Master's thesis

Morphological variation of the unionidae mussel *Anodonta anatina*

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ABSTRACT

Morphology of a given species can vary among individuals, morphology differences can be found between populations males can be different from females (sexual dimorphism) or e.g. with young and old individuals. The aim of the present study was to investigate morphological variability in the unionidae mussel *Anodonta anatina* originating from two populations. Measured variables included length, height, width, volume and dry mass of shell, dry mass of soft tissues and gill blades, and age, sex, estimation of glochidium larvae produced. The main results included faster growth of mussels in Lake Koijärvi than in Lake Päijänne (at the age of 4-5 years), which may reflect the higher productivity in Lake Koijärvi—and also faster growth of females in Lake Koijärvi (at the age of 4 years). In addition, shell was relatively higher in small individuals and in males than in bigger mussels or females. On the other hand, shell of big mussels (in Lake Koijärvi) and females was relatively wider in shape, and the shell volume was higher in females than in males. This sexual dimorphism could be due to space requirements of the glochidium larvae developed in female gills. The total dry mass of soft tissues (in both populations), as well as the mass of gill blades (in Lake Päijänne), was higher in females than among males, but the mass of body without gills was higher in males (in Lake Päijänne). The mass of shell compared to mass of soft parts did not differ between sexes in Koijärvi but in big-sized Päijänne mussels the males had heavier shells than females. These results indicate clear sexual dimorphism and population-dependent differences in morphology of *A. anatina*.

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TIIVISTELMÄ

Lajin sisällä voi olla vaihtelua morfologiassa niin yksilöiden kuin populaatioidenkin välillä. Koiraat ja naaraat voivat erota toisistaan (sukupuolidimorfia) tai eroja voi olla esimerkiksi nuorten ja vanhojen yksilöiden välillä. Tämän työn tarkoituksena oli tutkia pikkujärvisimpukan (*Anodonta anatina*, Unionidae) morfologisten piirteiden vaihtelua kahdessa simpukkapopulaatiossa. Mitatut muuttujat olivat kuoren pituus, korkeus, leveys, tilavuus ja kuivamassa, pehmytkudosten ja kidusten kuivamassa, ikä, sukupuoli ja glokidium-toukkamäärän arvio. Simpukoiden kasvu oli nopeampaa Kerimäen Koijärvessä kuin Päijänteellä (Pohjanlahti, Jyväskylä) 4- ja 5-vuotiailla yksilöillä—heijastaen todennäköisesti tuottavuuseroja järvien välillä—sekä nopeampaa naarailla Koijärvessä 4-vuotiailla.. Kuoren suhteellinen korkeus oli suurempi pienillä yksilöillä ja koirailla verrattuna isoihin yksilöihin ja naaraisiin. Toisaalta isot yksilöt (Koijärvessä) ja naaraat olivat suhteessa pituuteen leveämpiä eli muodoltaan paksumpia kuin pienet yksilöt ja koiraat ja lisäksi kuoren tilavuus oli naarailla suurempi kuin koirailla. Nämä sukupuolidimorfiset piirteet voivat selittyä glokidium-toukkien aiheuttamalla kohonneella tilan tarpeella naarassimpukoissa, jotka kantavat toukkia kiduksillaan kuoren sisällä. Pehmytkudosten kokonaiskuivamassa (molemmissa populaatioissa) sekä kidusten kuivamassa (Päijänteessä) oli suurempi naarailla kuin koirailla, mutta pehmytkudosten kuivamassa ilman kiduksia oli puolestaan korkeampi koirailla kuin naarailla (Koijärvessä). Lisäksi kuoren kuivamassaltaan Koijärven simpukoisssa ei ollut sukupuolieroja, mutta Päijänteessä isokokoisilla simpukoilla koirailla oli painavammat kuoret suhteessa pehmytkudosten kuivamassaan. Tulokset viittaavat selkeään sukupuolidimorfiaan ja populaatioiden välisiin eroihin pikkujärvisimpukan morfologiassa.

Contents

1. INTRODUCTION

Morphology of a given species varies among individuals. Morphological differences can be found between populations, but also within populations, e.g. between males and females (sexual dimorphism) or between young and old individuals. Recent study by Zieritz et al. (2011) indicates that *Rhipidocotyle sp*. (trmatodes) can modify the structure of the shell/morphology of the unionidae mussel (*Anodonta anatina*). In addition, they found morphological differences related to sex and habitat of *A. anatina*. However, there are limited amount of studies conducted about the shell morphology of Unionoida mussels. Unionoids are common and widespread freshwater mussels, inhabiting all continents except the Antarctic (Graf & Cummings 2006). *A. anatina* is a widespread European Unionidae species that inhabits both standing and flowing freshwater habitats, and usually reaches a life span of about 10 years (Økland 1963). *Anodonta* spp. typically start to reproduce at the age of four to five years (Heard 1975), though in some areas, *A. anatina* has been observed to reproduce as early as in its second year (e.g. Haukioja & Hakala, 1978).

Anadonta anatina is a member of the family of Unionidae and they are useful because their diversity, filtration ability and tropical link as higher predators. They make up 90% benthic invertebrate biomass and they are useful as their filter feeding ability decreases phytoplankton biomass (Marlene et al. 2005, Jerrine et al. 2005). In addition, their burrowing behavior leads them to be useful in river ecosystems (Throp e. al 1991). The enhanced grazing on phytoplankton and other suspended solids might shift lake status to another for example Koijärvi phytoplankton reach to the lake ecosystem type such as Päijänne less phytoplankton in suspension by grazing if they are enough in number at least in laboratory this is visible. Among those zebra mussel *Dressena polymorph Pallas* is known to improve water quality and had been implemented in the bio manipulation of lakes in Netherland and also promoted the decline blooms of toxic cyanobacteria in North America, (Frederic, 2010). The following mussels, blue mussels (*Mytilus edulis*) and two freshwater species of zebra mussel (*Dreisena polymorpha*) and the duck mussels (*Anodonta anatina*) were used to remove and concentrate water born pathogen by filtration and can be used in sanitary assessment of water quality (Frederic, 2010). The status of lakes can affect the status of the flesh and shell of the duck mussel. In our case the two lakes, Koijärvi and Päijänne status were used to find out those (Frederic, 2010).

According to (Esch et al. 1990), higher pH and alkalinity in Michigan waters contributed to a richer molluscan fauna. As we noticed, higher pH and less alkalinity observed in Koijärvi where the mussels *Anodonta anatina* fauna has been bigger and flourished than that of päijänne, it's also good to mention the turbidity and calcium contents were higher than the rest 14 lakes see in original table 1 in (Frederic, 2010). Indicating there is much enough amount of food and circulation of oxygen in Lake Koijärvi than päijänne. The mussels were in higher body shell size that might be due to the calcium and other nutrients easily availability to consumed and utilized by the clams. Other ambiotica (physical and chemical) factors contribute and affect the growth variation of mussels in the two lakes where the experiment was conducted.

The aim of this study was to investigate morphological variation—shell length, width, height and volume in the bivalve, *A. anatina* from two lake populations. The study sites, or populations, were Lake Päijänne (Jyväskylä) and Lake Koijärvi (Kerimäki). In addition, shell growth rate and dry weight of soft tissues, gills and shell of mussels were measured. The following questions were asked: Is there a difference in growth rate of mussels between the populations or between males and females? Do the relative height and width of shell differ between populations or in relation to size and sex of mussels? Are there any sex-related differences in the length-adjusted total dry mass or gill blade dry mass of mussels? The hypotheses were formed accordingly. First, mussel growth should be quicker in Lake Koijärvi since it is more productive than Lake Päijänne (Table 1). Second, shell of females should be relatively wider as the females brood glochidia larvae on their outer gill blades, which should require more space than a male gill blade—and therefore also the female shell should have bigger volume than that of the male shell. Third, because of the brooded glochidia larvae, also the total weight of female soft tissues and especially that of the gills should be higher than in the males.

The real analysis relies on three sex types those are castrated hermophardite, female and male. The female produce glocidium but also glocidium might grow in hermophardites also. The lenght growth of shell is high in females much more than in other sexes because females exhibit growth of shell in lenght than in width thicknesss since it requires much engery in width growth where the energy is limited for glocidium growth. In male both the shell sizes will grow, in shell width male show higer growth than females. The hight growth is also expected to grow well better in male than female.

2. BACKGROUND

2.1 Filter feeding in Uniondale

According to (Jerrine et al. 2005), the mussels were filter feeders, in river samples they consume by only opening their mouth to pass water but in lake they consume water to filter the food from it. In river samples the clams were infected by parasite (Figure 1). The feeding continuous to open chances to consume more parasites along with food they take in miracidia level.

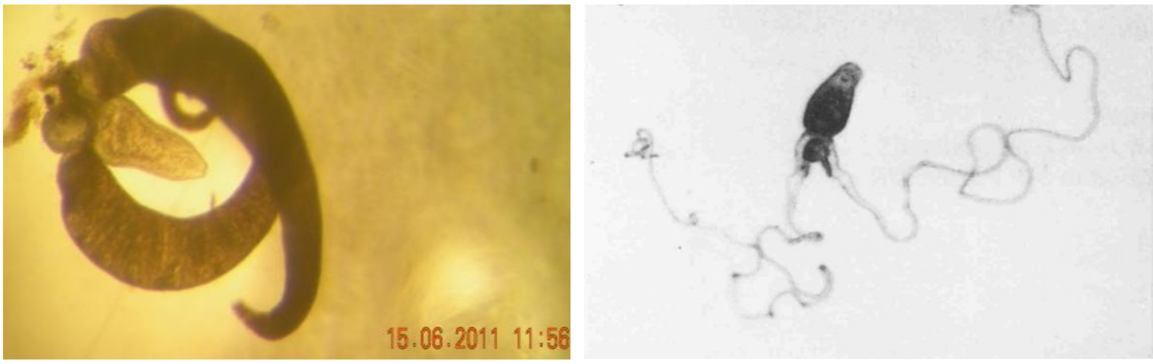


Figure 1. Picture showing *R. campanula* (left) and *R. fennica* (right) cercariae emerged from *Anodonta anatina* (*R. fennica* picture from Taskinen et al. 1991).

All Unioidea are said to be filter feeders. The foot, a tongue like organ used for digging is found in the middle of the anterior part of the Uniman. On both sides of the foot, there are two large morphological gills, which have some important functions (e.g. egg development) beside oxygen uptake. Cilia on the gills and mantle create water current and transport mucus to the mouth in front of the animal. The mucus together with the small particles trapped from the water are eaten. The following picture two shows the internal structures of unioidea. The uptake of water food filtrations said to be in the opposite side of the water flow so that they can regulate the amount of water to pass through and filter their food content, which seems common in most of *Anodonta* species. However, its energy consuming to suck the water against the water flow in rivers while in lakes it seems regular. Some species allows the river water flow to pass through them so that they can save energy of suction of water and as the water passes they filter their food. These kinds of species are *M. margaritiferia* and they regulate the amount of food taken by closing their shell to avoid the incoming water, (Bauer & Wächtler 2000).

The statuses of the living environment modify their digesting and feeding behaviour vice versa. If mussels are present in large numbers in a population, they can modify the algae compositions and microbial composition. The main source of their food is from suspended solids and sediment. Contaminations might have effect after polluted water is taken by mussels rather than direct sediment to mussel contamination. Isotope studies suggest that the food is mainly from dietary carbon derived from algae rather than benthic detritus or vegetation (Marlene et al., 2005). The fact shows that mussels can regulate and affect the phytoplankton content of the lake ecosystem. Due to their filtering power mussels are sometime used to filter water in aquaria and aqua culture tanks (Marlene et al., 2005). Some species are not selective on what they consume. Feeding is inhibited due to seasonally turbid or hyperoxic water. Strong correlation and peak of growth is observed in shell and somatic with phytoplankton's abundance, in mid dry seasons, eutrophication and food availability. There is not much relation between food uptake from organic detritus and even lesser peak when nutrient input increase at first rain in easily-wet seasons (Marlene et al., 2005).

The infrastructures are conditions are the best studied but still insufficiently understood is the habitat condition (Zieritz et al. 2011). August through March the time of production of glochidia in mussels they intent to product at age of four and life span usually 10 years' some time we found 12 years old ones. We presented the different characters that affect shell morphology, they are shell length, height, width and volume, mussel flesh dry weight, gills dry weight and shell weight, as well as sex and age of mussels. The next figure 2 shows the anatomy of clams among those the five main organs the foot, mantel, kidney, gonad and the digestive organs were in few mm taken for identification of infection.

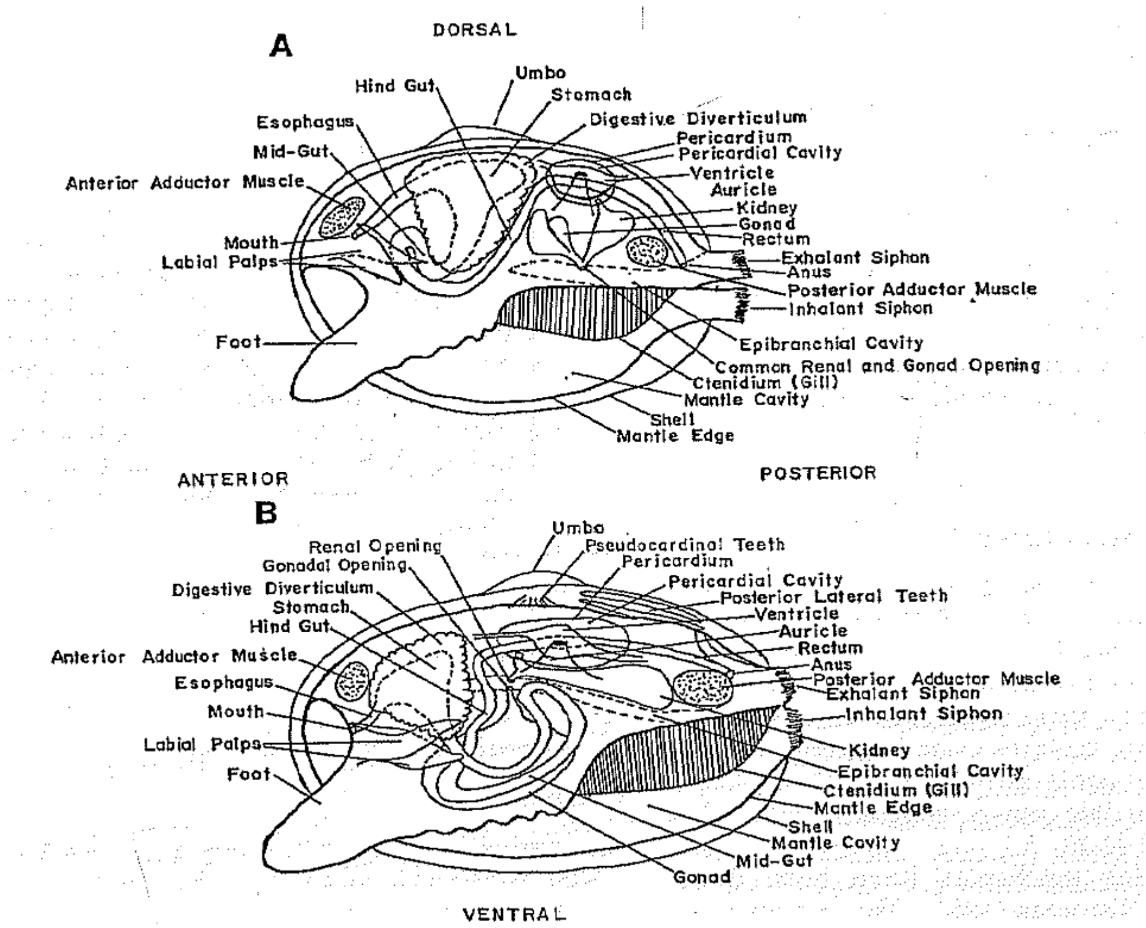


Figure 2. General internal system anatomy of organs that and organ systems of the soft tissues of (A) corbiculacean and (B) Unionacean fresh water bivalves. Borrowed from a book of (Thorp & Covich 1991))

3. MATERIAL AND METHODS

3.1. Study areas

Lake Koijärvi show higher productivity, conductivity and calcium content than Lake Päijänne (Table 1).

Table 1. Water quality parameters of the different Lakes, growth index i.e. length of the 3rd annulus (L3)/mm and number of 3-4 yr old clams included in growth measurements (N). Cond = conductivity (mS/m), Turbid= Turbidity (NTU), Chlo a = Chlorophyll a (µg/l), Tot. P = Total Phosphorus (µg/l), PO4-P = Phosphate phosphorus (µg/l), Tot. N = Total nitrogen (µg/l), Alk = Alkalinity (µg/l), Cal = Calcium (µg/L), (Sources: measurements by J. Taskinen from Koijärvi and by Arja Paloniemi from Päijänne)

Lake	pH	Cond (mS/m)	Turbid NTU	Colour (mgPt/l)	Tot. P (µg/l)	PO_4-P (µg/l)	Tot. N (µg/l)	Chlo a (µg/l)	Alk (µg/l)	Cal (µg/l)	N (µg/l)
Koijärvi	7.43	15.46	4.81	80.00	15.00	1.00	662.00	15.00	0.36	14.37	19
Päijänne	7.3	6.9	2.15	20.00	7.00	0.47	460.00	10.42	0.23	9.18	13.2

The area map of the two lakes, Koijärvi (figure 3) and Päijänne (figure 4) were taken from Retkikartta web site having them coordinates along pictorial maps. Lake Koijärvi is small shallow lake located in latitude of 61^0 53’ 34.8300” and longitude $29^0$11’46.5900” that have mussels bigger in size than that of päijänne where it’s located in latitude of 62^0 12’49.2720” and longitude $25^0$45’23.4420”. The sea levels also vary thus Koijärvi is at 88.4 m above sea level while päijänne is 78.2 m above sea level. The next map illustrates Lake Koijärvi and Päijänne, the first map show the Lake Koijärvi area in two small maps the maps are coordinate map type to show the clear drawings of the littoral area. Some section of Päijänne also illustrated below.

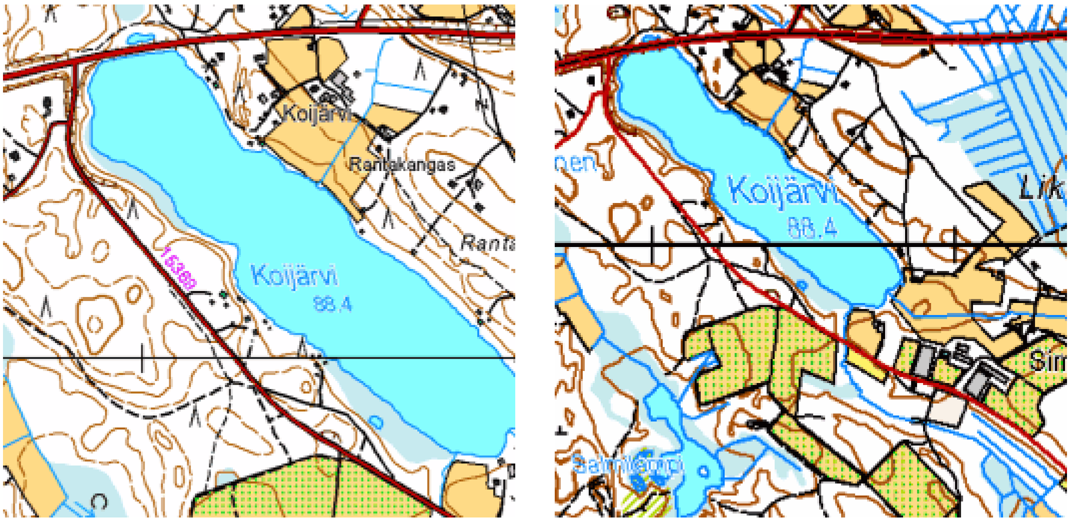


Figure 3. Lake Koijärvi looked at a coordinate maps

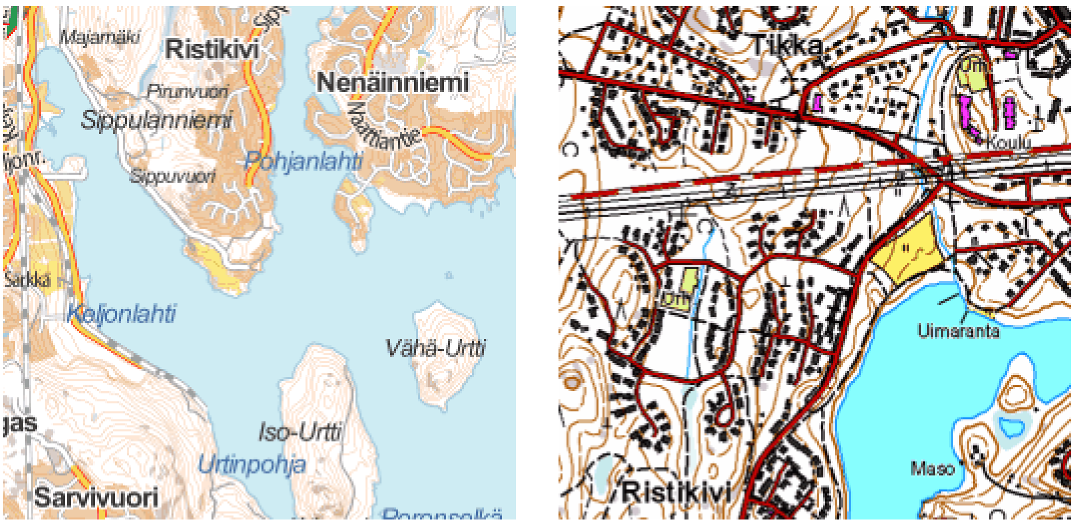


Figure 4. Lake Päijänne seen to at two sampling points

The mussels collected from Lake Koijärvi were bigger in size but were at same age level to Lake Päijänne.

Table 2. Locations, average ages and different length types most are in average. L= total average length, L3= average 3rd annuls and Lmax = the maximum length found among those.

Site	Latitude, longitude	sex	age	L	L3	Lmax
Koijärvi	61^0 53’ 34.8300” $29^0$11’46.5900”	all	4,75	82,05	58,86	99,075
Päijänne	62^0 12’49.2720” $25^0$45’23.4420”	all	4,1225	97,41	72,84	121,595

3.2. Sex determination and morphology measurement

Sex has been the base issue to evaluate in our study. Sex determination in our case was done by using dissection process to see the glochidium growth in female mussel. The mussel samples from Koijärvi and Päijänne were collected on appropriate day by Professor Jouni Taskinen then brought to stabilize and kept in Konnevesi Research Station . The samples were *Anodonta anatina* and *Unio tumidus*. Mussels were later brought to Jyväskylä then put in mesh iron foil box and kept in the lake Jyväsjärvi to give and stabilize their living condition before dissection process starts. They were kept in two numbered box not to mix koijärvi and Lake Päijänne samples. The analysis starts by taking 20 samples of mussel’s and one *Unio tumidus* at a time for dissection process.

The mussels were dissected and a few mm cut from main organ, the gonad was examined under pressed rectangle glass by using light microscope. To determine infection we observed thus few infections in both lakes. The analysis was done by my own except the dissection process to other principal methods. After identifying their infection level the glochidium percentage forecasted by looking the coverage of outer gill in both sides. Afterward with care not to lose glochidium with other soft body parts to be weighted, both outer gills together with glochidia were cut carefully and placed in tablet bottle size bottles. The rest of the body parts were collected and placed in another tablet look like bottle which was numbered. The dry weights of the flesh were done after drying them for appropriate time. The dissected shells were numbered from the inside using a pencil immediately before mixing them to avoid any loss. Those numbers shells were all washed by detergent to avoid any surfactant effect that will avoid and asymmetry in accuracy. Principally speaking the shell well washed to implicate the purity of shell without any flesh left in shell well all flesh must be collected to bottle of dry weight. But the sludge saliva look like structure causes in accuracy in shell accuracy volume measurement. Afterward the shells will give a proper procedure to find out their volume that took along with other methods three weeks’ time full usage.

The shell volume measured by using tap water and pouring full amount of water to half shell then decanting them to a young beaker then measures their volume in ml by using a pipette. Afterward the shell sizes measured were collected along with volume.

Then the shell dried and dry shell weights were discovered in the investigation procedure by using calibrated balance. The dried shell samples broken in to half of the shell in perpendicular to the length then at 3rd annulus the thickness of the shell identified. The volume repeated twice before hand for one half shells to get accurate average values and three times measurement done to get accurate thickness values. The analysis requires three appropriate procedures to conclude it one the shell measurements, second the flesh measurements and third the whole mussel measurements.

4. RESULTS

4.1 Growth (mean length at a given age)

Age-specific length of *A. anatina* indicated a faster growth of mussels in Lake Koijärvi (Fig. 5). Length differences were analysed in those age groups where the number of individuals for both populations was at least 3, i.e. 3-6 years old mussels (Table 3). Length difference between Koijärvi and Päijänne mussels was not significant at the age of 3 and 6 years (One-way ANOVA; $F_{1, 18} = 1.477$, $p = 0.240$ and $F_{1, 19} = 2.911$, $p = 0.104$) but was significant at the age of 4 and 5 years (One-way ANOVA; $F_{1, 36} = 21.132$, $p < 0.0001$ and $F_{1, 19} = 20.298$, $p < 0.0001$, respectively), with bigger mussels in lake Koijärvi than in Lake Päijänne. For this analysis, all mussel individuals included, both uninfected and those infected by the trematode *Rhipidocotyle fennica*.

When studying growth differences between sexes, only those age groups were included in which the number of males and females was at least 3 (Table 4). Age-specific lengths indicated a faster growth of female mussels in Lake Koijärvi (Fig. 5) but not in Lake Päijänne. Also in Lake Koijärvi, however, such a significant sex-difference was observed only in age class 4 years old mussels (One-way ANOVA; $F_{1, 14} = 8.667$, $p = 0.011$, Fig. B) but not in 2, 5 or 6 years old individuals (One-way ANOVA; $F_{1, 5} = 3.185$, $p = 0.134$, $F_{1, 11} = 0.166$, $p = 0.691$ and $F_{1, 12} = 0.022$, $p = 0.885$). In Lake Päijänne, One-way ANOVA statistics for age groups 3, 4 and 5 years old mussels were ($F_{1, 13} = 0.035$, $p = 0.854$, $F_{1, 17} = 0.156$ $p = 0.698$ and $F_{1, 5} = 0.235$, $p = 0.648$, respectively).

Table 3. Age-specific numbers of *Anodonta anatina* collected from Lake Koijärvi and Lake Päijänne (All mussels) and those infected by the trematode parasite *Rhipidocotyle fennica*.

Age	All mussels			Infected mussels		
	Koijärvi	Päijänne	Total	Koijärvi	Päijänne	Total
2	7	1	8	-	-	-
3	4	16	20	-	1	1
4	18	20	38	2	1	3
5	14	7	21	1	0	1
6	16	5	21	2	1	2
7	1	2	3	-	1	1
9	1	-	1	-	-	-
Total	61	51	112	5	3	8

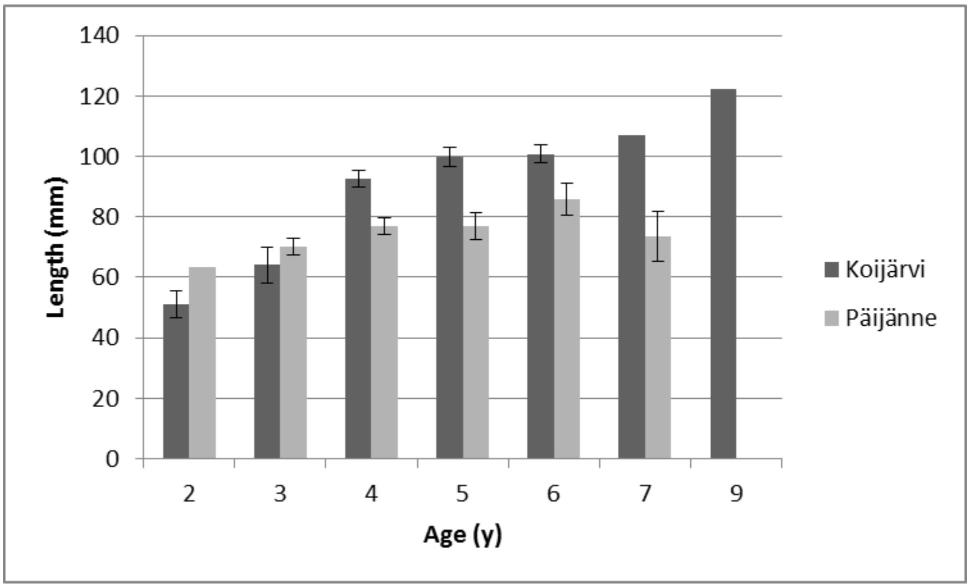


Figure 5. Age-specific mean (± s.e.) length of of *Anodonta anatina* collected from Lake Koijärvi and Lake Päijänne. All mussel individuals included, both uninfected and those infected by the trematode Rhipidocotyle fennica. For numbers of individuals, see Table 3.

Table 4. Age-specific numbers of males and females of *Anodonta anatina* collected from Lake Koijärvi and Lake Päijänne. Individuals infected by the trematode parasite *Rhipidocotyle fennica* were excluded.

Age	Lake Koijärvi			Lake Päijänne		
	Male	Female	Total	Male	Female	Total
2	4	3	7	1	-	1
3	4	0	4	7	8	15
4	5	11	16	7	12	19
5	3	10	13	3	4	7
6	3	11	14	2	3	5
7	1	-	1	-	1	1
9	-	1	1	-	-	-
Total	20	36	56	20	28	48

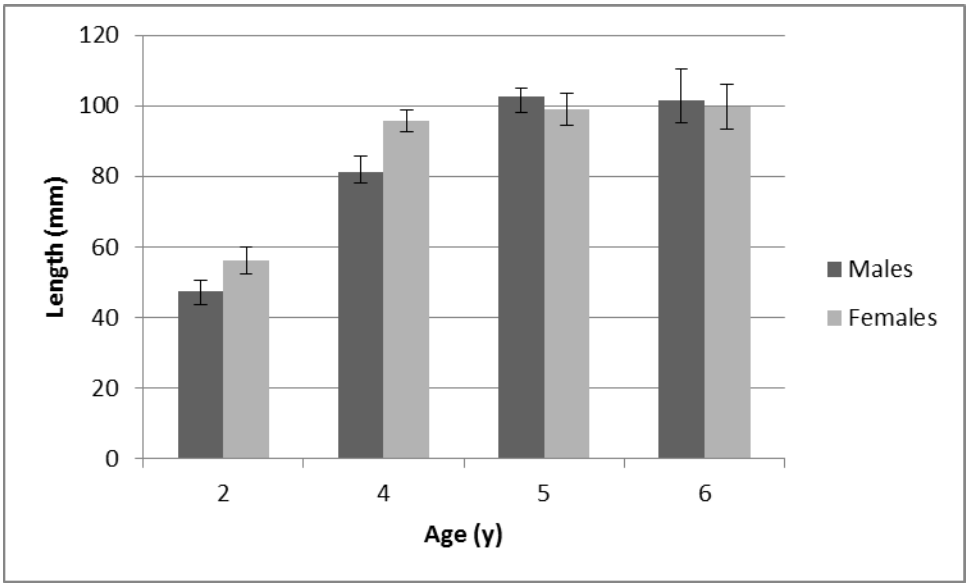


Figure 6. Age-specific mean (± s.e.) length of *Anodonta anatina* collected from Lake Koijärvi. Only individuals not infected by the trematode parasite *Rhipidocotyle fennica* and age groups containing at least 3 males and females were included. For numbers of individuals, see Table 4.

4.2 Relative height of shell (height/length)

To study the relative height, i.e. shell height in relation to length of mussels, first only those size groups were selected, in which mussels were found both in Lake Koijärvi sample and Lake Päijänne sample. Therefore, uninfected individuals between 50 and 100 mm in length were selected for the analysis of covariance (ANCOVA) in which shell height to length ratio was used as the response variable, sex as a fixed factor, population as a random factor and mussel length was used as a covariate. Results of ANCOVA indicated that the difference between populations was significant ($F_{1, 74} = 6.007$, $p = 0.017$) while the effect of sex was not ($F_{1, 74} = 0.059$, $p = 0.808$). The effect of covariate length was highly significant ($F_{1, 74} = 41.828$, $p < 0.0001$) indicating that in general, small mussels were relatively higher in shape (Fig. 7). Insignificant interaction term sex*length ($F_{1, 74} = 0.535$, $p = 0.467$) indicated that the effect of length on relative shell height was equal in males and females. However, the significant population*length interaction ($F_{1, 74} = 7.212$, $p = 0.009$) suggested that the effect of length on relative shell height was different in Lake Koijärvi and Lake Päijänne, which means different slopes of length-to-height-ratio against length (Fig. 7). Thus, the between-population differences can be interpreted so that among the small *A. anatina* Lake Päijänne mussels are relatively higher than Lake Koijärvi mussels, but among large mussels Lake Koijärvi individuals are relatively higher than Lake Päijänne individuals, i.e. Lake Koijärvi mussel lose their higher shape less at older ages compared to lake Päijänne (Fig. 7).

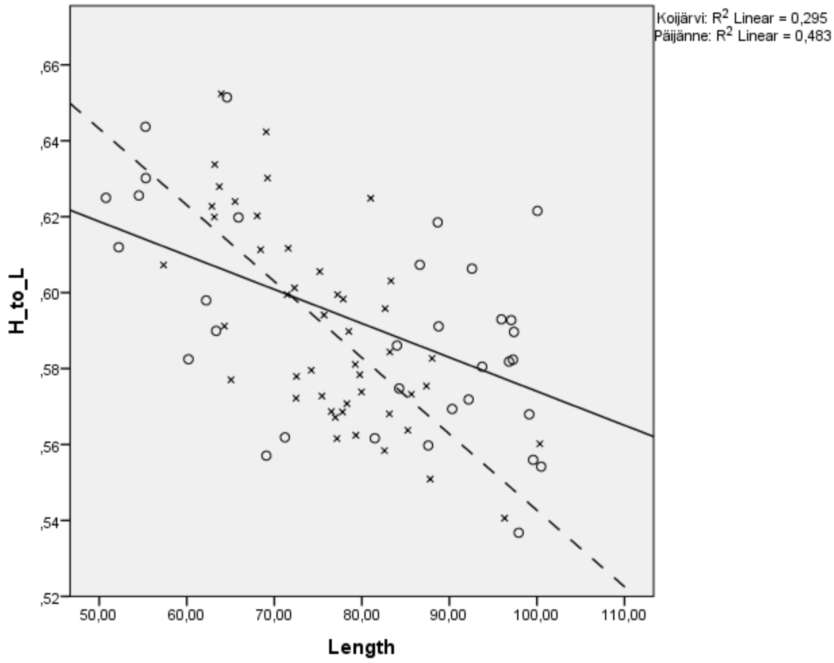
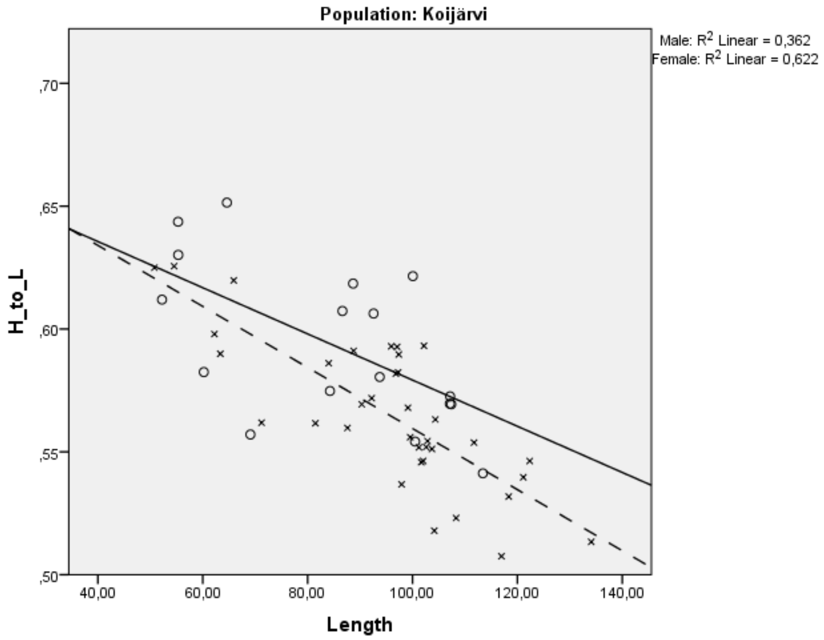


Figure 7. Relative height of shell (height to length ratio, y-axis) plotted against the length of mussels (x-axis, mm) in Lake Koijärvi (circles, solid line) and Lake Päijänne (crosses, dashed line) populations.

The possible sexual dimorphism in relative height of shell was further analyzed separately for Lake Koijärvi and Lake Päijänne by including all uninfected mussels 50 mm or larger in length. In such an approach ANCOVA was used where the response variable was shell height to shell length ratio and, sex being a fixed factor and mussel length a covariate. First also the length*sex interaction term was included in the analysis. As the interaction was found not significant—$F_{1, 52} = 0.890$, $p = 0.350$ and $F_{1, 52} = 0.145$, $p = 0.705$ for Lake Koijärvi and Lake Päijänne, respectively—indicating that the slopes of height-to-length/length relationship did not differ between males and females, the interaction term was excluded from the ANCOVA model. In Lake Koijärvi, a significant sex-difference was observed (ANCOVA, $F_{1, 52} = 6.158$, $p = 0.016$), and the effect of covariate length was also significant (ANCOVA, $F_{1, 52} = 54.426$, $p < 0.0001$). In Lake Päijänne, the sex-difference was also significant (ANCOVA, $F_{1, 46} = 5.922$, $p = 0.019$), as well as the covariate length (ANCOVA, $F_{1, 46} = 42.319$, $p < 0.0001$). Males were relatively higher in shape in both populations (Fig. 8).



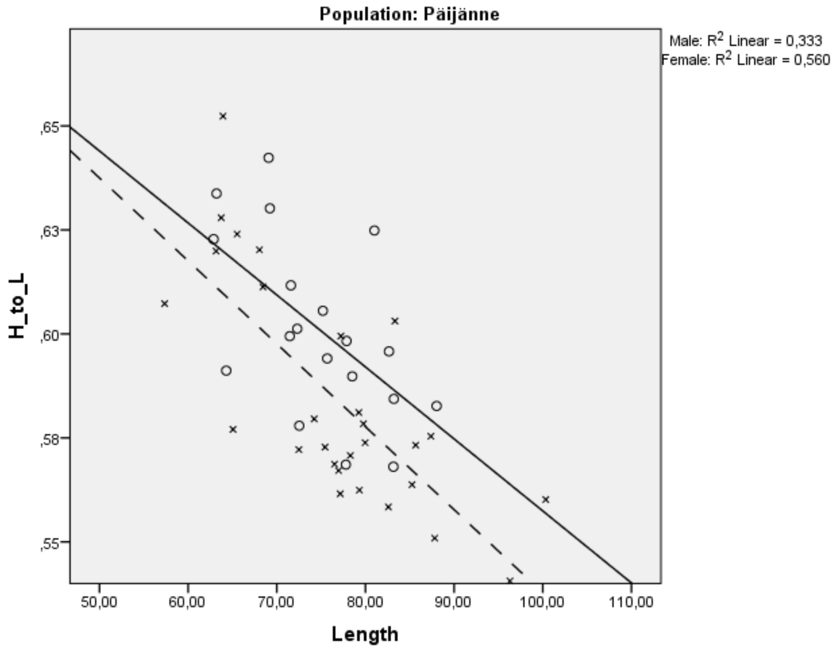


Figure 8. Relative height of shell (height to length ratio, y-axis) plotted against the length of mussels (x-axis, mm) in Lake Koijärvi population (upper picture) and in Lake Päijänne population (lower picture). Males are depicted with a circle and solid line. Females are depicted with a cross and dashed line.

4.3 Relative width of shell (width/length)

To study the relative width, i.e. shell width in relation to length of mussels, first only those size groups were selected, in which mussels were found both in Lake Koijärvi sample and

Lake Päijänne sample. Therefore, uninfected individuals between 50 and 100 mm were selected for the analysis of covariance (ANCOVA) in which shell width to length ratio was used as the response variable, sex as a fixed factor, population as a random factor and mussel length was used as a covariate. Results of ANCOVA indicated that the difference between populations was significant ($F_{1, 69}$ = 5.897, p = 0.018) while the effect of sex was not ($F_{1, 69}$ = 0.001, p = 0.969). The effect of covariate length was significant ($F_{1, 69}$ = 7.292, p = 0.009) indicating that in general, large mussels were relatively wider in shape (Fig. 7). Insignificant interaction term sex*length ($F_{1, 69}$ = 0.472, p = 0.494) indicated that the effect of length on relative shell width was equal in males and females. However, the significant population*length interaction ($F_{1, 69}$ = 7.164, p = 0.009) suggested that the effect of length on relative shell width was different in Lake Koijärvi and Lake Päijänne, which means different slopes of width-to-height-ratio against length (Fig. 9). Thus, the between-population differences can be interpreted so that in Lake Koijärvi mussels the relative width increases by mussel length but in Lake Päijänne mussels the relative width seems to be independent of mussel length when mussel length is between 50 and 100 mm (Fig. 9).

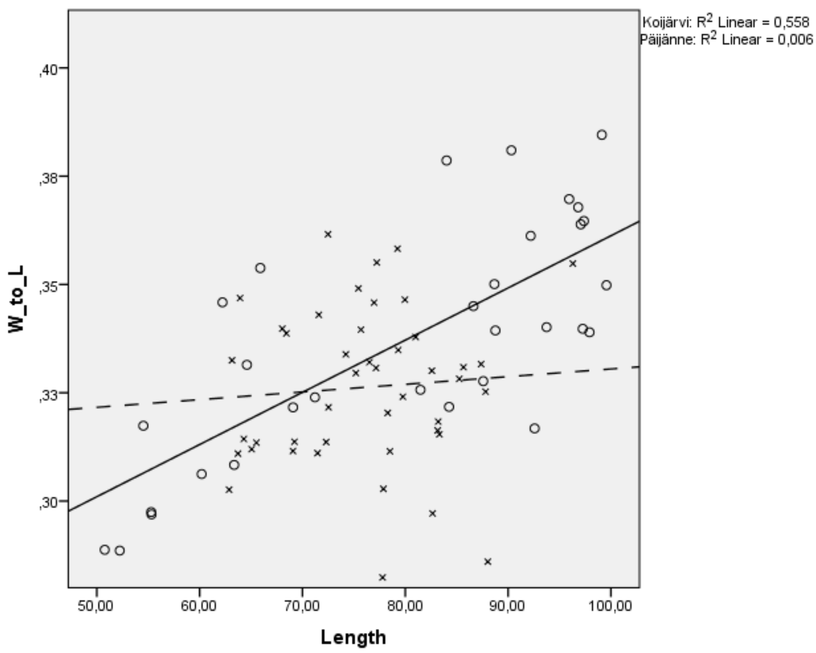
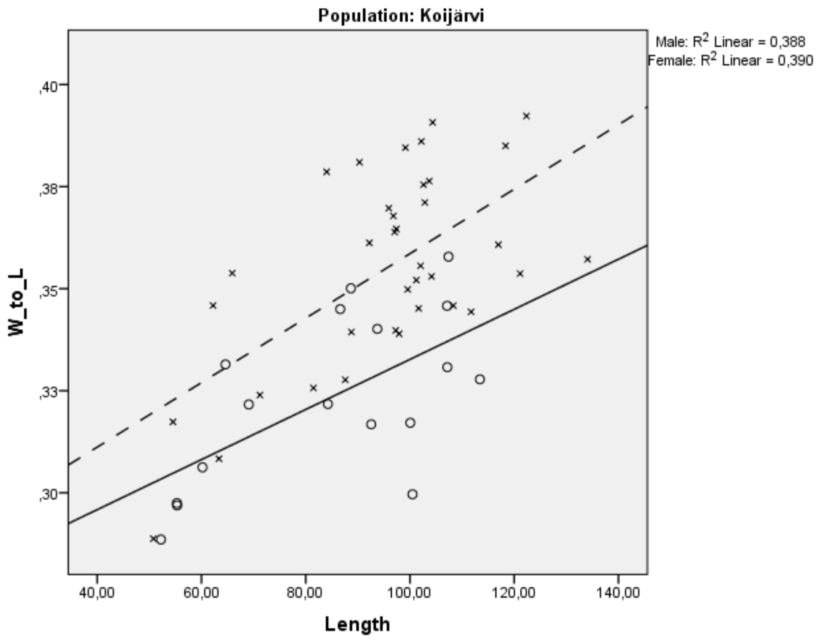


Figure 9. Relative width of shell (width-to-length ratio, y-axis) plotted against the length of mussels (x-axis, mm) in Lake Koijärvi (circles, solid line) and Lake Päijänne (crosses, dashed line) populations.

The possible sexual dimorphism in relative width of shell was further analyzed separately for Lake Koijärvi and Lake Päijänne by including all uninfected mussels 50 mm or larger in length. In such an approach ANCOVA was used where the response variable was shell width to shell length ratio and, sex being a fixed factor and mussel length a covariate. First also the length*sex interaction term was included in the analysis. As the interaction was found not significant—$F_{1, 52}$ = 0.417, p = 0.521 and $F_{1, 44}$ = 0.262, p = 0.611 for Lake Koijärvi and Lake Päijänne, respectively—indicating that the slopes of width-to-length/length relationship did not differ between males and females, the interaction term was excluded from the ANCOVA model. In Lake Koijärvi, a significant sex-difference was observed ($F_{1, 52} = 18.569$, $p < 0.0001$), and the effect of covariate length was also significant ($F_{1, 52} = 31.161$, $p < 0.0001$) (Fig. 10). In Lake Päijänne, the sex-difference was also highly significant ($F_{1, 44} = 15.868$, $p < 0.0001$), but the covariate length was not ($F_{1, 44} = 0.473$, $p = 0.496$). Females were relatively wider, inflated, in shape in both populations (Fig. 10).



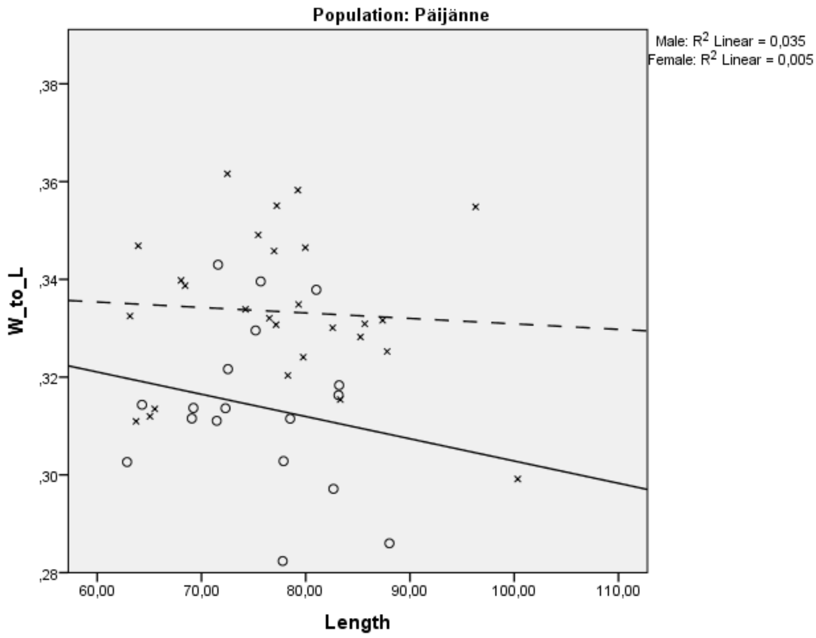


Figure 10. Relative width of shell (width-to-length ratio, y-axis) plotted against the length of mussels (x-axis, mm) in Lake Koijärvi population (upper picture) and in Lake Päijänne population (lower

picture). Males are depicted with a circle and solid line. Females are depicted with a cross and dashed line. In Lake Päijänne the slopes of lines did not differ from zero.

4.4 Relative mass (dry weight/length)

To study population specific differences in the relative mass, i.e. dry mass of the soft parts in relation to length of mussels, first only those size groups were selected, in which mussels were found both in Lake Koijärvi sample and Lake Päijänne sample. Therefore, uninfected individuals between 50 and 100 mm were selected for the analysis of covariance (ANCOVA) in which soft-tissue-dry-mass-to-length ratio was used as the response variable, sex as a fixed factor, population as a random factor and mussel length was used as a covariate. First, results of ANCOVA indicated that the interaction terms sex*length and populations*length were not statistically significant, and were excluded from the ANCOVA model. After this, the ANCOVA results suggested that the difference between populations was not significant ($F_{1, 56} = 0.363$, $p = 0.671$) while the effect of covariate length was ($F_{1, 56} = 118.821$, $p < 0.0001$). In addition the effect of sex, as well as sex*population interaction, were not significant ($F_{1, 56} = 10.996$, $p = 0.167$ and $F_{1, 56} = 0.894$, $p = 0.349$, respectively). Thus, Lake Koijärvi and Lake Päijänne populations did not differ from each other in the relative dry mass of mussels.

The possible sexual dimorphism in relative dry mass of soft parts was further analyzed separately for Lake Koijärvi and Lake Päijänne by including uninfected mussels in those size groups in which individuals were found both in males and females. Thus, from Lake Koijärvi, mussel from 50 to 114 mm were chosen, and from Lake Päijänne mussels from 57 to 89 mm in length were included. In such an approach ANCOVA was used where the response variable was soft-tissue-dry-mass-to-length ratio, sex was a fixed factor and mussel length a covariate. First also the length*sex interaction term was included in the analysis. As the interaction was found not significant in Lake Koijärvi ($F_{1, 32} = 0.545$, $p = 0.466$), it was excluded from the ANCOVA model. After that the ANCOVA results indicated that the effect of sex was only marginally significant ($F_{1, 32} = 3.346$, $p = 0.077$), while the effect of length remained highly significant ($F_{1, 32} = 89.099$, $p < 0.0001$). Thus, there was possibly a trend for a higher relative dry mass of soft parts females than in males in lake Koijärvi (Fig. 11). In Lake Päijänne the effect of sex ($F_{1, 39} = 4.445$, $p = 0.042$), the effect of length ($F_{1, 39} = 40.973$, $p < 0.0001$) and the sex*length interaction ($F_{1, 39} = 6.968$, $p = 0.012$), all were significant.

Thus, the relative dry mass was higher in females than in males (especially among the bigger mussels) and increased by mussel size in Lake Päijänne (Fig. 12).

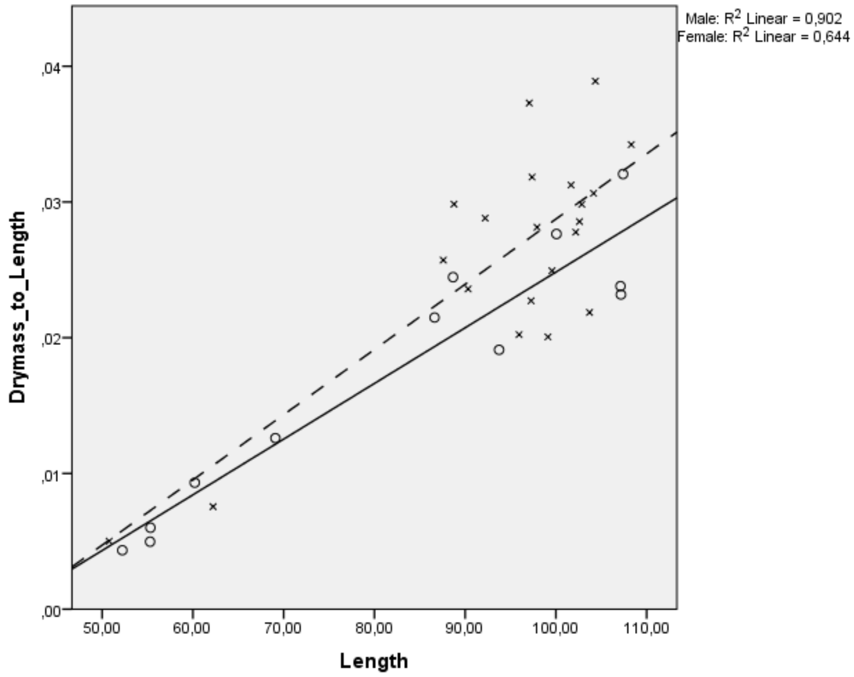


Figure 11. Relative dry mass of soft parts (soft-tissue-dry-mass-to-length ratio, y-axis) plotted against the length of mussels (x-axis, mm) in Lake Koijärvi population. Males are depicted with a circle and solid line. Females are depicted with a cross and dashed line.

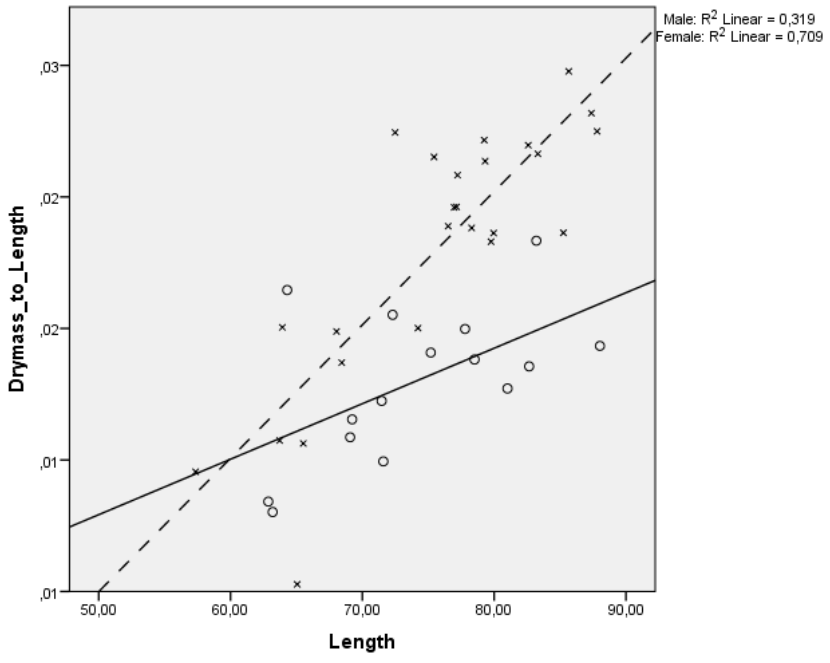


Figure 12. Relative dry mass of soft parts (soft-tissue-dry-mass-to-length ratio, y-axis) plotted against the length of mussels (x-axis, mm) in Lake Päijänne population Males are depicted with a circle and solid line. Females are depicted with a cross and dashed line.

4.5 Relative mass of gills (gills dry weight/length)

The possible sexual dimorphism in relative dry mass of gills was analyzed separately for Lake Koijärvi and Lake Päijänne by including uninfected mussels in those size groups in which individuals were found both in males and females. Thus, from Lake Koijärvi, mussel from 50 to 114 mm were chosen, and from Lake Päijänne mussels from 57 to 89 mm in length were included. In such an approach ANCOVA was used where the response variable was Log_{10}-transformed gills-dry-mass-to-length ratio, while sex was a fixed factor and mussel length the covariate. Also the length*sex interaction term was included in the ANCOVA model. In Lake Koijärvi the effect of covariate length was highly significant ($F_{1, 36} = 63.443$, $p < 0.0001$) but the main factor sex as such was not ($F_{1, 36} = 1.974$, $p = 0.169$).

However, there was a very significant interaction between sex and length ($F_{1, 36} = 10.527$, $p = 0.003$) indicating that the relative gill weight behaves differently in males and females with respect to length of mussel. Indeed, while the relative gill dry mass in small mussels seem to be equal in males and females, there is a remarkable difference in favor of females in the bigger (older) individuals in Lake Koijärvi (Fig 13). In Lake Päijänne, the length*sex interaction term was not significant ($F_{1, 42} = 2.992$, $p = 0.092$) and was excluded from the ANCOVA model. Consequently, results of ANCOVA indicated a highly significant effect of sex ($F_{1, 42} = 97.585$, $p < 0.0001$) as well as length ($F_{1, 42} = 11.111$, $p = 0.002$). Thus, the relative gill dry mass increased with mussel length in a similar manner both in males and females, and female gills were heavier than those of males in Lake Päijänne (Fig. 14)

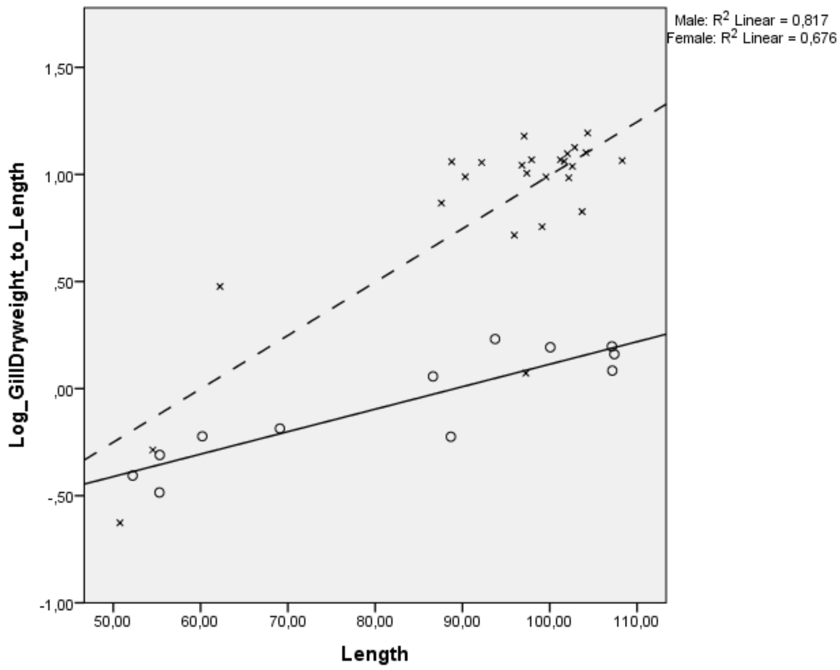


Figure 13. Relative dry mass of gills (gills-dry-mass-to-length ratio, y-axis) plotted against the length of mussels (x-axis, mm) in Lake Koijärvi population. Males are depicted with a circle and solid line. Females are depicted with a cross and dashed line.

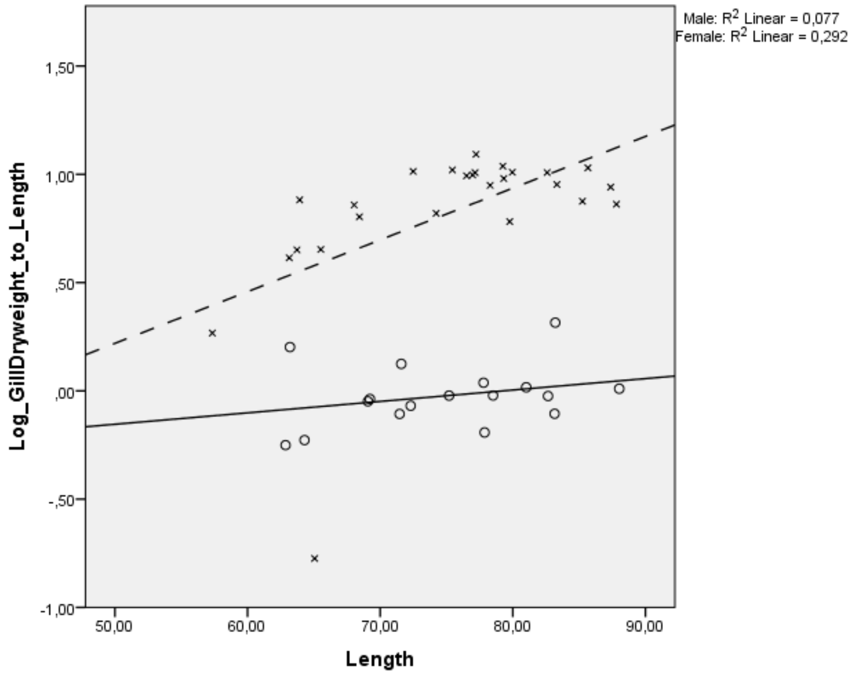


Figure 14. Relative dry mass of gills (gills-dry-mass-to-length ratio, y-axis) plotted against the length of mussels (x-axis, mm) in Lake Päijänne population. Males are depicted with a circle and solid line. Females are depicted with a cross and dashed line.

4.6 Relative mass without gills (body dry weight/length)

To study population specific differences in the relative body mass, i.e. dry mass of the soft parts without gills in relation to length of mussels, first only those size groups were selected, in which mussels were found both in Lake Koijärvi sample and Lake Päijänne sample. Therefore, uninfected individuals between 50 and 100 mm were selected for the analysis of covariance (ANCOVA) in which the Log_{10}-transformed soft-tissue-without-gills-dry-mass-to-length ratio was used as the response variable, sex as a fixed factor, population as a random factor and mussel length was used as a covariate. First, results of ANCOVA indicated that the interaction terms sex*length and populations*length were not statistically significant ($F_{1, 59} = 0.574$, $p = 0.452$ and $F_{1, 59} = 1.782$, $p = 0.187$, respectively), and were excluded from the ANCOVA model. After this, the ANCOVA results suggested that the difference between populations was not significant ($F_{1, 60} = 0.002$, $p = 0.973$). In addition, neither the effect of sex or sex*populations interaction was significant while the effect of covariate length was. Thus, Lake Koijärvi and Lake Päijänne populations did not differ from each other in the relative dry mass of body without gills.

The possible sexual dimorphism in the relative body mass, i.e. dry mass of the soft parts without gills in relation to length of mussels was further analyzed separately for Lake Koijärvi and Lake Päijänne by including uninfected mussels in those size groups in which individuals were found both in males and females. Thus, from Lake Koijärvi, mussel from 50 to 114 mm were chosen, and from Lake Päijänne mussels from 57 to 89 mm in length were included. In such an approach ANCOVA was used where the response variable was Log_{10}-transformed soft-tissue-without-gills-dry-mass-to-length ratio, while sex was a fixed factor and mussel length the covariate. Also the length*sex interaction term was included in the ANCOVA model. In Lake Koijärvi the interaction between sex and length was not significant ($F_{1, 35} = 2.621$, $p = 0.115$) and was thus excluded from the ANCOVA model. After this, ANCOVA results indicated the effect of length was highly significant but the effect of sex not ($F_{1, 42} = 2.992$, $p = 0.092$). Thus, in Lake Koijärvi, the body masses without gills did not differ between males and females. In lake Päijänne, similarly, the sex*length interactions was not significant, and was excluded from the model. After that the ANCOVA results indicated a highly significant effect of the factor sex and the covariate length ($F_{1, 41} = 18.935$, $p < 0.001$ and $F_{1, 4a} = 44.406$, $p < 0.0001$, respectively). So, in Lake Päijänne the body dry mass without gills was higher in males than in females and increased with mussel size (Fig. 15)

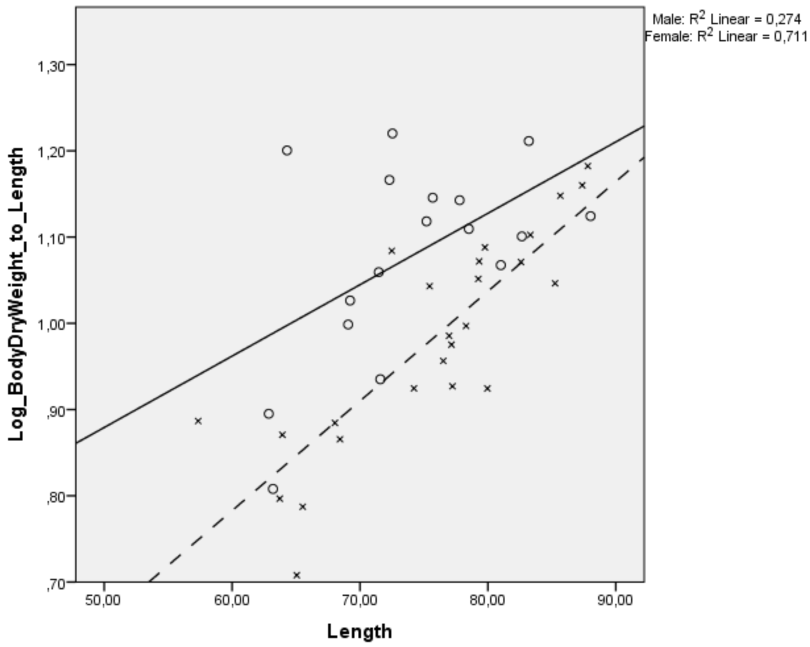


Figure 15. Relative dry mass of body without gills (dry-mass-of-soft-parts-without-gills-to-length ratio, y-axis) plotted against the length of mussels (x-axis, mm) in Lake Päijänne population. Males are depicted with a circle and solid line. Females are depicted with a cross and dashed line.

4.7 Relative shell volume (shell volume/length)

To examine the sexual dimorphism in the relative shell volume, i.e. volume of shell in relation to length of mussels was analyzed separately for Lake Koijärvi and Lake Päijänne by including uninfected mussels in those size groups in which individuals were found both in males and females. Thus, from Lake Koijärvi, mussel from 50 to 114 mm were chosen, and from Lake Päijänne mussels from 57 to 89 mm in length were included. In such an approach ANCOVA was used where the response variable shell volume-to-length ratio, while sex was a fixed factor and mussel length the covariate. Also the length*sex interaction term was included in the ANCOVA model. In Lake Koijärvi the interaction between sex and length was not significant ($F_{1, 47} = 0.040$, $p = 0.843$) and was thus excluded from the ANCOVA model. After this, ANCOVA results indicated both the effect of factor sex and covariate length were significant ($F_{1, 47} = 4.116$, $p = 0.048$ and $F_{1, 47} = 371.892$, $p < 0.0001$, respectively). Thus, in Lake Koijärvi, length-related shell volume was higher in females than in males, and increased with mussel size (Fig. 16). In Lake Päijänne, similarly, the sex*length interaction was insignificant ($F_{1, 43} = 0.450$, $p = 0.506$) and was therefore excluded from further analysis. After that, ANCOVA results suggested that the effect of length was highly significant ($F_{1, 43} = 30.336$, $p < 0.0001$) and the effect of sex marginally significant ($F_{1, 43} = 3.488$, $p = 0.048 = 0.069$). So, also in Lake Päijänne there was a trend for a higher relative shell volume in females than in males, and an increase with mussel size (Fig. 17)

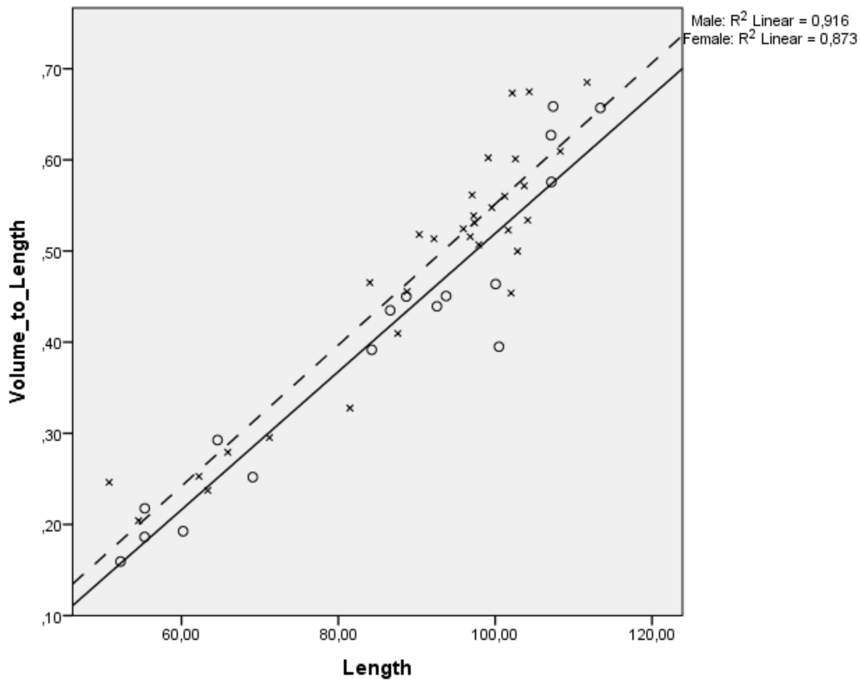


Figure 16. Relative shell volume (shell volume-to-length ratio, y-axis) plotted against the length of mussels (x-axis, mm) in Lake Koijärvi population. Males are depicted with a circle and solid line. Females are depicted with a cross and dashed line.

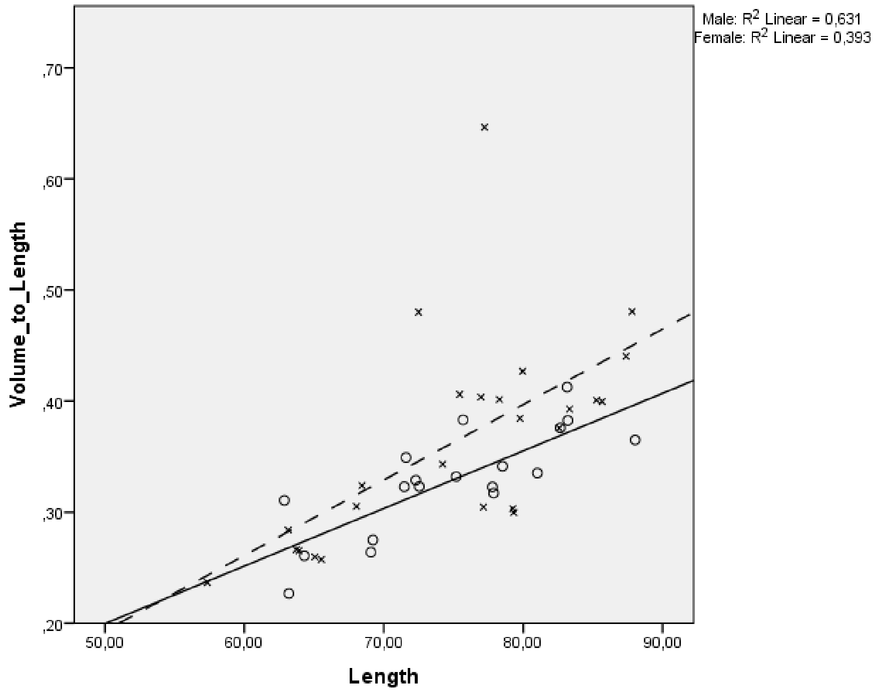


Figure 17. Relative shell volume (shell volume-to-length ratio, y-axis) plotted against the length of mussels (x-axis, mm) in Lake Päijänne population. Males are depicted with a circle and solid line. Females are depicted with a cross and dashed line.

4.8 Relative shell mass (shell weight/soft parts weight)

To study the sexual dimorphism in the relative shell weight, i.e. dry mass of the shell in relation to dry mass of the soft, Lake Koijärvi and Lake Päijänne materials were analysed separately. Uninfected mussels and those size groups in which individuals were found both in males and females were included. Thus, from Lake Koijärvi, mussel from 50 to 114 mm were chosen and from Lake Päijänne mussels from 57 to 89 mm in length were included. In such an approach ANCOVA was used where the response variable shell mass-to-length ratio, while sex was a fixed factor and mussel length the covariate. Also the length*sex interaction term was included in the ANCOVA model. In Lake Koijärvi neither the interaction between sex and length nor the effect of covariate length were significant ($F_{1, 31} = 2.329$, $p = 0.138$ and $F_{1, 31} = 1.437$, $p = 0.241$, respectively) and were thus excluded from the ANCOVA model. After this, ANCOVA results indicated that the effect of sex was not significant ($F_{1, 31} = 1.280$, $p = 0.267$). Thus, In Lake Koijärvi the relative weight of shell did not differ between males and females. In Lake Päijänne, on contrary, the sex*length interaction was significant ($F_{1, 38} = 6.268$, $p = 0.017$) although the covariate length as such was not ($F_{1, 38} = 0.262$, $p = 0.672$) In addition, the effect of factor sex was significant ($F_{1, 38} = 4.196$, $p = 0.048$). Therefore in Lake Päijänne there was a sex-difference so that among the bigger mussels the males had heavier shells related to the mass of soft parts than the females, but among small individuals such a difference did not exist (Fig. 18).

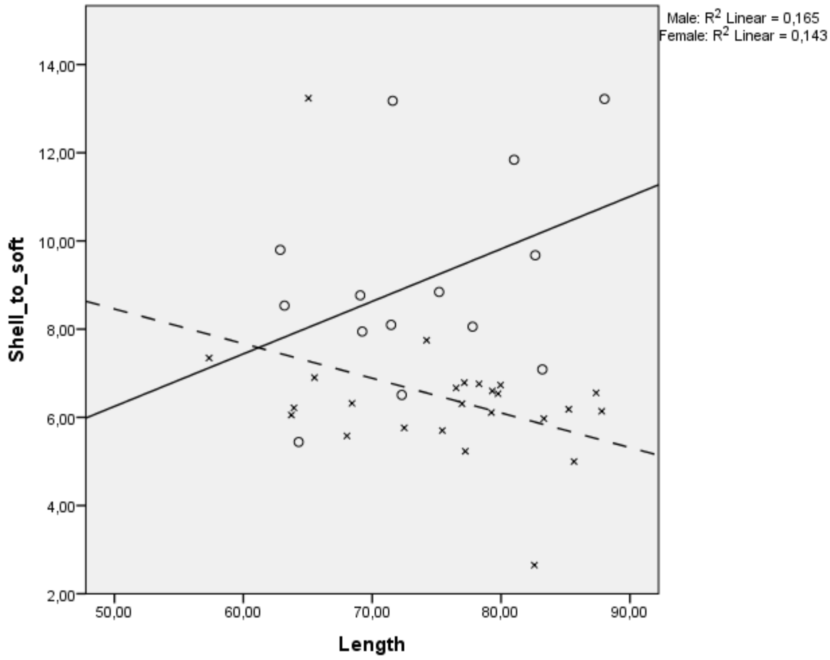


Figure 18. Relative shell weight (shell dry weight-to-soft parts dry weight, y-axis) plotted against the length of mussels (x-axis, mm) in Lake Päijänne population. Males are depicted with a circle and solid line. Females are depicted with a cross and dashed line.

DISCUSSION

Morphological variability was investigated and the measured variables were length, width, volume and dry mass of shell, dry mass of soft tissues and gill blades. Age, sex estimation and glochidium larvae produced were also investigated. Shells of big and female mussels were relative wider. Also, females had bigger shell volume than males. The volume wideness difference between males and females exhibited sexual dimorphism. Female's volume wideness is probably due to the space requirements for glochidium larvae. The total masses without gills were higher in males but the total dry mass of soft tissue with gills blades was higher in females. The clear dimorphisms were population dependent difference in morphology of *A. anatia*. In Koijärvi the mass of shell compared to soft tissue was higher in males than in Päijänne or females, this show the clear dimorphism.

Age specific lengths were noticed to have specific higher growth in Lake Koijärvi than Päijänne but was specific around 4-5 years old age. Faster growth in age specific were in females of Koijärvi but not Päijänne and such significant sex difference observed only in age 4 but not between the ages 2-6 years. Along in graph of length vs. age, it is clear to see at same age Koijärvi seem to have bigger mussel's growth than Päijänne also above age 4. The suitability of the lake Koijärvi exhibit clarity after production of glochidium meaning that females are in a more comfortable environment than male individuals. Until then the growth was better for both in Päijänne since the amount of food available is enough comfort young's. But when they grow the limitation exhibits. The numbers of female individuals considered were much higher in Koijärvi than Päijänne that might show clarity variations in growth inclination in Koijärvi, number of individuals in number might affect the overall look in Koijärvi to Päijänne study. But within Koijärvi, females were higher in length until age 4 where male's growth excide age 5 and 6. Thus shows that females start focusing in internal energy usage balance than shell growth after a certain age.

However the effect of length on relative shell height was different in Lake Koijärvi and Lake Päijänne. The different slopes of length to height ratio observed in Koijärvi mussels maybe be because they lose their higher shape less at older age than Päijänne mussels and also might be related to the continues available nutrient and food in lake Koijärvi. But the variation in small individuals implies that the growth requirement is much comfortable in Lake Päijänne that indicates the food matters much an interest point to focus onto considering growth variability in different lakes. The variation between small individuals may be investigated in further study. Not to forget the length in mm limitations related to age. Non-significant result was obtained in length to sex analysis and also height to length /length. In both populations, males were relative higher in shape showing a significant difference between sexes but were not significant in width of shell but the react of covariance length was significant meaning larger mussels were relative wider which is invisible in terms of sex vs. length indicating that the relative length on shell width was equal in males and females. The significant populations vs. length interaction suggest the effect of length in relative width was different in Lake Koijärvi and Lake Päijänne. In Lake Koijärvi mussels, the relative width increase with mussels length but in Lake Päijänne mussels, the relative width seems to be independent of mussel's variations in length in mussels between 50 and 100 mm. As noticed, there was no interaction in length vs. sex for both lakes. But width in length/length relationship did not differ between males and females; the interaction was excluded from the ANCOVA model. The significance of sex difference was observed in both lakes but the effect of covariance length was significant only in Lake Koijärvi, as expected females are relatively wider inflated, in shape in both lake populations.

The analysis of soft tissue dry mass to length ratio was conducted among those the interaction terms sex vs. length and population vs. length were not significant and were excluded form ANCOVA model. Afterward the ANCOVA result suggested that the difference between populations was not significant while the effect of covariate length was. The finding revealed that Lake Koijärvi and Lake Päijänne population are not different from each other in relative dry mass of mussels. The possible sexual dimorphism was used and found that it was not significant in Lake Koijärvi. There was a trend for a higher relative dry mass of females than in males in Koijärvi Lake. In a relative way in Lake Päijänne the effect of sex, length and sex vs. length interaction were significant. Suggesting the relative dry mass was higher in females than in males (especially among the bigger mussels) and increased by mussel site in Lake Päijänne. The transformations of gills dry mass to length ratio, while sex was a fixed factor and mussel length the covariate. In Lake Koijärvi the covariate in length there was the effect highly significant but the main factor sex as such was not. But very significant interaction between sex and length indicating that the relative gill weight behaves differently in males and females with respect to length of mussels. As noticed in this analysis females gills were heavier than those of males in Lake Päijänne but this might be due to the amount of glochidium present in gills of females. Further analysis required to compare male to female gills without glochidium content for both sex to show real variation in sex.

The selected 50 and 100 mm individuals of lakes transformation soft tissue without gills dry mass to length ratio was used as response variable and mussels length was used as a covariate. The ANCOVA suggests no significance between populations neither the effect of sex or sex population interactive were significant while the effect of covariate length was. Again both lakes were not differing from each other in dry mass of body without gills. From Lake Koijärvi for further analysis mussels from 50 to 114 mm were chosen and from lake Päijänne 57 to 89 mm in length included with infected mussels Log_{10} transformation soft tissue without gills dry mass to length ratio, while sex was a fixed factor and mussels length the covariate. No significant result was noticed in Lake Koijärvi in the interaction between sex and length. After this ANCOVAs result indicates the effect of length was highly significant but the effect of sex not. As noticed in Lake Päijänne the body dry mass without gills was higher in males than in females and increased with mussel size.

Shell volumes analysis included infected mussels. Thus from Koijärvi 50 to 114 mm were chosen and from Päijänne 57 to 89 mms in length were included. In Lake Koijärvi the interaction between the sex and length was not significant and was excluded from the ANCOVA model. As noticed, l in Lake Päijänne there was a trend for a higher relative shell volume in females than in males and this increased with mussel size. In Lake Koijärvi the relative weight of shell did not differ between males and females. In Lake Päijänne on contrary the sex length interaction was significant although the covariate length as sum was not. The effect of factor sex was significant therefore a significant variability was noticed amongst bigger female mussels but among small individuals such a difference did not exist. The real new investigation was to find out what nature hold as normal as noticed hidden variation. With additional investigations without glochidium the variability of sex can be investigated. The sex variation seems hidden but noticeable therefore additional subsequent study might be required.

The mussels size were noticed in different format but the inside shell volume must be noticed in different forms. The mussel variation in subsequent analysis will release more studies that expected to clarify the Finland lakes variations in morphology and sex variability in shell will be demonstrated along. Shell size might vary in size but the mussel in them similar in variability but the notice also shows their variation in one look. The mussels must be investigated for further understanding of well study to be overcome. Usually it is imposible to differentiate male and female *A. anatina* without seeing the occurrence of glochidia in marsupial gills of female mussels, or by dissecting the gonads of mussels. In the future, the present results might be utilized, for example, in separation of sexes of mussels by morphological measures, without need to scarify the mussel individual for microscopic gonad examination.

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REFERENCES

Arja, P., and Juhani H., 2004. Lake päijänne: mid congress excursion E5. Ebookbrowse.com/paijanne-pdf-d330188657.

Bauer, G.,& K.Wächtler (Eds.), 2000. Ecology and Evolution of the fresh water mussels Unionoida, Springer-Verlag Berlin Heidelberg, New York. ISBN 3-540-67268-0

Bernard, F. and T. K. Graczyk, 1997. Trematho biology; advanced in. CRC Press LLC. ISBN 0-8493-2645-1

Dytham, C., 2007. Choosing and Using Statistics, A Biologist Guide 2nd ed., Black Well Publisher, in USA. ISBN 978-1-4051-0243-8

Esch, G., A., Bush and J. Aho, 1990. Parasite communities: Patterns and Process. Chapman and Hall, 29 West 35th street, NY 10001. ISBN 0-412-33540-9

Frederic A. S., 12.8.2010. Relationship Between Water Qualtiy Parameters and Growth of The Freshwater Mussel Anodonta Anatina L. Master thesis published at Jyväskylä University.

Graf, DL., Cummings KS. 2006. Palaeoheterodont diversity (Mollusca: Trigonioida + Unionoida): what we know and what we wish we knew about freshwater mussel evolution. *Zool J Linn Soc* 148:343-394.

Haukioja, E., & Hakala T. 1978. Life-history evolution in *Anodonta piscinalis* (Mollusca, Pelecypoda) - correlation of parameters. Oecologia 35:253-266.

Heard, WH., 1975. Sexuality and other aspects of reproduction in *Anodonta* (Pelecypoda:Unionidae). Malacologia 15:81-103.

Jerrin, S., Nicolas et al., 2005. Form great lakes book (Marlene E. et. al.) Pathways of Food Uptake in Native (Unionidae) and introduction (Corbiculidae and Dreissenidae) Freshwater Bivalves. Great lakes science center 1451 green Rd. Ann Arvor, Michigan 48105.

John, M. G., & Cindy, J. Brunner, 2009. ‘Infection Diseases of Freshwater Mussels and other Freshwater Bivalve Mollusks’, published by Taylor and Francls group, LLC Auburn University, Auburn, Alabama, USA. ISSN: 1064-1262

Kalff, J., 2002. Book called-‘Limnology’, published by prentic-Hall, Inc. Upper Saddle River, NJ 07458. ISBN 0-13-033775-7

Kerbs, C. J., 1989. Title called Ecological Methodology, published by Harper and Row, New York. 654s.

Lewis, E. E., Campbell, J. F., Sukhdeo, M. V. K. (Editors), 2002. Behavioral Ecology of Parasites published by Cambridge, MA, USA: CABI Publishing, 2002. P. 1-12. http://site.ebrary.com/lib/jyvaskyla/Doc?id=10060562&ppg=21 Copyright© 2002

Marlene, E., C. Fabbro, R. S. Schneider and W. Foster, 2005. Journal of great lakes research: devoted to research on large lakes of the world and their watersheds. Volum 31, number 1. ISSN 0380-1330

Mäkelä, M., L. Soininen, S. Tumola & J. Öistämö, 2002. Technical formulas, published at Tammertkniikka, Hippoksenkatu 21, 33530 Tampere, Finland. ISBN: 951-9004-85-8

Økland, J., 1963. Notes on population density, age distribution, growth and habitat of *Anodonta piscinalis* Nilss. (Moll., Lamellibr.) in a eutrophic Norwegian lake. Nytt Magasin for Zoologi 11:19-43.

Stayer, D.L. and D. R. Smith, print in 2003. 'A guide to sampling freshwater mussel populations' published by American fisheries Society, Monograph 8, Bethesda, Maryland. ISBN 1-888569-50-6

Taskinen, J. received 13 August 1997; received in revised form 8 December 1997; accepted 19 December 1997. Influence of trematode parasite on the growth of a bivalve host in the field, published at Department of Biological and Environmental Sciences, University of Jyväskylä, P.O.Box 35, FIN-40351, Jyväskylä, Finland. ETH-Zürich, Experimental Ecology, ETH-Zentrum NW, CH-8092 Zürich, Switzerland. International Journal of Parasitology 28(1998) 599-602.

Taskinen, J., J. Jokela, P.Mutikainen and K. Kopp, 2005. Virulence of parasites in hosts under environmental stress: experiments with anoxia and starvation, published at Department of Biology, University of Oulu, P.O.Box 3000, FIN-90014 University of Oulu, Finland (jukka.jokela@oulu.fi) – J.Taskinen, Department of Biological and Environmental Sciences, University of Jyväskylä, P.O.Box 35, FIN-40351, Jyväskylä, Finland. –Oikos 108:156-164.

Taskinen, J., and E. Tellervo Valtonen, received March 15, 1994 and accepted January 30, 1995. Age-, size- and sex-specific infection *Anodonta Piscinails* (Bivalvia: Unionidae) with *Rhipidocotyle fennica* (Digenea: Bucephalidae) and its influence on host reproduction, University of Jyväskylä, Department of Biology, Yliopistonkatu 9, 40100, Jyväskylä, Finland.

Taskinen, J., T. Mäkelä & E. Tellervo Valtonen, received 15 February 1996, accepted 18 March 1996. Exploitation of *Anodonta Piscinails* (Bivalvia) by trematodes; parasite tactics and host longevity, published at Department of Biological and Environmental Sciences, University of Jyväskylä, P.O.Box 35, FIN-40531 Jyväskylä, Finland and Oulanka Biological Research Station, University of Oulu, 25 Kiutaköngäs, FIN-93999, Kuusamo, Finland. © Finnish Zoological and Botanical Publishing Board 1997. ISSN: 0003-455X.

Taskinen, J., E.T. Valtonen and T. Mäkelä, 1994. Quantity of sporocysts and seasonality of two *Rhipidocotyle Sp.* (Digenea: Bucephalidae) in *Anodonta Piscinalis* (Moullusca: Bivalvia), published at International Journal of Parasitology, vol. 24, no. 6, pp.877-886, 1994. Copyright © 1994 Australian Society for Parasitology, Elsevier Science Ltd. Printed in Great Britain. 0020-7519(94) E0032-1

Taskinen, J. E. Tellervo Valtonen and David I. Gibson, accepted 30 August, 1990. Studies on Bucephalid Digeneans parasitizing mollusks and fishes in Finland -I. Ecological data and experimental studies, published at Department of Biology, University of Jyväskylä, SF-40100, Finland, Department of Zoology, The Natural History Museum, London SW 7 5BD, UK. Systematic Parasitology 19:81-94, 1991. © 1991 Kluwer Academic publishers, printed in Netherlands.

Throp, J.H and Covich, A.P, 1991. 'Ecology and classification of Northern American Freshwater invertebrates', published by Acadamic Press, Inc., London, UK. ISBN 0-12-690645-9

Villi, E. & M. Heino, recieved 15 June 1994, accepted 16 September 1994. Valve movements of freshwater mussel *Anodonta anatina*: a reciprocal transplant experiment between two lakes, published at Department of Zoology, Division of Physiology, and Davison of Ecology P.O.Box 17, FIN-0014 University of Helsinki, Finland. © 1994 Finnish Zoological and Botanical Publishing Board, ISSN: 0003-455X.

Zieritz, A. David C. Aldridge, 22-Feb-2011. Sexual, habitat-constrained and parasite –induced dimorphism in the shell of a freshwater mussel (*Anodonta Anatina, Unionidae*), published at University of Cambridge, Department of Zoology, CB2 3EJ Cambridge, UK. Also found under Journal of Morphology, at John Wiley and sons publisher.

APPENDIX

<u>Data recording table</u>: the possible data's recorded are listed on the coming table-3 to easily identify the required parameter in laboratory work.

<u>Table 5:</u> the real data table used, table recording for laboratory results per two weeks interval at lab for *Anodonta anatia* and *Unio timidus* observations.

Mark number and date		Observation			Size and growth [mm]		Shape (elongation and inflation)					Density		Age [yr]	Sex ratio, one result per river	Cercariae count [Cn/l/day]		Water flow rate, temp & pH	
Mark	Date	Sex [m/f/h]	Colour	Infection [i/ui]	L_3	L_{max}	Width [mm]	Height [mm]	Length [mm]	SaA [mm^2]	SuA [mm^2]	Dry weight (DW) [g]	Volume [ml]	Winter curve count	[m/f]	Cn / 2L/d		RPM	T
																RC	RF		
K1																			
K2																			
K3																			
K4-K287																			
….																			
H1																			
H2																			
H3-H299																			
L1 … etc																			
Average																			

a) **<u>pictures taken at sampling and 24 hr interval counting</u>**

Figure 19: sampling area, sampling, 20 mussels tank control, 0.5 litter control and cercariae counting

b) **Picture taken during microbial counting and dissection**

Figure 20: 100 µm filtered water, microscope counting and dissection gills and glocidium observation