Institut für Parasitologie
der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Dr. med vet. Peter Deplazes

Arbeit unter Leitung von Dr. sc. nat. Daniel Hegglin

Age, season and spatio-temporal factors affecting the prevalence of *Echinococcus multilocularis* and *Taenia taeniaeformis* in *Arvicola terrestris*

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Pierre Edouard Burlet

Tierarzt
von Reichenburg, SZ

genehmigt auf Antrag von

Prof. Dr. med. vet. Peter Deplazes, Referent

Prof. Dr. Paul Torgerson, Korreferent

Zürich 2011

Inhaltsverzeichnis

Summary	2
Zusammenfassung	3
Age, season and spatio-temporal factors affecting the prevalence of *Echinococcus multilocularis* and *Taenia taeniaeformis* in *Arvicola terrestris*	4-31
Alter, Jahreszeit und räumlich-zeitliche Faktoren, welche die Prävalenz von *Echinococcus multilocularis* und *Taenia taeniaeformis* in *Arvicola terrestris* beeinflussen	32-45
Danksagungen	46

Summary

Taenia taeniaeformis and *Echinococcus multilocularis* both infect the water vole *Arvicola terrestris*. We investigated the effect of age, spatio-temporal and season-related factors on the prevalence of these parasites in *A. terrestris*. The absolute age of the voles was calculated based on their eye lens weights, and we included the mean day temperature and mean precipitation experienced by each individual as independent factors. Lenses were prepared immediately after trapping or frozen. Frozen lenses weighed at average 3.3% more than the unfrozen ones from the same animals. Applying a correction factor corrects this overestimation of age. Overall prevalences of *E. multilocularis* and *T. taeniaeformis* were 15.1% and 23.4%, respectively, in 856 *A. terrestris* trapped in the canton Zürich, Switzerland. Prevalences were lower in young than in older animals. 12 of 129 *E. multilocularis*-infected voles harboured protoscoleces. Strong spatio-temporal variations in prevalences of *E. multilocularis* were revealed. Low temperatures significantly correlated with the infection rate whereas precipitation was of lower importance. Significant spatial variations in prevalences were also identified for *T. taeniaeformis,* but no significant meterological factors. Our results suggest that the enhanced survival of *E. multilocularis* eggs under cold weather conditions determines the level of infection pressure on the intermediate hosts and possibly also the infection risk for human alveolar echincoccosis. Therefore, deworming foxes may be most efficient if conducted just before and during winter.

Zusammenfassung

Taenia taeniaeformis und *Echinococcus multilocularis* infizieren beide die Schermaus *Arvicola terrestris*. Wir untersuchten den Einfluss von Alter, räumlich-zeitlichen und jahreszeitlichen Faktoren auf die Prävalenz dieser Parasiten in *A. terrestris*. Das absolute Alter der Schermäuse wurde aufgrund des Linsengewichts ihrer Augen berechnet. Der Tagesdurchschnitt der Temperatur und der Niederschläge, die jedes Individuum durchmachte, wurden als unabhängige Faktoren betrachtet. Die Gesamtprävalenzen von *E. multilocularis* und *T. taeniaeformis* in 856 im Kanton Zürich, Schweiz, gefangenen *A. terrestris*, betrugen 15.1%, beziehungsweise 23.4%. Die Prävalenzen waren in jungen Tieren tiefer als in älteren und 12 von 129 *E. multilocularis* infizierten Tieren enthielten Protoscoleces. Starke zeitlich-räumliche Schwankungen der Prävalenz in *E. multilocularis* konnten nachgewiesen werden. Tiefe Temperaturen korrelierten signifikant mit der Infektionsrate, wohingegen Niederschläge von geringerer Bedeutung waren. Signifikante räumliche Veränderungen der Prävalenz wurden auch für *T. taeniaeformis* aufgezeigt, aber keine signifikanten meteorologischen Faktoren. Unsere Resultate deuten darauf hin, dass das höhere Überleben von *E. multilocularis* Eiern bei kalten Wetterbedingungen den Grad des Infektionsdrucks auf den Zwischenwirt und möglicherweise auch das Infektionsrisiko für die humane alveoläre Echinokokkose bestimmen. Folglich dürfte die Entwurmumg von Füchsen am effizientesten sein, wenn sie kurz vor und während des Winters durchgeführt wird.

Age, season and spatio-temporal factors affecting the prevalence of *Echinococcus multilocularis* and *Taenia taeniaeformis* in *Arvicola terrestris*

Pierre Burlet[1], Peter Deplazes[1], Daniel Hegglin[1§]

[1]Institute of Parasitology, University of Zurich, Winterthurerstr. 266a, CH-8057 Zurich, Switzerland

[§]Corresponding author

Email addresses:

PB: pierre.burlet@access.uzh.ch

PD: deplazesp@access.uzh.ch

DH: daniel.hegglin@swild.ch

Abstract

Background

Taenia taeniaeformis and the related zoonotic cestode *Echinococcus multilocularis* both infect the water vole *Arvicola terrestris*. We investigated the effect of age, spatio-temporal and season-related factors on the prevalence of these parasites in their shared intermediate host. The absolute age of the voles was calculated based on their eye lens weights, and we included the mean day temperature and mean precipitation experienced by each individual as independent factors.

Results

Overall prevalences of *E. multilocularis* and *T. taeniaeformis* were 15.1% and 23.4%, respectively, in 856 *A. terrestris* trapped in the canton Zürich, Switzerland. Prevalences were lower in young (≤ 3 months: *E. multilocularis* 7.6%, *T. taeniaeformis* 17.9%) than in older animals (>7 months: 32.6% and 34.8%). Only 12 of 129 *E. multilocularis*-infected voles harboured protoscoleces. Similar proportions of animals with several strobilocerci were found in *T. taeniaeformis* infected voles of <5 months and ≥5 months of age (12.8% and 11.9%). Multivariate analyses revealed strong spatio-temporal variations in prevalences of *E. multilocularis*. In one trapping area, prevalences varied on an exceptional high level of 40.6-78.5% during the whole study period. Low temperatures significantly correlated with the infection rate whereas precipitation was of lower importance. Significant spatial variations in prevalences were also identified for *Taenia taeniaeformis*. Although the trapping period and the meteorological factors temperature and precipitation were included in the best models for explaining the infection risk, their effects were not significant for this parasite.

Conclusions

Our results demonstrate that, besides temporal and spatial factors, low temperatures contribute to the risk of infection with *E. multilocularis*. This suggests that the enhanced survival of *E. multilocularis* eggs under cold weather conditions determines the level of infection pressure on the intermediate hosts and possibly also the infection risk for human alveolar echincoccosis (AE). Therefore, interventions against the zoonotic cestode *E. multilocularis* by deworming foxes may be most efficient if conducted just before and during winter.

Background

Population dynamics of organisms in temperate zones are generally shaped by seasonal variations. Parasites living within their hosts are protected from the direct impact of season-related factors like temperature or humidity but they usually have free living stages that can directly be affected by adverse environmental conditions. The understanding of how meteorological factors and seasonal changes affect the population dynamics of zoonotic parasites can contribute to better understand their epidemiology and to develop efficient control strategies.

In many parts of Europe, the zoonotic fox tapeworm *Echinococcus multilocularis* has benefited from increasing fox (*Vulpes vulpes*) populations and the invasion of foxes into urbanized areas during the last two decades [1-4]. In many cities of Switzerland, Germany and France, the life cycle of *E. multilocularis* is established in urban settings [2, 5-7]. As a consequence, the incidence of human alveolar echinococcosis (AE) has increased in Switzerland by a factor of 2.6 during the first five years of the 21st century as compared with the preceding five year period [8]. Human AE is an expensive disease to manage [9] and the frequently life-long treatment is very demanding for the affected patients. Therefore, there is a need to better understand the factors which affect the transmission dynamics of this parasite.

In experimental studies, it has been shown that the eggs of *E. multilocularis* can survive several months in a cold and humid environment, which is typical for winters in central Europe, but only a few days when exposed to dry and hot conditions prevailing in summers [10]. It therefore could be expected that *E. multilocularis* eggs excreted by foxes can accumulate during winter resulting in a higher infection pressure during this period compared to the rest of the year.

In Europe, the natural life cycle of *E. multilocularis* depends on the predator-prey relationship between foxes as the most important definitive hosts and *Arvicolidae* (voles), mainly the species *Microtus arvalis* and *Arvicola terrestris*, as intermediate hosts [11]. *Arvicola terrestris* and *M. arvalis* have a short life expectancy ranging from several months to rarely over 1 year [12]. Their population densities and structures are strongly affected by perennial cycles [13, 14] and seasonal changes. Population densities of voles are generally highest in autumn and lowest in spring when the age structure of populations is strongly shaped by a higher proportion of old animals due to reduced reproduction during winter [15-17]. To understand the seasonal variation in the epidemiology of *E.* multilocularis, it is therefore important to know to what extent different age classes of the intermediate hosts develop the infective stages (protoscoleces) for the final hosts.

We investigated the influence of temporal and spatial factors on the prevalence of *E. multilocularis* in *A. terrestris*, the most abundant intermediate host in the city of Zürich, Switzerland. Furthermore, we analysed how age affects the prevalence and the development of protoscoleces and whether low temperatures (as a proxy for the winter season) and high humidity correlate with infection risk. The same analyses were undertaken for *Taenia taeniaeformis*, another *taeniid* species with domestic cats as principal definitive host and *A.*

terrestris as a frequent intermediate host. Eggs of *Taenia* species have a similar resistance to freezing [18] and desiccation [19] as those of *E. multilocularis*, suggesting similarities concerning seasonal variations in the infection pressure on intermediate hosts.

Materials and methods

Study area and animals

The study was conducted in the periphery of the city of Zurich, Switzerland, and in the nearby municipality of Rifferswil (Fig. 1). From March 2007 to June 2008, a total of 856 *A. terrestris*, 252 animals in Zurich and 604 in Rifferswil, were collected. These animals were not trapped for the purpose of this study but rather in the framework of a continuous control program to avoid agricultural damages on grassland areas. Field workers used unbaited Topcat traps (Topcat GmBH, L'Auberson; Switzerland) and tongue traps (Hauptner Instrumente GmbH, Dietlikon, Switzerland), which were placed in vole galleries.

The voles were either dissected immediately after trapping or stored in a chest freezer at a constant temperature of -20ºC prior to dissection. Careful examination was performed at the opening of thoracic and peritoneal cavities, and organs, in particular the liver, were attentively examined for lesions. Metacestodes were collected and identified after morphological characteristics. *Taenia taeniaeformis* was determined by counting all lucent, round-shaped vesicles of 3-10 mm size. All other lesions with a diameter of > 3 mm were cut into small pieces and investigated for the presence of protoscoleces of *E. multilocularis*. If protoscoleces were present, the metacestode material was squashed in a sieve with 1 mm mesh size and washed with PBS. Protoscoleces were counted under a binocular microscope in a petri dish. If more than 100 protoscoleces were present, 3 diluted subsamples of 100 µl were counted microscopically and the total number was calculated. Visually unidentifiable lesions were further investigated after proteinase K digestion by a PCR specific for *E. multilocularis* [20].

Age determination of rodents

The absolute age was calculated by measuring the weight of dry crystalline lenses [21, 22] according to Burlet et al. [23]. In short, after dissection, eyes were put directly into formalin (10%) for fixation over a period of 4 weeks. Lenses were then removed from the eye, air-dried at +80°C over a period of 48 hours and immediately weighted. The age of individual voles was calculated by the formula $x=e^{\frac{y-1.858}{1.202}}$, where x is the age in months and y the eye lens weight in mg. As freezing increases lens weights of *A. terrestris* by 3.3%, lens weights of frozen lenses were divided by 1.033 to obtain a correct age estimate [23].

Determination of seasonal and climatic factors

The date of birth of each vole was calculated based on its age and trapping day. To analyse the influence of meteorological factors, the means of day temperature (measured 5 cm above ground) and of precipitation experienced by each individual was calculated (data source: Swiss Meteorological Institute MeteoSwiss; weather station Zürich Fluntern, 8°34'/47°23').

Statistical analyses

Prevalences of *E. multilocularis* and *T. taeniaeformis* were analysed using logistic regression models. The variables age (age of individuals expressed in months), period (time when an individual was trapped: March - June 2007, July – October 2007, November 2007 – February 2008, March – June 2008), area (trapping areas 1-4, Fig. 1), mean day temperature [°C] and mean precipitation per day of living [mm] were selected as independent variables for the modelling procedure.

Models were fitted using all possible combinations of the selected predictor variables. Best models were selected using Akaike´s information criterion (AIC, [24]) corrected for small samples sizes. Only models with ΔAICc < 2 compared to the model with the lowest AICc were selected. Akaike model weights were calculated [25] to determine the degree by which a model was supported by the data.

Logistic regressions were calculated using SPSS 17.0 [24]. Maximum likelihood estimate of k was used to calculate the degree of overdispersion of the number of protoscoleces in infected rodents. This parameter of negative binomial distribution tends towards 0 with increasing accumulation of parasites [26].

Results

Age determination revealed strong shifts in the age structure of the *A. terrestris* population over time (Fig. 2). During the first period (March-June 2007) the portion of animals older than 5 months (48.6%, CI 95% 36.9%-60.6%) was significantly higher than in the same period one year later (March-June 2008: 26.8%, 21.7%-32.5%). Mean day temperatures per month during the second year of the study (July 07 – June 08) were consistently (unless August 2007 and May 2008) higher than during the preceding year (in average 2.0°C).

Liver lesions were observed in 270 of 856 dissected *A. terrestris* (31.5%, CI 95% 28.4%-34.8%). The overall prevalence rate of *E. multilocularis* was 15.1% (12.7%-17.6%), and protoscoleces were found in 12 animals corresponding to 1.4% (0.7%-2.4%) of all studied animals or 9.3% (4.9%-15.7%) of the *E. multilocularis*-positive animals. Animals older than 7 months were more than 4-times more frequently infected than animals ≤ 3 months.

Furthermore, none of the animals under 3 months of age harboured protoscoleces (Table 1), and the youngest animal with protoscoleces was 3.2 months of age. In 10 animals the protoscolex burden was determined. The maximum likelihood estimate of k = 0.16 indicates a heavily overdispersed protoscolex burden. Four animals (40%) harboured between 61 and 568 protoscoleces (together 1057 protoscoleces), representing 0.2% of the total number, five animals had between 2492 and 67'550 protoscoleces, and the extrapolated number of protoscoleces was 451'540 in one animal, representing 73.8% of this parasite stage identified in this study. However, no relation between age and the number of protoscoleces was identified (Spearman R = -0.52, p=0.12; Fig. 3).

The overall prevalence of *T. taeniaeformis* was 23.4% (20.6%-26.3%). Animals older than 5 months were roughly 2-times more frequently infected than animals ≤ 3 months (Table 1). Most infected animals harboured one *T. taeniaeformis* strobilocercus but 25 out of 200 infected animals had 2-10 strobilocerci (Fig. 3). The proportion of multiple infections was not dependent on the age of the animals (Spearman R = 0.02, p=0.82): Seventeen of 133 (12.8%) infected animals of <5 months of age and 8 of 67 (11.9%) infected animals of ≥ 5 months of age had more than one strobilocercus. In total, five of the ten animals with known *E. multilocularis* protoscoleces burden were simultaneously infected with *T. taeniaeformis*, which is a significant higher proportion than expected by chance (Actus randomization test, p<0.05). Three of these five animals harboured 2, 8 and 10 strobilocerci and, interestingly, the animal with 10 strobilocerci also had the highest protoscoleces burden. In addition, 1.9% (1.1%-3.0%) of all animals were infected with *T. crassiceps*. Scoleces of this species were mostly found in subcutaneous cysts but also pleural cavities.

The model selection procedure for *E. multilocularis* infections revealed two best models (ΔAICc < 2; Table 2), containing 'age', 'period', 'area', 'mean day temperature' and 'mean precipitation'' as factors for explaining the prevalence (Table 3). Prevalence rates of *E.*

multilocularis differed strongly between the four trapping areas (Fig. 4a) and ranged between 11.2% (95% CI 12.7-27.2, area 3) and 60.7% (CI 40.6-78.5%, area 2). Furthermore, low temperatures significantly increased the infection risk (Table 3). The second best model suggests that higher precipitation is associated with a higher infection risk but this effect was not significant (Table 3).

The model selection procedure for *T. taeniaeformis* revealed 4 best models (ΔAICc < 2; Table 2) which include the factors 'age', 'period', 'area', 'mean day temperature' and 'mean precipitation' as factors explaining parasite prevalence (Table 3). In animals with ages of 5 months or higher, the prevalence was significantly higher than in juvenile animals (Table 1), and prevalences were lower in area 1 than in area 4 (Table 3 and Figure 4b). Although each of the three factors 'period', 'mean temperature' and 'mean precipitation' entered one of the four best models, the 95% confidence intervals of the odds ratios strongly overlapped the value 1 for all three factors.

Discussion

Prevalence of *E. multilocularis*

This study shows that the transmission dynamics of the two taeniid species *E. multilocularis* and *T. taeniaeformis* are significantly affected by spatial factors. The prevalence rates of *E. multilocularis* in *A. terrestris* were significantly higher (95% CI: 40.6-78.5%) in one study area as compared to the others and surpassed, to our knowledge, the highest ever reported prevalence of 39% in intermediate hosts in Central Europe [27]. This finding confirms the occurrence of micro-foci [28-30] with exceptional high *E. multilocularis* infection pressure in densely populated areas and possibly reflects the high fox densities in urban and periurban areas [2, 3, 31].

Transmission dynamics of the two investigated taeniid species can be affected by various host-related factors. In addition to the densities of final (foxes for *E. multilocularis* and domestic cats for *T. taeniaeformis*) and intermediate hosts [32-34], the predation activity of final hosts [17, 35] can play an important role in transmission [7, 36]. Furthermore, temporal fluctuations of prevalences can origin from shifts in the age structure of populations [37]. Once a metacestode has established, it can be detected for the rest of the rodent's life. Therefore, infections accumulate with increasing age in single vole generations and prevalences increase. In previous studies done in Zurich and the city of Geneva, *E. multilocularis* prevalences were 10.7 and 9.2%, in adult voles respectively, and 1.3% in subadults and juveniles [20, 38]. In this study, we also recorded a higher prevalence in adult voles. Furthermore, we documented an increase of prevalence rates over several age classes (Table 1).

The age structure of voles is closely related to seasonal factors. In early spring, old animals predominate as reproduction is low in winter [16, 17, 37]. Nevertheless, season-related age structure can vary considerably from year to year, as shown by our data with a higher proportion of old overwintering voles in spring 2007 (Fig. 2) after an extraordinary mild winter [39, 40]. As age strongly affects parasite prevalence, such temporal variations in the age structure over years can hamper the detection of seasonal variation in the infection pressure.

As shown in this study, the determination of the absolute age of intermediate hosts can help to overcome such methodological limitations. Based on this data, it was possible to calculate for each individual rodent to which temperatures it was exposed during its live. Hence, it could be demonstrated that low day temperatures typical for the winter season correlate with a higher infection rate. Absolute age estimates were also considered in the only study we are aware of that also described a clear season-related infection pattern of *E. multilocularis* [37].

As in our study for *A. terrestris*, seasonal patterns of infection were found for *M. arvalis* with highest prevalences of *E. multilocularis* in spring in old over-wintered animals which had acquired their infections in winter (from October to April).

Staubach et al. [41]demonstrated that *E. multilocularis*-infected foxes are more frequently found in areas with high soil moisture]. Correspondingly, our second best model explaining the prevalence of *E. multilocularis* in *A. terrestris* included the factor precipitation. However, the effect size of this factor alone was too small to demonstrate a clear relationship with the infection risk. Soil moisture is not only affected by precipitation but also by many other factors (e.g. temperature, vegetation growth and sun exposition [Hagan 1955]) and a direct measure of soil moisture could be a better predictor than the amount of precipitation to explain the infection pressure.

The fact that living during periods with low temperatures contributed significantly to the infection risk with *E. multilocularis* underlines the epidemiological relevance of the experimental study of Veit and colleagues [10] showing that *E. multilocularis* eggs survive for months under cold conditions but die within a few hot and dry days. Another aspect that possibly contributes to seasonal patterns in the parasite's transmission dynamics is a seasonal pattern of the predation on *A. terrestris* and other rodents by foxes [7, 17, 36]. These studies revealed higher predation rates in autumn with a larger availability of voles than in spring, when rodent density are generally considerably lower [7, 42]. The higher consumption possibly explains higher prevalence rates in foxes during winter months as revealed by several studies in high endemic areas [43, 44] and consequently, a higher contamination of the environment with *E. multilocularis* eggs.

The presence of protoscoleces was clearly related to the age of the investigated animals. The low number of animals with protoscoleces made it impossible to perform multivariable analyses in order to investigate seasonal effects on the prevalence of protoscoleces harbouring animals. However, it is expected that season-related changes in the age structure of the intermediate host population cause that there is a higher proportion of animals harbouring protoscoleces during winter and early spring, before the start of reproduction. This does not necessarily mean that foxes are exposed to a higher infection pressure during this period, as absolute density of voles and predation on rodents by foxes is considerably lower during spring and early summer [17, 36, 45].

Prevalences of *T. taeniaeformis*

Similar to *E. multilocularis*, *T. taeniaeformis* causes lifelong infections in intermediate hosts and is more prevalent in older animals [20, 38, 46-48]. Furthermore, eggs of different taeniid species have a similar resistance to environmental conditions as those of *E. multilocularis* [10, 19]. Accordingly, two other studies reported higher prevalence rates in overwintered intermediate hosts [15, 47]. In contrast,, we found no clear association of low temperatures and high precipitation with higher prevalence rates of *T. taeniaeformis*. The eggs of this parasite might be less sensitive to hot and dry weather condition due to the special defecation behavior of domestic cats which usually burry their droppings into loose soil where taeniid eggs are less exposed to adverse weather conditions. Further, the loose ground of the burrows of *A. terrestris* could be a good substrate for cats to defecate.

Contrary to Reperant and colleagues [38], we found significant spatial variations in the prevalence of *T. taeniaeformis*. However, in contrast to Le Pesteur [15], the observed pattern did not correspond to the spatial variation of *E. multilocularis* prevalence rates. Probably, these different spatial patterns are caused by different distributions of fox and cat densities. Interestingly, the lowest prevalence rates for *T. taeniaeformis* were found in an area (area 1) where many people walk their dogs and this might contribute to a lower presence of domestic cats.

Although 23% of all investigated animals were infected with *T. taeniaeformis*, only 13% of these 200 animals harbored more than one strobilocercus. Furthermore, increasing age was not linked to a higher amount of animals with more than one strobilocercus and the prevalences were similar in animals >7 months and those of 5 to ≤ 7 months (Table 1). This parasite has not a proliferative growth like *E. multilocularis* [49] and it is unlikely that it caused an increase of the mortality of infected intermediate hosts which could explain such an asymptotic increase of prevalence along the age classes. Possibly *T. taeniaeformis* was hyperendemic in *A. terrestris* and regulated by a concomitant immunity. Such regulations have been demonstrated with experimental *T. taeniaeformis* infections of rats [50, 51] and by epidemiological investigations of sheep and goats infected with *T. hydatigena* [52]. Interestingly, the fact that heavily *E. multilocularis*-infected voles had more *T. taeniaeformis* strobilocerci than expected indicates an immuno-suppression driven by *E. multilocularis* metacestode [53], which counter-acts the protective immune mechanisms against super-infections with *T. taeniaeformis*.

Conclusions

Our results demonstrate that the availability of absolute age estimates of intermediate hosts can be crucial to detect season-related variations in the infection pressure by taeniid species. As shown for *E. multilocularis*, infection pressure on voles can vary considerably within a small spatial scale and along different seasons. This knowledge possibly can also contribute to model spatial and temporal variation of the infection risk for human. Based on the presented results, we suggest that reducing the infection pressure on intermediate host and presumably as well on humans by the delivery of anthelmintic baits for foxes is more effective during the cold and humid winter season than during the rest of the year.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

PB did the laboratory work, analyzed the data, participated in the statistical analyses and drafted the manuscript. PD and DH designed and supervised the study and critically revised the manuscript. Additionally, DH contributed to data analyses and statistics. All authors read and approved the final manuscript.

Acknowledgements

We are grateful to Marianne Fritzsche from Grün Stadt Zürich and to Andreas Weidmann, Simon Franz and all other field workers that delivered the trapped rodents. We thank Alexander Mathis and Paul Torgerson for valuable comments on the manuscript and acknowledge the support of our research activities by the Swiss Federal Veterinary Office (Nr. 1.07.04). This work represents part of the dissertation of Pierre Burlet, veterinarian.

References

1. Chautan M, Pontier D, Artois M: **Role of rabies in recent demographic changes in Red Fox (*Vulpes vulpes*) populations in Europe.** In *1st Euro-American Mammal*

Congress; *Jul 19-24; Santiago Composte, Spain*. Museum Nat Hist Naturelle; 1998: 391-410.

2. Deplazes P, Hegglin D, Gloor S, Romig T: **Wilderness in the city: the urbanization of *Echinococcus multilocularis*.** *Trends Parasitol* 2004, **20:**77-84.
3. Gloor S, Bontadina F, Hegglin D, Deplazes P, Breitenmoser U: **The rise of urban fox populations in Switzerland.** *Mamm Biol* 2001, **66:**155-164.
4. Romig T, Thoma D, Weible AK: ***Echinococcus multilocularis* - a zoonosis of anthropogenic environments?** *J Helminthol* 2006, **80:**207-212.
5. Fischer C, Reperant LA, Weber JM, Hegglin D, Deplazes P: ***Echinococcus multilocularis* infections of rural, residential and urban foxes (*Vulpes vulpes*) in the canton of Geneva, Switzerland.** *Parasite* 2005, **12:**339-346.
6. König A, Romig T, Thoma D, Kellermann K: **Drastic increase in the prevalence in *Echinococcus multilocularis* in foxes *(Vulpes vulpes*) in southern Bavaria, Germany.** *Eur J Wildl Res* 2005, **51:**277-282.
7. Robardet E, Giraudoux P, Caillot C, Boue F, Cliquet F, Augot D, Barrat J: **Infection of foxes by *Echinococcus multilocularis* in urban and suburban areas of Nancy, France: Influence of feeding habits and environment.** *Parasite* 2008, **15:**77-85.
8. Schweiger A, Ammann RW, Candinas D, Clavien PA, Eckert J, Gottstein B, Halkic N, Muellhaupt B, Prinz BM, Reichen J, et al: **Human alveolar echinococcosis after fox population increase, Switzerland.** *Emerg Infect Dis* 2007, **13:**878-882.
9. Torgerson PR, Schweiger A, Deplazes P, Pohar M, Reichen J, Ammann RW, Tarr PE, Halkik N, Mullhaupt B: **Alveolar echinococcosis: from a deadly disease to a well-controlled infection. Relative survival and economic analysis in Switzerland over the last 35 years.** *J Hepatol* 2008, **49:**72-77.

10. Veit P, Bilger B, Schad V, Schafer J, Frank W, Lucius R: **Influence of environmental factors on the infectivity of *Echinococcus multilocularis* eggs.** *Parasitology* 1995, **110:**79-86.

11. Eckert J, Deplazes P: **Biological, epidemiological and clinical aspects of echinococcosis: a zoonosis of increasing concern.** *Clin Microbiol Rev* 2004, **17:**107-135.

12. Hausser J, Saucy F, Bourquin J-D, Longschamp C, Arlettaz R, Fumagalli L: *Säugetiere der Schweiz:Verbreitung – Biologie - Ökologie.* Birkhäuser Verlag, Basel, Schweizerische Gesellschaft für Wildtierbiologie; 1995.

13. Fichet-Calvet E, Pradier B, Quéré JP, Giraudoux P, Delattre P: **Landscape composition and vole outbreaks: evidence from an eight year study of *Arvicola terrestris*.** *0906-7590* 2000, **23:**659-668.

14. Giraudoux P, Delattre P, Habert M, Quéré JP, Deblay S, Defaut R, Duhamel R, Moissenet MF, Truchetet DSD: **Population dynamics of fossorial water vole (*Arvicola terrestris scherman*): A Land use and landscape perspective.** *Agric Ecosyst Environ* 1997, **66:**47-60.

15. Le Pesteur MH, Giraudoux P, Delattre P, Damange JP, Quere JP: **Spatiotemporal distribution of four species of cestodes in a landscape of mid-altitude mountains (Jura France).** *Ann Parasitol Hum Comp* 1992, **67:**155-160.

16. Morel J: **Le campagnol terrestre, *Arvicola terrestris (L.)*, en Suisse: biologie et systématique (mammalia, rodentia).** *PhD Thesis, Université de Lausanne* 1981**:**85.

17. Weber JM, Aubry S: **Predation by foxes, *Vulpes vulpes*, on the fossorial form of the water vole, *Arvicola terrestris scherman*, in western Switzerland.** *J Zool* 1993, **229:**553-559.

18. Lucker JT: **A test of the resistance of *Taenia saginata* eggs to freezing.** *J Parasitol* 1960, **46:**304-304.

19. Laws GF: **Physical factors influencing survival of taeniid eggs.** *Exp Parasitol* 1968, **22:**227-239.

20. Stieger C, Hegglin D, Schwarzenbach G, Mathis A, Deplazes P: **Spatial and temporal aspects of urban transmission of *Echinococcus multilocularis*.** *Parasitology* 2002, **124:**631-640.

21. Martinet L: **Détermination de l'âge chez le campagnol des champs (*Microtus arvalis pallas*) par la pesée du cristallin.** *Mammalia* 1966, **30:**425-430.

22. Morris P: **A review of mammalian age determination methods.** *Mamm Rev* 1972, **2:**69-103.

23. Burlet P, Hegglin D, Deplazes P: **Efficient age determination: how freezing affects eye lens weight of the small rodent species *Arvicola terrestris*.** *Eur J Wildl Res* 2010, **in press**.

24. Norusis MJ: *SPSS/PC + Advanced Statistics SPSS.* Chicago, IL, USA.; 1986.

25. Burnham K, Anderson D: *Model Selection and Inference: A Practical Information-theoretic Approach.* Springer-Verlag, New York; 1998.

26. Wilson K, Grenfell BT: **Generalized linear modelling for parasitologists.** *Parasitol Today* 1997, **13:**33-38.

27. Gottstein B, Saucy F, Wyss C, Siegenthaler M, Jacquier P, Schmitt M, Brossard M, Demierre G: **Investigations on a Swiss area highly endemic for *Echinococcus multilocularis*.** *Appl Parasitol* 1996, **37:**129-136.

28. Giraudoux P, Delattre P, Takahashi K, Raoul F, Quéré JP, Craig P, Vuitton D, In p, Press I: **Transmission ecology of *Echinococcus multilocularis* in wildlife: what can be learned from comparative studies and multiscale approaches? .** In *Cestode*

Zoonoses: Echinococcosis and Cysticercosis. Edited by Craig P, Pawlowski Z; 2002: 251-266

29. Tackmann K, Löschner U, Mix H, Staubach C, Thulke H-H, Conraths FJ: **Spatial distribution patterns of *Echinococcus multilocularis* (Leuckart 1863) (Cestode: *Cyclophylldea*: *Taeniidae*) among red foxes in an endemic focus in Brandenburg, Germany.** *Epidemiol Infect* 1998, **120:**101-109.

30. Hansen F, Jeltsch F, Tackmann K, Staubach C, Thulke HH: **Processes leading to a spatial aggregation of *Echinococcus multilocularis* in its natural intermediate host *Microtus arvalis*.** *Int J Parasitol* 2004, **34:**37-44.

31. Harris S: **The food of suburban foxes (*Vulpes vulpes*), with special reference to London.** *Mamm Rev* 1981, **11:**151-168.

32. Raoul F, Michelat D, Ordinaire M, Decote Y, Aubert M, Delattre P, Deplazes P, Giraudoux P: ***Echinococcus multilocularis*: secondary poisoning of fox population during a vole outbreak reduces environmental contamination in a high endemicity area.** *Int J Parasitol* 2003, **33:**945-954.

33. Saucy F: **Density dependence in time series of the fossorial form of the water vole, *Arvicola terrestris*.** *Oikos* 1994, **71:**381-392.

34. Viel JF, Giraudoux P, Abrial V, Bresson-Hadni S: **Water vole (*Arvicola terrestris scherman*) density as risk factor for human alveolar echinococcosis.** *Am J Trop Med Hyg* 1999, **61:**559-565.

35. Raoul F, Deplazes P, Rieffel D, Lambert J, Giraudoux P: **Predator dietary response to prey density variation and consequences for cestode transmission.** *Oecologia (Berlin)* 2010, **164:**129-139.

36. Hegglin D, Bontadina F, Contesse P, Gloor S, Deplazes P: **Plasticity of predation behaviour as a putative driving force for parasite life-cycle dynamics: the case of**

urban foxes and *Echinococcus multilocularis* tapeworm. *Func Ecol* 2007, **21:**552-560.

37. Delattre P, Pascal M, Le Pesteur M-H, Giraudoux P, Damange J-P: **Caractéristiques écologiques et épidemiologiques de l'*Echinococcus multilocularis* au cours d'un cycle complet des populations d'un hôte intermédiaire (*Microtus arvalis*).** *Can J Zool* 1988, **66:**2740-2750.

38. Reperant LA, Hegglin D, Tanner I, Fischer C, Deplazes P: **Rodents as shared indicators for zoonotic parasites of carnivores in urban environments.** *Parasitology* 2009, **136:**329-337.

39. Defila C: **Phänologischer Rückblick ins Jahr 2007.** *Agrarforschung* 2008, **15:**220-223.

40. Defila C: **Phänologischer Rückblick ins Jahr 2008.** *Agrarforschung* 2009, **16:**158-162.

41. Staubach C, Thulke HH, Tackmann K, Hugh-Jones M, Conraths FJ: **Geographic information system-aided analysis of factors associated with the spatial distribution of *Echinococcus multilocularis* infections of foxes.** *Am J Trop Med Hyg* 2001, **65:**943-948.

42. Weber JM, Dailly L: **Food habits and ranging behaviour of a group of farm cats (*Felis catus*) in a Swiss mountainous area.** *J Zool* 1998, **245:**234-237.

43. Hofer S, Gloor S, Muller U, Mathis A, Hegglin D, Deplazes P: **High prevalence of *Echinococcus multilocularis* in urban red foxes (*Vulpes vulpes*) and voles (*Arvicola terrestris*) in the city of Zurich, Switzerland.** *Parasitology* 2000, **120:**135-142.

44. Brossard M, Andreutti C, Siegenthaler M: **Infection of red foxes with *Echinococcus multilocularis* in western Switzerland.** *J Helminthol* 2007, **81:**369-376.

45. Yoneda M: **Influence of Red Fox Predation Upon a Local-Population of Small Rodents .III. Seasonal-Changes in Predation Pressure, Prey Preference and Predation Effect.** *Appl Entomol Zool* 1983, **18:**1-10.

46. Deter J, Berthier, K., Chaval Y., Cosson, J.F., Morand, S., Charbonnel, N.: **Influence of geographical scale on the detection of density dependence in the host parasite system, *Arvicola terrestris* and *Taenia taeniaeformis*.** *Parasitology* 2005**:**596-605.

47. Fichet-Calvet E, Giraudoux P, Quéré JP, Ashford RW, Delattre P: **Is the prevalence of *Taenia taeniaeformis* in *Microtus arvalis* dependent on population density?** *J Parasitol* 2003, **89:**1147-1152.

48. Theis JH, Schwab RG: **Seasonal prevalence of *Taenia taeniaeformis* - relationship to age, sex, reproduction and abundance of an intermediate host (*Peromyscus maniculatus*).** *J Wildl Dis* 1992, **28:**42-50.

49. Eckert J, Friedhoff KT, Zahner H, Deplazes P: *Lehrbuch der Parasitologie für die Tiermedizin.* Stuttgart, Germany: Enke Verlag; 2005.

50. Lightowlers MW: **Fact or hypothesis: concomitant immunity in taeniid cestode infections.** *Parasite Immunol*, **32:**582-589.

51. Miller HM: **Immunity of the albino rat to superinfestation with *Cysticercus fasciolaris*.** *J Prevent Medicin* 1931, **5:**453–464.

52. Torgerson PR, Williams DH, Abo-Shehada MN: **Modelling the prevalence of *Echinococcus* and *Taenia* species in small ruminants of different ages in northern Jordan.** *Vet Parasitol* 1998, **79:**35-51.

53. Mejri N, Hemphill A, Gottstein B: **Triggering and modulation of the host-parasite interplay by *Echinococcus multilocularis*: a review.** *Parasitology* 2010, **137:**557-568.

Tables

Table 1. Prevalence rates of taeniid infections in trapped *Arvicola terrestris* of different age classes. 95% confidence intervals are shown in brackets (N total = 856).

age class in months (N animals)	≤ 3 (N=436)		>3-5 (N=227)		>5-7 (N=101)		>7 (N=92)	
E. multilocularis	7.6	(5.3-10.5)	19.4	(14.5-25.1)	21.8	(14.2-31.1)	32.6	(23.2-43.2)
E. multilocularis protoscoleces	0.0	(0.0-0.7)	1.3	(0.3-3.8)	3.0	(0.6-8.4)	6.5	(2.4-13.7)
T. taeniaformis	17.9	(14.4-21.8)	24.2	(18.8-30.3)	34.7	(25.5-44.8)	34.8	(25.1-45.4)
T. crassiceps	0.9	(0.3-2.3)	2.6	(1.0-5.7)	5.0	(1.6-11.2)	0.0	(0.0-3.2)

Table 2. Factors affecting prevalences of *Echinococcus multilocularis* and *Taenia taeniaeformis*. All factors are shown that were included in the best models (ΔAICc<2, N = 856).

factors included in best models	AICc	ΔAICc	AICc weight
a) *E. multilocularis*:*			
age, period, area, mean day temperature	-244.04	0	0.57
age, period, area, mean day temperature, mean precipitation sum	-243.45	0.59	0.43
b) *T. taeniaeformis**:*			
age, area	51.08	0.00	0.42
age, area, mean day temperature	52.22	1.14	0.24
age, area, period, mean day temperature	52.75	1.67	0.18
age, area, mean precipitation sum	52.94	1.87	0.16

* Null model AICc = -131.22
** Null model AICc = 83.73

Table 3. Odds ratios of factors explaining prevalences of *Echinococcus multilocularis* and *Taenia taeniaeformis* in *Arvicola terrestris*. Shown are all odds ratios (OR) and 95% confidence intervals (95%CI) for the factors of the best models (ΔAICc < 2, N=856).

	E. multilocularis				*T. taeniaeformis* strobilocerci							
	best model		2nd best model		best model		2nd best model		3rd best model		4th best model	
Model factors	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Age	1.13	1.06-1.20	1.13	1.06-1.20	1.13	1.08-1.20	1.13	1.07-1.20	1.13	1.07-1.20	1.13	1.07-1.20
Period												
-Mar-Jun 07 vs Mar-Jun08	3.92	1.96-7.81	3.58	1.83-6.98					0.88	0.47-1.65		
-Jul-Oct 07 vs Mar-Jun08	1.70	0.75-3.89	1.70	0.75-3.83					0.95	0.63-1.45		
-Nov07-Feb08 vs Mar-Jun08	1.83	1.07-3.14	1.70	1.01-2.85					1.05	0.69-1.58		
Area												
-Area 1 vs 4	1.54	0.85-2.80	1.55	0.86-2.81	0.51	0.28-0.92	0.51	0.28-0.91	0.53	0.29-0.71	0.51	0.28-0.91
-Area 2 vs 4	8.60	3.60-20.50	8.63	3.61-20.66	1.05	0.45-2.46	1.09	0.46-2.55	1.07	0.45-2.51	1.06	0.45-2.48
-Area 3 vs 4	0.94	0.49-1.81	0.94	0.48-1.80	0.47	0.27-0.80	0.49	0.29-0.86	0.48	0.28-0.82	0.47	0.28-0.82
Mean day temperature	0.90	0.84-0.96	0.92	0.87-0.97			0.99	0.96-1.02				
Mean precipitation			1.13	0.90-1.43							0.98	0.88-1.09
Constant	0.09	-	0.11	-	0.22	-	0.26	-	0.22	-	0.24	-

Figure legends

Figure 1. Study areas in the canton of Zurich, Switzerland. Areas 1-3 are situated along the urban periphery of the city of Zurich, area 4 is located in the the municipality of Rifferswil. Number of investigated water voles (*Arvicola terrestris*): N = 99 (area 1), N = 28 (area 2), N = 125 (area 3), N = 604 (area 4).

Figure 2. Age pyramids of *Arvicola terrestris* in the canton of Zurich, Switzerland, in different seasons. The single pyramid segments represent the percentage of the population trapped during the corresponding period. Dark grey: males, light grey: females.

Figure 3. Numbers of *Echinococcus multilocularis* protoscoleces and *Taenia taeniaeformis* strobilocerci in trapped *Arvicola terrestris*.

Figure 4 Temporal prevalences of *Echinococcus multilocularis* and *Taenia taeniaeformis* strobilocerci in 4 study areas. a) Prevalences and 95% confidence intervals of *Echinococcus multilocularis* (undifferentiated and protoscoleces-containing metacestodes), b) prevalences and 95% confidence intervals of *Taenia taeniaeformis* strobilocerci. Overall prevalence rates per area are symbolised by circles. For *E. multilocularis,* the number of voles with protoscoleces and the total number of studied individuals are given above the associated bars (N total = 856 *Arvicola terrestris*). Study areas are shown in Fig. 1.

Figure 1

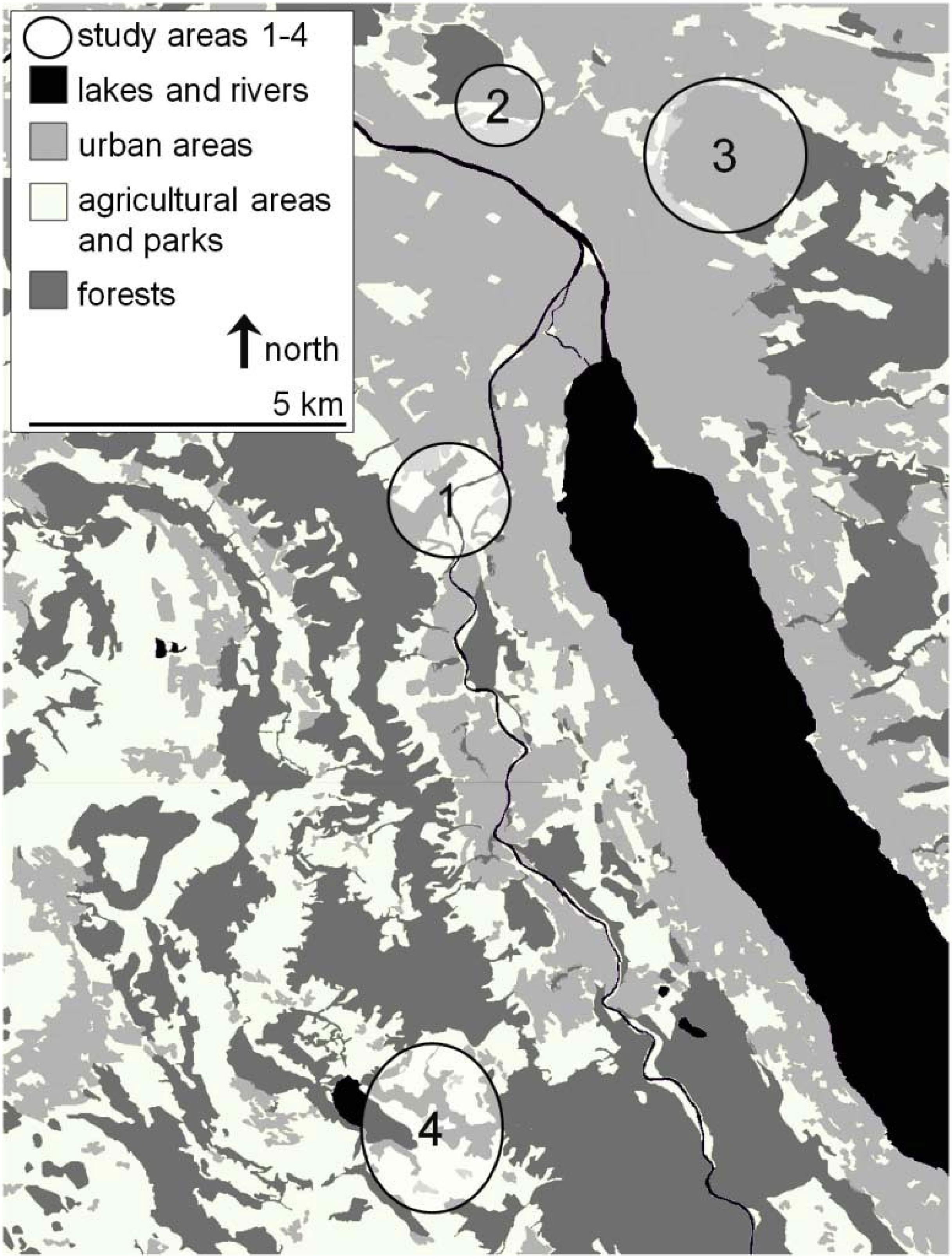
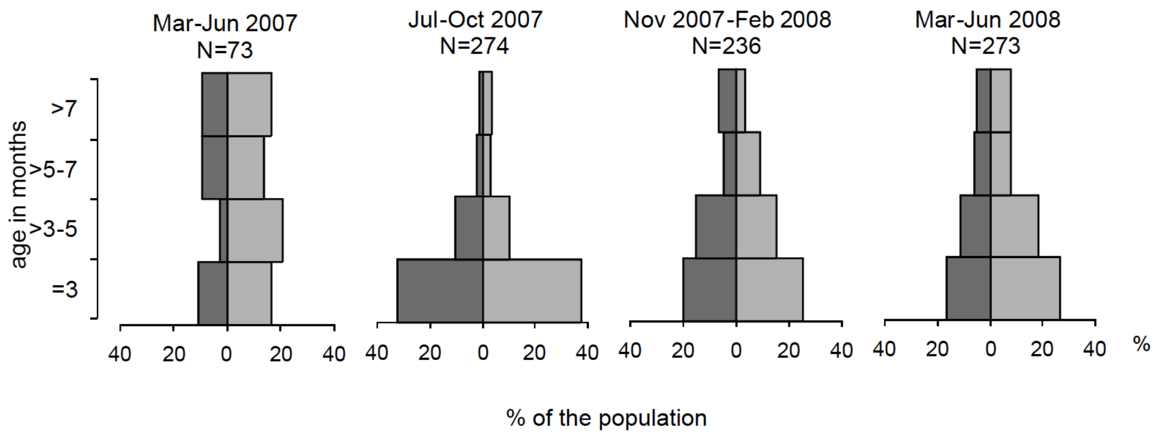

study areas 1-4
lakes and rivers
urban areas
agricultural areas and parks
forests
north
5 km
1
2
3
4

Figure 2



Mar-Jun 2007
N=73
Jul-Oct 2007
N=274
Nov 2007-Feb 2008
N=236
Mar-Jun 2008
N=273
age in months
>7
>5-7
>3-5
=3
40 20 0 20 40
%
% of the population

Figure 3

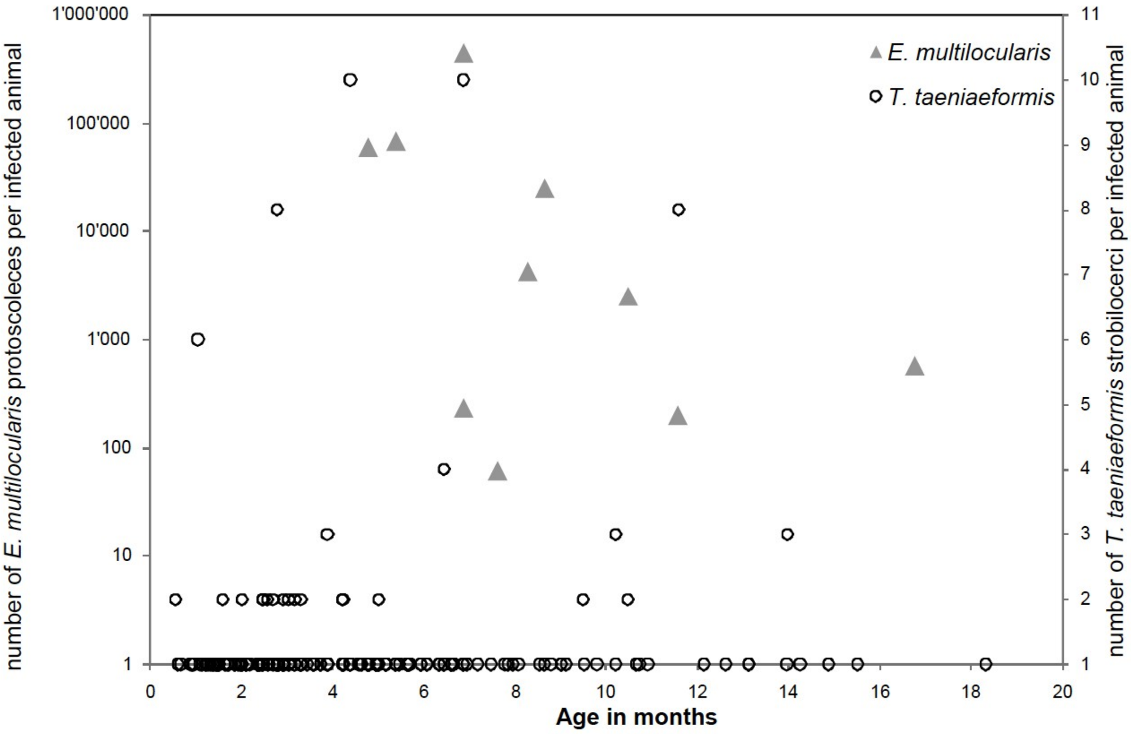
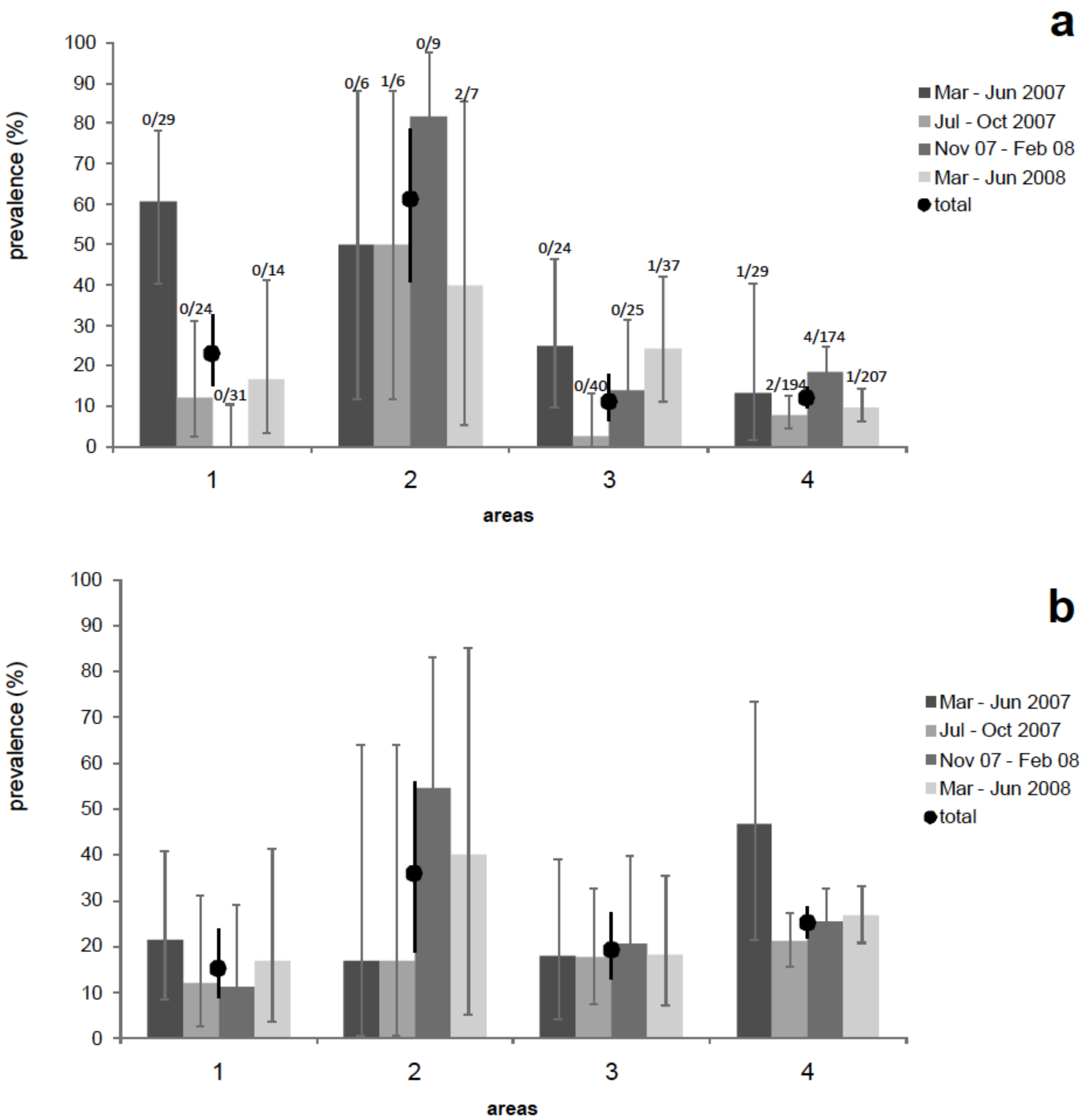

1'000'000
100'000
10'000
1'000
100
10
1
11
10
9
8
7
6
5
4
3
2
1
0
2
4
6
8
10
12
14
16
18
20
Age in months
number of E. multilocularis protoscoleces per infected animal
number of T. taeniaeformis strobilocerci per infected animal
E. multilocularis
T. taeniaeformis

Figure 431


a
prevalence (%)
100
90
80
70
60
50
40
30
20
10
0
0/29
0/24
0/31
0/14
0/6
1/6
0/9
2/7
0/24
0/40
0/25
1/37
1/29
2/194
4/174
1/207
1
2
3
4
areas
Mar - Jun 2007
Jul - Oct 2007
Nov 07 - Feb 08
Mar - Jun 2008
total
b
prevalence (%)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
areas
Mar - Jun 2007
Jul - Oct 2007
Nov 07 - Feb 08
Mar - Jun 2008
total

Efficient age determination: how freezing affects eye lens weight of the small rodent species *Arvicola terrestris*

Pierre Burlet[1], Peter Deplazes[1], Daniel Hegglin[1§]

[1]*Institute of Parasitology, University of Zurich, Winterthurerstr. 266a, CH-8057 Zurich, Switzerland*

[§]Corresponding author

tel. +41(0)44-635-85-01
fax +41(0)44-635-85-07
daniel.hegglin@swild.ch
http://www.uzh.ch/paras/

Abstract

Age determination of animals by measuring the weight of their eye lenses is a widely-used method in wildlife biology. In general, it is recommended to prepare lenses immediately after trapping to avoid errors in the age estimation due to decomposition of lens tissue. However, in many field studies, large numbers of animals need to be trapped over long periods of time, in huge areas and by many different field workers. Therefore, the immediate preparation of eye lenses imposes a considerable logistic constraint that could be avoided by prior freezing of trapped animals. To assess the impact of freezing, lens weights of frozen and unfrozen eyes of 114 *Arvicola terrestris* were compared pair-wise. The frozen lenses weighed at average 3.3% (95% CI: 2.4 – 4.1%) more than the unfrozen ones from the same animals. Freezing time, weight of lenses and mean temperature of the trapping day as an indicator of decomposition speed did not affect the freezing-induced weight increase. Age estimates based on weights of unfrozen lenses varied between 24 and 445 days. Estimates based on frozen lenses were systematically higher. Applying a constant correction factor of 1.033^{-1} for the weight of frozen lenses corrects this overestimation of age. We conclude that age determination with frozen lenses of small rodents can yield valid age estimates if a correction factor for freezing is applied. Thus, age determination can be organized much more efficiently in field studies, which is highly advantageous for many ecological, agricultural and epidemiological research projects.

Key words

age estimation, Arvicola terrestris, calibration, eye lens mass, population dynamic, voles

Introduction

Population dynamics of many rodent species are complex with interfering annual and perennial cycles (Krebs 1996). There is still a debate to what extent different extrinsic forces such as seasonality, food availability, predation and climatic changes (Hanski et al. 2001; Hörnfeldt et al. 2005; Tkadlec and Zejda 1998) and intrinsic factors such as population structure, social stress and maternal effects (Boonstra 1994; Inchausti and Ginzburg 1998; Oli and Dobson 2001) contribute to these cycles which are accompanied by considerable shifts in the age structure of these species (Cerqueira et al. 2006; Janova et al. 2003; Norrdahl and Korpimaki 2002). Efficient and precise methods for age determination are therefore valuable tools to investigate processes that affect the population dynamics of such species.

In wildlife biology, many different methods are used to determine the relative or absolute age of animals (Morris 1972). A common method is the age determination by measuring the weight of formalin-fixed and dried eye lenses (Morris 1972). Lens size increases steadily in a curvilinear manner by continuous proliferation of new lens fibres with a mass that is closely related to its age (Lord 1959; Tanikawa 1993). Although lens weight can be affected by other factors like gender (Janova et al. 2007) and season (Martinet and Spitz 1971; Pokrovskij 1971), the impact of these factors is far less pronounced than their impact on other age related parameters as size and body weight (Augusteyn 2008; Friend 1967). Therefore the weight of dried eye lenses is considered as a good marker for the absolute age if calibrated with animals of known age (Morris 1972).

Different authors recommend to prepare eye lenses immediately after trapping to avoid decomposition processes that could affect the age estimation (Friend 1967; Montgomery 1963; Rongstad 1966). However, in many field studies, large numbers of animals need to be trapped over long periods of time, in huge areas and by many different field workers who might neither have the training nor the facilities for the immediate preparation of eye lenses. Therefore, prior freezing of animals would increase the efficiency of material collection. However, conflicting results have been published documenting an impact of freezing on eye lens weight (Broekhuizen 1971; Montgomery 1963; Pelton 1970) or no effect (Friend 1967; Kauhala and Soveri 2001; Longhurst 1964; Millar and Iverson 1976).

The water vole *Arvicola terrestris* is a very abundant rodent species in Western and Central Europe (Görner and Hackethal 1988) and an important prey species (Weber et al. 2002). It is one of the most important agricultural pests (Morilhat et al. 2007). Furthermore, *A. terrestris* is an important intermediate host for the fox tapeworm *Echinococcus multilocularis* which causes alveolar echinococcosis, a severe human liver disease (Eckert and Deplazes 2004; Eckert et al. 2001). An efficient method for assessing the age structure in *A. terrestris* populations over time and space would contribute to investigations addressing different ecological, agricultural and epidemiological questions. In this study, we therefore analysed whether and to what extent freezing affects eye lens weights of this rodent species.

Methods

From January 2007 to August 2008, a total of 281 *A. terrestris* were trapped in the periphery of the city of Zurich (Switzerland), in the framework of a rodent control program in agriculture. Field workers used unbaited Topcat traps (Topcat GmBH, L'Auberson; Switzerland) and tongue traps (Hauptner Instrumente GmbH, Dietlikon, Switzerland) which were inserted into vole galleries in grassland areas. All rodents were brought to the laboratory directly after trapping and were not frozen before dissection.

To analyse the effect of freezing on eye lens weight, eyes of 131 animals were removed and fixed in formalin (10%) for 4 weeks, one of each pair directly after trapping and the others after a defined period in a chest freezer at constant temperature of -20ºC (sample A). All eyes of the remaining 150 animals were fixed without prior freezing (sample B). After the fixation, eyes were slit open and the lenses were removed by applying light pressure. Lenses were cleaned from remaining tissue, carefully dried with a soft paper towel, put into open vials and air-dried at +80°C in a hybridisation oven for 48 hours. To minimize the exposure to atmospheric moisture, individual lenses were removed separately from the oven just before weighting with a microbalance (AT260 Delta Range, Mettler Toledo, Greifensee, Switzerland). A subset of eight lens pairs was repeatedly weighed during a drying period of 168 hours (immediately, after 1, 2, 4, 8, 24, 28, 32, 48, 52, 120, 124, 128, 144, 148, 152, 168 hours) to identify the optimal drying time.

Highly asymmetric lens pairs (deviation from mean lens weight ratio > 1.5 interquartile ranges) were excluded from our analyses. Weights of frozen and unfrozen lenses were compared by a paired t-test. The weight ratio of each frozen lens to its unfrozen counterpart was used to assess the impact of lens weight, freezing time, day temperature (as an indicator for the decomposition speed after trapping) and gender on a possible freezing effect by performing multivariate linear regression analyses.

To calculate absolute ages, we used results from two studies (Boujard 1982; Morel 1981), where the relationship between absolute age and lens weight was calculated based on the data of *A. terrestris* individuals of known age. According to these studies, we use the equation

$x = e^{\frac{y-h}{m}}$, where x is the age in months, y the weight of a single eye lens in mg, m the slope and h the axis intercept. The authors calculated population-specific values for m and h for different populations in Switzerland (Morel 1981) and the adjacent French Jura mountains (Boujard 1982). However, these values were based on small sample sizes (sample sizes: 12-116 animals, median 33 animals) and might rather reflect random variations than population-specific differences, which are unlikely according to other studies (Augusteyn 2007, 2008).

Therefore, we considered taking the mean parameter values h = 1.858 and m = 1.202 as best approximation for estimating the absolute age.

All statistical tests were performed using SPSS software vs. 17.0.

Results

The mean weight of the 8 lens pairs that were oven-dried was 68% higher before drying than the final weight after 168 hours drying time. During the first day of the drying process, the relative weight dropped sharply. After 48 hours the mean weight of the lenses did not differ significantly from their final weight (mean deviation 0.3%, 95%-CI -0.4-1.0%). Therefore, all other lenses were dried for 48 hours.

The weight ratios of frozen to unfrozen lenses (sample A) showed a similar variation (SD 0.147) as the weight ratios of unfrozen lens pairs (sample B, SD 0.186, levene's test of homogeneity of variance: $F = 0.67$, $p = 0.41$). After removing all outliers (n= 17), 114 lens pairs remained for the pair-wise comparison of frozen and unfrozen lenses (sample A). The weight ratio of small lens pairs (frozen lens ≤ 3 mg) was higher (SD 0.049) than the ratio of larger lens pairs (frozen lens > 4 mg, SD 0.028, $F = 11.5$, $p = 0.001$). The frozen lenses weighed on average 3.3% more (95% CI: 2.4 – 4.1%) than the unfrozen ones of the same animals ($t = -8.12$, df 113, $p < 0.0001$). Multivariate linear regression models were constructed to predict the freezing-induced weight increase by freezing time (range: 27 to 251 days), mean day temperature (-3.3ºC to 19.4ºC), lens weight (1.6 to 5.1 mg) and gender. Models were built with all possible combinations of the independent variables, but no model revealed a significant influence of any of these factors.

Age estimates of the investigated animals based on weights of unfrozen lenses varied between 24 and 445 days. A comparison with the frozen lenses revealed that freezing causes an overestimation of age, e.g. by 39 days for one year old animals. Considering the average freezing-induced weight increase by applying a constant factor of 1.033^{-1} for the weight of frozen lenses corrects this overestimation (Fig. 1).

Discussion

The freezing of lenses resulted in moderate but significant higher weights which is in contradiction to other studies reporting either no (Friend 1967; Kauhala and Soveri 2001; Longhurst 1964; Millar and Iverson 1976) or a weight-reducing effect (Broekhuizen 1971; Montgomery 1963; Pelton 1970) of freezing. However, weight differences between frozen and unfrozen lenses in all these studies were moderate. Furthermore small sample sizes (Friend 1967; Millar and Iverson 1976) or a less sensitive approach of comparing groups of frozen and unfrozen animals instead of applying pair-wise comparisons (Kauhala and Soveri 2001) were used. A reason for the lower weights of frozen lenses found in some studies could be that much larger animals like e.g. racoons (Montgomery 1963) or leporid species (Broekhuizen 1971; Pelton 1970) were investigated. Such species need more time to thaw and therefore decomposition processes could progress further as compared to voles where the eyes can be removed and put into formalin directly after taking the animals out of the freezer. However, to avoid any effects of decomposition, we recommend freezing voles as fast as possible after trapping.

The unexpected increase of eye lens weights after freezing could be caused by increased incorporation of formaldehyde into freezing induced lesions providing a larger surface. The ratio of frozen to unfrozen lenses showed a higher variation for small eye lens pairs, which can be explained by the weighting precision of 0.1 mg. Therefore, the use of more sensitive balances could increase the precision of age estimates, especially for young animals. Further improvements could be achieved by calibration studies with animals of known age that investigate the potential effects of gender and season on lens growth rates as shown for Microtus by other studies (Janova et al. 2007; Martinet and Spitz 1971; Pokrovskij 1971)

The high amount of outliers (13% strongly asymmetric lenses) was probably caused by slight lens ruptures during the dissecting procedure (Friend1967), by injuries due to the subterraneous living of the species and by an asymmetric eye development in some individuals. The comparison with eye pairs of which neither of the two lenses were frozen (sample B) revealed that freezing did not affect lens weight variance and gives evidence that the observed asymmetries were not affected by the freezing procedure.

The freezing induced increase of lens weights was not affected by the weight of the lenses, the duration the lenses were stored in the freezer or the mean air temperature at the trapping day, the latter being regarded as indicator for speed of decomposition. Therefore, we suggest that the age of *A. terrestris* can be determined based on frozen lenses with the same precision as with unfrozen lenses by applying a constant correction factor of 1.033^{-1}. It can be expected that similar correction factors can be calculated for other small rodents and, as consequence, that age determination can be organized much more efficiently for population dynamic studies of small rodents that depend on analysing high number of animals.

Ethical standards

The authors declare that the study comply with the current laws of the country in which they were performed.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgements

We are grateful to Marianne Fritzsche from Grün Stadt Zürich and to Andreas Weidmann, Simon Franz and all other field workers that delivered the trapped rodents. We thank Alexander Mathis for valuable comments on the manuscript and acknowledge the support of our research activities by the Swiss Federal Veterinary Office (Nr. 1.07.04). This work represents part of the dissertation of Pierre Burlet, veterinarian.

References

Augusteyn RC (2007) On the relationship between rabbit age and lens dry weight: improved determination of the age of rabbits in the wild. Mol Vis 13:2030-2034.

Augusteyn RC (2008) Growth of the lens: in vitro observations. Clinical and Experimental Optometry 91:226-239.

Boonstra R (1994) Population cycles in microtines: the senescence hypothesis. Evol Ecol 8:196-219.

Boujard T (1982) Contribution à l'étude de la structure et de la cinétique d'une population de la forme fouisseuse du campagnol terrestre (*Arvicola terrestris scherman* (shaw)) dans le Doubs (France) pendant la phase pullulante du cycle. Laboratoire de la faune sauvage et de cynégétique, Université Pierre et Marie Curie Paris VI: Jouy-en-Josas. p 50.

Broekhuizen S (1971) Age determination and age composition of hare populations. Union Internationale des Biologistes du Gibier Xe congrés, Paris:477-489.

Cerqueira D, De Sousa B, Gabrion C, Giraudoux P, Quéré JP, Delattre P (2006) Cyclic changes in the population structure and reproductive pattern of the water vole, *Arvicola terrestris* Linnaeus, 1758. Mamm Biol 71:193-202.

Eckert J, Deplazes P (2004) Biological, epidemiological and clinical aspects of echinococcosis: a zoonosis of increasing concern. Clin Microbiol Rev 17:107-135.

Eckert J, Gemmel MA, Meslin F-X, Pawlowski ZS (2001) *WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern*. World Organisation for Animal Health and World Health Organization: Paris.

Friend M (1967) Some observations regarding eye-lens weight as a criterion of age in animals. N Y Fish Game J 14:91-121.

Görner M, Hackethal H (1988) *Säugetiere Europas*. Neumann Verlag: Radebeul, Leipzig.

Hanski I, Henttonen H, Korpimaki E, Oksanen L, Turchin P (2001) Small-rodent dynamics and predation. Ecology 82:1505-1520.

Hörnfeldt B, Hipkiss T, Eklund U (2005) Fading out of vole and predator cycles? Proceedings of the Royal Society B-Biological Sciences 272:2045-2049.

Inchausti P, Ginzburg LR (1998) Small mammals cycles in northern Europe: patterns and evidence for a maternal effect hypothesis. J Anim Ecol 67:180-194.

Janova E, Havelkov D, Tkadlec E (2007) Does reproduction accelerate the growth of eye lens mass in female voles? Belg J Zool 137:85-88.

Janova E, Heroldova M, Nesvadbova J, Bryja J, Tkadlec E (2003) Age variation in a fluctuating population of the common vole. Oecologia 137:527-532.

Kauhala K, Soveri T (2001) An evaluation of methods for distinguishing between juvenile and adult mountain hares *Lepus timidus*. Wildl Biol 7:295-300.

Krebs CJ (1996) Population cycles revisited. J Mammal 77:8-24.

Longhurst WM (1964) Evaluation of the eye lens technique for aging Columbian black-tailed deer. J Wildl Manage 28:773-784.

Lord RDJ (1959) The lens as an indicator of age in cottontail rabbits. J Wildl Manage 23:358-360.

Martinet L, Spitz F (1971) Variations Saisonnieres de la croissance et de la mortalite du campagnol des champs, *Microtus arvalis*. Role du photoperiodisme et de la vegetation sur ces variations. Mammalia 35:38-84.

Millar JS, Iverson SL (1976) Weight of the eye lens as an indicator of age in *Peromyscus*. Can Field-Nat 90:37-41.

Montgomery GG (1963) Freezing, decomposition, and racoon lens weights. The Journal of Wildlife management 27:481-483.

Morel J (1981) Le campagnol terrestre, *Arvicola terrestris (L.)*, en Suisse: biologie et systématique (mammalia, rodentia). University de Lausanne: Nyon. p 85.

Morilhat C, Bernard N, Bournais C, Meyer C, Lamboley C, Giraudoux P (2007) Responses of *Arvicola terrestris scherman* populations to agricultural practices, and to *Talpa europaea* abundance in eastern France. Agriculture, Ecosystems and Environment 122:392-398.

Morris P (1972) A review of mammalian age determination methods. Mamm Rev 2:69-103.

Norrdahl K, Korpimaki E (2002) Changes in population structure and reproduction during a 3-yr population cycle of voles. Oikos 96:331-345.

Oli MK, Dobson FS (2001) Population cycles in small mammals: The alpha-hypothesis. J Mammal 82:573-581.

Pelton MR (1970) Effects of freezing in weights of cottontail lenses. The Journal of Wildlife management 34:205-207.

Pokrovskij AV (1971) Seasonal changes in biological cycles in some rodents and the problem of absolute age determination. Acta Zool Fenn 8:94-96.

Rongstad OJ (1966) A cottontail rabbit lens-growth curve from Southern Wisconsin. J Wildl Manage 30:114-121.

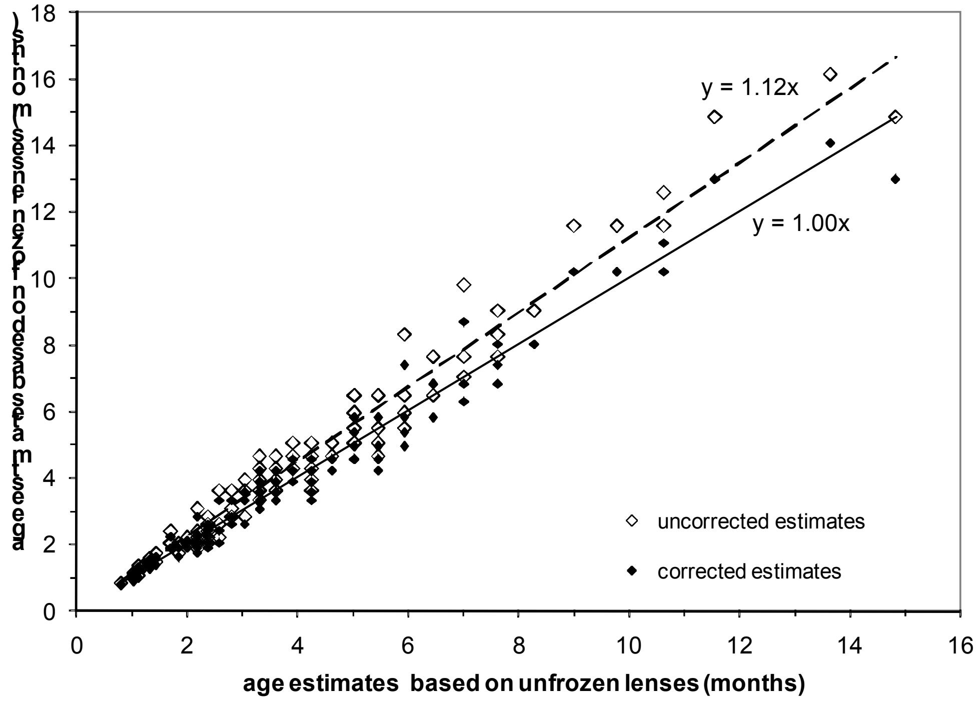
Tanikawa T (1993) An eye-lens weight curve for determining age in black rats, *Rattus rattus*. J Mamm Soc Japan 18:49-51.

Tkadlec E, Zejda J (1998) Small rodent population fluctuations: The effects of age structure and seasonality. Evol Ecol 12:191-210.

Weber JM, Aubry S, Ferrari N, Fischer C, Feller NL, Meia JS, Meyer S (2002) Population changes of different predators during a water vole cycle in a central European mountainous habitat. Ecography 25:95-101.

Fig. 1 Absolute age estimates for *Arvicola terrestris* and linear regressions calculated with unfrozen lenses and their frozen counterpart (n = 114). Blank diamonds and dashed line: age estimates of frozen lenses are calculated without applying a correction factor; solid diamonds and solid line: age estimates of frozen lenses were calculated after dividing the weight of the frozen lens by the factor 1.033.

Figure 1


age estimates based on frozen lenses (months)
age estimates based on unfrozen lenses (months)
y = 1.12x
y = 1.00x
uncorrected estimates
corrected estimates
0
2
4
6
8
10
12
14
16
18

Danksagungen

Bedanken möchte ich mich bei:

-Prof. Peter Deplazes und Dr. Daniel Hegglin für die gute Betreuung

-Prof Alexander Mathis und Prof. Paul Torgerson für die sorgfältige Begutachung der Arbeit

-Marianne Fritzsche von Grün Stadt Zürich

-Andreas Weidmann, Simon Franz und den anderen Feldarbeitern für das Fangen der Schermäusen

-Das Bundesamt für Veterinärwesen für die Unterstützung unserer Forschungen

-Meinen Mitdoktoranden und das ganze Team des Institut für Parasitologie

-Meinen Eltern, Brüdern, Grosseltern und allen anderen Personen, die immer für mich da waren.

Lebenslauf

Name	Pierre Burlet
Geburtsdatum	07.04.1979
Geburtsort	Zürich
Nationalität	CH
Heimatort	Reichenburg, Sz

04.1986 – 07.1992	Primarschule Zürich
08.1992 – 01.1999	Kantonsschule Wiedikon, Zürich (Typus B)
01.1999	Maturität Typ B
10.1999 – 11.2006	Studium der Veterinärmedizin an der Universität Zürich, Schweiz
07. – 11.2006	Staatsexamen an der Universität Zürich, Schweiz
02.2007 – 03.2011	Anfertigung der Dissertation unter Leitung von Prof. Dr. med. vet. Peter Deplazes und Dr. sc. nat. Daniel Hegglin am Institut für Parasitologie der Vetsuisse-Fakultät Universität
03. – 10. 2010	Praktizierender Kleintierarzt in der Tierklinik Schwarzmann in Rankweil, Österreich
Seit 01.02.2011	Praktizierender Kleintierzarzt in der Praxis Laufenburg und in der Tierklinik Am Sonnenberg in Möhlin