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Predation by invasive alien water frogs (*Pelophylax*) in Southwest Finland

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Biological invasions are a major cause of biodiversity loss worldwide. Water frogs (*Pelophylax* spp.) have expanded beyond their native range in Europe. In Finland, invasive water frog populations have established in the southwest, yet their dietary habits and potential impacts on native fauna remain poorly understood. In this study, water frogs were collected from two habitats in Southwest Finland during summer and autumn 2023 (n = 73) to investigate their diet. Intestinal contents were analysed using DNA metabarcoding with COI and 12S markers. Sequence data were processed into sequence variants and identified to taxa. Prey composition was analysed using redundancy analysis, similarity percentage analysis, and diversity metrics. A new metric, the Potential Biodiversity Pressure Index (PBPI), was developed and applied. DNA metabarcoding of 68 edible frogs (*Pelophylax* kl. *esculentus*) revealed a diverse diet of 104 prey taxa across 26 orders, dominated by Coleoptera, Diptera, Trichoptera, and Hymenoptera. Diet included terrestrial, semi-aquatic, and aquatic prey, indicating cross-habitat foraging. Native amphibians *Rana arvalis* were found in the diet. Redundancy analysis showed significant effects of site and season on prey composition, while size and sex were nonsignificant. PBPI highlighted elevated threat by edible frog predation on Trichoptera and Odonata. Edible frogs exhibit a generalist feeding strategy likely shaped by prey availability and seasonal variation. High PBPI on Odonata indicates potential impacts on this insect group of conservation concern. These findings underscore the frogs' role as an ecotonal predator and emphasize the need for monitoring and management of invasive water frog populations in Finland.

Key words: *Pelophylax*, water frogs, invasive alien species, predation, prey composition, DNA metabarcoding, Southwest Finland

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Haitalliset vieraslajit ovat yksi merkittävimmistä syistä biodiversiteetin vähenemiseen maailmanlaajuisesti. Sammakkoeläimistä erityisesti vihersammakot (*Pelophylax*-suvun lajit) ovat viime vuosina levinneet alkuperäisen esiintymisalueensa ulkopuolelle Euroopassa. Suomessa nämä haitalliset vieraslajit ovat vakiintuneet erityisesti Lounais-Suomessa, mutta niiden ravinnonkäytöstä ja vaikutuksista alkuperäisiin lajeihin tiedetään vasta hyvin vähän. Tässä tutkimuksessa vihersammakoita kerättiin kahdelta paikalta Lounais-Suomessa kesän ja syksyn 2023 aikana (n = 73). Suoliston sisältö analysoitiin DNA-massaviivakoodauksen avulla käyttäen kahta kohdealuetta (COI ja 12S). Sekvenssidata puhdistettiin ja analysoitiin sekvenssivarianteiksi, jotka tunnistettiin lajeihin. Saalisyhteisön koostumusta tarkasteltiin redundanssianalyysillä, prosentuaalisella samankaltaisuustestillä ja monimuotoisuusanalyysien avulla. Tutkimuksen aikana kehitettiin myös potentiaalinen biodiversiteettipaineen indeksi (PBPI), joka paljasti vihersammakoiden uhkaamat hyönteislahkot. Massaviivakoodaus 68 ruokasammakolta (*Pelophylax* kl. *esculentus*) paljasti monipuolisen ravinnon, johon kuului 104 saalistaksonia. Saalisyhteisöä hallitsivat kovakuoriaiset (Coleoptera), kaksisiipiset (Diptera), vesiperhoset (Trichoptera) ja pistiäiset (Hymenoptera). Ravinto koostui pääosin maalla esiintyvistä lajeista, mutta myös vesieliöitä löytyi näytteistä, mikä osoittaa ruokasammakoiden saalistusvaikutuksen molempiin elinympäristöihin. Ruokasammakon saaliissa havaittiin myös kotimaisia sammakkolajeja, kuten *Rana arvalis*. Redundanssianalyysi osoitti saalisyhteisön vaihtelevan merkittävästi paikasta ja vuodenajasta riippuen. Sen sijaan sammakon koko ja sukupuoli eivät vaikuttaneet tässä tutkimuksessa saalislajiyhteisön koostumukseen. PBPI-analyysi paljasti, että ruokasammakot kohdistavat suurinta suhteellista painetta vesiperhosiin (Trichoptera) ja sudenkorentoihin (Odonata). Ruokasammakot ovat moniruokaisia ja niiden ravinto määräytyy todennäköisesti paikallisen saalislajiston ja kausivaihtelun mukaan. Ruokasammakoiden aiheuttama korkea biodiversiteettipaine tiettyjä hyönteislahkoja kohtaan (PBPI) sekä niiden alkuperäisiin sammakkolajeihin kohdistama saalistus voivat vaikuttaa paikallisten hyönteispopulaatioiden rakenteeseen ja alkuperäisten sammakkolajien populaatioihin. Nämä havainnot korostavat vihersammakoiden roolia haitallisina vieraslajeina ja tarvetta seurata sekä hallita niiden populaatioita Suomessa.

Avainsanat: *Pelophylax*, vihersammakot, haitallinen vieraslaji, saalistus, saalislajisto, DNA-massaviivakoodaus, Varsinais-Suomi

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1 Introduction

1.1 Anthropocene extinction

The ongoing sixth mass extinction, also referred to as the Anthropocene extinction, is marked by the rapid loss of species, predominantly driven by human activities. This extinction event is comparable to the five previous mass extinctions in Earth's history, which were caused by natural catastrophes (Barnosky et al., 2011). According to the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), the main drivers of biodiversity loss are changes in land and sea use, direct exploitation of organisms, climate change, pollution, and invasive alien species (IPBES, 2019). Among these five principal drivers, the introduction of invasive alien species constitutes a major factor, frequently identified as a significant contributor to biodiversity decline (Clavero & García-Berthou, 2005; Didham et al., 2007; Ricciardi, 2004).

1.2 Invasive species

Invasive species are organisms that have been intentionally or unintentionally introduced by humans to new environments, often leading to substantial economic, social and ecological consequences (Pyšek & Richardson, 2010; Simberloff et al., 2013). Diagne et al. (2021) estimated that the minimum economic cost of biological invasions between 1970 and 2017 amounted to approximately 1.288 trillion U.S. dollars. Beyond economic impacts, invasive species also pose significant social challenges, affecting various aspects of human life and well-being. These impacts can be both direct and indirect, influencing for example health, cultural practices, and community well-being (Schindler et al., 2015; Shackleton et al., 2019). Furthermore, invasive species pose a significant threat to global biodiversity. Globally, 14% of critically endangered terrestrial vertebrate species are threatened by invasive species, with the percentage rising to 28% on islands (Dueñas et al., 2021).

Although invasive species are often viewed negatively, it is important to recognize that in certain contexts, invasive species may also exert positive effects, contingent on the perspective from which the issue is evaluated (Goodenough, 2010). Both the beneficial and detrimental impacts of invasive species should be considered in a balanced manner, with no inherent prioritization of one over the other (Vimercati et al., 2020). Taking the above factors into account, a case-by-case assessment is essential for a comprehensive understanding of the diverse ecological, economic, and social consequences of invasive species.

1.2.1 Ecological effects of invasive species

As discussed above, invasive species pose significant threats to ecosystems and biodiversity worldwide and their control can be challenging, especially if they have already become established in an area (Green & Grosholz, 2021; Mack et al., 2000). One of the most pronounced ecological impacts is biodiversity loss, as invasive species often drive native species to extinction through competition, predation, or the introduction of pathogens (Bissattini et al., 2019; Doherty et al., 2016; Vilcinskas, 2015). Additionally, invasive species can profoundly alter ecosystem functioning by changing the dynamics, composition, and structure of ecosystems (Vilà et al., 2006). These changes can disrupt critical ecological functions such as nutrient cycling, habitat maintenance, and the retention of soil and nitrogen (Lázaro-Lobo et al., 2023). These changes can destabilize ecological systems, reduce ecosystem resilience to environmental changes, and hinder restoration efforts, or, in some cases, facilitate the establishment of alternative stable states of the ecosystem (Chaffin et al., 2016). Moreover, invasive species can have cross-ecosystem effects, impacting not only the ecosystems they invade but also adjacent ecosystems (Peller & Altermatt, 2024). In this way, by altering nutrient cycles, disrupting trophic networks, and changing species interactions, invasive species can destabilize ecosystem balance and health, often leading to cascading effects across landscapes.

1.2.2 Competition between invasive and native species

Invasive species often outperform native species in terms of growth rates and reproductive success (Jaspers et al., 2018). This allows them to compete more effectively for limited resources such as food, shelter, and space (Perdereau et al., 2011; Smith, 2005). As a result, invasive species may ultimately drive native species to extinction by outcompeting them for essential resources, though such cases appear to be relatively rare (Davis, 2003; Gurevitch & Padilla, 2004). Moreover, the competitive advantage of invasive species can be further enhanced by their ability to tolerate novel enemies or escape from natural enemies, allowing them to sustain dominance even in hostile environments (Keane & Crawley, 2002). However, this alone does not fully account for their success (Colautti et al., 2004).

1.2.3 Predation by invasive species

In addition to competition, invasive species contribute more strongly to the extinction of native species through predation (Gurevitch & Padilla, 2004). Previous research has shown that alien predators tend to have roughly twice the impact on prey populations as native predators (Salo

et al., 2007). Invasive predators may directly prey on native species, leading to declines or even local extinctions (Doherty et al., 2016). For example, research has shown that invasive frog species prey on native amphibians, posing a threat to local populations (Pille et al., 2021; Wilson et al., 2018). Additionally, invasive species can indirectly impact native species by increasing the population of their natural predators, a phenomenon known as apparent competition, which can lead to greater predation pressure and further decline of native prey species (Pille et al., 2021; Wilson et al., 2018).

1.2.4 Introduction of diseases by invasive species

Another major threat posed by invasive species is the introduction of diseases to which native species have little or no resistance (Young et al., 2017). These diseases can spread from invasive species to native species, leading to significant population declines and, in some cases, the collapse of native species (Vilcinskas, 2015). Invasive species can alter disease dynamics by acting as hosts for native pathogens (spillback) or introducing new diseases to native species (spillover), which can affect local populations and disrupt ecosystems (Atkinson & Savage, 2023; Kelly et al., 2009). In some cases, diseases carried by invasive species have even been explored as potential tools for their biological control (McColl et al., 2016). Interestingly, invasive species themselves may sometimes be affected by the very diseases they introduce, but only if native species evolve resistance to these diseases (García-Ramos et al., 2015).

1.2.5 Additional factors

Invasive species can also alter the physical environment in ways that further harm native species. Invasive plants can modify habitats by altering soil characteristics and microclimates, changing ecosystem structure and making it less conducive to native species (Ruckli et al., 2013). These changes, when combined with the direct effects of invasive species, can create a vicious cycle of ecosystem degradation. Degraded habitats are more vulnerable to further invasions, and once invasive species become established, they can accelerate habitat loss and potentially drive native species to extinction (Marvier et al., 2004).

The combined effects of competition, predation, disease introduction, and habitat modification create a complex and multi-faceted challenge for conservation efforts. Addressing the ecological impacts of invasive species requires a comprehensive understanding of these mechanisms and their interactions with native ecosystems. This becomes even more

challenging when dealing with species that influence not just a single ecosystem but function across two different ecosystems simultaneously, as is the case with invasive amphibians.

1.3 Amphibians and invasive species

Amphibians represent a somewhat controversial group when it comes to invasive species. Freshwater habitats often face challenges from invasive alien species, which have significant detrimental effects, particularly on the vulnerable and declining native amphibian populations (Beebee & Griffiths, 2005; Falaschi et al., 2020). Currently, 16% of amphibians listed on the International Union for Conservation of Nature (IUCN) Red List are threatened by invasive alien species (Nunes et al., 2019). Since the 1980s, an increasing number of studies have highlighted the negative impact of invasive alien predators on native amphibian populations (Kats & Ferrer, 2003; Kraus, 2015). Although amphibians face threats from invasive species, anurans are among the most frequently reported invasive species (Bucciarelli et al., 2014; Kraus, 2015; Lever, 2003). Due to their ease of translocation, high reproductive rates, and generalist diet, invasive anurans have become a significant concern for conservation efforts (Pitt et al., 2005). Predator–prey interactions between invasive alien anurans and native species can affect organisms at various trophic levels, including those of higher, equivalent, or lower rank (Kraus, 2015). Research has shown that some amphibians are more likely to become invasive species than others, for example water frogs (*Pelophylax* Fitzinger, 1843, Ranidae Rafinesque, 1814) have a high potential to become invasive species (Denoël et al., 2022). In Europe, the animal trade has led to multiple invasions of water frogs (Pille et al., 2021).

1.4 Water frogs as invasive species

According to recent studies, the genus *Pelophylax*, commonly known as water frogs, includes 12 species (formerly around 25 species), excluding *P. demarchii* (Scortecci, 1929) due to being known from only a single specimen with uncertain provenance and ongoing doubts regarding its taxonomic validity, as well as a lack of information on its extent of occurrence, status, and ecological requirements, while *P. lateralis* (Boulenger, 1887), now classified under the family *Hylarana* Tschudi, 1838, is also excluded (Dufresnes et al., 2024; IUCN, 2015; Wu et al., 2025). The water frogs have been extensively studied in Europe due to their remarkable reproductive mechanism, where sympatric hybridization between genetically distinct parental species results in a wide variety of genetically viable hybrid forms (Radojičić et al., 2015). Notable species examples in Europe being *P. lessonae* (Camerano, 1882) (the pool frog), *P.*

ridibundus (Pallas, 1771) (the marsh frog), and their hybrid *P.* kl. *esculentus* (Linnaeus, 1758) (the edible frog) (Figure 1).

Figure 1. Edible frog (*Pelophylax* kl. *esculentus*) in the author's hand at Rusko gravel pit, 5 September 2025. Photo: Anssi Teräs.

Biological invasions by *Pelophylax* species, particularly *P. ridibundus* have been documented across Western Europe, posing threats to native populations and ecosystems (Dufresnes et al., 2024; Kolenda et al., 2024; Pille et al., 2021). The widespread movement of Anatolian (*P. r. bedriagae*) and Balkan (*P. r. ridibundus* and *P. r. kurtmuelleri*) marsh frogs across Europe, driven by the frog legs trade, has made these lineages a pervasive yet overlooked invasive species, enabling them to colonize habitats where they were previously absent and threatening other taxa, including native *Pelophylax* species and genotypes (Dufresnes et al., 2024). Understanding the ways in which invasive water frogs affect ecosystems is crucial for developing effective management and conservation strategies to mitigate their impacts.

1.4.1 Generalist feeding strategy of water frogs

Water frogs are semi-aquatic ambush predators that employ a "sit-and-wait" foraging strategy, actively hunting both diurnally and nocturnally (Cogălniceanu et al., 2001; Moore & Biewener, 2015). The effectiveness of this approach is influenced by variables such as prey availability, prey evasiveness, and the energetic demands of the predator (Moore & Biewener, 2015). In

accordance with the principles of optimal foraging theory, organisms utilizing this strategy must exhibit both generalist tendencies and opportunistic prey selection to maximize their net energy gain per unit time (Glaudas et al., 2019; MacArthur & Pianka, 1966).

As concluded, water frogs are generalist and opportunistic feeders, which enables them to prey on a broad range of organisms across various habitats. This adaptability allows water frogs to exert significant top-down control on pond and wetland communities, potentially leading to the decline of native species populations (Pille et al., 2024). The broad diet of water frogs further complicates their ecological impact, as it can disrupt food webs by targeting a diverse set of organisms, from the base of the trophic pyramid to higher-level consumers (Pille et al., 2024).

The diet of water frogs appears to be primarily terrestrial (e.g. Balint et al., 2010; Breka et al., 2024). However, the presence of a considerable amount of aquatic prey, which in certain environments may exceed terrestrial prey (Nicoara et al., 2005), indicates that they forage in both terrestrial and aquatic habitats. This highlights their role in operating within the ecotone and creating complex problems in the trophic network between aquatic and terrestrial ecosystems, which are typically highly diverse habitats in terms of biodiversity (Mollov, 2008).

1.4.2 Prey community composition

The introduction of invasive alien water frogs has led to direct ecological consequences, including their predation on native vertebrates and invertebrates (Balint et al., 2010; Breka et al., 2024; Çiçek & Ahmet, 2006; Dan et al., 2001; Fathinia et al., 2016; Karaica et al., 2016; Katsiyiannis & Tzoras, 2020; Mollov, 2008; Nicoara et al., 2005; Pesarakloo et al., 2017; Pille et al., 2021, 2023, 2024; Plitsi et al., 2016; Ruchin & Ryzhov, 2002).

For example, in southern France, stomach content analysis of marsh frogs revealed that 9% of their stomachs contained native amphibians, indicating a significant predatory impact on local amphibian populations (Pille et al., 2021). This predation is often size-dependent, with larger marsh frogs consuming larger native species such as tree frogs (Katsiyiannis & Tzoras, 2020; Pille et al., 2021). In the case of water frogs, smaller individuals primarily prey on tadpoles and small newts (e.g., larvae), while larger individuals consume a broader spectrum of amphibian prey, including adult native anurans, newts, and their larvae (Measey et al., 2015; Pille et al., 2021). From a conservation perspective, the broad predation of adult water frogs, which consume prey of all sizes, underscores their significant impact on native amphibian populations (Cohen et al., 1993; Pille et al., 2021). The water frogs, especially marsh frog, are also well-

documented for exhibiting cannibalistic behaviour under certain conditions (Çiçek & Ahmet, 2006; Cogălniceanu et al., 2001; Fathinia et al., 2016; Ivanov et al., 2024; Mollov, 2008; Nicoara et al., 2005; Plitsi et al., 2016; Ruchin & Ryzhov, 2002).

Most studies have demonstrated that the diet of water frogs predominantly comprises organisms from the phylum Arthropoda, particularly the class Insecta (Balint et al., 2010; Çiçek & Ahmet, 2006; Cogălniceanu et al., 2001; Fathinia et al., 2016; Karaica et al., 2016; Mollov, 2008; Nicoara et al., 2005; Pesarakloo et al., 2017; Plitsi et al., 2016; Ruchin & Ryzhov, 2002). These studies commonly utilize the stomach flushing method (Solé et al., 2005), which has inherent limitations, as prey identification is typically constrained to the order level (Fathinia et al., 2016; Karaica et al., 2016; Plitsi et al., 2016) and mostly to the family level (Balint et al., 2010; Cogălniceanu et al., 2001; Mollov, 2008; Pesarakloo et al., 2017), with genus- and species-level identification being relatively uncommon (Çiçek & Ahmet, 2006; Nicoara et al., 2005; Ruchin & Ryzhov, 2002), likely due to the partial digestion of stomach contents. Because the partially digested stomach contents might make some prey items easier to identify than others, and smaller prey may be more difficult to detect, larger prey could end up being overrepresented in the data. The most frequently recorded prey items belong to the orders Coleoptera (beetles), Hymenoptera (sawflies, wasps, bees and ants), and Diptera (flies) (Balint et al., 2010; Çiçek & Ahmet, 2006; Cogălniceanu et al., 2001; Fathinia et al., 2016; Karaica et al., 2016; Mollov, 2008; Nicoara et al., 2005; Pesarakloo et al., 2017; Plitsi et al., 2016; Ruchin & Ryzhov, 2002). In other amphibian species, prey composition analyses have been conducted using molecular methods (DNA metabarcoding), which has proven to be an effective tool for accurately mapping amphibian diets at the species level, making even the identification of small prey items possible (Marques et al., 2022; Pereira et al., 2021; Unger et al., 2020). To my knowledge, such studies have not previously been conducted on species within the genus *Pelophylax*, particularly in populations where the genus occurs as a non-native taxon. Although dietary studies have been carried out on this genus outside its natural range, none have, as far as I am aware, employed this specific methodology.

1.4.3 Seasonal variations in predation pressure

The predation pressure exerted by water frogs varies throughout the year, with some organisms being more vulnerable to predation during specific seasons. While marsh frogs consistently prey on aquatic organisms, their consumption of terrestrial prey fluctuates seasonally (Pille et al., 2023). This seasonal variation in predation pressure could affect native species that are

adapted to certain environmental conditions, particularly those that are seasonally abundant at the same time as water frogs are most active or those that occupy specific niches in the ecosystem (Pille et al., 2023). Consequently, the timing and intensity of water frog predation may exacerbate the vulnerability of native populations during critical life stages, such as breeding or juvenile development (Pille et al., 2021). During periods when arthropod prey is largely absent, predation pressure shifts toward alternative prey, such as other amphibians and their tadpoles, primarily at the beginning of spring (Pille et al., 2021). In warmer seasons (summer), increased prey availability and frog activity lead to higher prey diversity and counts, while cooler temperatures (autumn) result in reduced prey availability, decreased frog activity, and a decline in both prey diversity and counts (Bayrakcı & Çiçek, 2022).

1.4.4 Niche overlap and resource competition

One of the primary factors contributing to the ecological impact of invasive water frogs is their overlap in ecological niches with native amphibian species, as both share similar habitat preferences, particularly in wetland environments with specific water depths and types of aquatic vegetation (Pille et al., 2024). This overlap increases the potential for both predation and competition for limited resources, such as food, breeding sites, and space, all of which are influenced by these habitat characteristics (Pille et al., 2024). The invasion of water frogs into previously unoccupied habitats, with suitable vegetation and water depth, often leads to the displacement of native species and a shift in the overall composition of the local amphibian community, reducing both the abundance and diversity of native amphibians, especially in regions where invasive water frogs are introduced in high densities (Pille et al., 2024). This shift within the amphibian trophic level may lead to increased and novel forms of predation within the community, potentially altering the entire ecosystem.

1.4.5 Spread of the diseases

Ranavirus and Chytridiomycosis are two major infectious diseases that significantly impact amphibian populations globally, contributing to substantial mortality rates, population declines, and, in some cases, extinctions (Daszak et al., 2003). *Pelophylax* species have been identified as transmitters of both Ranavirus and Chytridiomycosis, contributing to the spread of these pathogens in amphibian populations (Ariel et al., 2009; Baláž et al., 2014).

Ranavirus is a significant cause of amphibian mortality, particularly in the common frog (*Rana temporaria*) in Britain (Cunningham et al., 2007). The virus leads to systemic haemorrhages

and skin ulcerations, resulting in high mortality rates (Cunningham et al., 2007). Ranavirus is transmitted through direct contact with infected individuals or contaminated water (North et al., 2015). The pathogen can affect various amphibians, with different strains showing a broad host range, including frogs and toads (North et al., 2015).

Chytridiomycosis is caused by the fungal pathogen *Batrachochytrium dendrobatidis* Longcore, Pessier & D.K. Nichols, 1999 (Bd), which has been linked to mass mortalities, population declines, and even extinctions in amphibian species worldwide (Webb & Waddle, 2022). *B. dendrobatidis* spreads through water and direct contact between individuals, thriving in cool, moist environments, which makes certain habitats more susceptible to outbreaks (Kriger et al., 2007).

1.4.6 Summary: impact of invasive water frogs outside their native habitat

The introduction of water frogs outside their native range poses a considerable threat to native amphibian populations, especially in regions already under stress from environmental factors such as habitat destruction, climate change, or disease. Through their generalist feeding strategy and broad environmental niche, water frogs exert significant predation pressure and resource competition, potentially leading to declines in native populations and alterations in prey community composition. This, combined with habitat overlap, exacerbates the decline of already endangered or small, isolated populations, and in some cases, may contribute to local extinctions. Effective management strategies are needed to mitigate these impacts, addressing both direct predation and the broader ecological consequences of habitat alteration caused by invasive water frogs, to protect native animal populations and preserve biodiversity.

1.5 Water frogs in Finland

Water frogs recorded in Finland are considered to be outside their native biogeographical range, according to current knowledge. The whole genus *Pelophylax* has been included in Finland's list of nationally harmful invasive species (Government Decree on the Management of Risks Caused by Invasive Alien Species 704/2019). The Finnish water frog species complex comprises two species, the marsh frog (*P. ridibundus*) and the pool frog (*P. lessonae*), as well as their hybrid, the edible frog (*P.* kl. *esculentus*) (Hoogesteger et al., 2013). *P. ridibundus* populations were previously found in the estuaries of the Vantaa and Porvoo rivers from the 1930s to the 1950s but became extinct in the 1960s (Suomalainen, 1941; Terhivuo, 1993). A subsequent observation of water frogs was recorded on the island of Ruissalo in Turku in 2008.

Initially, *P. ridibundus* was identified based on sound recordings from 2008, but after further examination, all captured individuals were determined to be *P.* kl. *esculentus* (Hoogesteger et al., 2013). The confirmation of *P. ridibundus* in Finland remains uncertain. In June 2013, a previously unidentified population of *P. lessonae* was discovered in the municipality of Kaarina in southwestern Finland (Hoogesteger et al., 2013). This species had not been reported in Finland prior to this discovery. Although this population had been known since 2009, it was only later identified as *P. lessonae* (Hoogesteger et al., 2013). In 2018, *P. lessonae* was confirmed in Finland using molecular techniques (Zeisset & Hoogesteger, 2018).

By 2012, the water frog species had been reported in approximately 50 locations within the Turku region (Hoogesteger et al., 2013). After that, the water frog species have rapidly proliferated in southwestern Finland, now occurring in multiple location unnaturally fast. They are believed to pose a threat to Finland's native amphibian species due to their loud croaking, which may interfere with the breeding of native amphibians. Additionally, when the tadpoles of native species hatch, they potentially become easy prey for adult water frogs, further exacerbating the threat to local amphibian populations. All above-mentioned is possible when considering other studies conducted on species within the genus and their feeding habits. However, to date, no studies or confirmations have been made in Finland regarding the impact of water frogs on native amphibians or other Finnish taxa (e.g., insects).

The dietary preferences of species and their trophic relationships play a central role in shaping ecological niches and structuring interactions among coexisting species (Lunghi et al., 2022). These aspects provide important insights into the life histories of anurans and the patterns of their population dynamics (Anderson et al., 1999). Moreover, examining feeding habits and trophic links is valuable for detecting changes in environmental conditions and habitats (Batista et al., 2011), as well as for informing conservation planning and management strategies for species at risk (Stuart et al., 2005). A comprehensive list of prey species would offer valuable ecological insights into the feeding habits of these invasive water frog species in non-native habitats. Such information could, for example, inform decision-making processes regarding the protection of species listed in Annex IV of the EU Habitats Directive.

In this study by utilizing the latest DNA metabarcoding and bioinformatic techniques, I am, for the first time, able to identify the prey species of water frogs present in Finland, even down to the species level. Insects and other animals preyed upon by these frogs can be identified, providing insights into the potential ecological threat they may pose to Finland's native fauna.

1.6 Research questions and hypothesis

As generalist and opportunistic feeders, water frogs are expected to have a prey community primarily composed of arthropods, particularly insect groups and other small invertebrates that can be readily ambushed. The most threatened insect groups by the water frog predation are likely to include semi-aquatic and aquatic insects, which are often encountered by frogs in their foraging habitats near the shoreline.

Secondly, due to their relatively large body size and predatory capacity, water frogs may also prey upon native amphibians, potentially exerting direct negative effects on local amphibian populations.

Finally, the composition of the prey community is expected to vary according to water frog species, population, individual size (snout–vent length, SVL), sex, and season. Such variation likely reflects differences in local prey availability, foraging behaviour, and ecological conditions across populations and time. Prey diversity and counts are expected to fluctuate seasonally, with higher values during summer and lower ones in autumn.

This thesis aims to answer the following study questions:

1. What does the prey community of water frogs (*Pelophylax* spp.) consist of in Southwest Finland, and which insect groups are the most threatened by the water frogs?
2. Do water frogs predate on native amphibian species?
3. Does the composition of the prey community vary according to water frog species, population, frog size (snout–vent length, SVL), sex, and season? How do prey diversity and prey counts change across seasons?

2 Materials and methods

2.1 Water frog samplings

For this study, water frogs were collected from two different locations to account for some spatial variation in the water frog distribution area in Southwest Finland. The study sites were Lieto, Pörintie, sandpit (Lat 60° 30' 5.165" Lon 22° 35' 21.760", WGS84) and Rusko, Soramontuntie, gravel pit (Lat 60° 31' 20.831" Lon 22° 13' 26.334", WGS84). Both study sites were selected because they represent the habitats commonly inhabited by water frogs in the region. Permits to collect frogs were granted for the project by the Centre for Economic Development, Transport and the Environment (Southwest Finland) and from private landowners. The genus *Pelophylax* is included in Finland's national list of invasive alien species (Government Decree on Managing the Risk Caused by Alien Species 704/2019), so special permits for euthanasia were not required.

The Lieto study site was sampled three times (13 June, 6 September, and 16 September 2023), and the Rusko study site was sampled twice (16 June and 31 August 2023) to incorporate some temporal variation and ensure sufficient data collection. A total of 73 water frogs of varying sizes were captured by nets. The nets used had a wooden shaft (95 cm in length), a net opening diameter of 30 cm, and a net depth of 70 cm, with a mesh size of 1 mm. Frogs were captured by active netting, where frogs were ambushed from vegetation and water. Netting was conducted between 9:00 a.m. and 4:00 p.m. on sunny days, as water frogs exhibit activity throughout both diurnal and nocturnal periods (Cogălniceanu et al., 2001). Each sampling session lasted approximately four hours, with a team of two people conducting opportunistic shoreline searches with nets to maximize capture efficiency.

After capture, the frogs were euthanized following the standard protocol recommended by the Central Animal Laboratory of the University of Turku (UTUCAL), using a solution of MS-222 (5 g/L, pH neutralized with bicarbonate). Frogs remained in the solution for one hour to ensure complete euthanasia (Leary et al., 2020). Euthanasia was confirmed by the loss of the righting reflex, cessation of opercular movements, and absence of withdrawal reflexes, following standardized amphibian euthanasia protocols. No secondary euthanasia method was required, as prolonged exposure to MS-222 (5 g/L for 1 hour) has been shown to ensure complete and irreversible cessation of brain function in amphibians (Leary et al., 2020), also observed during this study.

Table 1. External morphology characters of the *Pelophylax* species recorded in Finland.

External morphological characteristics distinguishing the three water frog species (*Pelophylax* kl. *esculentus*, *P. lessonae*, and *P. ridibundus*) recorded in Finland. We used the following table of morphological characteristics in this study to identify our focal species. The table summarizes key diagnostic traits including body size, coloration, hind limb proportions and characteristics, metatarsal tubercle morphology, and species-specific features relevant for visual identification in the field according to Dufresnes (2019) and Speybroeck et al. (2016).

Characteristic	***P.* kl. *esculentus***	***P. lessonae***	***P. ridibundus***
Adult size (SVL)	Variable in size	Smaller, 4–7 cm (rarely 8 cm)	Large, up to 15 cm
Colour	Bright green with dark spots, some brown variants	Bright green, grass-green or brown, with lemon-yellow on males in breeding season	Varies, typically green
Legs when the femora are flexed at a 90° angle	Long tibial bones whose medial edges touch	Short tibial bones whose medial edges do not touch	Long tibial bones that cross over each other
Hind leg length	Heel extends to in front of the eyes or to the snout, rarely past the tip of the snout	Heel extends only to the eyes	Long, might extend past the tip of the snout
Metatarsal tubercle shape	Medium, slightly prominent, usually less than half the length of the innermost toe of the hind leg	Large, prominent, nearly symmetrical half circle, more than half the length of the innermost toe of the hind leg	Small, less prominent, less than half the length of the innermost toe of the hind leg
Distinctive features in the field	Males have off-white or grey vocal sacs, resembles either *P. lessonae* or *P. ridibundus*, hard to identify in the field	Males have white vocal sacs, and brown coloration is common in Finland	Males have grey vocal sacs, larger size, long hind legs

In the laboratory, the species and sex of each specimen were determined, and snout-vent length (SVL) was measured using digital calliper (Goobay, 77001). Although the digital calliper had an accuracy of 0.01 mm, measurement values were rounded to the nearest 0.1 mm to maintain consistency with biological relevance and to account for the instrument's error limit of 30 µm (0.03 mm). Species identification was based on external morphological characteristics (Dufresnes, 2019; Speybroeck et al., 2016), which are summarized in Table 1. Frogs were divided into two size groups, small and large, based on their snout vent length (SVL), with an equal number of individuals in each group. The division was based to the length distribution of the captured individuals, ensuring that the groups represented the lower and upper halves of the population. This grouping was used in SIMPER (similarity percentage) analyses (see Section 2.6.4) to assess differences in diet composition between size classes, whereas SVL was treated as a continuous variable in other analyses. After measurements were taken, an autopsy was

performed. The small and large intestines of each frog were removed, and intestinal tissues were initially preserved in 99.5% ethanol and transferred to a freezer for long-term storage, ensuring DNA preservation for downstream molecular analysis (DNA metabarcoding of prey items). All prey taxa detected through metabarcoding were further categorized according to their primary habitat (terrestrial, semi-aquatic and aquatic), using information obtained from the biology section of the Laji.fi database. In all large individuals, the stomach was also opened, and any prey specimens identifiable to the species level were recorded as additional data for the study based on morphological characteristics. Visual prey identification was only performed on large individuals because their stomachs were easier to open, and the larger prey items found in them allowed for more reliable morphological identification, these prey items were not used in analysis. The sex of the frogs was confirmed during the autopsy by examining reproductive organs. Males were identified by the presence of testes, while females were identified by the presence of ovaries.

2.2 DNA extraction and quality assessment

To access prey DNA, intestine samples were cut open horizontally using small sterile scissors, and up to 150 mg of partially digested prey material (typically lighter samples, maximal sample size for prep kit is 150 mg) was scooped out with a small measurement spoon. All equipment was cleaned and flame-sterilized after each preparation to prevent contamination.

DNA was extracted using Zymo Research's Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (Zymo Research Corporation, 2021), following the manufacturer's protocol version 2.4.5. One empty tube was included as extraction control to test for cross-contamination during DNA extraction step. DNA was eluted into 100 microliters, and the quality was assessed with a Thermo Scientific NanoDrop microvolume spectrophotometer (ND-1000 UV/Vis), which measures light absorbance at specific wavelengths: DNA at 260 nm, proteins at 280 nm, and common extraction-derived contaminants (salts, organic compounds) around 230 nm. The instrument quantifies DNA concentration using absorbance at 260 nm and provides purity ratios (260/280 and 260/230) to detect contaminants. After quality assessment, DNA samples were sent to the Center for Evolutionary Applications at the University of Turku (CEA) for PCR-based NGS library preparation.

Table 2. Primers used in the study to amplify invertebrate and vertebrate DNA during PCR.

Primers used in the study for PCR amplification of invertebrate and vertebrate DNA (Leray and 12S-V5), including primer names, forward and reverse sequences, taxonomic targets, and expected fragment lengths.

Primer pair name	Forward primer name and sequence	Reverse primer name and sequence	Target taxa and genetic region	Target length
Leray	mlCOIintF, GGWACWGGWTGAACW GTWTAYCCYCC (Leray et al., 2013)	jgHCO2198, TAIACYTCIGGRTGIC CRAARAAYCA (Geller et al., 2013)	Animal mitochondrial COI gene	313 bp
12S-V5	12S-V5F, TAGAACAGGCTCCTCTA G (Riaz et al., 2011)	12S-V5R, TTAGATACCCCACTA TGC (Riaz et al., 2011)	Vertebrate 12S	98 bp

2.3 PCR amplification and controls

DNA was amplified in two separate polymerase chain reaction (PCR) with one primer pair in each reaction targeting vertebrate and arthropod prey species (Table 2). For each sample and target locus, two technical replicates of the PCR were conducted to ensure amplification consistency and detect potential stochastic effects in the reaction. The detailed thermal cycling conditions for each PCR protocol are presented in Tables 3–5. For contamination during PCR setup, each PCR run included a no-template control (NTC) containing all reaction components except DNA. A mock community sample was also included to evaluate primer performance (details in Table 6).

The first (locus-specific) PCR was performed in a total reaction volume of 10 µL per reaction. Each reaction contained 5.0 µL of MyTaq^{TM} HS Mix, 2X (Meridian Bioscience, Cincinnati, Ohio, USA) (final concentration: 1X), 0.6 µL of primer mix (forward and reverse) at a final concentration of 0.6 µM (0.3 µM each primer), and 2.4 µL of ultrapure water, and 2.0 µL of DNA template. For a 96-reaction setup, the master mix was prepared with the following reagent volumes: 241.9 µL of ultrapure water, 504.0 µL of MyTaq^{TM} HS Mix, 2X and 60.5 µL of primer mix, ensuring consistency across all reactions. The PCR conditions were optimized according to Bioname Ltd guidelines to achieve efficient amplification (Table 3 and 4).

Table 3. Detailed PCR program for Leray primer.

PCR program for the Leray primer (Leray-COI), showing the forward primer (mlCOIintF), reverse primer (jgHCO2198), denaturation, annealing, and extension temperatures, and the duration of each step.

PCR program for primers: Forward, mlCOIintF Reverse, jgHCO2198	**°C**	**Time**	**Name: Leray-COI**
Begin with:	95 °C	05:00	Initial denaturation
16 cycles of:	95 °C	00:10	Denaturation
	61* °C	00:30	Annealing
	** decreased 1 °C per cycle*		
	72 °C	01:00	Extension
20 cycles of:	95 °C	00:10	Denaturation
	46 °C	00:30	Annealing
	72 °C	01:00	Extension
1 cycle of:	72 °C	10:00	Final extension
Total time		1:21:40	
Real total time		2:06:00	

A mock community sample was included by Bioname Ltd in the study to assess primer efficiency, validate taxonomic assignments, and detect potential sequencing errors or contamination. The amplification efficiency of different primers may vary slightly between different prey species, the effect of which can be calibrated by mock community sample. The mock sample contained known DNA amounts from various insects (Table 6) from species absent in the study area.

Table 4. Detailed PCR program for 12S-V5 primer.

PCR program for the 12S-V5 primer (12S-V5F v2), showing the forward primer (tagF_12S-V5F), reverse primer (tagF_12S-V5R), denaturation, annealing, and extension temperatures, and the duration of each step.

PCR program for primers: Forward, tagF_12S-V5F Reverse, tagF_12S-V5R	**°C**	**Time**	**Name: 12S-V5F v2**
Begin with:	95 °C	10:00	Initial denaturation
35 cycles of:	95 °C	01:00	Denaturation
	45 °C	01:00	Annealing
	72 °C	01:00	Extension
Real total time		2:21:00	

2.4 Library PCR and sequencing

In the second stage, Illumina-compatible adapters were attached for sequencing, and dual indexes were added for sample identification. A second-round (library preparation) PCR was performed in a total reaction volume of 10 µL to amplify DNA libraries. Each reaction contained MQ-H_2O, MyTaq™ HS Red Mix, 2X (Meridian Bioscience, Cincinnati, Ohio, USA), the i7 reverse index and i5 forward index primer (stock aliquot concentrations 5 µM), and template DNA. For a 96-reaction setup, the master mix was prepared using 504.0 µL of MyTaq™ HS Red Mix (2X) and 100.8 µL of i7 reverse primer, ensuring uniformity across reactions. A volume of 6.0 µL of this master mix was dispensed into each well. The remaining 4.0 µL consisted of 1.0 µL of i5 forward primer (stock aliquot concentration 5 µM) and 3.0 µL of DNA template from the first PCR, added individually to each well. The final reaction included 5 µL MyTaq™ HS Red Mix (final concentration 1X) 1.0 µL i7 primer (0.5 µM), 1.0 µL i5 primer (0.5 µM), and 3.0 µL of template DNA. The PCR cycling conditions were optimized to achieve efficient library amplification (Table 5).

Table 5. Detailed PCR program for DNA library.

PCR program for DNA library preparation (Library-PCR), showing denaturation, annealing, and extension temperatures, and the duration of each step.

PCR program for indexing	°C	Time	Name: Library-PCR
Begin with:	95 °C	03:00	Initial denaturation
15 cycles of:	98 °C	00:20	Denaturation
	60 °C	00:15	Annealing
	72 °C	00:30	Extension
1 cycle of:	72 °C	03:00	Final extension
Total time		0:22:15	

A DNA library consists of DNA fragments with attached adapters that enable sequencing. Adapters provide priming sites for sequencing primers and allow fragments to bind to complementary oligonucleotides on the flow cell for cluster generation. Adapters contain index sequences (sometimes referred to as barcodes, but this is not the same as so called DNA barcode used for species identification) to distinguish samples. By introducing different combinations of forward and reverse indices for each reaction, it was possible to multiplex all samples in a single sequencing run. Success and fragment size of each reaction were verified using agarose gel electrophoresis. A 2% agarose gel, stained with MIDORI Green Advance (NIPPON

Genetics, Tokyo, Japan), was used and visualized under UV light. Expected fragment sizes were 504 bp for the Leray-COI primers and 273 bp for the 12S-V5 primers.

Table 6. Composition of mock community sample used in the study by Bioname Ltd.

Composition of the mock community sample constructed and provided by Bioname Ltd (Turku, Finland). The taxonomic hierarchy of mock organisms from class to species.

Class	Order	Family	Genus	Species
Insecta	Blattodea	Blattidae	*Shelfordella*	*Shelfordella* sp.
Insecta	Coleoptera	Tenebrionidae	*Pimeliinae gen.*	*Pimeliinae* sp.
Insecta	Hymenoptera	Ichneumonidae	*Pimpla*	*Pimpla* sp.
Insecta	Coleoptera	Cerambycidae	*Stromatium*	*Stromatium* sp.
Insecta	Hymenoptera	Formicidae	*Camponotus*	*Camponotus* sp.
Insecta	Orthoptera	Gryllidae	*Cardiodactylus*	*guttulus*
Insecta	Hymenoptera	Formicidae	*Odontomachus*	*Odontomachus* sp.

Once prepared, DNA libraries were sent to DNA analysis and research service company Bioname Ltd (Turku, Finland; www.bioname.fi), where library pools were purified using the GeneJet Gel Kit (Thermo Scientific, ref K0691) according to the manufacturer's instructions and then sequenced. Sequencing was performed on an Illumina NovaSeq6000 SP Flowcell (only part of the flow cell was used for this library) 2x250bp (Illumina Inc., San Diego, California, USA) with a PhiX control library (sequencing quality control) at the Turku Centre for Biotechnology, Finland.

2.5 Bioinformatics

Bioinformatics on the sequencing data was conducted by Bioname Ltd, and the bioinformatics pipeline they used was based on the methodology described by Kaunisto et al. (2020). The first step in the process involved merging and trimming the demultiplexed paired-end reads for quality using VSEARCH version 2.22.1 (Rognes et al., 2016) with the 'fastx_filter' command using truncation length 250 bp and 'fatq_maxee_rate' 0.05. Then, filtered R1 and R2 reads were merged with 'fastq_mergepairs' using default settings and 'fastq_allowmergestagger' option. The primers were then removed from the merged reads using CUTADAPT version 3.5 (Martin, 2011), applying a 20% mismatch rate for primer mismatches. The primer-specific minimum and maximum lengths were set to ±10 base pairs of the target length for each primer pair (Leray: min=303, max=323; 12S-V5: min=88, max=108). The reads were then collapsed into unique sequences, with singletons removed, also using VSEARCH. These unique reads were then

denoised, filtered for chimeras, and collapsed into sequence variants, here, ZOTUs (Zero-radius Operational Taxonomic Units), using the USEARCH version 11 UNOISE3 algorithm with its default (minsize=9, unoise_alpha=2) settings (Edgar & Flyvbjerg, 2015). The UNOISE algorithm is particularly effective at removing chimeras, PhiX sequences, and Illumina artifacts, as described by Edgar and Flyvbjerg (2015).

For the COI sequence variants (Leray), the next step involved assigning these variants to taxa using a custom database with the SINTAX algorithm (Edgar, 2010). Bioname's custom database was built by downloading public sequences from the BOLD database, adding sequences from their own mock community, and incorporating full taxonomy annotations. Taxa assignment to ZOTUs followed specific probability thresholds: species assignments were made with a probability of 0.65 or higher, while genus, family, order, class, and phylum assignments were made with probabilities of 0.60 or higher for each level.

These sequence variants were then matched to taxa using the GenBank nt database via a local BLAST search (Altschul et al., 1990), using default BLAST parameters, except for the word size, which was set to 30 (-word_size 30), and a minimum identity threshold of 90% (-perc_identity 90). The BLAST results for each ZOTU were retained based on the lowest e-value. The results from BLAST were further refined as follows: If only two top hits with equal scores were found, both taxa were retained in the results, separated by a slash ('/'). In cases where multiple species were assigned to the same genus, they were grouped under that genus. For instance, if a ZOTU1 was assigned equally to GenusA speciesA1, speciesA2, and speciesA3, the assignment would be listed as "GenusA spp." For cases where multiple taxa were assigned to different genera, they were grouped under the lowest common ancestor. For example, if a ZOTU was equally assigned to GenusA speciesA1, GenusA speciesA2, GenusB speciesB1, and GenusC1 speciesC1, it would be assigned to the family level, such as "Family1".

The data were collapsed by taxonomic assignment, so all reads associated with a given taxon were summarized within each sample. For both the Leray and 12S-V5 primer pairs, reads from both PCR replicates were combined, and only taxa detected in both replicates were retained. To address potential misassignments during the index demultiplexing process, often referred to as 'tag-jumping' or 'sample cross-talk', a conservative tag-jumping rate of 0.5% was applied (Drake et al., 2022; Koskinen et al., 2022; Parisy et al., 2023). Sequence variants with a proportion of reads less than this threshold was removed. For example, if the total read count for a sample

was 10,000, any ZOTU that had fewer than 50 reads (0.5% of 10,000) would be excluded from the sample.

Additionally, non-target taxa, such as fungi and plants, were filtered out. Taxa with low abundance (defined as fewer than two reads) were removed from the data. Both absolute sequence read counts and relative read abundances (proportional summaries of the counts) were also calculated, as described by (Deagle et al., 2019).

2.6 Analyses

All statistical analyses were conducted in R v4.4.3 (R Core Team, 2025) using the package vegan (Oksanen et al., 2025).

2.6.1 Potential biodiversity pressure index (PBPI)

To assess potential biodiversity pressure from frog predation, the Potential Biodiversity Pressure Index (PBPI) was developed. PBPI is calculated as

$$\text{PBPI} = \frac{P_d}{P_f}$$

where P_d is the proportion of species, individuals or metabarcoding reads of a given insect order in the frog's diet, and P_f is the proportion of species of that order in the Finnish insect fauna. PBPI values indicate relative biodiversity pressure, not dietary preference, prey abundance, or biomass. High PBPI values (>1) reflect high biodiversity pressure, meaning the order is disproportionately represented in the diet compared to what would be expected based on its species richness in Finland. Low PBPI values (<1) reflect low biodiversity pressure, indicating the order is underrepresented relative to its national species richness. The index is designed as a rapid, biodiversity-weighted assessment tool for conservation biology, providing a quick indication of insect orders that may be relatively more or less exposed to predation. See Appendix A2 for a more detailed explanation and conceptual background.

2.6.2 Species accumulation and richness estimation

Species accumulation curves were constructed to evaluate the rate at which species were detected across samples and to estimate sampling completeness. Curves were generated in R using the specaccum() function from the vegan package with the "random" method and 10,000 permutations to account for variation in sample order. Curves were visualized using ggplot2,

including shaded ribbons representing standard error around the mean species richness. Confidence intervals were calculated as ±1.96 × standard error.

Total species richness, including undetected species, was estimated with the Chao1 estimator (Chao, 1984) using the specpool() function. Observed richness, estimated total richness (Chao1), and the estimated number of undetected species were reported. It is important to note that singleton prey species were removed during the bioinformatics workflow and are therefore not included in the analyses. Consequently, the true prey species richness in this study may be higher than indicated by the current data, as Chao 1 estimates rely on the presence of singleton species.

2.6.3 Redundancy analysis (RDA)

To test the effects of frog size (SVL), sex, sampling site, and season on prey community composition, redundancy analysis (RDA) was conducted in R using the rda() function from the vegan package. Prey abundance data were Hellinger-transformed using decostand(method = "hellinger") to down-weight the influence of rare taxa and to make the data suitable for linear ordination.

The significance of each explanatory variable, while controlling for the others, was assessed with marginal permutation tests using anova.cca(..., by = "margin", permutations = 10000), and results were considered statistically significant at $\alpha = 0.05$. Multicollinearity among predictors was evaluated using variance inflation factors (vif.cca()), and variables with VIF values > 10 were excluded from the final models. Ordination plots were visually inspected to ensure that linear relationships between explanatory variables and ordination axes were reasonable and that the Hellinger transformation effectively improved linearity and reduced the influence of rare taxa.

2.6.4 Similarity percentage analysis (SIMPER)

To identify which prey taxa contributed most to community dissimilarities between groups (species, site, season, and size classes), SIMPER analyses were conducted in R using the simper() function with Bray-Curtis dissimilarities (10,000 permutations). The Bray–Curtis index is appropriate for ecological community data because it is non-Euclidean, ignores joint absences, and emphasizes differences in relative abundance.

SIMPER assumes that samples are independent and that the dissimilarity metric adequately represents ecological distances. Independence was ensured by treating each frog as a separate sampling unit, and the influence of rare taxa was minimized through prior data filtering. For interpretation, the cumulative contribution of individual prey taxa to overall dissimilarities was assessed, reporting the top taxa that together explained approximately 70% of the observed differences.

2.6.5 Prey diversity and counts

Shannon diversity (H′), a commonly used measure of species diversity accounting for both richness and evenness, was calculated for each frog individual from prey occurrence data using the diversity() function (vegan). For each season, mean Shannon diversity and total prey count per frog were summarized together with 95% confidence intervals (CI) to quantify the uncertainty of the mean. Total prey count per frog was estimated as the sum of sequence reads per individual.

Normality of the data was assessed using Shapiro–Wilk tests. Because some variables were not normally distributed, seasonal differences in Shannon diversity and prey count per frog were analysed using independent-samples Wilcoxon rank-sum tests (Mann–Whitney U tests). Data were formatted so that each individual frog represented a separate observation, with a categorical variable for season (summer or autumn).

This approach allowed testing whether prey diversity and consumption count differed significantly between seasons, while the use of mean ± 95% CI in figures provided a clear descriptive summary of seasonal trends.

2.7 Use of artificial intelligence (AI) in the thesis

Artificial intelligence (AI) was utilized in this thesis in accordance with the ethical and academic guidelines of the University of Turku (23 March 2023). The AI tool used was ChatGPT (OpenAI GPT-5, October 2025 version).

The AI was employed only as a supportive tool to improve the clarity, structure, and linguistic accuracy of the English text. It was not used to generate scientific content, data, analyses, or interpretations. All research design, theoretical framing, data handling, and conclusions were conducted independently by the author and the supervisors.

The AI-assisted sections were carefully reviewed and edited by the author to ensure the accuracy and integrity of the content. Thus, the intellectual and scientific responsibility for the thesis remains entirely with the author.

3 Results

3.1 Data metrics

3.1.1 DNA sequencing and bioinformatics

DNA pools comprised a total of 73 samples. Sequencing yielded 13.5 million raw reads for the COI locus and 17.3 million raw reads for the 12S rRNA locus. Variant calling (ZOTUs) generated 802 sequence variants for COI and 53 for 12S, all corresponding in length to the expected amplicon size for each locus. Most quality-filtered reads were successfully mapped to sequence variants. PCR blanks and negative controls contained only few reads, indicating no cross-contamination or reagent-derived contamination. Overall, most quality-controlled reads were taxonomically assigned, with the majority classified within the class Amphibia, primarily the family Ranidae (host), and a smaller proportion assigned to the class Insecta. After removing non-target taxa (e.g., fungi, plants) and low-abundance taxa (see Methods), all samples retained filtered reads, with a mean of 165,469 (SD ± 32,367) reads per sample for the Leray primers and 227,534 (SD ± 60,216) reads per sample for the 12S-V5 primers. PCR1 replicates with the 12S-V5 primer pair failed in 64 of 73 samples (88%) and were excluded from further analyses. Following the bioinformatics pipeline, 115 ZOTUs remained, corresponding to 104 prey taxa detected across the samples. In five individuals, intestinal contents yielded no prey reads after filtering, resulting in a final sample size of 68 frogs for dietary analysis.

3.1.2 Water frog and prey data

All individuals included in the dietary analysis were identified as the edible frog (*Pelophylax* kl. *esculentus*). Therefore, the observations specifically concern this species, but they can be assumed to also reflect the foraging behaviour of other species within the genus. Edible frogs were collected from two populations (Rusko and Lieto) during summer and autumn. Juveniles were observed only in autumn. Detailed sampling data, including sex and population composition, are presented in Figure 2.

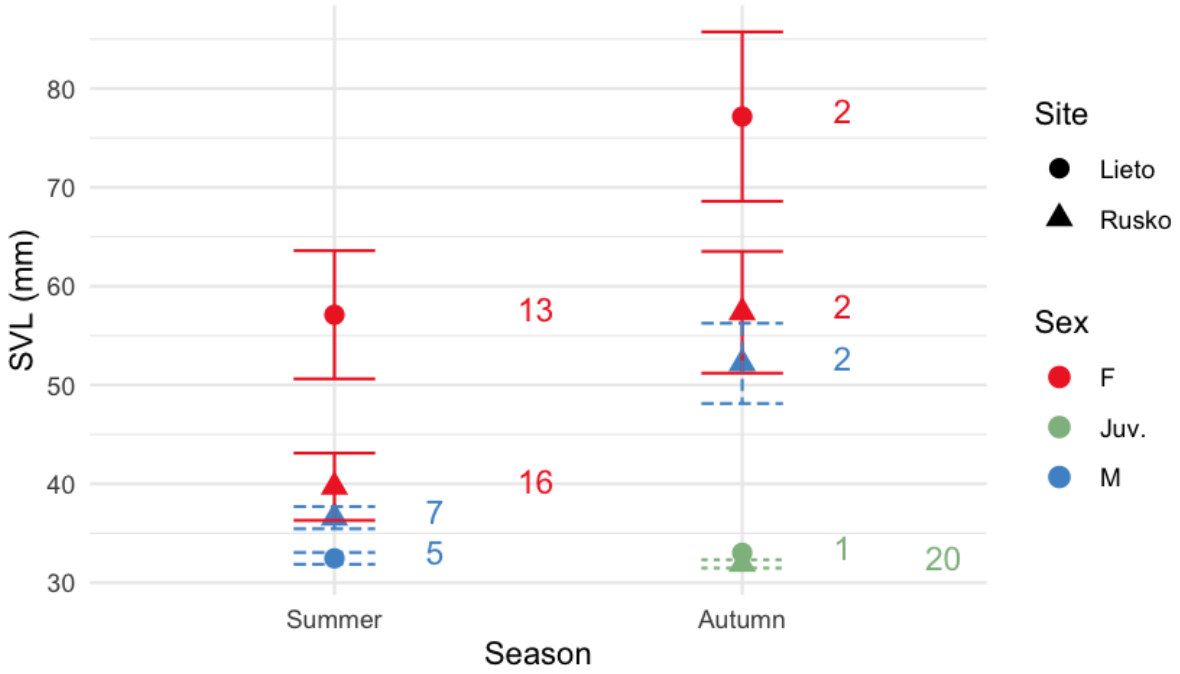

Figure 2. Overview of the sampled edible frogs (*Pelophylax* kl. *esculentus*), showing body size (SVL), sex, sampling sites, and variation of these traits between summer and autumn.

A species accumulation curve with 95% confidence intervals (CIs) was generated to assess prey taxon richness (Figure 3). The 95% CIs illustrate variability in estimated prey richness, with wider intervals at lower sample sizes. A Chao1 estimator yielded an observed number of 104 prey taxa and an estimated total of approximately 250 taxa, indicating that around 146 taxa were not observed in the current dataset.

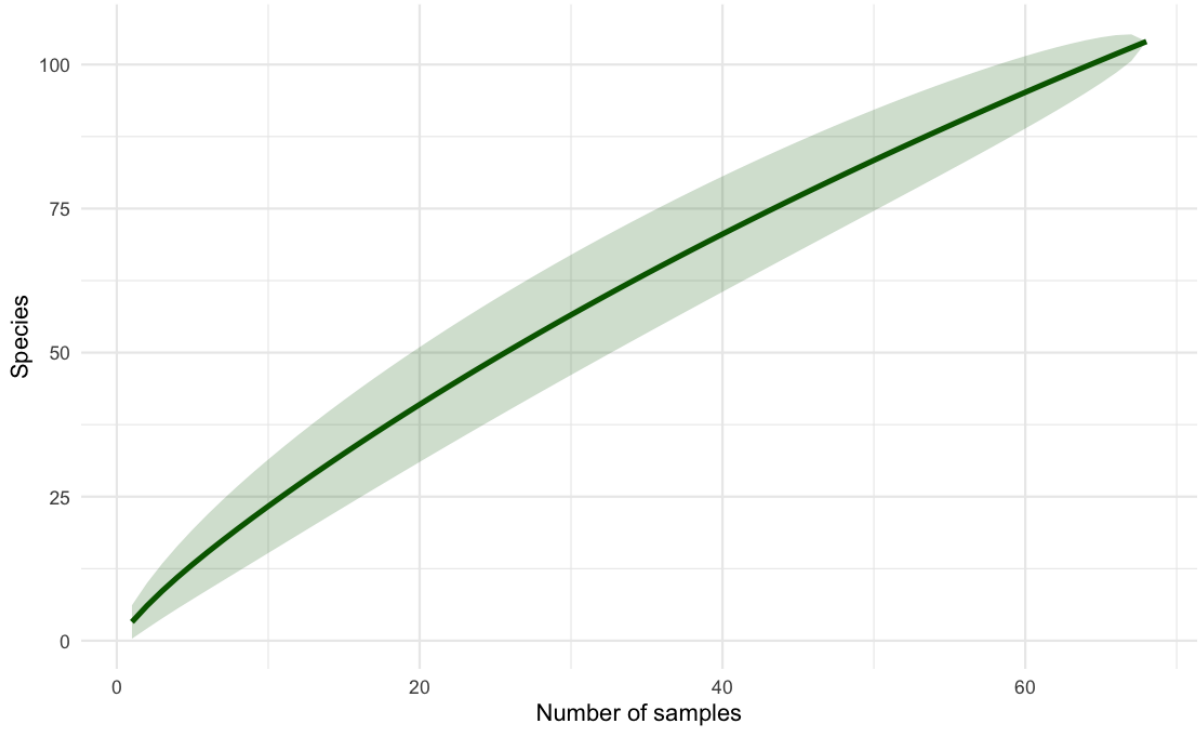

Figure 3. Species accumulation curve showing observed prey taxon richness with 95% confidence intervals (CIs).

3.2 Prey species

The composition of the prey community in edible frogs was examined across all 68 analysed individuals. A total of 104 prey taxa were detected (see Table A2 in Appendices for a full list of taxa).

At a higher taxonomic level (order; note that some minor groups represent other taxonomic levels), the most abundant prey groups were Coleoptera (42.65%), Diptera (15.66%), Trichoptera (9.18%), and Hymenoptera (8.13%). Additional minor prey orders included Podocopida, Rhabditida (probable secondary predation), Hemiptera, Stylommatophora, Odonata, and Crassiclitellata, while all remaining orders were observed at lower frequencies (Figure 4).

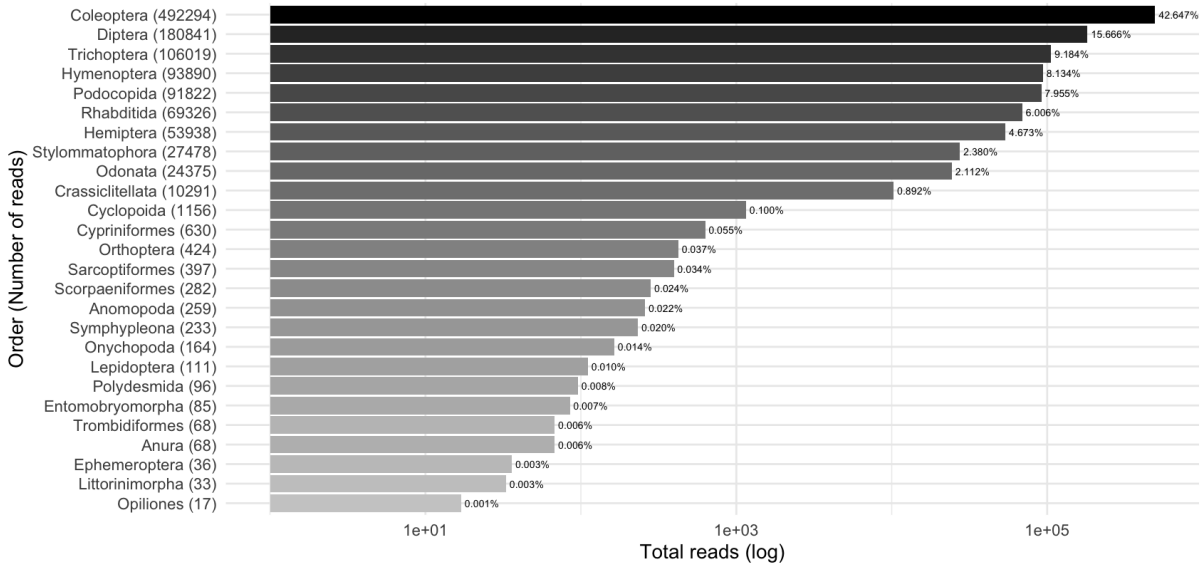

Figure 4. Relative abundance of prey taxa at the order level in the sampled edible frogs (*Pelophylax* kl. *esculentus*). The barplot shows total reads for each taxon (log-transformed on the x-axis), with the total read count indicated next to each taxon name on the y-axis and the relative proportion of each taxon in the total prey community indicated at the end of each bar.

The calculated PBPI values showed pronounced contrasts among insect orders (Table 7). The highest PBPI values were observed in Trichoptera and Odonata. Coleoptera also had a PBPI above 1. In contrast, Hymenoptera, Diptera, and Hemiptera had PBPI values below 1, while Lepidoptera, Orthoptera, and Ephemeroptera had the lowest values.

Table 7. Representation of insect orders in the edible frog diet relative to Finnish insect diversity.

Percentage of insect orders in the diet of *Pelophylax* kl. *esculentus* compared with their percentage within the Finnish insect fauna, with calculated PBPI values identifying orders potentially vulnerable to predation by water frogs (PBPI > 1 = elevated risk; PBPI < 1 = reduced risk).

Order	**Percentage of insects in edible frog diet**	**Percentage of all Finnish insect species**	**PBPI**
Coleoptera (Terrestrial)	≈51.7 %	≈15.6 %	3.31
Diptera (Semi-aquatic)	≈19 %	≈30.6 %	0.62
Trichoptera (Semi-aquatic)	≈11.1 %	≈0.88 %	12.61
Hymenoptera (Terrestrial)	≈9.9 %	≈31.8 %	0.31
Hemiptera (Terrestrial)	≈5.7 %	≈6.6 %	0.86
Odonata (Semi-aquatic)	≈2.6 %	≈0.26 %	10.0
Orthoptera (Terrestrial)	≈0.045 %	≈0.15 %	0.3
Lepidoptera (Terrestrial)	≈0.012 %	≈10.9 %	0.0011
Ephemeroptera (Aquatic)	≈0.004 %	≈0.23 %	0.017

The prey taxa were further categorized according to their primary habitat (for methodological details, see Section 2.1). Approximately 30% of prey taxa were aquatic, 9% semi-aquatic, and 61% terrestrial (Figure 5).

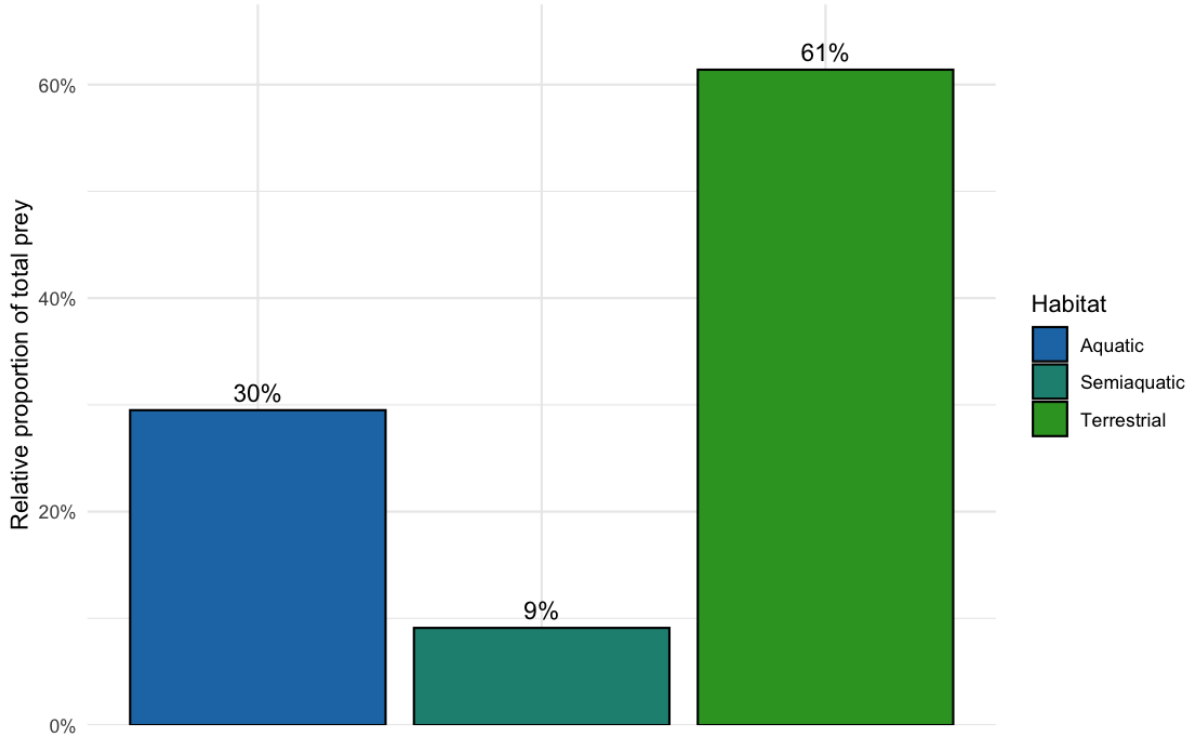

Figure 5. Habitat types of prey taxa detected in the study, showing the proportion of aquatic, semi-aquatic, and terrestrial taxa in edible frog (*Pelophylax* kl. *esculentus*) diet.

3.3 Predation on native amphibians

Prey DNA belonging to native amphibians were observed during the dietary analysis (Table A2 in Appendices). In summer, prey could be identified only to the family level (Ranidae) in one edible frog individual from Rusko with a snout–vent length (SVL) of 42.4 mm. In autumn, a smaller edible frog (SVL 29.9 mm) from Rusko contained DNA of a moor frog (*Rana arvalis*) in its intestinal content.

3.4 Factors affecting prey community composition

The effects of edible frog size (SVL), sex, sampling site, and season on prey community composition were assessed using redundancy analysis (RDA). Marginal permutation tests with 10,000 permutations indicated that sampling site ($F_{1,62} = 2.02$, $p < 0.001$) and season ($F_{1,62} = 2.39$, $p < 0.001$) significantly influenced prey community composition. In contrast, SVL ($F_{1,62} = 1.27$, $p = 0.093$) and sex ($F_{2,62} = 1.15$, $p = 0.128$) did not reach statistical significance. The

RDA model explained 16.79 % of the total variance, leaving 83.21 % as residual variance. The RDA biplot summarizing these results is presented in Figure 6.

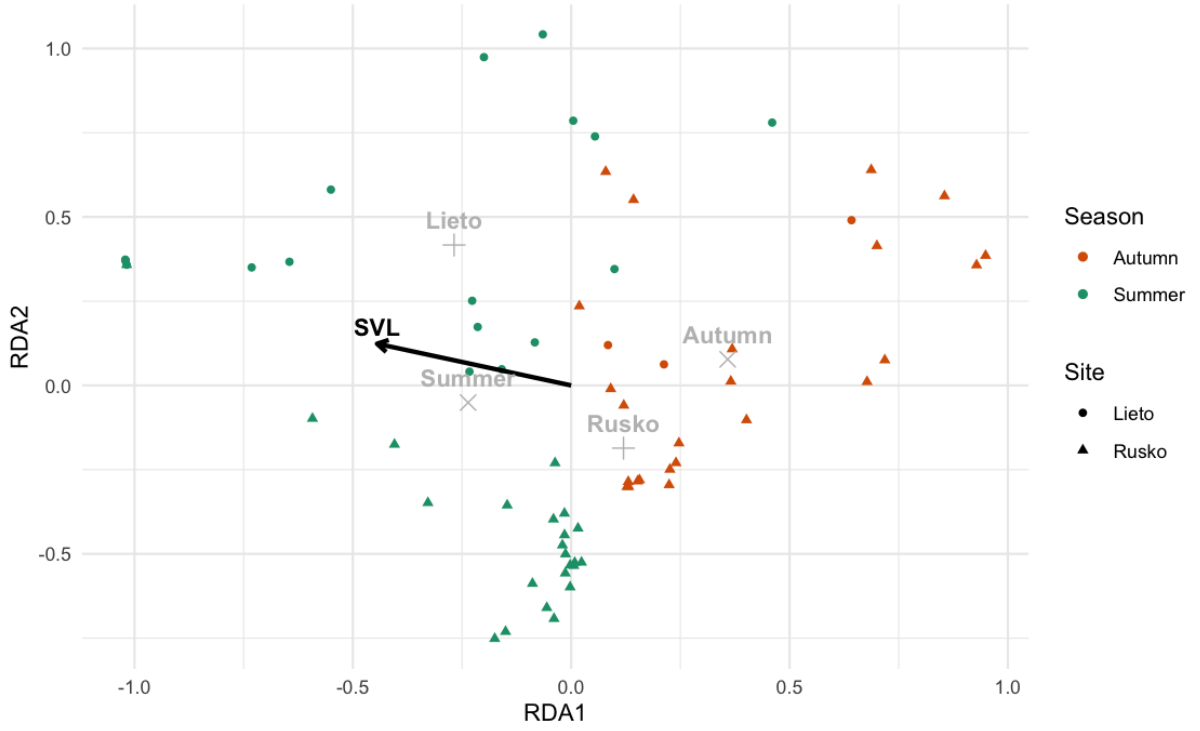

Figure 6. Redundancy analysis (RDA) biplot showing the effects of site, season and edible frog size (SVL) on prey community composition.

Shannon diversity (H′) per edible frog was slightly higher in summer (mean ± 95% CI: 0.675 ± 0.170, n = 41) than in autumn (0.567 ± 0.156, n = 27), but this difference was not statistically significant (Wilcoxon rank-sum test: W = 514, p = 0.625). Total prey count per edible frog, estimated as the sum of sequence reads, was higher in summer (mean ± 95% CI: 18,689 ± 9,498, n = 41) compared to autumn (14,374 ± 10,067, n = 27), but the difference was also not significant (Wilcoxon rank-sum test: W = 597, p = 0.590). Seasonal variation in both prey diversity and counts is illustrated in Figure 7.

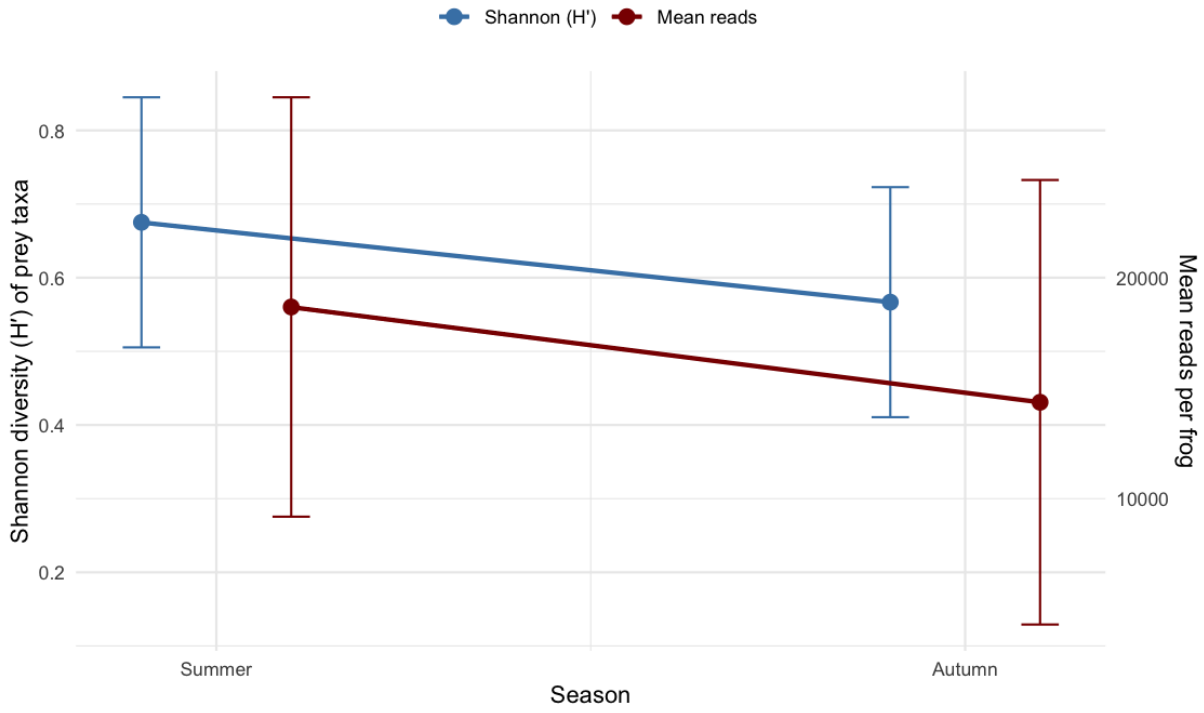

Figure 7. Seasonal variation in prey diversity and total prey count per edible frog. Mean values ± 95% confidence intervals (CI) are shown for Shannon diversity (H′) of prey taxa and mean sequence reads per frog.

3.5 Similarity percentage analysis (SIMPER)

SIMPER analyses (10,000 permutations) were used to identify the prey taxa contributing most to differences in prey community composition across sampling sites, seasons, and edible frog size (SVL). In the SIMPER plots, a species entirely associated with one group indicates that it occurs primarily or exclusively in that group and contributes substantially to the overall community dissimilarity from that group.

3.5.1 Sampling site differences

Comparisons between the Lieto and Rusko populations showed that dietary dissimilarities were primarily driven by a subset of key prey taxa. The taxa contributing most strongly included Chrysomelidae, *Lasius niger*, *Erioptera squalida*, Diptera, and *Donacia aquatica* (Table 8). The ten taxa with the highest contributions together explained approximately 48.5% of the total dissimilarity, and 23 taxa were required to reach 70% of the observed differences (Figure 8).

3.5.2 Seasonal differences

Seasonal contrasts between summer and autumn were dominated by Chrysomelidae, Diptera, *Lasius niger*, with additional contributions from *Camponotus herculeanus*, *Myrmica scabrinodis*, and other invertebrate taxa, ten taxa with highest contributions explaining approximately 42.8% of seasonal dissimilarity (Table 8). The 24 most influential taxa accounted for approximately 71% of the seasonal dissimilarity, based on cumulative contributions from the SIMPER analysis (Figure 8).

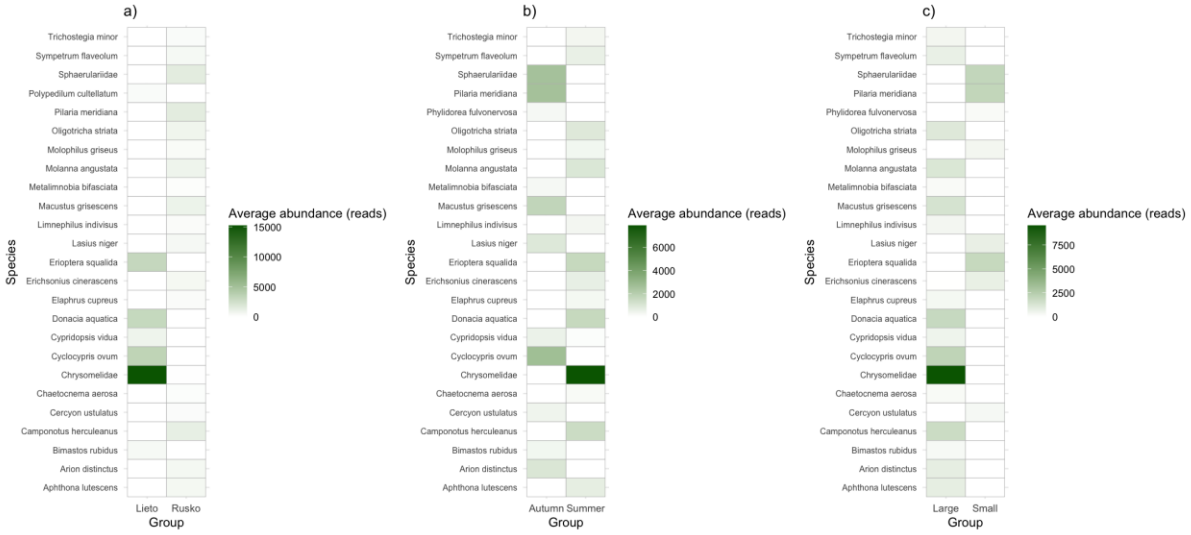

Figure 8. Heatmaps showing the contribution of prey taxa to dissimilarities in different SIMPER comparisons. Each heatmap summarizes the taxa (25 most) responsible for dissimilarities between groups: a) sampling sites, b) seasons, and c) edible frog size classes (large vs. small). Colour intensity represents the relative contribution of each taxon to the observed dissimilarity.

3.5.3 Size differences (SVL)

Differences between small and large edible frogs were associated with a limited set of prey taxa. The most influential contributors included Chrysomelidae, *Camponotus herculeanus*, *Lasius niger*, Diptera, and *Sympetrum flaveolum*, among others, ten taxa with highest contributions explaining approximately 41.2% of seasonal dissimilarity (Table 8). The 24 taxa with the highest contributions explained approximately 71% of the size-related dissimilarity, according to cumulative SIMPER contributions (Figure 8).

Table 8. Ten most taxa responsible for dissimilarities in different comparisons.

Rank of each taxon and its percentage contribution to dissimilarities between sites (Lieto vs. Rusko), seasons (summer vs. autumn), and predator size classes (large vs. small).

Rank	**Site (Lieto vs Rusko)**	**Contribution to Site dissimilarity (%)**	**Season (summer vs autumn)**	**Contribution to Season dissimilarity (%)**	**Size (large vs small)**	**Contribution to Size dissimilarity (%)**
1.	Chrysomelidae	≈17 %	Chrysomelidae	≈8.9 %	Chrysomelidae	≈11.4 %
2.	*Lasius niger*	≈4.6 %	Diptera	≈5.9 %	*Camponotus herculeanus*	≈5.1 %
3.	*Erioptera squalida*	≈4.4 %	*Lasius niger*	≈5.2 %	*Lasius niger*	≈4.4 %
4.	Diptera	≈4.1 %	*Camponotus herculeanus*	≈4.0 %	*Sympetrum flaveolum*	≈3.9%
5.	*Donacia aquatica*	≈3.9 %	*Myrmica scabrinodis*	≈3.7 %	*Macustus grisescens*	≈3.3 %
6.	*Cyclocypris ovum*	≈3.6 %	*Pilaria meridiana*	≈3.2 %	*Oligotricha striata*	≈2.7 %
7.	*Camponotus herculeanus*	≈3.2 %	*Megamelus notulus*	≈3.2 %	*Molanna angustata*	≈2.6 %
8.	*Bimastos rubidus*	≈3.2 %	Sphaerulariidae	≈3.1 %	*Erioptera squalida*	≈2.6 %
9.	*Myrmica scabrinodis*	≈2.3 %	*Macustus grisescens*	≈3.0 %	*Donacia aquatica*	≈2.5 %
10.	*Heterocerus fusculus*	≈2.2 %	*Cyclocypris ovum*	≈2.7 %	*Myrmica scabrinodis*	≈2.5 %
Total contribution		≈48.5 %		≈42.8 %		≈41.2 %

4 Discussion

4.1 Overview of key findings

In this study, I provide a temporal and spatial snapshot of the dietary composition of the edible frog (*Pelophylax* kl. *esculentus*) in Southwest Finland. Across 68 analysed individuals (Figure 2), metabarcoding revealed a moderately diverse diet comprising 104 prey taxa (Table A2 in Appendices), predominantly invertebrates from orders Coleoptera (beetles), Diptera (flies), Trichoptera (caddisflies), and Hymenoptera (sawflies, wasps, bees and ants), with smaller contributions from other groups (Figure 4). While the sampling did not fully capture all prey species (Figure 3), the results indicate that the diet is primarily terrestrial, complemented by a notable share of aquatic prey, reflecting foraging across both habitat types (Figure 5). This highlights the edible frog's role in linking aquatic and terrestrial trophic networks. Analysis using the potential biodiversity pressure index (PBPI) revealed that certain orders, particularly Trichoptera and Odonata, experience disproportionately high pressure relative to their national species richness, whereas other groups, such as Lepidoptera and Orthoptera, experience comparatively lower pressure (Table 7). This highlights insect taxa that may be more vulnerable to predation by edible frogs.

Predation on other amphibians was detected in two samples, indicating that while edible frogs may prey on native amphibians, such events do occur but were rare within the studied populations.

Community composition was significantly influenced by sampling site and season, while frog size and sex had little or no effect, as shown by RDA (Figure 6). SIMPER analyses identified a subset of prey taxa driving differences among populations, seasons, and size classes (Figure 8 and Table 8). Seasonal trends in prey diversity and counts indicated slightly higher values in summer compared to autumn, but these differences were not statistically significant (Figure 7).

Overall, edible frogs exhibit a flexible, generalist diet shaped likely by local prey availability and seasonal variation, with occasional predation on other amphibians and vertebrates. Generally, frogs function as generalist predators that consume a wide range of available prey. However, certain taxonomic or ecological groups appear to be more vulnerable to frog predation than others.

4.2 Prey community composition

Current dietary analyses of *Pelophylax* kl. *esculentus* revealed a prey community composition largely consistent with previous studies on water frogs. As expected, arthropods dominated the diet, with Coleoptera (43%), Diptera (16%), Trichoptera (9%), and Hymenoptera (8%) being the most abundant orders (Figure 4, Table A2 in Appendices). This pattern aligns with earlier reports indicating that insects constitute the bulk of water frog diets, with Coleoptera, Diptera, and Hymenoptera typically among the most frequently consumed taxa. However, the relatively high proportion of Trichoptera observed in this study was somewhat unexpected, as previous investigations have generally reported this order as a minor dietary component (Balint et al., 2010; Çiçek & Ahmet, 2006; Fathinia et al., 2016; Karaica et al., 2016; Mollov, 2008; Pesarakloo et al., 2017; Plitsi et al., 2016; Ruchin & Ryzhov, 2002).

The single most abundant prey taxon was the family Chrysomelidae, comprising 28% of all prey items (Table A2 in Appendices). These were most likely species of the genus *Donacia*, aquatic leaf beetles associated with emergent vegetation. A high representation of Chrysomelidae in the diet has also been reported in previous studies (e.g., Ruchin and Ryzhov, 2002), indicating that these beetles are a common and readily available prey resource for water frogs.

In the diet of edible frog, aquatic prey accounted for approximately 30% of the identified taxa, terrestrial prey represented 61%, and semi-aquatic taxa 9% (Figure 5). These findings correspond with previous observations (e.g. Balint et al., 2010; Breka et al., 2024) and highlight the role of edible frogs as ecotonal predators, linking aquatic and terrestrial food webs as mentioned also in previous studies (Mollov, 2008).

By applying the PBPI, I was able to demonstrate that frogs feed disproportionately on certain insect groups (Table 7). Trichoptera emerged as the most affected order, being consumed far more frequently than expected based on their modest representation in the Finnish insect fauna. This likely reflects their high abundance and accessibility in wetland environments where frogs typically forage. Odonata also showed strong overrepresentation, which may be also explained by their predictable occurrence near aquatic habitats. Coleoptera, a highly diverse and mostly terrestrial group, were likewise detected more than expected, possibly due to their abundance both in vegetation and on the ground (see above regarding Chrysomelidae).

On the other hand, Hymenoptera, Diptera, and Lepidoptera, despite representing a substantial proportion of the Finnish national insect fauna, were relatively rare in the intestinal contents. Hymenoptera and Lepidoptera, although diverse and abundant nationally, are generally not associated with aquatic habitats or wetlands. Diptera includes both aquatic and terrestrial species, but most of the national fauna is not closely linked to water bodies. These ecological preferences likely reduce the availability of these insect groups in the frogs' foraging environment, contributing to their lower relative representation in the diet.

Nevertheless, ants such as *Camponotus herculeanus* (5.1%) and *Lasius niger* (2.2%) were among the most abundant prey items of these frogs (Table A2 in Appendices). Ants are typically extremely numerous in the environment, occurring in large colonies that produce high local densities of individuals, which is also reflected in the data. Therefore, I do not consider frogs to pose any real threat to these hymenopterans, although I highlight their possible role in the diet of this invasive species. This aspect is further discussed in Section 4.5.

These results suggest that edible frogs may exert their strongest predation pressure on semi-aquatic insect orders, particularly Trichoptera and Odonata. This finding is ecologically significant, as for example dragonflies and damselflies are a group of high conservation concern at the European scale, with several species listed under the EU Habitats Directive and included in national red lists. The observation that Odonata constitute a one of the largest prey groups of *P.* kl. *esculentus* in the studied areas suggests that alien water frogs may impose additional pressure on populations already affected by habitat degradation and other anthropogenic stressors.

In summary, the prey community of water frogs in Southwest Finland is dominated by terrestrial and semi-aquatic insects, primarily Coleoptera, Diptera, Hymenoptera and Trichoptera. The potential biodiversity pressure index (PBPI) indicates that Trichoptera and Odonata experience the highest relative pressure and may therefore represent the most vulnerable prey groups.

4.3 Notable prey groups and species

4.3.1 Anura (frogs)

Edible frog's diet included representatives of the order Anura (Table A2 in Appendices). Within the samples, *Rana arvalis* and another occurrence at the family level (Ranidae) were detected.

In the latter sample, most reads were assigned to the genus *Pelophylax*, corresponding to the host species. In addition, a small number of reads were assigned only to the family level (Ranidae). As genus *Pelophylax* itself belongs to this family, it cannot be determined with certainty whether these reads reflect the host or a potential prey individual, although the latter remains a possibility. The raw data also suggested the presence of *Rana temporaria*, but this assignment was removed during the bioinformatic filtering process, because there were too few reads. Notably, these two Anura species are listed in Annex IV of the EU Habitats Directive. For broader interpretation of Anura records, see Section 4.4.

4.3.2 Coleoptera (beetles)

Coleoptera represented the largest prey group in the diet of the edible frog (Figure 4). Fifteen Coleoptera species are listed in Annex IV of the EU Habitats Directive, none of these were detected. However, the nationally vulnerable (VU) species *Chaetocnema aerosa* was identified from cut samples (Table A2 in Appendices). This taxon is included in Annex 6 of the Government Decree on Nature Conservation (1066/2023). The dominance of Coleoptera in the diet indicates that the invasive water frogs exert substantial pressure on this order, particularly on species inhabiting or closely associated with aquatic environments, such as members of the family Chrysomelidae. In addition, an adult *Cicindela hybrida* was identified in stomach contents outside the DNA-based dataset, demonstrating that even fast-flying beetle species can be captured by edible frogs.

4.3.3 Odonata (dragonflies and damselflies)

Odonata represented the ninth most abundant prey order (Figure 4). Although six odonate species occurring in Finland are listed in Annex IV of the EU Habitats Directive, none of these were detected in the metabarcoding dataset. Notably, for example *Leucorrhinia pectoralis* and *Leucorrhinia albifrons*, two of the Annex IV species, were observed flying in the study area during summer fieldwork in Lieto, indicating their local presence even though they were not found among the prey. In addition, remains of other dragonfly species not detected by metabarcoding were identified from body and wing parts in the stomachs of large individuals while preparing the sample in the laboratory, including imago stages of *Cordulia aenea* and *Libellula quadrimaculata*. Finally, metabarcoding of summer samples revealed two species of the genus *Sympetrum*, which are adults in autumn (Table A2 in Appendices). In light of these findings, these results indicate that Odonata constitute a dietary resource across their multiple

life stages. Newly emerged adults appear particularly vulnerable to predation, as their limited mobility immediately after emergence makes them especially susceptible to capture by frogs. This insect order is likely one of the most threatened by invasive water frogs (Table 7; see Section 4.2).

4.3.4 Trichoptera (caddisflies)

Trichoptera ranked among the four most consumed prey orders by the edible frog (Figure 4, Table A2 in Appendices). Previous studies, which did not employ molecular methods for prey species identification, have generally reported this group as a minor component of the diet (Balint et al., 2010; Çiçek & Ahmet, 2006; Fathinia et al., 2016; Karaica et al., 2016; Mollov, 2008; Pesarakloo et al., 2017; Plitsi et al., 2016; Ruchin & Ryzhov, 2002), whereas in this study it represented a relatively large proportion. In Finland, eight Trichoptera species are listed as threatened in the national red list (Hyvärinen et al., 2019), highlighting the potential conservation relevance of predation on this order, although none of these threatened species were detected in our material.

4.4 Predation on native amphibians

Approximately 3% of the focal water frogs' prey DNA consisted of other amphibians, confirming the occasional predatory impact of water frogs on sympatric amphibians. In my study, a moor frog (*Rana arvalis*) was detected in the gut of a small individual collected in autumn, whereas during summer, native Ranidae were identified only at the family level (see 4.3.1). These observations are consistent with previous reports of *Pelophylax* predation on other amphibians (Pille et al., 2021). Given the relatively small sample size, it is plausible that during periods of lower arthropod availability, water frogs may shift their predation toward alternative prey, including native amphibians. In addition to the findings of Pille et al. (2021), we detected native amphibians consumed in autumn, indicating that there may be two peak periods of amphibian predation by *Pelophylax*, one in spring and one in autumn.

Although molecular methods do not reliably detect cannibalism, such behaviour was observed in captivity: when a large female (SVL 85.7 mm) and a small juvenile (SVL 27.7 mm) were held together prior to euthanasia, the larger female consumed the smaller individual. This observation aligns with previous studies (Çiçek & Ahmet, 2006; Cogălniceanu et al., 2001; Fathinia et al., 2016; Ivanov et al., 2024; Mollov, 2008; Nicoara et al., 2005; Plitsi et al., 2016; Ruchin & Ryzhov, 2002), indicating that cannibalism likely occurs in *Pelophylax* kl. *esculentus*

in Southwest Finland. The observation of a small juvenile edible frog (SVL 29.9 mm) consuming *R. arvalis* in autumn also suggests true intraguild predation (IGP), although the relative sizes of the frogs cannot be confirmed. Predation on similarly sized amphibians has previously been documented within the genus *Pelophylax* (Ivanov et al., 2024).

These findings demonstrate that water frogs prey upon native amphibians, confirming direct at least infrequent predatory interactions within the studied populations.

4.5 Variation in predation

Redundancy analysis (RDA) indicated that sampling site and season significantly shaped prey community composition, whereas frog size and sex had little or no statistically significant effect (Figure 6). SIMPER analyses showed that dietary differences among populations, seasons, and frog size classes were primarily driven by a small subset of prey taxa (Figure 8, Table 8), with Chrysomelidae, *Lasius niger*, Diptera, and *Camponotus herculeanus* repeatedly contributing most to dissimilarity. This indicates that variation in frog diets is structured around a few dominant prey types rather than being randomly distributed across all available prey in the concise review of dietary aspects in this study.

Local habitat features and resource distribution directly influence prey availability. For example, pond with abundant ant nests may increase encounter rates with ants, while ponds with extensive aquatic vegetation provide suitable habitat for Chrysomelidae, explaining possibly their higher occurrence among prey items. Such spatially structured variation indicates that local ecological conditions, such as vegetation type, microclimate, and the distribution of invertebrate colonies, are likely key drivers of the observed dietary differentiation.

Peaks in prey abundance may drive dietary differences across seasons, reflecting temporal niche variation and episodic resource pulses in freshwater habitats (Jensen et al., 2024; Yang et al., 2008). Although prey availability was not directly measured, seasonal patterns suggest a potential tendency toward higher prey diversity and counts in summer compared to autumn (Figure 7). However, these differences were not statistically significant and should therefore be interpreted with caution. This pattern is nevertheless broadly consistent with previous observations (Bayrakcı & Çiçek, 2022; Pille et al., 2021, 2023) suggesting that frogs may adjust their diet toward alternative prey during periods of reduced arthropod availability, reflecting flexible foraging behaviour. Cooler autumn temperatures likely limit both prey availability and foraging activity, contributing to observed seasonal differences.

Size-related dietary variation may result from the greater energetic demands of larger frogs, consistent with metabolic scaling theory, leading them to consume more prey or higher counts of energy-rich taxa (Brose et al., 2019). In these habitats, the Chrysomelidae consumed are most likely of the genus *Donacia*, which are medium-sized beetles, while *Camponotus herculeanus* is the largest of Finland's ant species. These taxa also appear to be preferentially associated with larger frogs (Figure 8), further indicating the role of frog size in shaping diet composition.

Sex had no significant influence on diet composition, indicating that male, female and juvenile frogs exploit prey communities in largely similar ways under the studied conditions. However, it should be noted that the limited sample size in the present dataset may have reduced the ability to detect subtle patterns, and with a larger number of individuals both sex and size effects could potentially emerge.

From an ecological perspective, the concentration of dietary differentiation on a few abundant prey groups aligns with optimal foraging theory (MacArthur & Pianka, 1966; Pyke, 2019) and suggests that these taxa may function as keystone prey, shaping ecological interactions and energy flow within freshwater ecosystems (Estes et al., 2011; Power et al., 1996). By preying on these key species, edible frogs may indirectly influence broader ecosystem processes. It is also important to note that this study examined frog diet through a short observational window, and a larger dataset could reveal similar patterns or potentially uncover different ones.

In summary, prey composition varied significantly among sites and seasons, whereas frog size and sex had no detectable influence. Prey diversity and prey counts appeared to be higher in summer than in autumn, although these differences were not statistically significant.

4.6 Impacts of water frogs on native species and potential management

My results reinforce the view that invasive alien water frogs are generalist predators with broad dietary niches, capable of exploiting a wide range of aquatic and terrestrial prey, particularly insects. Their diet includes taxa listed in the EU Habitats Directive and largely reflects local prey availability, as they feed opportunistically on whatever organisms are most abundant or accessible in their environment. The detection of native amphibians as prey, even in small individuals, highlights their potential to impact native populations, particularly vulnerable species during seasonal peaks in predation pressure. Observed spatial and temporal variation in diet suggests that water frogs may adaptively shift their feeding patterns in response to local prey availability, which can exacerbate impacts on native species that rely on similar resources.

The edible frog appears to have effectively occupied an empty ecological niche (shoreline sit-and-wait strategy), at least in sand and gravel pits, where they exhibit almost continuous predatory activity during their active season. At the Lieto study site on 16 September 2023, during late evening observations, medium-sized common frog (*Rana temporaria*) was seen hunting along the shoreline at times when most *Pelophylax* individuals were silent and likely already entered hibernation. This indicates that native frogs also use these habitats, although their activity periods might be temporally separated from those of *Pelophylax*.

While generalist predators such as frogs are expected to exploit abundant prey opportunistically, the strong signal for Odonata in the diet may reflect not only availability near aquatic habitats but also a genuine foraging preference. However, without a dedicated dietary selection analysis this cannot be confirmed with certainty. In the context of invasive or introduced frog populations, such a preference could elevate risks for sensitive insect taxa. Thus, the role of edible frogs as ecotonal predators may extend beyond simply linking aquatic and terrestrial food webs, potentially imposing selective pressures on insect groups of conservation concern. From a conservation management perspective, these findings underscore the importance of monitoring edible frog populations in areas where threatened or protected Odonata occur. Targeted surveys assessing frog diet alongside Odonata population dynamics could help clarify the extent of trophic impacts. Where evidence of strong local predation pressure exists, management actions such as limiting the spread of edible frog populations or enhancing habitat conditions for Odonata may be warranted to mitigate potential long-term risks.

At last, management of established populations of water frogs is challenging. The most effective control methods likely involve targeting reproduction, such as removing frog spawn (with care to avoid harming native species) or removing large adult females annually. In the estuaries of the Vantaa and Porvoo rivers, hunting the marsh frog (*Pelophylax ridibundus*) appears to have been an effective method for reducing local populations, eventually leading to the extinction of the species in Finland (Suomalainen, 1941; Terhivuo, 1993).

4.7 Limitations and future directions

Despite providing a detailed snapshot of the edible frog's diet, this study has several limitations that should be considered when interpreting the results. The difference between the observed and estimated total prey richness suggests that additional sampling could reveal further taxa (Figure 3). This highlights that the current dataset may not capture the full dietary diversity,

and caution is needed when interpreting patterns based solely on the observed taxa. The sample size, although substantial (68 individuals), represents only a single year, two seasons and two sites (Figure 2), which limits the ability to generalize temporal patterns across multiple years or to capture interannual variation in prey availability. Additionally, the study area is restricted to Southwest Finland, so spatial extrapolation to other regions or habitat types should be done cautiously.

Second, metabarcoding inherently carries biases that may affect the detection of certain prey taxa. Primer specificity, differential amplification efficiency, and variation in DNA degradation among prey types can lead to the under- or overrepresentation of some taxa. For example, soft-bodied prey may be under-detected due to rapid DNA degradation during digestion, whereas hard-bodied arthropods with chitinous exoskeletons may persist longer and therefore be overrepresented in metabarcoding data. Furthermore, the method cannot reliably distinguish between primary predation and secondary ingestion (e.g., prey consumed by other prey items), which may inflate apparent predation on some taxa. The use of blocking primers (not applied in this study) would likely be beneficial in this type of research, as they can improve the accuracy of prey species detection while reducing the amplification of host/predator DNA.

Third, while this study captured dietary composition across size classes, it provides limited insight into individual-level variation in prey choice, foraging behaviour, or energetic requirements. Frog sex and size were not strongly associated with diet in this study, but a larger dataset could reveal subtle patterns not detectable in the current dataset. In future studies, incorporating stomach flushing and traditional morphological identification of prey items would complement metabarcoding data, providing a more complete picture of dietary composition and validating molecular results.

For future research, several avenues could enhance understanding of edible frog feeding ecology. Longitudinal studies spanning multiple years and additional seasons would allow assessment of interannual variability and more robust conclusions regarding seasonal patterns. Combining metabarcoding with direct observation, stomach flushing, and morphological prey identification could improve prey detection accuracy and quantify ingestion rates more reliably. Investigating prey availability and activity patterns in the environment would allow formal tests of prey selectivity and functional responses. Expanding the study to multiple populations across varied habitat types could clarify how local environmental conditions and anthropogenic impacts shape diet composition.

In addition to foraging ecology and prey composition, future studies on edible frogs in Finland should address broader ecological and biogeographical questions. Little is currently known about the origin and introduction pathways of these invasive frog populations, or how they have established in anthropogenic habitats such as sand pits and quarries. Understanding the mechanisms behind their unusually rapid spread to new environments would provide valuable insights into invasion dynamics and habitat suitability.

Moreover, future work should not only focus on the potential negative impacts of these frogs but also consider their role within local food webs. For instance, edible frogs may serve as prey for various bird species (e.g., herons), thus contributing to ecosystem energy flow. Across all study sites, grass snake (*Natrix natrix*) was observed, and in Rusko an individual was verified attempting to predate water frogs. This snake species has also been reported as increasingly common in the Botanic Garden of the University of Turku, particularly around the pond area where water frogs occur in large numbers; it used to be quite rarely seen there in the past (T. Andersson, pers. comm., 20 Nov. 2025). In the 2000 national Red List assessment of Finland, grass snake was classified as Vulnerable, in 2010 it was listed as Near Threatened, and by 2019 it was considered Least Concern due to improved population viability. Investigating such trophic interactions could clarify whether edible frog exerts net positive or negative effects on native biodiversity. While invasive water frogs may provide additional prey for grass snakes, potentially benefiting local predators, this can lead to apparent competition, whereby increased snake populations impose higher predation pressure on native amphibians, potentially negatively affecting their population dynamics.

Finally, disease ecology warrants dedicated attention. Screening for pathogens such as *Batrachochytrium dendrobatidis* (chytridiomycosis) and Ranavirus is essential to evaluate whether these populations may act as reservoirs or vectors that could threaten native amphibian communities. Assessing infection prevalence and potential transmission dynamics would help predict the long-term ecological consequences of edible frog invasions in Finnish ecosystems. During this study, none of the sampled edible frogs exhibited any visible external or internal signs of disease, and all individuals appeared healthy.

4.8 Conclusions

To conclude, the results provide the most detailed assessment to date of the trophic ecology of *Pelophylax* kl. *esculentus* in Southwest Finland's boreal freshwater ecosystems. The invasive water frog exhibits a broad, generalist diet dominated by arthropods, with occasional predation

on native amphibians. Dietary patterns are shaped primarily by spatial and seasonal factors, reflecting local prey structure and environmental conditions rather than frog morphology or sex. Despite methodological and temporal limitations, the present findings establish a robust ecological baseline for understanding how invasive water frogs integrate into, and potentially alter, native food webs in Southwest Finland.

The study highlights specific prey groups, particularly Trichoptera and Odonata, that may experience disproportionate pressure by water frogs, raising questions about indirect effects on biodiversity and ecosystem function. Consequently, *P.* kl. *esculentus* should be viewed not only as a consumer within aquatic–terrestrial ecotones but also as a potential modifier of community dynamics. Future work linking dietary data with prey availability, habitat characteristics, and population monitoring will be essential for evaluating the long-term ecological and conservation consequences of these expanding frog populations in Finland.

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Appendices

Appendix 1. Conceptual background and additional explanation of PBPI

An important consideration for interpreting PBPI is species richness within each insect order. Orders with many species are more likely to contribute at least some individuals or species to observed records, simply because more species are available. This increases the probability that at least one species from a species-rich order appears in the data. Conversely, orders with fewer species have a lower baseline probability of being represented. Variation in insect body size may also explain why some datasets show deviations from the expected relationship between species richness and observations. Additionally, certain groups may be more frequently observed or collected due to collector preferences or hobbyist interests e.g. Lepidoptera.

To test this assumption, I used data from the Finnish Laji.fi database, which compiles species occurrence records from human-collected datasets, species richness of groups and size information of different insect species (retrieved 17 November 2025). Because the number of species and observations vary over several orders of magnitude, both variables were log10-transformed to normalize the distribution and stabilize variance, making statistical relationships easier to detect. Analysis of log-transformed data revealed a strong positive correlation between species richness and the number of observations ($r = 0.78$, $p < 0.001$). Similarly, maximum body size (from largest species of the order) was positively correlated with observations ($r = 0.71$, $p < 0.001$). Multiple regression confirmed that both species richness and maximum body length independently and positively influence the number of records (species slope = 0.78, $p < 0.001$; max body size slope = 1.68, $p < 0.001$; model $R^2 = 0.85$, $F_{(2,20)} = 57.92$, $p < 0.001$). These results indicate that patterns of recorded observations in Finland are shaped by both species diversity and maximum body size (Figure A1).

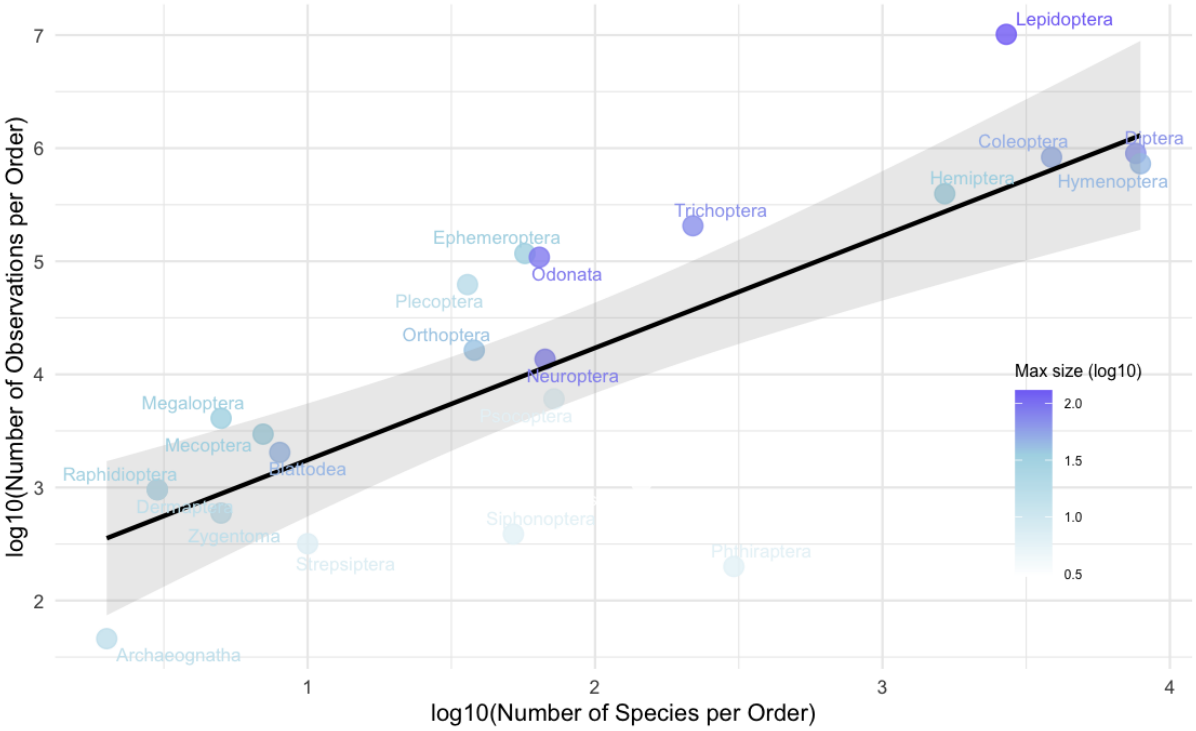

Figure A1. Relationship between insect order species richness, maximum body size, and the number of observations displayed on log10-transformed axes. Each point represents an insect order. The x-axis shows species richness, and point colour denotes the maximum body length within the order. Both axes are log10-transformed to accommodate the broad range of values. The fitted regression line illustrates the positive association between species richness and observation frequency. Multiple regression indicates that both species richness and maximum body size independently increase detectability ($R^2 = 0.85$, $F_{(2,20)} = 57.92$, $p < 0.001$). Data originate from the Laji.fi database (retrieved 17 November 2025).

Based on the above observation, PBPI is grounded in the assumption that species richness reflects the visibility of a taxonomic group in collections or samplings. Species-rich groups are more likely to appear in the data because they have more potential contributors. This implies that in collections, larger taxonomic groups tend to yield more individuals than smaller groups, even if individual species are equally abundant, this of course depend on collection methods and collector. Therefore, species richness serves as a natural normalizing measure, allowing us to assess which groups are relatively more or less exposed to predation or overrepresented in samples. Predators, including sit-and-wait types, can be viewed as “collectors” that capture what they encounter, but their foraging is not random: habitat structure, prey availability, and behaviour influence which prey are accessible. This non-randomness is a key consideration when interpreting PBPI. If we perform a PBPI analysis using Laji.fi data, Lepidoptera appear to experience high biodiversity pressure (Table A1). This is likely because humans, as

"predators," actively observe them. However, unlike sit-and-wait predators, humans do not capture prey passively but actively target specific taxa while mostly ignoring others. This is important to consider when interpreting PBPI results, because PBPI only reflects whether a prey taxon was captured more or less than expected, and does not indicate whether the predator is actively selecting that taxon.

Table A1. Representation of insect order observation numbers relative to Finnish insect diversity.

All insect orders in Finland and their relative species richness to all insect fauna P_f and number of observations of every insect order relative to all observations of insects P_d. Calculated PBPI values indicating biodiversity pressure in the data. Data retrieved from Laji.fi database 17 November 2025. The table presents rounded values, while all calculations were based on precise numbers.

Order	**P_f (%)**	**P_d (%)**	**PBPI**
Diptera	≈30.642%	≈6.643%	≈0.217
Mecoptera	≈0.028%	≈0.021%	≈0.774
Siphonoptera	≈0.209%	≈0.002%	≈0.013
Lepidoptera	≈10.865%	≈74.911%	≈6.894
Trichoptera	≈0.882%	≈1.52%	≈1.723
Coleoptera	≈15.615%	≈6.141%	≈0.393
Strepsiptera	≈0.04%	≈0.002%	≈0.058
Neuroptera	≈0.269%	≈0.1%	≈0.371
Megaloptera	≈0.02%	≈0.03%	≈1.499
Raphidioptera	≈0.012%	≈0.007%	≈0.582
Hymenoptera	≈31.822%	≈5.374%	≈0.168
Phthiraptera	≈1.224%	≈0.001%	≈0.001
Psocoptera	≈0.29%	≈0.044%	≈0.154
Hemiptera	≈6.635%	≈2.917%	≈0.439
Thysanoptera	≈0.584%	≈0.008%	≈0.014
Blattodea	≈0.032%	≈0.015%	≈0.467
Orthoptera	≈0.153%	≈0.12%	≈0.787
Plecoptera	≈0.145%	≈0.459%	≈3.166
Dermaptera	≈0.012%	≈0.007%	≈0.583
Ephemeroptera	≈0.229%	≈0.864%	≈3.762
Odonata	≈0.257%	≈0.801%	≈3.108
Zygentoma	≈0.02%	≈0.004%	≈0.217
Archaeognatha	≈0.008%	≈0.0003%	≈0.042

PBPI provides a biodiversity-weighted perspective on the relative exposure of insect orders to for example predation. PBPI is calculated by comparing the proportion of an order in, for

example, a predator's diet to its relative species richness in area (e.g., country), and its interpretation is influenced by taxonomic diversity at the order level. This index works best in regions, such as Finland, where insect species are well known, as accurate knowledge of species richness is essential for the index to function. PBPI is calculated as

$$\text{PBPI} = \frac{P_d}{P_f}$$

where P_d is the proportion of species, individuals or metabarcoding reads of a given insect order in e.g. frog's diet, and P_f is the proportion of species of that order in the Finnish insect fauna. PBPI values indicate relative biodiversity pressure, not dietary preference, prey abundance, or biomass. High PBPI values (>1) reflect higher biodiversity pressure, meaning the order is disproportionately represented in the diet compared to what would be expected based on its species richness in Finland. Low PBPI values (<1) reflect lower biodiversity pressure, indicating the order is underrepresented relative to its national species richness.

The index does not consider how insects are distributed within the predator's habitat. Its purpose is to identify taxonomic groups that may warrant attention when assessing potential impacts of a predator. For example, in this study, Odonata appear to be at higher potential risk if frog populations continue to grow. In contrast, Lepidoptera may appear less concerning, as they are underrepresented in the diet in this small-scale assessment.

The idea behind PBPI is to provide a quick evaluation without requiring extensive prey availability analyses, offering an initial indication of which (prey) groups may deserve further attention. PBPI is at this stage a prototype and requires further development. In this thesis, the index is applied for the first time and should not yet be regarded as a fully validated method; in the future, its assumptions will be tested using passive traps that do not attract prey, with the expectation that the relationship between species richness and the number of representatives per order is maintained.

Appendix 2. Prey species list

Table A2. Prey taxa observed in this study, showing the lowest taxonomic level identified using molecular methods, the corresponding order, total reads, and the proportion of all prey taxa.

Taxon	Order	Total reads	Percent of all prey taxa
Scapholeberis mucronata	Anomopoda	119	0.010%
Ceriodaphnia quadrangula	Anomopoda	62	0.005%
Alonella excisa	Anomopoda	54	0.005%
Ceriodaphnia	Anomopoda	24	0.002%
Rana arvalis	Anura	44	0.004%
Ranidae	Anura	24	0.002%
Chrysomelidae	Coleoptera	323,721	28.044%
Donacia aquatica	Coleoptera	64,911	5.623%
Aphthona lutescens	Coleoptera	28,025	2.428%
Erichsonius cinerascens	Coleoptera	25,723	2.228%
Elaphrus cupreus	Coleoptera	12,165	1.054%
Cercyon ustulatus	Coleoptera	11,517	0.998%
Chaetocnema aerosa	Coleoptera	7,950	0.689%
Aphthona	Coleoptera	5,825	0.505%
Anotylus rugosus	Coleoptera	4,962	0.430%
Chaetarthria seminulum	Coleoptera	3,063	0.265%
Heterocerus fusculus	Coleoptera	1,754	0.152%
Schistoglossa aubei	Coleoptera	1,450	0.126%
Plateumaris discolor	Coleoptera	616	0.053%
Helochares obscurus	Coleoptera	181	0.016%
Cercyon convexiusculus	Coleoptera	133	0.012%
Philonthus corvinus	Coleoptera	102	0.009%
Stenus melanarius	Coleoptera	101	0.009%
Galerucella lineola	Coleoptera	44	0.004%
Notoxus monoceros	Coleoptera	27	0.002%
Stenus europaeus	Coleoptera	24	0.002%
Bimastos rubidus	Crassiclitellata	9,908	0.858%
Dendrobaena octaedra	Crassiclitellata	383	0.033%
Mesocyclops leuckarti	Cyclopoida	1,043	0.090%
Thermocyclops oithonoides	Cyclopoida	80	0.007%
Eucyclops serrulatus	Cyclopoida	33	0.003%
Carassius carassius	Cypriniformes	630	0.055%
Pilaria meridiana	Diptera	69,803	6.047%
Erioptera squalida	Diptera	65,097	5.639%

Molophilus griseus	Diptera	14,808	1.283%
Metalimnobia bifasciata	Diptera	7,480	0.648%
Phylidorea fulvonervosa	Diptera	7,285	0.631%
Polypedilum cultellatum	Diptera	6,194	0.537%
Diptera	Diptera	2,493	0.216%
Gymnopternus metallicus	Diptera	2,469	0.214%
Dicranomyia ventralis	Diptera	1,765	0.153%
Anopheles messeae	Diptera	987	0.086%
Platycheirus clypeatus	Diptera	727	0.063%
Melanostoma	Diptera	488	0.042%
Omisus caledonicus	Diptera	471	0.041%
Gymnopternus silvestris	Diptera	172	0.015%
Cecidomyiidae	Diptera	117	0.010%
Diamesa chorea	Diptera	93	0.008%
Micropsectra insignilobus	Diptera	62	0.005%
Orthocladius telochaetus	Diptera	55	0.005%
Limnophyes	Diptera	51	0.004%
Carcelia reclinata	Diptera	38	0.003%
Metriocnemus beringensis	Diptera	37	0.003%
Scathophaga litorea	Diptera	27	0.002%
Chaoborus flavicans	Diptera	24	0.002%
Forcipomyia knockensis	Diptera	22	0.002%
Halocladius variabilis	Diptera	21	0.002%
Paracladopelma	Diptera	21	0.002%
Tanytarsus lugens	Diptera	20	0.002%
Heterotrissocladius changi	Diptera	14	0.001%
Isotomurus palustris	Entomobryomorpha	85	0.007%
Caenis horaria	Ephemeroptera	36	0.003%
Macustus grisescens	Hemiptera	46,543	4.032%
Megamelus notulus	Hemiptera	3,953	0.342%
Javesella dubia	Hemiptera	1,747	0.151%
Microvelia reticulata	Hemiptera	1,024	0.089%
Hemiptera	Hemiptera	586	0.051%
Psyllidae	Hemiptera	85	0.007%
Camponotus herculeanus	Hymenoptera	59,008	5.112%
Lasius niger	Hymenoptera	25,434	2.203%
Myrmica scabrinodis	Hymenoptera	4,823	0.418%
Lasius platythorax	Hymenoptera	3,515	0.305%

Myrmica rubra	Hymenoptera	635	0.055%
Hymenoptera	Hymenoptera	239	0.021%
Myrmica sabuleti	Hymenoptera	127	0.011%
Lasius alienus	Hymenoptera	60	0.005%
Formicidae	Hymenoptera	49	0.004%
Pammene populana	Lepidoptera	90	0.008%
Lepidoptera	Lepidoptera	21	0.002%
Littorinimorpha	Littorinimorpha	33	0.003%
Sympetrum flaveolum	Odonata	24,320	2.107%
Sympetrum danae	Odonata	55	0.005%
Polyphemus pediculus	Onychopoda	164	0.014%
Lacinius ephippiatus	Opiliones	17	0.001%
Tetrigidae	Orthoptera	374	0.032%
Orthoptera	Orthoptera	50	0.004%
Cyclocypris ovum	Podocopida	73,001	6.324%
Cypridopsis vidua	Podocopida	18,723	1.622%
Cyprididae	Podocopida	98	0.008%
Polydesmida	Polydesmida	96	0.008%
Sphaerulariidae	Rhabditida	69,284	6.002%
Rhabditida	Rhabditida	42	0.004%
Punctoribates hexagonus	Sarcoptiformes	290	0.025%
Sarcoptiformes	Sarcoptiformes	107	0.009%
Pungitius pungitius	Scorpaeniformes	282	0.024%
Arion distinctus	Stylommatophora	27,478	2.380%
Ptenothrix atra	Symphypleona	233	0.020%
Molanna angustata	Trichoptera	40,988	3.551%
Oligotricha striata	Trichoptera	37,638	3.261%
Trichostegia minor	Trichoptera	13,991	1.212%
Limnephilus indivisus	Trichoptera	13,126	1.137%
Glyphotaelius pellucidus	Trichoptera	233	0.020%
Limnephilus stigma	Trichoptera	43	0.004%
Arrenurus tricuspidator	Trombidiformes	68	0.006%