Master's thesis

SHELL MORPHOLOGY OF THE UNIONID MUSSELS (*Anodonta anatina*, *Unio pictorum* and *U. tumidus*) IN RELATION TO GENDER AND TREMATODE PARASITISM

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International Aquatic Masters Programme

07.03.2015

ABSTRACT

Freshwater mussels (Unionoida) show great variability in shell morphology which can be associated with differences in habitat, sex and/or parasite infection. Three unionid mussel species, (*Anodonta anatina*, *Unio pictorum* and *Unio tumidus*) were collected from two sites Lake Saravesi, (Laukaa) and Lake Kuivasjarvi, (Oulu), between May - October 2012. Intraspecific trends that were associated with the mussels' sex and/or parasites infection were studied and observations were made on how sex and parasite infection could have a role in shells morphological characters/patterns. The study revealed a range of morphometric differences among and across populations, as well as, within and between species. The hypothesis that female growth would decrease relative to males at adult ages due to energetic cost of glochidium larvae production did not receive unequivocal support. In addition, the hypothesis of adult female shells being wider (more inflated) than that of males due to space requirements by carried glochidia was also supported only in *A. anatina* of Lake Kuivasjärvi but not in Lake Saravesi, or in other mussel species. Male *A. anatina* were higher than females in Lake Saravesi, but not in Lake Kuivasjärvi, or in other mussel species, except for *U. pictorum*, in which females were higher than males among the large individuals. Between species, *A. anatina* had the highest shell, followed by *U. tumidus* and *U. pictorum*, while *U. tumidus* had the widest shell, followed by *U. pictorum* and *A. anatina*. The hypothesis of trematode infected mussels being wider due to space requirements by growing parasites was supported partly: mussels infected by *R. fennica* were, indeed, wider than the uninfected individuals, but for mussels infected with *R. campanula* this pattern was not found. On the other hand, *R. campanula*–infected individuals were relatively longer (more elongated) than the uninfected mussels. This may indicate a parasite species specific interaction between parasitism and host mussel shell morphology.

JYVÄSKYLÄN YLIOPISTO, Matemaattis-luonnontieteellinen tiedekunta

TIIVISTELMÄ

Unionidae-heimon simpukoilla on suurta vaihtelua kuoren morfologiassa, liittyen esimerkiksi habitaattiin, sukupuuoleen tai loisinfektioihin. Tässä työssä kerättiin kolmen simpukkalajin (*Anodonta anatina*, *Unio pictorum* and *Unio tumidus*) edustajia Laukaan Saravedestä ja Oulun Kuivasjärvestä toukokukun ja lokakuun 2012 välisenä aikana, ja kuoren pituus, korkeus ja paksuus sekä kuoren tilavuus mitattiin. Hypoteesille, että glokidium-toukkien tuottaminen alentaisi naaraiden kasvunopeutta suhteessa koiraisiin ei saatu tukea. Lajeja vertailtaessa todettiin, että *A. anatina* −simpukalla kuoren suhteellinen korkeus oli suurin, *U. tumidus* − lajilla toiseksi suurin ja *U. pictorum* −lajilla pienin. Hypoteesille, että naaraaat olisivat muodoltaan leveämpiä glokidium-toukkien vaatiman tilan takia saatiini tukea vain Kuivasjärven *A. anatina* −lajilla, muttei samalla lajilla Saravedessä eikä *Unio*-lajeilla. Sukupuolten välisiä eroja kuoren suhteellisessa korkeudessa löytyi Saraveden *A. anatina* − lajilla (koiraat suhteessa korkeampia kuin koiraat) sekä *U. pictorum* −lajilla (naraat muodoltaan korkeampia kuin koiraat vanhemmissa ikäluokissa).Trematoda-loisia esiintyi vain *A. anatina* −simpukalla (havaitut loislajit: *Rhipidocotyle fennica* ja *R. campanula*). Hypoteesi, että trematoda-loisittujen simpukoiden kuori olisi leveämpi kuin ei-loisittujen simpukoiden (jotta loisella olisi enemmän tilaa kehittyä), sai tukea *R. fennica* −lajin osalta; *R. fennica* − loisittujen yksilöiden kuoren suhteellinen leveys oli suurempi kuin ei-loisittujen, mutta *R. campanula* −loisen kohdalla vastaavaa havaintoa ei tehty. Sen sijaan *R. campanula* −loisittujen yksilöiden kuoren suhteellinen korkeus oli pienempi kuin ei-loisittujen simpukoiden. Toisin sanoen *R. campanula* − loisittujen simpukoiden kuri oli dorsoventraalisesti litistynyt, eli pitkulaisempi. Tutkimus paljasti sukupuoleen ja trematoda-loisintaan liittyviä muutoksia/eroja simpukoiden kuoren muodossa ja herätti mielenkiintoisia uusia kysymyksiä esimerkiksi loislajien erilaisista vaikutuksista tai yhteyksistä isäntäsimpukan kuoren morfologiaan.

Contents

1. INTRODUCTION

The interpretation of different morphological patterns of animal species has been a subject of scientific interest as far back as the 18th Century (Darwin 1859). Not only is this vital for taxonomic research, understanding the factors that determine variation in morphology is very important as this potentially contains a range of information about an individual's biological and ecological characteristics, and freshwater mussels, (unionids) are ideal for this purpose. Ecophenotypic morphological trends have assisted in habitat requirements identification of endangered species (Zieritz & Aldridge 2009). On the other hand, nonecophenotypic morphological trends could be useful for reconstruction of sex ratios, for example, Quiles & Monchot (2004) and Berger *et al*. (2001) used dimorphic morphological patterns to ascertain sex ratios of ungulate and bear populations. Regrettably, our poor understanding of sexual and parasite-induced differences in unionids shell morphology has made it inappropriate for us to use similar applications in the shell morphology of freshwater mussels. However, the idea that freshwater mussels serve as the first intermediate host to trematode parasites Taskinen *et al*. (1991) makes them to be very important especially in this study which focuses on how parasites can influence their shell morphology. The striking phenotypic variability in freshwater mussel shells (i.e. size and form) has long been a nightmare to taxonomists, resulting in enormous lists of taxonomic synonyms for many species. Such variability could be attributed to unionids ability to change phenotype in response to changing environmental conditions, and thus can be interpreted as an adaptation to the unpredictable and changing nature of freshwater habitats or genetic variability (e.g. Zieritz & Aldridge 2011). Differences in shell morphology amongst unionids can vary between males and females, old and young individuals, species, between and among populations (Zieritz & Aldridge 2011). Also, infestations by *Rhipidocotyle* trematodes have been shown to alter sagittal and lateral shell shape of freshwater mussel, *A. anatina* (Zieritz & Aldridge 2011).

1.1. Freshwater mussels (unoinids)

Freshwater mussels (Bivalvia: Unionoida) are common, widespread and inhabit all continents except the Antarctic (Graf & Cummings 2006). Their oldest known representatives' date as far back as the Triassic (Watters 2001) and their typically high degree of variation in shell morphology potentially contain a range of information about the biological and ecological characteristics of the individual. Freshwater mussels' produces hard parts that persist in sedimentary deposits and as such can be used in the reconstruction of ancient populations and species. Freshwater mussels shell morphology could be of special importance in these respects. Sex, trematode infection and indirect habitat effect could influence shell morphology of the freshwater mussel, *A. anatina,* e.g. (Zieritz & Aldrige 2011) found female *A. anatina* mussels to be thinner and elongated than the males and this trend varied between populations. However, limited knowledge on which factors cause which trends in shell form makes intraspecific morphological differences in unioinids to be rarely used for redrawing both past and present characteristics (Claassen 1998). Presumably the best studied although not sufficiently understood type of intraspecific variation in the shell morphology of unionids is that which deals with the conditions of the habitat (Hornbach *et al*. 2010, Zieritz *et al*. 2010).

1.2. Life cycle of freshwater mussels

The life cycle of freshwater mussels of the family Unionidae involves four distinct stages: 1) the fertilized egg, 2) the young or glochidium in the brood sac of the female mussel,

3) the glochidium in the parasitic stage on a fish or salamander and 4) the adult, free-living stage within a shell, (Haag & Warren 1997), (Fig. 1). Mussels have two separate sexes although a few species are hermaphroditic. In many species the two sexes can be distinguished by the shape of the shell. The male sex cells are released into the water and are taken into the female's body through the in-current siphon. They are then transported to the gills where the eggs are fertilized. The gills now serve as brood pouches as well as respiratory organs. Next, the eggs develop into glochidium, possessing only partially developed organs. At this point they are discharged from the female through the ex-current siphon into the water column. If they are to survive, they must be taken into the mouth of a passing fish and attach themselves to its gills, (Fig. 1). The glochidium remains embedded for 1 to 6 weeks in the tissues of the host fish, not increasing in size, but developing adult structures. Some species of mussels parasitize only one species of fish, while others are not so particular. This parasitism usually does not harm the host, (fish). At the end of the 3rd stage, the young clam dislodges itself from the fish and falls to the bottom. If a suitable substrate is encountered, the young will survive and grow to adult.

Glochidia in the gills of fish
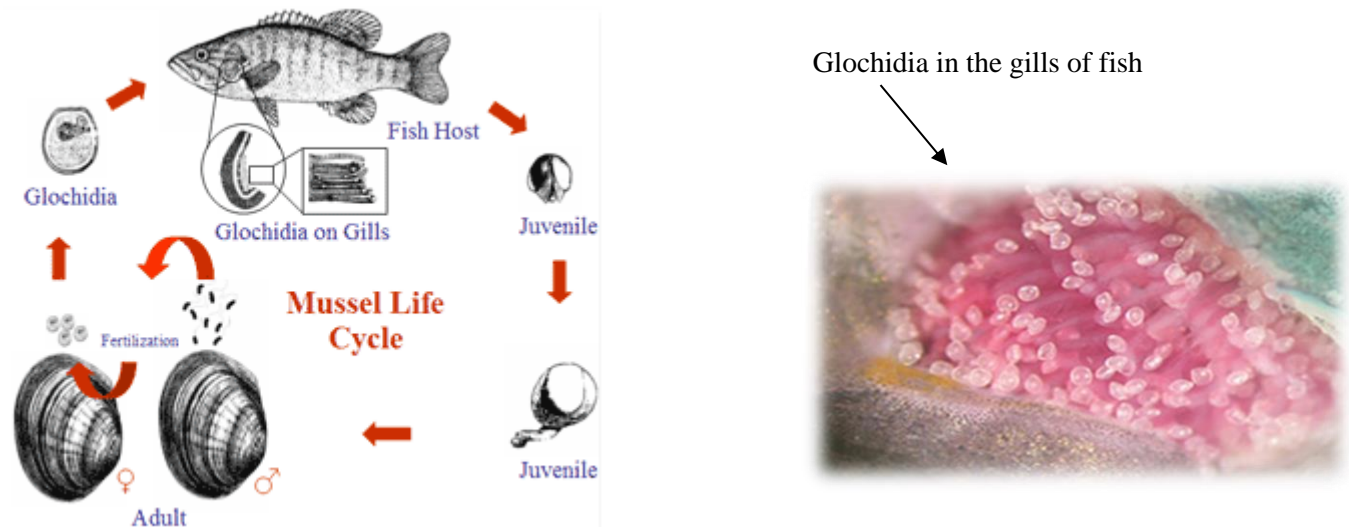
Figure 1. Life cycle of freshwater mussel and glochidia in the gills of fish (host). Source: https://www.google.fi/search?q=life+cycle+of+freshwater+mussels&biw=1366&bih=643&source=lnms&tbm=isch&sa=X&ei=9N7jVLTbCcTNygOb54DoDA&sqi=2&ved=0CAYQ_AUoAQ

1.3. Trematode parasites

Trematodes otherwise known as flukes are parasitic in nature and belong to the phylum Platyhelminthes. The digenetic trematodes are known to have an estimated number of approximately 25,000 species (Esch *et al*. 2002). Attachment of the parasite to the host and locomotion is aided by such features as the external suckers around the mouth and/or the additional ventral sucker. Trematode infections are known to be occurring worldwide.

1.3.1. Life cycle of *Rhipidocotyle* trematodes

Rhipidocotyle fennica and *R. campanula* are two *Rhipidocotyle* trematodes with a complex life cycle requiring more than one host for its completion. Both species undergo asexual reproduction in their common first intermediate host, (mussel), to produce cercariae.

The cyprinid fish, roach (*Rutilus rutilus*) is the second intermediate host and both parasitic species exist as mertacercariae in the cyprinid fish. *R. fennica* penetrates to fins of the fish while *R. campanula* penetrates to the fish gills (Taskinen *et al*. 1991). In the final host *R. fennica* exist as adult worm in pike, (*Esox* lucius) while *R. campanula* exist as adult worm in European perch, (*Perca fluvatilis*), (Taskinen 1992). The adult worms undergo asexual reproduction and releases eggs. The eggs then hatch to miracidia larvae in water and finds mussels. Miracidia then develops to mother sporocysts which produce cercariae that are shed into the water (Fig.2). These two species could have an influence on the shell morphology of its host (*A. anatina*), e.g the shells of *A. anatina* females infected by *R. campanula* were thinner and more elongated than the males (Zieritz & Aldridge 2011).

1.4. Shell dimorphism in freshwater mussels

1.4.1. The shell of freshwater mussels
The name Bivalvia – two valved mollusks differentiates mussels from all other mollusks, and the mussel shell is usually in two distinct halves. The variability in mussels shell's form could be a criterion to classify bivalves. These shell forms could be like a sheath, wedge or oval. The shell halves can in some instances exhibit some symmetry as can be observed in river mussels along the sides of a river or the shell halves could be quite different from one another as can be seen in marine oysters (*Ostrea*) along a sea coast. The hinge, where the ligament holds the shell together may be armed with teeth that hold the shell halves in position (Fig. 3). The hinge teeth are also different in different groups and could therefore be used for identification. The shell of the bivalve protects the soft body mass of a mussel. Calciferous cells that are found at the edges of the mantle continuously secretes calcium carbonate material in rings (umbones) around the shell's edges, which is the reason why a bivalve shell continuously grows in its habitat.

1.4.1.1. Sex induced shell dimorphism
Sexual dimorphism has been recorded for unionid mussels (Gofas 2001, Zieritz & Alridge 2011) as well as for marine (Raven 1990), freshwater (Minton & Wang 2011) and terrestrial snails (Kenchington & Glass 1998). Sexual size and/or shape dimorphism in broadcast spawners such as freshwater mussels may be observed as a result of consecutive hermaphroditism and/or their sexes' anatomical differences (Zieritz & Alridge 2011). For example, in several marine bivalves, protandric or protogynous consecutive hermaphroditism leads to size dimorphism of sexes in several families (Loosanoff 1936, Saleudddin 1965, Morton 1976), although hermaphroditism is rare in unionoids (Bloomer 1934, McIvor & Aldridge 2007). A typical pronounced sexual shape dimorphism is exhibited by most members of the unionid tribe Lampsilini (Kirtland 1834, Heard & Guckert 1970, Kotrla & James 1987). Lampsilines exhibit a unique character in using only the posterior portions of their outer gill demibranchs as marsupia (heterogenous condition), which enlarge both laterally and ventrally when gravid, resulting in female shells with more evenly rounded posterior margins and more inflated ventro-posterior regions than males (Kotrla & James 1987). Freshwater mussels in Europe have homogenous marsupials and are thought not to portray considerable sexual shell dimorphism. Brander (1954) made a description of male shells of *Pseudanodonta complanata* as more elongated and ovally shaped than the more trapezoid females. Contrary to this view, (Siebold 1837) found that females of *A. cygnae* and *A. anatina* were more elongated than males. There are also some unionids shell characters that have been considered to exhibit

sexual dimorphism, such as the shell's material composition (Cameron *et al*. 1979), as well as shell thickness (Jass & Glenn 2004).
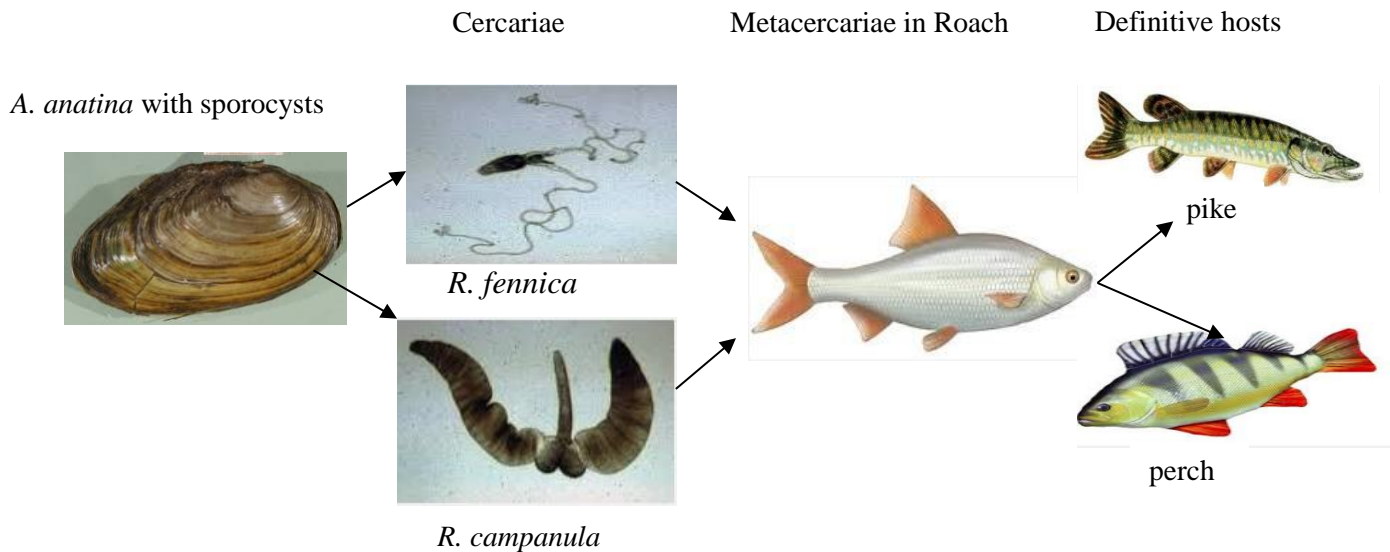

Figure 2. Life cycle of Rhipidocotyle species parasites (Taskinen *et al*. 1992)

1.4.1.2. Parasite induced shell dimorphism

Parasitism has been recognized as one of a number of stress factors that may affect the host by lowering the ability of the host to adapt to changing environmental conditions (Williams & Jones 1994). Freshwater mussels are known to harbor a number of internal and external parasites including bacteria, ciliates, viruses, trematodes, nematodes, hirudineans, oligochaetes, chironomids, mites and copepods (Grizzle & Brunner 2009, Taskinen 1998). Parasite infestation may cause alteration in shell size and shape of *A. anatina* (Zieritz & Aldridge 2011). The effect of parasitism on the morphology of unionids is poorly studied. However, infection by *R. fennica* and *R. campanula* has been linked with decreased reproduction in *A. anatina* and *A. anatina* shell growth has been shown to be slowed during heavy infestation by *R. fennica* (Taskinen 1998). This parasitic infestation can lead to reduced reproductive output and even castration of the host (Taskinen & Valtonen 1995, Gangloff *et al*. 2008) since the host's gonads are replaced with fibrosis or sporocysts, and this may in turn affect the development of dimorphic characters in the bivalve's shell. During periods of host stress such as anoxia or starvation, infestation by *R.campanula* can eventually lead to an increase in mortality rates in populations of *A. anatina* (Jokela *et al*. 2005).

1.4.1.3. Habitat induced shell dimorphism

Freshwater habitats with favorable conditions (water bodies with enough dissolved oxygen, benthic micro flora and fauna availability as food, and/or constant water flow) have been proven to host fecund mussels with more swollen gills and consequently more inflated shells of gravid females compared to less fecund populations (Peglar 1993). These requirements make unionoids in favorable habitats to exhibit significant dimorphic patterns (Aldridge 1999). Similar habitat influenced dimorphic patterns have been observed in other animal taxa such as beetles (Stillwell & Fox 2007) and gastropods (Goodwin & Fish 1977).
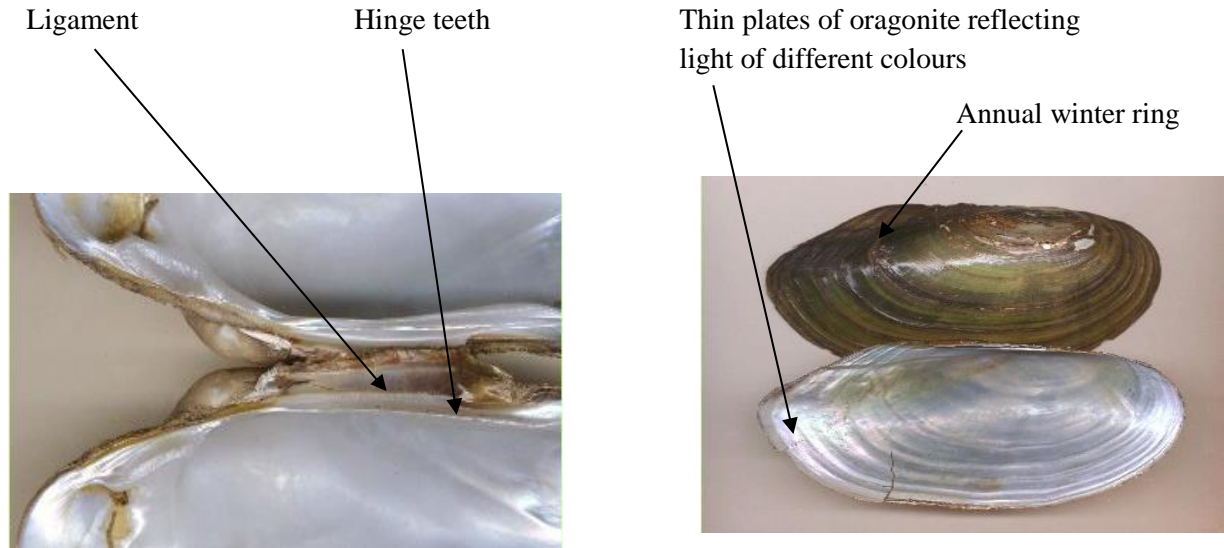
Ligament   Hinge teeth   Thin plates of oragonite reflecting
light of different colours

Annual winter ring

Figure. 3 The ligament, hinge teeth, oragonite plates and annual winter rings of a freshwater mussel. Source: M. Kohl: European Unionaceans (viewed: 18/02/2015).

Under favorable conditions, some unionid populations tend to be gravid when they are just 2 years old, which is a comparatively early age of maturity for anodontines (Heard 1975) but may help explain the sexual dimorphism recorded amongst the population. Therefore, inter-populational variation of life history traits such as length of incubation and/or timing of glochidial release, age/size of maturity (e.g. Bauer 2001, Hochwald 2001, Haag & Stanton 2003) might have an effect on female shell bulging and should be taken into account when describing sexual dimorphic patterns in unionid populations. Although still not well understood, possibly the best studied type of intraspecific variation in unionids shell morphology is that which deals with habitat conditions (e.g. Hornbach *et al*. 2010, Zieritz *et al*. 2010). Such ecophenotypic trends can help in providing knowledge of reconstructing palaeoenvironments and/or endangered species' habitat requirements (Zieritz & Aldridge 2009).

1.5. Aims and hypothesis

The aim of the study was to investigate to what extent sex can induce shell differences among three Finnish freshwater mussel species (*A. anatina, U. pictorum* and *U. tumidus*) collected from different sites.

We hypothesized that there will be no difference in shell length between younger (≤ 3 years) males and females but at the age of ≥ 4 years, males will be longer than females since from this age females start to reproduce. This could be because at younger ages female mussels invest all of their energy resources on growth. But as they become fecund with increasing age, some of their energy reserves are diverted into production of glochidial larvae as opposed to the males that produce only sperm.

It was also hypothesized that adult female shells will have a greater relative shell width and volume compared to the males to accommodate marsupial gills.

Also the aim was to investigate if and to what extent *Rhipidocotyle* trematodes can cause variation in the shell morphology of freshwater mussel, *A. anatina*. It was hypothesized that infected *A. anatina* will have a greater relative shell width compared to the uninfected

individuals. This could be because infected *A. anatina* mussels will contain sporocysts in their gonads that will lead to an increase in the mussels' soft tissues thereby leading to inflated shells to accommodate the parasites.

2. MATERIALS AND METHODS

2.1. Study area

The study sites consist of Lake Saravesi 62° 25′ N 025° 57′ E in Laukaa, Central Finland, and Lake Kuivasjarvi 65° 01′ N 025° 28′ E in Oulu, Northern Finland (300 km away from Lake Saravesi) (Fig. 4). Lake Saravesi is eutrophic, small (7.8 km²) and has a mean depth of 5.5 m while Lake Kuivasjarvi is hypereutrophic, smaller (0.84 km²), shallow, and with a mean depth of 1.9 m. Maximum water temperatures (18 – 20 °C) are reached at the end of July or the beginning of August for Lake Saravesi while for Lake Kuivasjarvi, they occur in July (Taskinen *et al*. 1991).
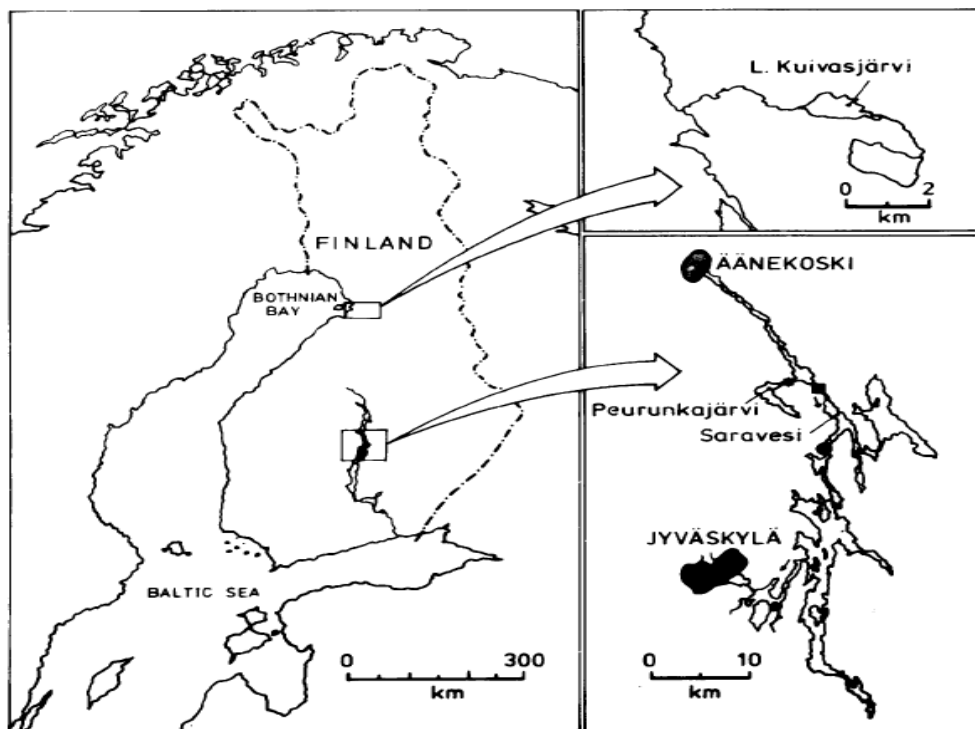
Figure 4. Map of Finland showing study sites, from (Taskinen *et al*. 1991)

2.2. Collection and cercarial shedding monitoring

Freshwater mussels (*A. anatina*, *U. pictorum* and *U. tumidus*) (Fig.5), were collected monthly by bottom dredge between May – October 2012. Immediately after collection, clams were monitored for cercarial emergence in the laboratory by individually placing each clam in a 3 liters transparent plastic box (length 26.5 cm, breadth 19 cm and height 13.6 cm) filled with 2 liters of filtered lake water. After 24 hours, each box was visually inspected for cercarial emergence. For clams shedding cercariae, shed cercariae were identified using the method described by (Taskinen *et al*. 1991). After each monitoring, each clam was dissected

and a piece of gonad pressed between two glass plates and observed under a light microscope to determine sex and parasite infection status of the clam. The same protocol was followed throughout the study period.

A. anatina

U. tumidus

U. pictorum

Figure 5. Study species. Source: https://www.google.fi/search?q=anodonta+anatina+unio+tumidus+unio+pictorum&biw=1366&bih=643&source=lnms&tbm=isch&sa=X&ei=5HLkVItl46DIA_qggogG&ved=0CAYQ_AUoAQ#tbm=isch&q=freshwater+mussels&imgdii=   (viewed 18/02/2015)

2.3. Morphological measurements

For shell shape analysis, three shell dimensions length (*L*), width (*W*) and height (*H*) for each clam was measured to ± 0.01 mm using a digital caliper (Fig. 6). Shell dry weight (*DW*) was measured to ± 0.01 g by using a chemical balance (Fig. 6). Shell volume (V) was measured by first using a wash bottle to fill water into the shell. The water was then transferred from the shell to a petri dish. The petri dish with the water in it was then placed on a chemical balance. The readings (excluding tare weight of the petri dish) were recorded to ± 0.01 g, after which this weight was later converted to volumetric measurements to the nearest ± 0.01 cm^3. To determine the age, the annual winter rings (Fig. 3), displayed on the shell were counted according to (Aldridge 1999).

2.4. Data analysis

Data was analyzed using the SPSS software version 15.0. Differences in age-specific lengths between mussel species and collection sites were analyzed using one-way ANOVA. Relative differences (i.e. pair wise comparison) between lakes for shell mean heights was analyzed using descriptive statistics, while differences between males and females for growth (mean shell length at the age of 3) was analyzed using the ANCOVA. Shell height differences between pairs of the three mussel species was analyzed by using the Mann-Withney test.

Males and females pair wise differences in shell mean heights, as well as *Rhipidocotyle* infected and uninfected mussels from the two lakes was analyzed by using the ANCOVA.

| Length | Width | Height |

Figure 6. Morphological measurements

Mussels shell width differences between lakes were analyzed using the two - way ANOVA. Then pair wise comparison on shell width for both *Rhipidocotyle* species infected and uninfected *A. anatina* was analyzed using one - way ANCOVA. Pair wise comparison of volume differences between mussel species and lakes was equally analyzed using two - way ANCOVA.

3. RESULTS

3.1. Description of the materials

All three mussel species (*A. anatina*, *U. pictorum* and *U. tumidus*) were found from Lake Saravesi, while only *A. anatina* was found from Lake Kuivasjarvi. Numbers of mussels studied, ages and shell length are given in Table 1. In total, 418 and 593 mussel individuals were studied from Lake Saravesi and Lake Kuivasjärvi, respectively (Table 1).

Table 1. Number (N), mean age and mean shell length at the age of 3 years (L3, a proxy of growth rate) of unionid mussels (*A. anantina*, *Unio pictorum* and *Unio tumidus*) studied from Lake Saravesi, Laukaa, and Lake Kuivasjärvi, Oulu, in summer 2012. Means are given with ± S.D. and range (min-max).

	N	Age (years)	Length (mm)	L3 (mm)
Lake Saravesi				
A. anatina	78	6.3 ± 2.3 (3-13)	65.8 ± 11.6 (38.8-97.4)	48.5 ± 7.5 (35.5-65.5)
U. pictorum	304	10.5 ± 3.9 (3-19)	84.8 ± 13.0 (37.9-116.3)	47.0 ± 8.8 (22.6-68.1)
U. tumidus	99	7.1 ± 2.3 (3-13)	64.6 ± 13.2 (34.3-95.6)	38.4 ± 10.7 (20.5-60.7)
Lake Kuivasjärvi				
A. anatina	593	5.6 ± 1.7 (3-13)	61.9 ± 10.7 (31.4-87.7)	48.5 ± 6.4 (29.8-65.5)

3.1.2. Trematodes (*Rhipidocotyle* species) occurrence in the study sites

Rhipidocotyle species infections were found in *A. anatina* mussels of both lakes (Table 2); meanwhile, the infection levels in *U. pictorum* and *U. tumidus* were so low, so these mussels were excluded from the analysis. However, low infection levels of *U. pictorum* with

R. campanula were observed in mussels at the age of 7 and 14 years old. The proportions (percentages) of uninfected and *Rhipidocotyle* sp. infection on *A. anatina* from both lakes are shown in Table 2.

Table 2. Number (n) and proportion (%) of uninfected and trematode (*R. campanula and R. fennica*) infected mussels (*A. anatina*) collected from Lake Saravesi and Lake Kuivasjarvi in summer 2012.

Site	Species	Age (yrs)	n	Uninfected (%)	Infected (%)		Total (%)
					R. campanula	*R. fennica*	
L. Saravesi	*A. anatina*	2	5	5(100.0)	0(0.0)	0(0.0)	100.0
		3	9	7(77.8)	1(11.1)	1(11.1)	100.0
		4	8	7(87.5)	0(0.0)	1(12.5)	100.0
		5	11	9(81.8)	0(0.0)	2(18.2)	100.0
		6	15	4(26.7)	3(20.0)	8(53.3)	100.0
		7	9	5(55.6)	0(0.0)	4(44.4)	100.0
		8	7	3(43.0)	2(28.5)	2(28.5)	100.0
		9	5	3(75.0)	1(12.5)	1(12.5)	100.0
		10	3	1(33.3)	0(0.0)	2(66.7)	100.0
		11	2	0(0.0)	0(0.0)	2(100.0)	100.0
		13	1	1(100.0)	0(0.0)	0(0.0)	100.0
Total			45	45(60.0)	7(9.3)	23(30.7)	100.0
L. Kuivasjarvi	*A. anatina*	1	1	1(100.0)	0(0.0)	0(0.0)	100.0
		2	56	56(100.0)	0(0.0)	0(0.0)	100.0
		3	74	74(100.0)	0(0.0)	0(0.0)	100.0
		4	48	45(93.8)	3(6.2)	0(0.0)	100.0
		5	109	104(95.4)	5(4.6)	0(0.0)	100.0
		6	197	183(92.9)	12(6.1)	2(1.0)	100.0
		7	57	52(91.2)	4(7.0)	1(1.8)	100.0
		8	18	17(94.4)	1(5.6)	0(0.0)	100.0
		9	9	9(100.0)	0(0.0)	0(0.0)	100.0
		10	13	13(100.0)	0(0.0)	0(0.0)	100.0
		11	5	5(100.0)	0(0.0)	0(0.0)	100.0
		12	3	3(100.0)	0(0.0)	0(0.0)	100.0
		13	1	1(100.0)	0(0.0)	0(0.0)	100.0
Total			591	563(95.3)	25(4.2)	3(0.5)	100.0

3.2. Growth, mean lengths by age

3.2.1. Differences between lakes

Differences in mussel growth were first studied by including only individuals that were not infected by trematode parasites. When mussel growth was measured using shells at the age of 3 years (L3), there was no difference in the growth rate of *A. anatina* between Lake Saravesi (Mean ± S.D. 48.5 ± 7.5) and Lake Kuivasjärvi (Mean ± S.D. 48.5 ± 6.4). For other mussel species, the between lake comparison was not possible.

3.2.2. Differences between males and females

Comparison was made in terms of absolute shell mean lengths of males and females *A. anatina*, *U.pictorum* and *U. tumidus* from L. Saravesi with males and females *A. anatina* from L. Kuivasjärvi. Reseonable number of individuals (n ≥ 3) for *A. anatina* from Lake Saravesi was available only for mussels aged 5 years. So, other age groups were left out of statistical analyses (Fig.7). In L. Saravesi, the mean lengths of female *A. anatina* mussels at the age of 5 were found to be longer than the males. However this difference was not statistically significant (ANOVA $F_{1, 7}=4.859$, P=0.063) (Fig.7). For *U. pictorum*, between the ages of 5 - 7 years, the mean length of females seemed to be longer than those of the males. But this difference was only significant between individuals at the age of 5 (ANOVA, $F_{1, 27}=5.927$, P=0.022). However at older age groups (8 - 9 years, 10 - 13 years and 15 - 17 years), the males became longer than the females, but these results were significant only for mussels at the age of 13 and 17 years (ANOVA, $F_{1,23}=8.062$, P=0.009 and $F_{1,13}=5.872$, P=0.031) respectively (Fig.8). For *U. tumidus*, there was no significant difference between sexes at all ages, (Fig.9).

Shell mean lengths results of *A. anatina* from Lake Kuivasjarvi revealed that female shells were longer than the males almost at all ages but statistically significant only for 2 and 8 years old mussels (ANOVA, $F_{1,54}=7.307$, P=0.009 and $F_{1,15}=16.272$, P=0.001) respectively (Fig. 10).

3.2.3. Differences between parasite infected and uninfected individuals

Differences in mussels' lengths between *R. campanula* infected and uninfected individuals was analyzed using the ANOVA model, with mussel length as the dependent variable and infection status as the fixed factor. Mussels infected with *R. fennica* and *Phyllodistomum* sp. were not included, as well as mussels with concurrent infections with *R. campanula* and the other parasite species. ANOVA was performed separately for Lake Saravesi and Lake Kuivasjärvi. In Saravesi, most of the age groups were such that the number of *R. campanula*-infected individuals was very low.
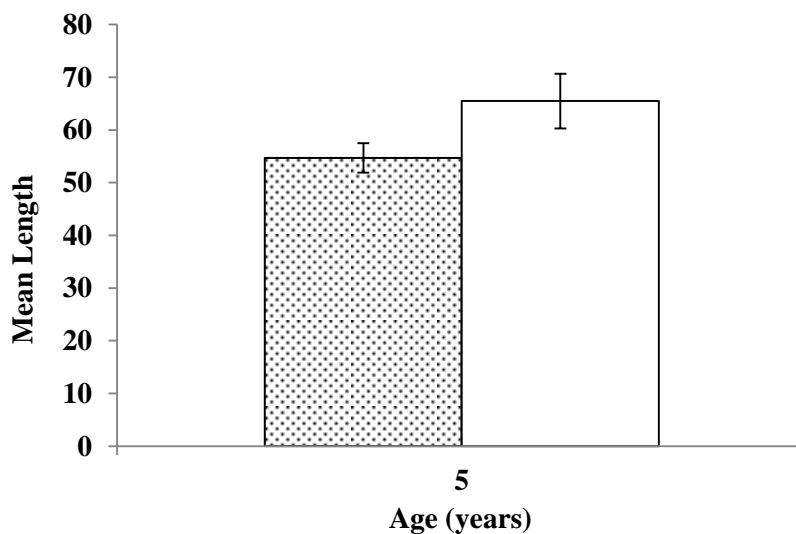

Figure 7. Shell mean lengths (mm) ± S.E of uninfected males and females *A. anatina* from L. Saravesi. Filled bar = males, open bar = females. Age groups with (n ≥ 3) included. Thus only 5 yrs old mussels were included
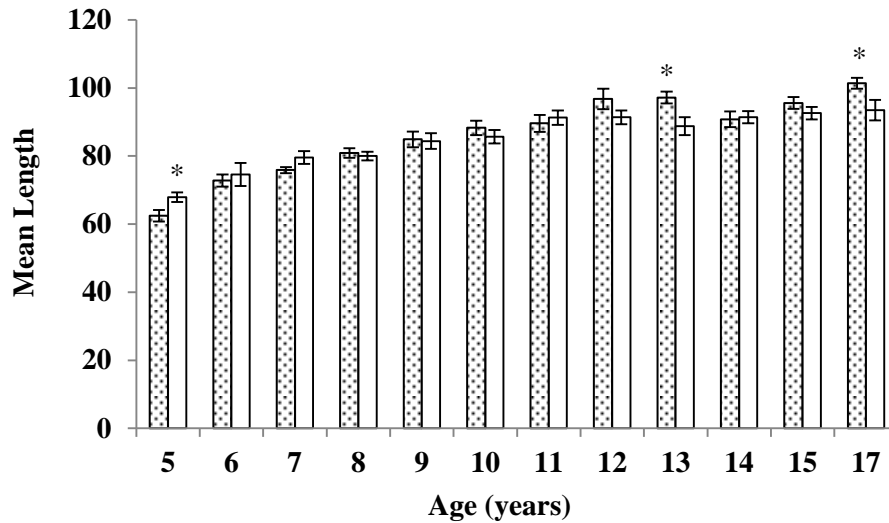
Figure 8. Shell mean lengths (mm) ±S.E of uninfected males and females *U. pictorum* from L. Saravesi. Filled bars = males, open bars = females. Age groups with (n ≥ 3) included only. Asterisks indicate statistically significant ($P < 0.05$) differences in age group 5 years old females, where females were longer and for 13 and 17 years old males, where males were longer.
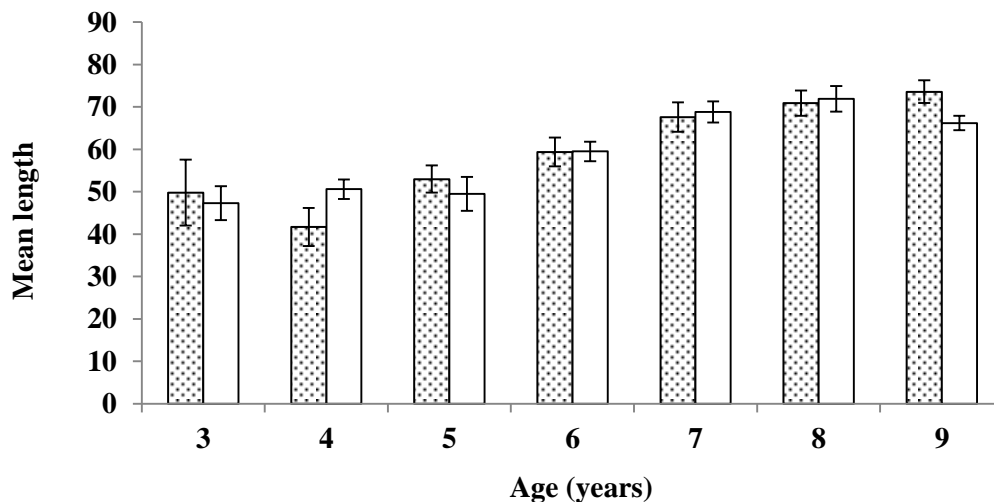

Figure 9. Shell mean lengths (mm) ± S.E of uninfected males and females *U. tumidus* from L. Saravesi. Filled bars = males, open bars = females. Age groups with (n ≥ 3) included only

In fact, there was only one age group in which the number of both infected and uninfected mussels was ≥ 3, namely 6 years old mussels. In this age group, the mean ± S.E. shell length of infected (n = 3) and uninfected (n = 4) mussels was 58.2 ± 6.3 mm and 63.4 ± 2.6 mm, respectively, but there was no statistically significant difference in length of *R. campanula*-infected and uninfected individuals ($F_{1,6} = 0.729$, p = 0.432).

In Lake Kuivasjarvi, the age groups in which the number of both infected and uninfected mussels was ≥ 3 were 4, 5, 6 and 7 years, and these age groups were selected for the ANOVA,

in which mussel length was used as the dependent variable and age (4, 5, 6 and 7 years), as well as infection status as fixed factors.

ANOVA results indicated that in Lake Kuivasjärvi *A. anatina*, the effect of infection status was statistically significant ($F_{1, 408} = 4.518$, $p = 0.034$), as well as the effect of mussel age ($F_{3, 408} = 17.598$, $p < 0.001$), but the interaction between age and infection status was not significant ($F_{3, 408} = 0.312$, $p = 0.817$). Thus, the *R. campanula*-infected mussels were consistently bigger than the uninfected ones in all age groups 4 - 7 years (Fig. 11).
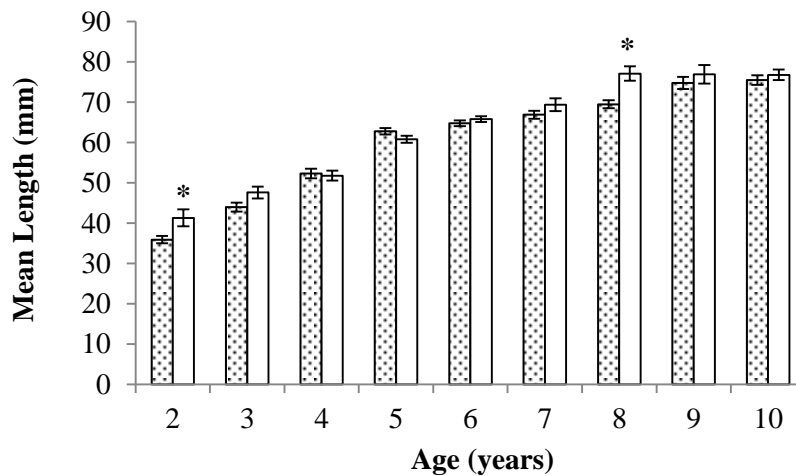

Figure 10. Age specific mean lengths (mm) ±S.E of *A. anatina* from Lake Kuivasjärvi. Filled bars = males, open bars = females. Age groups with (n ≥ 3) included only. Asterisks indicate statistically significant (*P* < 0.05) differences between males and females in age groups 2 and 8 years old.

Second, the possible connection between mussel length and *R. fennica* infection was studied in Lake Saravesi, since the prevalence of *R. fennica* infection was too low in Lake Kuivasjärvi. Mussels infected with *R. campanula* and *Phyllodistomum* sp. were not included, as well as mussels with concurrent infections with *R. campanula* and other parasite species. The age groups in which the number of both infected and uninfected mussels was ≥ 3 were 6 and 7 years, and these age groups were selected for ANOVA analysis, in which mussel length was used as the dependent variable and age, as well as infection status as fixed factors. Mean ± S.E. length of infected (n = 8) and uninfected (n = 4) mussels was 63.9 ± 6.4 mm and 63.4 ± 5.2 mm, respectively, in the age group 6 years, while the corresponding values for the 7 years old mussels were 68.1 ± 5.6 (n = 4) and 70.7 ± 6.1 (n = 5), respectively. ANOVA results indicated that in *A. anatina* of Lake Saravesi the effect of infection status was statistically not significant ($F_{1, 21} = 0.159$, $p = 0.695$), but the effect of mussel age was significant ($F_{1, 21} = 4.551$, $p = 0.048$), while the interaction between age and infection status was not significant ($F_{1, 21} = 0.315$, $p = 0.582$). Thus, the *R. fennica*-infected mussels did not differ from uninfected individuals in their length.
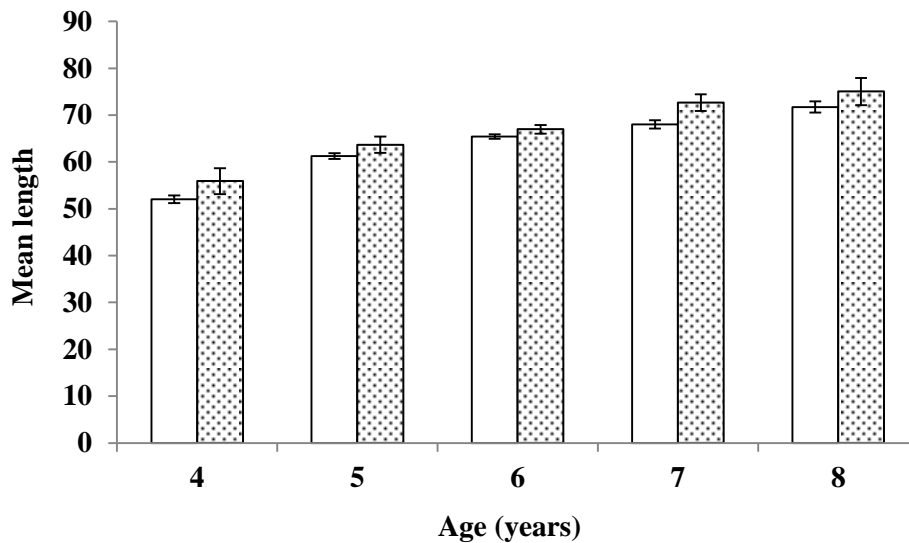
Figure 11. Age specific mean ± S.E. length (mm) of *Rhipidocotyle campanula*-infected (filled bars) and uninfected (open bars) *Anodonta anatina* from Lake Kuivasjärvi at different age groups where the number of both infected and uninfected mussels was ≥ 3.

3.3. Relative height of shell (height-to-length ratio)

3.3.1. Differences between lakes in relative shell height

Relative shell height in *A. anatina* from Lake Saravesi vs. Lake Kuivasjärvi was studied excluding the trematode-infected individuals. There was no difference in the relative shell height between the two lakes in *A. anatina* (Fig. 12).
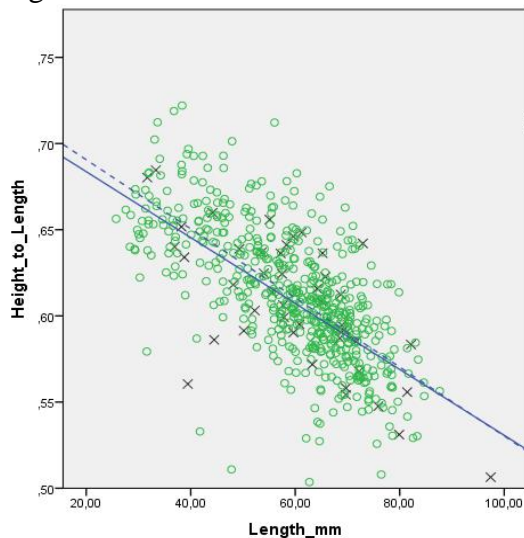

Figure 12. Relative shell height-to-length ratio (mm) of *A. anatina* mussels from Lake Saravesi (crosses, solid line) and Lake Kuivasjärvi (circles, dotted lines).

3.3.2. Differences between species in relative shell height

Relative shell heights of *A. anatina*, *U. pictorum* and *U. tumidus* with trematode infected individuals excluded, from highest to lowest shell height was in the order of *A. anatina*, *U. tumidus* and *U. pictorum* respectively (Fig. 13). Results of Mann-Withney U test indicated that there was a difference between *A. anatina* and *U. pictorum* (p < 0.001) and between *U. pictorum* and *U. tumidus* (p < 0.001), as well as between *A. anatina* and *U. tumidus* (p < 0.001) with *A. anatina* showing the highest relative shell height. In *A. anatina*, the relative shell height decreased with length of shell (Fig. 13), as shown by the statistically significant, negative correlation between relative shell height and shell length (Pearson correlation coefficient = -0.690, n = 45, p < 0.001). In *U. tumidus*, the relative shell height did not correlate with mussel length (Fig. 13), (Pearson correlation coefficient = -0.117, n = 98, p < 0.081). In contrast, the relative shell height of *U. pictorum* increased slightly with shell length (Fig. 13) (Pearson correlation coefficient = 0.136, n = 299, p = 0.018). These results mean that *A. anatina* shell is high when compared to length, but their relative height decreases rapidly with size of the mussels. *U. tumidus* shell height in relation to shell length is clearly lower than in *A. anatina* and is more or less unchanged from small to large individuals. *U. pictorum* had the lowest relative shell height among the three species, and its relative shell height differed from the other unionids so that it increased by mussel size.
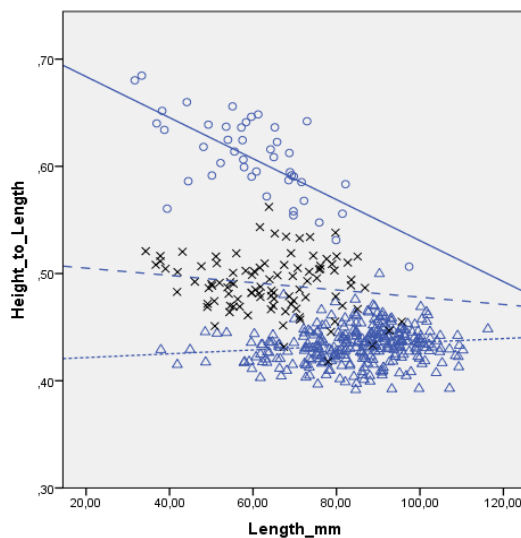

Figure 13. Relative shell height (mm) of *Anodonta anatina* (circles, solid line), *Unio pictorum* (triangles, dotted line) and *Unio tumidus* (crosses, broken line) from Lake Saravesi.

3.3.3. Differences between males and females in relative shell height

In the analysis of shell height differences between male and female *A. anatina*, trematode-infected and immature mussels (< 3 years) were excluded. ANCOVA analysis was performed with shell height-to-length ratio as the dependent variable, lakes (Saravesi and Kuivasjärvi) as a random factor, sex as a fixed factor and shell length as a covariate, including all possible interaction terms, was used. The results revealed that mussel gender and length had a highly significant effect on the relative shell height (ANCOVA; $F_{1, 592}$ = 25.07, p < 0.001) and (ANCOVA; $F_{1, 592}$ = 588.6, p < 0.001) respectively, but the effect of lake was non-

significant (ANCOVA; $F_{1, 592}$ = 0.909, p < 0.341). Thus, in both lakes the relative shell height was higher for males and decreased by shell length (Fig. 14 and 15). Estimated mean length-adjusted relative shell heights ± S.E. for males and females were 0.616 ± 0.002 and 0.605 ± 0.002, respectively, in the combined data of Lake Saravesi and Lake Kuivasjärvi. So, male *A. anatina* had relatively slightly higher shells.

Figure 14. Relative shell height (mm) of *Anodonta anatina* males (circles, solid line) and females (crosses, dotted line) in Lake Saravesi.
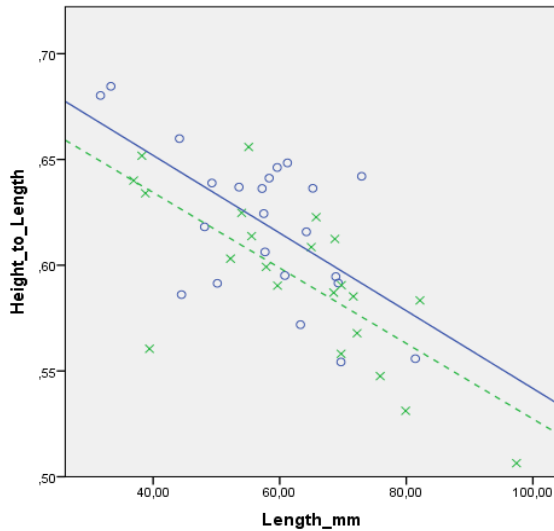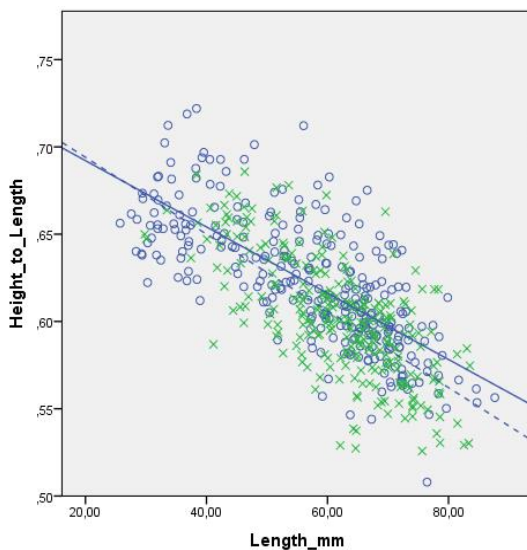

Figure 15. Relative shell height (mm) of *Anodonta anatina* males (circles, solid line) and females (crosses, dotted line) in Lake Kuivasjärvi.

Relative shell height differences between males and females *U. pictorum* (Lake Saravesi) were also studied so that trematode-infected mussels and immature individuals (<

3years old) were excluded. First, ANCOVA model with shell height-to-length ratio as the dependent variable and sex as a fixed factor and shell length as a covariate, including the interaction terms, was used. Results revealed that the interaction between shell length and sex was significant ($F_{1, 294} = 9.730$, p = 0.002), indicating that the slopes differed between sexes. Therefore, the use of shell length as a covariate in the analysis was not justified. Next, ANCOVA with only the covariate, shell length, was applied separately for males and females. For males, the effect of shell length on the relative shell height was not significant ($F_{1, 160} = 0.213$, p = 0.645) but for females its effect was highly significant ($F_{1, 134} = 17.321$, p < 0.001). Thus, in males the relative shell height does not change with mussel size, but in females the relative shell height increases with mussel length. When analyzed over the whole data, relative shell height of *U. pictorum* did not differ between males and females (ANOVA, $F_{1, 296} = 0.767$, p = 0.382). In large *U. pictorum* (> 90 mm) the mean ± S.D relative shell height was significantly lower in males (0.433 ± 0.178, n = 70) than in females (0.439 ± 0.015, n = 42) (ANOVA, $F_{1, 110} = 3.965$, p = 0.049) (Fig.16).
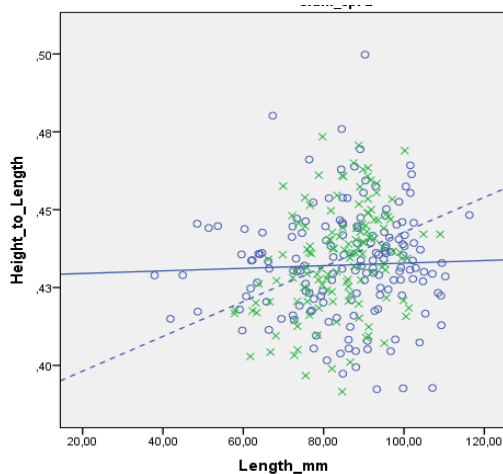

Figure 16. Relative shell height (mm) of *Unio pictorum* males (circles, solid line) and females (crosses, dotted line) in Lake Saravesi.

In the case of *U. tumidus* (Lake Saravesi), also ANCOVA model with shell height-to-length ratio as the dependent variable and sex (males, females) as a fixed factor and shell length as a covariate, including the interaction terms, was used. The interaction between sex and length of mussel was not significant ($F_{1, 94} = 0.002$, p = 0.962), indicating equal slopes and justifying the use of shell length as a covariate. When continued with a full factorial model, ANCOVA results indicated that the effect of sex on the relative shell height in *U. tumidus* was not significant ($F_{1, 95} = 0.533$, p = 0.467) (Fig.17).

3.3.4. Differences between *R. campanula* infected and uninfected mussels in relative shell height

First, the association between *R. campanula* parasite and relative shell height of *A. anatina* was analysed, so that uninfected and those mussels that were infected by *R. campanula*, only, were included. Since the previous analyses (above) indicated that *A. anatina* from Lake Saravesi and Lake Kuivasjärvi are morphologically almost identical with respect to relative shell height in relation to shell length (Fig.13), the ANCOVA model in which the both lakes

were included was first tried. Relative shell height was the dependent variable, lakes (Saravesi, Kuivasjärvi) was used as random factor and infection status (infected by *R. campanula*, not infected by any other parasite) as a fixed factor, shell length as a covariate, and the first model included all possible interaction terms.
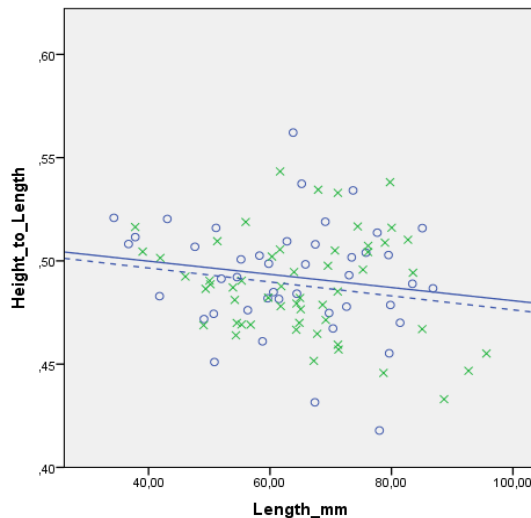

Figure 17. Relative shell height (mm) of *Unio tumidus* males (circles, solid line) and females (crosses, dotted line) in Lake Saravesi.

It turned out that the interactions, shell length x lake, shell length x infection status and shell length x lake x infection status were not significant, indicating that the slopes did not differ between lakes or with respect to infection status, which justified the use of shell length as a covariate in the analysis. Next, a full factorial ANCOVA model was used, with an outcome that the interaction between infection status and lake was not significant ($F_{1, 626} = 1.070$, p = 0.301), indicating that the effect of infection status on the relative shell height did not differ between lakes. Thus, this interaction term was excluded from the final ANCOVA model, which had only the main effects lake and infection status and the covariate. Results of this ANCOVA indicated a non-significant effect of lake on the relative shell height ($F_{1, 627} = 0.462$, p = 0.497), as well as non-significant effect of infection status ($F_{1, 627} = 0.422$, p = 0.516), while the effect of mussel length was highly significant ($F_{1, 627} = 638.14$, p < 0.001). Thus, in both lakes the relative shell height was not related to infection by *R. campanula* (Fig.18 and 19). The result did not change when studied separately for males and females.

3.3.5. Differences between *R. fennica* infected and uninfected mussels

The procedure was then applied to the other bucephalid trematode, *R. fennica* (uninfected and mussels infected by *R. fennica* only, were included) in Lake Saravesi. ANCOVA results indicated that the effect of infection status was not significant in *R. fennica* either ($F_{1, 67} = 0.001$, p = 0.971) and that the interaction between infection status and mussel length was also nonsignificant ($F_{1, 67} = 0.006$, p = 0.939), while the effect of mussel length was highly significant ($F_{1, 67} = 25.431$, p < 0.001). Thus, the relative shell height was not related to infection by *R. fennica* either. The result did not change when studied separately for males and females (Fig.20).

Figure 18. Relative shell height (mm) of *Anodonta anatina* infected by the trematode parasite *Rhipidocotyle campanula* (crosses, solid line) and uninfected mussels (circles, dotted line) in Lake Saravesi.
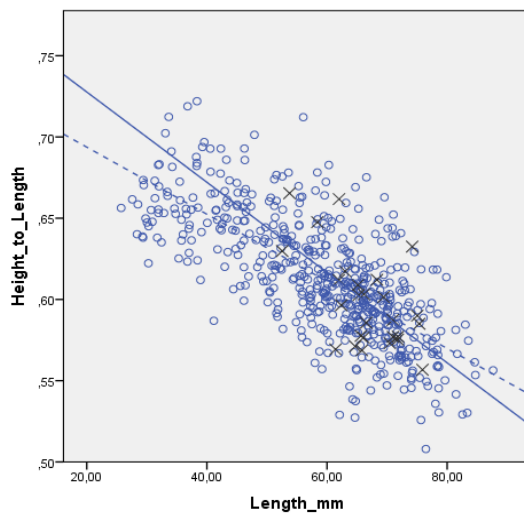
Figure 19. Relative shell height (mm) of *Anodonta anatina* infected by the trematode parasite *Rhipidocotyle campanula* (crosses, solid line) and uninfected mussels (circles, dotted line) in Lake Kuivasjärvi.

3.4. Relative width of shell (width-to-length ratio)

3.4.1. Differences between lakes in relative shell width
Differences between lakes in the relative shell width (width-to-length ratio) were analyzed using *A. anatina* and by including only uninfected individuals.
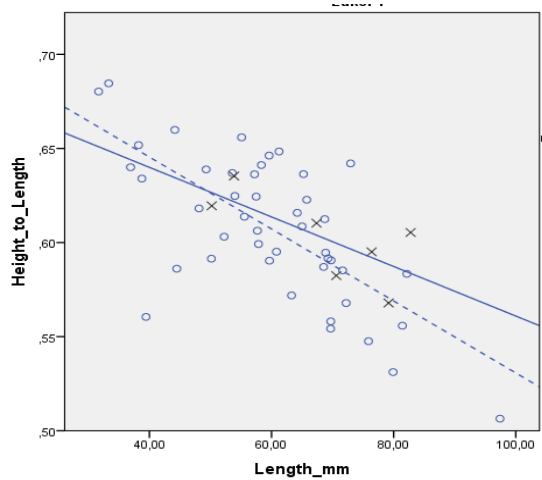
Figure 20. Relative shell height (mm) of *Anodonta anatina* infected by the trematode parasite *Rhipidocotyle fennica* (triangles, solid line) and uninfected mussels (circles, dotted line) in Lake Saravesi.
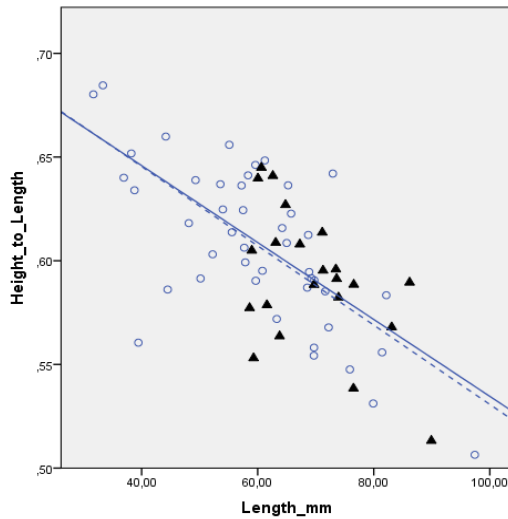
ANCOVA model with shell width-to-length ratio as the dependent variable and lake as a random factor and shell length as a covariate, including the interaction terms, was used. Results of ANCOVA suggested that the effect of lake was significant ($F_{1, 601} = 6.444$, p = 0.011), as well as the effect of shell length ($F_{1, 601} = 118.96$, p < 0.001), while the interaction between these two was not ($F_{1, 601} = 2.248$, p = 0.134). Therefore it can be stated that *A. anatina* shells are relatively wider in Lake Kuivasjärvi than in Lake Saravesi, and that there is a consistent increase in the relative shell width with size of mussel in both lakes (Fig. 21). Length-adjusted mean ± S.E. relative shell width was 0.322 ± 0.001 in Lake Kuivasjärvi, and 0.305 ± 0.003 in Lake Saravesi.

3.4.2. Differences between species in relative shell width

Differences in shell width between mussel species in Lake Saravesi were studied by excluding trematode-infected individuals. Relative shell widths of *A. anatina*, *U. pictorum* and *U. tumidus* differed clearly from each other. Shell of *U. tumidus* was the widest, *A. anatina* was the slimmest (at least when they were small) and shell of *U. pictorum* was in between (Fig. 22). Due to their clearly different patterns with respect to mussel size, the relative shell width was analyzed separately for each species. In *U. tumidus*, the relative shell width decreased with length of shell, as indicated by the significant, negative correlation between relative shell width and shell length (Pearson correlation coefficient = -0.406, n = 98, p < 0.001) (Fig.22). In contrast, the relative shell width of *A. anatina* increased with shell length (Fig. 22), as suggested by the statistically significant, positive correlation between relative shell width and shell length (Pearson correlation coefficient = 0.739, n = 45, p < 0.001). In *U. pictorum*, the relative shell width was unrelated to mussel length (Fig 23), Pearson correlation coefficient = -0.031, n = 301, p = 0.595). These results mean that *U. tumidus* mussels are wide, inflated, when compared to length, but their relative width decreases with size, whereas the opposite is true for *A. anatina*.
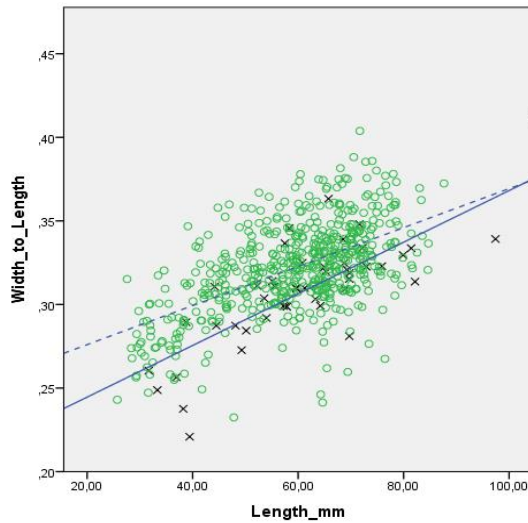
Figure 21. Relative shell width (mm) (width-to-length ratio plotted against shell length) of *Anodonta anatina* in Lake Saravesi (crosses, solid line) and Lake Kuivasjärvi (circles, dotted line).

Thus, while there is a very large difference between these two species when they are small (from about 30 mm to 50 mm), they have fairly equal relative shell width when mussels are large (from about 70 to 100 mm). Unlike *U. tumidus* and *A. anatina*, where there is considerable variation, *U. pictorum* mussels do not change their shell shape when they grow.
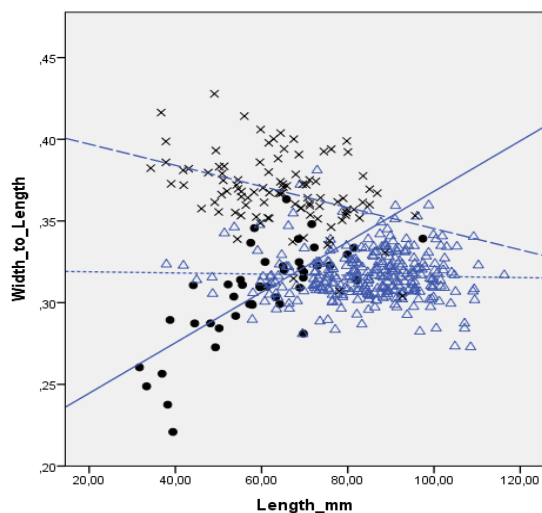
Figure 22. Relative shell width (mm) of *Anodonta anatina* (dots, solid line), *Unio pictorum* (triangles, dotted line) and *Unio tumidus* (crosses, broken line) from Lake Saravesi.

3.4.3. Differences between sexes in relative shell width

Relative shell width differences between males and females of *A. anatina* were studied so that trematode-infected mussels and immature individuals (< 3 years old) were excluded. First, ANCOVA model with shell width-to-length ratio as the dependent variable, lakes (Saravesi, Kuivasjärvi) as a random factor, sex (males, females) as a fixed factor and shell length as a covariate, including all possible interaction terms, was used. Outcome of this was

that the interaction shell length x lake, shell length x sex and shell length x lake x sex were not significant indicating that the slopes did not differ between lakes or sexes. Next a full factorial ANCOVA model was used, and the results revealed that the main effects of sex and lake were not significant ($F_{1, 597} = 0.462$, $p = 0.619$ and $F_{1, 597} = 6.201$, $p = 0.243$, respectively), but the effect of shell length was significant ($F_{1, 597} = 271.06$, $p < 0.001$), as well as the interaction between sex and lake were significant ($F_{1, 597} = 4.129$, $p = 0.043$). This indicates that the effect of sex was different in Lake Saravesi and Lake Kuivasjärvi. Therefore ANCOVA was applied to the lakes separately. It was found that in Lake Saravesi the effect of mussel sex was not significant ($F_{1, 45} = 0.514$, $p = 0.477$) (Fig. 23), while in Lake Kuivasjärvi it was highly significant ($F_{1, 557} = 38.702$, $p < 0.001$) (Fig. 24.). In Lake Kuivasjärvi the females were relatively wider as the length-adjusted mean $\pm$ S.E. relative shell width in males and females was 0.316 ± 0.001 and 0.328 ± 0.001, respectively (Fig. 24).

Relative shell width differences between sexes of *U. pictorum* and *U. tumidus* (Lake Saravesi) were studied so that trematode-infected mussels and immature individuals (< 3 years) were excluded, using the procedure described above in case of the relative shell width. ANCOVA results indicated that the effect of mussel gender on shell width was not significant for either species, while the shell width was significantly affected by mussel length in *U. tumidus* (Fig. 25 and Fig. 26).
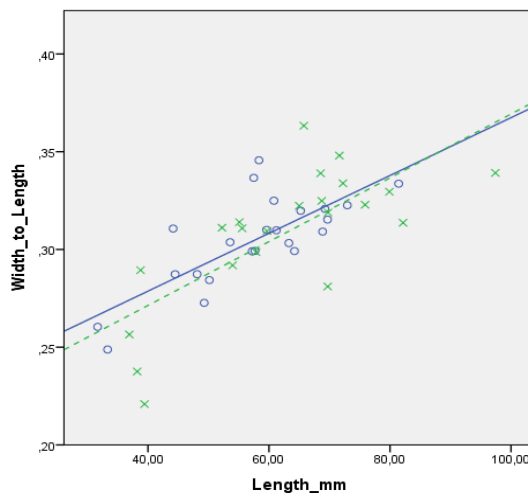

Figure 23. Relative shell width (mm) of *Anodonta anatina* males (circles, solid line) and females (crosses, dotted line) in Lake Saravesi material.

3.4.4. Differences between *R. campanula* infected and uninfected mussels in relative shell width

To study the effect of *R. campanula* infection on the relative shell width of *A. anatina*, the uninfected and individuals infected with only *R. campanula* (concurrent infections with other parasite species were excluded). Mussels 50 mm or longer were selected in the analyses to include size groups that represented both infected and uninfected mussels. An ANCOVA model in which the both lakes were included was first applied. Relative shell width was the dependent variable, lake (Saravesi, Kuivasjärvi) was used as a random factor and infection status (infected by *R. campanula*, not infected) as a fixed factor, shell length as a covariate, and the first model included all possible interaction terms. It turned out that the interactions

shell length x lake, shell length x infection status and shell length x lake x infection status were not significant, indicating that the slopes did not differ between lakes or with respect to infection status, which justified the use of shell length as a covariate in the analysis. Next, a full factorial ANCOVA model was used, with an outcome that the effect of lake ($F_{1,\,493} = 1.596$, $p = 0.426$), effect of infection status ($F_{1,\,493} = 0.004$, $p = 0.962$), and the interaction between infection status and lake ($F_{1,\,493} = 1.136$, $p = 0.287$), all were non-significant, while the positive effect of shell length was highly significant ($F_{1,\,493} = 39.404$, $p < 0.001$) (Fig. 27), indicating that the effect of infection status on the relative shell width did not differ between lakes. Thus, the lake x infection status interaction term was excluded from the final ANCOVA model, which had only the main effects lake, infection status and the covariate. Results of this ANCOVA indicated a non-significant effect of lake on the relative shell width ($F_{1,\,627} = 0.462$, $p = 0.497$), as well as non-significant effect of infection status ($F_{1,\,627} = 0.422$, $p = 0.516$), while the effect of mussel length was highly significant ($F_{1,\,627} = 638.14$, $p < 0.001$). Thus, in both lakes the relative shell width was not related to infection by *R. campanula*. This was also studied separately for males and females to avoid the possible effect of mussel gender, but the result did not change.
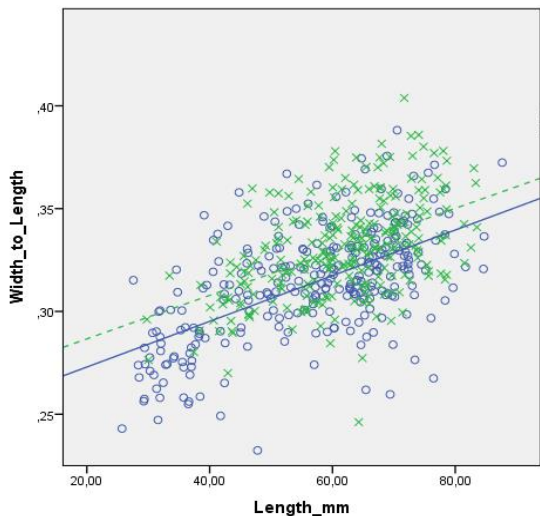

Figure 24. Relative shell width (mm) of *Anodonta anatina* males (circles, solid line) and females (crosses, dotted line) in Lake Kuivasjärvi material.

3.4.5. Differences between *R. fennica*-infected and uninfected mussels in relative shell width

To study the effect of *R. fennica* infection on the relative shell width of *A. anatina* from Lake Saravesi, uninfected and individuals infected with only *R. fennica* were included (concurrent infections with other parasite species were excluded). Mussels ≥ 55 mm long were selected for the analyses to have representative size groups for both infected and uninfected mussels. An ANCOVA model in which the relative shell width was the dependent variable and infection status (infected by *R. fennica*, not infected by any other parasites) as a fixed factor, and shell length as a covariate, and the first model included also the interaction between the covariate and infection status. The interaction, shell length x infection status was not significant, indicating that the slopes did not differ between infected and uninfected, which

justified the use of shell length as a covariate in the analysis. Next, a full factorial ANCOVA model was used, with an outcome that the effect of infection status was significant ($F_{1,\,54}$ = 12.489, p = 0.001) but the effect of covariate length was only marginally significant ($F_{1,\,54}$ = 3.813, p = 0.056). Thus, the individuals infected by *R. fennica* were wider than the uninfected mussels (Fig. 29). Length-adjusted mean ± S.E. relative shell width in *R. fennica* infected mussels was 0.320 ± 0.003 while in uninfected mussels it was 0.336 ± 0.003. When the ananlysis was done separately for males (n = 30) and females (n = 22), the higher relative width in infected individuals was observed in males but not among female mussels.
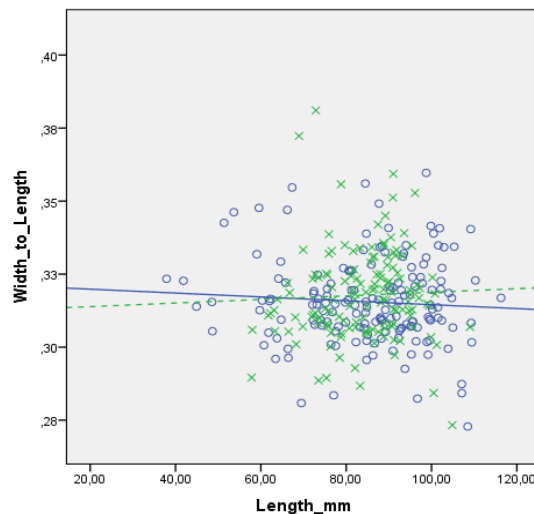

Figure 25. Relative shell width (mm) of *Unio pictorum* males (circles, solid line) and females (crosses, broken line) in Lake Saravesi material.

3.5. Relative volume of shell (volume-to-length ratio against length square)

3.5.1. Differences between lakes and species in relative shell volume

Relative shell volumes of *A. anatina*, *U. pictorum* and *U. tumidus* in Lake Saravesi are given in (Fig. 30) as plotted against length squared, shell volume meaning here the space covered by the shell (not the volume of the shell tissue itself). Trematode-infected individuals were excluded before analyses. Differences between mussel species were analyzed with ANCOVA, with shell volume-to-length ratio as the dependent variable, mussel species as a fixed factor, and square of shell length as a covariate. First the ANCOVA model included, in addition to the main effects species and length^2, also the interaction between those two. ANCOVA result revealed that the interaction between mussel species and the covariate length^2 was clearly significant ($F_{2,\,444}$ = 16.010, p < 0.001), suggesting that the slopes (effect of covariate) were different in different species. To determine which species differed from each other, the analysis was repeated using *A. anatina* and *U. tumidus*. ANCOVA results revealed that the interaction between species and the covariate was in this case not significant ($F_{1,\,143}$ = 0.007, p = 0.935), suggesting that the slopes (effect of covariate) were identical in using *A. anatina* and *U. tumidus*. This means that it was the species *U. pictorum* that had a different relationship of shell volume-to-length with the covariate, i.e. the increase of shell volume-to-length with respect to length was not as steep in *U. pictorum* as in *A. anatina* and *U. tumidus* (Fig. 29).
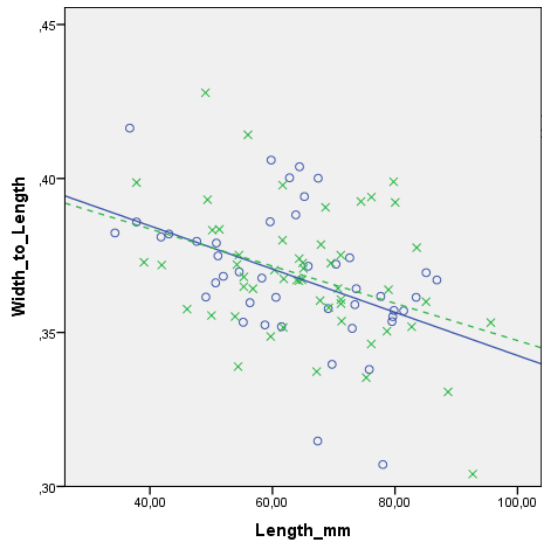
Figure 26. Relative shell width (mm) of *Unio tumidus* males (circles, solid line) and females (crosses, broken line) in Lake Saravesi material.
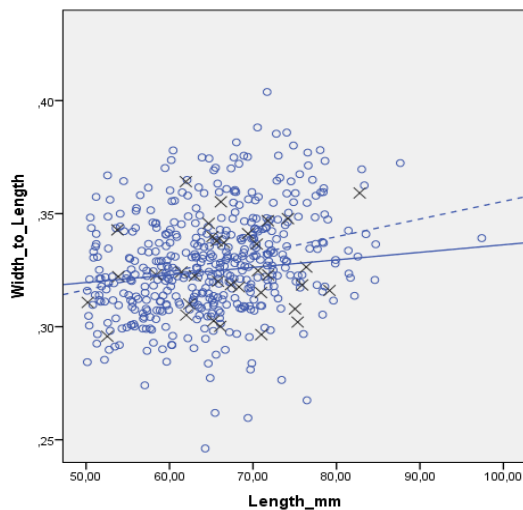
Figure 27. Relative shell width (mm) of uninfected (circles, broken line) and *R. campanula* infected (crosses, solid line) *A. anatina* in the combined material of Lake Saravesi and Lake Kuivasjärvi.

The ANCOVA was continued with *A. anatina* and *U. tumidus* by applying a full factorial model, which suggested that both the effect of species ($F_{1, 143} = 41.969$, $p < 0.001$) and the effect of the covariate ($F_{1, 143} = 1129.9$, $p < 0.001$) were statistically highly significant. This means that the relative shell volume (volume-to-length) was higher in *A. anatina* than in *U. tumidus*, and that in both species the relative shell volume increased linearly with square of length. Mean ± S.E. length-adjusted volume-to-length ratios for *A. anatina* and *U. tumidus* were 0.290 ± 0.005 and 0.254 ± 0.003, respectively.
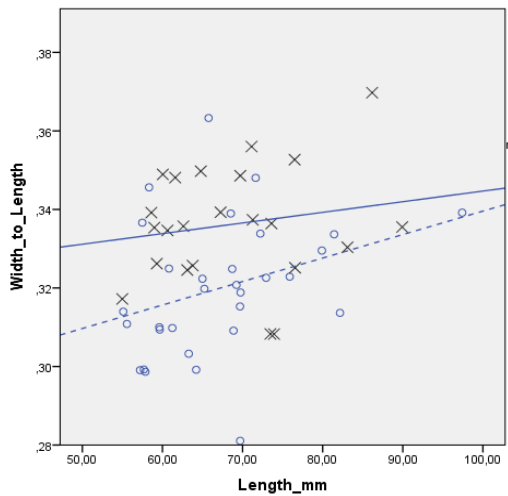
Fig.28. Relative shell width (mm) of uninfected (circles, broken line) and *R. fennica* infected (crosses, solid line) in ≥ 55 mm long *A. anatina* from Lake Saravesi.

However, length-related volume was the smallest in *U. pictorum*. This was illustrated by analyzing differences between the species in length groups of 60 - 70 mm individuals using one way ANOVA with shell volume-to-length as the dependent variable and mussel species as factor. ANOVA results revealed a highly significant effect of mussel species ($F_{2, 68} = 41.205$, p < 0.001). Post Hoc tests suggested clear differences between all species (p < 0.001 in all pairwise comparisons). Mean ±S.E. volume-to-length ratios for *A. anatina* (n = 14), *U. tumidus* (n = 29) and *U. pictorum* (n = 28) were 0.315 ± 0.009 and 0.266 ± 0.006 and 0.231 ± 0.004, respectively.

Figure 29. Relative shell volume (volume to length squared) of *Anodonta anatina* (circles, solid line), *Unio pictorum* (triangles, dotted line) and *Unio tumidus* (crosses, broken line) from Lake Saravesi.

4. DISCUSSION

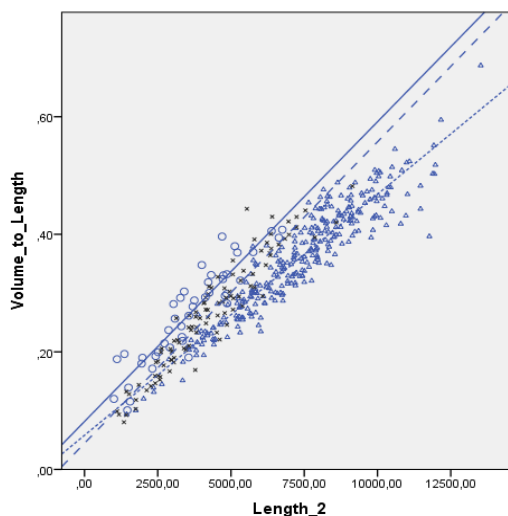This study was aimed at investigating how sex and trematode parasites could have an influence on the shell morphology of three European freshwater unionoid mussel species. The findings of this work could have its application in the taxonomy and conservation of mussels which are already endangered and becoming rare species.

A series of morphological patterns was observed across and within populations, as well as within and between species that were collected from the two study sites.

4.1. Sexual dimorphic patterns and between species differences

As expected from the hypothesis that at younger ages (≤ 3 years), female mussels will have the same lengths as males, while at older ages (≥ 4 years), male mussels will become longer than the females, the study supports this trend among the lake Saravesi population of *U. pictorum* (Fig. 8) but not in *A. anatina* of Lake Kuivasjärvi (Fig. 10). This outcome is in line with the work of Brander (1954) who made a description of male shells of *Pseudanodonta complanata* to be more longer and ovally shaped than the more trapezoid females but a contrary view to this was that of Siebold (1837) who found females of *A. cygnae* and *A. anatina* to be more longer than males. Nonetheless, among the Lake Saravesi *U. tumidus* mussels, no such trend was observed (Fig.9). Furthermore, a different trend was observed in Lake Kuivasjarvi *A. anatina* mussels (Fig.10), whereby females were longer than males at all age groups but statistically significant ($P < 0.05$) in age groups of 2 and 8. The trend of males becoming longer than the females at older ages could be explained by the idea that fecundity in mussels (unionoids) increases with size (Bauer 1998, Hochwald 2001, Haag & Stanton 2003). Brooding in females (e.g *A. anatina*) starts from the age of 4, and brooding females are known to be responsible for the provision of calcium (Silverman *et al*., 1985, 1987) and nutrients (Wood 1974) to their eggs and glochidia which are formed in their marsupia water channels. By implication, the nourishing and production of offspring by gravid female mussels will lead to the depletion of these resources (nutrients and/or calcium) that otherwise could have been used for shell growth thus inducing this type of sexual dimorphism amongst the mussel population (Downing & Downing 1993, Mclvor & Aldridge 2007).

This study revealed that female *A. anatina* mussels were (wider) more inflated than the males (Fig. 24). It is probable that this could be as a result of altered shell width growth of females during the period when they are gravid, as proven by Tankersley & Dimock (1992) that gravid marsupial gills in long-term brooding *Pyganodon cat-aracta* swell to nearly thirty times their non-brooding width.

There was equally a clear statistical difference in shell width (inflation) across species, whereby the shells of *U. tumidus* were widest, *A. anatina* had the smallest shell width, while that of *U. pictorum* was in-between that of *U. tumidus* and *A. anatina* (Fig.22).

Shell volume also varied across species with highest to lowest relative shell volume following the order of *A. anatina*, *U. tumidus* and then *U. pictorum* (Fig.29). These differences in relative shell volumes could be because *A. anatina* shell was wider and higher than in the other species. May be *A. anatina* invests more if its energy resources for the growth of its soft tissues, thus higher shell volume, as opposed to *U. tumidus* and *U. pictorum* that may invest more of their energy on the growth of hard shells. The investment of energy on the growth of shells has been proven to limit the growth of soft tissues of unoinoids (Currey 1988).

4.2. Trematodes

This study revealed a high prevalence of *R. fennica* (51.1%) and low prevalence of *R. campanula* (15.6%) in *A. anatina* from Lake Saravesi. Only *R. campanula* (4.4%) was found in *A. anatina* from Lake Kuivasjärvi. These results are in line with the study of Taskinen *et al*. (1991) that also reported a high prevalence of *R. fennica* (33.2%) and low prevalence of *R. campanula* (1%) in *A.anatina* from Lake Saravesi, as well as only *R. campanula* with prevalence of 5% in *A. anatina* from Lake Kuivasjärvi.

In the *A. anatina* population at lake Saravesi, there was no significant difference in shell length between the infected and uninfected mussels. However, in Lake Kuivasjärvi, *R. campanula* infected mussels were significantly longer than uninfected mussels (Fig. 11). The trematodes infection observed in large mussels could be as a result of their higher filtering rates. The enhanced growth that was observed in individuals that were infected with trematodes from Lake Kuivasjärvi could be either the parasites adaptation to increase the available space in the host shell or a non-adaptive byproduct of host castration or a host counter adaptation to improve reproductive output (see McCarthy *et al*. 2004).

This study hypothesized that, trematodes (*R. fennica*/*R. campanula*) infected mussels will have wider shells compared to their uninfected counterparts. However, only in *R. fennica* there was a statistical effect on the shell width in Lake Saravesi (Fig. 29). Here, *R. fennica* infected *A. anatina* were wider than uninfected individuals. This effect could be due to parasite reproduction in the gonads of mussels leading to the bulging of mussel's soft tissues, as such resulting to more inflated shell growth to accommodate the sporocysts. Trematode parasitism can slow overall growth rates and reduce reproductive output and physiological condition of unionoids (Jokela *et al*. 1993, Gangloff *et al*. 2008, Taskinen 1998). Even though reduced fecundity could be expected to result in decreased swelling of marsupial gills and obviously, relatively narrower shells, the data of this work suggest that infected mussels of both sexes tended to be relatively more inflated than uninfected ones, at least for *R. fennica* infected *A. anatina* from Lake Saravesi.

5. CONCLUSION

Based on the results of this finding, we can conclude that there is some difference in shell morphology between male and female mussels in terms of height and width. Results of the findings also revealed a difference between trematode infected and uninfected mussels in terms of shell length and width. *R. campanula* infected mussels were longer than the uninfected in Lake Kuivasjarvi and *R. fennica* infected mussels were wider than the uninfected in Lake Saravesi.

It is recommended that further research on mussel morphology should take into consideration the fact that life history traits which occurs because of variation within, between and across mussel populations such as size/age of maturity, incubation length and/or timing of glochidial release might also influence female shell bulging and should be considered when depicting sexual dimorphic patterns in unionoid populations (Bauer 2001, Hochwald 2001, Haag & Stanton 2003).

Choo Jocelyn for making time out of her busy schedule to make positive criticisms and guidance whenever I needed her assistance. I also thank Prof. Jouni Taskinen and Mah Choo Jocelyn for proofreading this work both at the preliminary and final stages.

I wish to dedicate this work to my late father, Moteka Adolf Otte and son, Njimerie Walters Lokili, who were called by the Lord Almighty and as a result have not live to rip the fruits of my educational pursuit. I wish to acknowledge the material and moral support from friends; Kodia, Prince, Martin, Thomas, Besong, Henry, Polain, Haja, Jocelyn, and others I have not mentioned herein during the time of grief for my late father and son.

My warmest gratitude goes to my beloved mother who has always made it a duty that we know each other's wellbeing, as well as keeping me informed on my family's welfare.

Finally, my endless appreciation goes to God for making it possible for me to go through this academic achievement.

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