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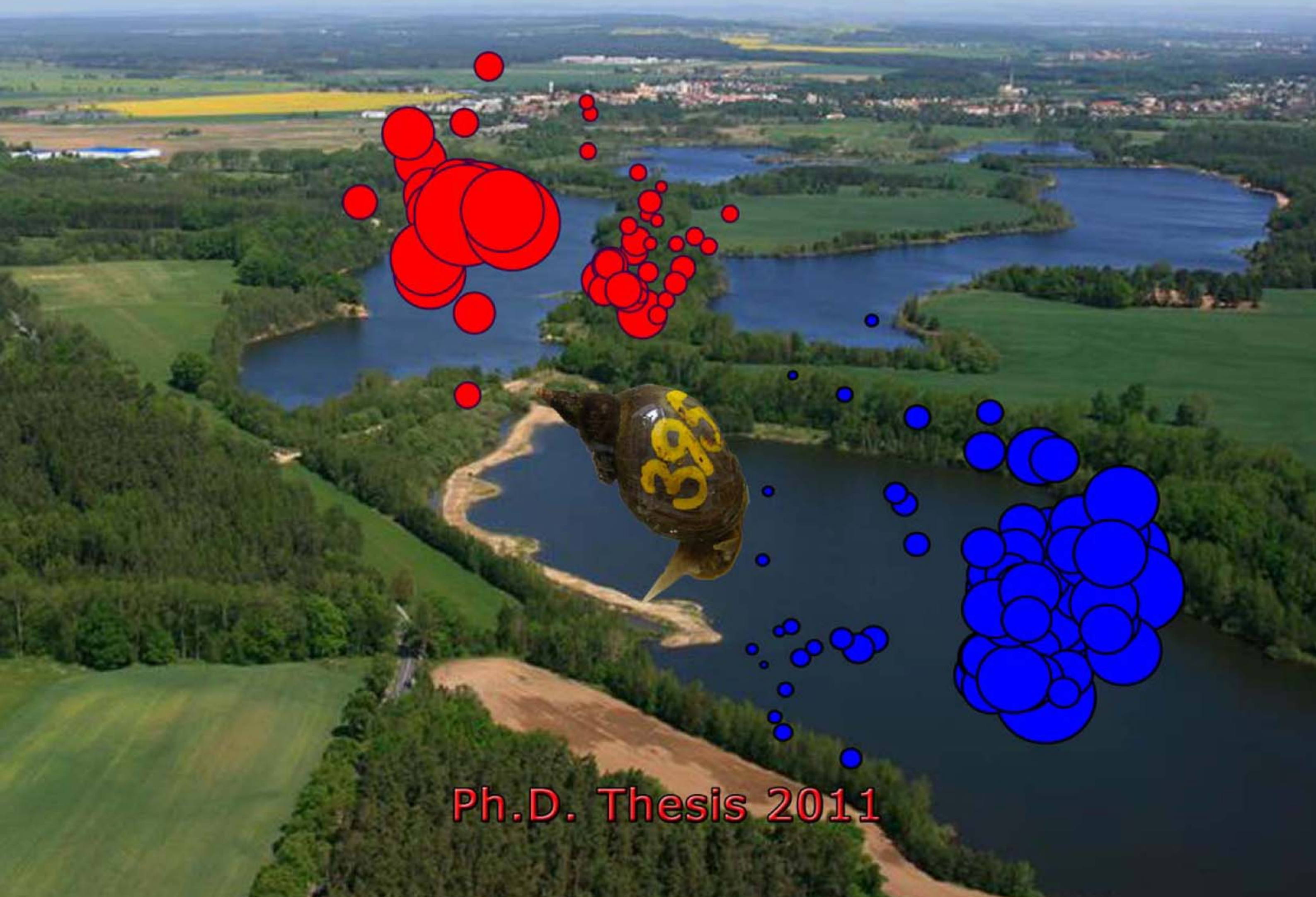
Miroslava Soldánová



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Faculty of Science, Department of Parasitology

# COMPOSITION AND STRUCTURE OF LARVAL TREMATODE COMMUNITIES IN MODEL FRESHWATER PULMONATE GASTROPODS IN EUTROPHIC ENVIRONMENTS IN CENTRAL EUROPE

Ph.D. Thesis

Mgr. Miroslava Soldánová

Supervisor: Dr. Aneta Kostadinova, Ph.D.

Consultant: RNDr. Anna Faltýnková, Ph.D.

Department of Helminthology, Institute of Parasitology, Biology Centre,
Academy of Sciences of the Czech Republic

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## ANNOTATION

This work applies advanced sampling (mark-release-recapture) and comparative approaches addressing the patterns in composition, structure and variability of larval trematode communities in three species of gastropod molluscs (*Lymnaea stagnalis*, *Planorbarius corneus* and *Radix auricularia*) at two nested scales of community organisation in typical Central European eutrophic environments. Hypothesis-testing with the application of null-model analyses, logistic regression modelling and multivariate randomisation techniques, revealed determinants of transmission rates, levels of infection and community structure in freshwater snail hosts in Central Europe and elucidated the mechanisms linking the spatial and temporal environmental variability with the action of complex community assembly rules in freshwater pulmonate snails.

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## DECLARATION

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České Budějovice, 12th April, 2011 Miroslava Soldánová

# AUTHOR'S CONTRIBUTION TO THE PAPERS

I The author conceived the study, carried out the sampling, parasite screening and identification, performed statistical analyses and drafted the manuscript. Overall author's contribution is c. 90%.

II The author conceived the study, carried out the sampling, parasite screening and identification, participated in the statistical analyses and drafted the manuscript. Overall author's contribution is c. 80%.

III The author designed the field study, carried out the sampling, parasite screening and identification, participated in the statistical analyses and data interpretation, wrote the first draft of the manuscript and incorporated co-authors' amendments and revisions. Overall author's contribution is c. 50%.

IV The author participated in the study design and fieldwork, supervised parasite identification, data handling and cohort analysis, and contributed to the final draft of the manuscript. Overall author's contribution is c. 40%.

V The author took main part in conceiving the study and sampling design, carried out the sampling, parasite screening and identification, data interpretation and drafting the manuscript. Overall author's contribution is c. 75%.

Agreement of the co-authors

John Barrett

Rebecca Brown

Anna Faltýnková

Aneta Kostadinova

Armand Kuris

Ana Pérez-del-Olmo

Kevin Lafferty

Tomáš Scholz

Christian Selbach

Bernd Sures

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# SUMMARY

Infracommunities of larval trematodes within mollusc hosts provide an excellent study system to explore interspecific interactions and non-random patterns of species distribution and community assembly and to assess whether the mechanisms creating these patterns affect the higher levels of community organisation. However, in spite of the recently accumulated evidence for the effect of spatial and temporal variability on component communities, a rigorous test of the competitive exclusion hypothesis using original infracommunity data and quantitative assessment of the scale effects (*i.e.* spatial and temporal heterogeneity) has been carried out on a single marine snail-trematode system. This study aimed (i) to provide novel data on the spatial and temporal variation in the composition and structure of larval trematode communities in selected model species of pulmonate snails in the freshwater productive environments typical of Central Europe; and (ii) to attempt a parasite community approach for assessing effects of man-induced environmental degradation on snail-trematode associations in selected eutrophic water bodies in Central Europe. A combination of advanced sampling (mark-release-recapture technique) and comparative approaches (within- and cross-host taxon assessment) to trematode communities in the model snail species, *Lymnaea stagnalis*, *Radix auricularia* and *Planorbarius corneus*, yielded abundant novel data on the composition, structure and variability of larval trematode communities at three nested scales in typical Central European eutrophic environments. Hypothesis testing *via* null-model analyses, logistic regression modelling and multivariate randomisation techniques, helped the advancement of our understanding of the patterns of composition and structure of trematode communities in freshwater snail hosts and depicting the mechanisms linking habitat variability with the action of complex community assembly rules in freshwater pulmonate snails.

The results of the thesis comprise five original papers.

## Paper I

Small-scale temporal and spatial patterns of the composition and structure of trematode communities in Lymnaea staganlis sampled by a mark-release-recapture technique in five fishponds in South Bohemia between 2006-2008, were analysed using prevalence data for individual parasite species and functional/taxonomic groupings in 83 distinct snail population samples (6,534 snails; 8,908 capture events). The five eutrophic fishponds provided excellent environments for the development of species-rich and abundant trematode communities. A total of 14 species utilising six transmission pathways was found. Nine prevalent species were consistently present in component communities, but had differential contribution to the parasite flow in the five ponds resulting in significant contrasting patterns of community similarity and the prevalence of the three major transmission guilds driving this similarity. Multivariate analisis of similarities revealed effect of locality and season on community structure, and a significant strong differentiation of communities in relation to pond size context (i.e. small, medium-sized and large) and pond function context (i.e. rearing and hibernation ponds). Component communities split into two groups: (i) those from the large pond (dispersal-dependent or ‘pulsed transmission’ communities) dominated by duck and gull generalists with active miracidial transmission; and (ii) those from the smaller ponds (continual presence or ‘press transmission’ communities) dominated by two plagiorchioidean species infecting snails via egg ingestion. Three hypotheses were suggested for the remarkable differences in larval trematode flow in the similar and closely located eutrophic ponds: (i) species-specific differences in parasite colonisation potential displayed by an ‘active-passive’ dichotomy in miracidial transmission strategies of the species; (ii) top-down effects of pond context on transmission pathways of the trematodes; and (iii) competition as an important mechanism in eutrophic environments with a bottom-up effect on component community structure.

## Paper II

High recruitment rates of multiple species and hierarchical competition are key to competitive exclusion model of community assembly in larval trematode communities in molluscs. Eutrophic environments provide conditions for speeding up trematode transmission and this would increase the strength of interspecific interactions. The first assessment for a pulmonate snail host, and for highly productive aquatic environments, of the rates of colonisation and extinction at the level of individual snail host patches, was carried out to test these predictions. Using a unique large dataset (individual infection histories of 1,471 native sentinel snails) from a mark-recapture study of *Lymnaea stagnalis* in six eutrophic fishponds in South Bohemia the study demonstrated extraordinarily rapid colonisation by trematodes of a snail host, thus meeting the assumptions of the competitive exclusion model. A total of 652 colonisation events were detected in 580 of the sentinels. The overall average duration of exposure of the sentinels in the field until the detection of infection change was 59 days but changes occurred earlier in 363 cases. Overall annual colonisation rates ranged from 242-502% $yr^{-1}$ so that odds of trematode establishment in an individual snail in the ponds studied are 2-5 times per year. Extinction rates were substantially lower than colonisation rates and, therefore, would not result in turnover rates high enough to significantly affect prevalence patterns in the snail populations. At the species level, analyses on sample-based estimates of probabilities of colonisation revealed that shared species traits associated with transmission and competitive abilities determine the limits of colonisation abilities. Colonisation rates were exceedingly higher for the species transmitted to the snail passively *via* eggs. There was a significant effect of species competitive abilities on colonisation rates due to subordinate species being substantially better colonisers than both strong and weak dominants, a pattern consistent with the predictions of the competition-colonisation trade-off hypothesis. These results suggest that, at the extraordinarily high trematode colonisation potential in the area studied, the spatial and temporal patterns of intraspecific heterogeneity in recruitment may provide conditions for intensification of interspecific interactions so that complex community assembly rules may be involved.

## Paper III

The competitive relationships among 14 larval trematode species infecting *Lymnaea stagnalis*, and the importance of structuring effect of environmental (spatial and temporal) heterogeneity and competition on trematode infra- and component communities were examined using application of null-model analyses. Snails collected in three-week periods between 2006-2008 from seven fishponds in South Bohemia in the Czech Republic by a mark-release-recapture method were screened individually for cercarial emission (7,623 snails marked and released; 10,382 capture events). Long-term investigation into infracommunity dynamics revealed changes in trematode infections and high rate of species co-occurrences (7%), *i.e.* simultaneous infections with two or more species within an individual host, particularly double infections (6.8%). Dominance hierarchy was postulated based on the infection sequence changes from one species to another, single/double to double/triple infections or loss of species from a double infection, and on indirect evidence. Seven top dominant species with putatively similar competitive abilities (six redial and one sporocyst species) dominated over sporocyst only trematode species. Three mechanisms of community assembly presumably determined interspecific dominance: the direct interference competition, indirect exploitative competition and/or the effect of prior occupancy. A statistical analysis based on null-model using observed prevalences resulted in fewer observed double infections than would be expected by chance and indicated a non-random structure of trematode communities. The first assessment using sequential null-model analysis for trematode communities in freshwater systems in Europe revealed interplay between environmental heterogeneity in recruitment and competition among parasites. Species isolation effect, which decreased the likelihood of species encounter and thereby interactions, resulted from differential community composition and structure in the largest pond. Temporal heterogeneity in recruitment contributed to the structure of parasite assemblages by intensifying interactions within snail host. Finally, interspecific competition was the major mechanism explaining the paucity of observed double infections indicating that trematode infracommunities in *L. stagnalis* are interactive. The additive effect of competition on component community structure was demonstrated by the exclusion of substantial proportion of interacting trematodes (11.2%).

## Paper IV

Small-scale temporal and spatial variability in composition and structure of larval trematode communities in *Lymnaea stagnalis* and *Planorbarius corneus* was studied in two small nearby fish ponds in South Bohemia (Czech Republic). Quantitative data for the snail populations and parasite composition and prevalence in three distinct seasonal samples of each host species were used to address the question of how much heterogeneity in component community structure can be detected in relation to small-scale temporal and spatial variability. The hypothesis of a seasonal change in both snail population structure and levels of parasitism with twin infection peaks, and the hypothesis of substantial concordant homogeneity in community composition and structure in both host-parasite systems due to the close proximity of the ponds, were tested. A total of 1,513 snails of all available sizes (717 *L. stagnali*s and 796 *P. corneus*) was examined in spring, summer and autumn 2009. Snail size-frequency data were modelled as a mixture of overlapping lognormal distributions to estimate the number, mean sizes and relative abundance of the snail cohorts in each sample. The levels of parasitism in the populations of both hosts were high (overall prevalence 37.1 and 55.4%, respectively). *L. stagnalis* was parasitized predominantly by allogenic species maturing in a wide range of birds (12 spp.) whereas *P. corneus* was infected by relatively more species completing their life cycles in micromammals (7 spp.). Communities in both hosts exhibited a congruent pattern of seasonal change in overall infection rates and community composition with lower levels of infection in spring. Both temporal and spatial variation was closely related to the structure of snail populations, and no significant differentiation of community composition with respect to pond was observed. Patterns of richness and similarity of component communities in *L. stagnalis* and *P. corneus* were compared with continental and regional trematode faunas of these hosts using published data for Central Europe (3 and 2 inventories, respectively) and fishponds in Třeboň (12 and 8 inventories, respectively). Comparisons revealed overall congruent patterns of decreased richness and similarity and increased variability at the smaller scales in both host-parasite systems. The relative compositional homogeneity of larval communities in both snail hosts irrespective of scale suggests that historical data at small to medium regional scales may provide useful estimates of past richness and composition of larval trematode communities in these snail hosts.

## Paper V

Communities of larval trematodes in two lymnaeid species, *Radix auricularia* and *Lymnaea stagnalis,* in four man-made interconnected reservoirs (Hengsteysee, Harkortsee, Kemnader See and Baldeneysee) on the River Ruhr (North Rhine-Westphalia, Germany) were described focusing on among- and within-reservoir variations in parasite prevalence and component community composition and structure. Trematode communities in *Radix auricularia* and *Lymnaea stagnalis* were species-rich and abundant (12 and 6 species, respectively spp.). The lake-adapted *R. auricularia* dominated numerically over *L. stagnalis* and played a major role in the trematode transmission in the reservoir system. Both host-parasite systems were dominated by bird parasites (13 out of 15 species) characteristic for eutrophic water bodies. Logistic regression analysis identified two environmental variables, the oxygen content and pH of the water, as important determinants of the probability of infection, in addition to snail size. Between-reservoir comparisons indicated an advanced eutrophication at Baldeneysee and Hengsteysee and the small-scale within-reservoir variations of component communities provided evidence that larval trematodes may have reflected spatial bird aggregations (infection 'hot spots'). Two life history groupings of dominant species, the 'cyprinid' and 'anatid' parasites, that depict two aspects of progressive eutrophication in this mature reservoir system, were identified. Overall results suggest that trematode communities in the lake-adapted *R. auricularia* are better suited for monitoring the effect of environmental change on host-parasite associations in the reservoir system on the Ruhr River and other similar systems due to the important role of this host in trematode transmission in lakes. Whereas variations in trematode community diversity and abundance may indicate the degree of eutrophication on a larger scale (among reservoirs), the infection rates of the two life history groups of dominant species, the 'cyprinid' and 'anatid' assemblages, may be particularly useful in depicting environmental variability, eutrophication effects and infection 'hot spots' on smaller spatial scales.

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# 1. GENERAL INTRODUCTION

Parasitism has evolved from a free-living ancestral lifestyle on many independent occasions in several taxonomic groups of animals (Poulin, 1998; de Meeûs & Renaud, 2002). The evolution of the host-parasite relationship has lead to a great diversification of parasites involving a wide variety of life cycles and morphological, physiological and behavioural adaptations that help coping with physical and biotic environmental challenges. Almost all free-living organisms are hosts to parasites and almost all major groups of animals have parasitic members. It is likely that more than half of the species of organisms on earth utilize parasitic way of life (Price, 1980; Poulin & Morand, 2000). A close relationship between two organisms in which one (the parasite) lives in or on, and at the expense of the other (the host), and causing it some harm, is the classical definition of parasitism. In a broader sense, a parasite depends on the host that provides some benefit (usually food) but the parasite does not always damage the host. However, many other definitions of parasitism exist and generally none of them is completely satisfactory since always exceptions can be found among intimate relationships between organisms and the border between several types of associations related to parasitism such as mutualism and commensalism overlap (Rohde, 2005).

This is not the case of digenean trematodes, a widespread and large entirely parasitic group within the phylum Platyhelminthes. So far, approximately 25,000 species of trematodes, many of veterinary and medical importance, are known to parasitize a broad range of vertebrate definite hosts harbouring the adult sexual stage (Esch et al., 2002; Sukhdeo & Sukhdeo, 2004). A characteristic feature of trematodes is a complex life-cycle involving both free-living (miracidium, cercaria) and parasitic (sporocyst, redia, metacercaria, adult) developmental stages (Fig. 1). The immense diversity of life-cycles ranges from one involving a single host species to a multiple-host species complex cycle involving up to four hosts, mutually connected through various trophic relations (*e.g.* Combes, 2001; Combes et al., 2002; Galaktionov & Dobrovolskij, 2003). The typical trematode life-cycle requires three hosts for successful completion: a vertebrate definitive host (usually fish, birds and mammals) in which sexual reproduction takes place, an invertebrate first intermediate host (almost exclusively molluscs) in which asexual multiplication occurs, and a second intermediate host (different groups of vertebrates and invertebrates) which serves as a transmission vector to the definitive host (Fig. 1) (Galaktionov & Dobrovolskij, 2003).

Molluscs become infected after eating eggs that are released with the faeces of the definitive host, or after penetration by free-swimming miracidia hatched from eggs. In the snail host, trematodes multiply *via* asexual reproduction to produce large quantities of morphologically distinctive larvae, sporocysts and/or rediae, and cercariae. Depending on the trematode species, genetically identical cercariae are produced in either sporocysts or rediae. After their release from the snail, cercariae disperse in the external environment where they usually actively seek out and penetrate the next host. Once inside, cercariae transform into metacercariae that are trophically transmitted to definitive hosts after ingestion of the second intermediate host. The life-cycle is completed by maturation, sexual reproduction and egg production of the adult stage. Both free-living mobile stages, miracidium and cercariae, do not feed and posses limited energy supplies, which makes their life span very short. This short transmission opportunity is compensated by the great diversity of behaviours related to the processes of host-finding and host-recognition that include the ability to respond to the physical or chemical stimuli of the environment or emanating from the host (reviewed by Combes et al., 1994; Haas, 1994; Haas et al., 1995). However, behavioural and morphological adaptations differ remarkably between these two infective stages depending on the use of different hosts. The target host organisms for miracidia are almost always molluscs that are relatively immobile, whereas the target hosts for cercariae belong to diverse classes of invertebrates and vertebrates, *i.e.* mobile hosts (Combes et al., 2002).

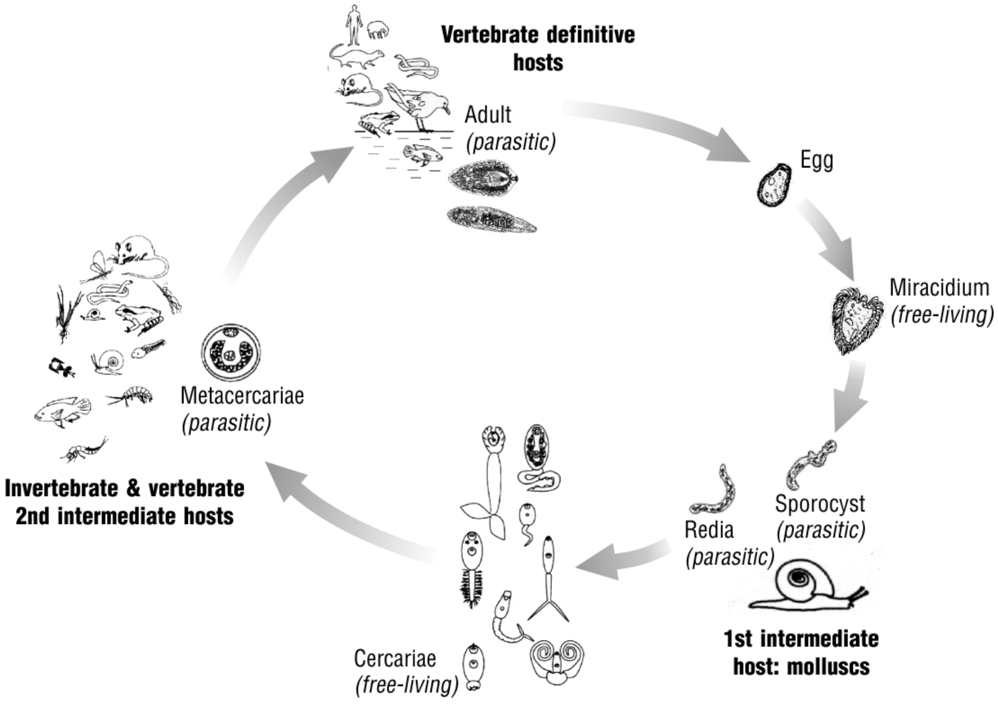

Fig. 1. Typical trematode life-cycle (modified after Combes et al., 2002 and Sukhdeo & Sukhdeo, 2004).

Due to the sequential use of different host species throughout complex life-cycles, trematode diversity and abundance in snails is inherently linked to host diversity and abundance and reflects the dynamics of the trophic web at the ecosystem level (*e.g.* Marcogliese, 2003; Hechinger & Lafferty, 2005; Fredensborg et al., 2006; Lafferty et al., 2006, 2008). Trematodes are common and abundant in marine and freshwater habitats throughout the world and important elements of food webs (Lafferty et al., 2006; Kuris et al., 2008; Lafferty et al., 2008). Trematode larvae are essential components of ecosystems subsuming substantial fraction of biomass with levels comparable to some free-living species (Kuris et al., 2008) and may exert strong influence on the structure, dynamics and function of food webs (Hechinger & Lafferty, 2005; Lafferty et al., 2006, 2008; Hechinger et al., 2007;). Kuris et al. (2008) estimated that the annual production of free-swimming trematode transmission stages in the Carpinteria Salt Marsh represented biomass greater than that of other parasites and even birds. They concluded that the presence and abundance of a certain type of organism in a given territory may be dependent on the activity of trematodes.

Intramolluscan trematode larval stages are functionally important components of aquatic communities with significant effects on host survival, reproduction, growth and competitive ability, thus making trematode parasitism a key factor regulating snail populations (Lafferty et al., 2006, 2008; Miura et al., 2006). Trematode larvae form dense populations in the tissues of the snail hepatopancreas and gonad, often reaching about half of the tissue volume of the infected snail host (Kuris & Lafferty, 1994, 2005; Lafferty & Kuris 2009); these organs may be completely eliminated by larval feeding activities. Host castration, an important intensity-independent effect, is perhaps the most striking and well described impact of trematodes on their snail hosts associated with alteration of host's fecundity and somatic growth (*e.g.* Sousa, 1983; Minchella, 1985; see Sorensen & Minchella, 2001 for a review). Castration involves either complete destruction or prevention of development of snail's gonad which is sometimes followed by the enhanced growth of infected snails, known as gigantism, due to the energy allocation of the recourses into growth rather than to energetically costly reproduction. Castrated snails are biologically dead since they no longer produce offspring (Kuris, 1973; Sousa, 1983; Lafferty, 1993).

Although operating at the level of individual snails, host castration may result in severe depression of the reproductive output of the snail populations; infected snails also suffer from decreased survival, associated with excessive energy demands or tissue damage caused by the intramolluscan larvae (Bayne & Loker, 1987) and higher mortality when exposed to environmental stress (*e.g.* Jensen et al., 1996). On the other hand, parasite-induced snail gigantism could significantly alter the size-structure, habitat and resource use, and intraspecific competitive interactions of the host population (Miura et al., 2006). These non-lethal effects can alter energy transfer in freshwater food webs and this may have a substantial ecological impact at the ecosystem level.

## 1.1. TREMATODE COMMUNITIES IN SNAILS: EXCELLENT MODEL SYSTEMS FOR ECOLOGICAL RESEARCH

Because parasite assemblages comprise potentially interacting species and inhabit discrete delimited habitats (the hosts) that are patchy and spatially partitioned, parasite communities are excellent and attractive model systems for testing community assembly hypotheses *via* comparative analyses in search for non-random patterns and the ecological mechanisms causing these patterns (Holmes & Price, 1986; Esch et al., 1990; Sousa 1994; Poulin, 1998). The search for structure in parasite communities is enhanced by their hierarchical organisation, so that comparative analyses of patterns in the distribution and abundance of parasites can be carried out at several nested spatial scales.

Parasites live as populations that are divided into metapopulations because they are found in spatially discontinuous habitats (usually the hosts). This is reflected in the theoretical framework for nested hierarchical organization of parasite populations and communities developed more than two decades ago (Esch et al., 1975; Margolis et al., 1982; Holmes & Price, 1986; Esch et al., 1990; Bush et al., 1997) the latter paralleling the former. This hierarchical approach, evolved from studies at both population and community level, has become widely accepted in terms of better understanding and interpreting of the populations and communities of parasites (Esch et al., 2002). Based upon the conceptual work cited above, the currently accepted definitions of the terms used throughout the text are provided below.

A population of parasites comprises all of the individuals of a single parasite species at a particular place at a particular time. Individual hosts are equivalent to habitat patches harbouring infrapopulations of several metapopulations of different parasite species, so that parasite population dynamics in a host population is equivalent to patch colonisation and extinction. Colonization of an individual host represents the establishment of a parasite population in an unoccupied patch (*i.e.* uninfected host individual) or an already occupied patch (*i.e.* when the host individual is re-infected). Local extinction of each patch can be the result of recovery from infection (self-cure *sensu* Fernandez & Esch, 1991a; Esch &

Fernandez, 1994), eviction due to competitive exclusion, parasite-induced mortality or the natural death of the host or the entire parasite population.

Table 1 shows the spatial scales applied and the terms describing the hierarchical structure of parasite communities in the present study. All community data are acquired at the level of infracommunities which are replicate samples of the next hierarchical level, component communities, that represent the local pools of parasite species from which infracommunities can acquire species (Poulin, 2005). An important difference exists between the structure and dynamics of these two levels of community organisation, particularly created by the distinct period of time, in which they persist in an ecosystem. Infracommunities are usually short-lived with life span equal to that of the host, whereas component communities last longer, at least a few host generations (Poulin, 1998). Local parasite fauna corresponds to a higher scale (temporal rather than spatial in the present study; Table 1). The composition and structure of local parasite faunas are dependent on historical and zoogeographic factors and environmental filters acting on parasite dispersal (Holmes, 1990; Esch et al., 1990; Guégan et al., 2005). Although parasite faunas are generated by biological processes, they are viewed as artificial units. Theoretically, the maximum number of parasite species that can be found in a population of a host species equals the number of species in the parasite fauna. However, component communities rarely reach the richness of the parasite fauna (Poulin, 1998).

Table 1. Terms describing the hierarchical structure of parasite communities at the scales of study and their definitions.

| **Spatial scale** | **Term** | **Definition** |
|---|---|---|
| Host individual | Infracommunity | All of the infrapopulations of parasites of different species within an individual host |
| Host population | Component community | All of the infracommunities of parasites of all species in a host population sample in a given locality at one point in time |
| Host species in an ecosystem | Local parasite fauna | All parasites of all species exploiting a given host species in a local ecosystem |

In addition to the general attributes of parasite communities as suitable models for ecological research, such as hosts being discrete habitat units and replicate samples amendable to powerful statistical tests, trematode communities in gastropods possess some unique characteristics. First, snails are widespread and abundant so that large samples of replicate infracommunities can be collected. Secondly, snails can be sampled in a blind fashion with respect to infection thus increasing the power of statistical assessments of randomly sampled infracommunities. Thirdly, many snail-trematode systems are characterised by high diversity thus increasing the probability of interspecific encounter and interactions. Fourthly, snail populations are amendable to field manipulation *e.g.* mark-release-recapture, use of sentinel snail experiments, so that communities can be studied using the most powerful community ecology methodologies (Kuris, 1990; Kuris & Lafferty, 1994). Finally, two system-specific features appear to be most important for exploring patterns in community assembly. Communities of larval trematodes within mollusc hosts provide an excellent study system to explore interspecific interactions due to the high potential for monopolization of resources (both trophic and spatial) within individual snail host patches by dense infrapopulations resulting from asexual reproduction (Sousa, 1993, 1994; Kuris & Lafferty, 1994; Lafferty et al., 1994).

Snail-trematode systems have long been the focus of medical, evolutionary and ecological research, resulting in a substantial body of literature on factors affecting snail-trematode associations and communities (reviewed by Esch & Fernandez, 1994; Esch et al., 2001). However, until recently, studies on these systems did not appear well-represented with respect to the development of theories concerning parasite community structure, compared to communities of vertebrate parasites, especially fish parasites (Sousa, 1994). The major recent advances in the study of trematode community structure are reviewed briefly here, focusing on observed patterns and hypotheses suggested for the processes generating these patterns.

## 1.2. PATTERNS AND PROCESSES SHAPING COMMUNITIES IN SNAIL HOSTS: INFRACOMMUNITIES

A key question in parasite community ecology has been whether the parasite species in each infracommunity are random subsets of the ones found in the component community (Guégan et al., 2005; Poulin, 1998, 2005). If the species composition of infracommunities departs from that expected by chance alone, then the infracommunities are most likely structured by one or more ecological processes (Poulin, 2005). As indicated in the previous section, infracommunities of larval trematodes within mollusc hosts provide an excellent study system to explore interspecific interactions and non-random patterns of species distribution. In contrast to vertebrate-macroparasite systems, where parasite populations within individual vertebrate hosts can increase only as a result of additional colonisation by infective larvae, a single miracidium (a free-living dispersive trematode larva) gives rise *via* asexual reproduction to thousands larvae of the next generations (rediae or sporocysts), thus resulting in substantially denser parasite populations in individual snails. Further, snails as patches of habitat for parasites are much smaller and offer lower diversity of microhabitats for colonization than vertebrate hosts. Since most larval trematodes target snail hepatopancreas and gonad where they multiply asexually, the potential for monopolization of resources (both trophic and spatial) by a single species is higher in individual snail than in vertebrate host patches (Sousa, 1993, 1994). Thus, interspecific competition, considered as one of the main forces that structure infracommunities, is more likely to occur if two or more trematode species infect the same snail host (*e.g.* Kuris, 1990, Sousa, 1990, 1994; Kuris & Lafferty, 1994, 2005; Lafferty et al., 1994). It is not, therefore, surprising that larval trematode communities in snails represent the only study system for which a direct link has been established between a non-random patterns of species co-occurrences observed in natural communities with specific data on interspecific competition obtained from the same or similar systems (Kuris, 1990; Lafferty et al., 1994).

Two lines of research carried out in the early 20 century have stimulated studies leading to this discovery. Both faunistic inventories on snail-trematode systems originating early in the 20th century (*e.g.* Sewell, 1922; Dubois, 1929; Cort et al., 1937; Martin, 1955; Ewers 1960; Bourns, 1963; Vernberg et al., 1969; Robson & Williams, 1970; Vaes, 1979; Walker, 1979) and more recent ecological surveys of natural snail populations in both freshwater (Goater et al., 1989; Fernandez & Esch, 1991a,b) and marine trematode systems (Kuris 1990, Sousa, 1990, 1993; Lafferty et al., 1994; see also references in Kuris & Lafferty, 1994) have recognised the variation in the frequency of multiple infections, *i.e.* simultaneous infections of an individual snail with two or more species. The vast majority of these surveys demonstrates that multiple infections occur typically less frequently than might be expected by chance, thus suggesting that competitive interactions may account for this recurrent pattern of low infracommunity richness. This has evoked a series of studies focused on the patterns of species distribution within individual molluscan hosts.

The second line of research, that has been largely influential to the developing of testable hypotheses, stems from the observations of Wesenberg-Lund (1934) who demonstrated that rediae of some echinostomatid species prey upon heretospecific rediae or sporocysts. In an extensive series of experimental studies on three freshwater snail-trematode systems (the lymnaeid *Lymnaea rugibinosa* and the planorbids *Indoplanorbis exustus* and *Biomphalaria glabrata*), Lie and colleagues have substantially contributed to clarify the nature of interspesific interactions among larval trematodes (reviewed by Lie et al., 1968; Lim & Heyneman, 1972; Kuris, 1990; Esch & Fernandez, 1994; Kuris & Lafferty, 1994). Lie and colleagues described frequent direct predation by dominant species with redial larval stages on subordinate species as well as suppression of the development of subordinate species by species with only sporocyst larval stages and that prior occupancy determines interspecific dominance in some species combinations. They also revealed that the competitive relationships among various trematode species are of hierarchical character (Lie et al., 1965; Lie & Umathevy, 1965a,b; Lie & Basch, 1967; Jeyarasasingman et al., 1972; Lie et al.,1976).

Redial predatory behaviour studied in experimental conditions has thus provided a solid baseline for the establishment of dominance hierarchies of competitive interspecific interactions among larval trematodes in three snail-trematode systems: (i) the marine prosobranch *Cerithidea californica* (see Kuris, 1990, modified by Lafferty et al., 1994); (ii) the freshwater planorbid *Helisoma anceps* (see Fernandez & Esch, 1991a); and (iii) the freshwater lymnaeid *L. rugibinosa* (see Kuris, 1990). These lines of research outlined the key question in community ecology studies on snail-trematode models, *i.e.* whether larval trematodes are assembled at random or communities are structured by more complex community assembly rules (*i.e.* direct interference competition; indirect exploitative competition; and priority effects). Subsequent research on patterns and potential mechanisms shaping larval trematode communities (Kuris, 1990; Sousa, 1990, 1992, 1993; Fernandez & Esch, 1991a,b; Williams & Esch, 1991; Kuris & Lafferty, 1994; Lafferty et al., 1994) has resulted in the emergence of two schools of thought supporting different hypotheses for the depauperate nature of infracommunities in snail-trematode systems. The debate revolved around the relative roles of spatial (and temporal) heterogeneity as isolating processes versus intramolluscan competition as structuring forces (Esch et al., 2002; Kuris & Lafferty, 2005).

One school of thought developed the competitive exclusion hypothesis stating that within the individual host, trematode communities are structured primarily *via* antagonistic interactions (direct interference competition) that are hierarchical in nature. Kuris (1990) examined at the infracommunity level the patterns of association among trematodes in *C. californica* studied by Martin (1955). He used a simple null model first suggested by Cort et al. (1937) and based on species prevalences in the component community, to generate the expected number of double infections and test for departures from randomness separately for each species pair. Kuris (1990) also proposed a method for quantitative assessment of scale effects, *i.e.* the contribution of temporal (or spatial) heterogeneity to the pattern of interspecific association observed at a larger scale, provided direct evidence for interspecific interactions in *C. californica* host-parasite system, postulated a dominance hierarchy for trematodes parasitizing this marine snail and proposed a method for estimation of the potential impact of competitively dominant on subordinate species. He estimated considerable effect on the latter and suggested three conditions for competition to be important: (i) a high overall prevalence; (ii) an existing dominance hierarchy between potential competitors; and (iii) a predominance of interference competition. An independent study on the same snail-trematode system provided partial support for the deterministic assembly rules at the level of individual hosts (Sousa, 1993).

Lafferty et al. (1994) developed a method that allows assessment of the effects of temporal and spatial heterogeneity in parasite recruitment in conjunction with competition hypothesis. They parameterized the null model with the prevalence that each species would have achieved if no interactions have occurred (*i.e.* pre-interactive prevalence). Applying their method to communities in *C. californica*, these authors revealed that interspecific competition was the most significant structuring factor and that spatial heterogeneity intesifies species co-occurrences. Lafferty et al. (1994) suggested a three-step process that determines the composition of a larval trematode community: (i) the potential for co-occurrences is defined by the diversity and abundance of trematodes recruiting in an area; (ii) the difference between initial and potential number of co-occurrences is determined by the spatial and temporal heterogeneity; and (iii) the number of multiple-species infections that persist is reduced by competition according to a hierarchy of dominance.

Kuris & Lafferty (1994) tested the predictions of the competitive exclusion hypothesis applying the null-model analysis method of Lafferty et al. (1994) in a meta-analysis on 62 snail-trematode datasets and found that both sources of heterogeneity generally significantly intensify the potential for competitive interactions. They estimated that on average 10% of the trematode infections were lost to competition and concluded that this is important structuring process and that infracommunity interactions are additive at the component community level. Finally, Kuris & Lafferty (2005) stressed that the hypotheses of spatial (and temporal) heterogeneity as isolating processes *versus* intramolluscan competition as structuring forces are not mutually exclusive.

In contrast, the authors forming the concurrent school who studied short-lived freshwater pulmonate snails *H. anceps* and *Physa gyrina*, held the view that interspecific interactions, although occurring within individual snails, are unimportant in determining larval community assembly within the local snail populations (reviewed in Esch & Fernandez, 1994; Esch et al., 2001, 2002). Esch and colleagues carried out detailed field and experimental studies on the two snail-trematode systems in Charlie's pond, a small eutrophic impoundment in North Carolina and revealed differences in infracommunities in the two snail hosts, which they attributed to the different life histories and vagility of the snails. Thus, the observed frequency of multiple infections in *H. anceps* was low and hierarchical dominancesubordinance relationships were revealed in laboratory and field experiments (Fernandez & Esch, 1991a). On the other hand,  about 20% of examined *P. gyrina* harboured double and triple infections but experimental study showed no evidence for dominance (Snyder & Esch, 1993). These studies have led the authors to conclude that a combination of spatial and temporal factors affecting the distribution of infective stages, snail population dynamics (life span, mortality, recruitment, size structure), habitat characteristics and the nature of infective stages (eggs or miracidia) were responsible for the establishment of depauperate infracommunities in their systems (Fernandez & Esch, 1991a,b; Williams & Esch, 1991; Snyder & Esch, 1993; Sapp & Esch, 1994; Esch et al., 2002).

Esch et al. (2002) concluded that the complexity of parasite flow within an ecosystem requires attention if competitive processes are not strong enough to influence the structure of the component communities. This view was supported by Curtis (2002) who, based on own extensive studies on trematode communities in the marine prosobranch, *Ilyanassa obsoleta*, and a comparative analysis with two other marine snail host-parasite systems, *Littorina littorea* and *C. californica*, suggested that external factors involving history and the ecological characteristics of parasites and hosts are the key determinants of the structure of trematode component communities in marine snail hosts. Sousa's studies (1990, 1993) on infra- and component communities in *C. californica* tend to support Curtis's (2002) suggestions. Sousa (1993) concluded that although interspecific competition may prevent species co-existence within infracommunities, it has low impact on the structure of component communities.

A basic assumption of the competitive exclusion hypothesis is that individual snails are synchronously colonized by miracidia of multiple species resulting in an initial increase of community richness. Strong hierarchical asymmetrical interactions between co-infecting trematodes would then lead to exclusion of competitively subordinate species thus minimizing species richness of infections that persist in older snails (Sousa, 1990, 1993; Esch et al., 2001, 2002). However, field data supporting this assumption are scarce. Thus there are no data from freshwater systems and existing knowledge is based on from mark-recapture studies on two marine hosts, *C. californica* (see Sousa, 1993) and *I. obsoleta* (see Curtis, 1996, 2003; Curtis & Tanner, 1999). The main goal of these authors was to test the validity of the assumptions for hypotheses of community assembly in natural systems *via* quantification of recruitment and extinction probabilities. They found that the rates of trematode recruitment are markedly low (under 5% $yr^{-1}$, *i.e.* an infracommunity might experience colonisation once in 20 years. Sousa (1993) also observed generally low annual rates of invasion and exclusion of established trematode infections in *C. californica*, that also exhibited site-specific variation. Assuming that established infections persist during the life span of the host, Curtis (2002) concluded that infections persist along with the long life span of these snail hosts, and the high prevalence is a result of a slow accumulation over time rather than rapid colonization.

## 1.3. PATTERNS AND PROCESSES SHAPING COMMUNITIES IN SNAIL HOSTS: COMPONENT COMMUNITIES

The hierarchical framework for parasite communities depicted in section 1.1. offers an advantage in the search for interconnection between processes acting at different scales. Scale-dependency is considered an important determinant of the establishment and organisation of trematode component communities (Kuris, 1990; Esch et al., 2001). Recent research at this scale has been focused predominantly on the spatial patterns of variation of the distribution of trematodes and the mechanisms determining their recruitment into host populations (Esch & Fernandez, 1993; Poulin, 2005).

The main questions addressed in relation to the spatial mechanisms have been: (i) Do interspecific interactions affect component community structure at a regional scale (*i.e.* within single regional pools); and (ii) How does spatial heterogeneity at a local scale (*i.e.* within an ecosystem) affect component community structure? In other words, examination of the spatial patterns in larval trematode communities in snail populations addressed the more general question (and the continued debate) over the additivity of infracommunity interactions at the component community level.

Kuris (1990) hypothesised that competition may be important locally but driven by stochastic factors on a global scale. However, our knowledge at large biogeographical scales is very limited. Early reviews suggest relatively homogeneous distribution of parasites in marine molluscs over large spatial scales (Cheng, 1967; Lauckner, 1980). However, the only study on spatial occurrence patterns in two mollusc-trematode systems, *L. littorea* and *Hydrobia ulvae*, over large geographical scales in Europe revealed that trematode distribution exhibits more structure than expected from the large-scale dispersal abilities of their bird and fish final hosts (Thieltges et al., 2009). This finding shows an agreement with Kuris's (1990) prediction.

The predominance of bird parasites in the rich and abundant marine mollusc-trematode systems has led to the hypothesis that bird vagility may link parasite communities in intermediate hosts that inhabit spatially separated sites at a regional scale (Kuris, 1990; Sousa, 1993). This hypothesis has been tested at a regional scale in two studies. Curtis (1997) examined component communities in *I. obsoleta* at nine sites in the area of Delaware Bay and found nested pattern of trematode occurrence in *I. obsoleta* among sites at a regional scale. He suggested that this pattern is due to the nested pattern of occurrence among sites of the final bird hosts. On the other hand, Esch et al. (2001) did not find significant nested pattern in the trematode composition on the much smaller spatial scale (five sites in less than a quarter of km at Carpinteria Salt Marsh) of the study of Lafferty et al. (1994); this supports the suggestion of Kuris (1990) for the local importance of competition.

The effects on the heterogeneity in trematode recruitment of differential habitat use by birds have since been addressed in a number of studies at small to medium spatial scales. The small spatial scale study of Smith (2001) on trematode recruitment into the population of the marine snail, *Cerithidea scalariformis*, in Florida mangrove swamps, was the first to show explicitly the link between bird abundance and trematode prevalence in snails. She detected a causal chain of correlations supported by regressions between perch density, bird abundance, bird dropping density, and parasite prevalence in snails. Further, in an experiment with sentinel snails, Smith (2001) found evidence suggesting that snail movement or behaviour may influence the dispersion of parasites from the foci of transmission and thus either homogenize or intensify spatial variation in the patterns of infection. By applying the model of Lafferty et al. (1994) to her field data, she found that perch density served to intensify trematode species interactions, resulting in a substantial loss of subordinate species to competitively dominant species.

Skirnisson et al. (2004) studied by correlational and principal component analyses the influence of several abiotic and biotic variables on the distribution of trematode infections in *Hydrobia ventrosa* in 12 ponds on the Melabakkar Salt Marsh in Iceland. They assumed that these factors influenced attractiveness of the habitats for definitive hosts, and concluded that the observed spatial heterogeneity in infections in *H. ventrosa* is almost exclusively determined by the species composition, abundance, and behaviour of the final bird hosts inhabiting the area.

Hechinger & Lafferty (2005) quantified birds at fine spatial scales in two types of habitat in Carpinteria Salt Marsh (California) using timelapse videography, and related bird communities to larval trematode communities in the snail populations (*C. californica*) sampled at the same small spatial scales. They found significant positive correlations in species richness, species heterogeneity and abundance between the final bird hosts and trematodes in the snail host. Furthermore, these authors have shown that bird abundance and species richness are both positively and significantly correlated with the proportion of trematodes lost to interspecific competition in snail populations. They suggested that the uneven use of the wetland ecosystem by birds drives the spatial heterogeneity in infection that causes this intensification of interspecific competition.

Lafferty et al. (2005) studied trematodes in *Batillaria minima* from mangrove habitats at two estuaries in Puerto Rican salt marshes. They revealed association between bird abundance and parasitism similar to those observed in caged sentinel snails by Smith (2001) but in free-ranging snails and suggested that, in contrast to the latter study, snail movement does not fully obscure associations between habitat and parasitism.

Fredensborg et al. (2006) examined natural spatial variation of larval trematodes in the populations of *Zeacumantus subcarinatus* across 12 bays in Otago Harbour (New Zealand) and revealed that trematode prevalence in snails was positively correlated with bird abundance across bays. However, they found no evidence that trematode prevalence reflected the spatial distribution of birds at lower scales (within bays) and suggested that abiotic factors may override the differential input of trematode eggs from birds.

Kube et al. (2002b) studied component communities of larval trematodes infecting the mudsnail *Hydrobia ventrosa* in coastal lagoons of the southern Baltic Sea in relation to the structure of waterfowl communities. They failed to demonstrate a significant relationship between trematode abundance in snail hosts and bird abundance and attributed this to the lack of host specificity in most trematode species in their system. Kube et al. (2002b) suggested that the low host specificity of the trematodes in combination with the enormous waterfowl diversity in the coastal lagoons might explain the stability of the prevalence patterns of the component trematode communities in *H. ventrosa*.

Byers et al. (2008) examined trematode prevalence in *Littorina littorea* at 28 intertidal sites in New England, USA and quantified a number of physical and biological variables for the sites. Using a hierarchical, mixed-effects model, they found that the primary factors associated with trematode prevalence are the abundance of final bird hosts (gulls) and snail size (a proxy for time of exposure). Byers et al. (2008) failed to detect regional spatial gradients in either trematode prevalence or independent environmental variables and suggested that trematode prevalence might be predominantly determined by local site characteristics favouring high gull abundance.

Granovitch et al. (2000) surveyed trematode infections in 27 spatially separated populations of *Littorina saxatilis* and *L. obtusata* in two regions of Kandalaksha Bay of the White Sea between 1984-1994 (plus two heavily infected populations sampled annually over a 16 yr period). They observed a close association only between three ecologically and morphologically similar trematode species (*Microphallus* spp.) with respect to the spatial distribution and temporal dynamics. However, the long-term changes in prevalence in the two heavily infected populations suggested asynchronous and uncorrelated dynamics of infection with the three *Microphallus* spp.

A more pronounced effect of spatial heterogeneity at a local scale (*i.e.* within an ecosystem) on patterns of infection with larval trematodes has been demonstrated in studies by Esch and colleagues (reviewed by Esch & Fernandez, 1994; Sapp & Esch, 1994; Esch et al., 2001, 2002) on the freshwater snail-trematode systems at Charlie's Pond. These studies also provide the main evidence for temporal variability in trematode prevalence (but see Kube, 2002a below). Esch's team developed a successful marking technique that allowed them to carry out longitudinal collecting protocols in the study of the snail population biology in conjunction with trematode component community dynamics (reviewed by Esch & Fernandez, 1994).

Williams & Esch (1991) using a mark-release-recapture protocol found that vagility of *H. anceps* is low, resulting in lower infection with trematodes recruited *via* ingestion of eggs. They also found higher trematode prevalence in shallow water, which was attributed to a single frog parasite, and explained this with the distribution of the final frog hosts. Williams & Esch (1991) concluded that transmission probability of the parasite is enhanced by the behaviour of the final hosts in conjunction with the mode of infection.

Fernandez & Esch (1991a,b) applied the approach of Williams & Esch (1991) to *H. anceps* in the same pond and suggested that not all snails have the same probability of being infected due to the collective action of several factors such as the behaviour of the definitive hosts, the structure of the habitat, the nature of infective stages (eggs, miracidia) and the dynamics of the snail population (mortality, recruitment and size structure). In contrast, communities in *P. gyrina* at the same pond, although dependent on the spatial distribution of the infective stages, exhibited homogenisation due to the high vagility of the snails which reduced the effect of the microhabitat variability in the distribution of trematode infective stages (Snyder & Esch, 1993).

Recently, Faltýnková et al. (2008) examined the structure of trematode communities in *Valvata macrostoma* at two sites in very close proximity (c. 50–70 m apart) in Lake Konnevesi (Finland). They found marked differences in species composition and prevalence between the shallow littoral and the deep offshore site, with high species diversity in the shallow site and concluded that differences in trematode infection of molluscs can emerge at very narrow spatial and temporal scales.

Temporal variations have also been subject to studies in relation to their effect on trematode prevalence and patterns in community composition and structure. Esch et al. (2001) suggested a dichotomy between freshwater and marine systems due to the differential effect of the seasonal/annual variations on component communities, depending on the longevity of both snail and trematode populations. They hypothesised that short-lived hosts (multiple cohorts per season; mostly freshwater) experience stronger effects of processes on ecological time-scales than in long-lived hosts (multiple seasons per cohort; mostly freshwater). Twin infection peaks, a characteristic pattern in temperate systems, where the input of infective stages is seasonal, have been described in many systems (reviewed in Esch & Fernandez, 1994) and were attributed mostly to the snail mortality and larval recruitment (Snyder & Esch 1993; Esch & Fernandez; 1994; Esch et al., 2001). Kube et al. (2002a) also related the temporal variations in trematode prevalence observed in the marine *Hydrobia ventrosa*-trematode system in the coastal lagoons of the southern Baltic Sea to the life history traits of the snail host with high population turnover within the two-year life span. Temporal variations were found to be less important in other marine snail-trematode systems (Kuris, 1990).

Temporal variations of infection may also be influenced by the distribution and abundance of bird definitive hosts because most of them are migratory and their occurrence is limited to a particular time of the year. For instance, definitive host abundance at some time creates temporal pulses in trematode recruitment resulting in a high prevalence in snail populations. Thus heterogeneity in the abundance of definitive hosts over time and space may either intensify recruitment or act as an isolating factor. For example, Kuris & Lafferty (1994) estimated that spatial and temporal aspects (along with snail size) contributed to about 20% to community structure *via* intensification in recruitment.

## 1.4. LARVAL TREMATODES AS INDICATORS OF BIODIVERSITY, ECOSYSTEM HEALTH AND ENVIRONMENTAL PERTURBATION

As indicated above, for successful completion of the life-cycle in the definitive hosts trematodes require first, second and sometimes third intermediate hosts, which ensure parasite transmission through trophic relations. Trematode diversity and abundance in snails thus reflect associations with a wide range of free-living hosts taking part in the life-cycle within an ecosystem and can, therefore inform on host diversity, abundance and the dynamics of the trophic web at the ecosystem level (*e.g.* Marcogliese, 2003; Hechinger & Lafferty, 2005; Fredensborg et al., 2006; Lafferty et al., 2006, 2008; Hechinger et al., 2007; see also Huspeni et al. 2005 and references therein). Indeed, recent studies have demonstrated association between the diversity and abundance of larval trematode communities in snails and the diversity and abundance of bird communities (*e.g.* Smith, 2001; Hechinger & Lafferty, 2005; Fredensborg et al., 2006) and even a positive linkage with their second intermediate hosts (Hechinger et al., 2007). Since many final hosts are highly mobile (*e.g.* birds, fish, mammals), sampling of larval trematode stages in their first intermediate hosts (snails) represents an easy and cost-efficient approach to obtain information on spatial patterns in abundance of host populations in aquatic habitats, and may thus serve as a useful tool in conservation, *e.g.* Byers et al. (2010) estimated the variations in abundance of the diamondback terrapins, *Malaclemys terrapin*, from trematode prevalence in snails *I. obsoleta*. Empirical evidence is being accumulated that trematodes in their first intermediate hosts can serve as bioindicators of the condition of free-living animal communities in diverse ecosystems (Huspeni et al., 2005; Hechinger et al., 2007 and references therein; Hechinger et al., 2008).

Further, it has been suggested that the prevalence of trematodes declines with habitat degradation (Robson & Williams, 1970; Pohley, 1976) but comparative studies in support of this suggestion are still few. Cort et al. (1960) were the first to link the decline in larval trematode diversity and species richness in a Michigan lake over 20 years. They suggested that increased human disturbance reducing the populations of the final bird hosts over that time may have caused this decline, and Keas & Blankespoor (1997) observed continued declines in prevalence at re-sampling the same sites. Lafferty (1993) detected fine-scale variation in prevalence between populations with respect to disturbance in a salt marsh. Bustnes & Galaktionov (1999) and Bustnes et al. (2000) have recorded high prevalences of trematodes in snail populations at degraded dump sites that attract gulls.

More recently, snail-trematode systems have been used to assess anthropogenic change (reviewed by Morley, 2007) *e.g.* the effect of wetland restoration projects (Huspeni & Lafferty 2004; Morley & Lewis, 2007). Trematode communities in gastropods have also been suggested as candidate useful indicators of the environmental impacts of global warming (Poulin, 2006; Poulin & Mouritsen, 2006). In a recent review on trematode parasites as estuarine indicators, Huspeni et al. (2005) provided supporting evidence of larval trematodes as bioindicators and technical suggestions for their inclusion in monitoring programs in estuarine evnironments; some of them are applicable to freshwater systems.

Eutrophication of aquatic ecosystems is considered as one of the most profound forms of anthropogenic change with a great impact on both parasite and host communities, and ultimately on food webs. Human activities associated with agriculture, livestock or urbanization, increase the input of nutrients (nitrogen or/and phosphorus) into ecosystems and generate high eutrophication levels in the aquatic environment. Eutrophication has frequently been suggested as being associated with increased parasitism in fishes and invertebrates (Lafferty, 1997; Lafferty & Kuris, 1999; Marcogliese, 2005) and recent theoretical work suggests a positive association of eutrophication with the impact of infectious disease (Lafferty & Holt, 2003). High eutrophication of water bodies, associated with colonization by snails and nesting ducks, is suggested as an important factor leading to re-emergence of cercarial dermatitis (caused by *Trichobilharzia* spp., see Kolářová et al., 2010 and references therein).

Recent intensive field and experimental work both, have provided evidence that eutrophication increases transmission rates of the trematode *Ribeiroia ondatrae* to the second intermediate hosts, frog larvae, *via* nutrient-mediated increases in the density of the first intermediate host, *Planorbella trivolvis* (syn. *Helisoma trivolvis*) and cercarial production (Johnson et al., 2002, 2004; Johnson & Chase, 2004; Johnson et al., 2007; see also McKenzie & Townsend, 2007 for a review). Skelly et al. (2006) and Johnson & McKenzie (2009) have suggested that infections in amphibians with echinostomatid metacercariae (species of *Echinostoma* and/or *Echinoparyphium*) increase due to similar nutrient-mediated mechanisms.

The only long-term study in perhaps the most explored freshwater ecosystem, Charlie's Pond, provides a "negative" example in support of the hypothesis of productivity-mediated increase in trematode parasitism. Negovetich & Esch (2007) carried out a comparative assessment of the data on the size and fecundity of *H. anceps* and trematode infection and community composition obtained in 1984 and 20 years later (1984 *vs* 2002, 2005, and 2006). They observed a trend of reduction in snail size and fecundity and the prevalence of the most abundant trematode at the first sampling year, *Halipegus occidualis.* Negovetich & Esch (2007) suggested that the loss of emergent vegetation resulting in reduced periphyton production (food supply for snails) has affected the snail population and this has led to size-mediated differences in the prevalence of *H. occidualis*. They hypothesised that the lower prevalences of this species with high competitive abilities may have in turn, permitted an increase in prevalence of other subordinate species, resulting in an increase in species richness of the trematode component community. The successional changes observed at Charlie's pond thus tend to support the hypothesis of productivity effects on both snail and trematode populations.

To **summarise**, two pervasive patterns have emerged from the studies on snail-trematode systems: (i) low levels of trematode co-existence in individual snail host patches; and (ii) spatial and temporal variation in prevalence. Recent research towards understanding of the processes that determine trematode community structure in marine snails has resulted in the formulation of the competitive exclusion (Kuris, 1990) and accumulation-coexistence hypotheses (Curtis, 2003) of community assembly. On the other hand, research on freshwater host-parasite systems has led to the hypothesis that interspecific interactions within individual snails are unimportant in determining larval community assembly within the local snail populations and that external factors determining the parasite flow within an ecosystem are key determinants of the structure of trematode component communities in snail hosts (Esch & Fernandez, 1994). However, inferences for competition from patterns in parasite community structure in snail-trematode systems appear to be contingent on the hosts studied, and may therefore reflect fundamental differences between the freshwater and marine snails (predominantly pulmonate *vs* predominantly prosobranch) or host life span (short-lived *vs* long lived, respectively).

Further, in spite of the evidence accumulated recently for the effect of spatial and temporal variability on component communities, a rigorous test of the competitive exclusion hypothesis using original infracommunity data and quantitative assessment of the scale effects (*i.e.* spatial and temporal heterogeneity) has been carried out on a single marine snail-trematode system (*C. californica*). Freshwater snail-trematode systems are largely unexplored since existing knowledge on trematode community structure in freshwater snails is based on studies from a single ecosystem, a small eutrophic pond with specific and rather poor fauna of final hosts. This raises the question if the findings of these studies are of general importance, *i.e.* applicable to other freshwater snail-trematode systems. Althouh the rates at which trematodes colonise individual snails may define the patterns of species co-occurrence and subsequent interactions, and thus represent an important prerequisite for the competitive exclusion hypothesis, they have been assessed on marine snail-trematode systems only (*C. californica* and *I. obsoleta*) and the outcomes indicate that these rates are very low.

Nutrient rich freshwater ecosystems provide conditions for an increase in abundance of the intermediate and definitive hosts for trematodes and this may result in increased parasite recruitment, transmission, and infection levels. However, the evidence for association between eutrophication and enhanced parasitism is so far based on assessment of single species population dynamics. This raises the question on how would these changes translate into the composition and structure of larval trematode communities. Unravelling determinants of community structure in parasites in eutrophic environments can largely benefit from a comparative approach in which several populations of a single snail host species are investigated simultaneously. However, quantitative information on the composition and structure of larval communities in snails within productive freshwater ecosystems in Europe, is sorely lacking.

## 1.5. THIS STUDY

The present study was initially designed to provide novel information at the community level, for the freshwater snail *Lymnaea stagnalis* by applying a mark-release-recapture technique on mature, and thus most epidemiologically important cohorts, in seven ponds in Třeboň. The large taxonomically consistent dataset gained in the course of the relatively long-term study on *L. stagnalis* afforded comparative assessment of the structure of the trematode communities in this host at two nested scales of community organisation (*i.e.* infracommunities and component communities). Furthermore, the dataset enabled a search for non-random patterns of community assembly and to assess the contribution of the major processes responsible for community organisation in freshwater snail-trematode systems.

With the progress of data analysis, however, the study expanded since we felt additional questions need to be addressed. First, we wanted to know if the patterns of small-scale variability detected in communities in *L. stagnalis* also apply when considering all snail cohorts. Secondly, we asked whether communities in another abundant sympatric snail host in the area of study, *Planorbarius corneus*, would exhibit concordant patterns of variation. This has led to a short-term simultaneous examination of communities in both hosts in two of the ponds. Thirdly, we wanted to know whether communities in *L. stagnalis* would exhibit similar characteristics in the large eutrophic lakes in Central Europe. This has led, through collaboration with colleagues at the University of Duisburg-Essen, to a comparative study at four mature man-made reservoirs on the River Ruhr, which revealed that another freshwater snail, *Radix auricularia*, plays a major role in trematode transmission in lakes. Finally, we addressed the question if the existing data on infections in the two common and abundant freshwater snails in Europe, *L. stagnalis* and *P. corneus*, would be useful as historical baselines for community richness and structure in future assessments of anthropogenic change.

Collectively, the examination of large snail samples yielded abundant data on parasite communities in the three snail hosts from typical Central European eutrophic environments. The present work therefore, attempts to add to the current knowledge on parasite communities in freshwater snails by addressing the following questions regarding the composition and structure of parasite communities in the model host species:

- What are the characteristics of larval trematode communities in freshwater snails in eutrophic ponds and lakes in Central Europe?
- What are the rates of trematode colonisation and extinction in individual snail host patches in productive environments?

- Are there recognisable patterns of spatial and temporal variation in component community composition and structure at small regional scales?
- Are trematode communities structured by complex assembly rules or are they just random assemblages resulting from stochastic processes?
- Can large-scale historical faunistic data be used to estimate community richness and structure in the past?

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# 2. AIM AND OBJECTIVES

The **aim** of the study is two-fold: (i) to provide novel data on the spatial and temporal variation in the composition and structure of larval trematode communities in selected model species of pulmonate snails in the freshwater productive environments typical of Central Europe; and (ii) to attempt a parasite community approach for assessing effects of man-induced environmental degradation on snail-trematode associations in selected eutrophic water bodies in Central Europe.

The following **objectives** were targeted in the investigation:

**2.1.** Delineation of the composition of component communities in *Lymnaea stagnalis* in seven eutrophic ponds in South Bohemia by the application of a mark-recapture sampling design. Quantification of long-term temporal and spatial patterns of the structure of trematode communities. Comparative assessment of the spatio-temporal patterns in community structure to test of the hypothesis that pond context, *i.e.* size, function and management of the ponds, determines the transmission dynamics of parasite populations and component community composition and structure.

**2.2.** Testing the prediction that trematode colonisation rates meet the assumptions of a competitive exclusion hypothesis using the data on the dynamics of infracommunity composition over time in *L. stagnalis* system. Assessment of differences in colonisation ability at the species level. Test of the uneven transmission hypothesis and the competition-colonisation trade-off hypothesis.

**2.3.** Establishment of a novel dominance hierarchy of trematodes parasitising *L. stagnalis* in Europe using direct evidence for interspecific interactions from the large infracommunity dataset obtained in the mark-recapture study. Assessment of the relative importance of environmental heterogeneity and competition as community assembly mechanisms at both scales of community organization, infra- and component communities using null model analyses.

**2.4.** Evaluation of the short-term small-scale variability in the composition and structure of the component communities of larval trematodes in *L. stagnalis* and *Planorbarius corneus* using quantitative data for both, snail and parasite populations in two of the model ponds studied to test the hypothesis of substantial concordant homogeneity in community composition and structure at small spatial and temporal scales.

**2. 5.** Comparison of the patterns of richness and similarity of continental and regional faunas of *L. stagnalis* and *P. corneus* with the novel component community data for the two snail hosts addressing the question as to whether larger-scale faunistic data may serve as consistent estimates of community richness and structure in these host-parasite systems.

**2.6.** Assessment of parasite response to anthropogenic pressures in urbanised areas typical on a European scale by application of a community approach to the diversity of larval parasites in the lymnaeids *Radix auricularia* and *L. stagnalis*, in relation to hydrological conditions and water quality parameters in four man-made interconnected reservoirs of the Ruhr River (North Rhine-Westphalia).

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# 3. RESEARCH PAPERS

## 3.1. Paper I

### Parasites in a man-made landscape: contrasting patterns of trematode flow in a fishpond area in Central Europe

**Soldánová, M.**, Faltýnková, A., Scholz, T. & Kostadinova, A.

*Parasitology* (2011) 138 (in press)

#### ABSTRACT

We have explored a large body of novel data focusing on small-scale temporal and spatial patterns in the composition and structure of larval trematode communities in *Lymnaea stagnalis* (L.) froma typical Central European agricultural landscape. The 5 eutrophic fishponds studied provide excellent environments for the development of species-rich and abundant trematode communities. Nine prevalent species were consistently present in component communities, but had differential contribution to the parasite flow in the 5 ponds resulting in significant contrasting patterns of community similarity and the prevalence of the 3 major transmission guilds driving this similarity. Component communities split into 2 groups: (i) those from the large pond dominated by anatid and larid generalists with active miracidial transmission; and (ii) those from the smaller ponds dominated by 2 plagiorchioideans infecting snails via egg ingestion. We put forward 3 hypotheses for the remarkable differences in larval trematode flow in the similar and closely located eutrophic ponds: (i) species-specific differences in parasite colonisation potential displayed by an 'active-passive' dichotomy in miracidial transmission strategies of the species; (ii) top-down effects of pond context on transmission pathways of the trematodes; and (iii) competition as an important mechanism in eutrophic environments with a bottom-up effect on component community structure.

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# Rapid colonisation of *Lymnaea stagnalis* by larval trematodes in eutrophic ponds in Central Europe

**Soldánová, M.** & Kostadinova, A.

*International Journal for Parasitology* (under review)

## ABSTRACT

High recruitment rates of multiple species and hierarchical competition are key to competitive exclusion model of community assembly in larval trematode communities in molluscs. Eutrophic environments provide conditions for speeding up trematode transmission and this would increase the strength of interspecific interactions. To test these predictions, we provide the first assessment for a pulmonate snail host, and for highly productive aquatic environments, of the rates of colonisation and extinction at the level of individual snail host patches, of a large guild of trematode species. Using a unique large dataset from a relatively long-term mark-recapture study of *Lymnaea stagnalis* in six eutrophic fishponds in Central Europe, we demonstrate extraordinarily rapid colonisation by trematodes of a snail host thus meeting the assumptions of the competitive exclusion model. Overall annual colonisation rates ranged from 242-502% $yr^{-1}$ so that odds of trematode establishment in an individual snail in these ponds are 2-5 times per year. Extinction rates were substantially lower than colonisation rates and, therefore, would not result in turnover rates high enough to significantly affect prevalence patterns in the snail populations. At the species level, analyses on sample-based estimates of probabilities of colonisation revealed that shared species traits associated with transmission and competitive abilities determine the limits of colonisation abilities. Colonisation rates were exceedingly higher for the species transmitted to the snail passively *via* eggs. There was a significant effect of species competitive abilities on colonisation rates due to subordinate species being substantially better colonisers than both strong and weak dominants, a pattern consistent with the predictions of the competition-colonisation trade-off hypothesis. Our results suggest that, at the extraordinarily high trematode colonisation potential in the area studied, the spatial and temporal patterns of intraspecific heterogeneity in recruitment may provide conditions for intensification of interspecific interactions so that complex community assembly rules may be involved.

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## 3.3. Paper III

# The role of environmental heterogeneity and competition in structuring trematode communities in the great pond snail *Lymnaea stagnalis* (L.)

**Soldánová, M.**, Kuris, A. M., Scholz, T. & Lafferty K. D.

(manuscript in advanced preparation; target journal: *Journal of Parasitology*)

## ABSTRACT

Sequential analytical approach based on null models was applied to assess the importance of two sources of environmental heterogeneity (spatial and temporal) and competition on the structure of larval trematodes infra- and component communities in the pulmonate snail *Lymnaea stagnalis*. A mark-release-recapture method was used to monitor changes in trematode infections over time. A total of 7,623 snails (10,382 capture events) was sampled regularly in seven fishponds in two close areas of Jindřichův Hradec and Třeboň in South Bohemia (Czech Republic) from August 2006 to October 2008. Fourteen trematode species, high overall infection levels (average 39%) and high frequency of multiple infections (7%) comprising 16 types of double and 3 types of triple species combinations, were recorded throughout the study. Putative competitive relationships among trematodes were organized in a dominance hierarchy based on direct and indirect evidence for direct interference competition, indirect exploitative competition, and/or effects of prior occupancy. Seven top dominant species with putatively similar competitive abilities (six redial and one sporocyst species) dominated over other trematodes possessing only sporocysts in their life cycle. This first analysis for a freshwater snail-trematode system revealed the structuring effect of spatial and temporal heterogeneity in parasite recruitment on trematode communities, the former decreasing and the latter intensifying the potential for species encounter, and thereby interactions. Competition among trematodes significantly reduced species co-occurrences due to the negative impact of the dominant species on the abundance of subordinate species. The importance of the structuring effect of competition on component communities results from exclusion of substantial proportion of interacting trematodes. The comparative analysis using two null models confirmed that the null model that operates with observed prevalences underestimates the expected frequencies of co-occurrences.

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## 3.4. Paper IV

# Small-scale to large-scale and back: larval trematodes in *Lymnaea stagnalis* and *Planorbarius corneus* in Central Europe

Brown, R., **Soldánová, M.**, Barrett, J. & Kostadinova, A.

*Parasitology Research* (2011) 108: 137-150

## ABSTRACT

We examined the small-scale temporal and spatial variability in composition and structure of larval trematode communities in *Lymnaea stagnalis* and *Planorbarius corneus* in two fish ponds in the Czech Republic and compared the patterns of richness and similarity to continental and regional trematode faunas of these hosts. The levels of parasitism in the populations of both hosts were high, the former parasitized predominantly by allogenic species maturing in a wide range of birds and the latter infected by relatively more species completing their life cycles in micromammals. Communities in both hosts exhibited a congruent pattern of seasonal change in overall infection rates and community composition with lower levels of infection in spring. Both temporal and spatial variation was closely related to the structure of snail populations, and no significant differentiation of community composition with respect to pond was observed. Comparisons with large-scale inventories revealed overall congruent patterns of decreased richness and similarity and increased variability at the smaller scales in both host-parasite systems. The relative compositional homogeneity of larval communities in both snail hosts irrespective of scale suggests that historical data at small to medium regional scales may provide useful estimates of past richness and composition of larval trematode communities in these snail hosts.

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## 3.5. Paper V

# Larval trematode communities in *Radix auricularia* and *Lymnaea stagnalis* in a reservoir system of the Ruhr River

**Soldánová, M.**, Selbach, C., Sures, B., Kostadinova, A. & Perez-del-Olmo, A.

*Parasites & Vectors* (2010) 3: 56

## ABSTRACT

**Background:** Analysis of the data available from traditional faunistic approaches to mollusc-trematode systems covering large spatial and/or temporal scales in Europe convinced us that a parasite community approach in well-defined aquatic ecosystems is essential for the substantial advancement of our understanding of the parasite response to anthropogenic pressures in urbanised areas which are typical on a European scale. Here we describe communities of larval trematodes in two lymnaeid species, *Radix auricularia* and *Lymnaea stagnalis* in four man-made interconnected reservoirs of the Ruhr River (Germany) focusing on among- and within-reservoir variations in parasite prevalence and component community composition and structure.

**Results:** The mature reservoir system on the Ruhr River provides an excellent environment for the development of species-rich and abundant trematode communities in *Radix auricularia* (12 species) and *Lymnaea stagnalis* (6 species). The lake-adapted *R. auricularia* dominated numerically over *L. stagnalis* and played a major role in the trematode transmission in the reservoir system. Both host-parasite systems were dominated by bird parasites (13 out of 15 species) characteristic for eutrophic water bodies. In addition to snail size, two environmental variables, the oxygen content and pH of the water, were identified as important determinants of the probability of infection. Between-reservoir comparisons indicated an advanced eutrophication at Baldeneysee and Hengsteysee and the small-scale within-reservoir variations of component communities provided evidence that larval trematodes may have reflected spatial bird aggregations (infection 'hot spots'). Two life history groupings of dominant species, the 'cyprinid' and 'anatid' parasites, that depict two aspects of progressive eutrophication in this mature reservoir system, were identified.

**Conclusions:** We conclude that trematode communities in the lake-adapted *R. auricularia* are better suited for monitoring the effect of environmental change on host-parasite associations in the reservoir system on the Ruhr River and other similar systems due to the important role of this host in trematode transmission in lakes. Whereas variations in trematode community diversity and abundance may indicate the degree of eutrophication on a larger scale (among reservoirs), the infection rates of the two life history groups of dominant species, the 'cyprinid' and 'anatid' assemblages, may be particularly useful in depicting environmental variability, eutrophication effects and infection 'hot spots' on smaller spatial scales.

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# 4. CONCLUDING REMARKS AND FUTURE PROSPECTS

Profiting from the application of from both, advanced sampling methods (mark-release-recapture) and comparative approaches (within- and cross-host taxon assessment), this study yielded abundant novel data on the composition, structure and variability of larval trematode communities at three nested scales in typical Central European eutrophic environments. The model freshwater snail host species, the lymnaeids *Lymnaea stagnalis* and *Radix auricularia* and the planorbid *Planorbarius corneus*, represent the most suitable trematode intermediate hosts with a wide Palaearctic distribution. Since they collectively encompass trematode diversity at the ecosystem level over large spatial scales, the delineation of larval trematode community structure in these hosts from well-defined ecosystems would advance further ecological research and the use of communities as indicators of environmental change. Further, the hypothesis driven approaches with the application of null-model analyses, logistic regression modelling and multivariate randomisation techniques, helped the advancement of our understanding of the patterns of composition and structure of trematode communities in freshwater snail hosts by depicting the mechanisms linking habitat variability with the action of complex community assembly rules in freshwater pulmonates.

The study has captured exhaustively the diversity of the trematode species parasitizing *L. stagnalis* (16 species), *R. auricularia* (12 species) and *P. corneus* (7 species) in Europe's wetlands. These diverse species assemblages utilise diverse transmission pathways and a wide range of final vertebrate hosts. The species composition of component communities indicated different roles of the lymnaeid and planorbid hosts in trematode transmission thus portraying the relationships between two different final host components of aquatic ecosystems, the more diverse and vagile bird communities and the poorer and more confined to the water micromammal/amphibian communities. This was supported by the higher compositional homogenisation *vs* differentiation of the local faunas in *L. stagnalis* and *P. corneus*, respectively. On the other hand, the finding of concordant homogeneity in the composition and structure of component communities in these two host species at fine spatital scales indicated the importance of the habitat-associated structure of the snail populations.

The delineation of community composition and structure in the model snail hosts at the component level demonstrated, that the productive ecosystems studied in the Czech Republic and Germany provide excellent environments for the development of species-rich and abundant trematode communities in the first intermediate hosts as evidenced by the high transmission rates and overall prevalence levels. The notable differentiation in community composition and structure among localities revealed in the two study systems, *i.e. Lymnaea*-pond system in the Czech Republic and *Radix*-lake system in Germany, depicted the action of top-down effects of habitat context (*i.e.* size, function/management, and productivity and water quality, respectively) on trematode transmission pathways and abundance. The patterns of parasite flow were particularly different in the ponds in South Bohemia as evidenced by the significant contrasting patterns of community similarity and the prevalence of the three major trematode transmission guilds between the large pond (Vlkovský) and the remaining smaller ponds. Although the relatively long-term study of communities in *L. stagnalis* in South Bohemia has led us to consider the significance of top-down effects, we tested the alternative hypothesis for competition as an important mechanism in eutrophic environments with a bottom-up effect on component community structure.

This first assessment for a pulmonate snail host, and the first for highly productive aquatic environments, of the rates of colonisation and extinction at the level of individual snail host, of a large guild of trematode species, demonstarted extraordinarily rapid colonisation by trematodes thus meeting the assumptions of the competitive exclusion model. The substantially lower estimates for trematode extinction rates suggested low species turnover rates also in support of the competitive hypothesis. As predicted from the observed variations in component community structure, the study also revealed a significant effect of species competitive abilities on colonisation rates due to subordinate species being substantially better colonisers than competitively dominant species, a pattern consistent with the predictions of the competition-colonisation trade-off hypothesis. These results suggested that the spatial and temporal patterns of intraspecific heterogeneity in recruitment in the area studied, provide conditions for intensification of interspecific interactions so that complex community assembly rules may be involved.

This question was addressed by the application of a sequential null-model analyses of the effects of spatial and temporal heterogeneity in conjunction with the competition hypothesis. The present study is the first to postulate a dominance hierarchy for trematodes in a freshwater snail using direct field evidence based on original taxonomically consistent data, gathered at the level of the individual snail hosts. The results demonstrated, for the first time on a freshwater snail-trematode system, a significant bottom-up structuring effect of competition at the infracommunity level and provided evidence for its additivity at the component community level.

Where to from here? It is not unusual and even sometimes tempting, an exhaustive study on one system to be followed by explorative research on similar systems using the same methodological approach. Perhaps a mark-recapture study on a planorbid, *e.g. Planorbarius corneus* or *Planorbis planorbis*, would be influential for confirmation or disproval of the competition hypothesis on yet another freshwater snail system. We, however, feel that "going up" the scales of the present study would lead to fruitful discoveries.

"Going up" on a geographic scale with the knowledge gained at the small spatial scales of the present study, would fertilize the search for large-scale geographical patterns in trematode distribution and abundance, a prerequisite for predictive distribution modelling approaches at the forefront of research on emerging wildlife diseases.

"Going up" on a lower geographic scale relies to the "applied" direction of mollusc-trematode research, *i.e.* the use of trematode communities as tools for detecting free-living animal diversity, food-web structure and biomonitoring environmental change, is likely to yield valuable results. The dominance hierarchy developed in the present study will be a valuable tool for calculating the pre-interactive prevalences of the trematode species in communities in these assessments. As revealed by the pilot study in the four Ruhr lakes in Germany, a quantitative trematode community approach holds a promise for further generalisations. Particularly valuable in relation to habitat productivity is the finding, that infection rates of the two life history groups of dominant species, the 'cyprinid' and 'anatid' assemblages may depict environmental variability and eutrophication effects on smaller spatial scales. This first study relating environmental variables with the outcomes of trematode transmission at the level of individual snails in nature, stresses the need of considering water quality variables in biomonitoring programs.

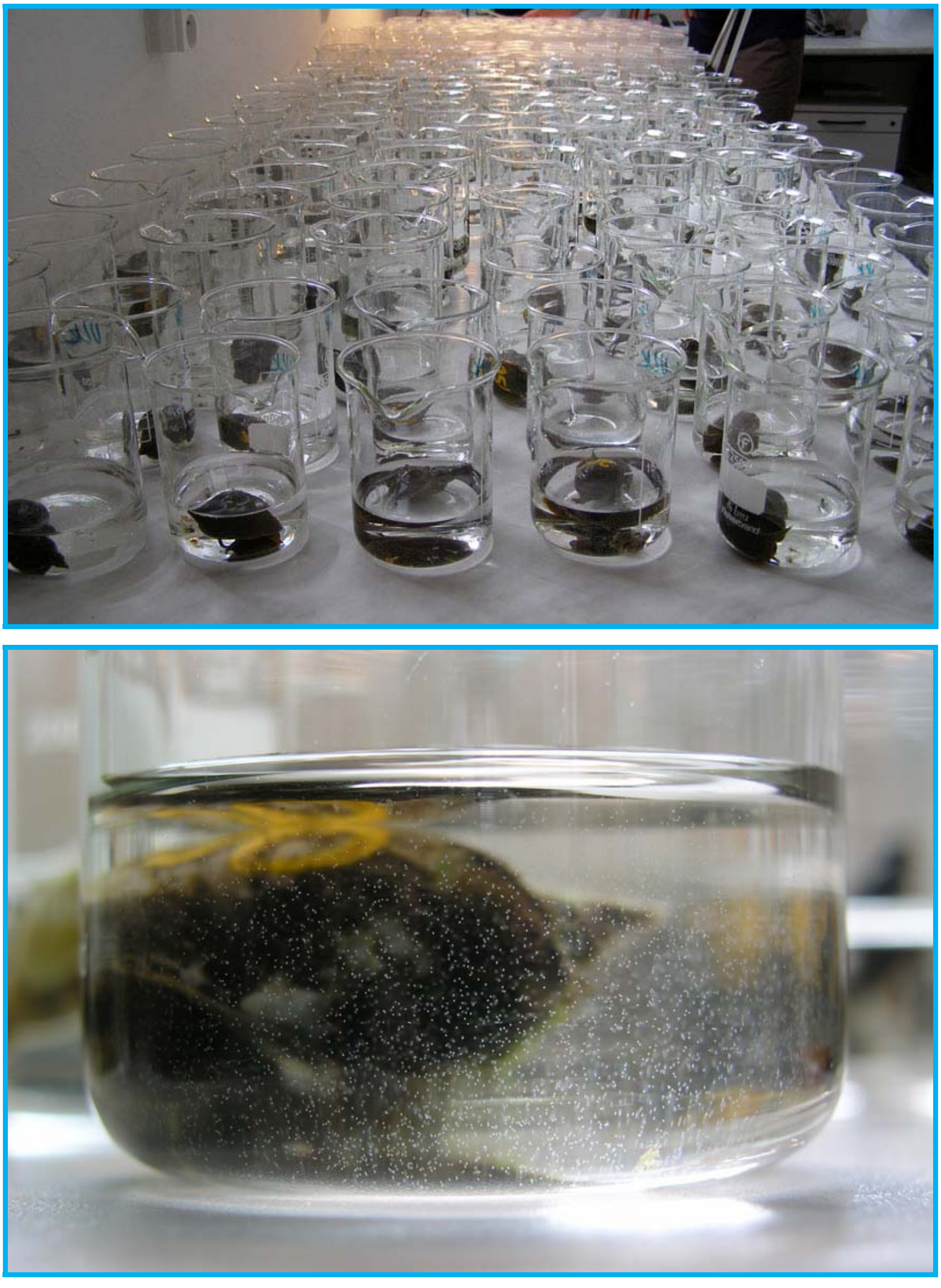
"Going up" on a host scale aspect relates to unravelling the complexity of the parasite flow within an ecosystem, a largely unexplored field on a European scale. Of particular concern should be conservation issues in the European wetlands, predominantly man-made environments supporting high animal diversity. The contrasting patterns parasite flow in the study ponds in Třeboň Basin Biosphere Reserve and Protected Landscape Area (BR/PLA), and the significant effect of competition by the dominant echinostomatid trematodes on these patterns, indicate that the bottom-up processes could be transferred to the upper levels of the trophic chains. Thus, if a habitat provides good conditions for birds, this may be bad news for amphibians (intermediate or usually dead-end hosts for echinostomatids) because of the potential of heavy infections in their populations as a by-product of the high transmission rates. Reserves serve as “hot-spots” for both the diversity and population abundance of birds, fishes and amphibians (intermediate and final hosts for trematodes) and may thus represent trematode infection “hot-spots”. However, our knowledge of the complex host-parasite interactions at the ecosystem level in reserves is inexistent, and again, the evidence for their significance comes from studies in disparate ecosystems, hosts and parasites in North America. This is a promising line of research on emerging diseases in wildlife.

There is a lot to learn from snails, the seemingly insignificant inhabitants of Europe’s wetlands holding a multitude of interactions within their tiny shells.

# 5. APPENDICES

## Appendix I

Snail screening for patent trematode infections (cercarial emission) and labelling used in the mark-recapture-release method applied to examine larval trematode communities.



# Appendix II

Sampling sites at the fishponds in the area of Třeboň (**V**, Vlkovký; **HS**, Hluboký Sax; **HH**, Hluboký u Hamru) and Jindřichův Hradec (**Z**, Zavadil; **VD**, Velký Dvorecký; **B**, Bartoňovský; and **S**, Špitálský) in South Bohemia (Czech Republic).

Pond **V** (Vlkovský)

Pond **HS** (Hluboký Sax)
before the restoration project
(October 2006)

Pond **HS** (Hluboký Sax)
during the restoration project
(October 2007)

Pond **HS** (Hluboký Sax)
after the restoration project
(August 2008)

Pond **HH** (Hluboký u Hamru)

Pond **Z** (Zavadil)

Pond **VD** (Velký Dvorecký)

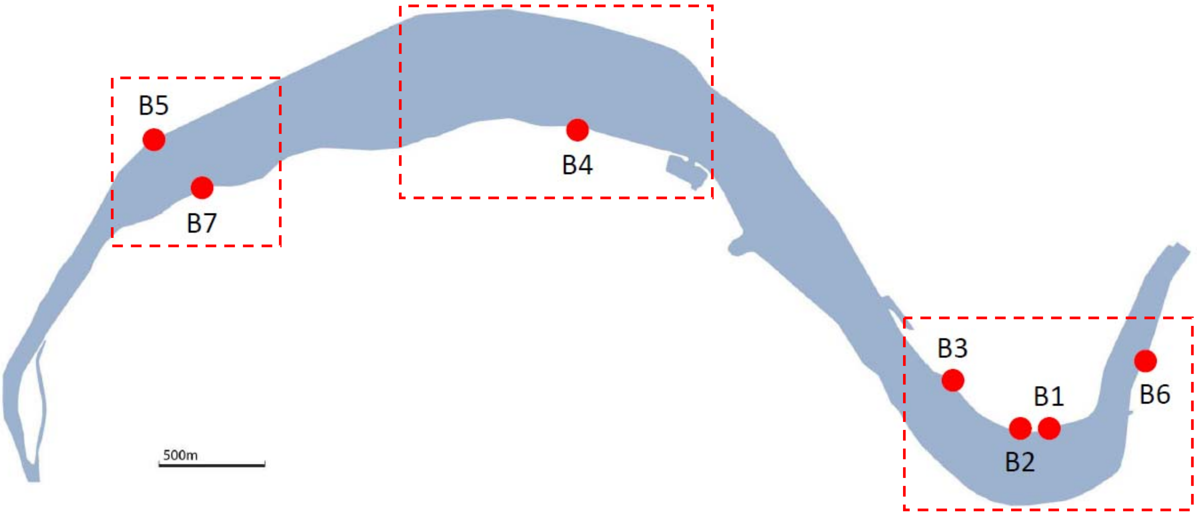
Pond **B** (Bartoňovský)

Pond **S** (Špitálský)

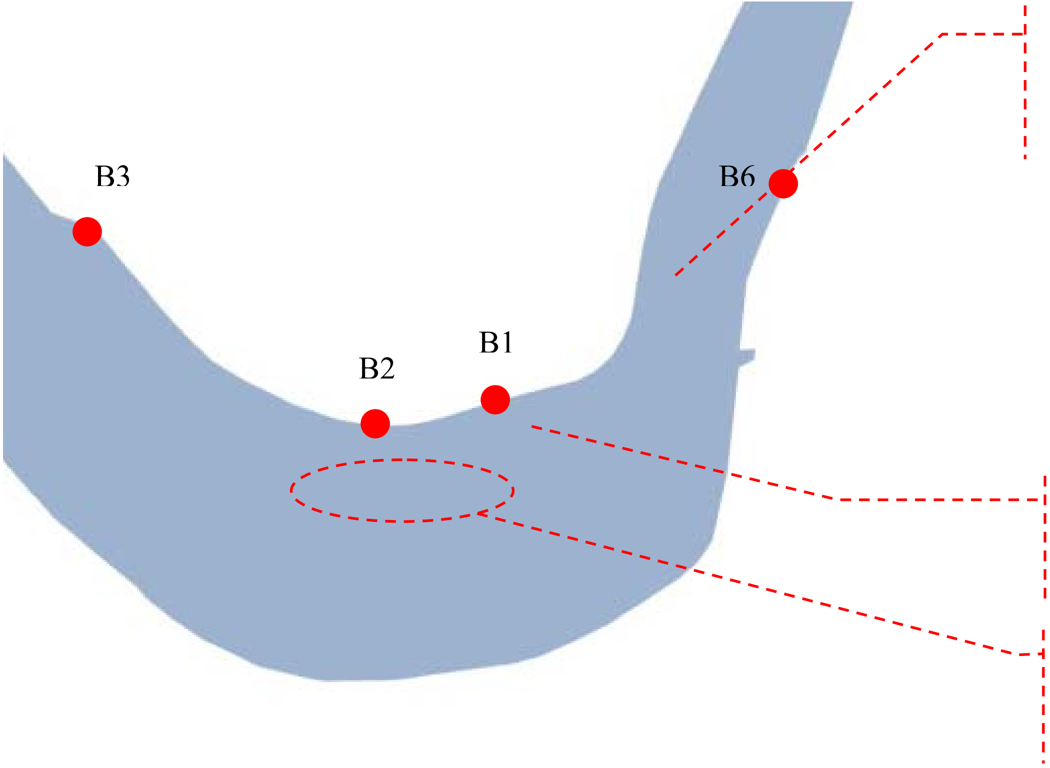
## Appendix III

**Soldanova et al. (2010), Parasites & Vectors, 3: 56. Additional file 1.** Maps of the four reservoirs on the Ruhr River with indication of the sampling sites, bird aggregations and photos.

# Baldeneysee



### Sites B1, B2, B3 and B6

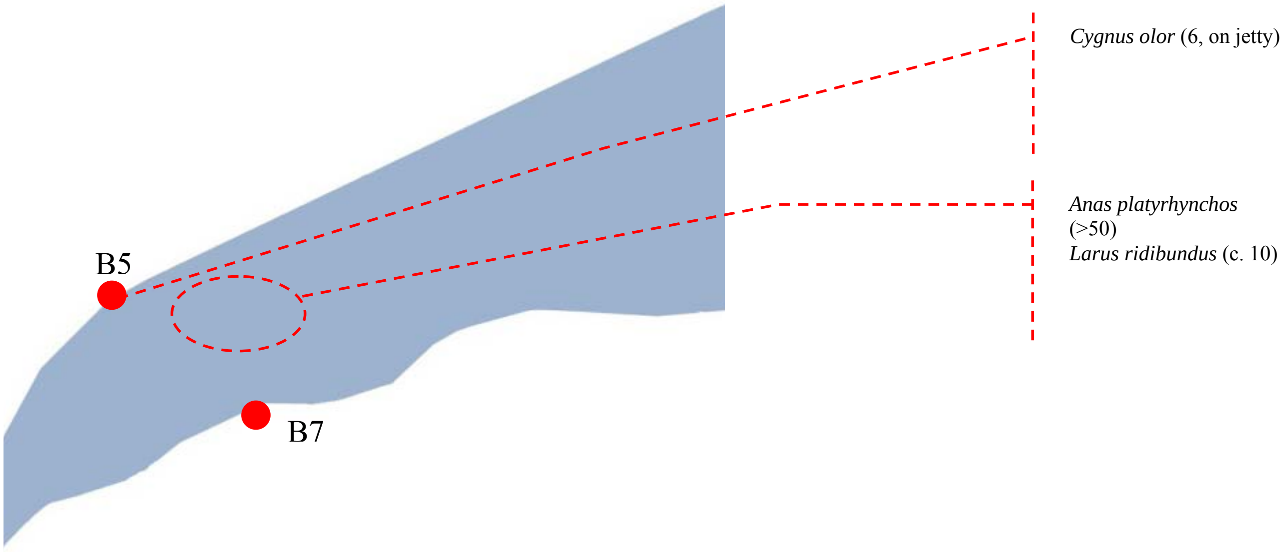


*Anas platyrhynchos* (c. 20)
*Tachybaptus ruficollis* (1)

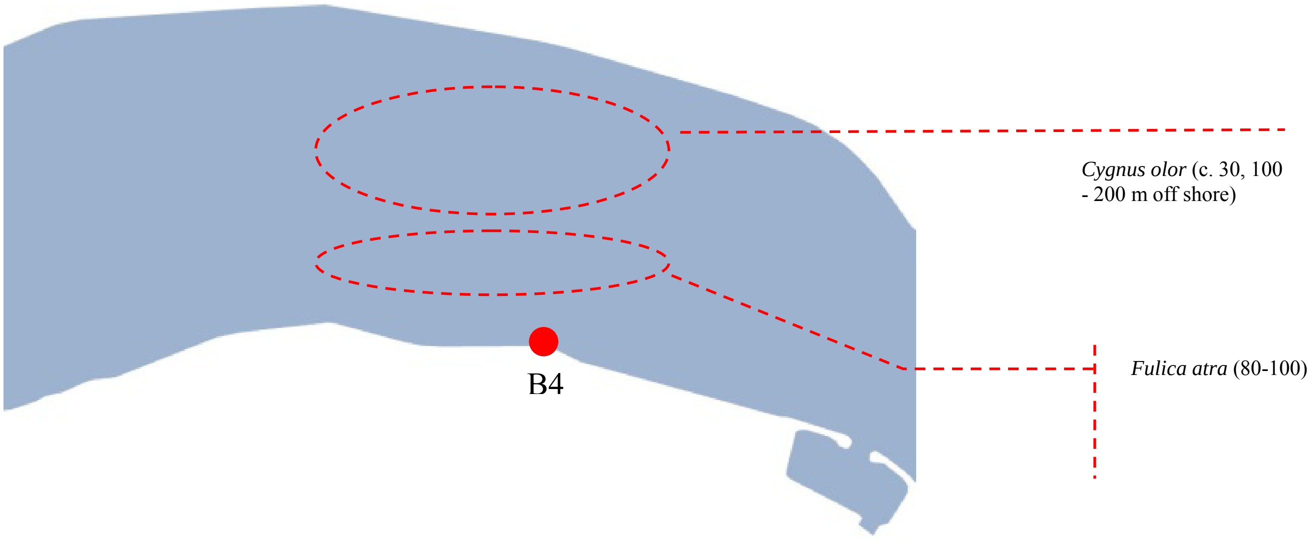
*Phalacrocorax carbo* (6)

*Fulica atra* (>60)
*Cygnus olor* (2)
*Anas platyrhynchos* (<15)

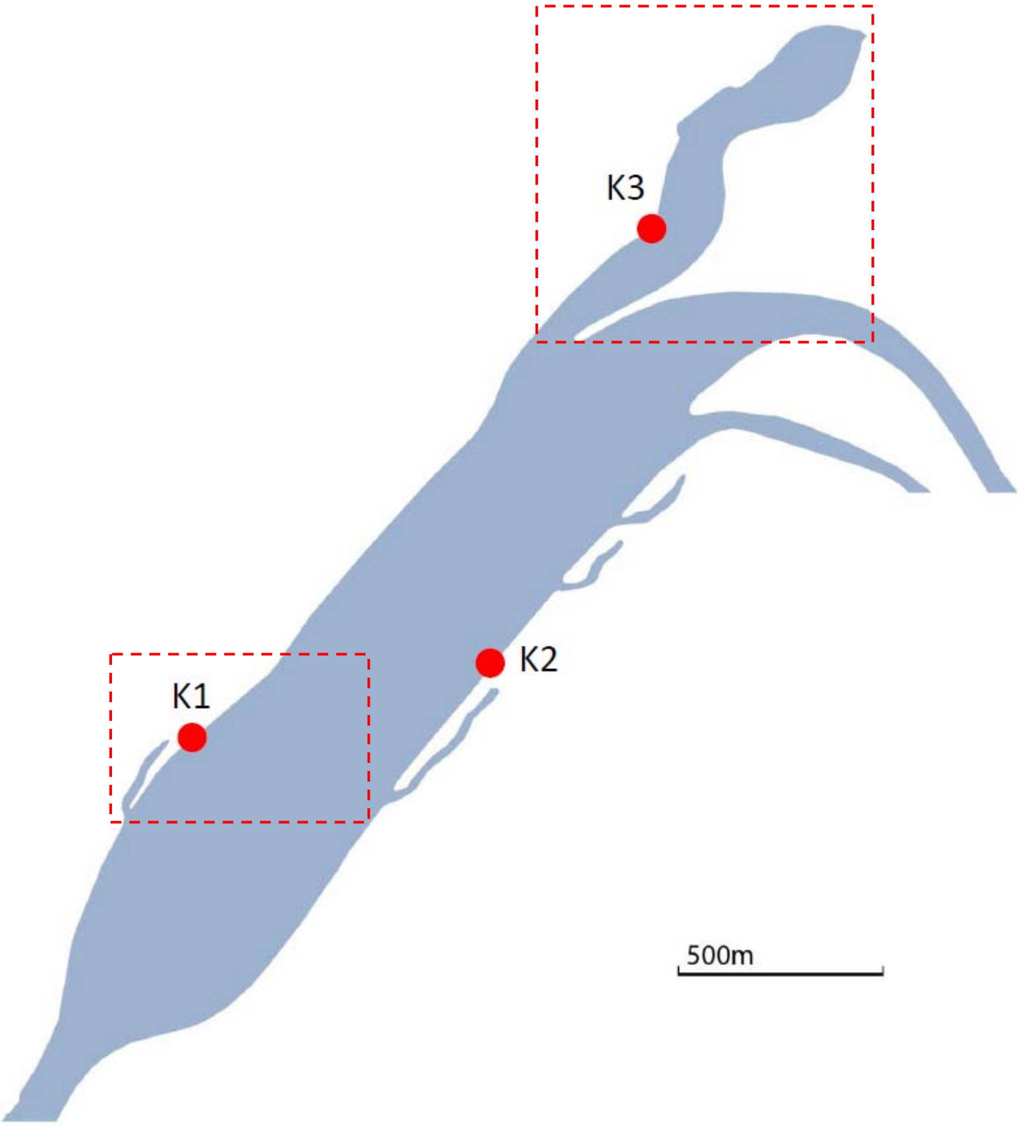
## Sites B5 and B7



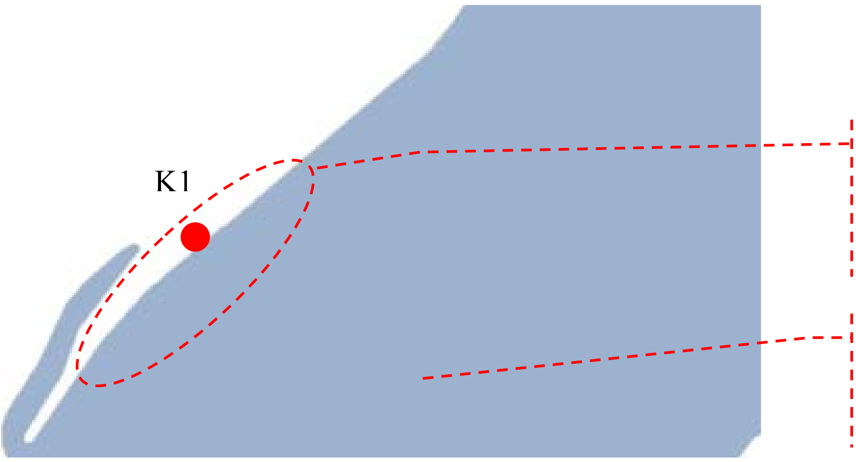
## Site B4



# Kemnader See



## Site K1

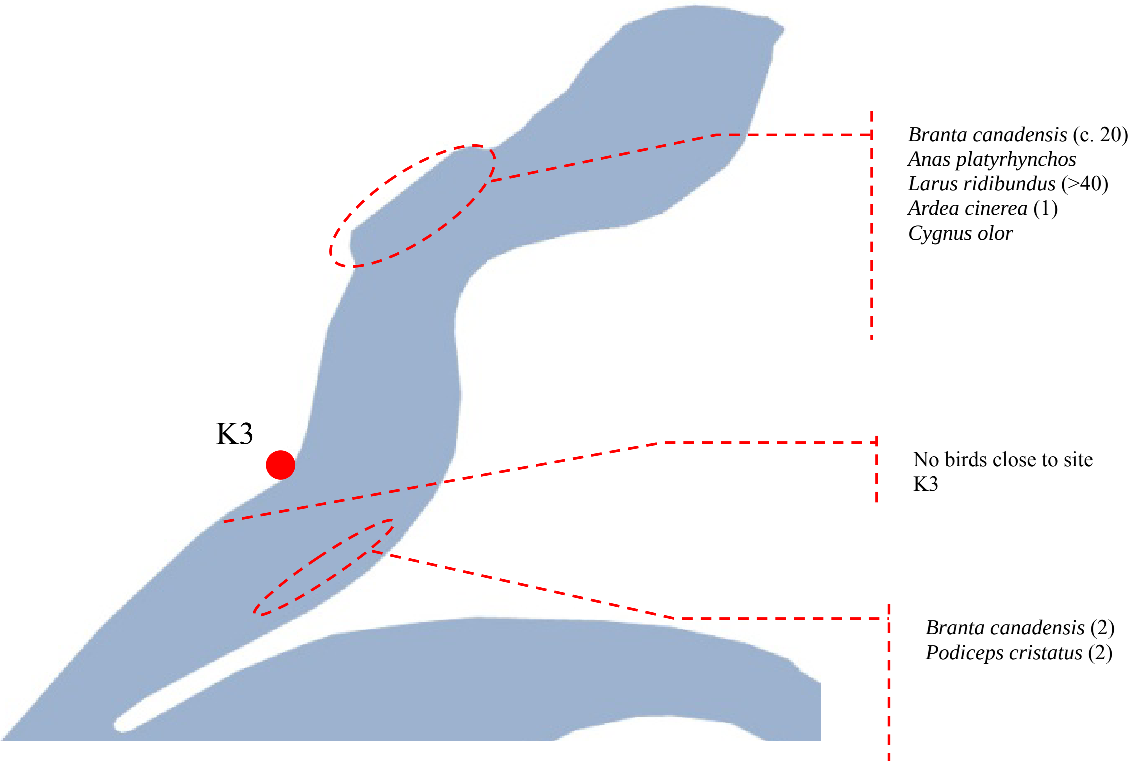


*Anas platyrhynchos*
*Alopochen aegyptiacus*
*Fulica atra*
*Cygnus olor* (3)

*Fulica atra* (>50, on the reservoir)

*Anas platyrhynchos*, *Alopochen aegyptiacus*, *Cygnus olor* and *Fulica atra* on a jetty close to K1

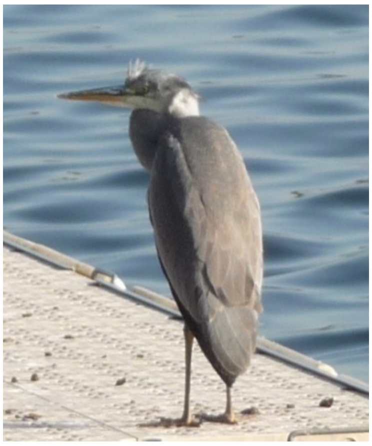
## Site K3



*Podiceps cristatus* close to K3

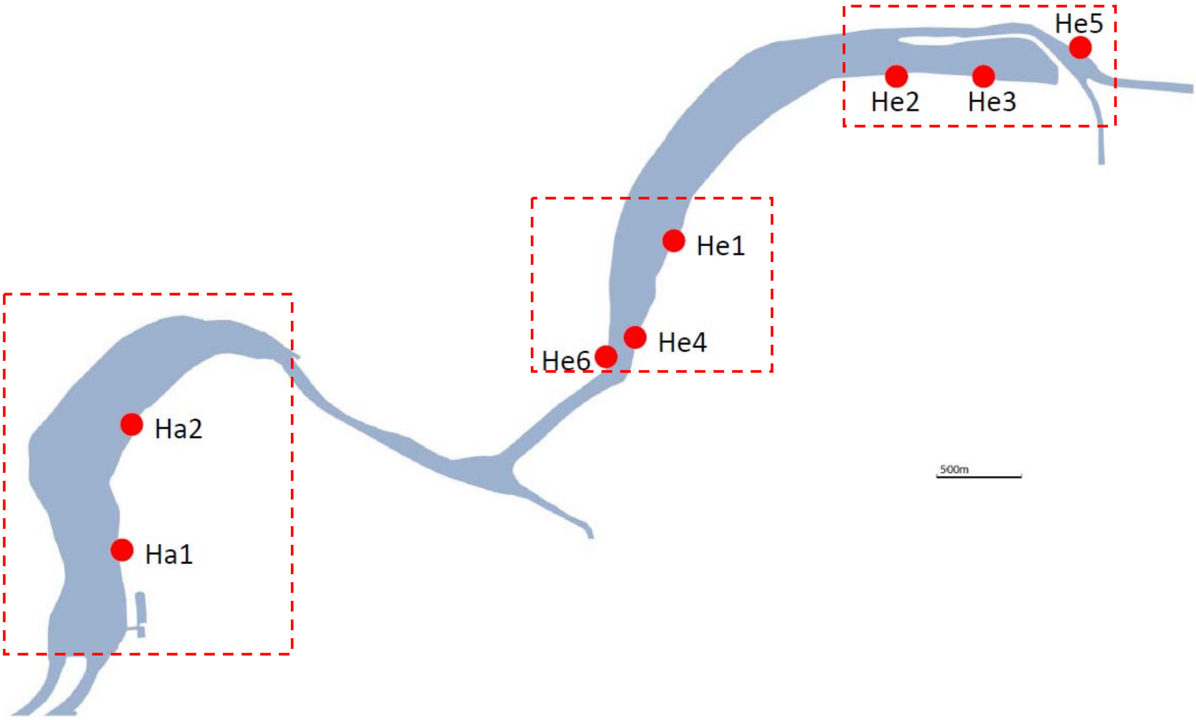
*Branta canadensis* close to site K3

*Larus ridibundus* on a jetty close to site K3

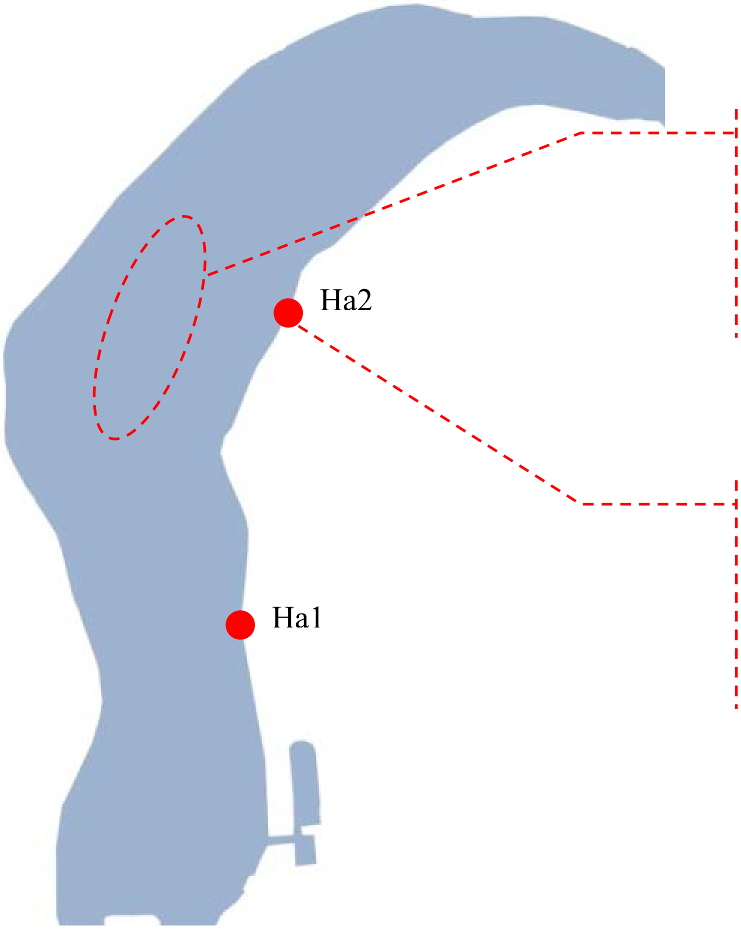


*Ardea cinerea* on jetty close to site K3

## Harkortsee and Hengsteysee



### Sites Ha1 and Ha2



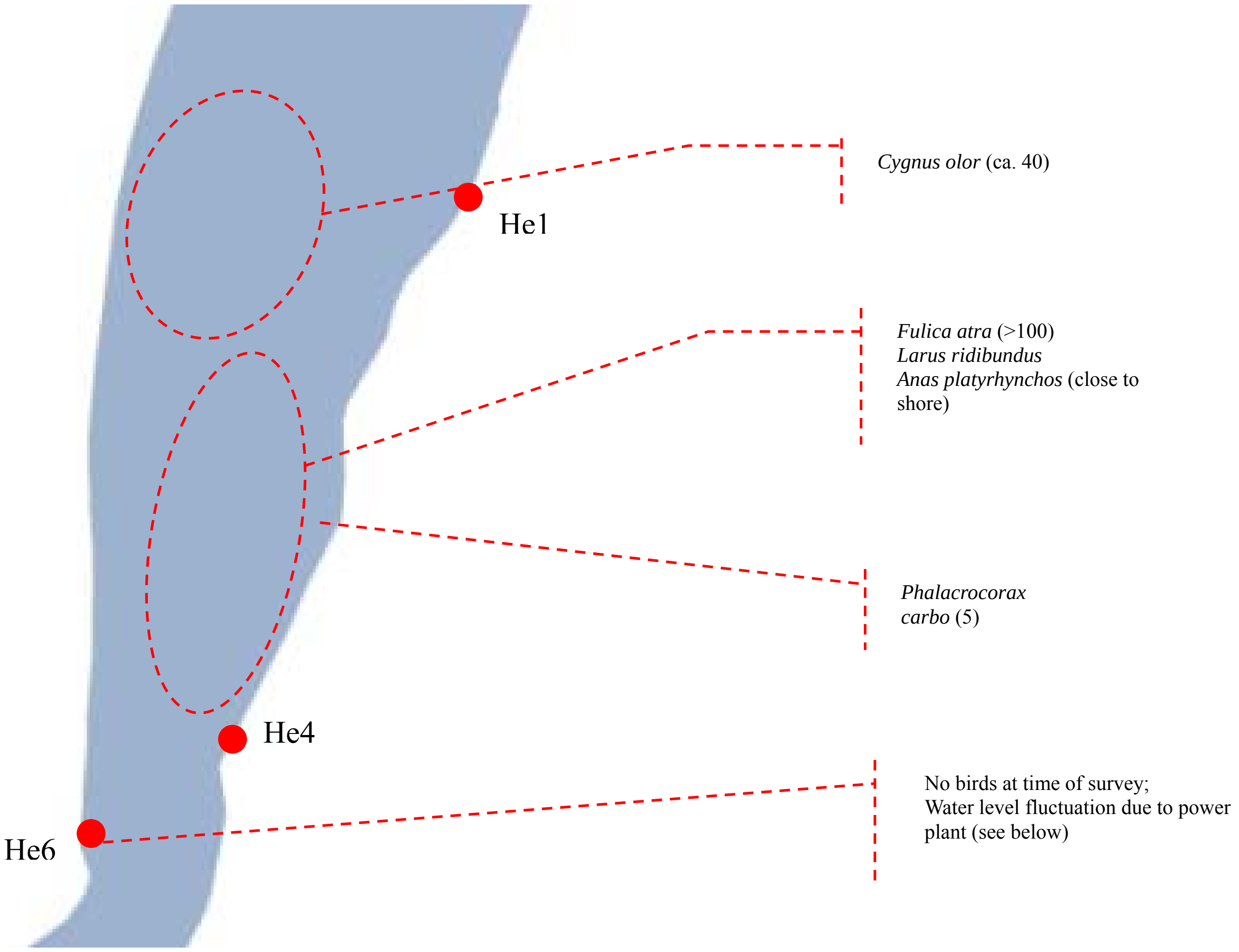
*Fulica atra* (>100, close to shore)
*Anas platyrhynchos*
*Larus ridibundus*
*Cygnus olor* (2)
*Podiceps cristatus*

No birds close to sampling sites Ha1 and Ha2 at time of survey

*Branta canadensis* and *Phalacrocorax carbo* common in this area according to residents

*Anas platyrhynchos* and *Fulica atra* on Harkortsee

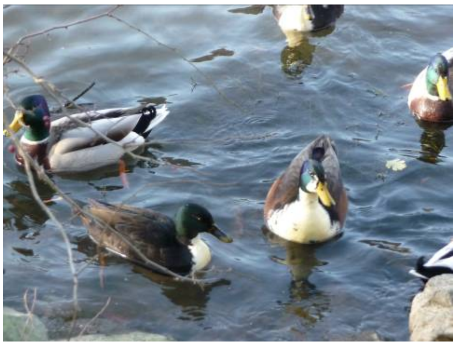
## Sites He1, He4 and He6



*Phalacrocorax carbo* on Hengsteysee (between sites He1 and He4)

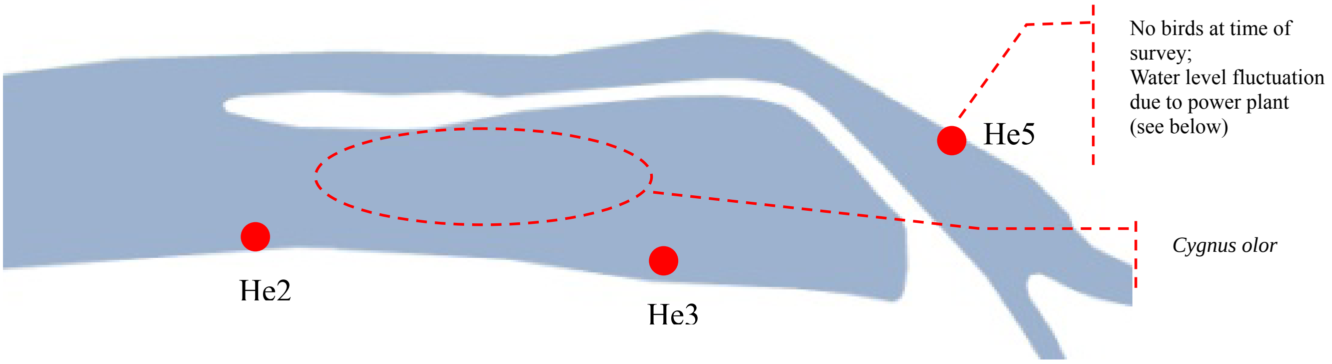
*Cygnus olor*, *Phalacrocorax carbo*, *Fulica atra* and ducks close to site He1

Domestic duck close to site He4

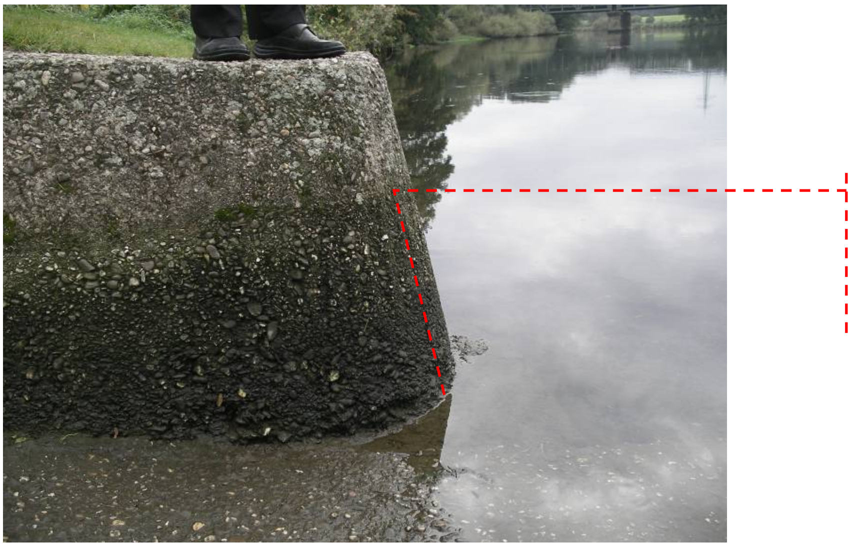


*Anas platyrhynchos* and domestic ducks close to site He4

## Sites He2, He3 and He5



*Cygnus olor*, *Fulica atra* and *Phalacrocorax carbo* close to sites He2 and He3



Water level fluctuation
due to operation of the pumped-storage hydropower plant at
Hengsteysee

Site He5