survivorship. In this study, I have shown that growth rings in caudal vertebrae are annual in nature, apparently induced by hibernation, and therefore theoretically suitable for determining age of living snakes. However, numerous practical problems make this method difficult to apply with accuracy. Nonetheless, it is clear that size and age are not closely related in garter snakes and that any complete analysis of their demography will demand further attention to separating age-specific from size-specific effects.

Size-frequency data are occasionally used to define year classes (Parker and Plummer 1987), and the first few year classes could be defined from the T. elegans data, but groups beyond 2 years are impossible to define, as the bulk of the snakes of 350 mm SVL and longer are all mixed together. Age groups have been crudely estimated for T. sirtalis in Manitoba by Gregory (1977), but only by using body size. He found considerable overlap of size classes, especially in females, that made division into discrete classes difficult. The youngest snakes in the denning populations were possibly in their second year, and individual males and females were found three years after their original marking, so at least four age–groups were presumed to be present. This conclusion is consistent with my results, as the youngest T. sirtalis that I examined had two annuli and the oldest had six, for five age groups. The decrease in average SVL from one female age group to the next could indicate greater longevity in snakes that are smaller at maturity (or have slower growth rates), but the small sample size precludes any definite conclusion of this kind and the decrease is not seen in the T. elegans sample.

Adult female T. sirtalis in Manitoba grow faster than adult males (Gregory 1977), and juvenile females have been found to grow faster than males starting in the first month after birth (Crews et al. 1985). The adult female T. elegans at Qualicum are on average larger for a given age than the males, which is consistent with other populations of garter snakes (e.g. Kephart 1981); early growth is similar between the two sexes, as shown by Gregory and Prelypchan (1994). The growth rate generally slows after maturity is reached, then slows again after about the fifth hibernation. There appears to be only a rough correlation between the age of a snake and its size, especially after maturity is reached. The plot of SVL against number of annuli shows a roughly triangular shape for males and females, so that the oldest snakes are not the longest; as mentioned for the T. sirtalis above, this could indicate that the slower-growing snakes live longer.

In general, the female T. elegans seem to live longer than the males or older females are caught more often than older males. The high number of 2 to 4 year old males and 3 to 5 year old females in the sample is probably due to sampling bias; reproductive males and females are easier to find and catch, the males at the den sites in the spring and fall and the gravid females basking in the middle of summer. Parker and Plummer (1987), looking at medium-sized viviparous colubrids in general, found that females mature at about 3 years of age, while males are a year younger at maturity. The T. sirtalis sample also suggests that the males mature at 2 years and the females at 3; the snakes were collected at the den site during emergence, and the only snakes that would be there at that time are mature ones trying to mate. The sample (although admittedly limited) does not have any males younger than 2 or any females younger than 3.

A linear regression predicting the number of rest lines from SVL for the prickly forest skink (Gnypetoscincus queenslandiae) was statistically significant (Cunningham 1993), and other studies seem to have found significant correlations between number of annuli and size or age (e.g. Verrell and Francillon 1986, for male Triturus vulgaris but not for females). I also found a broad positive relationship with significant correlation between number of annuli and SVL and mass for T. elegans, but not for T. sirtalis. Manitoba T. sirtalis have a large amount of variation in growth rates between individuals and between years (Gregory 1977), which is typical for most reptile species, but the main reason for the lack of correlation in this sample is probably the small sample size. The correlation coefficient, although significant, is not very high (around 0.4 or 0.5), and it is doubtful that the relationship could be used to accurately predict age from SVL for T. elegans. The correlation was slightly stronger for mass than for SVL, but measured weight for an individual snake could vary according to how recently that snake had eaten, and the size of the meal, so again I do not think that weight could be used to estimate age.

Previous studies that have tried to validate the use of skeletochronology to determine the age of amphibians and reptiles have obtained mixed results. Hemelaar and Van Gelder (1980) verified that one growth ring was formed per year in Bufo bufo, Castanet and Cheylan (1979) used known-age specimens to verify the technique for the turtles Testudo hermanni and T. graeca, Castanet and Naulleau (1974) verified growth marks for Vipera aspis, and Castanet (1978) verified annuli for Lacerta lepida and L. viridis. However, Griffiths (1962) found that the number of bands at the two ends of the same ectopterygoid differed, and felt that the bands did not indicate annual growth marks. There was significant variation between the number of annuli counted in the ectopterygoid and vertebra annuli in the T. sirtalis I examined, but I feel that was mainly due to remodelling and secondary bone in the middle of the ectopterygoid, obliterating annuli that were visible in the vertebra; where the number of annuli differed, usually the number in the vertebra was higher than the number in the ectopterygoid. This remodelling occurred to an even greater degree in the ribs, and most of the ribs were completely useless for counting annuli. Mandibles and ribs of crocodiles were also found to be not suitable for skeletochronology due to the secondary bone, and the number of annuli was variable between these parts of the skeleton and long bones and osteoderms (Hutton 1986). I compared the structures directly after assigning counts of annuli, and I could trace strong patterns of annuli between the ectopterygoids and the vertebrae and between sections of the same structure. For this reason, I feel that even though the results of this statistical test showed significant difference, the annuli are generally consistent throughout an individual.

It is uncertain whether reproductive events have an effect on the number of annuli that are recorded in the bones of females. I did not see any particular spacing patterns between the annuli of females that would indicate this, but it is something to consider when looking at the results of this study. The female T. sirtalis showed greater differences in counts of annuli between vertebrae and ectopterygoids, either due to their greater age allowing more variability in counting, or possibly due to reproduction events that were counted as annuli in the vertebrae but not detected in the ectopterygoids. Bone marking of a population of snakes over several years with OTC or another label would help to resolve this question; if certain years show two growth marks, and the individuals were known to be gravid in those years, then reproductive marks could be confirmed.

Reproductive life tables could not be accurately constructed for the female T. elegans, as no correlation could be made between age and number of offspring in the snakes kept in the laboratory. The calculation of fecundity (b_x) assumes annual reproduction by individual females, which might not be the case for this population of T. elegans. The four females for which I have information from both years were gravid in either 1994 or 1995, not both, and Gregory (1977) inferred that female T. sirtalis from Manitoba followed a biennial reproductive cycle. The life table does show that the youngest age classes have the highest mortality rate, but I expected to see a proportionately much higher mortality rate for neonates than for the other age classes. There is probably a greater proportion of neonates in the population than what I calculated for the life table; the ratio of mature to immature snakes was 6.8:1, compared to 2.4:1 found by Kephart (1981). An increased number of individuals in the 0 age category would also affect the calculation of the reproductive rate of the population, bringing it down to a more logical number; the calculated value is extremely high and therefore probably inaccurate. The main assumption in constructing the life tables (and for the Peterson index used to calculate population size) is that of equal catchability; every individual in the population has an equal chance of being caught, and each age class has equal representation. It is almost impossible to avoid sampling bias in most population studies, and snakes are definitely impossible to sample without bias, for reasons discussed below.

Tail injuries have been recorded for as many as 32% of snakes in a population of

Thamnophis sirtalis fitchi (Jayne and Bennett 1989), while another population of the same species had about 10% of the individuals with some tail loss. The proportion of T. elegans and T. ordinoides at Qualicum that had already lost some portion of their tails was similar to the latter figure. Female T. elegans had the highest percentage, and were the most numerous in my sample. I found that the size, sex, and species of snake caught seemed to vary with the time of year, the time of day, the specific site being searched, and the weather conditions. Large, gravid female T. elegans were the easiest to find and catch for several reasons: 1) gravid females are more likely to be found basking in the open than nongravid females, juveniles, or males (e.g. Gregory 1977); 2) larger snakes are easier to spot at a distance and more likely to be caught (more body length to grab); 3) I noticed that T. ordinoides are more secretive and stay closer to cover than T. elegans, so are harder to find. Female T. elegans probably also have a higher rate of tail loss than the other groups because they are more exposed to predation through their reproductive status. The deviation of the observed sex ratio from 1:1 is most likely also due to the greater catchability of gravid females.

The neonate T. elegans in the hibernation experiment that were fed as much as they could eat over the winter grew to a much greater length than the other groups, and had a series of wide, light bands in the vertebrae that I thought could be related to the number of ecdysis events. The number of bands only roughly matched the number of shedding events, but Boiga irregularis in captivity showed clear growth marks related to ecdysis and not feeding events (Collins and Rodda 1994), so I conclude that this is the most likely source of the bands; the neonates were fed three times every week, so feeding events either would not be recorded in the bone, or would create many more annuli than the number observed. Those snakes that were kept out of hibernation all winter but fed a small amount also showed the wide bands but did not grow as large. They were probably fed too much to completely arrest their growth, but a hibernation event is apparently needed to form a definite annulus. The snakes that were placed in hibernation twice over the winter had two dark rings that were lighter than the annulus seen in the snakes in hibernation over the entire winter, but were still darker than the bands in the other two groups.

The results of the oxytetracycline experiment were disappointing, as fisheries biologists have been using this technique to label structures in fish with much success (e.g. Tzeng and Yu 1989). I might have had better success with greater concentrations of OTC, either in one large dose or as several doses over a week, but my main concern was for the health of the snakes. As the OTC component of my experiment was relatively minor, I felt it important to err on the conservative side and avoid side effects from overdoses of OTC (which can include reduction in growth rate and reduced mineralization in embryos and juveniles (Johnson 1964)). Alternative bone labels, such as alzarin red, are also known to affect growth rate (Johnson 1964), and are generally used when the animal will eventually be sacrificed anyway. The few samples that did work served to indicate that the annulus seen in the stained sections was indeed produced over the hibernation period of November 1994 to May 1995.

The known-age snakes in the laboratory were all neonates or 3 or 4 years old in November 1994, so the skeletochronology technique can be considered validated for the 0 to 1, 3 to 4, and 4 to 5 age classes in T. elegans. The recaptured snakes allowed me to tentatively validate age classes 0 to 1, 3 to 4, 5 to 6, and 6 to 7 for T. ordinoides and 3 to 4, 4 to 5, 5 to 6, and 6 to 7 for T. elegans. Beamish and McFarlane (1983) stress that all age groups in the population being examined must be validated, especially in the case of harvested populations. Older age groups are more difficult to age with accuracy, as the outermost rings may be crowded together and the innermost rings may be lost through resorption. Errors in counts of annuli will be biased towards younger ages, and this can have major impacts on the management of the species, especially when population growth rates are based on age-specific fecundity and survivorship (Beamish and McFarlane 1983). I found that in some of the samples the annuli could be counted more than one way, and the number could be, for example, either 4 or 8, depending on whether I counted all the bands as annuli or only the darker ones. Ideally, this study would continue for several more years in the field and in the laboratory, to examine mark-recapture and artificial hibernation results in a greater range of age classes.

Recaptures of T. elegans and T. ordinoides in the field prove that one annulus is formed for each hibernation period. However, even when the samples are compared directly and the extra annulus can be seen at the edge of the recapture samples, the separate counts do not always produce a difference of one annulus (although for the entire group of recapture samples the difference was not significantly different from one). This underscores the difficulty in counting annuli consistently; what I count as an annulus in one sample I may not count in another, no matter how I try to standardize the technique. As I looked at more and more samples (over 400 altogether), I developed criteria for counting annuli. The lack of significant difference between the first and second readings that I did was somewhat surprising, as I felt that my criteria for counting annuli had changed over the course of the study. Just under half of the re-read samples did have differing numbers of annuli assigned to them, so even though the result was not statistically significant, I was not absolutely precise in my readings. The second reader did not have the experience in looking at growth rings that I did, but the ages were still not significantly different, which indicates that although it becomes easier to count annuli as more samples are examined, the basic technique is not difficult to acquire. The difference in counts between two readers was significantly different for female Iguana iguana (Zug and Rand 1987), but good agreement between a naive and an experienced reader was found for Triturus vulgaris (Verrell and Francillon 1986), although not statistically tested. More comparisons of this kind are definitely needed in herpetological studies using skeletochronology; too often, researchers rely on verification of the growth rings in other species, and do not bother to test their precision in counting the growth rings (e.g. Chinsamy et al. 1995).

Fisheries biologists have been using skeletochronology for decades to determine the age of fish, and samples are sent to aging laboratories where people who have read thousands of samples assign ages to the fish. In some laboratories, readers specialize in a particular species, and become very familiar with the appearance of annuli in that species. The Ageing Unit at the Alaska Fisheries Science Center (Seattle, Washington) determines ages of all of the otolith samples collected on the west coast of the United States, and 20% of all readings are re-read by an independent reader for precision quality control (Kimura and

Lyons 1991). This level of expertise and application of statistical analyses is not yet apparent in the field of herpetology. Herpetologists determined to use skeletochronological techniques on their frogs, salamanders, lizards, or snakes should apply the lessons that fisheries biologists have learned to their own research to avoid the problems that have appeared from applying skeletochronological data to management situations.

Conclusion:

The skeletochronological technique is labour-intensive; many samples need to be examined to get an accurate picture of the population, the reading of the samples must be standardized between samples and between readers or readings, and most important, the technique must be validated for each age class with mark-recapture, the examination of known-age individuals or labelling. This means that this technique is not useful for short-term studies, but could be an important part of a large, long-term study that involves mark-recapture over more than two or three years. Skeletochronology appears to work very well on amphibian and lizard toes and long bones; the structure of those bones leads to clear, concentric rings that are easily read. Unfortunately, the only structure that it is possible to examine in snakes without killing them is the caudal vertebra. Although the centrum and the neural arch show annuli, the bands vary in appearance as they are followed around the vertebra, and can be difficult to count with confidence. This does not mean that the technique is any less valid for snakes than for lizards and amphibians, but that it is more difficult to obtain accurate and precise results. Another consideration is that although the sampling of caudal vertebrae allows relatively nondestructive sampling, it is not feasible for snakes that depend on the tail tip for defence (e.g. rattlesnakes) or have a blunt tail that would leave a very large open wound if the end was cut off (e.g. the boids).

It is likely that many life-history parameters of garter snakes are more dependent on size than on age, but in order to prove this, the age of the individuals must be compared to the size and the parameters related to each. I have shown that, with some effort, skeletochronology can be used to determine the ages of garter snakes, although the accuracy must be more firmly established and aging criteria better defined. Using the ages

I obtained through this technique, I have shown that age and size are not closely related in garter snakes. I hope that future studies take these results as an indication that it is dangerous to make assumptions about these kinds of relationships without testing them.

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