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EFFECTS OF TEMPERATURE ON MARBLED CRAYFISH (*PROCAMBARUS VIRGINALIS*, LYKO 2017) INVASION ECOLOGY

Master's Thesis

Chair of Hydrobiology and Fishery

Supervisors: Fabio Ercoli, PhD
Paul Teesalu, MSc

Tartu 2021

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Abstract of Master´s Thesis

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Curriculum: Fisheries and applied ecology

Title: Effects of temperature on marbled crayfish (*Procambarus virginalis*, Lyko 2017) invasion ecology

Pages: 45
Figures: 10
Tables: 4
Appendixes: 1

Department: Chair of Hydrobiology and Fishery

Field of research: Hydrobiology, water ecology, marine biology, limnology (B260)

Supervisors: Dr. Fabio Ercoli, MSc Paul Teesalu

Place and date: Tartu 2021

Biological invasions are crucial issues worldwide and marbled crayfish (*Procambarus virginalis*, Lyko 2017) is one of the examples of freshwater invaders spreading across Europe and beyond. Marbled crayfish has high growth rate and reproduces via parthenogenesis, making it unique among other decapod species. Even though it is a warm water species, they can resist colder temperatures and are highly adaptable. Furthermore, it is also a vector for *Aphanomyces astaci*, causing crayfish plague. Due to its high adaptability, fast growth and reproduction, since one individual is enough to start a new population, it is important to understand its invasion ecology in order to implement better management plan in the new invaded ecosystems.

In this thesis the main goal was to assess whether the temperature is the main factor of marbled crayfish establishment and distribution in the artificially warm outflow channel of Balti Power Plant in Narva. We hypothesised to find temperature gradient along the channel which reflects marbled crayfish distribution and trophic niche. We analysed marbled crayfish and their potential food sources for stable carbon (^{13}C) and nitrogen (^{15}N) isotopes to assess its trophic niche. Temperature data did not show the gradient in the channel, however the channel was significantly warmer than Narva Reservoir. Stable isotope results showed change in marbled crayfish diets and trophic niche along the channel and between seasons, indicating a shift from enriched carbon to depleted carbon values from head to mouth of the channel and a shift from high to lower trophic level from spring and summer to autumn. Moreover, results showed diets being similar in head and middle of channel where crayfish mostly rely on macroinvertebrates and macrophytes, while in the mouth of the channel diet seems to shift more towards periphyton. Diet also changed from protein-rich in spring to vegetation-based diet in autumn. Based on the results, temperature had important role in the marbled crayfish establishment in the invaded channel, however the distribution of its population might have been affected by ecological aspects rather than temperature, like better food availability in the head of the channel.

Keywords: Invasive species, trophic niche, temperature, *Procambarus virginalis*, stable isotopes.

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Juhendaja(d): Dr. Fabio Ercoli, MSc Paul Teesalu
Kaitsmiskoht ja aasta: Tartu 2021</td></tr>
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Invasiivsete võõrliikide levimine on oluliseks teemaks kogu maailmas ning marmorvähk (*Procambarus virginalis*, Lyko 2017) on üks magevee võõrliikidest, mis levib hetkel üle Euroopa ja mujalgi. Neil on suur kasvukiirus ning paljunemine partenogeneesi kaudu muudab marmorvähid unikaalseteks teiste liikide seas. Tegu on küll soojalembese liigiga, kuid nad suudavad vastu pidada ka külmematele temperatuuridele ning on kõrge kohanemisvõimega. Lisaks võivad nad levitada *Aphanomyces astaci*, põhjustades vähikatku. Tänu kiirele kohanemisvõimele, kasvule ning paljunemisele on oluline mõista marmorvähi invasiooni ökoloogiat. Käesoleva töö põhieesmärgiks oli hinnata, kas Balti Soojuselektrijaama kanalist leitud marmorvähkide populatsiooni leviku jaoks on temperatuur olulisim faktor. Hüpoteesiks oli, et leiame kanalist temperatuuri gradiendi, mis peegeldub populatsiooni tiheduses ning ökoloogilises niššis. Selleks analüüsisime marmorvähkide ja nende potensiaalsete toiduallikate süsiniku ja lämmastiku stabiilseid isotoope (^{13}C ja ^{15}N). Temperatuuriandmed kanalis gradienti ei näidanud, küll aga saime kinnitust, et kanal on oluliselt soojem Narva veehoidlast. Stabliilsete isotoopide analüüsi tulemused näitasid ökoloogilise nišši muutust kanali algusest kanali suudmesse liikudes. Tulemused näitasid, et marmorvähkide toit on sarnane kanali ülemises ja keskosas, kus põhiliselt toituvad nad suurselgrootutest ja makrofüütidest. Kanali alumises osas oli põhiliseks toiduobjektiks perifüüton. Muutusi oli ka näha ka erinevate aastaaegade vahel. Kevadel oli põhiliseks toiduobjektiks suurselgrootud kuid sügisel näitasid tulemused taimestiku põhisemat toitumist. Tulemused näitasid, et temperatuuril on oluline roll populatsiooni asutamisel, kuid ei mõjutanud populatsiooni levikut kanalis. Populatsiooni jaotumine kanalis võib olla mõjutatud teistest ökoloogilistest aspektidest, nagu näiteks parem toidukättesaadavus kanali alguses.

Märksõnad: invasiivsed ligiid, ökoloogiline nišš, temperatuur, *Procambarus virginalis*, stabiilsed isotoobid

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1. INTRODUCTION

Biological invasions are crucial issues worldwide and it is an important topic in freshwater ecosystems. Introduction of non-native crayfish species are an example of freshwater invaders threatening native crayfish species and ecosystems (Kouba *et al*. 2014). Currently, in Estonia there are three non-native crayfish species: signal crayfish (*Pacifastacus leniusculus*), spiny cheek crayfish (*Faxonius limosus*) and marbled crayfish (*Procambarus virginalis*).

Marbled crayfish was discovered in the pet trade in Germany in the 1990's (Scholtz *et al.* 2003, Lukhaup 2001) and since that the species became highly invasive spreading across European countries and beyond. Currently there are regulations in the European Union that prohibits the trade, import and keeping the marbled crayfish (Hossain *et al.* 2018). However, despite regulations, marbled crayfish is still spreading.

Marbled crayfish is a warm water species with the optimal temperature being 18-25°C (Vogt 2008). However, there are evidence that the species can survive in colder temperatures (Veselý *et al*. 2015, Kaldre *et al*. 2016). It is the only decapod species that reproduces via parthenogenesis (Vogt 2008). All specimens are female, and one individual is enough to start a new population in the invaded habitat (Jones *et al.* 2008). This characteristic along with its high adaptability, rapid reproduction and growth rate, allow marbled crayfish to spread and establish populations in many habitats, making the species highly invasive. Moreover, it is also a vector for *Aphanomyces astaci* which causes crayfish plague, extremely dangerous for native crayfish species.

Marbled crayfish was found in Estonia for the first time in 2017 in the Balti Power Plant channel of Narva Reservoir. The main aim of this thesis was to study the invasion ecology and population dynamics of marbled crayfish in the cooling water channel of Narva Power Plant. In particular, the goals of this thesis were: (i) to test whether the temperature is the main factor of marbled crayfish establishment and distribution in the channel, and (ii) whether the trophic niche of marbled crayfish differs between head and mouth of the channel according to its distribution. We hypothesised that the temperature in the channel is warmer respect to Narva Reservoir, providing a more suitable habitat for the establishment and growth of marbled crayfish. We also hypothesised the existence of a temperature gradient from head (warmer) to mouth (cooler) of the channel that reflects the marbled crayfish distribution and affects its trophic niche.

The author would like to thank supervisors Dr. Fabio Ercoli and MSc Paul Teesalu for their input and knowledge, Dr. Katrin Kaldre and MSc Margo Hurt for the fieldwork, and MSc Toomas Kõiv for providing temperature loggers.

2. LITERATURE OVERVIEW

2.1 Origin and distribution

Marbled crayfish was initially discovered in the 1990s in the German aquarium trade (Scholtz *et al.* 2003, Lukhaup 2001). Due to its distinctive colour pattern it became known as marmorkrebs, which translates as „marbled crayfish“ (Scholtz *et al.* 2003). It is suggested that marbled crayfish originated from the species *Procambarus fallax* and is the parthenogenic form of it (Martin *et al*. 2010). *P. fallax* has its native habitat range in the southern parts of Florida and Georgia and it is reasonable to assume that marbled crayfish also originates from south-eastern United States, even though there have been no reports of marbled crayfish populations (Chucholl 2010). It is also considered that marbled crayfish is a possible hybrid of the *P. fallax* and another species of the same genus (Martin *et al*. 2016). As of now marbled crayfish is considered an independent species (Lyko 2017).

Due to its beautiful colour pattern marbled crayfish became popular among aquaria hobbyists and was commonly sold in pet shops and online (Chucholl 2013; Lipták *et al.* 2015). As a result of its asexual reproduction, home aquaria can get overpopulated and result in the sale or disposal of redundant individuals (Peay 2009; Patoka *et al.* 2014, 2016a). Released into the wild, this crayfish species can lead to populations in natural ecosystems (Faulkes *et al.* 2012; Patoka *et al.* 2016b). Even releasing a single individual might lead to a new established population due to its parthenogenic reproduction mode (Marten *et al.* 2004, Jones *et al.* 2009).

European Commission Regulations (EU Regulation No. 1143/2014 and Commission Implementing Regulation No. 2016/1141), currently prohibits the import, trade, keeping and breeding of marbled crayfish in the European Union (Hossain *et al.* 2018). However, despite the prohibitions, marbled crayfish continue to expand its invasive range.

The first marbled crayfish caught in the wild was in late 2003 in southwestern Germany (Marten *et al.* 2004). Afterwards there were more reports from other areas of Germany (Chucholl *et al.* 2010; Martin *et al.* 2010). Most of populations are large and presumably well established, suggesting a lag between introduction and detection (Chucholl 2014). Considering the lag, it is likely that the trend of increasing population is a result of introductions, which occurred several years ago, around the time in which marbled crayfish became popular among aquarists (Chucholl 2014). Parthenogenesis and high spawning rate are great advantages in establishing new wild populations (Chucholl *et al.* 2012).

In 2012, 15 marbled crayfish records were known in Europe and most of them were reported in Germany. Marbled crayfish were found in various freshwater habitats such as rivers, brooks, canals, natural and artificial lakes and ponds (Chucholl *et al.* 2012). Since then, the species has spread all over Europe and beyond.

Currently marbled crayfish has been discovered in the Netherlands (Soes *et al.* 2010), Italy (Marzano *et al.* 2009), Slovakia (Janský *et* al. 2010), Sweden (Bohman *et al.* 2013), Croatia (Cvitanic 2017), Belgium (Scheers *et al.* 2021), Malta (Deidun *et al.* 2018), Ukraine (Novitsky *et* al. 2016), Hungary (Lőkkös *et al.* 2016), Romania (Pârvulescu *et al.* 2017), Czech Republic (Patoka *et al.* 2016b), France (Grandjean *et al.* 2021), and in Estonia (Ercoli *et al.* 2019). Besides Europe, populations of marbled crayfish have been found in Madagascar (Jones *et al.* 2009) and Japan (Kawai *et al.* 2010).

2.2 Life cycle and reproduction

Marbled crayfish possesses r-selected characteristics; it matures early, grows fast, has high fecundity and can reproduce several times a year (Jones *et al.* 2009; Seitz *et al.* 2005). Among the decapod crustacean species, marbled crayfish is the only one able to reproduce by obligatory apomictic parthenogenesis (Scholtz *et al.* 2003; Vogt *et al.* 2008), which is the development of oocytes without fertilization and meiosis (Scholtz *et al.* 2003; Vogt *et al.* 2004). Their offspring conserve the original genetic information and are genetically identical clones of their mother (Martin *et al.* 2007), but their morphology can vary substantially (Vogt *et al.* 2008, Martin *et al.* 2010). Populations in Madagascar are genetically homogeneous and very similar to the oldest known stock found in Germany in 1995, which supports that global marbled crayfish population represents a single clone (Gutekunst *et al*. 2018).

Marbled crayfish grows step-wise by moulting and goes under approximately 25 moulting cycles in its lifetime. The body length (without chelae) for adult animals is 4-8 cm and weight

1.5-15 g, but very large individuals can reach 12 cm and weigh more than 25 g (Vogt 2011). Typical lifespan of marbled crayfish is 2-3 years, but the maximum reported lifespan is 1610 days (Vogt 2010). Under laboratory conditions they can complete up to seven reproductive cycles during that time (Vogt 2010). Marbled crayfish develop directly within the eggs which are carried under the mothers pleon (Vogt 2011). During unfavourable conditions ovigerous marbled crayfish can leave the water for a period of time to ventilate the brood (Vogt 2013). The eggs need about 27 days to hatch, at a temperature around 20 °C (Seitz *et al*. 2005). Life cycle of marbled crayfish can be divided into four phases:

1) The embryonic phase which begins with oviposition and ends 17-28 days later with hatching (Vogt 2008).
2) Juvenile phase includes 7-8 stages (Vogt 2008), during which they go under two moulting events (Vogt *et al*. 2004, Kozák *et al.* 2015). Juveniles stay often close to their mother for several weeks and are fully developed after the second moult (Seitz 2001; Vogt 2008).
3) Adolescent phase during which they become marmorated and display visible female sexual characteristics (Vogt *et al.* 2004).
4) Adult phase that span from first spawning to death (Vogt 2010).

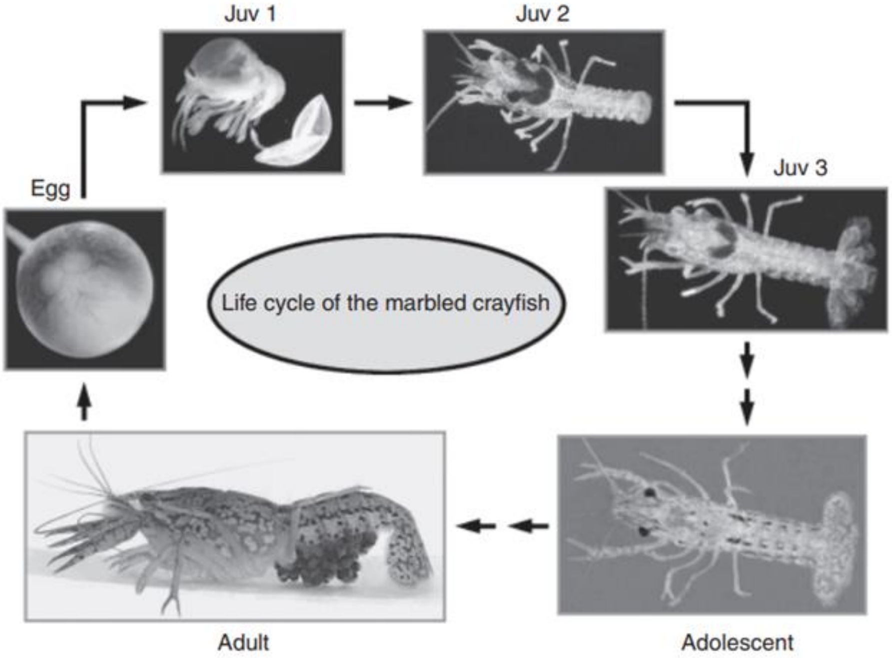

Figure 1. Life cycle of the marbled crayfish (Vogt 2008).

Usually the first reproduction occurs after 5-6 months after hatching (Vogt 2008). Clutch size increases with the size and age of the female (Vogt 2011). Values reported both in laboratory and free-living populations range from 416-731 eggs (Seitz *et al.* 2005; Jones *et al.* 2009; Chucholl *et al.* 2010; Lipták *et al.* 2017). However, in wild populations, reproduction timing remains poorly studied (Liptak *et al.* 2016, 2017).

2.3 Ecology and invasion ecology

The marbled crayfish is a highly potential invader (Jimenez *et al.* 2010; Kawai *et al*. 2016). It can survive low temperatures during winter (Vesely *et al.* 2015), and dry periods by burrowing (Kouba *et al.* 2016). There have been reports of marbled crayfish in temperate and tropical waters as well (Jones *et al.* 2009, Martin *et al.* 2010) which shows its tolerance to a wide range of temperatures (Hossain 2018). It also shows competitiveness against other crayfish species when size matched individuals are faced with each other (Jimenez *et al*. 2011). However, claw size is important in a fight with similar size animal (Snedden 1990; Garvey et al. 1994) and males often have larger claws, thus putting all female marbled crayfish species at a disadvantage (Jimenez et al. 2011).

Crayfish detect the presence of predators through olfactory organs and avoid predators by seeking shelter (Blake *et al*. 1993) through rapid backward movement by repeated flexing of the abdomen (Wine *et al.* 1972; Herberholz *et al.* 2004). Marbled crayfish appear to be calmer in stressful conditions (Kawai *et al.* 2016). Crayfish mainly use chemical or visual communication signals rather than physical combat, to avoid higher energy costs (Breithaupt *et al.* 2011). Marbled crayfish shows similar agonistic behaviour that has been observed in other crayfish species (Luna *et al.* 2009), crabs (Sneddon *et al.* 2000) and lobsters (Kravitz 2000). Agonistic encounters of marbled crayfish consist of temporal sequences of threat displays, restrained fighting and brief periods of unrestrained combat (Luna *et al.* 2009).

Invasive crayfish species are usually able to move over land (Peay *et al.* 2010; Puky 2014), while native species are mainly bound to a life in permanent water bodies (Holdich 2002; Kozák *et al*. 2015; Kouba *et al.* 2016). Marbled crayfish can be considered as highly resistant species (Chucholl 2014, Kouba *et al.* 2016) and has been frequently reported on land during migration or found dead in places that are distant from waterbodies (Chucholl *et al.* 2012).

In different parts of the world, crayfish invasions have caused local extinctions of native crayfish and resulted in changes in aquatic ecosystems (Rodriguez *et al.* 2005; Rosenthal *et al*. 2006). In Madagascar marbled crayfish was identified as a particularly dangerous invader (Gutekunst *et al.* 2018) because it may threaten rural livelihoods (Jones *et* al. 2009). Indeed, due to releasing one or few individuals, they multiplied at an enormous speed and invaded habitats such as rice fields, rivers, lakes and swamps in many areas of the country (Jones *et al.* 2009, Kawai *et al.* 2010). Jones *et al.* 2009 interviewed people in Madagascar about marbled crayfish presence and they reported being able to harvest many kilograms in few hours. Vendors also said that they have kept crayfish alive for 3 days without water. Many fishermen also said that marbled crayfish had a strong negative impact on fishing in their local area.

2.4 Marbled crayfish role in the food web of freshwater ecosystems

Marbled crayfish utilizes a wide range of food sources that likely impacts and modifies the structure of the food web. Detritus, both autochthonous and allochthonous, is the most important food source for marbled crayfish, while zoobenthos, algae and macrophytes are utilized to a lesser extent (Liptak *et al*. 2019). Because it processes allochthonous and autochthonous matter, marbled crayfish serves as an important decomposer in the ecosystem.

Marbled crayfish can be also omnivorous and predator as well as an important food source for organisms at higher trophic levels like predatory fish. Thus, it can be considered as a keystone species with ability to transport the energy from the bottom of the food web to top predators (Liptak *et al*. 2019), potentially causing strong changes in the invaded ecosystems, similarly to the red swamp crayfish (Geiger *et al.* 2005).

The utilization of a wide range of food sources and the ability to exploit an ecosystem at various trophic levels may contribute to marbled crayfish successful establishment under a wide range of conditions (Liptak *et al.* 2019).

2.5 Marbled crayfish vector of pathogens

North-American origin crayfish species are vectors of the *Aphanomyces astaci* (Oomycetes) (Kozubíková *et al.* 2009), which is considered among the world's one hundred most invasive species (Lowe *et al.* 2000). It causes crayfish plague, which is dangerous to native crayfish populations in Europe (Unestam 1972; Aquiloni *et al.* 2010). Five genotypes (A, B, C, D, E) have been recognized (Svoboda *et al.* 2017). In crayfish plague epidemics in Europe and Japan genotype groups A, B, D and E have been identified (Viljamaa-Dirks *et al.* 2013; Kozubíková *et al*. 2014; Rezinciuc *et al.* 2014). North-American origin species are generally resistant to the crayfish plague (Cerenius *et al.* 2003). Marbled crayfish has been confirmed as a host of the genotype group D strain, originally isolated from the red swamp crayfish (Keller *et al.* 2014). Moreover, it has been also speculated that marbled crayfish may soon host other strains (Hossain 2018). In addition to crayfish plague, detection of rickettsial and coccidian-like organisms in the ovary and further organs of marbled crayfish, can be dangerous for native European crayfish species (Vogt *et al.* 2004).

2.6 Role of temperature in marbled crayfish ecology

The metabolic processes of ectotherm species are influenced by temperature and recent research shows that temperature may play a determining role on nutrient acquisition (Carreira *et al.* 2017). Climate change may provide invasive alien species (IAS) with new suitable habitats (Carreira *et al.* 2017), changing their trophic impacts as they optimize nutrient intake for higher temperatures (Carreira *et al.* 2017).

Higher temperatures favour the assimilation of smaller and structurally less complex nutrients like carbohydrates over complex nutrients like proteins (Carreira *et al.* 2017). Protein to carbohydrate assimilation ratio of crayfish, shifts to a greater assimilation of carbohydrates at higher temperatures (Croll *et al.* 2007). Higher temperatures promote greater increase in respiration than in growth, and increase the demand for carbon over nitrogen (Karl *et al.* 2008; Forster *et al.* 2011). Omnivorous ectotherms should optimize energy intake by avoiding diets that are protein rich and increase herbivory at higher temperatures (Carreira *et al.* 2017).

Carreira *et al.* (2017) concluded an experiment where diet choices of *P. clarkii* were investigated during a heatwave using ^{13}C and ^{15}N stable isotopes. Results suggested a decreased efficiency of herbivorous diets at lower temperatures and decreased efficiency of carnivorous diets at higher temperatures. Experiment also showed a decreased performance in crayfish during a long heat wave, suggesting that long summer heat waves may have negative effects and are unlikely to boost *P. clarkii* populations.

In general, marbled crayfish are best cultured at 18-25 °C but can survive temperatures lower than 8 °C and higher than 30 °C for weeks. However, under these conditions mortality increases and reproduction stops (Vogt *et al.* 2004). Seitz *et al.* 2005 showed that majority of marbled crayfish kept in low temperatures (8-10°C) survived more than 40 days and individuals can molt at temperatures below 10 °C when the temperature is gradually decreasing (Seitz *et al.* 2005; Kaldre *et al.* 2016).

Higher temperatures shorten the molt cycle and lead to faster growth (Hartnoll 2001). Marbled crayfish growth is strongly dependent on the temperature, highest at 25 °C, and lowest at 15 °C. Experiment showed that length and total weight increase by 17.5 mm and 1700 mg at 25 °C, whereas same parameters increased 7 mm and 100 mg at 15°C (Seitz *et al.* 2005).

With good conditions, marbled crayfish can grow up to 12 cm in total body length. Highest growth rate has been observed at 25°C and highest survival rate at 20°C (Seitz *et al.* 2005; Vogt 2008). Haubrock *et al.* 2019 induced thermic shock to crayfish by taking them from room temperature (20 °C) to colder temperatures, imitating release into natural waterbodies by pet owners. One individual died after 21 days of being kept at 6 °C in water and two others died after 63 days. Every individual kept at 4 °C died within 49 days and crayfishes kept in 2 °C died in two weeks. It has been also seen that carapace length did not have an effect on survival rates and that individuals kept at 2 °C did not show any foraging activity. However, Pfeiffer 2005 and Veselý *et al.* 2015 provided evidence that marbled crayfish can survive the winter season as well as other outdoor experiments have shown that marbled crayfish are able to survive winters in Northern Europe (Kaldre *et al.* 2016). Marbled crayfish was seen surviving under ice (Pfeiffer 2005) and in Sweden, were caught from a river which had constant flow and water temperature 0-0.1°C during winter (Bohman *et al*. 2013). However, due to the climate in Northern Europe, it is possible that reproduction will not occur and adult crayfish can survive cold temperatures by lowering its activity and slowing down body functions (Bohman *et al.* 2013).

Experiments conducted by Kaldre *et al*. 2016, showed temperature effects on marbled crayfish in Nordic climate using outdoor tanks. They found that marbled crayfish could survive less than one week at 1-2 °C, and that longer periods of time caused high mortality. However, they also found that larger individuals seemed to be more tolerant of lower temperatures. Some individuals survived three months at an average temperature of below 5 °C. They also indicated that, due to low water temperatures in Estonian freshwaters, marbled crayfish cannot reproduce all year long, but at least once a year.

Nevertheless, results based on laboratory studies are not fully conclusive and research conducted in nature, that investigate the effect of temperature on invasive marbled crayfish, would be critical for a better understanding of its invasion strategy and ecology.

2.7 Marbled crayfish in Estonia

In September 2017 field sampling of biota was carried out in Narva Reservoir and in the outflow channel of Balti Power Plant, in Estonia (Figure 2). Six marbled crayfish specimens were found among samples from the middle part of the channel (Ercoli *et al.* 2019).

At that time marbled crayfish were sold in aquarium shops in Estonia, so it is most likely that the marbled crayfish has been introduced into the Narva water system by humans. Natural immigration is unlikely because, to our knowledge, there is no data about the presence of marbled crayfish in Estonia nor in Russia so far (Ercoli *et al.* 2019). In spring and summer 2018 further sampling was carried out in order to assess population density along the whole channel and an established population was found. (Ercoli *et al* 2019).

During the sampling it was found that sampling sites closer to the Balti Power Plant (head channel) had higher CPUE (Catch Per Unit Effort), while sites further away from Power Plant (in the middle and mouth of the channel) indicated lower CPUE. This evidence suggested that the distribution of marbled crayfish population in the channel was most likely due to the water temperature regulated by the power plant. In such condition the water would have been warmer in the head and colder in the mouth of the channel. In 2019 marbled crayfish were sampled again in the channel in spring, summer and autumn indicating the same CPUE pattern found in 2018.

The same CPUE pattern found in both years led to the hypotheses of this thesis. We hypothesised that there is a temperature gradient along the channel and that the CPUE and trophic niche of marbled crayfish were affected by temperature.

3. MATERIALS AND METHODS

3.1 Study sites and temperatures data

Field sampling was carried out in the outflow channel of the cooling system of the Balti Power Plant, in Narva Reservoir (Figure 2). The channel is approximately 7 Km long and has a mean depth around 2 m. To record the temperatures during seasons 8 temperature loggers (A1, A2, B1, B2, C1, C2, D1, D2), HOBO Pendant® Temperature/Light 64K Data Logger, were placed in the channel at approximately half meter from the bottom. The loggers were placed in different sites in order to cover three different sections of the channel, the head, middle and mouth (Figure 2). Unfortunately only 4 loggers were found at the end of the study (Figure 2). In addition to the temperature in the channel, water temperatures from Narva Reservoir (site Kulgu) and outside the mouth of channel, (Figure 2) as well as air temperatures were provided by Estonian Weather Service and by Narva monitoring program.

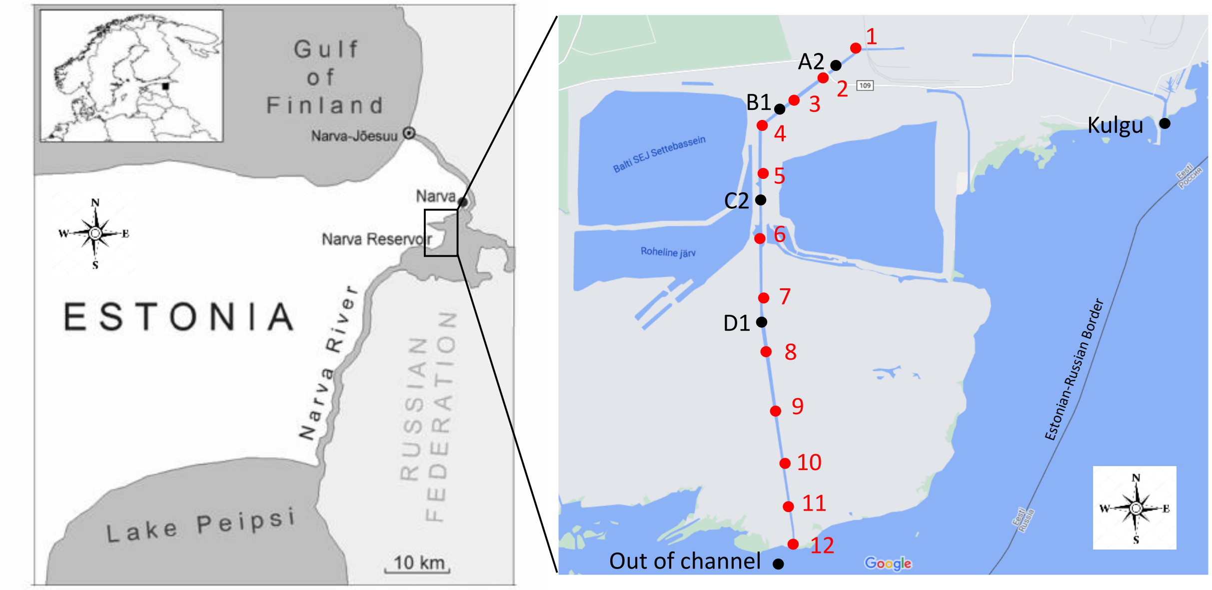

Figure 2. Sampling sites (red dots from 1 to 12) and locations of temperature loggers (black dots, A2, B1, C2, D1) along the outflow channel of the cooling system of the Balti Power Plant in Narva Reservoir.

3.2 Marbled crayfish and food sources sampling

In order to assess population density and structure of marbled crayfish sampling was carried out following same protocol as in Ercoli *et al*. 2019, along the whole channel during spring and summer 2018, spring, summer and autumn 2019 and spring and summer 2020. 100 cylindrical crayfish traps with 10 mm mesh size (“Mjärde Lini”) were placed as separate lines consisting of 10 traps per line in each of the 12 sites (2018) and 11 sites (2019 and 2020) selected in the channel (Figure 2) and kept in the water overnight. Frozen cyprinid fish were used as bait. For every trapping session at each site, Capture Per Unit Effort (CPUE) was calculated per line, which was consisting of 10 traps each. 20 artificial refuge traps (ART) were used in 2018 and 2019 in addition to the ordinary traps (Green et al. 2018), for catching small crayfish individuals (Ercoli *et al*. 2019). Crayfish individuals were grouped in three subpopulations to represent head, from site 1 to 4, middle, from 5 to 8, and mouth of the channel from 9 to 12 (2018) and from 9 to 11 (2019 and 2020) (Figure 2).

Three replicates of macroinvertebrates, macrophytes, periphyton and detritus were collected as potential food sources in each section of channel and season. Macroinvertebrates were collected using kick-net, with a mesh size of 0.05 mm and by hand, while macrophytes (*Phragmites australis; Nuphar lutea, Equisetum fluviatile, Myriophyllum sp. and Potamogeton natans*) and detritus, which primarily consisted of dead tree leaves, were collected by hand. Periphyton was sampled gently brushing the rocks and cobbles surfaces. All samples were placed into cool box after collection and then transferred to a laboratory and kept in freezer (-22 °C) until further processing for carbon (^{13}C) and nitrogen (^{15}N) stable isotope analysis (SIA).

3.4 Samples preparation for stable isotopes analyses

A total of 180 individuals of marbled crayfish were caught from the channel and prepared for stable isotope analysis. Crayfish were defrosted and carapace length (CL) and weight were measured to nearest mm using calliper, and to nearest 0.1 grams using microbalance, respectively. A piece of abdominal muscle tissue was taken, as recommended by Stenroth *et al.* (2006), from the tail of each marbled crayfish individuals. All the tools (scalpel, tweezers) and working place were cleaned by using ethanol between every sample. The flesh samples were placed in glass tubes covered with parafilm (Figure 3). Macroinvertebrate samples were identified mainly at the order taxonomic level (Odonata, Ephemeroptera, *Dreissena polymorpha*, Coleoptera, Chironomidae, Tricoptera and Gastropods) and placed into glass tubes as well as samples of detritus and macrophytes. All samples were put in the freeze dryer for 48 hours, except macrophytes and detritus which were dried for 48 hours at 60 °C in oven. All samples were successively grounded to a fine homogeneous powder. Grounded animal samples were weighed into tin caps in the amount of 0.6 mg while plant samples (detritus, macrophytes and periphyton) were weighed at 1.0 mg.

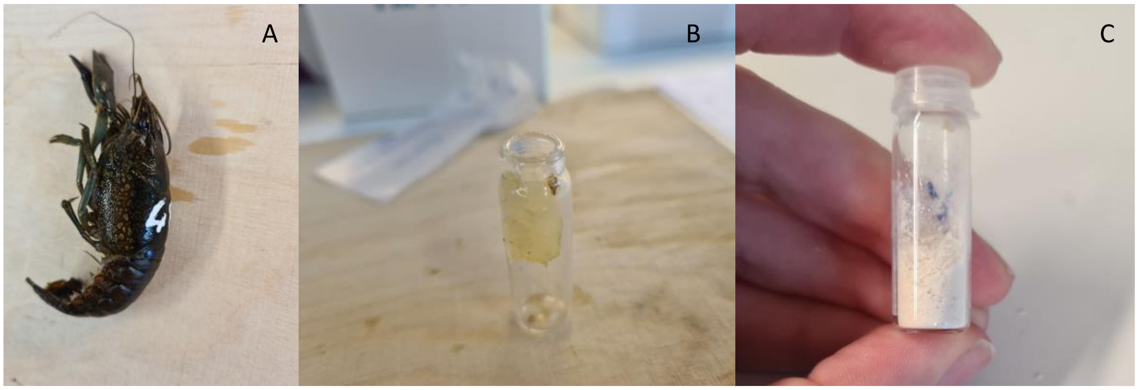

Figure 3. Crayfish samples preparation for stable isotope analyses. Marbled crayfish (A), piece of abdominal muscle tissue taken from marbled crayfish and placed into glass tube (B) and freeze-dried grounded sample (C).

3.5 Stable isotopes analyses

All the samples were analyzed for carbon and nitrogen stable isotopes with a FlashEA1112 elemental analyser coupled to a Thermo Finnigan DELTAplus Advantage continuous flow isotope ratio mass spectrometer (Thermo Electron Corporation, Waltham, MA, U.S.A.) at Jyväskylä University in Finland. Stable isotope values of carbon and nitrogen are expressed in delta notation as parts per thousand (‰) according to:

$$\delta X = (\mathrm{Rsample} / \mathrm{Rstandard} - 1) \times 1000$$

Where X is either carbon or nitrogen isotopes, and R is the ratio of heavy to light isotope of carbon or nitrogen. Reference materials used were internal standards of known relationship to the international standards of Vienna Pee Dee, including belemnite for carbon isotopes and atmospheric nitrogen for nitrogen isotopes. Stable isotope ratios are expressed as parts per thousand (‰) delta values relative to the international standards for carbon and nitrogen. White muscle tissue of northern pike (*Esox lucius* L.) (for animal based samples) and birch leaves (*Betula pendula* L.) (for detritus and periphyton) with known isotopic compositions were used as internal working standards to ensure precision of the analyses. One standard sample was run repeatedly after every six samples in each sequence. Standard deviations within reference samples in each sequence were less than 0.1 ‰ for carbon and 0.2 ‰ for nitrogen in pike and in birch leaf samples.

3.6 Statistical analyses

Data were checked for normality prior to statistical tests. Since normality assumptions were not met, we used non parametric Kruskal-Wallis test and, if significant differences were found, Wilcoxon-Mann-Whitney was employed to test for differences in temperatures, crayfish length and weight, stable isotope of ^{13}C and nitrogen ^{15}N, between sites and seasons. To quantify the differences between trophic niche widths of marbled crayfish subpopulations in the head, middle and mouth of the channel, we used Jackson *et al.* 2012 method, where SIBER (Stable Isotope Bayesian Ellipses in R) was employed to illustrate trophic niche in a bi-plot space and areas of each trophic niche are expressed in ‰^{2}. Statistical analysis and trophic niche models were run using R- program, version 4.0.5 (R Development and core team 2021).

4. RESULTS

In spring 2018, a total of 63 marbled crayfish were caught from the channel, with a mean CPUE of 0.8 ± 0.6 SD (Standard Deviation) (Figure 4), mean carapace length (CL) and weight of 39 mm ± 6 SD and 16g ± 8 SD , respectively. During summer 2018, a total of 20 individuals were caught with a mean CPUE of 0.2 ± 0.2 SD (Figure 4) and mean CL and weight being 42 mm ± 6 and 19 g ± 8, respectively. In spring 2019, 30 crayfishes were caught with a mean CPUE of 1.4 ± 0.3 SD (Figure 4), mean CL of 39 mm ± 7 SD and weight 14 g ± 8 SD. During summer a total of 38 animals were caught with mean CPUE 0,7 ± 0.8 SD (Figure 4), mean CL 39 mm ± 7 SD and mean weight 14 g ± 6 SD. In the late autumn of 2019, 29 individuals were caught, with a mean CPUE of 1.9 ± 0.9 (Figure 4), mean CL 36 mm ± 8 SD and mean weight 12g ± 8 SD. It is important to note that during this season, no marbled crayfish were caught from the mouth of the channel (Table 1).

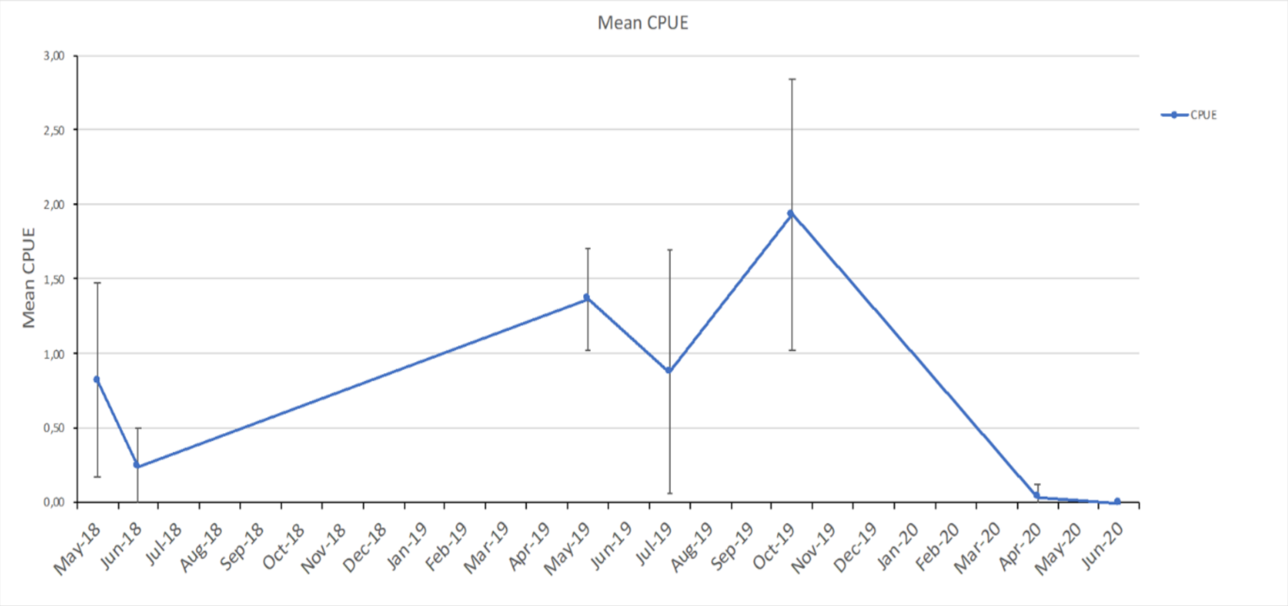

Figure 4. Mean CPUE values from all seasons.

Mean CPUE in spring 2018 was higher than in summer 2018 (Figure 4). In spring 2019 mean CPUE was again higher than summer but started to increase again in autumn (Figure 4). In spring 2020 CPUE sharply declined and in summer no crayfish were caught in the channel (Figure 4). However, during each season CPUE significantly decline (p-value < 0.001) along the channel from head to the mouth (Table 1).

Table 1. Mean CPUE ± Standard deviation values at different seasons in head, middle and mouth of the channel.

Sections of channel	**Seasons**				
	Spring 2018	**Summer 2018**	**Spring 2019**	**Summer 2019**	**Autumn 2019**
Head	1.4 ± 0.46	0.45 ± 0.26	1.27 ± 0.12	1.4 ± 0.9	2.67 ± 0.61
Middle	0.6 ± 0.44	0.18 ± 0.13	1.5 ± 0.51	0.53 ± 0.28	1.20 ± 0.35
Mouth	0.3 ± 0.23	0.03 ± 0.04	1.3 ± 0.01	0.3 ± 0.2	0.0

Length and weight mean values were calculated from each sections of the channel and seasons (Table 2). Length and weight did not show any significant differences neither between sections (p-value = 0.77, p-value = 0.74, respectively) nor between seasons (p-values = 0.12, p-value = 0.14, respectively). Length and weight values from all sections and seasons are shown in appendix 1

Table 2. Mean values of carapace length and weight ± standard deviation of marbled crayfish individuals caught in the head, middle and mouth of the channel during different seasons.

Seasons	**Head of channel**		**Middle of channel**		**Mouth of channel**	
	Length (mm)	weight (g)	Length (mm)	weight (g)	Length (mm)	weight (g)
Spring 18	41.93 ± 3.0	17.79 ± 5.1	38.15 ± 6	13.25 ± 7	41.33 ± 8.1	17.87 ± 9
Summer 18	41.56 ± 4.8	18.02 ± 7.2	43.85 ± 6.3	21.62 ± 8.3	39.15 ± 8.8	15.95 ± 11.5
Spring 19	36.84 ± 5.4	11.52 ± 5.4	40.78 ± 6.5	16.25 ± 8.3	41.28 ± 7.5	17.16 ± 8.7
Summer 19	40.47 ± 4.3	14.9 ± 4.4	41.12 ± 6.3	16.08 ± 6.2	32.31 ± 4.8	8.15 ± 3.9
Autumn 19	38.56 ± 3.9	13.35 ± 5.3	34.93 ± 8.9	10.97 ± 8.7	0	0

4.1 Temperature in the channel

Temperatures obtained from the out of channel site was excluded from the analyses since showed temperatures only from summer. Consequently Kulgu was included as representing of temperature of Narva Reservoir. Any significant difference in temperatures were found between sites A2, B1, C2, D1, indicating the absence of temperature gradient along the channel (Figure 5, Table 3). However, data showed that water temperatures in the channel (from each site) were significantly warmer than in Narva Reservoir (Kulgu) ($p\text{-value} < 0.001$) (Figure 5, Table 3).

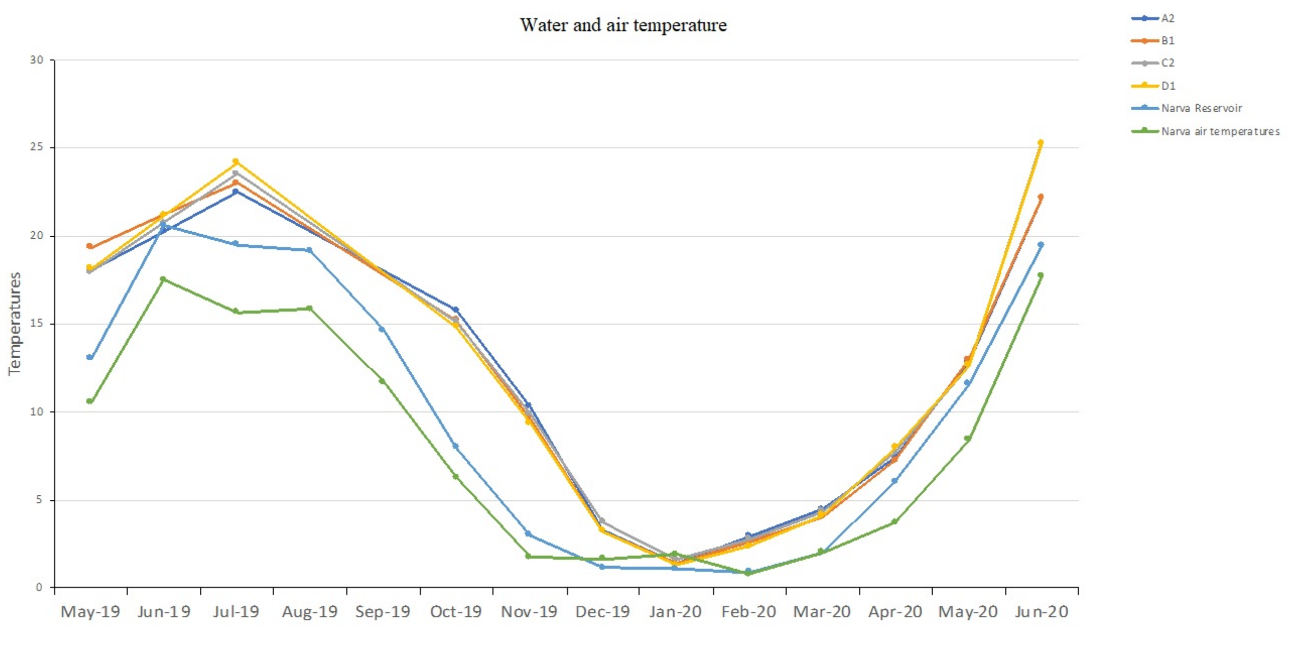

Figure 5. Water and air temperatures in the channel and Narva Reservoir from May 2019 to June 2020.

Table 3. Results of Wilcoxon comparison tests. Significant p-values at $p < 0.05$.

Sites comparison	**p-values**
Site A2-B1	p-value = 0.611
Site A2-C2	p-value = 0.643
Site A2-D1	p-value = 0.619
Site B1-C2	p-value = 0.321
Site B1-D1	p-value = 0.997
Site Kulgu-A2	p-value < 0.001***
Site Kulgu-B1	p-value < 0.001***
Site Kulgu-C2	p-value < 0.001***
Site Kulgu-D1	p-value < 0.001***

4.2 Stable isotopes and trophic niches

Stable isotopes results show that marbled crayfish change its diets and trophic niches along the channel and between seasons. Mean values of $\delta^{13}C$ and $\delta^{15}N$ in the head of channel was -27.00 and 10.79, while in middle and mouth of the channel were -27.25 and 10.58, and -28.65 and 11.13 respectively (Figure 6). Results show a shift from enriched to depleted carbon isotope values from head to mouth of the channel reflecting also a shift in trophic niche (Figure 6, Figure 7). Carbon and nitrogen stable isotopes values of crayfish individuals from all sections and seasons are shown in appendix 1.

In general, marbled crayfish show large variation in $\delta^{13}C$, indicating a wide utilization of food sources along the channel. Its $\delta^{13}C$ values showed significant differences between the head and mouth and between middle and mouth of the channel, but not between head and middle (Table 4). In general, in the head and middle of the channel the diet of marbled crayfish is fairly similar and consisted mostly of macroinvertebrates and macrophytes (Figure 6). However, diet of marbled crayfish in the mouth differs significantly from head and middle of the channel, relying mostly on periphyton and less on macroinvertebrates (Figure 6). Detritus was not important for the diet of marbled crayfish in the channel (Figure 6).

Table 4. Results of Wilcoxon tests in nitrogen and carbon stable isotopes values of marble crayfish between sections. P-values significant at $p < 0.05$.

Stable isotopes	Sections comparison	p-values
$\delta^{15}N$	Location (middle-mouth)	p-value= 0.002 **
	Location (middle-head)	p-value = 0.076
	Location (head-mouth)	p-value = 0.346
$\delta^{13}C$	Location (middle-mouth)	p-value < 0.001***
	Location (middle-head)	p-value = 0.317
	Location (head-mouth)	p-value < 0.001***

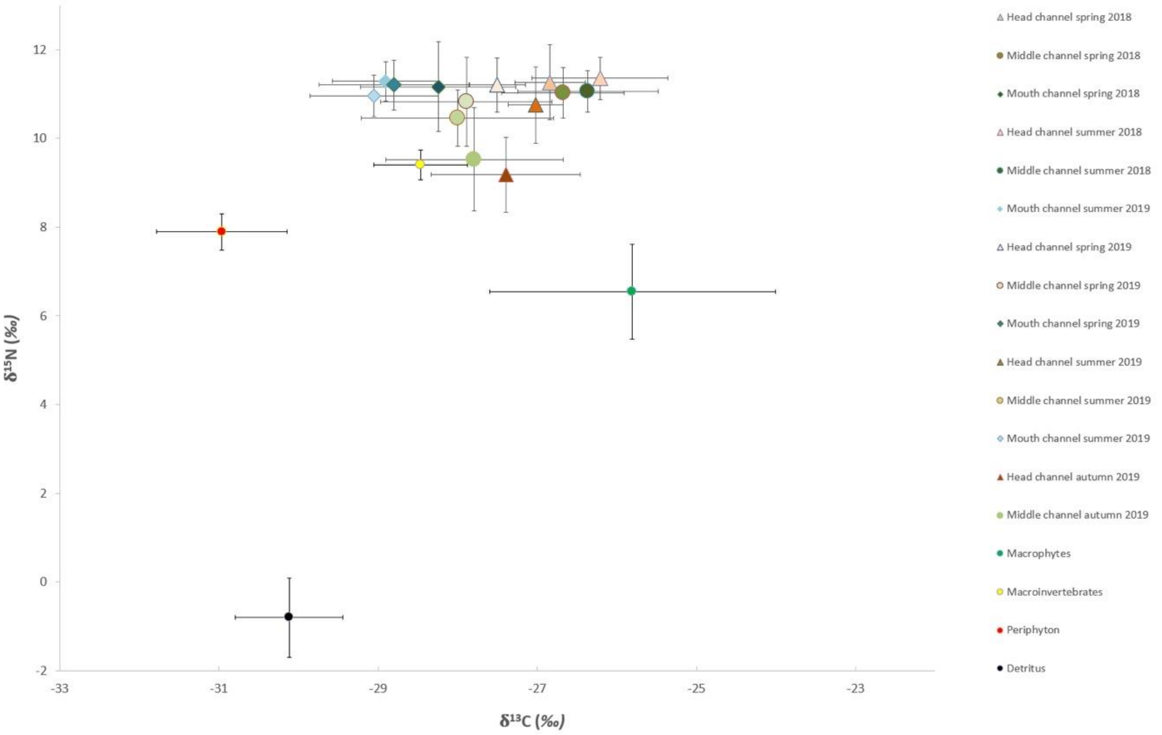

Figure 6. Mean values of $\delta^{13}C$ and $\delta^{15}N$ ± standard deviation of marbled crayfish individuals and their putative food sources from 2018 and 2019 at different section of channel (head, middle and mouth).

Results also indicated significant differences in $\delta^{13}C$ and $\delta^{15}N$ between seasons. In particular between spring and summer for $\delta^{13}C$ (p-value = 0.003), and between spring and summer, spring and autumn and summer and autumn for $\delta^{15}N$ (p-value < 0.001, p-value < 0.001, p-value < 0.001) mostly due to the lower $\delta^{15}N$ values recorded in autumn 2019. Indeed, during autumn 2019 marbled crayfish individuals indicate lower trophic position shifting their diet more towards macrophytes (Figure 6).

SIBER model results indicated overlapping of trophic niches in the head and middle of the channel supporting that marbled crayfish species was using same food sources in these two channel sections (Figure 7). However, in agreement with stable isotopes results, trophic niche in the mouth is placed far away from middle and head of the channel, indicating different food source use (Figure 7).

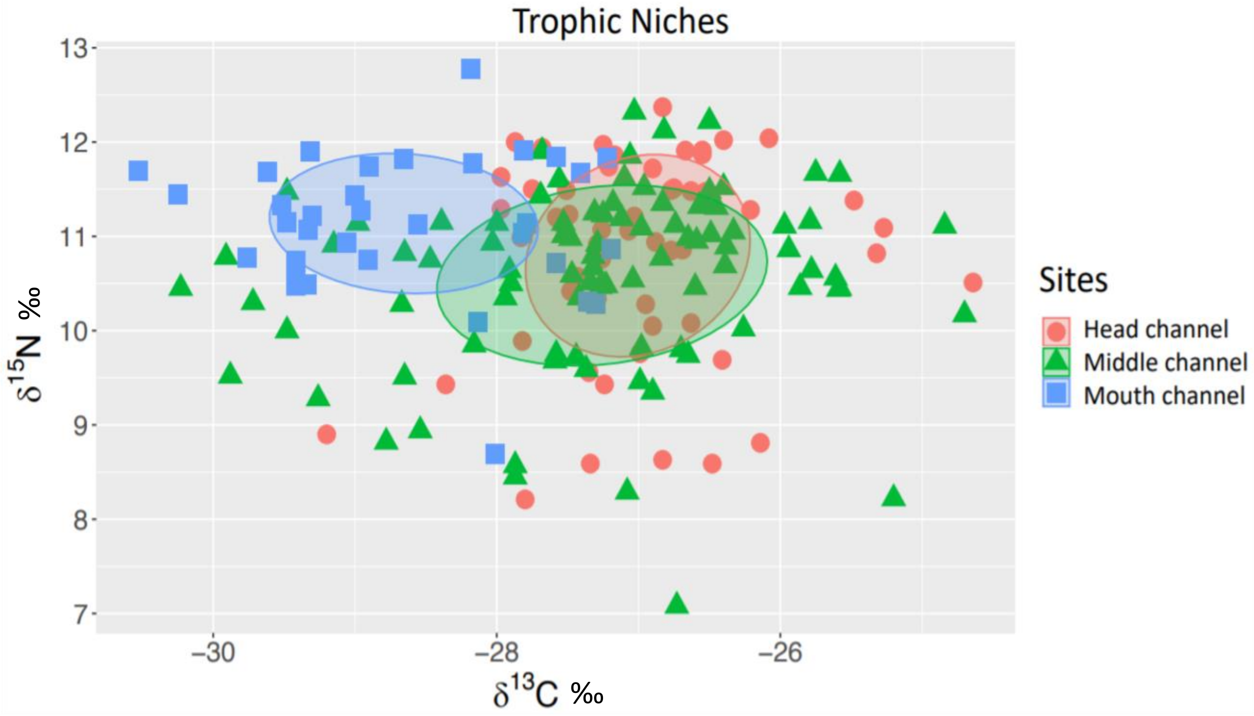

Figure 7. Trophic niches of marbled crayfish subpopulations caught in the three sections of channel (head, middle and mouth) are represented by ellipses. Dots, squares and triangles represent marbled crayfish individuals according to nitrogen and carbon isotope values (‰) and different sections.

Standard ellipse areas, by SIBER models, of trophic niches of head and mouth subpopulations were similar (2.56 ‰^{2}, 2.12 ‰^{2}) while trophic niche of middle was slightly wider (3.37 ‰^{2}) (Figure 8) due to a wider range of δ^{13}C and δ^{15}N values.

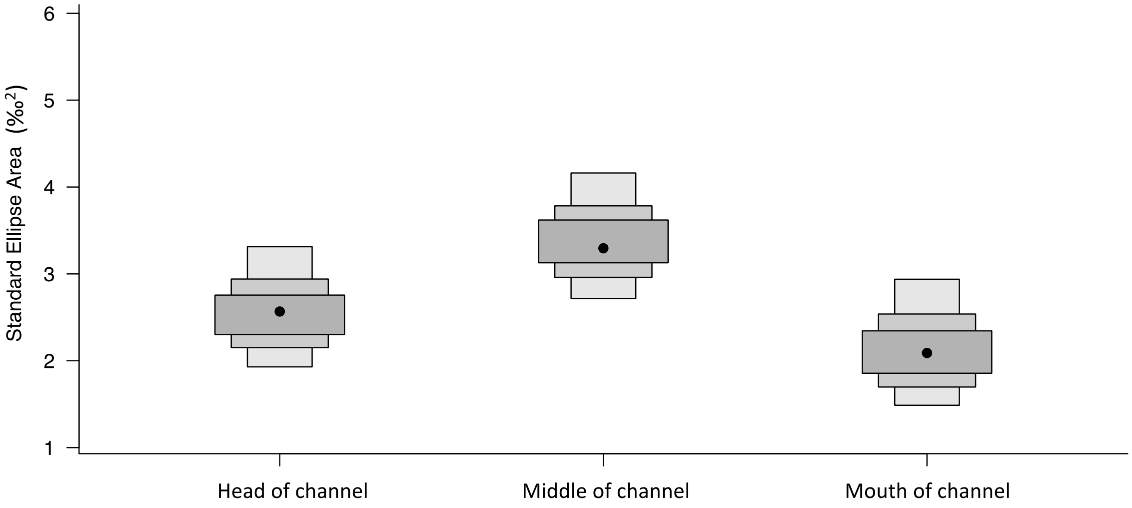

Figure 8. Mean values of Standard Ellipse Area (black dots) of each marbled crayfish subpopulations sampled in head, middle and mouth of the channel. Dark grey, light grey and outer light grey boxes represent 50%, 75% and 95% confidence intervals.

Trophic niches also differs from season to season (Figure 9). In spring and summer 2018 and 2019, trophic niches show similar trophic level, but different δ^{13}C (Figure 9). Results also show that trophic niches in spring and summer 2018 and 2019 displayed higher trophic level than autumn trophic niche, suggesting a more protein-rich diet in spring and summer and a more herbivory diet in autumn (Figure 9).

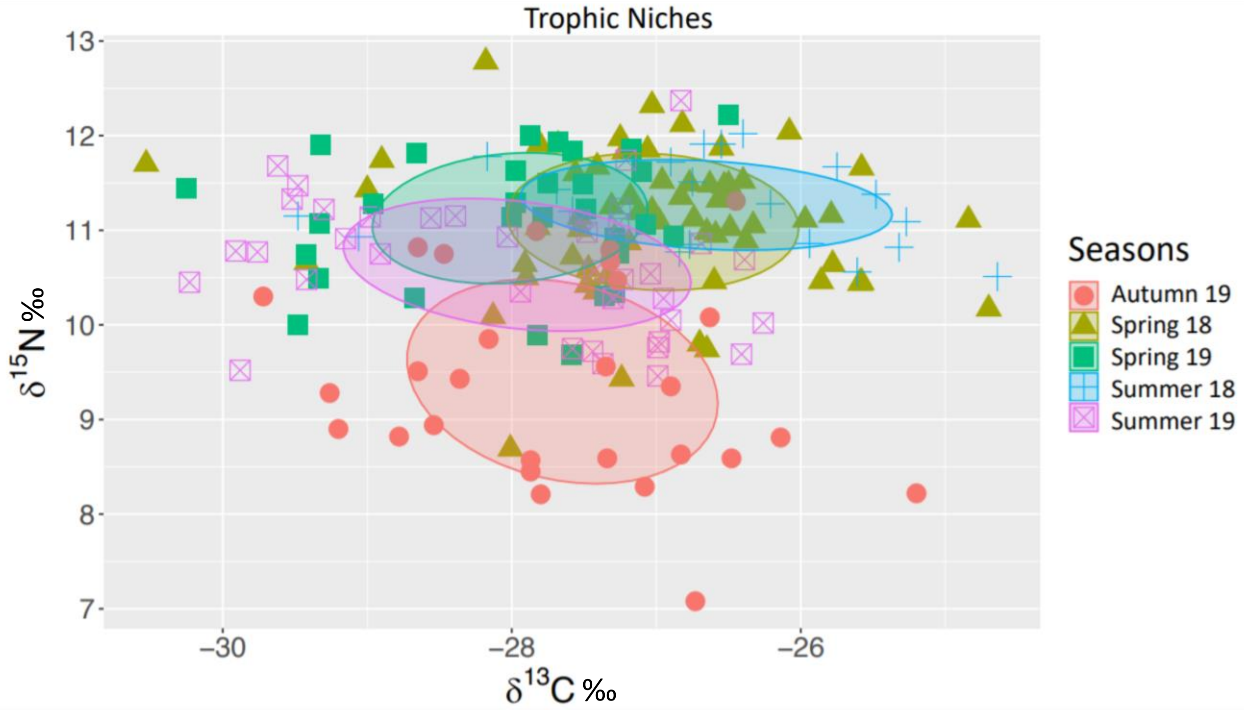

Figure 9. Trophic niches of marbled crayfish caught at different seasons are represented by ellipses. Points represent marbled crayfish individuals according to nitrogen and carbon isotope values (‰) and different seasons.

Standard ellipse areas of trophic niches of spring and summer 2018 and 2019 were fairly similar (2.21 ‰^{2}, 1.79 ‰^{2}, 1.97 ‰^{2}, 2.46 ‰^{2}) while trophic niche of autumn 2019 was slightly wider (3.42 ‰^{2}) (Figure 10), mostly due to wider range of δ^{15}N values.

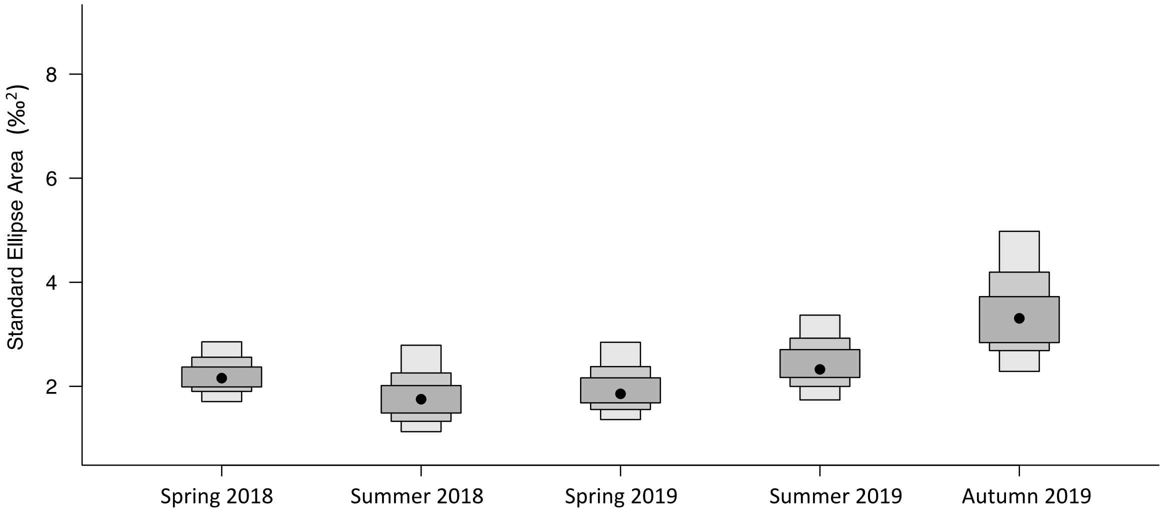

Figure 10. Mean values of Standard Ellipse Area (black dots) of marbled crayfish sampled in different seasons. Dark grey, light grey and outer light grey boxes represent 50%, 75% and 95% confidence intervals.

5. DISCUSSION

Contrary to our hypothesis results did not show a temperature gradient along the channel. The average temperatures between the studied sites showed no significant differences. However, as we hypothesised, water temperature, from each site in the outflow channel of Balti Power Plant, was significantly warmer than in Narva Reservoir. The daily average of air temperatures in Narva and the water temperature in Narva Reservoir shown fairly close values. However, water temperature in the channel was significantly warmer than in the Narva Reservoir proving that the power plant is affecting the temperature in the channel, making the habitat more suitable for marbled crayfish.

The optimal temperature range for marbled crayfish is 18-25°C (Vogt 2008), a range that were recorded in the channel. However, optimal temperature was recorded both in the channel as well as in Narva Reservoir, with temperatures staying on the lower side of the optimal range. Nevertheless, in Narva Reservoir the optimal water temperature was reached during summer months (June-August) with the highest temperature of 21°C (2018-2020), while temperature in the channel stayed in their optimal range from May to September with the highest temperature reaching 25°C. Longer period of high temperatures provide marbled crayfish with better conditions for growth and reproducing. Highest growth rate in laboratory conditions has been observed at 25°C (Seitz *et al.* 2005; Vogt 2008). Moreover, temperature and size are directly connected to reproduction. Thus, with the temperatures in the channel that stayed higher for longer period, gave to marbled crayfish population longer time to reproduce and growth, compared to Narva Reservoir. Although it would need further studies to investigate more on marbled crayfish reproduction in natural condition, our results suggest that it is likely that in the artificially warm channel, marbled crayfish could reproduce twice a year. This would have provided more suitable conditions for the establishment and growth of marbled crayfish population in the channel.

Only few marbled crayfish individuals were found outside the outflow channel during previous monitoring (Margo Hurt, pers. comm.), which supports the hypothesis that due to warmer temperature, the channel provides better habitat for marbled crayfish. However, although temperature is important factor for the population establishment, we did not find the temperature gradient along the channel, suggesting that temperature is not involved in the population distribution as we hypothesised.

CPUE decreased from head to mouth of the channel in both 2018 and 2019, during each season and, in autumn 2019, no marbled crayfish were caught from the mouth of the channel. However, the absence of temperature gradient in the channel suggests that there are other reasons behind the decreasing CPUE values from the head to mouth of the channel.

Stable isotope results showed differences in trophic niche and diet from head to mouth of the channel, which supports our hypothesis that trophic niche of marbled crayfish differs between the two sections, but was not due to the temperature gradient. Stable isotopes results also showed that differences in trophic niche and diet were due to a wide use of the food sources in the channel indicted by a substantial wide range of $\delta^{13}C$ values in crayfish individuals from head to mouth of the channel. Nevertheless, trophic niche of head and middle of channel strongly overlap, indicating same food sources use, consisted mostly of macroinvertebrates and macrophytes. However, trophic niche in the mouth of the channel is placed separately, showing substantial lower carbon isotopes values and indicating different food source use, consisted mostly of periphyton and to a lesser extent of macroinvertebrates. We believe that this difference were due to a different food source availability between head and mouth of channel. However, the lack of quantitative samples in this study make our hypothesis difficult to be tested. Surprisingly, contrary to the results found in Liptak *et al.* 2019, detritus was not an important food source for marbled crayfish in the studied channel.

We also noted a seasonal shift of trophic niches. Results showed trophic niches occupying higher trophic level during spring and summer seasons and lower in autumn, suggesting that marbled crayfish rely more on protein-rich diet in spring and summer, but switch to more herbivory diet in autumn. This might be due to the natural cycle of macroinvertebrates being less abundant during autumn.

The sharp decline in CPUE after autumn 2019 with only a couple of individuals found in the channel might be due to the cold temperatures during winter. Kaldre *et al.* (2016) showed in outdoor experiments that some individuals can survive three months at an average temperature of 5°C. They also noted that larger individuals seemed to be more tolerant of lower temperatures. The water temperature in the channel stayed below 5°C for 4 months from December 2019 to March 2020, which according to Kaldre *et al*. (2016) experiment results, it might has caused high mortality rates.

Kaldre *et al.* (2016) also found that marbled crayfish could survive less than one week at 1-2 °C and Haubrock *et al*. 2019 indicated that foraging activity stops at these temperatures. Data from the Balti Power Plant channel showed water temperatures reached low degrees during winter 2019-2020, with the lowest temperatures being less than 1°C. Data also showed that average temperature were under 2°C for 30 days straight from December 2019 to January 2020, and for 23 days in February, dropping down to less than 1°C for 17 days. Thus, it is likely that most of the crayfish in the channel died due to cold temperatures.

Unfortunately, we do not have water temperature data from the channel during previous winter 2018-2019. However, air temperatures and water temperatures in Narva Reservoir did not show colder winter from December 2018 to March 2019. Consequently, it might be that the abundance of marbled crayfish was high in spring 2019 due to the Power Plant activity. However, since we could not get the information from the Balti Power Plant neither about when the Power Plant was working nor the water temperatures from that period, we can only assume that the more mild winter 2018-2019 was the reason for the higher population abundance in spring 2019 compared to the spring 2020.

It is also possible that crayfish sampling had an impact on the population abundance in the channel. Adults crayfish can survive winter by lowering their activity and slowing down body functions (Bohman *et al*. 2013). Moreover, experiments have also showed that larger animals were more resistant to cold temperatures (Kaldre *et al*. 2016). Crayfish sampling was taken two times in 2018, three times in 2019 and again two times in 2020. Yet, the use of refuge traps ARTs were not so effective as expected since only few individuals were caught from them (Ercoli *et al.* 2019). This could mean that high percent of only adult marbled crayfish were caught from the channel, leaving the crayfish population composed mostly by juveniles which could not withstand the winter cold temperatures. It is possible then, that sampling, along with cold winter temperatures, had an impact on the population abundance.

In conclusion, based on our results we found that temperature is most likely the reason why marbled crayfish population established in the channel and has not yet spread in Narva Reservoir. However, in the channel temperature did not have a main role in the distribution of marbled crayfish population, which instead was likely affected by a different food source availability, showed by different trophic niche and diet along the channel. This might suggests that the reason why the CPUE declined from head to mouth, was probably due to a better choice of food sources in the head of the channel. Marbled crayfish has high adaptability towards wide range of temperatures and habitats and can strongly change food web structure of invaded ecosystems. Fluctuations of abundance are common in invasive species population establishment. We believe that, despite any specimens were found in 2020, and since one marbled crayfish individual is enough to start a new population, it is possible that abundance could start rising again in the near future. We also believe that increasing of temperature due to climate change might support the spreading of marbled crayfish outside the channel. However, further research are needed for understanding marbled crayfish reproduction and food source availability in the channel to predict its next invasion strategies.

SUMMARY

Marbled crayfish is a highly invasive species and unique among other decapod species due to its parthenogenetic reproduction. This species has been discovered in the outflow channel of Balti Power Plant in Narva Reservoir. In this thesis we studied its population distribution along the channel and its trophic niche using stable isotopes analyses of carbon and nitrogen. In particular, we used temperature data from different sections of the channel (head, middle and mouth) and from Narva Reservoir, to assess the role of temperature in the establishment, distribution and trophic niche of marbled crayfish in the channel. Marbled crayfish and its potential food sources (macroinvertebrates, macrophytes, periphyton and detritus) were sampled during spring and summer 2018 and spring, summer and autumn 2019.

Contrary to our hypothesis, results did not show significant differences in temperatures along the channel. However, temperature in the channel was significantly warmer than in Narva Reservoir, providing a more suitable habitat for the establishment and growth of marbled crayfish. CPUE values decreased significantly from head to mouth of the channel and sharply decreased after autumn 2019 in all sections. According to temperatures data, the sharp decreasing might be due to the low temperatures during previous winter when temperature dropping down to less than 2 °C.

Stable isotope results showed differences between the head and mouth of the channel and between seasons. Carbon isotope values shifted from enriched to depleted, from head to mouth of the channel, with mean $\delta\ ^{13}C$ value of -27,00 and -28,65 respectively. Nitrogen values differ in middle and mouth of the channel showing lower trophic level in the middle due to the lower nitrogen isotopes values registered in autumn. In the head and middle of the channel the diets were similar and consisted mostly of macroinvertebrates and macrophytes, however individuals in the end of the channel rely more on periphyton and at lesser extent on macroinvertebrates. Diet changed from more protein-rich diet in spring and summer, to a more herbivory diet in the autumn, most likely due to a less availability of macroinvertebrates during autumn. Trophic niches also indicated spatial and seasonal variation in line with stable isotopes results. While

trophic niches in head and middle of the channel were overlapping, indicating similar food source use, head and mouth were placed separately, showing different food source utilization. In spring and summer trophic niches showed higher trophic level compared to autumn in which marbled crayfish indicated a less consume of macroinvertebrates.

In conclusion, we did not find a temperature gradient in the channel as we had hypothesised. However, we did find that temperature in the channel was significantly warmer than in Narva Reservoir, which provides more suitable habitat for marbled crayfish. Consequently, temperature has important role in establishment of marbled crayfish population, but our results indicate it is not the main factor for the population distribution in the channel. Based on our results, distribution might be due to better food availability, for example macroinvertebrates, in the head of the channel.

ÜLDKOKKUVÕTE

Temperatuuri mõju invasiivse marmorvähi levikule

Marmorvähk (*Procambarus virginalis*) on üks kolmest Eestis leitud võõrvähiliigist. Tegu on invasiivse liigiga, kes on eriline oma partenogeense paljunemise poolest. Käesolevas magistritöös uurisime liigi populatsiooni jaotumist mööda kanalit ning ökoloogilist nišši. Selleks kasutasime temperatuuriandmeid kanali erinevatest osadest ning Narva veehoidlast (Kulgu sadamast). Marmorvähi isendid püüti 2018. aasta kevadel ning suvel ning 2019. aasta kevadel, suvel ning hilissügisel. Lisaks koguti nende potensiaalseid toiduobjekte (suurselgrootud, makrofüüdid, perifüüton ja detriit). Kogutud proovid valmistasin ette stabiilsete isotoopide analüüsiks. Töös on kasutatud lämmastiku ja süsiniku stabiilsete isotoopide analüüsi, et uurida, kuidas muutub ökoloogiline nišš mööda kanalit.

Vastupidiselt töös püstitatud hüpoteesile ei näidanud tulemused kanalis temperatuuri gradienti. Küll aga saime kinnitust, et vee temperatuur soojuselektrijaama väljavoolu kanalis oli oluliselt kõrgem kui Narva veehoidlas ($p < 0.001$), võimaldades seega paremaid tingimusi populatsiooni asutamiseks ning kiiremaks kasvuks. Arvukus vähenes kanali algusest suudmesse ja 2019. aastal oli arvukuses järsk langus. Temperatuuriandmed viitavad, et järsk arvukuse vähenemine võib olla tingitud külmast temperatuurist, kui veetemperatuur langes nädalateks alla 2 °C.

Isotoopide analüüs näitab erinevust kanali algusest kanali suudme poole liikudes. Kanali alguse ja keskosa ökoloogiline nišš kattub, kuid kanali suue on oluliselt erinev. Süsiniku väärtused langevad kanali algusest (keskmine $\delta^{13}C$ väärtus -27,00) suudmesse (-28,65). Lämmastiku väärtused näitasid olulist erinevust kanali keskosa ning suudme vahel ($p=0,0021$).

Stabiilsete isotoopide tulemused näitasid muutusi marmorvähi ökoloogilises niššis ning toidus. Muutusi oli näha kanali alguse ning suudme vahel ning samuti eri aastaaegadel. Kanali algus ning keskosa olid üldjoontes sarnased ning vähkide põhilisteks toiduobjektideks olid suurselgrootud ning makrofüüdid. Kanali suudmes, aga koosnes nende toit põhiliselt perifüütonist. Muutusi oli märgata ka eri aastaaegade vahel. Kevadel ning suvel toitusid marmorvähid rohkem suurselgrootutest ning sügisel on märgata üleminekut taimestiku põhisemaks.

Kokkuvõtteks võib öelda, et kanalist temperatuurigradienti me ei leidnud. Küll aga näitasid tulemused, et temperatuur oli kanalis oluliselt kõrgem kui Narva veehoidlas, tänu millele on soojuselektrijaama väljavoolu kanal marmorvähkidele sobivam elupaik. Temperatuuril on oluline roll populatsiooni asutamisel, kuid meie tulemuste põhjal ei mõjuta see populatsiooni tihedust mööda kanalit. Stabiilsete isotoopide tulemused näitasid ökoloogilise nišši muutusi kanali algusest suudmesse liikudes. Tulemuste põhjal võime öelda, et populatsiooni tihedust kanalis mõjutavad teised ökoloogilised aspektid, nagu näiteks parem toidu kättesaadavus kanali ülemises osas.

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APPENDIXES

Appendix 1. Carbon and nitrogen stable isotopes, length and weight values of marbled crayfish individuals from different sections and seasons.

Seasons	Sections	$\delta^{13}C$	$\delta^{15}N$	Length	Weight
Spring 2018	head	-27.24	9.43	42.90	24.98
Spring 2018	head	-26.53	11.47	45.26	20.08
Spring 2018	head	-26.55	11.87	41.63	15.72
Spring 2018	head	-27.25	11.97	40.79	15.82
Spring 2018	head	-26.63	11.48	37.25	10.33
Spring 2018	head	-26.08	12.04	44.06	21.71
Spring 2018	head	-27.48	10.42	36.52	9.42
Spring 2018	head	-26.77	11.49	43.86	19.67
Spring 2018	head	-27.03	11.21	45.06	22.36
Spring 2018	middle	-26.59	10.95	39.65	13.54
Spring 2018	middle	-27.53	11.01	43.64	19.16
Spring 2018	middle	-24.70	10.17	52.94	35.35
Spring 2018	middle	-26.60	10.46	40.02	16.32
Spring 2018	middle	-26.65	10.98	40.39	18.40
Spring 2018	middle	-26.70	9.80	36.51	10.53
Spring 2018	middle	-27.03	12.32	37.49	11.93
Spring 2018	middle	-27.12	11.19	37.37	9.97
Spring 2018	middle	-26.98	11.09	47.46	24.64
Spring 2018	middle	-24.84	11.11	39.06	12.97
Spring 2018	middle	-27.42	10.35	38.83	11.45
Spring 2018	middle	-27.90	10.50	40.47	13.23
Spring 2018	middle	-26.82	12.12	38.39	11.26
Spring 2018	middle	-27.34	10.51	33.15	7.41
Spring 2018	middle	-27.56	11.60	29.58	4.93

Spring 2018	middle	-26.49	11.02	35.96	9.07
Spring 2018	middle	-26.33	11.05	36.32	9.66
Spring 2018	middle	-25.59	10.44	33.51	7.22
Spring 2018	middle	-26.65	9.74	27.25	5.26
Spring 2018	middle	-26.74	11.12	31.72	6.32
Spring 2018	middle	-27.06	11.85	45.02	20.38
Spring 2018	middle	-26.57	11.32	41.46	19.23
Spring 2018	middle	-26.50	11.48	40.46	15.62
Spring 2018	middle	-25.79	11.16	47.66	29.59
Spring 2018	middle	-27.53	11.14	46.57	25.06
Spring 2018	middle	-27.18	11.35	42.07	16.43
Spring 2018	middle	-25.97	11.11	43.09	15.84
Spring 2018	middle	-25.58	10.45	38.87	13.51
Spring 2018	middle	-25.58	11.66	47.66	22.09
Spring 2018	middle	-25.78	10.64	31.75	6.13
Spring 2018	middle	-26.96	11.52	33.15	7.15
Spring 2018	middle	-26.38	10.89	37.49	10.08
Spring 2018	middle	-27.91	10.64	30.02	5.72
Spring 2018	middle	-27.29	10.92	33.63	7.62
Spring 2018	middle	-25.86	10.46	36.43	10.27
Spring 2018	middle	-26.53	11.33	37.51	13.52
Spring 2018	middle	-26.40	11.52	29.64	5.29
Spring 2018	middle	-26.83	11.35	28.94	5.43
Spring 2018	middle	-27.68	11.90	40.90	11.96
Spring 2018	middle	-27.47	10.59	28.08	4.15
Spring 2018	middle	-27.31	11.25	43.86	19.67
Spring 2018	mouth	-27.41	11.67	46.81	23.19
Spring 2018	mouth	-29.42	10.66	36.76	10.49

Spring 2018	mouth	-27.81	11.91	51.76	32.36
Spring 2018	mouth	-29.00	11.43	42.10	17.80
Spring 2018	mouth	-27.58	10.72	41.68	16.79
Spring 2018	mouth	-28.13	10.09	37.33	12.23
Spring 2018	mouth	-28.18	12.78	38.75	13.63
Spring 2018	mouth	-27.22	11.83	53.33	31.16
Spring 2018	mouth	-27.82	11.03	43.76	18.94
Spring 2018	mouth	-28.90	11.74	39.37	14.81
Spring 2018	mouth	-27.19	10.87	51.26	31.44
Spring 2018	mouth	-28.01	8.69	30.81	6.05
Spring 2018	mouth	-30.53	11.70	23.58	3.45
Summer 2018	head	-25.27	11.09	50.98	35.90
Summer 2018	head	-26.40	12.02	51.43	29.55
Summer 2018	head	-27.58	11.20	42.27	19.23
Summer 2018	head	-25.32	10.82	43.37	19.20
Summer 2018	head	-24.64	10.51	40.93	16.44
Summer 2018	head	-26.21	11.28	40.18	16.34
Summer 2018	head	-26.55	11.91	41.50	16.14
Summer 2018	head	-26.75	11.51	36.47	11.91
Summer 2018	head	-25.48	11.38	39.57	16.56
Summer 2018	head	-26.67	11.91	38.26	12.23
Summer 2018	head	-26.90	11.72	36.19	11.12
Summer 2018	head	-26.77	10.85	37.51	11.59
Summer 2018	middle	-25.94	10.86	37.33	12.23
Summer 2018	middle	-25.75	11.67	48.79	30.39
Summer 2018	middle	-26.84	10.77	49.73	28.91
Summer 2018	middle	-27.69	11.43	48.23	25.41
Summer 2018	middle	-25.61	10.56	35.17	11.18

Summer 2018	mouth	-28.17	11.78	36.02	10.02
Summer 2018	mouth	-29.48	11.15	51.21	32.06
Summer 2018	mouth	-29.06	10.93	30.23	5.76
Spring 2019	head	-27.87	12.00	26.70	3.90
Spring 2019	head	-27.49	11.23	43.30	18.90
Spring 2019	head	-27.97	11.29	33.20	7.30
Spring 2019	head	-27.51	11.49	34.70	8.10
Spring 2019	head	-27.97	11.63	31.50	6.70
Spring 2019	head	-27.26	10.76	31.00	5.80
Spring 2019	head	-27.75	11.50	36.20	9.80
Spring 2019	head	-27.68	11.94	46.20	21.50
Spring 2019	head	-27.07	11.06	40.50	15.20
Spring 2019	head	-27.82	9.89	33.40	7.50
Spring 2019	head	-27.29	10.34	35.90	9.80
Spring 2019	head	-27.29	10.92	43.90	18.10
Spring 2019	head	-27.17	11.86	41.50	17.70
Spring 2019	head	-26.88	10.94	37.70	11.00
Spring 2019	middle	-26.50	12.22	43.40	19.00
Spring 2019	middle	-27.10	11.62	46.10	23.10
Spring 2019	middle	-27.59	9.68	39.20	12.20
Spring 2019	middle	-29.48	10.00	32.50	6.80
Spring 2019	middle	-28.00	11.14	50.30	29.20
Spring 2019	middle	-28.67	10.28	33.20	7.20
Spring 2019	mouth	-29.42	10.74	49.80	27.10
Spring 2019	mouth	-29.33	11.07	28.60	4.50
Spring 2019	mouth	-27.36	10.31	50.30	27.70
Spring 2019	mouth	-29.34	10.49	34.20	8.40
Spring 2019	mouth	-29.32	11.90	32.10	6.50

Spring 2019	mouth	-27.58	11.84	51.80	30.10
Spring 2019	mouth	-28.96	11.28	40.80	15.90
Spring 2019	mouth	-27.79	11.14	44.00	21.10
Spring 2019	mouth	-30.25	11.44	40.50	14.70
Spring 2019	mouth	-28.66	11.82	40.70	15.60
Summer 2019	head	-27.26	11.07	44.80	19.30
Summer 2019	head	-26.41	9.69	38.50	11.80
Summer 2019	head	-26.99	9.76	40.60	14.30
Summer 2019	head	-26.69	10.86	35.30	10.30
Summer 2019	head	-26.83	12.37	44.30	18.70
Summer 2019	head	-27.53	11.11	35.90	10.30
Summer 2019	head	-27.21	11.74	48.30	22.90
Summer 2019	head	-27.43	10.57	42.50	17.80
Summer 2019	head	-26.90	10.05	34.80	9.00
Summer 2019	head	-26.95	10.28	39.70	14.60
Summer 2019	middle	-26.99	9.46	39.00	14.00
Summer 2019	middle	-26.39	10.69	48.60	22.70
Summer 2019	middle	-26.98	9.82	39.80	14.40
Summer 2019	middle	-27.23	10.48	42.60	17.40
Summer 2019	middle	-27.37	9.59	43.40	17.30
Summer 2019	middle	-27.94	10.35	41.50	16.50
Summer 2019	middle	-27.44	9.72	36.30	11.00
Summer 2019	middle	-27.25	11.23	49.20	24.00
Summer 2019	middle	-27.48	10.98	48.10	23.30
Summer 2019	middle	-29.88	9.52	25.10	2.90
Summer 2019	middle	-29.15	10.91	39.00	10.50
Summer 2019	middle	-27.58	9.75	42.20	17.10
Summer 2019	middle	-27.04	10.54	41.30	16.60

Summer 2019	middle	-28.98	11.14	46.80	23.30
Summer 2019	middle	-28.39	11.15	45.10	21.60
Summer 2019	middle	-29.91	10.78	32.20	7.40
Summer 2019	middle	-26.26	10.02	43.10	18.50
Summer 2019	middle	-29.48	11.47	49.70	25.30
Summer 2019	middle	-28.03	10.93	38.80	12.10
Summer 2019	middle	-30.23	10.45	30.50	5.70
Summer 2019	mouth	-29.42	10.48	32.40	7.20
Summer 2019	mouth	-27.30	10.28	40.10	15.40
Summer 2019	mouth	-29.30	11.22	38.80	13.20
Summer 2019	mouth	-29.52	11.33	26.80	4.20
Summer 2019	mouth	-28.91	10.75	32.40	7.90
Summer 2019	mouth	-29.76	10.77	26.90	4.10
Summer 2019	mouth	-29.62	11.68	27.80	4.50
Summer 2019	mouth	-28.56	11.13	33.30	8.70
Autumn 2019	head	-27.80	8.21	40.30	14.90
Autumn 2019	head	-26.14	8.81	36.80	11.80
Autumn 2019	head	-27.35	9.56	37.20	11.40
Autumn 2019	head	-29.20	8.90	39.80	14.70
Autumn 2019	head	-26.63	10.08	38.70	12.50
Autumn 2019	head	-28.36	9.43	38.30	12.40
Autumn 2019	head	-26.48	8.59	35.60	9.50
Autumn 2019	head	-27.83	10.99	48.90	28.20
Autumn 2019	head	-26.83	8.63	34.50	8.10
Autumn 2019	head	-27.34	8.59	35.50	10.00
Autumn 2019	middle	-26.90	9.35	36.70	10.30
Autumn 2019	middle	-27.87	8.57	29.00	5.10
Autumn 2019	middle	-28.54	8.94	28.50	4.60

Autumn 2019	middle	-27.32	10.67	45.70	19.20
Autumn 2019	middle	-27.87	8.45	27.30	4.20
Autumn 2019	middle	-27.27	10.47	41.40	13.70
Autumn 2019	middle	-29.26	9.28	24.90	3.00
Autumn 2019	middle	-28.16	9.85	24.30	3.10
Autumn 2019	middle	-25.20	8.22	40.80	15.80
Autumn 2019	middle	-27.32	10.79	44.50	20.30
Autumn 2019	middle	-27.08	8.29	31.40	3.80
Autumn 2019	middle	-28.65	10.82	41.60	16.90
Autumn 2019	middle	-26.73	7.08	26.10	3.40
Autumn 2019	middle	-28.78	8.82	27.30	3.90
Autumn 2019	middle	-27.35	10.17	47.30	23.60
Autumn 2019	middle	-26.45	11.31	51.60	32.80
Autumn 2019	middle	-28.47	10.75	43.60	18.20
Autumn 2019	middle	-29.72	10.30	26.60	3.60
Autumn 2019	middle	-28.65	9.51	25.00	3.00

Lihtlitsents lõputöö salvestamiseks ja üldsusele kättesaadavaks tegemiseks ning juhendaja(te) kinnitus lõputöö kaitsmisele lubamise kohta

Mina, Johanna-Maria Muuga,
(sünnipäev 27/02/1995)

1. annan Eesti Maaülikoolile tasuta loa (lihtlitsentsi) enda loodud lõputöö
Temperatuuri mõju invasiivse marmorvähi levikule,
mille juhendaja on Fabio Ercoli ja Paul Teesalu,
   1.1. salvestamiseks säilitamise eesmärgil,
   1.2. digiarhiivi DSpace lisamiseks ja
   1.3. veebikeskkonnas üldsusele kättesaadavaks tegemiseks

kuni autoriõiguse kehtivuse tähtaja lõppemiseni;

2. olen teadlik, et punktis 1 nimetatud õigused jäävad alles ka autorile;

3. kinnitan, et lihtlitsentsi andmisega ei rikuta teiste isikute intellektuaalomandi ega isikuandmete kaitse seadusest tulenevaid õigusi.

Lõputöö autor ________________________________
allkiri

Tartu, 25.05.2021

Juhendaja(te) kinnitus lõputöö kaitsmisele lubamise kohta

Luban lõputöö kaitsmisele.

________________________________ ____________________
(*juhendaja nimi ja allkiri*) (*kuupäev*)

________________________________ ____________________
(*juhendaja nimi ja allkiri*) (*kuupäev)*