# Biological Synopsis of the Marbled Crayfish (*Procambarus virginalis*)

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Aquatic Invasive Species Program
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# BIOLOGICAL SYNOPSIS OF THE MARBLED CRAYFISH (*PROCAMBARUS VIRGINALIS*)

by

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The Marbled Crayfish (*Procambarus virginalis)* is the first described asexual decapod crustacean and an emerging invasive threat to Canadian freshwater ecosystems. The species is suspected to have originated within the aquarium trade when the breeding of two Slough Crayfish (*Procambarus fallax*) produced a triploid offspring. Since its discovery in the wild over two decades ago, the Marbled Crayfish has been found throughout eastern and central Europe, Japan, Israel, as well as Madagascar—where it has established multiple reproducing populations. Recently, two North American feral populations were discovered in a southern Ontario stormwater management pond near Lake Ontario and a privately owned pond in Yarmouth County, Nova Scotia. Other feral populations may remain to be discovered, as suggested by the capture of single individuals in the wild in New York State. As a prerequisite for conducting a risk analysis of future invasions of Canadian inland waters, this biological synopsis summarizes information on the Marbled Crayfish's current worldwide distribution, biological traits, natural history, and impacts. Owing to the popularity of Marbled Crayfish among aquarium hobbyists, the release of unwanted pets is the most probable mechanism of introduction. Given the capacity of Marbled Crayfish to reproduce prolifically and parthenogenically, a single released individual could lead to the establishment of a feral population. Despite all individual Marbled Crayfish being genetic clones, epigenetic variation allows the species to be adaptable to different environments. Previous invasion history, diet studies, competition experiments, and documented burrowing behaviour (although not yet observed in Canada) suggest that the Marbled Crayfish can compete with native crayfishes and degrade invaded habitats.

**RÉSUMÉ**

Van Mierlo, V., Crew, A., Avlijaš, S., and Ricciardi, A. 2025. Biological Synopsis of the Marbled Crayfish (*Procambarus virginalis*). Can. Manuscr. Rep. Fish. Aquat. Sci. 3311: vi + 39 p. https://doi.org/10.60825/3gd2-9d79

L'écrevisse marbrée (*Procambarus virginalis)*, qui est le premier crustacé décapode asexué à avoir été décrit, est une espèce envahissante représentant une menace éventuelle pour les écosystèmes d'eau douce du Canada. Il est présumé qu'elle provient du commerce d'espèces destinées aux aquariums, dans le cadre duquel la reproduction de deux écrevisses des marécages (*Procambarus fallax*) aurait produit un descendant triploïde. Depuis sa découverte à l'état sauvage il y a plus de deux décennies, l'écrevisse marbrée a été observée à travers l'est et le centre de l'Europe, au Japon, en Israël, ainsi qu'à Madagascar, où diverses populations reproductrices sont établies. Récemment, en Amérique du Nord, deux populations férales ont été découvertes dans un bassin de gestion des eaux pluviales du sud de l'Ontario, à proximité du lac Ontario, et dans un étang privé du comté de Yarmouth, en Nouvelle-Écosse. La capture d'écrevisses marbrées individuelles à l'état sauvage dans l'État de New York indique que d'autres populations férales pourraient être découvertes. Le présent sommaire biologique, dont la publication est préalable à la réalisation d'une analyse des risques liés aux invasions futures dans les eaux intérieures du Canada, résume les renseignements sur la répartition actuelle à l'échelle mondiale, les caractéristiques biologiques, l'histoire naturelle et les répercussions de l'écrevisse marbrée. En raison de la popularité de l'espèce chez les aquariophiles, la libération de spécimens domestiques non désirés représente le mécanisme d'introduction le plus probable. Compte tenu de la capacité de l'espèce à se reproduire de manière prolifique et parthénogénétique, un seul individu libéré pourrait mener à l'établissement d'une population férale. Même si toutes les écrevisses marbrées sont des clones génétiques, la variation épigénétique permet à l'espèce de s'adapter à différents environnements. Les invasions passées, les études sur le régime alimentaire, les expériences liées à la compétition et le comportement fouisseur documenté (même s'il n'a pas encore été observé au Canada) indiquent que l'écrevisse marbrée peut entrer en compétition avec des écrevisses indigènes et dégrader les milieux qu'elle envahit.

## 1.0. INTRODUCTION

The Marbled Crayfish (*Procambarus virginalis*), the first described asexual species of decapod crustacean, has been identified as one of the 'worst' alien species in Europe (Nentwig et al., 2018). This species is thought to have originated from the sexually reproducing Slough Crayfish (*Procambarus fallax*) through autotriploidy and consists of genetically identical individuals that reproduce through parthenogenesis (Martin, Dorn, et al., 2010; Vogt, 2015). Speciation of the Marbled Crayfish most likely occurred in captivity sometime between the late 1980s and early 1990s (Legrand et al., 2023; Vogt et al., 2015).

The first detection of a live Marbled Crayfish in natural waters occurred in Germany in 2003 (Marten et al., 2004). To date, it has been documented in over a half dozen European countries (Chucholl et al., 2012; Hossain et al., 2018), Israel (Carneiro et al., 2023), the island nations of Madagascar and Japan (Deidun et al., 2018; Jones et al., 2009; Kawai and Takahata, 2010), and most recently in Canada—the first documentation of the species in North American inland waters (Hamr, 2024). Its occurrence in the wild is attributed primarily to the disposal of unwanted pets acquired commercially (Chucholl, 2013; Patoka et al., 2014).

Owing to its fast growth, early maturation, high fecundity, and environmental adaptability, there has been warranted concern over its potential introduction and establishment in North America (Jones et al., 2009; Seitz et al., 2005), including Canada (Faulkes, 2010). In October 2021, a citizen found and reported the first feral Marbled Crayfish individual in a field adjacent to an urban stormwater management pond in City View Park in Burlington, Ontario (iNaturalist, 2021). The following August, the Ontario Federation of Anglers and Hunters and Trent University positively detected Marbled Crayfish in this pond via environmental DNA analysis. A working group composed of government agencies, researchers, and non-governmental organizations, including the City of Burlington, Ontario Ministry of Natural Resources, and Fisheries and Oceans Canada (DFO), responded and attempted eradication of the population by dewatering the pond during the winters of 2022, 2023, and 2024. Although eradication efforts continue (as of November 2025), the population is considered to be the first established and reproducing population of feral Marbled Crayfish in North America (Hamr, 2024). In October 2023, a single live individual was trapped by DFO in a waterbody in Yarmouth County, Nova Scotia (Hamr, 2024). This population has since been confirmed by DFO to be a reproducing population, with hundreds of adult and juvenile individuals being trapped in the same location. Based on these findings, it is possible that more populations have yet to be discovered in Canadian freshwaters. In response to this emerging threat and to help inform a risk assessment, a biological synopsis of the Marbled Crayfish is presented here. See Table 1 for a summary of key Marbled Crayfish knowledge gaps, gleaned from this synopsis.

## 1.1. NAME AND CLASSIFICATION

The taxonomic classification of *P. virginalis* given by Lyko (2017) is based on its species traits, reproductive incompatibility, and substantial genetic differences compared to *P. fallax*, the parent species, and to the Everglades Crayfish (*Procambarus alleni*), a species endemic to Florida, despite their morphological similarity. Below is a classification scheme from CABI Invasive Species Compendium (Chucholl, 2011) and Lyko (2017).

Domain: Eukaryota
Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Crustacea
Class: Malacostraca
Subclass: Eumalacostraca
Order: Decapoda
Suborder: Pleocyemata
Superfamily: Astacoidea, Latreille
Family: Cambaridae, Hobbs
Genus and Species: *Procambarus virginalis* (Lyko, 2017)
Common English name: Marbled Crayfish
Common French name: L'Écrevisse marbrée
Other name used in literature: Marmorkrebs (from German)
Local common names: Mramorni rak (Croatia), Gambero marmorato (Italy), marmerkreeft (Netherlands), foza orana or orana vahiny (Madagascar)

## 1.2. DESCRIPTION

Marbled Crayfish is an evolutionarily young species, estimated to have arisen at some point between the mid 1980s and early 1990s (Legrand et al., 2023; Vogt, 2019; Vogt et al., 2015) as a result of the mating of two *P. fallax* individuals (Martin et al., 2010a). One *P. fallax* parent contributed a diploid gamete (with two copies of each chromosome) and the other contributed a normal haploid gamete, to produce the triploid *P. virginalis* individual from which all Marbled Crayfish descend via parthenogenesis (Gutekunst et al., 2018; Vogt et al., 2018).

Although Marbled Crayfish inherited its genome directly from *P. fallax*, triploidization introduced significant genetic and epigenetic changes which led to differences in adaptability and life history (Maiakovska et al., 2021; Vogt et al., 2019). One such difference is a ~20% reduction in methylation of Marbled Crayfish DNA (Vogt et al., 2015), including reduced methylation of housekeeping genes (Gatzmann et al., 2018) which enhances the expression variability of genes (Vogt et al., 2019). The balance of literature on the subject of Marbled Crayfish methylation for gene expression supports the notion that Marbled Crayfish establish epigenetic ecotypes through the expression of different phenotypic traits by way of gene methylation (Vogt, 2022). This is supported by recent studies that have shown that Marbled Crayfish individuals captured from four locations that differed in pH, water hardness, and three dissolved elements possessed location-specific methylation signatures in the hepatopancreatic tissue (Tönges et al., 2021). Differences in methylation signatures were also observed between field and laboratory colonies, as well as two laboratory colonies that were reared at different water temperatures (Tönges et al., 2021).

Unique epigenetic ecotypes and methylation signatures can arise from a variety of sources and at different stages of the Marbled Crayfish life cycle. First, environmental rearing conditions contribute to unique methylation signatures and associated phenotypic characteristics. One study found that differing temperatures and oxygen levels during juvenile stages of development had significant effects on the adult physiology of Marbled Crayfish, including carapace length and heart rate, indicating that environmental cues during development modulate phenotypic plasticity in genetically identical individuals (Göpel and Burggren, 2024). Second, methylation signatures can change within the lifespan of a single mature individual. This was observed by Venkatesh et al. (2023) when laboratory-raised adult Marbled Crayfish were transferred into an aquaculture environment and significant changes in methylation signatures were seen in as little as a single month post-relocation. Third, Marbled Crayfish methylation signatures can also be inherited generationally. This was seen in a laboratory study where exposure of Marbled Crayfish mothers to genotoxic chemicals resulted in genome methylation of the unexposed F1 that was similar to that of exposed mother crayfish, suggesting that generational experiences impact the epigenetics of subsequent generations (Marçal et al., 2021). Lastly, stochastic developmental variation (SDV), a type of random and non-environmental path of epimutations, can also have an effect on different phenotypes in genetically identical individuals raised in identical environmental conditions; both epigenetic mechanisms (SDV and environmental rearing conditions) could result in observed plasticity in Marbled Crayfish (Göpel and Burggren, 2024).

An approximately 10% reduction of the haploid genome size compared to *P. fallax* likely has additional consequences for gene regulation (Vogt et al., 2019). These changes appear to be responsible for the enhanced ability of the new species to adapt to cold environments, and its increased fitness traits, including larger size, longer lifespan, and higher fecundity compared to the parental *P. fallax* species.

Heterozygosity, which can buffer the effects of deleterious mutations, is also a property of the Marbled Crayfish genome, as evidenced by the divergence between the genotypes contributed by the two parental *P. fallax* individuals (Gutekunst et al., 2018). Therefore, despite its monoclonal nature resulting from asexual reproduction, the Marbled Crayfish does not seem to have impaired adaptability, and its fitness is enhanced beyond the direct benefits of parthenogenesis.

### 1.2.1 Visual appearance and identification of the species

Marbled Crayfish is named after the characteristic speckled colouration of its carapace, which is most prominent on the branchiostegal region. The speckled colouration is unique to Marbled Crayfish and *P. fallax* with the exception of two individuals that were found in Italy (Kamburska et al., 2024). These two crayfish possessed the classic marbled colour morph and chelae size and shape (small and narrow) typically associated with Marbled Crayfish, but were genetically identified as Red Swamp Crayfish (*Procambarus clarkii*) (Kamburska et al., 2024). Additionally, the White River Crayfish (*Procambarus acutus*), which was recently detected in Thornhill, Ontario (iNaturalist, 2024), have a chelae shape and open areola similar to that of Marbled Crayfish. Therefore, identification of Marbled Crayfish might be challenging if *P. clarkii* and/or *P. acutus* are also present in the waterbody.

The marbled pattern of lighter spots on a darker background in Marbled Crayfish (Figure 1) is epigenetically determined and varies between individuals (Vogt et al., 2018). The coloration ranges from brown to red to blue and seems to depend on rearing conditions and food type (Vogt et al., 2004). Body coloration of some individuals from the Burlington, Ontario population were dark brown or black which could partially conceal their distinctive speckled pattern to a lay observer (unpublished data). Individuals of this species generally range in size from 2.5–10 cm but may achieve a maximum body length of 12–13 cm, corresponding to an estimated body weight of approximately 35 g (Vogt et al., 2018). Ovigerous Marbled Crayfish, referred to as "in berry", can have exceptionally large clutch sizes varying from ~50 to >500 eggs per clutch (Figure 2) (Lyko, 2017).

Detailed accounts of Marbled Crayfish morphology, distinguishing characteristics, and development have been described in the literature (Kawai et al., 2009; Lyko, 2017; Vogt et al. 2018). Three key identifying features for Marbled Crayfish allow it to be differentiated from other crayfishes, except for *P. fallax* (Vogt et al., 2018). The first feature is the presence of a freely movable, bell-shaped annulus ventralis with an S-shaped sinus. The annulus is located on the underside of the cephalothorax between the fourth and fifth pairs of pereiopods (Figure 3). Secondly, Marbled Crayfish can be recognized by the well-developed tubercles on the mesial surface of the chela palm (Figure 4). Finally, in this species the dactyl is distinctly longer than the mesial margin of the palm (Figure 4). Visually, Marbled Crayfish are indistinguishable from females of their parent species, *P. fallax*. Absence of male individuals and a high proportion of individuals larger than 7 cm total length (TL) in a population can suggest the species is *P. virginalis* rather than *P. fallax* (Vogt et al., 2018). The identification can be confirmed by genetic analysis of microsatellite PclG-02: in *P. fallax* it is mono or di-allelic with fragment lengths varying between individuals, while in *P. virginalis* it is tri-allelic with fragment lengths of 267, 271, and 303 base pairs (Vogt et al., 2018).

## 2.0. DISTRIBUTION

### 2.1. ORIGIN

It is hypothesized that Marbled Crayfish arose in captivity, although the exact location remains unknown (Scholtz et al., 2003). While it is possible for triploid parthenogenic crayfish to appear in natural systems, Marbled Crayfish is absent from the entire native range of its parental species, which extends throughout most of Florida and a small area of southern Georgia (Vogt et al., 2018). The first detection of the triploid parthenogenic individuals was in the German aquarium trade in 1995 (Scholtz et al., 2003); however, integrated analysis of clonal genome evolution estimated the speciation date of Marbled Crayfish to be between 1986 and 1990 (Legrand et al., 2023). It is therefore highly probable that Marbled Crayfish arose in captivity, possibly as a result of exposure to heat or cold shock during transportation or storage of the animals (Vogt et al., 2018).

There is disagreement in the literature regarding the exact geographical origin of Marbled Crayfish. Microsatellite analysis comparing Marbled Crayfish to *P. fallax* from 10 different natural sites spanning across their native range suggests that the most likely provenance of the lineage is in the Santa Fe River basin, northern Florida (Vogt et al., 2018). In contrast, when compared to the mitochondrial genome, whole genome sequencing of *P. fallax* from 23 sites throughout Florida indicated strongly that Marbled Crayfish originated from the Everglades subpopulation in southern Florida (Gutekunst et al., 2021). What is agreed upon in the literature is that all Marbled Crayfish are monoclonal with genomic analysis of multiple European and Malagasy populations revealing a consistently low number of single nucleotide variants between populations on different continents (Maiakovska et al., 2021). Regardless of their true origin, due to the epigenetic and genetic changes described in section 1.2, the range inhabited by *P. fallax* is unlikely to be a good predictor of the areas Marbled Crayfish can inhabit.

## 2.2. NON-NATIVE DISTRIBUTION AND ABUNDANCE

The first documented occurrence of Marbled Crayfish in the wild was in 2003 when researchers found juvenile Marbled Crayfish in a dredging pool, 1 km away from the Rhine River in southern Germany (Marten et al., 2004). To date, most reports of Marbled Crayfish in Europe are from Germany (Chucholl et al., 2012); however, since its initial discovery, it has been reported throughout eastern and central Europe, with observations rapidly accumulating since the early 2000s (Figure 5). There are now nearly 40 documented invasions in the region (Figure 6) with single individuals and/or established populations in the following countries: the Netherlands, Czech Republic, Croatia, Estonia, Italy, Hungary, Slovakia, Ukraine, Romania, Sweden, the island of Malta, France (Aluma et al., 2023), Belgium, Poland, and most recently, Spain (Aluma et al., 2023; Bohman et al., 2013; Collas, 2019; Deidun et al., 2018; Ercoli et al., 2019; Grandjean et al., 2021; Janský and Mutkovič, 2010; Lipták et al., 2016; Lőkkös et al., 2016; Maciaszek et al., 2022; Marzano-Nonnis, 2009; Novitsky et al., 2016; Pârvulescu et al., 2017; Patoka et al., 2016; Samardžić et al., 2014; Sánchez et al., 2024; Sanna et al., 2021; Scheers et al., 2021; Soes and van Eekelen, 2006; Son et al., 2020; Vojkovská et al., 2014; Weiperth et al., 2020). Marbled Crayfish have also been documented in two multi-national river systems, the Rhine and the Danube rivers, highlighting their potential for rapid spread (Chucholl et al., 2012; Lipták et al., 2016, 2017).

Marbled Crayfish have been reported for the first time in the Middle East with two recently documented established populations in Israel (Carneiro et al., 2023). It has also been reported in two locations in Japan (Figure 6). The first detection was in 2006, when a single crayfish was found in a river near Sapporo (Kawai and Takahata, 2010, as cited in Vogt, 2018) and the second report was in 2016, when a single Marbled Crayfish was found in the outflow stream of a canal along the Shigenobu River near Matsuyama (Usio et al., 2017), although the authors mention that another specimen had been found earlier in the same region (Usio et al., 2017, as cited in Vogt, 2018). In both cases the introductions were associated with the aquarium trade.

Arguably, the most successful introduction of Marbled Crayfish to date has been in Madagascar (Figure 6) where it spread rapidly and is now found in very high abundances (Gutekunst et al., 2018; Jones et al., 2009). Although the origin of the initial introduction is unknown, the species was first detected in food markets in Madagascar's capital city, Antananarivo, in 2005 (Andriantsoa et al., 2019; Jones et al., 2009). Field surveys and interviews with locals determined that the species had been established in the wild since at least 2003 (Jones et al., 2009; Kawai et al., 2009). Since its discovery, Marbled Crayfish has spread swiftly across the island, increasing its dispersal area from $10^3$ $km^2$ to $>10^5$ $km^2$ within a decade (Gutekunst et al., 2018).

There are no documented observations of Marbled Crayfish in South America. Excluding Canada, Marbled Crayfish have been observed in North America in New York State, USA (Figure 6). Individual Marbled Crayfish have been observed in three discrete waterbodies: two in the Bronx (iNaturalist, 2022 and 2023a) and one in Ridgefield, New York (iNaturalist, 2023b). At time of publication, it is unclear if these are established populations.

## 2.3. DISTRIBUTION IN CANADA

Marbled Crayfish were first observed in Canada in City View Park in Burlington, Ontario, in October 2021 (iNaturalist, 2021; Hamr, 2024; Ontario Ministry of Natural Resources, 2024); members of the Marbled Crayfish Working Group subsequently confirmed this to be a reproducing population in July 2023. It is the first known established population of Marbled Crayfish in the wild in North America, and was likely the result of an aquarium release. In 2023, a single Marbled Crayfish was collected by DFO staff from a privately owned pond in Yarmouth, Nova Scotia (Hamr, 2024); hundreds of individuals of different age classes have since been collected in the same location. Although not officially confirmed at time of publication, this may be the second established Canadian population of Marbled Crayfish.

Despite being banned from sale or distribution in Ontario, Alberta, and Saskatchewan, Marbled Crayfish can be purchased by aquarium hobbyists from retailers in most provinces and territories, as well as through online platforms or classified ads (e.g., Kijiji) (Faulkes, 2018). A challenge to tracking the spread of captive live specimens is that two thirds of crayfish sales in North America are conducted "in person" (Faulkes, 2013). Compared to online sales that are shipped directly to the customer, these "in-person" sales can be made at a neutral public location; hobbyists may also travel long distances to aquarium retail stores, making it difficult to determine the live specimen's end location.

No climate match analysis has been conducted for Canadian natural waters; however, a recent Ecological Risk Screening Summary, using the Risk Assessment Mapping Program, calculated the climate match between the current geographic range of Marbled Crayfish and the United States (U.S. Fish and Wildlife Service, 2023). This analysis determined that under current climate scenarios there is moderate to high predicted likelihood of establishment for Marbled Crayfish in the American areas of the Great Lakes region; moderate matches in the central, northeast, and mid-Atlantic regions; and a low climate match along the Pacific coast and in the southern Appalachians (Figure 7).

A Maxent habitat suitability model for North America suggested that Marbled Crayfish are most likely to establish in the south-eastern and south-central portions of the United States, and not likely to establish in Canada (Feria and Faulkes, 2011). However, the model was trained using the distribution of the parental species *P. fallax*, the Madagascar population of Marbled Crayfish, and the seven occurrences of Marbled Crayfish in Europe (five in Germany, one in Italy, and one in the Netherlands) that had been documented prior to October 2010. The model can therefore be expected to underestimate habitat suitability for Marbled Crayfish in temperate areas.

Presently, there are no adequate quantitative models that inform the extent and location of suitable Marbled Crayfish habitat and climate in Canada. To address this knowledge gap, models for Canada should consider future climate scenarios as climate warming may facilitate and expand Marbled Crayfish establishment, particularly in cold, temperate regions (Haubrock et al., 2019).

The qualitative risk of Marbled Crayfish invasion in 21 district freshwater ecoregions of Canada, assessed using the Canadian Marine Invasive Screening Tool (CMIST) (Drolet et al., 2016), estimated that the Laurentian Great Lakes ecoregion is at highest risk (greatest CMIST score = 3.81) of Marbled Crayfish invasion (Brown and Therriault, 2022). The ecoregion of next greatest risk is the Alaska and Canadian Pacific Coastal ecoregion (Brown and Therriault, 2022). Interestingly, the results also suggest that Marbled Crayfish are of generally lower relative risk of invasion and had the lowest likelihood of invasion across all ecoregions when compared to seven other potential invasive crayfishes (Brown and Therriault, 2022). However, the authors suggest that the overall low invasive risk and potential impact estimated by the tool could be a result of limited literature regarding the ecological impacts of Marbled Crayfish on receiving ecosystems and because Marbled Crayfish are generally moved by a single vector (aquarium release) which may have resulted in an underestimated CMIST score (Brown and Therriault, 2022).

# 3.0. BIOLOGY AND NATURAL HISTORY

## 3.1. AGE AND GROWTH

Marbled Crayfish are considered to be an *r*-selected species, owing to their rapid growth, early maturation, high fecundity, short embryogenesis, and frequent spawning events (Hossain et al., 2018). The mean life span of Marbled Crayfish is ~700 days, whereas the oldest Marbled Crayfish ever recorded had a lifespan of 1610 days or ~4.5 years (Vogt, 2010).

As described by Vogt et al. (2004), the species life cycle is categorized into five periods: 1) embryonic development; 2) brood care; 3) the period of differentiation of the reproductive organs, which lasts until an individual reaches 2 cm TL; 4) the gonadal maturation period from 2–4 cm TL; and 5) the reproducing period of adults from 4–12 cm TL. Embryonic development generally lasts 2–3 weeks (Vogt and Tolley, 2004) and the brooding period another 2–5 weeks, depending on the environmental conditions and the individual female (Seitz et al., 2005; Vogt, 2008; Vogt et al., 2004; Vogt and Tolley, 2004). Each period is further differentiated into distinct stages and phases, with the embryonic period containing 10 stages, brooding period containing two phases (passive and active), and the juvenile phase containing approximately 7–8 stages. Lastly, the adult phase spans from first oviposition (which can occur as early as 110 days of age; Kouba et al., 2021) to death, and includes several reproductive cycles and moults (Vogt, 2008).

Growth of Marbled Crayfish is not isometric (Seitz et al., 2005) and thus resembles other species such as the Noble Crayfish (*Astacus astacus*), Signal Crayfish (*Pacifastacus leniusculus)*, and Spinycheek Crayfish (*Faxonius limosus)* (Hossain et al., 2018). However, all Marbled Crayfish are genetically identical clones and as such their growth is highly dependent on rearing conditions and prey quality (Kaldre, Haugjärv, et al., 2015). In a favourable environment, Marbled Crayfish can grow to ~12–13 cm in total body length (Seitz et al., 2005; Vogt et al., 2018) and experience their greatest growth period as juveniles (Vogt et al., 2018).

## 3.2. PHYSIOLOGICAL TOLERANCES

### 3.2.1 Salinity

The only study to examine salinity tolerance in Marbled Crayfish found that it has low survival, reduced growth, and is unable to reproduce in experimental conditions when exposed to salinity levels as low as 6 ppt (Veselý et al., 2017). This contrasts with other North American crayfish such as the Spinycheek and Signal crayfishes, which reproduce at salinities of 7 ppt, and the Red Swamp Crayfish, which reproduces at 25 ppt. There were no statistically significant differences between the 6 ppt, 9 ppt, 12 ppt, 15 ppt, and 18 ppt treatments; however, two crayfish (one at 9 ppt and the other at 12 ppt) survived until the end of the 155-day experiment. Mortality in the experiment was associated with moulting events and was likely a result of osmotic stress. Although Marbled Crayfish were clearly impaired by saline conditions, most individuals survived at least medium-term (~80 days), suggesting that the species may have an opportunity to adapt (Veselý et al., 2017).

### 3.2.2 Temperature

Despite having a temperature optimum (18–25°C) that resembles that of warmwater species, the Marbled Crayfish can withstand a broad range of temperatures (Veselý et al., 2015). It had initially been suggested that low winter temperatures would prevent the establishment of the species in temperate areas in Europe (Martin et al., 2010b), a conclusion based on the absence of documented stable populations in Europe, and the fact that, at the time, all records of the species on the continent consisted of single individuals. However, in 2010, Chucholl and Pfeiffer (2010) documented the first instance of an established Marbled Crayfish population in Lake Moosweiher (Germany), in which it was found throughout the lake at comparable or greater densities than the co-occurring invasive Spinycheek Crayfish (Chucholl and Pfeiffer, 2010), which originates from temperate regions of North America, and is considered to be the most successful invasive crayfish species in Europe (Holdich, 2002). Thereafter, additional self-sustaining populations were described in Slovakia (Janský and Mutkovič, 2010; see Chucholl et al., 2012 for review), Germany, northern Italy (Kouba et al., 2014), Czech Republic (Patoka et al., 2016), Poland (Maciaszek et al., 2022), as well as a potential population in Sweden (Bohman et al., 2013), refuting the notion that the species cannot establish in temperate regions. The range of temperatures at which Marbled Crayfish has been found in the wild is between 0°C and 38°C, and it currently occupies tropical to cold temperate climates—unlike its parental species *P. fallax*, which exists only in subtropical to warm temperate zones (Vogt et al., 2019).

At least five studies have examined the effects of temperature on Marbled Crayfish under controlled laboratory conditions. A study conducted at four temperatures (15°C, 20°C, 25°C, and 30°C) found that Marbled Crayfish grew fastest at 25°C and has maximum survival at 20°C, with increased mortality both at higher and lower temperatures (Seitz et al., 2005). Survival was high among individuals exposed to temperatures between 8°C and 10°C for at least 40 days, although reproduction halted below 15°C (Seitz et al. 2005). Additionally, Sheppard et al. (2024) reported that Marbled Crayfish exhibit more frequent and longer lasting reproduction at 26°C compared to a lower temperature (18.5°C). Another study (Veselý et al., 2015) tested overwinter survival of Marbled Crayfish by exposing a group of them to 2–3°C for 90 days, mimicking conditions in lentic ecosystems of temperate Europe; only one of 15 Marbled Crayfish survived the experiment. A similar experiment conducted outdoors (Kaldre et al., 2015) exposed 25 large (42.2 ± 6.4 mm mean TL) and 25 small (31.6 ± 2.8 mm mean TL) Marbled Crayfish to water temperatures under 10°C for five months. During the three coldest months of the experiment, the mean temperature was 5°C, including 27 days when the temperature was below 2°C. Mortality peaked during the 2°C period, especially for the small crayfish (16 of 25 individuals died). At the end of the experiment 60% of large crayfish were alive, compared to 8% of small crayfish. Crayfish became less active and stopped feeding when the temperature was below 10°C, though two small crayfish moulted during this period. All surviving crayfish started reproducing within weeks of being transferred into 20°C water at the end of the experiment (Kaldre et al., 2015). Finally, Haubrock et al. (2019) mimicked the release of pet crayfish into natural waterbodies in winter by suddenly moving Marbled Crayfish acclimated to 20°C into water cooled to 6°C, 4°C, and 2°C, respectively. Most crayfish survived at 6°C, with one of the 20 dying after 21 days, and two others at 63 days. At 4°C all individuals died within 49 days, and at 2°C mortality was 100% within two weeks. All animals continued to forage at 6°C, while foraging ceased at the two lower temperatures. One aspect that was not addressed by any of the experiments is the role that burrowing behaviour of the crayfish may have on increasing survival at low temperatures, which could facilitate the establishment of the species in habitats suitable for constructing burrows (Kouba et al., 2016).

### 3.2.3 Nitrate

One controlled laboratory study investigated and compared the effects of three environmentally relevant and three high concentrations of nitrate on Marbled Crayfish and Noble Crayfish embryo survival and sublethal effects (Laurenz et al., 2020b). Marbled Crayfish embryos experienced significantly reduced survival starting at 14 mg/L which is a common concentration found in German surface waters. By contrast, *A. astacus* only began to show similarly significant reduced embryo survival at 62.5 mg/L. However, the half-maximal effective concentration of nitrate on Marbled Crayfish hatching rate was seven times greater over a third of the time compared to *A. astacus* (Laurenz et al., 2020b). These findings suggest that Marbled Crayfish embryos are equally or more susceptible to environmental nitrate than their native counterparts over longer periods of time.

### 3.2.4 Other variables

To date, no experiments have been conducted to test the physiological tolerances of Marbled Crayfish to natural variables other than salinity, temperature, and nitrate. However, some ranges can be inferred from conditions measured in the waterbodies where they have been captured. Across the invaded range, Marbled Crayfish have been found in acidic (pH 3.9) to slightly alkaline (pH 8.4) waterbodies (Tönges et al., 2021; Vogt et al., 2019). In Hungary they have been recorded from a waterbody with dissolved oxygen levels as low as 2.4 $mg \cdot L^{-1}$ and saturation of 31% (Lőkkös et al., 2016). Vogt et al. (2019) report air breathing behaviour by Marbled Crayfish where the crayfish lays laterally on the water surface and propels a mixture of water and air through its gills, which the authors conclude is evidence of their ability to survive in waters with low oxygen concentration. Anecdotal reports documented by Jones et al. (2009) assert that market vendors have kept individual crayfish of this species alive without water for up to three days. Specific conductivity of the water in lakes supporting populations of the crayfish ranges from 299 $\mu S \cdot cm^{-1}$ (in Lake Moosweiher, Germany; Vogt et al., 2019) to 769 $\mu S \cdot cm^{-1}$ (in Lake Hévíz, Hungary; Lőkkös et al., 2016). Waterbodies supporting established Marbled Crayfish populations in Germany and Madagascar ranged in concentration of runoff pollutants including minerals such as manganese (0–4,792 µg/L), iron (0–2,249 µg/L), and aluminum (0–2,967 µg/L) (Tönges et al., 2021), further supporting their tolerance to a wide range of environmental conditions.

In the last five years, several controlled laboratory studies have investigated the effects of various synthetic chemicals on Marbled Crayfish survival, growth, and development. These studies tested Marbled Crayfish tolerance to commonly used herbicides, insecticides, and pharmaceutical drugs found in European surface waters. Marbled Crayfish exhibit mixed levels of tolerance to environmentally relevant concentrations of commonly used herbicides (Laurenz et al., 2020a,c; Velisek et al., 2020a,b). Marbled Crayfish exhibited gill anomalies and significantly reduced survival, growth, and development at all tested concentrations (range: 3.2–22 μg/L) of the herbicide metazachlor and its major metabolite compared to control groups (Velisek et al., 2020a). Laboratory experiments showed no significant effects of environmentally relevant concentrations (2.7 μg/L) of chloridazon, an agricultural herbicide, on mortality, growth, development, behaviour, or histopathy of Marbled Crayfish (Velisek et al., 2020b), yet a higher concentration (>135 μg/L) did significantly affect development, growth (27 μg/L), behaviour (270 μg/L), and histopathy (>135 μg/L) (Velisek et al., 2020b). Laurenz et al. (2020c) further found that, regardless of the concentration treatment of Terbuthylazine used, there was no significant effect of the herbicide on Marbled Crayfish embryonic development time, weight at hatching, or survival rate. This was in stark contrast to the results for *A. astacus* which exhibited significantly slowed embryonic development, reduced weight at hatching, and lowered rates of survival at concentrations of ≥1.6 mg/L (Laurenz et al., 2020c).

The only study that has investigated Marbled Crayfish tolerance to insecticide found that the species can tolerate exposure to various concentrations of the insecticide neonicotinoid thiacloprid for 28 days under different temperature regimes. However, exposure to neonicotinoid thiacloprid resulted in significant differences in physiological parameters (e.g., concentration of ammonia in blood) between treatment and control crayfish, indicating that there is a physiological cost to exposure (Stara et al., 2021).

Two studies have found Marbled Crayfish to be negatively affected by environmentally relevant concentrations of pharmaceuticals. Location and time spent under shelter were significantly modified when exposed to a combination of four antidepressants and methamphetamine at environmentally relevant concentrations (~1 μg $L^{-1}$) (Hossain et al., 2021). Additionally, Marbled Crayfish embryo survival rates were significantly lower and embryonic development was significantly slower at environmentally relevant concentrations (≥10.24 mg/L and ≥0.16 mg/L) of the pharmaceutical anti-inflammatory drug Diclofenac (Laurenz et al., 2020a).

### 3.3. REPRODUCTION

Marbled Crayfish reproduction is unique among decapod crustacean species but is relatively less complex because it involves obligatory apomictic parthenogenesis, which yields genetically identical offspring, or maternal clones (Martin et al., 2007; Martin and Scholtz, 2012; Scholtz et al., 2003; Vogt, 2008). The benefit to invasion success of their reproductive strategy is that a single individual could be sufficient to establish a wild population.

In laboratory conditions Marbled Crayfish can lay eggs in any month of the year with peaks of egg-laying observed around the spring and autumn equinoxes (Vogt, 2015; Vogt et al., 2019). A similar pattern was observed in the field in Lake Šoderica

(Croatia) where individuals with eggs in their ovaries can be found throughout the year but individuals with ventral eggs or brooding juveniles were only detected at high abundances in June and October/November (Cvitanić, 2017). A follow-up study captured gravid and non-gravid feral Marbled Crayfish monthly over nine months from Lake Šoderica and transferred them to a laboratory colony where their reproductive metrics were measured (Dobrović et al., 2021). They again found continuous presence of gravid females with apparent peaks in reproduction occurring twice in a continuous seven-month period with the first being in late spring/early summer and the other in mid-fall (Dobrović et al., 2021). They also found that ovarian egg number and number of hatched juveniles were significantly positively correlated with crayfish body size and condition (Dobrović et al., 2021). Individuals spawn 1–3 times per year, most often twice a year (Vogt et al., 2019), and spawn more frequently at warmer water temperatures (26°C) compared to cooler water temperatures (18°C) (Sheppard et al., 2024). They begin to reproduce as young as 110 days old, at a total body length of 4 cm (Kouba et al., 2021; Vogt, 2018), which is early in comparison with other crayfish species (Hossain et al., 2018; Kouba et al., 2021). Clutch sizes can be quite large, with maximum values reported from laboratory cultures and free-living populations ranging from 416 to 731 eggs (Chucholl and Pfeiffer, 2010; Jones et al., 2009; Lipták et al., 2017; Seitz et al., 2005; Vogt et al., 2015); these are significantly larger clutch sizes than those of its parent species, *P. fallax* (Vogt et al., 2019). The magnitude of Marbled Crayfish reproduction becomes evident when examining wild populations. Lipták et al. (2017) found that the eggs and brooded juveniles of a group of 27 berried females exceeded 11,000. The species is also able to reproduce late in life; the latest spawning ever recorded occurred at day 1530 (~4.2 years) and the maximum number of clutches per female observed during one lifetime is seven (Vogt, 2010). Recent work provides insight into the high level of parental care that Marbled Crayfish provide for their young, post release and under adverse environmental conditions (Kaur et al., 2024). Laboratory experiments revealed that low water levels and starvation extended the window of maternal care by fourfold, including prolonging attachment time to increase the protection period of juveniles (Kaur et al., 2024).

### 3.4. FEEDING AND DIET

There is limited research on the feeding of Marbled Crayfish in the wild, and most research has focused on feeding experiments and rearing conditions for Marbled Crayfish as aquarium pets or as model organisms for research (Jimenez and Faulkes, 2010; Kaldre, Meženin, et al., 2015).

Like other *Procambarus* species (Gherardi and Barbaresi, 2007), Marbled Crayfish are omnivores with the ability to consume a broad range of benthic invertebrates, aquatic plants, and algal organisms (Sheppard and Ricciardi, 2025). In Madagascar, the stomachs of dissected Marbled Crayfish contained mostly vegetable matter (Kawai et al., 2009); whereas, in Malta, the species was observed to consume a wide range of taxa, including the gastropod Garden Snail (*Cornu aspersum*), tadpoles of the Painted Frog (*Discoglossus pictus*) and Bedriaga's Frog (*Pelophylax bedriagae*), adults of the Western Mosquitofish (*Gambusia affinis*), nymphs of the Scarlet Dragonfly (*Crocothemis erythraea*), and larvae of the Band-eyed Drone Fly (*Eristalinus taeniops*) (Deidun et al., 2018). Most of this feeding took place early in the morning, as crayfishes were observed scavenging under reeds and vegetation; they were found to be mostly inactive later in the day (Deidun et al., 2018). A stable isotope study conducted in a gravel pit in Leopoldov (Slovakia) also found that Marbled Crayfish was omnivorous, with detritus being the most important nutrient source, and zoobenthos, algae, and macrophytes being consumed to a lesser extent (Lipták et al., 2019). In Hungary, a stable isotope study found that Marbled Crayfish fed primarily on detritus and algae (cumulative 64% of diet) as well as on zoobenthos and macrophytes (cumulative 35% of diet) when in sympatry with Spinycheek Crayfish in a stream in the Danube River system (Veselý et al., 2021). However, lab food preference experiments on Marbled Crayfish captured from a wild established population in Lake Moosweiher, Germany found that Marbled Crayfish preferred protein-rich food sources (invertebrates) over plant tissues (Linzmaier et al., 2020).

While Marbled Crayfish and other species are thought to have severely reduced or insignificant feeding rates while gravid (Little, 1976), a recent study showed that gravid Marbled Crayfish consume benthic prey (chironomid larvae) readily but at a lower rate than non-gravid individuals. The functional response of gravid individuals was unaffected by temperature change across the range of 18–26°C, whereas the maximum feeding rates of non-gravid individuals were reduced at 26°C compared with 18°C (Sheppard et al., 2024).

### 3.5. HABITAT

In Europe, Marbled Crayfish have been detected in lentic and lotic freshwater habitats, including brooks, rivers, canals, ditches, reservoirs, natural and artificial lakes, and ponds (Chucholl et al., 2012; Ercoli et al., 2019; Maciaszek et al., 2022; Marten et al., 2004; Martin et al. 2010b), though most established populations are found in lentic habitats (Chucholl et al., 2012). In Madagascar, Marbled Crayfish have been reported from a variety of diverse natural freshwater habitats, including rivers, lakes, and swamps (Andriantsoa et al., 2019; Gutekunst et al., 2018), as well as secondary habitats such as brick pits, drainage ditches, rice fields, and fish ponds (Jones et al., 2009). In the Middle East, established populations of Marbled Crayfish have been found in a freshwater spring-fed basin originating in a rocky cave and a spring-fed pool in an intermittent stream (Carneiro et al., 2023; Yanai et al., 2024). In Canada, the only known established population exists in an urban pond within a municipal park (Hamr, 2024; Ontario Ministry of Natural Resources, 2024). Established populations of Marbled Crayfish have yet to be reported in Japan.

One potential reason for their success in lentic environments is their ability to use muddy substrates as refugia; they have been found buried deep in the mud of pools with no surface water remaining (Jones et al., 2009), and they will retreat into the mud when disturbed (Deidun et al., 2018). Kouba et al. (2016) examined the ability of three native and five invasive crayfishes in Europe to construct vertical burrows in a humid, sandy-clay substrate under a simulated drought condition. They found that the native European crayfishes and one North American crayfish, *P. leniusculus,* did not construct vertical burrows at all. Of the species that created vertical burrows, Marbled Crayfish was able to construct larger and deeper burrows than either the Common Yabby (*Cherax destructor*) or the Spinycheek Crayfish and was surpassed only by the Red Swamp Crayfish. The ability to withstand desiccation of a waterbody through the construction of burrows into the hyporheic zone might play a significant role in the success of Marbled Crayfish establishment in environments subjected to highly fluctuating precipitation patterns, including drought, especially under climate change (Kouba et al., 2016).

## 3.6. INTERSPECIFIC INTERACTIONS

### 3.6.1 Other crayfishes

The competitive behaviour of Marbled Crayfish has been compared to other invasive crayfishes (Chucholl and Pfeiffer, 2010; Chucholl and Chucholl, 2021; Fořt et al., 2019; Hossain et al., 2019; Jimenez and Faulkes, 2011; Kouba et al., 2021, 2021; Linzmaier et al., 2018). Chucholl and Pfeiffer (2010) reported that the species was able to establish a population in Germany despite the presence of another highly invasive North American species, the Spinycheek Crayfish. Linzmaier et al. (2018) found that Marbled Crayfish were on average more aggressive than *F. limosus*, even when paired against larger opponents. Experiments on antagonistic interactions between Marbled Crayfish and the Red Swamp Crayfish, a congener native to southeastern North America, and one of the most aggressive invasive crayfish species, revealed that both species were equally likely to win dominance contests, although there were some issues with size-matching crayfish in the trials (Jimenez and Faulkes, 2011). A follow-up study confirmed that size-matched Marbled Crayfish are significantly more successful in establishing dominance than *P. clarkii* (Hossain et al., 2019). Fořt et al. (2019) examined agonistic encounters among Marbled Crayfish, the North American species *P. leniusculus* (native to B.C.), and the Australian species *C. destructor*, and found that Marbled Crayfish was the least aggressive and least successful in contests amongst these species.

When compared with other species, Marbled Crayfish's relative competition for nutritional resources varied. A functional response experiment found that the Spinycheek Crayfish had higher maximum feeding rates than Marbled Crayfish, but the reduced feeding by Marbled Crayfish may have been a consequence of some individuals reproducing during the experiment (Linzmaier and Jeschke, 2020). In another functional response experiment, Marbled Crayfish had a greater functional feeding rate than both the Calico Crayfish (*Faxonius immunis*) and Spinycheek Crayfish, but a lower feeding rate than the Noble Crayfish and the Signal Crayfish on gammarid prey. Marbled Crayfish feeding rate was greater than only *A. astacus* on Zebra Mussel (*Dreissena polymorpha*) prey items (Chucholl and Chucholl, 2021). The Marbled Crayfish's functional response ratio value was intermediate compared to that of the other four crayfishes, suggesting relatively moderate per capita effects on the tested nutritional resources (Chucholl and Chucholl, 2021). The authors acknowledge, however, that ecological impact is difficult to predict from a laboratory experimental study that exclusively used mature male crayfish, with the exception of Marbled Crayfish, which are all female (Chucholl and Chucholl, 2021).

A stable isotopic study in Hungary found that Marbled Crayfish was more carnivorous than the Spinycheek Crayfish when both species co-occurred exclusively with each other. When in sympatry with Red Swamp Crayfish, both Marbled Crayfish and Spinycheek Crayfish had narrower and more overlapping trophic niches; *P. clarkii* was more carnivorous than the other two species and preyed upon crayfish and fish (Veselý et al., 2021).

Experiments comparing reproductive age, survival, and growth of juvenile Marbled Crayfish in sympatry with four other crayfishes (*F. limosus, P. leniusculus, P. clarkii*, and *C. destructor*) found that Marbled Crayfish was the earliest to reach reproductive maturity, and it exhibited faster growth and greater rates of survival than both *F. limosus* and *P. leniusculus* but slower growth and lower rates of survival than *P. clarkii* and *C. destructor* in mixed stock treatments (Kouba et al., 2021).

### 3.6.2 Invertebrates

One study has investigated the interactions between Marbled Crayfish and non-crayfish invertebrate species: in laboratory experiments, an invasive amphipod, Killer Shrimp (*Dikerogammarus villosus*), was found to readily prey upon Marbled Crayfish eggs and juveniles, even when the latter were attached to the mother's body (Roje et al., 2020). When exposed to Killer Shrimp, juvenile Marbled Crayfish suffered higher predation than a comparable species, the Signal Crayfish. Conversely, adult Marbled Crayfish preyed upon *D. villosus* with higher attack rates and shorter handling times than did *P. leniusculus*. Despite their reciprocal predation pressure, Marbled Crayfish and Killer Shrimp were deemed unlikely to extirpate one another (Roje et al., 2020).

### 3.6.3 Vertebrates

Marbled Crayfish display evasive behaviours in the presence of fishes and they are readily preyed upon by native and invasive fishes (Kaur et al., 2023; Musil et al., 2023; Roje et al., 2021). In laboratory experiments, the Round Goby (*Neogobius melanostomus*) consumed all individual Marbled Crayfish for which they were not gape-limited and also injured or killed some larger crayfish that they could not ingest (Roje et al., 2021). Further, the Round Goby consumed larger crayfish when at lower temperatures (17$^{o}$C) and did not show a size preference at higher temperatures (23–26$^{o}$C); overall, the fish consumed half of their body weight in crayfish per day (Roje et al., 2021). Conceivably, such predation could locally reduce Marbled Crayfish populations in the Great Lakes basin, where the Round Goby is abundant and widespread. Additionally, Marbled Crayfish with no previous predator experience exhibited a significantly reduced feeding rate (up to 47% less) on sea lice prey when exposed to only an olfactory cue (i.e., no visual cue permitted) of the Round Goby, indicating that Marbled Crayfish possess innate predator-avoidance behaviours. The same study found that this response was unaffected by the presence or absence of the alarm cue given off by conspecifics (Musil et al., 2023)—a finding that is inconsistent with previous literature, possibly owing to lack of consideration of behavioural plasticity, differences in environment of origin or body size, and previous experience with predators in their study (Musil et al., 2023). Juvenile Marbled Crayfish exhibited reduced feeding and increased time spent in sheltering zones of experimental arenas when exposed to predator cues of Pumpkinseed (*Lepomis gibbosus*) (Kaur et al., 2023). These responses were strongest in juveniles that had prior exposure to *L. gibbosus* when exposed to a combined olfactory cue (water from a tank where *L. gibbosus* was actively feeding on conspecifics) compared to the single olfactory cue (water from a tank containing *L. gibbosus* at rest) or plain water control treatments (Kaur et al., 2023).

### 3.7. MOVEMENT AND MIGRATION

Human transport of Marbled Crayfish into new areas is a consequence of its popularity as an aquarium species (see section 4; Chucholl et al., 2012). The species can subsequently spread to new waterbodies through natural dispersal. Gutekunst et al. (2018) note that the rapid spread of the crayfish in Madagascar is facilitated by a dense network of freshwater habitats. Frequent observations of the species traveling over land, including up to 100 m from a lake, suggest that this behaviour is an inherent dispersal mechanism (Chucholl et al., 2012). Marbled Crayfish could possibly hitchhike on waterfowl as well, as evidenced by a study of its congener, the Red Swamp Crayfish, which demonstrated that recently-hatched crayfish are able to cling to a moving duck such that it could survive being transported to new waterbodies (Águas et al., 2014).

### 3.8. DISEASES AND PARASITES

Marbled Crayfish is a known carrier of the crayfish plague pathogen, *Aphanomyces astaci*, and its infection was confirmed both in captivity and in natural populations (Ercoli et al., 2019; Keller et al., 2014). Laboratory inoculation of Marbled Crayfish with *A. astaci* revealed that Marbled Crayfish have very strong resistance to the disease while carrying it at high levels in their tissues (Francesconi et al., 2021). Multiple infections with high concentrations of *A. astaci* and sustained nutrition stress resulted in only 53% mortality in 10 weeks, indicating that Marbled Crayfish are highly resistant to the crayfish plague even under sub-optimal conditions (Dobrović et al., 2022). These findings support the notion of Marbled Crayfish potentially carrying and spreading the disease to vulnerable populations (Dobrović et al., 2022; Francesconi et al., 2021). Although the pathogen is present throughout North America, Marbled Crayfish may act as an additional reservoir. Other potentially pathogenic organisms described on Marbled Crayfish include: a coccidian; a *Rickettsia*-like organism; peritrichous ciliates; and mites (Longshaw et al., 2012; Vogt et al., 2004).

## 4.0. USE BY HUMANS

Marbled Crayfish is the most popular crayfish species in the North American pet trade, accounting for roughly half of all individual crayfish sold (Faulkes, 2015). Its popularity among aquarium hobbyists is considered to be a driving factor of its spread in Europe (Chucholl, 2013, 2015; Gherardi, 2011; Kouba et al., 2014; Patoka et al., 2014). Parthenogenic reproduction of the species can lead to aquariums becoming overpopulated and prompting hobbyists to either give animals away or dump them into natural waterbodies or urban ponds (Kouba et al., 2014; Patoka et al., 2014). The species has also been increasing in popularity as a live food for predatory aquarium fish and ornamental turtles (Chucholl et al., 2012). It might also be used as fishing bait, as are many other crayfish species (DiStefano et al., 2009).

Marbled Crayfish can provide an efficient source of human dietary protein and research-grade protein products (Andriantsoa et al., 2019; Jones et al., 2009; Roy et al., 2023). Whole body homogenization of Marbled Crayfish and Red Swamp Crayfish revealed greater concentrations of branched-chain amino acids, especially leucine, than cow's milk-derived casein—the most concentrated known animal source (Roy et al., 2023). The results must still be validated internationally, but leucine from *Procambarus* crayfishes could be used in biomedical research and nutritional supplements (Roy et al., 2023). In Madagascar, the species is an important source of protein for human consumption; it is farmed on a small scale in rice fields and sold in local markets (Andriantsoa et al., 2019; Jones et al., 2009). Socio-economic perceptions of the Marbled Crayfish in Madagascar are mixed, with it both being positively accepted as an inexpensive source of animal protein that contributes to food security in impoverished communities, while also recognized as having detrimental effects on rice agriculture and fishing (Andriantsoa et al., 2020).

Marbled Crayfish might also have a positive impact on human health in Madagascar and similar tropical regions, as laboratory experiments indicate that the crayfish readily consumes the freshwater snail *Biomphalaria pfeifferi*, which carries the trematodes that can cause schistosomiasis (Faiad et al., 2023). Marbled Crayfish consumption of infected snails in areas of Madagascar where natural predators are absent and schistosomiasis is abundant in the human population suggests that it could reduce transmission of this disease to humans (Faiad et al., 2023); however, research is needed to quantify the population-level impact of crayfish predation on the snail.

Because all individuals of the species are genetically identical, and they are relatively easy to culture, Marbled Crayfish have also been a popular model organism in a variety of research fields including developmental biology, neurobiology, epigenetics, stem cell biology, ethology, biogerontology, toxicology, ecology, and evolutionary biology (Hossain et al., 2018). Specifically, Stein et al. (2022) argue that Marbled Crayfish is an excellent model organism for genetic and molecular research on neuron function because of its highly sequenced genome and parthenogenic reproduction. Marbled Crayfish have also been used as a proxy for the response of aquatic fauna to chemical pollutants and pharmaceuticals (Hossain et al., 2021; Stara et al., 2021; Velisek et al., 2020a). However, Laurenz et al. (2020c) warn that using Marbled

Crayfish as a model organism to understand the effects of toxins on non-target freshwater invertebrates may be ill-advised. They found that the direct comparison of developmental parameters between Marbled Crayfish and the Noble Crayfish show a vast difference in response to the herbicide Terbuthylazine, such that if Marbled Crayfish response to the chemical were tested without another crayfish species for comparison, the results would have been misleading.

# 5.0. IMPACTS ASSOCIATED WITH INTRODUCTION

## 5.1. IMPACTS ON WATER QUALITY AND HABITAT

Marbled Crayfish exhibit burrowing behaviour (Kouba et al., 2016). Although there are presently no studies examining the environmental impacts of this activity for Marbled Crayfish, studies on other invasive crayfishes illustrate the potential consequences for water clarity and bank stability. For example, burrowing behaviour by *P. leniusculus*, which constructs less extensive burrows than Marbled Crayfish (Kouba et al., 2016), has been shown to increase ambient turbidity, cause periodic bank collapse events, and accelerate riverbank erosion overall (Harvey et al., 2014; Faller et al., 2016). As omnivores, Marbled Crayfish can consume macrophytes, although at a lower rate than their congener, the Red Swamp Crayfish (Sheppard and Ricciardi, 2025); when present in high abundances, it is conceivable that their shredding activities will have an impact on riparian vegetation.

## 5.2. IMPACTS ON FAUNA

In Madagascar, predation by Marbled Crayfish has apparently caused local reductions in a species of snail, *Biomphalaria pfeifferi* (Andriantsoa et al., 2019). Marbled Crayfish can impose impacts on invertebrate prey even after the crayfish sustain significant injury (Soto et al., 2023; Toutain et al., 2024). Functional response experiments revealed that the loss of one or both claws had no significant effect on the Marbled Crayfish rate of attack or consumption of live *Chironomus plumosus* larvae (Soto et al., 2023). In another functional response study, the absence of one or both claws resulted in reduced attack rates and increased handling time by crayfish feeding on more hard-bodied prey (i.e., *Gammarus fossarum*). However, single clawed and clawless Marbled Crayfish were still able to kill and consume some *G. fossarum*, indicating the resilience of their effects on food webs (Toutain et al., 2024).

Due to its aggressive behaviour (see section 3.6.1), omnivorous diet (section 3.4), and because it is a carrier of the crayfish plague (section 3.8), there are concerns that Marbled Crayfish may displace more sensitive native crayfish species such as those in the genus *Astacoides*, endemic to Madagascar, all of which are listed as endangered (Kawai et al., 2009). There are similar concerns for native European crayfishes (Hossain et al., 2018; Lőkkös et al., 2016; Longshaw et al., 2012). A study of the trophic role of Marbled Crayfish in a gravel pit hypothesizes that the broad diet of this species is likely to modify food web structures (Lipták et al., 2019). Marbled Crayfish have similar or higher levels of aggression than two highly invasive North

American species of crayfish, *F. limosus* and *P. clarkii* (Hossain et al., 2019; Linzmaier et al., 2018), and could compete with these species. Few studies have evaluated the direct interactions between Marbled Crayfish and native crayfishes of North America; however, one laboratory experimental study found that Marbled Crayfish are antagonistically dominant over female Calico Crayfish of similar size, indicating that Marbled Crayfish could outcompete Calico Crayfish for resources (Hossain et al., 2020). Effects of Marbled Crayfish on native crayfish biodiversity in Canada remains a key knowledge gap at time of publication.

## SUMMARY

Information on the invasion history of the Marbled Crayfish in various regions provides insight on its capacity to establish, spread, and exert impacts in North American inland waters. Over the past 20 years, the species has been reported in several countries spanning multiple ecoregions, suggesting widespread intentional introductions and its inherent invasiveness. The importation of Marbled Crayfish as pets is the principal pathway of introduction and transfer across countries. At present, there are only two verified populations of Marbled Crayfish established in Canadian waters, but live individuals continue to be in the possession of aquarium hobbyists and aquarium retailers throughout North America. Based on its invasion history in temperate European waters, and recent discoveries near Lake Ontario and in the Yarmouth watershed, conditions for the establishment of Marbled Crayfish in natural systems in Canada are deemed adequate, at least in southern areas. We predict that future surveys will confirm the presence of Marbled Crayfish populations in other Canadian waterbodies, most likely in ponds and storm drainages around major urban centers. There is a paucity of data on the impacts of Marbled Crayfish invasion on biodiversity, food webs, and ecosystem function; however, both its extraordinary reproductive capacity and the impacts of *Procambarus* congeners suggest that the Marbled Crayfish poses a potentially significant invasion threat.

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## TABLES AND FIGURES

**Table 1:** Summary of key knowledge gaps.

| | |
|---|---|
| **Vectors and Distribution** | Quantitative data on occurrence and abundance in legal and illegal live trade in Canada (e.g., pet suppliers, retailers, and online sales). |
| | Occurrence and abundance in Canada's inland waterbodies (especially urban ponds, canals, and stormwater drainage systems). |
| | Suitable habitat and climate match models for Canada, both at present conditions and those projected under climate warming. |
| **Physiology and Reproduction** | Tolerance to hypoxia, pH, aerial exposure, and starvation. |
| | Frequency and magnitude of reproductive events (e.g., spawning frequency and clutch sizes) in the wild. |
| **Ecological impacts** | Effects on native biodiversity in Canada, especially on native crayfishes. |
| | Changes to trophic ecology under warming temperatures: how temperature exposure and acclimation affect resource preference (i.e., animals versus macrophytes as food sources). |
| | Impacts on ecosystem function (e.g., nutrient and contaminant cycling) and food web structure. |
| | Impacts on physical habitat (e.g., erosion of stream banks and damage to wetlands) resulting from burrowing behaviour and macrophyte shredding. |

**Figure 1:** Images of Marbled Crayfish displaying variable carapace colouration. The individual on the bottom is brooding juveniles. Images by Sabine Bailey, McGill University.

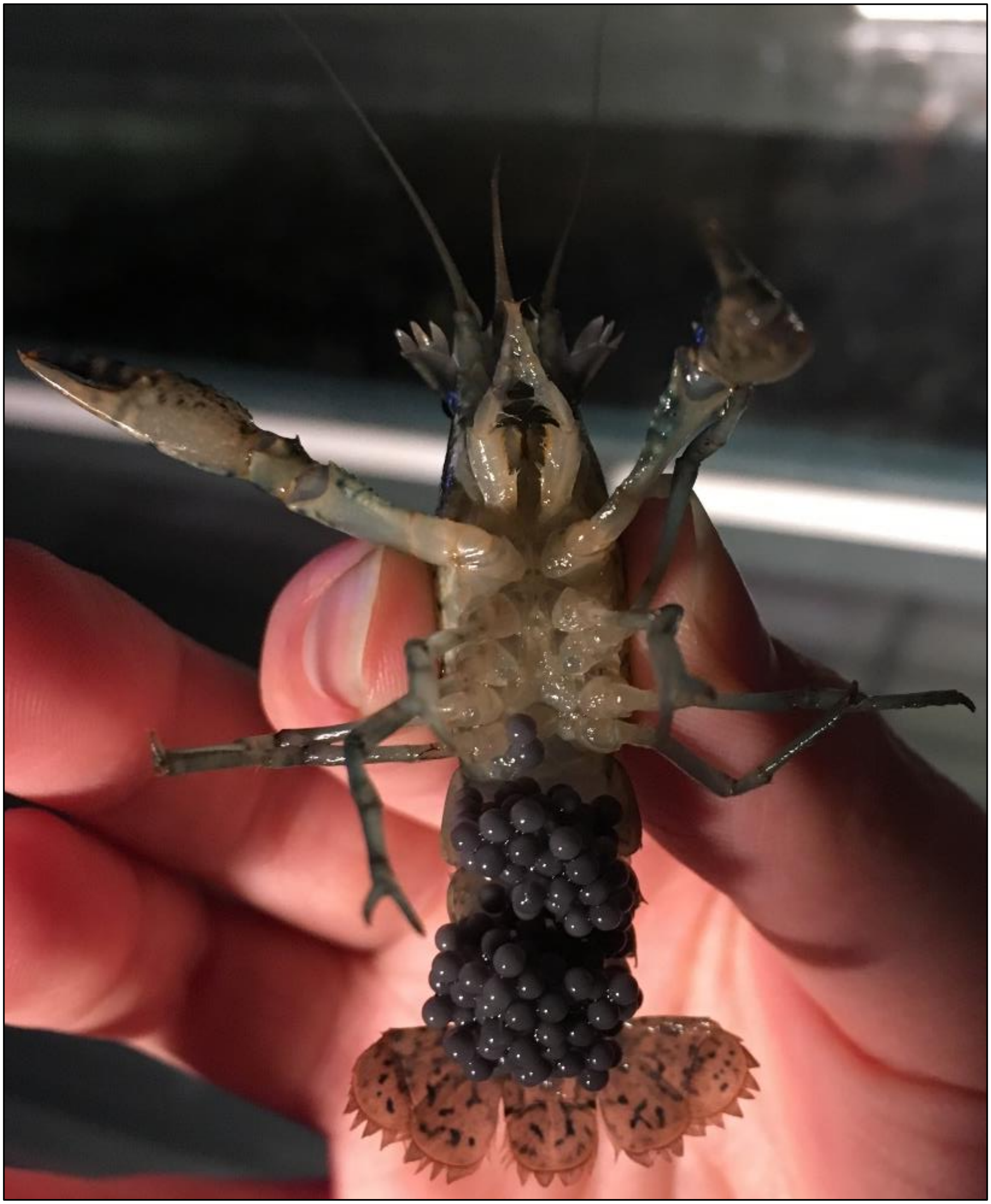

**Figure 2:** Ventral side of a Marbled Crayfish in berry. Image by Sabine Bailey, McGill University.

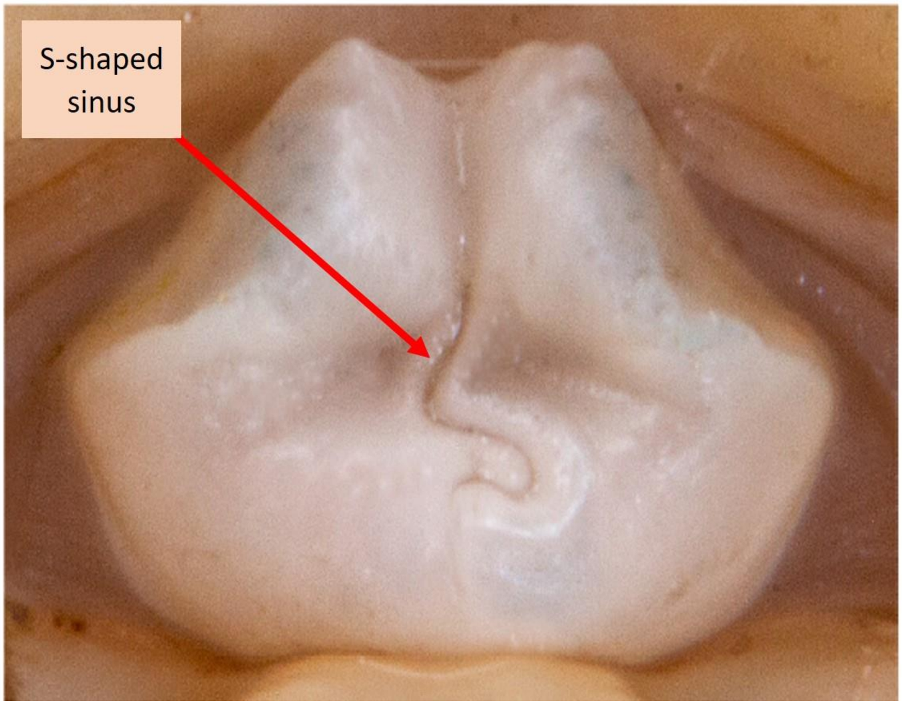


**Figure 3:** Photograph of the annulus ventralis of a mature Marbled Crayfish from a wild population. Adapted from Vogt et al. (2018).


**Figure 4:** Photograph of the right chela (ch) of a Marbled Crayfish, including the palm and dactyl. Red arrow points to row of tubercles on mesial margin of palm. Adapted from Vogt et al. (2018).

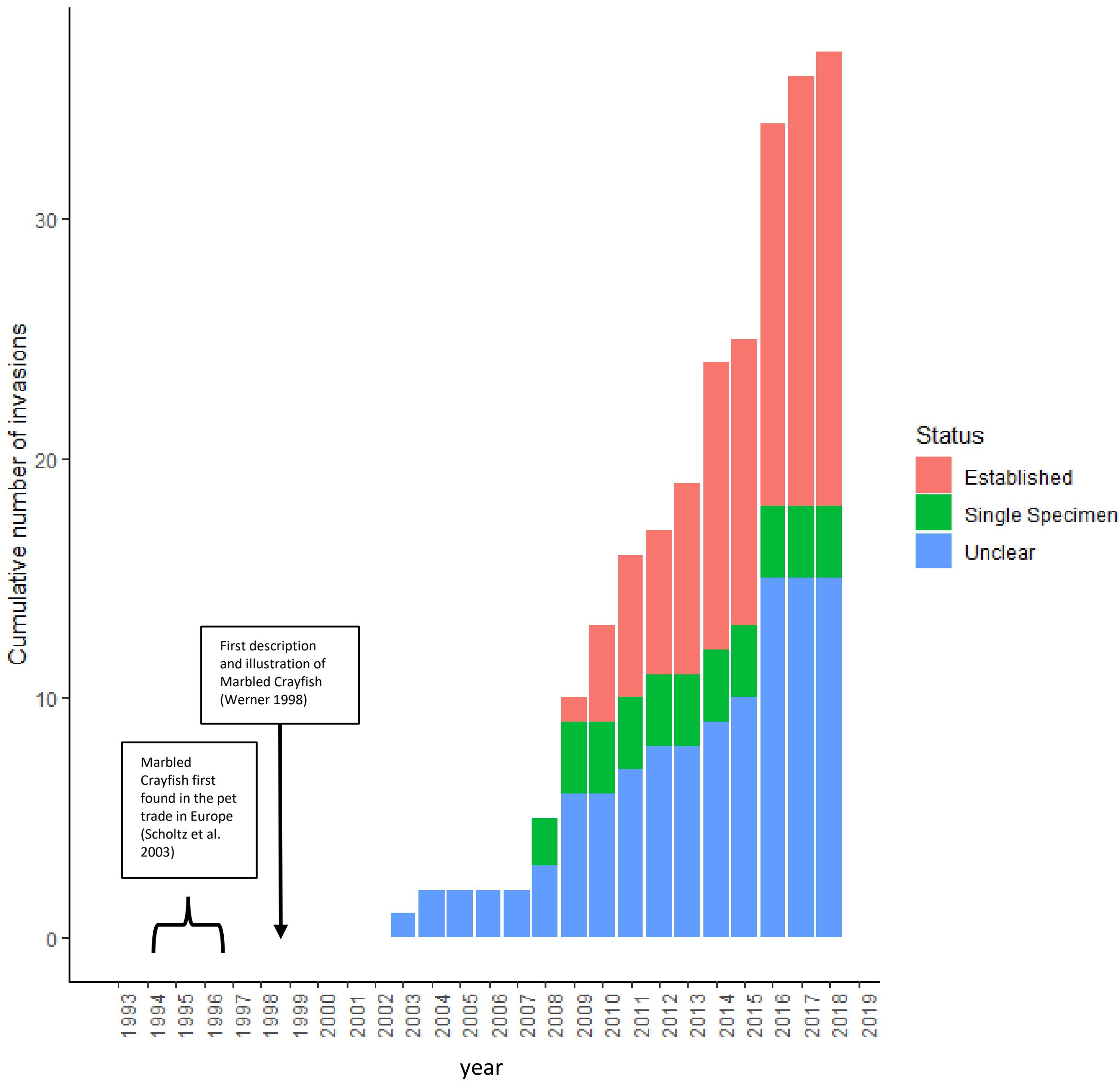


**Figure 5:** Important events and cumulative number of European Marbled Crayfish records in relation to time. Adapted from Chucholl et al. (2012).

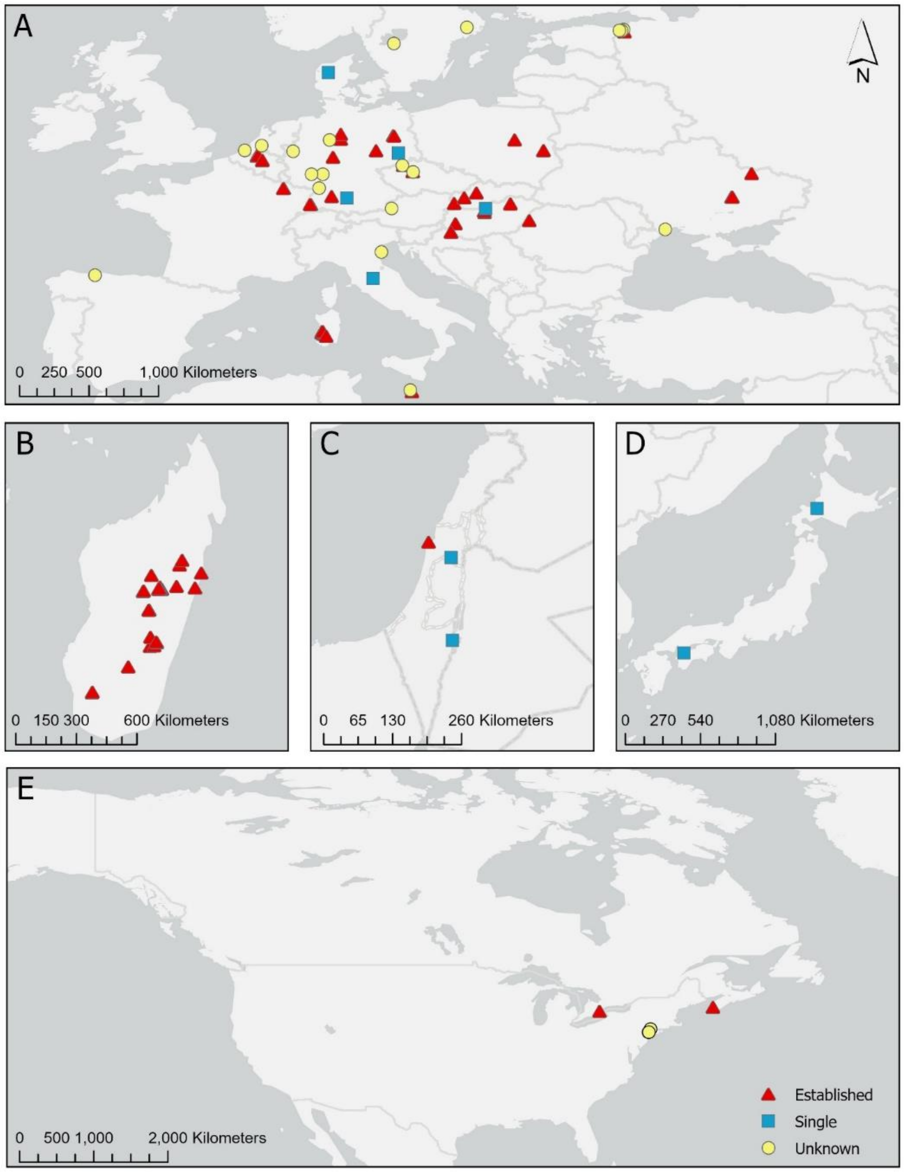


**Figure 6:** Occurrence of *P. virginalis* in Europe (A), Madagascar (B), Israel (C), Japan (D), and North America (E). Red triangles represent established populations, yellow circles are populations of unknown status, and blue squares are reports of single individuals. Information to create the map was compiled from Aluma et al. (2023); Andriantsoa et al. (2019); Bláha et al. (2022); Bohman et al. (2013); Carneiro et al. (2023); Chucholl et al. (2012); Chucholl and Pfeiffer (2010); Collas (2019); Cvitanić (2017); Deidun et al. (2018); Dobrović et al. (2021); Ercoli et al. (2019); Gatzmann et al. (2018); Grandjean et al. (2021); iNaturalist (2022, 2023a, and 2023b); Janský and Mutkovič (2010); Jones et al. (2009); Kawai et al. (2009); Kawai and Takahata (2010); Linzmaier (2016); Linzmaier et al. (2020); Lipták et al. (2016, 2017); Lőkkös et al. (2016); Maciaszek et al. (2022); Marten et al. (2004); Martin et al. (2010b); Marzano-Nonnis (2009); Mojžišová et al. (2022); Novitsky et al. (2016); Obermüller (2018); Pârvulescu et al. (2017); Patoka et al. (2016); Samardžić et al. (2014); Sánchez et al. (2024); Sanna et al. (2021); Scheers et al. (2021); Soes (2016); Soes and van Eekelen (2006); Son et al. (2020); Usio et al. (2017; Veselý et al. (2021); Vojkovská et al. (2014); Weiperth et al. (2020); Wenande (2019); and Yanai et al. (2024).

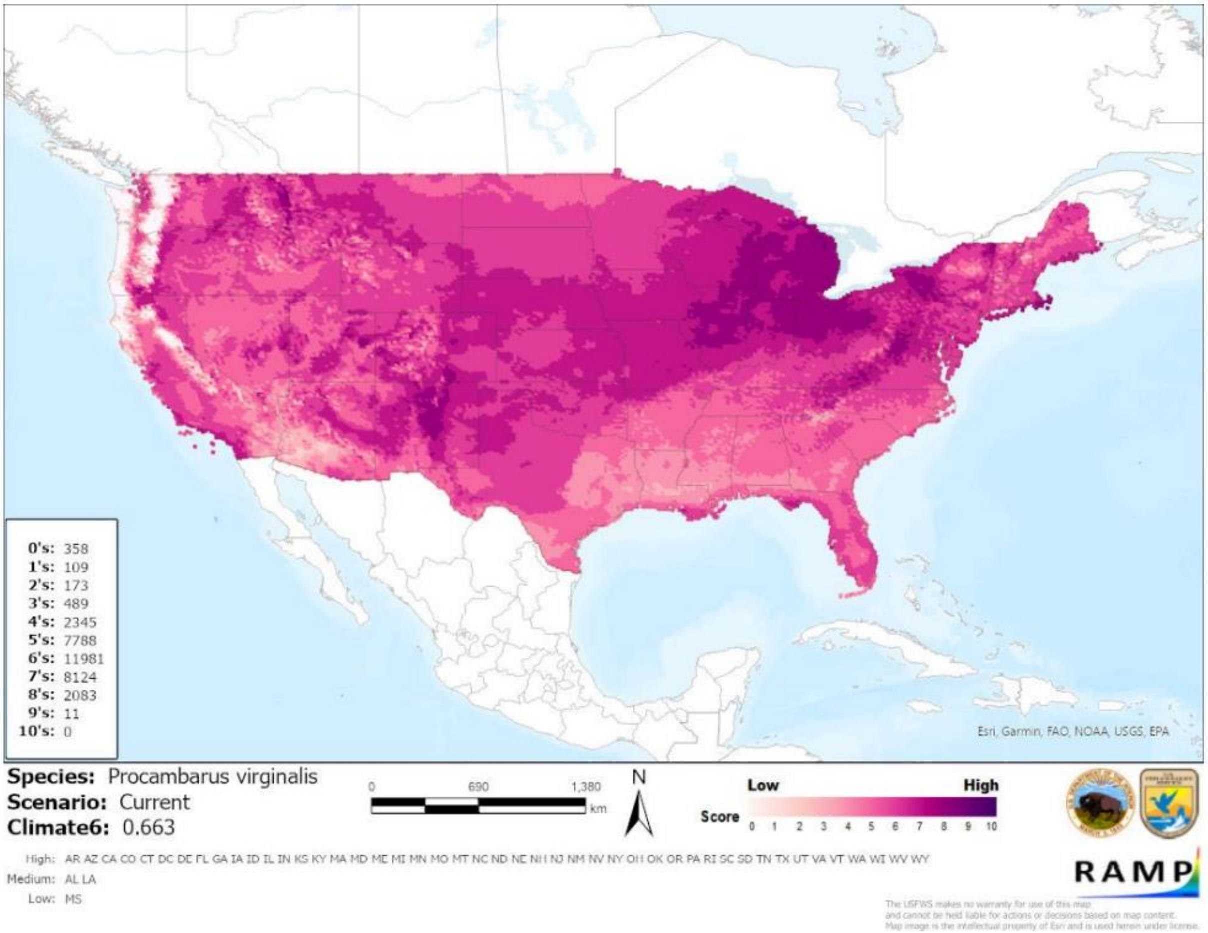


**Figure 7:** Map illustrating the climate matching analysis for Marbled Crayfish in the United States. Reproduced from U.S. Fish and Wildlife Service (2023).