

**Master's thesis**

**Interannual variation in littoral benthic  
macroinvertebrate communities under contrasting  
crayfish predation in a large boreal lake**

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Keywords: Alien species, freshwater predation, long term studies, *Pacifastacus leniusculus*

### **ABSTRACT**

Long term studies are widely used nowadays in many fields of ecology. For instance, records over years represent a valuable tool to monitor and comprehend environmental changes. In freshwater ecology, long term studies are often applied in restoration effect assessments using a before-after-control-impact design. Little is known about the long term effects of freshwater predators on their prey, for example of how crayfish as omnivorous predators affect macroinvertebrate prey communities. Bottom substrate of stony littoral shores in three sites of Lake Päijänne, in Central Finland, were sampled for macroinvertebrates (MI) during five consecutive years and identified mainly to species. Of the three sites one was without crayfish, a second with a newly-introduced signal crayfish population, and a third one with a well-established signal crayfish population. Annual crayfish catching was also performed at the sites. Crayfish establishment was not yet successful as there was no population development after the introduction. Therefore, MI density and taxa richness showed no clear trend, though there were differences among years after the introduction of the predator. Snail densities and species richness increased one year after the introduction and later decreased significantly. No change was observed at the control site in density and taxa richness of MI and snails over years whereas at the crayfish site, there was a significant increase in total density and taxa richness in the last year. This might result from decreased crayfish abundance in the last two study years. Ordination analysis showed no clear interannual directional change in community composition, but the sites were clearly differentiated. The study showed that the effects of established signal crayfish populations on MI communities are fairly consistent in time, but that crayfish establishment and manifestation of effects on MI may require considerable time in large boreal lake littorals.

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**TIIVISTELMÄ**

Pitkiä aikasarjoja hyödynnetään nykyisin laajalti ekologissa tutkimuksissa. Monien vuosien ajan kerätyt havainnot tarjoavat arvokkaan keinon esimerkiksi seurata ja ymmärtää ympäristömuutoksia. Sisävesiekologiassa pitkäaikaistutkimuksia sovelletaan usein hoitotoimien vaikutusten arvioinnissa käyttäen ennen–jälkeen–kontrolli–käsittely -asetelmaa. Melko vähän tiedetään petojen pitkäaikaisvaikutuksista saalispopulaatioihin makeissa vesissä, esimerkiksi siitä miten ravut kaikkiruokaisina petoina vaikuttavat saaliina olevien makroskooppisten vesiselkärangattomien populaatioihin. Tässä tutkimuksessa kerättiin viitenä peräkkäisenä vuotena kivikkorantojen pohjaeläinyhteisönäytteet kolmesta eri paikasta Päijänteeltä. Yhdessä paikoista ei ollut rapuja lainkaan, toiseen paikkaan istutettiin täplärapuja tutkimusjakson alussa ja kolmannessa paikassa oli jo hyvin asettunut täplärapukanta. Myös koeravustukset tehtiin kullakin paikalla vuosittain. Istutettu rapukanta ei vielä asettunut tutkimusjaksolla. Tämän vuoksi pohjaeläinten runsaudessa ja lajimäärässäkään ei tapahtunut suuntautunutta muutosta, vaikkakin vuosien välinen vaihtelu saalistajan istutuksen jälkeen oli merkittävää. Kontrollipaikan pohjaeläintiheydessä ja lajimäärässä ei ollut vuosien välisiä eroja, mutta rapupaikalla tiheys ja lajimäärä olivat merkittävästi aiempaa suurempia viimeisenä tutkimusvuotena. Tämä saattoi johtua rapujen vähenemisestä kahtena viimeisenä vuonna. Ordinaatioanalyysi ei osoittanut johdonmukaisia ajallisia muutoksia pohjaeläinyhteisöjen lajikoostumuksessa, mutta selkeät erot paikkojen välillä. Tutkimus osoitti että vakiintuneen täplärapukannan vaikutus kivikkorannan pohjaeläinyhteisöön on ajallisesti melko vakaa, mutta että rapukannan asettuminen ja pohjaeläimistövaikutusten ilmeneminen istutuksen jälkeen vaativat huomattavan ajan suurissa boreaalisisä järvissä.

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## 1. INTRODUCTION

Long-term and interannual studies are an important way of investigating and understanding environmental phenomena that take place along longer time periods and hence cannot be observed right away. In addition, there is the importance of constant contemporary monitoring, collection and recording of present data that will help recognizing ongoing trends and serve as background for further research in years to come. Understanding global phenomena, such as climate change and animal migration, heavily relies on regular collection and recording of precious data stored for years and sometimes centuries. In freshwater ecosystems, many rivers and lakes have been widely studied and monitored on interannual basis. For instance, Louhi *et al.* (2011) studied how successful have restoration programs been in Finnish streams, by evaluating the effectiveness of restoration in improving breeding of brown trout, using both a before-after-control-impact method and a space-time substitution design in almost 20 years observation period. In United States of America, Bêche *et al.* (2006) investigated how seasonal variations influence species composition and community structure of macroinvertebrates in a river within a period of 6-19 years. The aim was to find out whether the annual environmental variation affects macroinvertebrate (MI) community traits over years and how. In a shorter period, Suurkuukka *et al.* (2012) studied the changes of lake littoral MI communities among three years. They combined space and time parameters with data from three lakes in Southern Finland concluding that predicting patterns of MI abundance and species richness over time and space for dominant species are actually predictable. In running waters, infrastructures such as dams have been controversial over time and study of their environmental impact has been of great importance in the long term to understand for example what happens to MI communities downstream of a constructed dam (Vinson 2001, Stanley & Luebke 2002) or how MI communities change after dams have been destroyed (Renöfält *et al.* 2013). Long term studies in aquatic environment have also been conducted by palaeolimnological techniques, to trace back past temperatures and other environmental conditions that prevailed at a time when *in situ* observation could not be possible (e.g. Korhola *et al.* 2002, Verbruggen *et al.* 2011). In all the above cases, a common driver to carry research emerges, which is the need to better understand environmental response, often plant and animal community change in relation to a given factor or a combination of factors. Accidental or deliberate introduction of nonindigenous species to new ecosystems is one of such factors of interest that also have important effects on plant and animal communities.

Originating from North America, the signal crayfish *Pacifastacus leniusculus* Dana is nowadays a well-established invasive species in freshwater ecosystems throughout Europe. The species was introduced in late 1900s in countries such as Finland and Sweden to compensate for the population collapse of native species, the noble crayfish *Astacus astacus* (Lowery & Holdich 1988, Westman 1995). This invasive species has often found favorable conditions in its new habitats and competes with many native species (Westman & Savolainen 2001, Griffiths *et al.* 2004). Noble crayfish population in Finland has dramatically dropped for the last decades and been progressively replaced by signal crayfish. The reason for this is linked with the competitive advantages the invasive species has over the native one owing to its faster growth rate, earlier female sexual maturity (Abrahamsson 1971, Savolainen *et al.* 1997), and in particular, better resistance to the crayfish plague *Aphanomyces astaci* (Edgerton *et al.* 2004, Bohman *et al.* 2006). Noble crayfish is not the only species suffering from introduction of signal crayfish to new habitats. Signal crayfish also potentially has a negative impact on fish by preying on

young individuals and eggs, thereby threatening fish populations and related fisheries and recreational activities often associated to fishes such as trout and salmon (Griffiths *et al.* 2004, Peay *et al.* 2009). Signal crayfish can also affect benthic fish community composition and density by outcompeting and feeding on them (Guan & Wiles 1997) even though there are also contrasting results from large boreal lakes (Ruokonen *et al.* 2012). It is known that signal crayfish affects MI communities directly and indirectly with typically greatest effects on snails (Nyström *et al.* 1999, 2001; Nyström & Pérez 1998, Nyström *et al.* 1999). Few field studies have been conducted to document the development of effect of signal crayfish predation on littoral MI communities in time, and on the other hand to investigate if interannual MI community variability is affected by crayfish.

The second-largest lake of Finland, Lake Päijänne, was selected to study how MI communities of stony littorals vary inter-annually across sites with a contrasting crayfish status and with an emphasis on density and species richness of MI and of snails in particular. More specifically I evaluated if the MI community differed between a site with and another without crayfish and if this difference was consistent in time. Moreover, I monitored a site with a newly introduced crayfish population, with the expectation that total MI and snail density and taxa richness will decrease over the years as a consequence of crayfish predation.

## 2. MATERIAL AND METHODS

### 2.1. Study sites and sampling

The study was carried out in Lake Päijänne, which is situated in Central Finland and is the second largest lake of the country. MI samples were collected every year from 2007 to 2011 during the first half of August, at three sites from north to south: i) Saalahti, (wherein a crayfish population was introduced in 2007), ii) Kuhmoinen (non-crayfish site) and iii) Nyystölä, having a well-established crayfish population since the early 1990s (Figure 1). All the sites were selected to be sufficiently similar (stony littoral zones) to each other in the major environmental conditions (Bjurström *et al.* 2009). Three replicates of stony bottom substrates of littoral shores were sampled for MI at one meter depth using a 0.5 m x 0.5 m frame and a centrifugal pump on a boat (Figure 2). Animals retained on a 500 µm sieve were immediately transferred into labelled boxes containing 70 % ethanol for later laboratory sorting and identification. Sorting was performed using sharp tweezers and a laboratory lamp Schott KL1500 with adjustable light intensity. Animals were sorted on a white-background plastic plate about 50 cm x 30 cm in dimensions. Sample aliquots were taken using a tablespoon and washed under gentle tap water flow on a 50 µm mesh size strainer to eliminate ethanol before spreading on the plate. Identification was carried out under a dissecting microscope with identification keys as reference, mostly to species level for taxa such as Hirudinea (Figure 3B), Tricoptera (Figure 3A, C, F), Coleoptera (Figure 3D), Plecoptera (Figure 3H) and Ephemeroptera (Figure 3G) whereas a few taxa were identified to higher taxonomic units, such as Acarina (Figure 3E). I processed the samples from years 2009, 2010 and 2011 whereas data of 2007 and 2008 were obtained from previous studies in the same project. Crayfish catching was carried out every year end of July along the shore, at ~2 m depth using roach (*Rutilus rutilus*) as bait in 25 cylindrical crayfish traps distanced by 5 m from each other (Ruokonen *et al.* 2012). The traps were placed in the evening, collected the next morning and crayfish counted. Scuba diving also permitted to visually confirm the absence of crayfish in the control site. Catch Per Unit of Effort (CPUE) is widely used in fisheries to estimate harvest and it was also used in this

study (number of crayfish individuals trap<sup>-1</sup> night<sup>-1</sup>) since it represents a good appreciation of crayfish abundance (Skurdal *et al.* 1993, 1995).

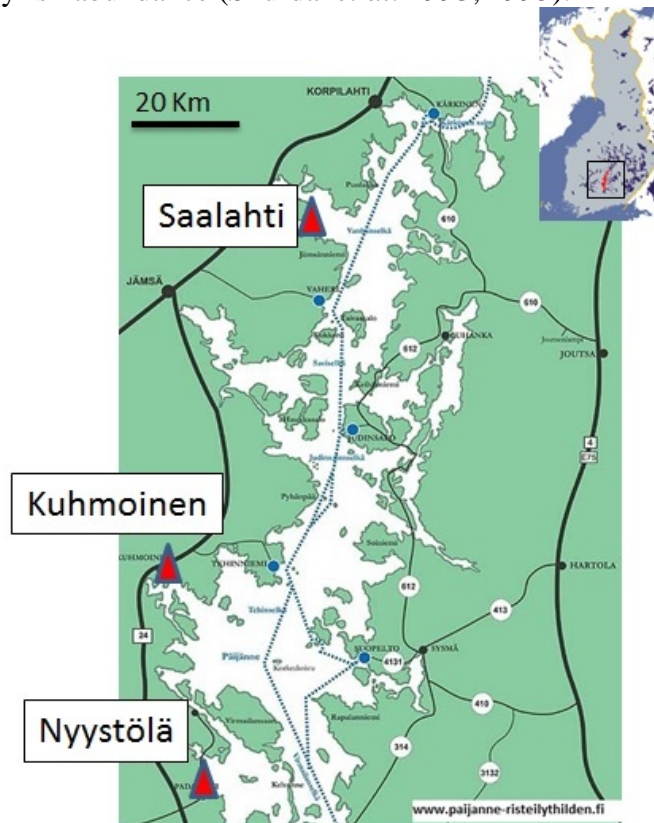


Figure 1. Map of the study sites. Saalahti (newly-introduced crayfish population), Kuhmoinen (control site with no crayfish) and Nyystölä (crayfish site).



Figure 2. Sampling equipment and machinery: Centrifugal pump (A), the boat (B), snorkeling diver with 0.5 m x 0.5 m frame and pumping up all particles and animals in that surface area (C) and collection bucket equipped with 500 µm sieve (D). Photographs from Kimmo Sivonen.



## 2.2. Data analysis

Total macroinvertebrate and snail densities and species richness were calculated for each site and year. IBM SPSS Statistics 22 was used to analyze year and site effect on MI density and taxon richness (number of taxa per replicate) both as separate fixed factors and in combination (Two-way ANOVA) using the log-transformed data from three replicates of each year. In addition, ordination analysis was carried out by Non Metric Multidimensional Scaling (NMS) using PC-ORD Version 4.0 (McCune & Mefford 1999), to illustrate variation in MI community composition among sites and years.

MRBP (Blocked Multi-Response Permutation Procedures), year as a blocking factor, was also used to test if the MI assemblages differed between sites. Multi response permutation procedures (MRPP) and multi response blocked procedures (MRBP) are data dependent distribution-free statistical techniques that utilize Euclidian distance to compare a priori groups using multivariate data (here species abundances) which may not be normally distributed and therefore may not be analyzed using conventional multivariate analysis of variance (Biondini *et al.* 1998, Cai 2006).

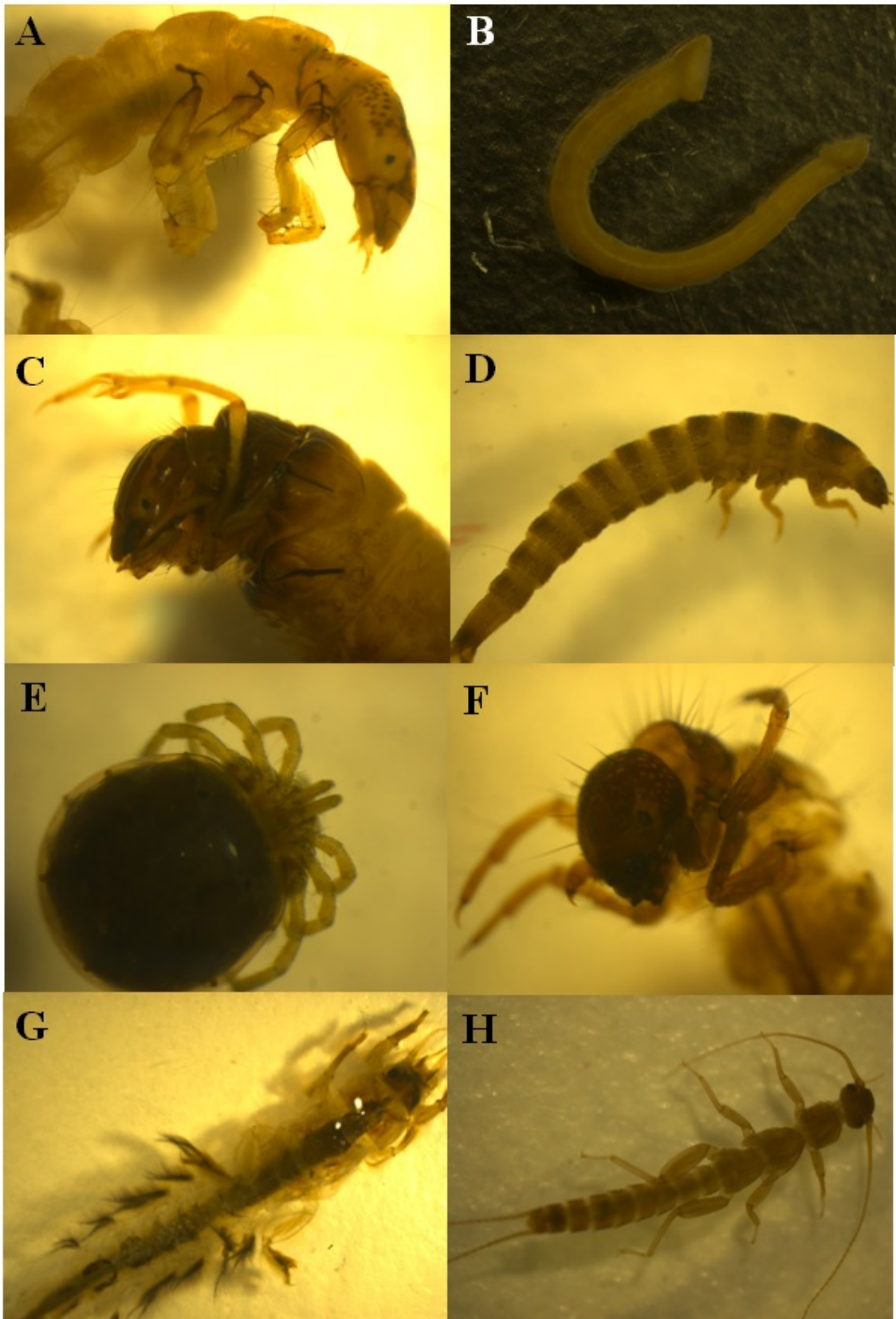


Figure 3. Some common macroinvertebrate taxa identified in the study: *Polycentropus flavomaculatus*, Tricoptera (A); *Piscicola geometra*, Hirudinea (B); *Ceraclea sp.*, Tricoptera (C); *Oulimnius tuberculatus*, Coleoptera (D); Acarina (E); *Lepidostoma hirtum*, Tricoptera (F); *Ephemerella vulgata*, Ephemeroptera (G); *Leuctra fusca*, Plecoptera (H).

### 3. RESULTS

#### 3.1. Crayfish catches

The highest crayfish catches were at Nyystölä (CPUE = 9 individuals trap<sup>-1</sup> night<sup>-1</sup> in 2009) followed by Saalahti (maximum CPUE = 0.53 in 2008) and Kuhmoinen, where the only crayfish recorded was in 2011, CPUE = 0.04 (Figure 4). At Nyystölä, the catch increased from 2007 to 2009 (CPUE 4, 8 and 9 respectively), followed by a sharp drop in 2010 (2.40) to remain almost similar in 2011 (Figure 4).

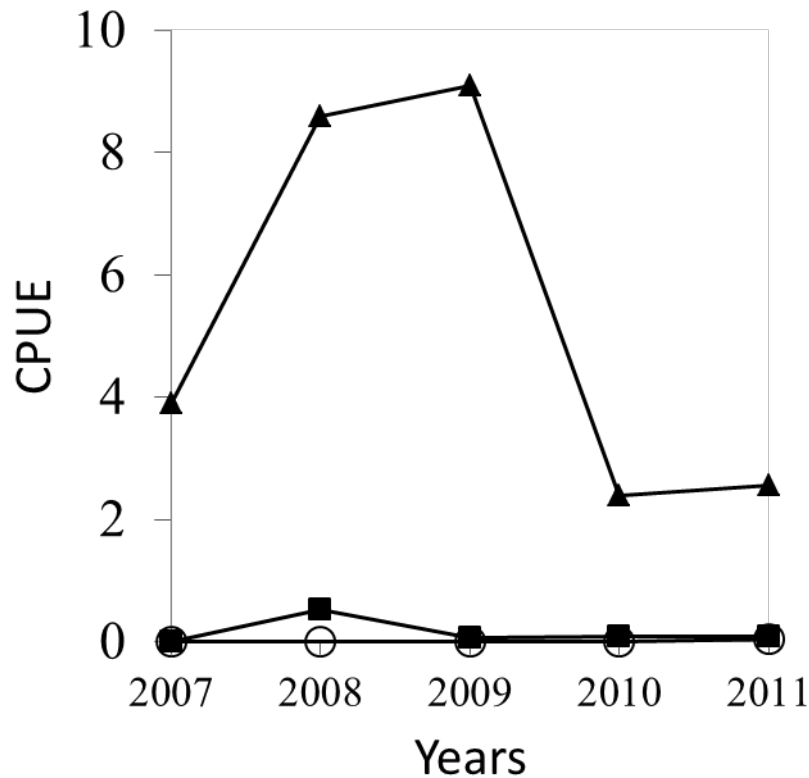


Figure 4. Crayfish catch per unit of effort (CPUE, individuals trap<sup>-1</sup> night<sup>-1</sup>) at Saalahti (squares), Kuhmoinen (dots) and Nyystölä (triangles) in years 2007-2011.

#### 3.2. Macroinvertebrate densities

Two-way ANOVA revealed a significant total macroinvertebrate (MI) density difference among years ( $F = 8.48$ ,  $p < 0.001$ ) and sites ( $F = 14.95$ ,  $p < 0.001$ ), and the interaction too ( $F = 3.79$ ,  $p = 0.004$ ). More precisely, at Saalahti (Figure 5A), there were differences in densities among years ( $F = 5.20$ ,  $p = 0.016$ ), as 2010 had significantly lower densities than 2009 and 2008 ( $p = 0.035$  and  $p = 0.041$  respectively). At Kuhmoinen, no difference was found ( $F = 2.49$ ;  $p = 0.110$ ) (Figure 5B), whereas there was a significant difference among years in MI densities at Nyystölä, crayfish site ( $F = 5.85$  and  $p = 0.011$ ) (Figure 5C), where however, only 2011 had nearly significantly higher densities than 2007 ( $p = 0.06$ ), based on Tukey HSD test.

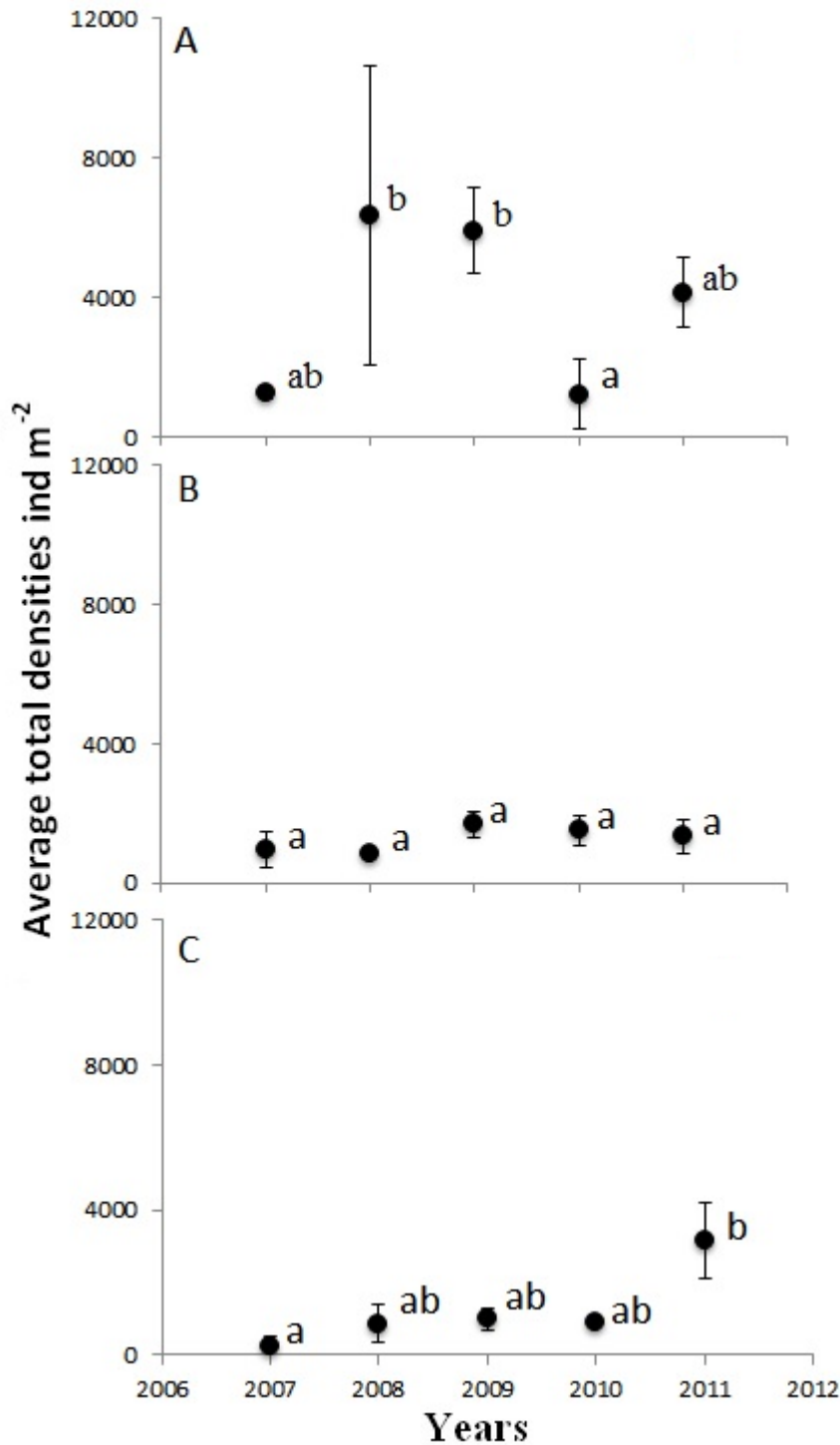


Figure 5. Interannual variation in mean macroinvertebrate densities at Saalahti (A), Kuhmoinen (B) and Nyystölä (C). Error bars represent standard deviation and years with similar letters were not significantly different in MI density from each other, based on oneway Anova coupled with Tukey HSD post hoc test.

### 3.3. Macroinvertebrate taxon richness

Taxon richness (number of taxa per replicate) was significantly affected by time ( $F = 4.88$ ,  $p = 0.004$ ) and site ( $F = 12.09$ ,  $p < 0.001$ ), but with no significant interaction ( $F = 1.85$ ,  $p = 0.107$ ). At the introduction and control sites, there was no significant difference in taxon richness among years ( $F = 1.27$ ,  $p = 0.343$  and  $F = 3.23$ ,  $p = 0.06$  respectively),

but there was a difference at the crayfish site ( $F = 3.90$ ,  $p = 0.01$ ), (Figures 6: A, B, and C respectively). At the latter site, the difference was between 2007 and 2011 which had a significantly higher taxa richness ( $p = 0.004$ ). There was a general increase in taxa richness in all the sites in the course of the study period, even not statistically significant.

### **3.4. Abundance, species richness and composition of snail assemblages**

Time ( $F = 8.95$ ,  $p < 0.001$ ) and site ( $F = 7162.77$ ,  $p < 0.001$ ) and the combination of years and site had a significant effect on snail densities ( $F = 11.78$ ,  $p < 0.001$ ) based on Two-way ANOVA. The differences at the introduction site ( $F = 7.38$ ,  $p = 0.005$ ) was 2010 having significantly lower snail density than 2008 ( $p = 0.013$ ) (Figure 7A). No snail density change was observed at the control site ( $F = 2.76$ ,  $p = 0.087$ ) (Figure 7B) and there was a change at the crayfish site ( $F = 23.12$ ,  $p < 0.001$ ), with 2011 only having significantly higher densities than all other years, namely 2007 ( $p < 0.001$ ), 2008 ( $p = 0.002$ ), 2009 ( $p < 0.001$ ) and 2010 ( $p < 0.001$ ), (Figure 7C).

The same test applied to snail species richness also showed that there was a significant time ( $F = 6.24$ ,  $p = 0.001$ ) and site effect ( $F = 142.13$ ,  $p < 0.001$ ) on snails species richness, with significant interaction ( $F = 7.93$ ,  $p < 0.001$ ). In details, snail species richness was different among years at the introduction site ( $F = 4.81$ ,  $p = 0.020$ ). Year 2008 had higher species richness than 2007 and 2011 ( $p = 0.048$  and  $p = 0.041$  respectively), (Figure 7A). No difference was significant at the control site ( $F = 2.04$ ,  $p = 0.164$ ) (Figure 7B), and there was a significant difference at the crayfish site ( $F = 17.77$ ,  $p < 0.001$ ), where only year 2011 had a significantly higher snail density than all the other sites, namely 2007 ( $p < 0.001$ ), 2008 ( $p = 0.007$ ), 2009 ( $p < 0.001$ ) and 2010 ( $p = 0.001$ ), (Figure 7C).

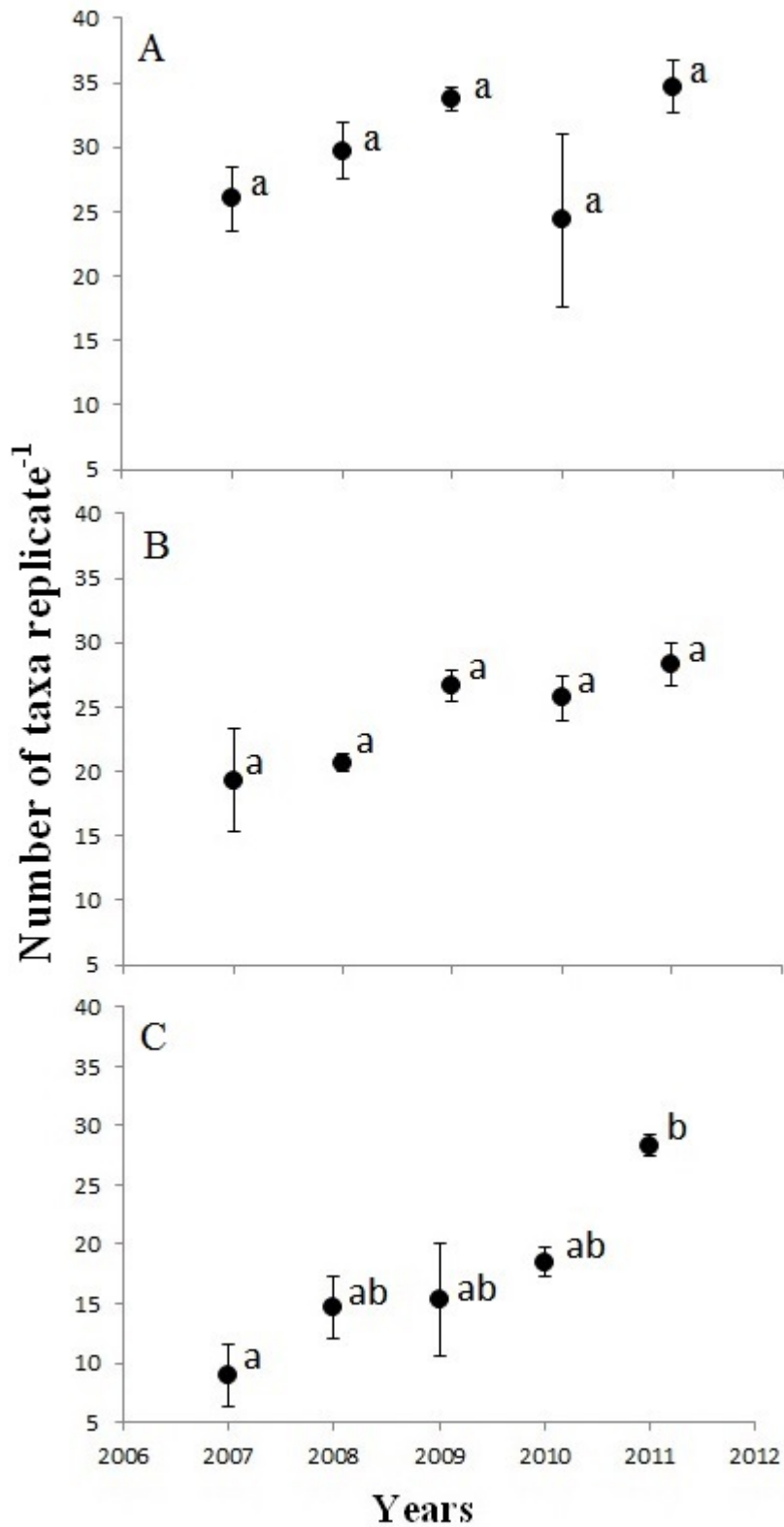


Figure 6. Interannual variation in macroinvertebrate taxon richness (number of taxa replicate<sup>-1</sup>) at Saalahti (A), Kuhmoinen (B) and Nyystölä (C) in years 2007-2011. Error bars represent standard error and years with similar letters were not different.

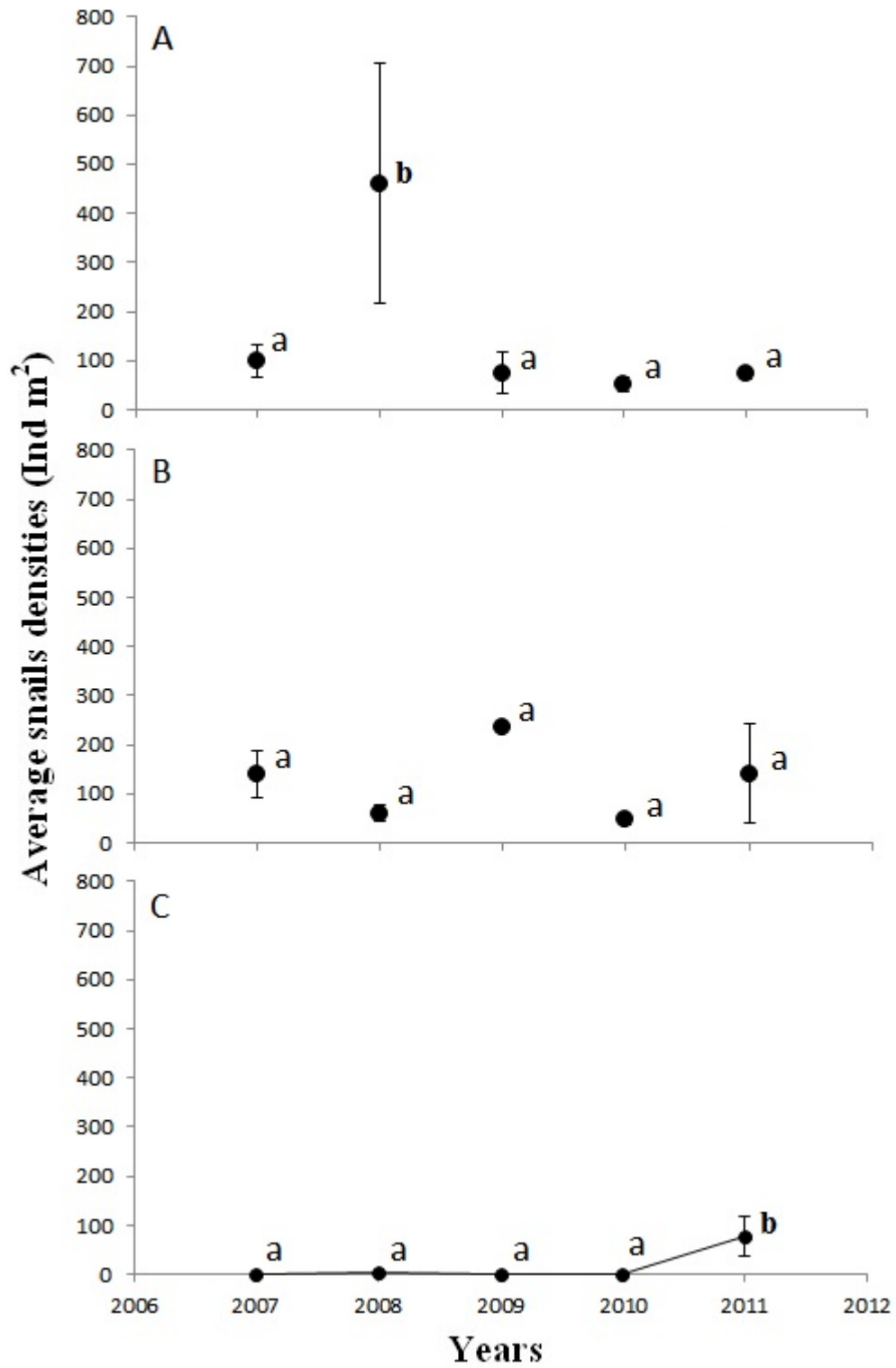


Figure 7. Interannual variation in snail densities at Saalahti (A), Kuhmoinen (B) and Nyystölä (C) in years 2007-2011, error bars represent standard error, years with similar letter were not different based on Oneway Anova coupled with Tukey HSD post hoc test.

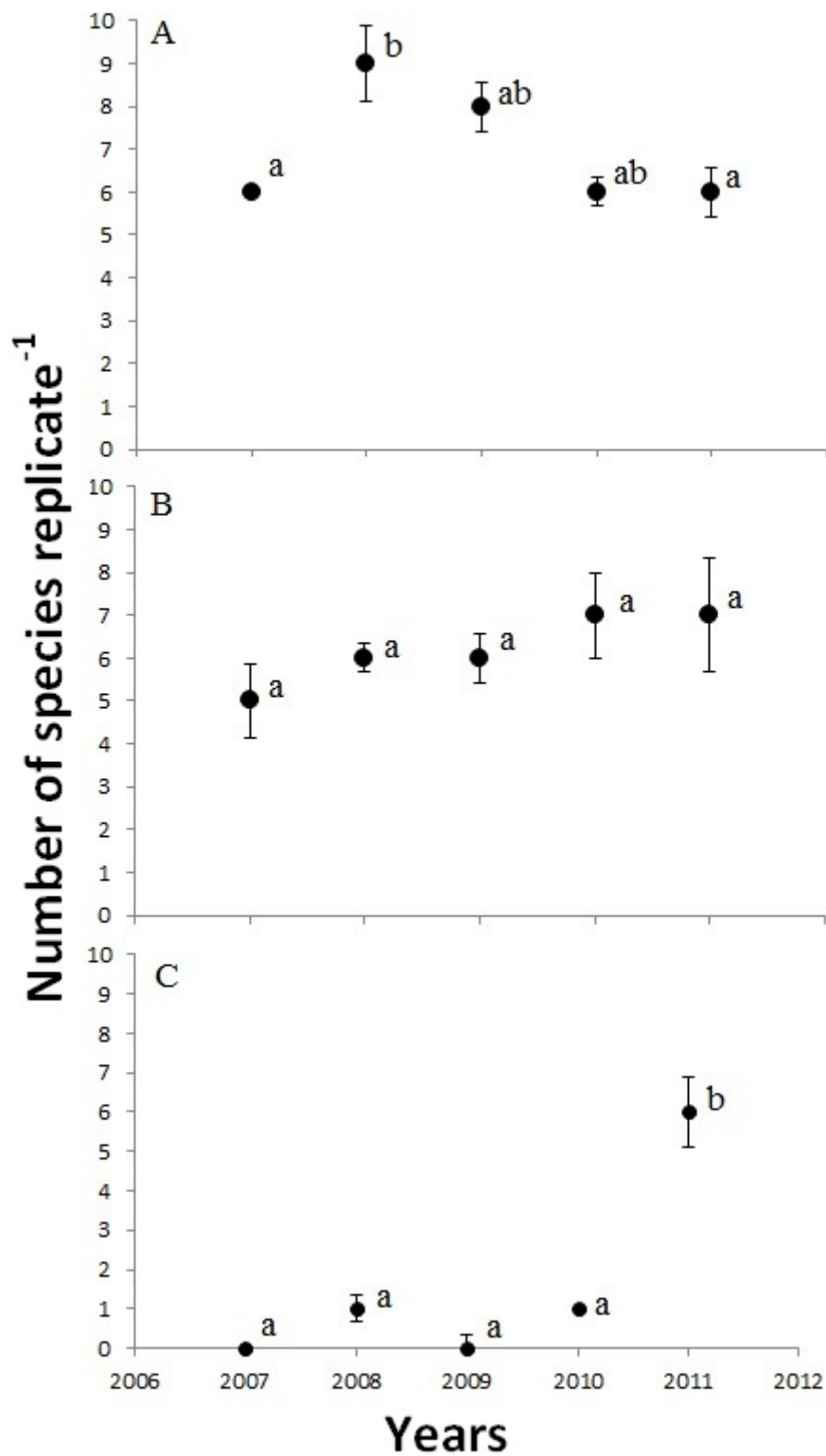


Figure 8. Interannual variation in snail species richness (number of species replicate<sup>-1</sup>) at Saalahti (A), Kuhmoinen (B) and Nyystölä (C) in years 2007-2011, error bars represent standard error, years with similar letter were not different based on Oneway Anova coupled with Tukey HSD post hoc test.



All sites combined, thirteen species of snails were identified, and the most abundant were *Armiger crista*, *Bathymorphalus contortus*, *Bithynia tentaculata*, *Gyraulus albus* and *Radix balthica*, together representing 90 % of snail abundance. At Saalahti exactly the same species were the most abundant and represented 93 % of total at this site. Kuhmoinen had *Bithynia tentaculata*, *Gyraulus albus* and *Radix balthica* as most abundant species (87 %) and lastly *Bithynia tentaculata*, *Gyraulus albus* and *Acroloxus lacustris* dominated at Nyystölä (84 %) (Figure 9).

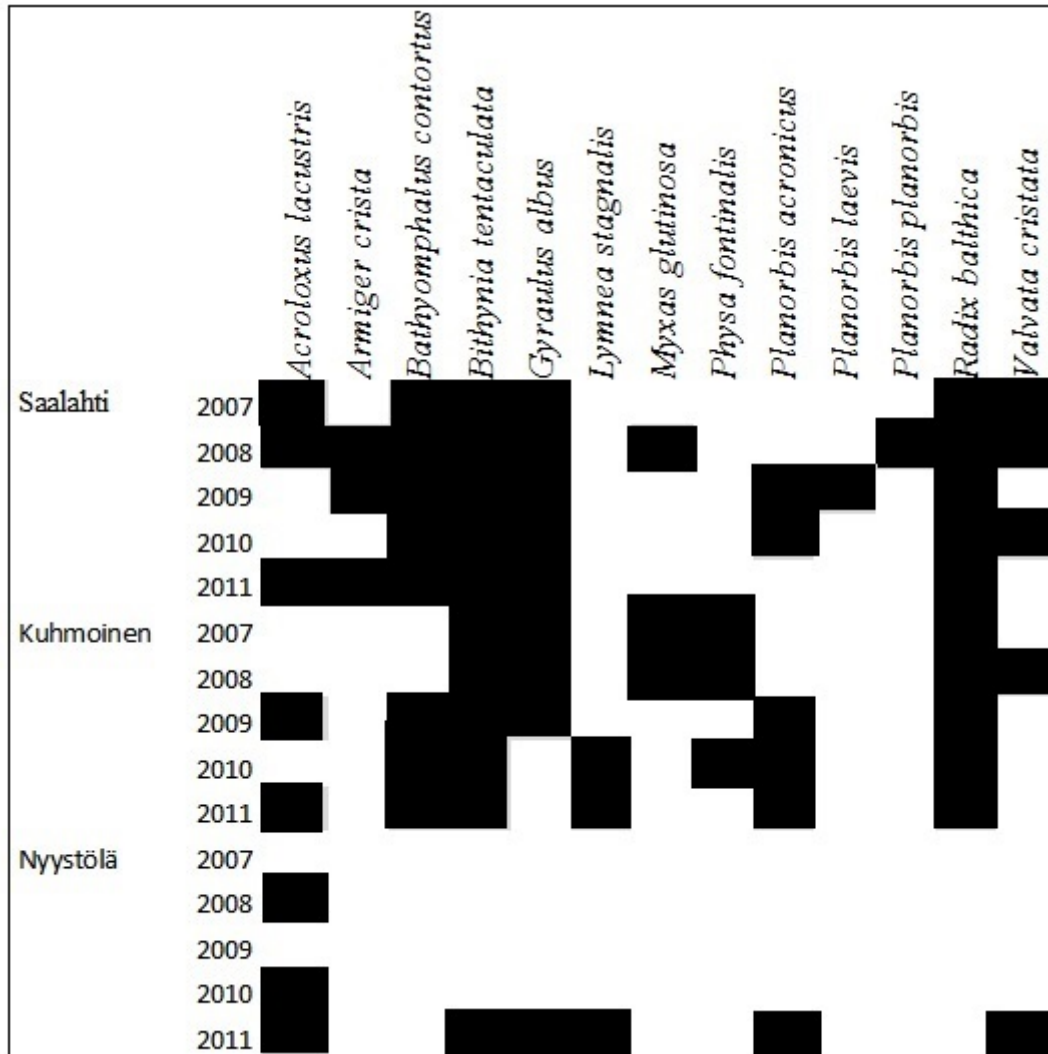


Figure 9. Distribution of Gastropoda at Saalahti (crayfish introduction site), Kuhmoinen (non-crayfish site) and Nyystölä (crayfish site). Shaded cells mean presence and white cells absence of the given species in the corresponding year and site.

Out of the 13 species of snails identified during the whole study, 2 (*Lymnea stagnalis* and *Physa fontinalis*) were not found at Saalahti whatever the year, 3 were missing from Kuhmoinen and 7 from Nyystölä. At Saalahti, 4 species (*Bathymorphalus contortus*, *Bithynia tentaculata*, *Gyraulus albus* and *Radix balthica*) were found every year, at Kuhmoinen such species were 3 (*Bithynia tentaculata*, *Gyraulus albus* and *Radix balthica*) and none at Nyystölä where *Acroloxus lacustris* was found in 2008, 2010 and 2011, and all other species only in 2011 (Figure 9).

### 3.5. Ordination analysis

In the NMS ordination space (Figure 9), Nyystölä was well separated from the two other sites on axis 2 and was between Saalahti and Kuhmoinen on axis 3 whereas all sites overlapped on axis 1. Combining sites and years, MRBP analysis suggests that the three sites were significantly different from each other in species composition: Saalahti vs Kuhmoinen ( $p = 0.016$ ,  $A = 0.135$ ), Saalahti vs Nyystölä ( $p = 0.015$ ,  $A = 0.2$ ) and Kuhmoinen vs Nyystölä ( $p = 0.015$ ,  $A = 0.234$ ). Community compositional variation seemed to be greatest in Nyystölä, with directional pattern.

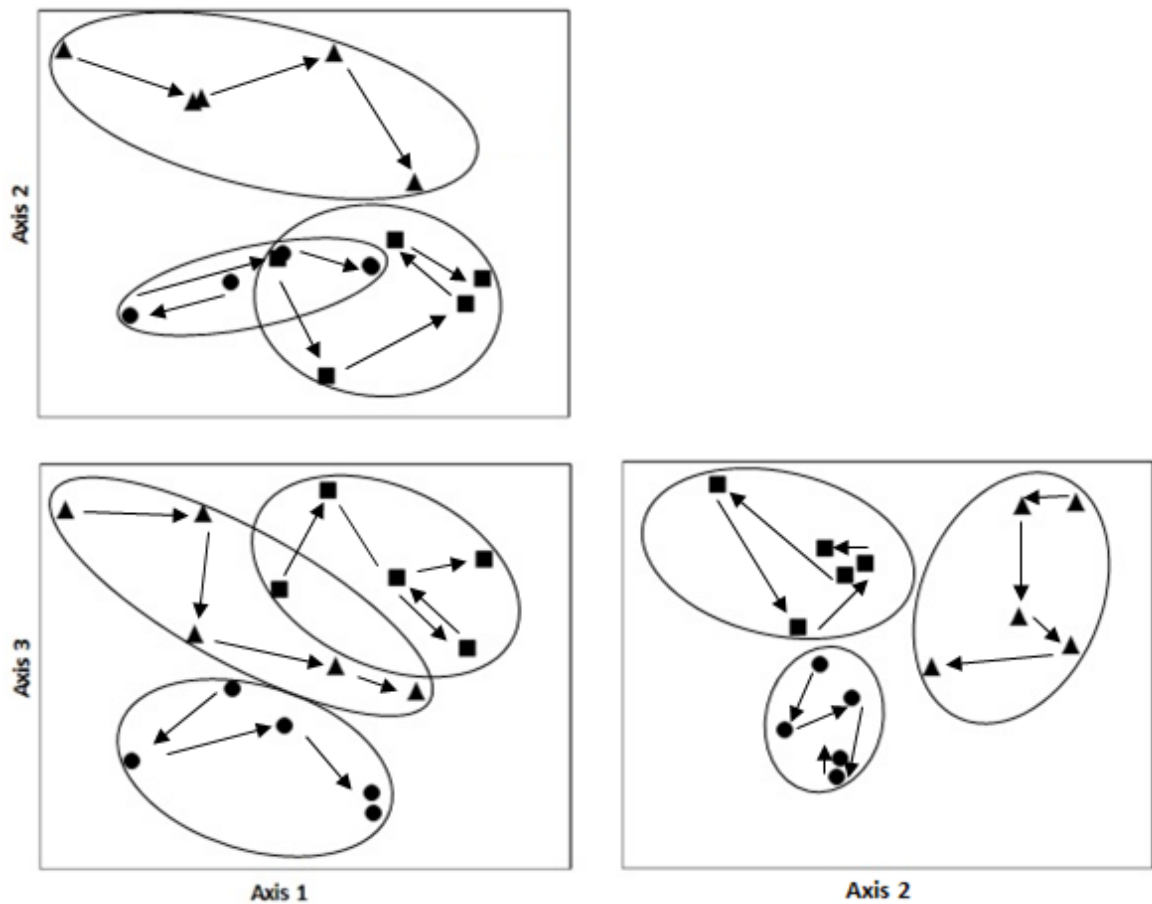


Figure 10. NMS ordination graphs showing grouping of sites Saalahti (squares) Kuhmoinen (dots) and Nyystölä (triangles) by their littoral macroinvertebrate composition. Arrows show direction from 2007 to 2011 within each site.

#### 4. DISCUSSION

Crayfish catches were very low at the introduction site (Saalahti), with a maximum of 0.5 CPUE in 2008 and almost nothing caught in the other years. This suggests very low crayfish population in this site and also that introduction has not yet been successful. Consequently we could not expect significant predation effect of crayfish on MI population with such low crayfish abundance. The reason for this failure is unknown but could be the unfavorable lake bottom substrate and the low introduction density combined. In an intensive aquaculture laboratory experiment aimed at investigating the effect of tank bottom on signal crayfish growth, survival and cheliped condition, Savolainen *et al.* (2003) found that signal crayfish grow better on loose gravel than on glued gravel and bare tank-bottom (in plastic PVC). However, typical habitats of well-established signal crayfish populations are quite similar to that of Saalahti. Westman *et al.* (1999) monitored *P. leniusculus* population in lake Iso-Majajärvi in central Finland for about 25 years following the introduction in 1969. Establishment of crayfish population was very slow and the slowness was imputed to environmental conditions such as water acidity and calcium content which are very important in crayfish chelae formation process. To better understand signal crayfish introduction in freshwater ecosystems, Sahlin *et al.* (2010) used mathematical and statistical models to predict establishment success of signal crayfish in 165 signal crayfish populations in Swedish lakes and running waters. The models compiled several variables such as temperature, length of winter, latitude and altitude. Major findings were that probability of establishment is very low one year following first introduction (25 - 30 %) and relatively high after 10 years (55 to 70 %), even if he later discussed that most populations in the 44 lakes were established after 2 – 3 years. A more recent study still in Sweden focusing on signal crayfish population collapse in 44 lakes found that the growth/lag period (period when the population increases after first introduction) was about 15 years (Sandström *et al.* 2014). These two studies combined suggest that it might be too early to expect any viable crayfish population at Saalahti within such a short time (5 years) after introduction. At the control site (Kuhmoinen), the single crayfish captured in the last year of the experiment (2011) suggests that the site is becoming colonized by crayfish migrating from other parts of the lake. Kuhmoinen and Nyystölä are distant of about 80 km and signal crayfish migration has been evaluated to be about 600 m year<sup>-1</sup> in lake Karisjärvi, a small isolated lake on Northwestern shore of lake Päijänne in central Finland (Kirjavainen & Westman 1999). Therefore it is possible in the future to have more crayfish in this site which was unoccupied by crayfish during the present study. The highest catches, as expected were at Nyystölä (crayfish site) but the rapid drop in 2010, followed by similarly low abundance the next year may be due to high trapping pressure, but more likely due to unfavorable weather conditions at some point of the crayfish life cycle, either during these years or previous years, causing decreased recruitment of catchable crayfish. It was therefore even expected that there was also significant change in the prey (MI) population in 2011, especially in snail population.

The fact that total macroinvertebrate taxa richness and densities at the introduction site did not steadily decrease as expected might be a proof that the high annual variability is not due to any crayfish predation effect. Not only the lack of any clear trend over the years in taxa richness and density, but also their inconsistent variability supports the idea of factors external to crayfish predation affecting these variables, despite decrease observed in 2010 for both variables. Therefore, despite crayfish introduction in 2007, density variation cannot be attributed to crayfish predation. The crayfish site (Nyystölä) showed a similar invariability in total density and species richness to the control site.

Before 2010, the population seemed to be in 'equilibrium' and density was the same every year. However, the drop in predator density from 2009 to 2010 apparently induced a rapid increase in total MI density and species richness as shown in Figures 6 and 7.

Snail community variability reflected total MI variability to certain extent. No significant interannual density differences were found at the control site and at the introduction site (Figure 5, A & B and Figure 3, A & B). They also both had a significantly higher MI density in 2011 compared to other years, but snail densities were similar among 2008, 2009 and 2010 and yet differed between 2007 and 2011. With few exceptions, snail densities were highest at Saalahti every year compared to other sites, which is contrasting because the introduction of crayfish should have made Saalahti to have less snails than the control site. The idea of crayfish introduction failure is further comforted by this irregular variation.

Despite the fact that all sites were discriminated in ordination analysis, there was no consistent direction in interannual community change. MRPP p-values on one hand and community structure graph on NMS on the other hand, indicate close similarity between Saalahti and Kuhmoinen compared to Nyystölä, suggesting no significant predation effect at the introduction site. Yearly variations were 'random' at Saalahti and Kuhmoinen, suggesting that macroinvertebrate community at the introduction site is not conveying towards a certain equilibrium similar to that of a crayfish site and/or that factors other than signal crayfish predation may be affecting their sparse and unpredictable interannual variation. In the case of a successful crayfish development at Saalahti, we could have expected MI community therein to be gradually changing over time to resemble that of Nyystölä which has already reached a relatively stable state of crayfish and MI populations. Therefore, given the conclusions of Sahlin *et al.* (2010) and Sandström *et al.* (2014), the period 2017 – 2022 could be a reasonable time to expect to see an established crayfish population at Saalahti and get a more convincing idea of their impact on macroinvertebrate communities in the long term since it takes about 10 to 15 years for signal crayfish population to effectively settle in lakes.

It is important to point out some limitations about the present study. The fact that identification has been practically impossible with some groups of invertebrates that may have otherwise yielded some important information is one of those limitations. For instance abundant and diverse chironomid assemblages provide valuable information about long term change in freshwater ecosystems. An example of dramatic crayfish predation effect on chironomids communities in streams is provided by Usio & Townsend (2002, 2004); who found that among all chironomid taxa, Southern koura, *Paranephrops zealandicus* affected primarily large and medium size larvae of the subfamily Tanypodinae. Smaller sized Chironominae were also affected by the predator but to a lesser extent. Furthermore, substrate type (leaf or rock) also influenced the predation effect on chironomids, leaf preys on leaf substrate experiencing more dramatic predation than those on rocky substrate. Therefore going further in chironomids identification might have revealed more valuable information.

Another group that has not been fully identified in this study is Oligochaeta. They play an important role in trophic interactions and lake ecosystems. Though much is known about their diversity, distribution and effect of toxins on their community (Martin *et al.* 2008, Seys *et al.* 1999, West *et al.* 1997), few is known about Oligochaeta long term community change under predation of signal crayfish, further identification of this group of macroinvertebrates could therefore yield more information considering also that crayfish

affect Oligochaeta community by bioturbation and fine sediment displacement (Usio & Townsend (2004).

The present interannual study reveals numerous facts even if they are not directly linked to its primary purpose which was the effect of crayfish predation. The results herein represent a solid ground for future studies when crayfish population will have completely established. Sampling repeated in a similar way using similar methodology and locations will therefore be a worth continuation of this work and may provide valuable comparative results for better understand interannual effect of signal crayfish on lake macroinvertebrates in large boreal lakes.

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