DOTTORATO DI RICERCA IN ETOLOGIA ED ECOLOGIA ANIMALE E ANTROPOLOGIA
(XXV CICLO)

Interactions between two invasive alien species, *Procambarus clarkii* and *Dreissena polymorpha,* in the aquatic ecosystems of central Italy

Tesi di
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Dedicated to Francesca Gherardi

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ABSTRACT

Recently, a highly invasive alien species, the zebra mussel *Dreissena polymorpha*, has colonized some Italian aquatic ecosystems, previously colonized by another highly invasive alien species – the North American crayfish *Procambarus clarkii*. This is the first time that these two species coexist.

The elucidation of the newly established relations between both species is an excellent opportunity for applied research in invasion ecology, as both species present strong ecological and economic impacts. To evaluate the relative importance of their interactions, in this thesis we studied the frequency and density of zebra mussel ***fouling*** of individuals of *P. clarkii* and studied the ***predation*** by *P. clarkii* on *D. polymorpha*, with the final purpose of understanding whether crayfish can control, and in what conditions, the zebra mussel populations (***modelling***).

Dispersal and colonization of substrates by *D. polymorpha* are facilitated by its life cycle, characterized by both the existence of planktonic stages and the ability to abandon byssum at any time and crawl over the substratum to find new sites.

In the ***fouling*** study we recorded observations of *P. clarkii* fouling by *D. polymorpha* in Lake Trasimeno and in the laboratory. The results agree with previous reports that stated that zebra mussels can be found, if only occasionally, on most parts of the crayfish body, with a general preference for the ventral surface, and in this case, the pereopods.

The frequency of colonization on *P. clarkii* in the wild varied from 0 to 4%, much lower than the values observed in other crayfish. Adults of both sexes were colonized equally. The occurrence of multiple zebra mussels on individual crayfish were unexpectedly high; this may be related to poor crayfish condition and may also explain the only colonization of a live crayfish individual recorded in the laboratory

Both laboratory and field studies have shown that some crayfish species are effective predators of zebra mussels. However, their effective impacts on zebra mussel populations may depend on a number of other factors, including the availability of alternative prey. In this thesis, we analyzed the size-selective predation of *P. clarkii* on *D. polymorpha* in the presence and absence of alternative prey (*Physella acuta)*, particularly assessing the relevance of the estimated profitability of both preys.

The ***predation*** study confirmed that *D. polymorpha* represents a novel prey resource for *P. clarkii*, even in the presence of an alternative prey. *D. polymorpha* may even be a preferred prey, especially the juvenile stages, or individuals of small size (5-10 mm – developmental stage $\mathbf{n_1}$). However, the estimation of the profitability of a prey species was not useful to predict the predation behaviour when two types of prey are available.

The functional response of a predator is a key factor regulating the population dynamics of predator-prey systems.

The goals of ***moodeling*** study were to identify the consumption rates of *Dreissena polymorpha* juvenile stages by *Procambarus clarkii*, to determine the predator functional response, to evaluate the effects of predator satiation on the intensity of predation, and to model the impacts of predation by this crayfish on zebra mussel populations.

When presented with a range of mussel abundances, individual *P. clarkii* showed a functional response of the type II, which is characterized by a higher predation the lower the initial abundance of available prey.

P. clarkii also exhibited a satiation effect, diminishing its consumption rate from 33 mussels/day to 6 mussels/day over a 6-day period.

The combined effect of the functional response and predator satiation allowed the production of a model that predicts the complete consumption of local populations of up to 150/160 juveniles by one single crayfish over the period of one month. This impact may be important in low-density or at equilibrium populations of *D. polymorpha*, and will be greater the larger the synchronization between the prey reproductive cycle and the activity period of the predator.

One of the main objectives of the study of the interactions between these two invasive species is to understand whether predation by crayfish can somehow hinder the introduction (colonization) of *D. polymorpha*. Based on the results presented, it will be important to assess how different densities of crayfish may affect the populations of *D. polymorpha*, taking into account the possibility of interference competition between individual crayfishes, as well as the effects of the presence of alternative prey. The effects of satiation should also be studied further, as it is still unknown if crayfish may completely stop eating zebra mussels after a long period.

This thesis confirmed the existence of negative interactions between the two invasive species in the aquatic ecosystems of Central Italy, which developed within a short period of time, in less than a decade of coexistence.

Keywords: biological invasion; invasive alien species; *Procambarus clarkii; Dreissena polymorpha*; predator-prey interaction; profitability; fouling interaction; inland water; Italy

CHAPTER 1

GENERAL INTRODUCTION

1.1 *INVASIVE ALIEN SPECIES*

Invasive alien species (IAS) are species introduced outside their natural range, whose populations grow successfully and get established in the new habitat, often becoming a nuisance and a threat to native biota (Parker et al. 1999; Richardson et al. 2000; Lockwood et al. 2007). IAS are amongst the world's most significant, least controlled and reversible causes of human impacts on the planet's ecosystems (Lockwood et al. 2007). Today, there is a large consensus that they are one of the main threats to biodiversity and the second leading cause of local extinctions (Millenium Ecosystem Assessment 2005). Inland waters are particularly vulnerable to biological invasions and they experience a rate of biodiversity loss (Sala et al. 2000; Gherardi 2007a) that even matches that of tropical forests (Ricciardi and Rasmussen 1999).

Invasive species can affect native communities in different ways. In some cases invasion can cause considerable loss of biodiversity through competitive interactions and predations (Vitousek et al. 1996; Ruiz et al. 1997; Crooks 2002). Specifically, in fresh waters the extinction of indigenous species is becoming frequent as aggressive species invade degraded ecosystems. Endemic freshwater fish and amphibians are the taxa most vulnerable to intentional introductions throughout the world (Ilhéu et al. 2007).

The rate of invasion by freshwater alien species is increasing worldwide (Ricciardi 2006; Keller et al. 2009), with crustaceans and mollusks representing some of the most successful and high impact invaders (Van der Velde et al. 2000). Introduced crayfish cause irreversible changes to the ecology of invaded freshwater bodies (Hobbs et al. 1989), often leading to decreases in biodiversity (Crawford et al. 2006). Crayfish are opportunist omnivores that act as ecosystem engineers in invaded areas, due to their central position in the food webs (they are considered key stone species) and to the magnitude of the effects of their benthic activity (Charlebois and Lamberti 1996; Lodge et al. 2000; Creed and Reed 2004; Geiger et al. 2005). Crayfish consume a large variety of items, as detritus, algae, plants, invertebrates (including other crayfish), and vertebrates (Ilhéu and Bernardo 1993a, 1995; Momot 1995; Smart et al. 2002; Crehuet et al. 2007).

Among mollusks, species that suspension-feed on phytoplankton and seston, graze on periphyton, or browse on vascular plants are particularly invasive. They can develop massive populations in freshwater bodies, consuming so much primary production that they heavily affect the amount and community composition of primary producers, with consequences on every part of the ecosystem (Strayer 2010).

An important issue in the biological invasion discipline is the interactions among invasive species and native species. Introduced species may simply decrease the abundance of all native prey or produce differential effects on different prey (Parker et al. 1999). The potential for invasive species to influence community structure has widely recognized over the past few decades (Crooks 2002; Hoffmeister et al. 2005; Levine et al. 2003). Successful invaders often affect trophic interactions of an ecosystem (Shea and Chesson 2002).

Many studies have examined the interactions between native and invasive species (Dardi et al. 1996; Meekins and McCarthy 1999; Byers 2000; Seabloom et al. 2003; Cruz et al. 2006), highlighting the negative impacts (Human and Gordon 1996; Callaway and Aschehoug 2000). Due to the high rates of species introductions, many systems harbour several invasive species that can also interact with each other, potentially mitigating or exacerbating their impacts on native communities (Griffen et al. 2008). Understanding the ecological impact of introduced species is more difficult when several invaders are present in the same system, because their combined effect may be completely different from the invasion of a single species (Simon and Townsend 2003; Preisser and Elkinton 2008). Individual and cumulative effects of interactions between invaders in an ecosystem add another level of complexity to our attempts to understand the dynamics of biological invasions and to manage them.

Some studies so far have demonstrated different interaction outcomes that might arise from the presence of multiple invaders, ranging from negative (Perry et al. 2000; Riel et al. 2009; La Pierre et al. 2010) to positive (Ricciardi 2001; Adams et al. 2003; Jordan et al. 2008) or neutral effects (Cope and Winterbourn 2004).

Multiple invaders can interfere with each other in such a way as to attenuate their impacts (Vance-Chalcraft and Soluk 2005; Griffen et al. 2008). An invader may negatively affect another through competition and/or predation, reducing or minimizing their joint impacts on native biota (Ross et al. 2004). In other cases, invaders may have no effect on others alien species (Cope and Winterbourn 2004) or, through a variety of positive interactions, several invaders can produce synergistic effects (Simberloff and Von Holle 1999; Richardson et al. 2000; Ricciardi 2005), favour interactions, increasing their ecological impacts and promoting establishment and spread (Ricciardi 2001; O'Dowd et al. 2003; Grosholz 2005), so that the impact of several species together becomes greater than the sum of the impacts of the single species (Simberloff and Von Hall 1999).

These new interactions can influence native communities, but also the success and impact of invasive species (Robinson and Wellborn 1988; Baltz and Moyle 1993; Reusch 1998; Crawley et al. 1999; Byers 2002; de Rivera et al. 2005). However, studies on the interactions among co-invading alien species are today scanty (Ermgassen and Aldridge 2011), while they could provide important insights on their combined impacts on native species (Griffen et al. 2008).

1.2 *INTERACTIONS BETWEEN INVASIVE ALIEN SPECIES*

Aizen et al. (2008) suggested that the existence of differential interactions among invaders might take place in the most advanced stages of invasion, because at the early stages invasive species are integrated in the webs by interacting mostly with native ones.

As above, when multiple invaders co-occur and interact, they may either influence each other positively, resulting in an invasion meltdown, or they may interact negatively, inhibiting each other's success (Simberloff and Von Holle 1999).

1.2.1 *Facilitative interactions and invasional meltdowns*

The interactions between two or more invasive species have the potential to lead to synergistic impacts on the recipient ecosystem, wherein the impacts of a group of alien species are greater than the summed impacts of the individual species. The outcome of species introductions could result in accelerating rates of invasion, driving ecosystems to be invaded by greater numbers of species, each invasive species having the potential to facilitate further invasions or enhance impacts of other alien species (Von Holle 2011).

For example, once established, some invaders can alter habitat conditions in favour of other invaders, thereby creating a positive feedback system that accelerates the accumulation of non-indigenous species and their synergistic impacts. This defines the *invasional meltdown* model, which emphasizes facilitative (rather than antagonistic) interactions among introduced species (Simberloff and Von Holle 1999; Ricciardi 2001).

The *invasional meltdown* model has important implications for ecosystem management and biodiversity (Ricciardi 2001). If an increased frequency of introductions makes an ecosystem more vulnerable to further invasions, then efforts to reduce the rate of invasion become useless. This refutes the criticism that resources allocated toward prevention are wasted if some future invasions are inevitable.

Although *invasional meltdown* may increase local alfa-diversity through the increase of introduced species, the continuing replacement of endemic species by widespread invaders will inevitably reduce diversity among habitats and among regions (Rahel 2000). *Invasional meltdowns* will thus accelerate the homogenization of ecosystems (Ricciardi 2001).

Positive interactions among alien species could greatly worsen the problem of invasions, but they are poorly studied, mostly limited to plant-pollinator and dispersal interactions (Simberloff and Von Holle 1999; Richardson et al. 2000). Adams et al. (2003) showed the first experimental evidence of facilitation between two introduced vertebrates and supports the *invasional meltdown* hypothesis; they found that invasion of bullfrogs is facilitated by the presence of co-evolved non-native fish, which increase tadpole survival by reducing predatory macroinvertebrate densities.

1.2.2 ***Agonistic interactions or "invasional antagonism"***

An invasive species can also alter the potential impacts of a previously established invader. For example, the European weed *Pastinaca sativa* rapidly increased its toxicity in response to the introduction of one of its natural herbivores, two centuries after the weed has become established in North America (Zangerl and Berenbaum 2005). The consequences of multiple invaders may also depend on the order of their introduction (Robinson and Dickerson 1987).

Negative interactions may be expected when species use similar resources or have a similar ecological roles (Lawton and Brown 1993; Rosenfeld 2002). So, most previous studies of negative interactions among invasive animals have focused on taxonomically related species, rather than phylogenetically distant species occupying similar niches. For example, some experiments were conducted to determine if invasive round gobies (*Neogobius melanostomus*) suffer from aggressive attacks, alter their behaviour or use of shelter, or are outcompeted for food in the presence of the alien crayfish *Orconectes limosus*. Round gobies initiated the majority of aggressive encounters, while crayfish often responded by fleeing, suggesting that crayfish are likely to suffer energetic costs and an increased risk of predation in the presence of round gobies (Church 2009).

Another example of negative interaction between invasive species is the work of Hudina et al. (2011) involving two alien crayfish, *Orconectes limosus* and *Pacifastacus leniusculus*. While both species are detrimental to native ecosystems, the ability of *P. leniusculus* to withstand competition pressure from another successful invasive species underscores its potential to establish dominant populations.

When negative interactions between invaders become sufficiently intense, agonistic exclusion of one of the species may occur with a possible replacement of one invader by another (Griffen et al. 2011), as in the case of the interaction between the crabs *Carcinus maenas* and *Hemigrapsus sanguineus.* The European green crab *C. maenas* invaded the east coast of North America in the early 1800s. As omnivorous predator, it has had widespread consumptive and non consumptive impacts on the native community. Two decades ago, a second predatory crab (*H. sanguineus*) invaded the same region (Griffen et al. 2008). The arrival of *H. sanguineus* resulted in the rapid disappearance of *C. maenas* from rocky intertidal sites within just 3 years (Kraemer et al. 2007; Lohrer and Whitlatch 2002)**.** Further, *H. sanguineus* consumed settling *C. maenas* postlarvae and this consumption has been claimed for the decline of *C. maenas* (Lohrer and Whitlatch 2002).

The impacts of replacing one invader with another depend on the impacts of the individual species involved (Griffen et al. 2011), but the most important is that the negative interactions between invasive species can alter their impacts in areas where they coexist (Jensen et al. 2002; Griffen and Byers 2006; Griffen et al. 2008).

1.2.3 *Predator-prey interactions – Functional Responses*

A central goal in ecology is to understand and predict how the consumption rate of a predator changes with its prey density, because it links the dynamics of the prey and the predator and therefore strongly determines the dynamics of communities (Rudolf 2008). Knowledge of negative interactions, like predator-prey interaction involving alien species, has conservation and economic importance because these species have the potential to interfere with ecosystems and human activities (Lodge and Shrader-Frechette 2003).

The biotic resistance to predation depends on at least four factors: prey propagule pressure (the number of individuals initially introduced to an area), prey reproductive rate, predator abundance, and the functional response of the predator to the prey, that is, the relationship between resource density and consumer uptake rate (Twardochleb et al. 2012). Determining the shape and parameters of the functional response of a consumer to a novel prey can shed light on the potential for biotic resistance at different densities of the invader and predator (Maron and Vila 2001).

The functional response quantifies trophic relations and, in particular, gives insights into predator behaviour and its effects on prey populations (Holling 1959). Being the rapid exploitation of resources commonly associated with high-impact invaders (Strayer et al. 1999; Byers et al. 2002; Johnson et al. 2008; Morrison and Hay 2011), valuable insights into their ecological impact could be obtained from the assessment of their functional response.

Recently, it was suggested that introduced predators damaging native communities might display higher functional responses than similar native species (Bollache et al. 2008), but only few authors have investigated the predatory functional response in different species as a potential predictor of invader effects (Bollache et al. 2008; Hoff and Bolens 2004; Radford et al. 2007).

Modelling the predator functional responses is typically carried out adopting one of the three following models: type I, type II and type III. In a basic type I functional response, prey consumption rises linearly with increasing prey density. The type II functional response features a deceleration in prey consumption with increasing prey density towards a plateau. The type III functional responses have a similar plateau to type II functional responses, but at low prey density there is acceleration in prey consumption as prey density increases, forming an “S-shaped” curve (Haddaway et al. 2012).

The shape of the functional response curve is dependent on several characteristics, such as encounter rate, capture efficiency and handling time (Holling 1965; Hassel 1978). These characteristics may differ within a species as a consequence of predator or prey size, interference among predators or presence of alternative prey (Chesson 1989; Safina 1990; Tripet and Perrin 1994; Elliott 2003).

This novel use for functional responses has important implications for invasion ecology; it elucidates the role of native predators in resisting invasions, and, by quantifying invasion thresholds, it may help managers predict the outcomes of species introductions and biocontrol efforts (Twardochleb et al. 2012).

1.3 *STUDY SPECIES*

The study species, *Procambarus clarkii* and *Dreissena polymorpha*, are considered two of the world’s most invasive species and were listed among the “100 of the worst” IAS in Europe (DAISIE 2013).

1.3.1 *The zebra mussel, Dreissena polymorpha (Pallas, 1771)*

1.3.1.1 General description

The zebra mussel, *Dreissena polymorpha,* is an increasing invasive Ponto-Caspian bivalve (Karatayev et al. 2002). During the last two centuries, it has invaded a wide variety of lakes and watercourses on both sides of the Atlantic (fig. 1.1.) (Higgins and Vander Zanden 2010).

Where it has established, it occurs in densities not normally reached by any native species and usually dominates the benthic fauna in terms of biomass (Ward and Ricciardi 2007). Indeed, once they are introduced into a new area, zebra mussels often form dense aggregations, reaching densities of up to 700.000 individuals m^{-2} (Pimentel et al. 2000). This species causes economic costs, not only due to the effects exerted on the invaded habitats: in USA $3.1 billion are spent each decade to clear blocked intake pipes (Vitousek et al. 1996). In addition, economic resources are required to help affected ecosystems return to their "underlying element of organization and constancy" (Wagner 1993).

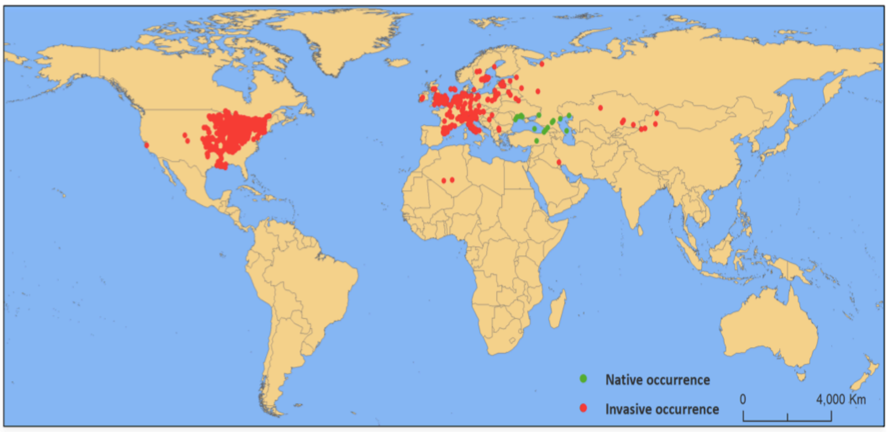

Figure 1.1. Distribution map of *Dreissena polymorpha* (Gonçalves and Capinha, *unpublished*).

Zebra mussel life cycle (fig. 1.2.) includes a free-swimming larval stage (veliger) that, after spending several days in the water column, attaches to substratum by byssal threads and metamorphoses into a sessile adult. The planktonic stage is relatively short (few weeks) compared with the zebra mussel lifespan (few years). During the last moments of the veliger stage, *D. polymorpha* individuals develop the byssum, that allows attachment to a solid substrate (Casagrandi et al. 2007). Adult mussels retain the ability to abandon their byssi at any time and crawl over substratum to find a new site (Ackerman et al. 1994).

The juvenile stage begins after settlement and ends when mussels become sexually mature and produce eggs and sperm (Casagrandi et al. 2007). Sexual maturity and first spawning seem to be correlated with a shell length of about 7-9 mm, independent of mussel age (Jantz and Neumann 1998). The analysis of size-frequency distributions and the study of growth in experimental cages showed that zebra mussel's lifespan may vary from a minimum of 2 years to a maximum of 8 years (Karatayev et al. 2006). This lifespan is dependent on local conditions, being generally shorter in warmer waters (Stanczykowska, 1977 in Casagrandi et al. 2007).

These life-history characteristics, unique among freshwater bivalves and the extraordinary high fecundity (Borcherding 1991) in combination with human-mediated dispersal mechanisms have facilitated its rapid spread (Carlton 1993; Johnson and Carlton 1996). The main pathways of the expansion in the range of zebra mussel are through inland navigation, particularly since the opening of new waterways between eastern and central Europe at the beginning of the 1800s, the transfer of animals for stocking in farms and the introduction into lakes of mussels attached to boat hulls. Larvae and adults may be carried in ballast water or as fouling on ship and boat hulls, navigation buoys, fishing vessel wells, as well as by transport of timber or river gravel, fish stocking water and fishing equipment (Reynolds and Donohoe 2001)**.**

Druses can range in size from 2 cm to more than 10 cm, in diameter and can consist of 10's to 100's of individuals. Druses are not permanently fixed to the substrate, but they provide "mobile" patches of hard substrate consisting of shell surfaces and interstices suitable for use by other benthic organisms (Botts et al. 1996).

1.3.1.2 Ecological impacts

The zebra mussel is a relevant species for its both negative and positive effects on the ecosystem (van Nes et al. 2008).

The ecological impact of zebra mussels in aquatic ecosystems depends largely on their filtration rate (clearance rate) of primary producers present in the water column (Nadaffi et al. 2007).

Zebra mussels can become an important filter feeder in the invaded habitats and can graze selectively on phytoplankton at the base of food web. Zebra mussels' grazing can dramatically reduce phytoplankton biomass, which can have strong effects on higher trophic levels, altering energy flow, community structure, and ecosystem function of lakes (MacIsaac 1996; Idrisi et al. 2001; Ward and Ricciardi 2007).

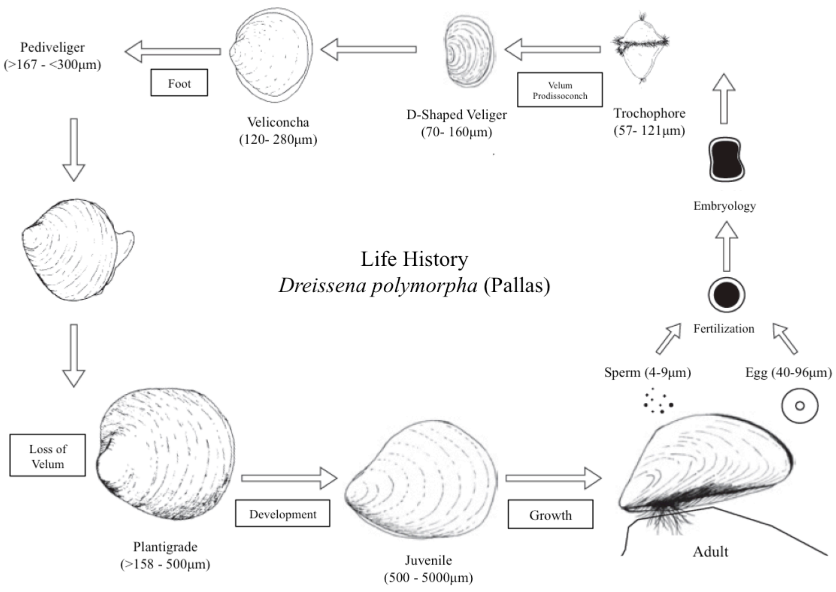

Figure 1.2. The life history of *Dreissena polymorpha* (adapted of Ackerman et al. 1994 and CEAM 2008)

Zebra mussels have also been shown to improve water transparency, filter microzooplankton, overgrow native bivalves, and compete with other grazers (MacIsaac 1996; Karatayev et al. 1997; Strayer et al. 1999).

Dreissena polymorpha, introduced to the Great Lakes in the mid-1980s, has had devastating ecological impacts (Lowe and Pillsbury 1995; MacIsaac 1996; Dermott and Kerec 1997; Ricciardi et al. 1998). Reeders et al. (1993) recorded a 46% decline in phytoplankton volume in a pond containing *Dreissena* relative to a control pond. MacIsaac (1996) suggests that this drastic energy shift may greatly affect invertebrate communities.

This mussel also serves as a sediment trap by converting large amounts of seston into excreted feces, creating a soft substrate of rich organic material highly suitable for deposit-feeding infaunal species (Leppäkoski 1984). An experimental study on the Saint Lawrence River (Ricciardi et al. 1997) showed great change in composition and numbers of macroinvertebrates on hard substrates colonized by the zebra mussel (Simberlof and Von Hall 1999).

Zebra mussels may also generate a mutual facilitation with macrophytes, including introduced ones, in shallow, light-limited waters (MacIsaac 1996). High mussel abundance increases water clarity, which in turn promotes macrophyte growth. The macrophyte growth, in turn, provides additional settling substrate for *Dreissena* (Lewandowski1982) and can help disperse zebra mussels between water bodies (Johnson and Carlton 1996). Eurasian water milfoil (*Myriophyllum spicatum*), an invasive aquatic weed, has greatly increased after the *Dreissena* invasion (MacIsaac 1996).

This invasive mussel have positive effects on other macronivertebrates because mussel shells increase surface area of hard substrate and create structurally complex substrates that provide smaller macroinvertebrates with refuge from predation and other disturbances (Botts et al. 1996; Stewart et al. 1998). Additionally, filter-feeding *Dreissena* may increase benthic organic matter by depositing pseudofeces and feces and by stimulating benthic algal production through excreted nutrients and increased benthic light levels (Lowe and Pillsbury, 1995) These sources of benthic organic matter are valuable food and habitat for other benthic macroinvertebrates (Lamberti 1996) and can, also, increase the diversity and abundance of invertebrate prey for benthivorous fishes (Thayer et al. 1997).

Although shells of older zebra mussels or their close proximity are the most preferred locations for mussel settlement (Griffiths et al. 1989; Mörtl and Rothhaupt 2003), limited availability of substrates in habitats with dense populations of *D. polymorpha* leads to colonization of alternative substrates, including mobile hard-shelled animals (Ďuriš et al. 2007).

1.3.1.3 Zebra mussel fouling on invertebrates

Unionids can provide the most abundant source of hard substratum for colonization by *D. polymorpha* (Lewandowski 1976; Hebert et al. 1989; Lyakhnovich et al. 1994; Ricciardi et al. 1995; Schloesser *et al*. 1996; Sousa et al. 2011), as well as Odonata larvae (Tucker and Camerer 1994; McCauley and Wehrly 2007; Fincke et al. 2009; Hughes 2010, Hughes and Fincke 2012) have been documented.

The colonization of crayfish bodies by zebra mussel is certainly an unusual phenomenon, although it has been occasionally reported in some species: *Astacus astacus* (Kulmatycki 1932), *Astacus leptodactylus* (Sebestyén 1938; Lamanova 1971), *Orconectus limosus* (Laurent and Suscillon 1962; Laurent 1994; Smietana 1996; Lajtner et al. 2005; Ďuriš et al. 2006; Ďuriš et al. 2007), *Orconectus rusticus* (Brazner and Jensen 2000), *Orconectes luteus* (Wetzel *et al*. 2004) and *Orconectes pardalotus* (Wetzal et al. 2005).

Studies have found that in few years zebra mussels can kill off entire unionid populations (Schloesser et al. 1996). Along with food competition and preventing opening for respiration, impaired movement has been implicated as a probable cause of mortality in unionids, following zebra mussel infestation (Ricciardi et al. 1995; Schloesser et al. 1996). Odonata larvae with zebra mussels attached to the ventral surface are likely to be destabilized and may have difficulty burrowing into sediments, affecting prey capture and predator avoidance (McCauley and Wehrly 2007). The impacts on crayfish still remain only speculative.

1.3.1.4 Predation on zebra mussels

Invasive zebra mussels from trochophore and veliger stages to adults (Molloy et al. 1997; Thorp et al. 1998; Magoulick and Lewis 2002; Eggleton et al. 2004) are preyed by copepods (Liebig and Vanderpleog 1995), fishes (Prejs et al. 1990; Nagelkerke and Sibbing 1996; Molloy et al. 1997; Magoulick and Lewis 2002), diving birds (21 species) (Molloy et al. 1997), turtles (Serrouya et al. 1995) and crayfish (Piesik 1974; Love and Savino 1993; MacIsaac 1994; Martin and Corkum 1994; Perry et al. 1997; Schreiber et al. 1998; Reynolds and Donohoe 2001). Other predators of zebra mussels include coelenterates, annelids, and rodents (Molloy et al. 1997).

In Polish lake, roach, with their strong pharyngeal denticles, feed almost exclusively (95-100 %) on zebra mussels (Mackie and Schloesser 1996) and their predation impact may be significant. Diving ducks may also consume zebra mussels in huge numbers, although they have had no measurable impact on mussel numbers (Hamilton et al. 1994; de Knock and Bowmer1993).

Decapod crustaceans have been shown to feed on zebra mussels in laboratory and field studies, mainly selecting zebra mussels of small to medium size classes. Marine crabs may be effective predators on adult zebra mussels where both coexist in estuaries (Molloy et al. 1994; Boles and Lipcius 1994).

In North America, Love and Savino (1993) showed that the crayfish *Orconectes virilis* was an effective predator on zebra mussels, even if it had a preference for other, more easily manipulated foods, if available. In Europe, the introduced *O. limosus* can be an effective predator of zebra mussels. Piesik (1974) calculated that this crayfish could take up to 100 small (1-5 mm) zebra mussels daily in the river Odra (central Europe), but Love and Savino (1993) suggest this may be affected by the lack of other available food sources.

MacIsaac (1994) showed that *Orconectes propinquus* displayed a strong numerical preference for small (3-5 mm) zebra mussels. However, in this laboratory-based study, larger zebra mussels were attacked more often than smaller individuals, the lower predation rates merely representing rejection after unsuccessful predation attempts. Survival of zebra mussels also increased when the crayfish were offered alternative macrophyte foods.

Perry et al. (2000) conducted a experiment to test the hypothesis if the invasive crayfish *O. rusticus* could significantly reduce the recruitment of zebra mussels in streams: their results suggest that streams with moderate to high densities of crayfish will experience slower invasion by zebra mussels and lower final densities of zebra mussels.

1.3.2. *The red swamp crayfish Procambarus clarkii (Girard, 1852)*

1.3.2.1 General description

The red swamp crayfish, *Procambarus clarkii*, is native to the central south of the USA (Louisiana) and northeastern Mexico. It is now established in every continent (fig. 1.3) except Australia and Antarctica, invading even remote islands like the Azores, Canaries and Hawaii as a result of human-mediated translocation (Hobbs et al. 1989; Huner 2002; Souty-Grosset et al. 2006). The red swamp crayfish was first imported into Europe in 1973 when Spain intentionally introduced the species for commercial production (Hasburgo-Lorena 1979) and rapidly established self-sustaining populations, becoming the most abundant crayfish species in the Iberian Peninsula. The first illegal introductions into France and Italy occurred soon after its introduction to Spain (Gutiérrez-Yurrita et al. 1999; Huner 2002; Gherardi 2006). *Procambarus clarkii* has also become popular as an ornamental aquarium species, and many populations in Central Europe originated from pet releases (Dehus et al. 1999; Soes and van Eekelen 2006; Dümpelmann et al. 2009). Today, *P. clarkii* is widespread and abundant throughout South-Western Europe, Northern and Central Italy, maintaining also reproducing populations in the Netherlands, Belgium, Great Britain, Germany, Switzerland and Austria (Chucholl 2011).

1.3.2.2 Ecological impacts

The success of this crayfish is mainly due to its ecological plasticity, which allowed its introduction and stabilization in diversified environments (Hobbs et al. 1989; Huner and Barr 1991).

It is a prolific and aggressive species, it tolerates low quality environmental conditions (low oxygen concentrations, drought periods and a wide range of water salinity and acidity) and it is well adapted to survive in areas with drastic, seasonal fluctuations in water levels by digging deep burrows (Barbaresi and Gherardi 2000).

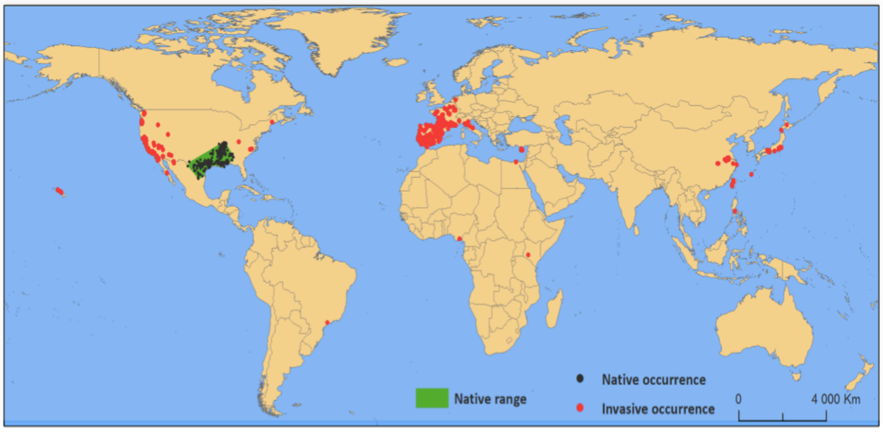

Figure 1.3. Distribution map of *Procambarus clarkii* (Capinha et al. 2013).

Hobbs et al. (1989) reported that the majority of *P. clarkii* introductions have had negative consequences. This species is a polytrophic omnivore, and its ecological impact on native ecosystems can be devastating (Rodríguez et al. 2005; Gherardi 2006; Souty-Grosset et al. 2006). It is especially considered a pest in agricultural areas, such as rice paddies, where it damages young rice plants as well as irrigation systems and dams (Anastácio et al. 2000; Gherardi 2006; Souty-Grosset et al. 2006).

The species has dramatic effects on the community by depleting all food sources available because of its voracity and ability to shift its diet (Huner and Barr 1991; Ilhéu and Bernardo 1993b; 1995; Gutiérrez-Yurrita et al. 1998). Juveniles may prefer more varied diets (particularly animals), because they have greater energetic needs for growth, or they may simply be better predators than adults (Correia 2002).

Their feeding habits could lead to changes in food webs and even disappearance of some species, and it may have a potential impact on macrophytes, invertebrates and small vertebrates (Geiger et al. 2005; Cruz et al. 2006; Souty-Grosset et al. 2006; Gherardi 2007b; Dana and Ortega 2010).

Red swamp crayfish have been repeatedly blamed as responsible for the local extinction of several snail species; an example is the coincidence between the extinction of two species of gastropods – *Lymnaea peregra* and *L. stagnalis* – in freshwater marshes of the

Donaña National Park (Spain) and the introduction of the red swamp crayfish (Montes et al. 1993).

Recently, Cruz and Rebelo (2005) pointed out this alien species as a predator of egg masses and larvae of all the amphibian species of southwest Iberian Peninsula. In some Californian streams, *P. clarkii* predation on eggs, larvae and adults of the newts *Taricha torosa* has been reported by Diamond (1996) as a factor leading to the disappearance of the species, which does not have mechanisms of defense against the new predator. Gherardi et al. (2001) compared the predation of the invasive red swamp crayfish *P. clarkii* on different species of tadpoles with that of a native crayfish, *Austropotamobius pallipes*, and found that the invasive crayfish caught them more rapidly and appeared to be faster in switching to different prey than the native species.

In Italy, the coexistence of *A. pallipes* and *P. clarkii* is reported in some places, like the Province of Bologna while *P. clarkii* shares some streams with the freshwater crab *Potamon fluviatile* in the Province of La Spezia (Barbaresi and Gherardi 2000) and *P. clarkii* outcompetes with indigenous crayfish, in particular *A. pallipes*, being dominant in aggressive interactions (Dardi et al. 1996; Gherardi and Cioni 2004).

This species has also a negative impact to native fauna as it contributes to the spread of numerous parasitic helminths of vertebrates (Sogandares-Bernal 1965), as well as the crayfish plague fungus (it may harbour the fungus within its body in a chronic or latent infection and thus may function as a vector for the disease into Europe; Huner and Lindqvist 1995).

Though no quantitative study has been yet made, the appearance of high densities of *P. clarkii* has been considered responsible for the increase in the number of bird species, like the egrets and herons *Botarus stellaris*, *Egretta garzetta* and *Ardea purpurea*, together with cormorants, recorded in some European areas, as in the Ebro region (Spain) and Massaciuccoli Lake (Tuscany-Italy) (Barbaresi and Gherardi 2000), all the species being predators of crayfish.

1.3.2.3 Red swamp crayfish as an opportunistic predator

Due to its role as an invasive species in many areas, the diet of *P. clarkii* has been studied in depth, a main issue being whether the species is an opportunistic or a selective feeder. Ilhéu and Bernardo (1993b) studied its diet by checking the gut contents of 164 animals collected with traps in several locations in Alentejo (southern Portugal). Although their results suggested a preferential consumption of vegetal material rather than other types of food, laboratory experiments revealed a crayfish preference for benthic invertebrates. A similar result was obtained by Gutiérrez-Yurrita et al. (1998) in analyzing *P. clarkii* stomachs (502) in the lower Guadalquivir Basin (southern Spain). Moreover, the analysis of 80 additional stomachs from the same geographic area confirmed previous observations and showed that food item diversity in crayfish stomachs is correlated with prey availability (Alcorlo et al. 2004).

Correia (2002, 2003) also found that crayfish resource use depends on the availability of aquatic prey, but in the Portuguese studied rice field populations there was a reverse dominance of food items in crayfish guts, with those of animal origin, not plants or detritus, being the most abundant.

Although *P. clarkii* is widely recognized to be omnivorous, it seems that shifts in diet might occur during its ontogenetic development (Correia 2003) with juveniles showing preference for food of animal origin and adults behaving mainly as herbivores (Ilhéu and Bernardo 1993a, Montes et al. 1993).

Ilhéu and Bernardo (1993b, 1995), Cronin et al. (2002), and Gherardi and Barbaresi (2007) used choice tests in which alternative food items were offered in similar quantities to crayfish and their preferences were evaluated according to the amount of resources left after some time. *P. clarkii's* feeding decisions seemed not to be associated with either the availability of plants in the habitat or their occurrence in the gut contents of wild crayfish. Neither did their ingested biomass depend on the organic content or assimilation efficiency.

Other studies suggested that feeding preferences of the species often depend on factors other than nutritional value and digestibility, because *P. clarkii* preferred *Urtica* sp. to earthworms, notwithstanding the higher nutritional value and assimilation efficiency of the latter (Gherardi and Barbaresi 2007).

Laboratory studies also showed *P. clakii* preys upon tadpoles of several indigenous species of *Rana* from Italy (Gherardi et al. 2001; Renai and Gherardi 2004), along with embryos and tadpoles of *Bufo bufo* and larvae of *Triturus vulgaris* (Renai and Gherardi 2004). Cruz and Rebelo (2005) found a very high consumption rate of amphibian egg masses by the red swamp crayfish in mesocosm experiments, even when alternative plant food items were made available.

1.4 *THE TWO INVASIVE SPECIES IN THE AQUATIC ECOSYSTEMS OF CENTRAL ITALY*

Animal xenodiversity in Italy is continuously increasing. Gherardi et al. (2008) found 112 alien species (64 invertebrates and 48 vertebrates) that contribute for about 2% of the inland-water species richness. The large majority of alien species entered Italy as a direct or indirect effect of human intervention (Gherardi et al. 2008), as the case for *P. clarkii* and *D. polymorpha*.

P. clarkii's resistance to the crayfish plague was the main reason of its introduction into Europe in 1973 and then into Italy (Gherardi 2006).

In Tuscany (Gherardi et al. 1999a), *P. clarkii* is especially abundant in the Lake of Massaciuccoli and in the surrounding area: in 1993, a stock of crayfish cultivated in a farm poured into the lake after a flood or was released into it when the farm went bankrupt (Gherardi et al. 1999b). In any case, most populations of *P. clarkii* living in the wild in Tuscany during the following years appear to have originated from human translocations from the Lake of Massaciuccoli (Gherardi et al. 1999b; Barbaresi and Gherardi 2000).

Thus, *P. clarkii* is to be found in the streams of the Versilia coast (the provinces of Lucca, Massa and Carrara) up to the Magra River (in the neighbouring region of Liguria) in the province of Genoa, in channels and ponds of the Bientina marshland (Lucca and Pisa provinces), in irrigation ditches of Osmannoro and Sesto Fiorentino (the suburbs of Florence), in low-flowing rivers of Pistoia and Prato provinces, and in the 1800-ha wide Fucecchio humid area. Other regions with published records of breeding populations of *P. clarkii* are the Marches and Latium, in particular the provinces of Rome and Rieti (Barbaresi and Gherardi 2000).

P. clarkii was recorded for the first time in Lake Trasimeno (Umbria, Central Italy) in 2000 (Dörr et al. 2001).

In Italy, the species seems to be physiologically resistant to low temperatures, being found in several cold lakes of Abruzzo at high altitudes (Barbaresi and Gherardi 2000).

The first established population of *D. polymorpha* in Italy was reported by Giusti and Oppi (1972) in Lake Garda (with recreational boats from Central Europe as possible vector). Since then, this bivalve has been spreading almost all over North Italy in various river basins with an Adriatic drainage (Cianfanelli et al. 2007), while the central southern Italian inland waters were known to have been colonized in two sites in Molise (Bodon et al. 2005) and in only one site in a Tyrrhenian catchment (Lake Trasimeno - Umbria, Spilinga et al. 2000).

In 2003, a new population was found in the artificial Lake Pavana (Lori and Cianfanelii 2006), being the first record of the zebra mussel in Tuscany. Lake Pavana belongs to a group of hydroelectric reservoirs located in the south of Emilia Romagna and north Tuscany (Lakes Brasimone, Santa Maria and Suviana), where *D. polymorpha* has been present since 1999 (Cianfanelli et al. 2007). Lake Suviana and Lake Pavana are linked by pipes that can occasionally pump water in both directions (Regione Toscana 2005); therefore we could hypothesize the transfer of *D. polymorpha*, probably through its planktonic larvae (Cianfanelli et al. 2010).

In 2005, it was found in the Lake Bilancino (5.10 km^2) in Florence Province, where an abundant population is present (Lori and Cianfanelli 2006).

1.5 *SCOPE AND GOALS OF THE THESIS*

Aquatic ecosystems in Italy that were already occupied by established populations of *P. clarkii*, when the zebra mussel arrived (Aquiloni et al. 2010). This is the first location in the world where these two invasive species coexist and it is important to study their interaction.

Once both species are established, there is also a possibility that zebra mussels may be transported upstream attached to crayfish (Brazner and Jensen 2000; Ďuriš et al. 2007). However, the ability of zebra mussels to attach to hard surfaces may also make life difficult for crayfish, hampering their movements and slowing their feeding rate.

My main goal was to understand the possible interactions between this two invasive alien species, *Procambarus clarkii* and *Dreissena polymorpha,* in the freshwater ecosystems of central Italy, in order to predict the future interactions between these two species in South-western European regions that will probably be colonized in the near future by *D. polymorpha*.

In particular, my specific goals were:

- to observe the frequency and density of zebra mussel colonization of individuals of *P. clarkia* (***Chapter 2***);
- to study the selective predation by *P. clarkii* on *D. polymorpha*, in the presence of alternative prey (*Physella acuta*) (***Chapter 3;4***);
- to evaluate the possibility of control of *D. polymorpha* by *P. clarkii* through differential predation of the small individuals (***Chapter 5***).

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CHAPTER 2

FOULING OF INVASIVE RED SWAMP CRAYFISH, *PROCAMBARUS CLARKII* (GIRARD, 1852) BY THE ZEBRA MUSSEL, *DREISSENA POLYMORPHA* (PALLAS, 1771) IN LAKE TRASIMENO, ITALY

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Abstract

Observations of *Procambarus clarkii* fouling, by alien zebra mussels (*Dreissena polymorpha*) in Lake Trasimeno (and in the laboratory) are recorded. Results agree with previous reports that zebra mussels can be found, if only occasionally, on most parts of the swamp crayfish body, with a general preference for the ventral surface, and in this case, the pereopods. The frequency of colonization on *P. clarkii* in the wild varied from 0 to 4%; much lower than other crayfish species (e.g. 65% in *Orconectes limosus* or 12-24% in *O. rusticus*). Adults of both sexes were colonized equally. Multiple zebra mussels on individual crayfish were unexpectedly high; this may be related to poor crayfish condition and may also explain the only colonization of a live crayfish individual recorded in the laboratory.

Keywords: *Dreissena polymorpha; Procambarus clarkii*; invasive species; colonization; fouling; Lake Trasimeno; Italy

2.1 *INTRODUCTION*

Zebra mussel, *Dreissena polymorpha* (Pallas, 1771) is an ever-expanding invasive Ponto-Caspian bivalve (Karatayev et al. 2002). During the last two centuries it has invaded large regions of Europe through rivers and artificial channels (Bij de Vaate et al. 2002). Its life cycle includes a planktonic larval (veliger) phase. After several days in the water column, it attaches to a substratum by byssal threads and there metamorphoses into an adult. In Lake Trasimeno (43°08’N 12°06’E, Umbria, central Italy), only one period of successful reproduction exists, from June to July (Lancione and Gaino 2006). Adult mussels retain the ability of abandoning their byssi at any time and may crawl over the substratum to find a new suitable home (Ackerman et al. 1994).

Typically, shells of conspecifics are the preferred location for veliger settlement (Mörtl and Rothhaupt 2003), but where dense populations of zebra mussels exist, they may colonize alternative substrates, including mobile, hard-shelled organisms (Ďuriš et al. 2007).

Zebra mussel colonization of invertebrates, particularly unionid mussels (Schloesser et al. 1996; Sousa et al. 2011) and odonate larvae (Weihrauch and Borcherding 2002; McCauley and Wehrly 2007) has already been documented. Colonization or fouling of crayfish species has only occasionally been reported, e.g., *Astacus astacus* (Kulmatycki 1932), *Astacus leptodactylus* (Lamanova 1971), *Orconectes limosus* (Ďuriš et al. 2007), *Orconectes rusticus* (Brazner and Jensen 2000), *Orconectes luteus* (Wetzel et al. 2004) and in the last decade *Orconectes pardalotus* (Wetzel et al. 2005).

The first record of *D. polymorpha* in Lake Trasimeno dated from the year 2000, and refers to sporadic findings of adults, as well as the first zebra mussel fouling on *Procambarus clarkii* (Spilinga et al. 2000). Presently, *D. polymorpha* density is 200 000 individuals/m^2 (Lancione and Gaino 2006), an abundance comparable to North American lakes (Nalepa and Schloesser 1993).

The invasive red swamp crayfish *Procambarus clarkii* (Girard, 1852) is a large, prolific, aggressive species (Barbaresi and Gherardi 2000), has many established populations in Italy (Aquiloni et al. 2010) and is present in large quantities, since 2000, in Lake Trasimeno, an optimal habitat for this species (Dörr et al. 2006).

Observations of *P. clarkii* fouling by *D. polymorpha* in Lake Trasimeno, as well as in a laboratory experiment are reported.

2.2 *METHODS*

In the wild, colonization data was documented from crayfish collected by professional fishermen in Lake Trasimeno. Crayfish were captured on six occasions with baited traps at a depth of approx. 1.5 m, over two summers 2011-2012 (table 2.1). Crayfish with mussels attached were measured and sexed; attached mussels were also measured (shell length).

In the laboratory, four crayfish were introduced singly into four aquaria (with 4 L dechlorinated tap water), and each was left for two weeks in the presence of 150 zebra mussels (shell length 5-15 mm).

2.3 RESULTS AND DISCUSSION

Overall abundance of attached zebra mussels was low; a total of 19 individuals on 12 crayfish (7 males and 5 females) in 2011, and 14 individuals on 10 crayfish (3 males and 7 females) in 2012 (table 2.1). The proportion of each sex amongst colonized individuals did not differ from the normal sex ratio at Trasimeno (which is of 1.15:1 in favour of females; Dörr et al. 2006) (G=0.731; *p*<0.75).

Table 2.1 Number and size of zebra mussels attached to *P. clarkii,* in Lake Trasimeno, during the summer periods 2011 and 2012.

	2011				2012	
	June 1	**June 13**	**July 28**	**September 13**	**June 19**	**July 28**
N° of crayfish examined	200	187	279	168	526	508
N° of crayfish with attached mussels	0	2	3	7	10	0
Total number of mussels attached	0	3	3	13	14	0
% crayfish with attached mussels	0	1.07	1.075	4.17	1.9	0
Range mussel size (mm)	0	2.3-3.8	2.9-4.3	3.1-8.8	1.5-3.3	0

Table 2.2 Distribution (%) of attached zebra mussels on crayfish.

Zebra mussels attached on	Frequency %	
	2011	2012
Eyes	8.3	10
Mouth area	8.3	30
Abdomen (ventral)	25	30
Chelae	8.3	0
Genital area	0	10
Pereopods	66.7	40
Telson & uropods	41.7	20

Colonized crayfish presented mean total length of 9.89 (±1.53) cm in 2011 and 8.56 (±1.15) cm in 2012. *P. clarkii* at Trasimeno is characterized by having a relatively large body size (8.4 cm to 13.5 cm total length) (Dörr et al. 2006).

The majority of zebra mussels attached to *P. clarkii* were small (< 5 mm long), corresponding to juveniles less than one year old (Ackerman et al. 1994). Larger sizes were observed in September.

Despite the rarity of the phenomenon (0 to 4.17% of crayfish with attached mussels; table 2.1), 32% of crayfish colonized had more than one mussel. There was a clear preference for the ventral surface of the crayfish, namely the pereopods and uropods/telson (table 2.2).

In laboratory, after a period of 5 days, only one mussel was found attached to a male crayfish measuring 30.7 mm, in the proximity of the antennules (fig. 2.1A). Post death, 15 days after the start of the experiment, the crayfish was colonized overnight by 18 mussels, on its ventral side (fig. 2.1B).

Ďuriš et al. (2007) show strong seasonality variation in the percentage of the crayfish *O. limosus* colonized by zebra mussels, exceeding 75% in spring and early summer, but sharply dropping to 0-20% in August, post molting (Ďuriš et al. 2007). This effect was not found in this instance; as the summer progressed, both the number and size of the colonizing zebra mussels increased. This is most likely related to the crayfish moulting at Trasimeno, that takes place from April to July (Dörr et al. 2006).

The rapid colonization of a dead *P. clarkii* in the laboratory shows that the exoskeleton of this species is a good substratum for the attachment of *D. polymorpha*. However, the overall proportion of colonized crayfish at Trasimeno was very low, compared to observations on *O. limosus* (17-94%) (Ďuriš et al. 2007) and *O. rusticus* (Brazner and Jensen 2000). This low frequency of colonization cannot be explained by molting (see above) or by *P. clarkii*'s burrowing habits, as the individuals tested in the laboratory did not molt and could not burrow. The intensity of grooming in *P. clarkii* is also quite low, when compared with other species (Bauer 2002). However it is not possible to exclude an increase of individual grooming after being colonized, as this behaviour was not examined during the experiment". Furthermore, the overall high activity rate of this crayfish may simply preclude a secure establishment of *D. polymorpha.*

Observations made in this study, confirmed reports by Ďuriš et al. (2006, 2007), that zebra mussels are occasionally found on most parts of the crayfish body, with a preference for the ventral surface, and particularly in our case, the pereopods. According to Smietana (1996) young specimens of zebra mussels that settle on crayfish find favourable living conditions, especially those attached to the upper parts of the body, but this preference can apparently vary among species. The observation of preferential ventral colonization on *O. limosus* by Ďuriš et al. (2006) differs significantly from data of Brazner and Jensen (2000) on epibiosis on *O. rusticus*, which refers to the chelae, telson, uropods, and carapace as the most heavily colonized parts.

The negative effects of zebra mussel attachment on odonate larvae include reduced mobility, foraging success, larval mobility, hydrodynamic destabilization and difficulty in burrowing into sediments (Hughes and Fincke 2012). Prey capture and predator avoidance may also be affected (McCauley and Wehrly 2007). The effects of zebra mussels on crayfish condition remain speculative.

The death of the single individual that was colonized in the laboratory may imply that its defences were somehow affected.

Individual vulnerability may also explain the relatively high frequency of multiple mussels on single crayfish at Trasimeno. Zebra mussel colonization may be a consequence of individual poor condition, and not its cause.

Figure 2.1 *Procambarus clarkii* adult male alive (A) and dead (B) with zebra mussels attached, in laboratory aquarium.

2.4 *Acknowledgements*

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CHAPTER 3

PREDATION BY *PROCAMBARUS CLARKII* ON THE INVASIVE ZEBRA MUSSEL *DREISSENA POLYMORPHA*: COMPARISON WITH AN ALTERNATIVE PREY

Vera Gonçalves, Elena Tricarico, Rui Rebelo and Francesca Gherardi

(In review to Freshwater Crayfish)

***Note**:*

Here we report the results of a preliminary laboratory study of *Procambarus clarkii* predation upon *Dreissena polymorpha* and an alternative prey, *Physella acuta*.

The study was presented at the Neobiota 2010 Conference Biological Invasions in a Changing World – from Science to Management. From these first results, a more in depth study was developed (see ***Chapter 4***).

Abstract

Both laboratory and field studies have shown that some crayfish species are effective predators of zebra mussels. However, their effective impacts on zebra mussel populations may depend on a number of other factors, including the availability of alternative prey. In this study, we analyzed the size-selective predation of the invasive red swamp crayfish *Procambarus clarkii* on zebra mussel (*Dreissena polymorpha*) and alternative mollusk prey (*Physella acuta).*

These preliminary results indicate that *P. clarkii* selects the size of its mollusk prey, with a preference for small (5-10 mm) to medium (10-15 mm) size-classes; however, there were no significant differences between the handling times of both prey, as well as of the different size classes. Our results show that the differential vulnerability of bivalves vs. gastropods may be dependent on prey size, which thus makes the crayfish's choice between prey with different shell hardness or body shape more complex than previously thought.

Key words: *Dreissena polymorpha*; *Physella acuta*; invasive alien species; predation; *Procambarus clarkii*

3.1 *INTRODUCTION*

Ecosystems are often invaded by more than one alien species, and understanding the interactions among the newcomers is important when further invasions are predicted and appropriate strategies for their management are to be chosen. Simberloff and Von Holle (1999) suggested that the interactions among invaders could lead to the phenomenon of *invasional meltdown*. According to the authors, non-indigenous species facilitate one another's invasion in various ways, potentially leading to an accelerating increase in expansions of invasive species and in their impact; as a consequence, an ecosystem could collapse under the impact of an integrated set of invaders. There are several documented examples of this phenomenon, particularly in freshwater ecosystems (Ricciardi 2001). Obviously, understanding and predicting *invasional meltdowns* has important implications for the management of ecosystems and the conservation of biodiversity (Ricciardi 2001).

The red swamp crayfish *Procambarus clarkii* (Girard) is a highly diffused alien species with many established populations in

Italy (Aquiloni et al. 2010). This species is native to the south-central United States (Louisiana) and north-eastern Mexico and has been introduced widely for aquaculture purposes (Gherardi and Holdich 1999). This is a large, prolific, aggressive species that is well adapted to live in areas with extreme fluctuations in water levels. It is widely recognized that its introduction generally yielded minimal economic benefits, but had negative consequences on the habitats colonized (Hobbs et al. 1989), where it has been responsible for biodiversity losses (Cruz et al. 2006).

Recently, populations of a second well-known alien invader, the zebra mussel *Dreissena polymorpha* (Pallas), have been found in Tuscany (Central Italy) (Lori and Cianfanelli 2006). The zebra mussel is a freshwater, filter-feeding bivalve, native to the rivers flowing into the Black and Caspian seas (Reynolds and Donohoe 2001). Its high reproductive output, filtering capability and seasonal production of free-swimming veliger larvae have enabled this species to invade an unexploited niche in freshwater systems, ultimately causing severe ecological and economic damage around the world. Once established, zebra mussels have proven nearly impossible to remove. The control of this species is therefore noteworthy (Reynolds and Donohoe 2001), considering also that *D. polymorpha* is continuously spreading in south-western Europe (Lori and Cianfanelli 2006).

Two crayfish species (*Orconectes virilis* (Hagen) and *Orconectes propinquus* (Girard)) readily consume large numbers of zebra mussel (Love and Savino 1993; Mac Isaac 1994). However, predation rates on zebra mussels decrease in the presence of an alternative prey species (Love and Savino 1993; Mac Isaac 1994; Perry et al. 1997). Other studies also suggest that crayfish species may be important predators on zebra mussels because: 1) crayfish reduce native mollusk populations (Lodge et al. 1994); 2) crayfish are common in many lakes and streams vulnerable to invasion by zebra mussels (Strayer 1991), and (3) both crayfish (Lodge et al. 1994) and zebra mussels (Dorgelo 1993) may share the same lake microhabitats.

Predicting the future direct and indirect interactions between *P. clarkii* and *D. polymorpha* is important for the conservation of the highly threatened freshwater systems in southern Europe.

Here we report some preliminary results of a laboratory study aimed at comparing the intensity of *P. clarkii*'s predation on *D.*

polymorpha and on an alternative prey species that shares the same habitat as the mussel, the freshwater gastropod *Physella acuta* (Draparnaud, 1805). *Procambarus clarkii* is a voracious predator of gastropods (Montes et al. 1993), and particularly skilled at capturing and consuming *P. acuta,* as documented by Renai and Gherardi (2004).

3.2 *MATERIALS AND METHODS*

Two hundred individuals of *D. polymorpha* and 150 individuals of *P. acuta* were collected from the Lake Bilancino (43^{o}58’41”N, 11°16’54”E), 40 km from Florence (central Italy), and 200 individuals of *P. clarkii* were collected from ditches in Sesto Fiorentino (43°48’50”N, 11^{o}11’41”E), 3 km from Florence. All animals were transported to the laboratory at the Department of Biology in Florence; and the experiment was conducted in July and August 2010.

Upon their arrival in the laboratory, crayfish were measured using a vernier calliper (cephalothorax length excluding rostrum, CL, averaging 36.7 mm), their sex was determined, and injuries were recorded. Only crayfish with intact pereopods were used in the experiments. To avoid pseudoreplication each crayfish was used only once in the following predation experiments.

Test crayfish were maintained in individual plastic tanks (26.5 × 16.2 × 15 cm) containing 1 L of well water at a temperature ranging from 19 – 25°C and were fed *ad libitum* with larvae of *Calliphora* sp. Water was changed once per week. Prior to the experiment, food was withheld from crayfish for 7 days.

Experiments were conducted in glass aquaria (45 × 25 × 27 cm) containing 5 L of well water and maintained as outlined above. Initially, the aquarium was divided into two sides by a removable, opaque plastic divider. Each test crayfish was introduced into one side, and either one *D. polymorpha* or *P. acuta* was added to the other side. After 15 min of acclimation, the divider was removed and the observations started.

The tested *D. polymorpha* belonged to one of three size-classes of shell length: *Small* (5-10 mm), *Medium* (10-15 mm), and *Large* (15-20 mm). The tested *P. acuta* belonged to one of two size-classes of shell length: *Small* (5-10 mm) and *Medium* (10-15 mm).

Each trial was videotaped for one hour and analysed by an experienced observer. We only used the observations from the crayfish that handled the offered mollusc, thereby excluding the individuals that showed no interest in the prey provided, and that could be starting moulting. For each trial, we measured the time required by the crayfish to open the mollusc and then the time it took to eat it. We calculated handling time as the sum of eating and opening times (Hughes and Seed 1981; Mistri 2004). A total of 10 replicates were run for each gender of crayfish and for each combination crayfish-prey size class.

The ability to prey upon each prey size category was analysed comparing the number or eaten and not-eaten prey with the G test for *D. polymorpha* size- classes and the Binomial test for *P. acuta* size-classes.

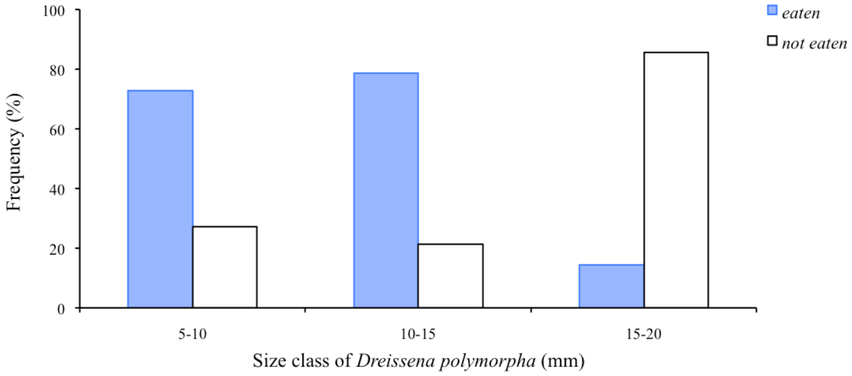
Due to the lack of homoacedasticity of the data, we used non-parametric Kruskal-Wallis tests to compare the handling times for the three size-classes of *D. polymorpha*, and Mann-Whitney U-tests for the comparisons between the two size-classes of *P. acuta* or between the two species.

3.3 ***RESULTS***

A preference for the smaller size-classes of both *D. polymorpha* and *P. acuta* (fig. 3.1a and 3.1b) was evident with a significant difference between species: the preferred size of *D. polymorpha* was the *Medium* size-class (G = 6.85; $p < 0.05$) and in *P. acuta* the *Small* size-class (Binomial test $p = 0.02$).

No significant difference between species was found for opening times (U = 23.00; $p = 0.61$). Opening times did not differ among the two size-classes of *P. acuta* (U = 152.5; $p = 0.151$) and the three size-classes of *D. polymorpha* (U = 19.50; $p = 0.07$). For the *Small* size-classes, eating time differed significantly between species (U = 8.00; $p < 0.05$) (fig. 3.2), with *D. polymorpha* requiring more time to be eaten.

A nearly significant difference between species was found for handling times displayed for *Small* size-classes (U = 9.00; $p = 0.053$) (fig. 3.3). No difference was evident between the two size-classes of *D. polymorpha* (U = 22.00; $p = 0.11$) (fig. 3.4).



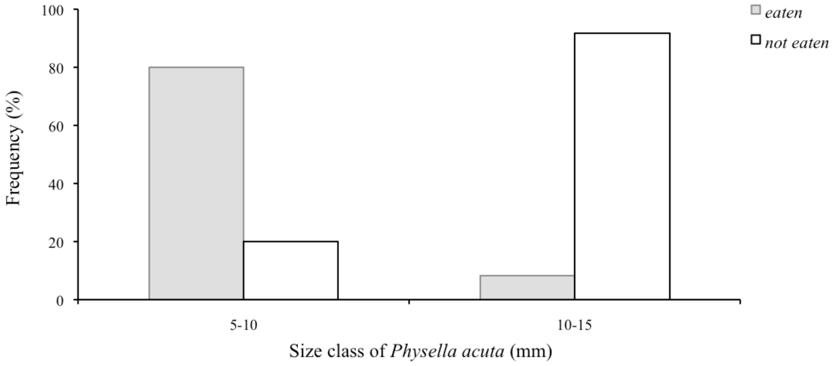


Figure 3.1. Comparison among the three, and between the two, size-classes (shell length) of *D. polymorpha* (a) and *P. acuta* (b), respectively, for the frequency of eaten and not eaten prey. Size-classes: *Small* = 5-10 mm; *Medium* = 10 -15 mm; *Large* = 15 -20 mm.

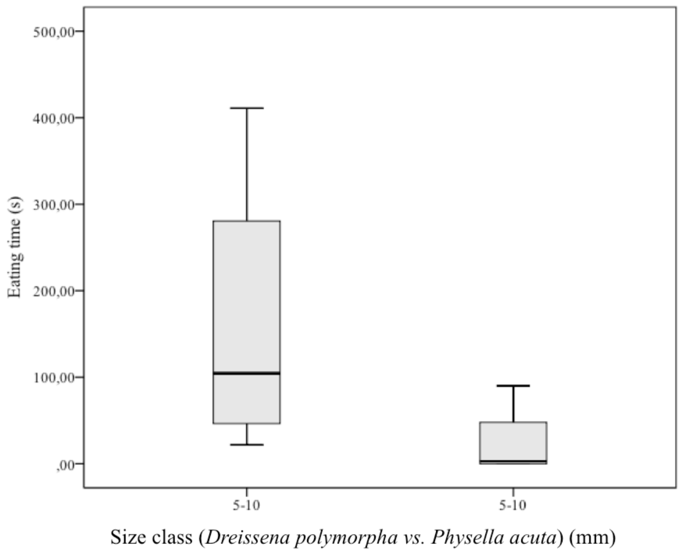


Figure 3.2. Eating time for the *Small* size-class (5-10 mm) of *D. polymorpha* (n=8) and *P. acuta* (n=7).

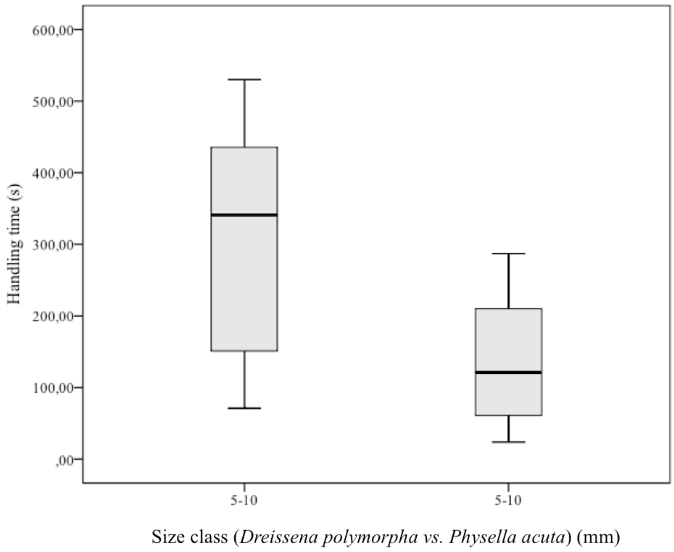


Figure 3.3. Handling time for the *Small* size-class (5-10 mm) of *D. polymorpha* (n=8) and *P. acuta* (n=6).

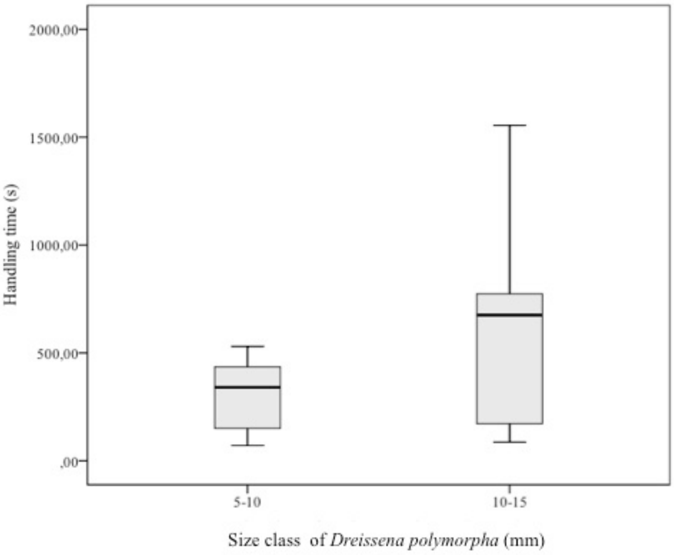


Figure 3.4. Comparison of handling times between the two size-classes of *D. polymorpha;* Size-classes: *Small* = 5-10 mm (n=8); *Medium* = 10 - 15 mm (n=10).

3.4 ***DISCUSSION***

These preliminary results indicate that *P. clarkii* selects the size of its mollusc prey, with a preference for small- to- medium size-classes. This confirms a previous study showing that some crayfish species are effective predators on *D. polymorpha* but usually select individuals of small-medium size-classes (MacIsaac 1994).

Differences in the size-classes of the molluscs consumed by crayfish may also reflect the relative ease of handling them (Martin and Corkum 1994). For instance, Alexander and Covich (1991) showed that the ingestion of snails (*Physella virgata* (Gould)) by the crayfish *Procambarus simulans* (Faxon) decreased with increasing handling time. Other studies have also shown that crayfish prefer prey that requires low handling times (Love and Savino 1993). On the contrary, in our study the handling time was greater for the *D. polymorpha Medium* size-class (10-15 mm), which seems to be the preferred class of prey. A novelty of our results is to have shown that the differential vulnerability of bivalves vs. gastropods may be dependent on prey size, which thus makes the crayfish's choice between prey with different shell hardness or body shape more complex than previously thought. Future research will focus on the crayfish multiple choice among the different size-classes of both prey species.

3.5 *Acknowledgements*

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CHAPTER 4

BIVALVE OR GASTROPOD? USING PROFITABILITY ESTIMATES TO PREDICT PREY CHOICE BY *P. CLARKII*

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Abstract

Recently, a highly invasive alien species, the zebra mussel *Dreissena polymorpha*, has colonized some Italian aquatic ecosystems, previously colonized by another highly invasive alien species – the North american crayfish *Procambarus clarkii*. This is the first time that these two species coexist, so predicting the future interactions between this two invasive species is crucial. To evaluate the relative importance of their interactions, we studied prey size selection and prey choice by *P. clarkii* on *D. polymorpha* in the presence and absence of alternative prey – the freshwater snail *Physella acuta*.

We followed an optimability-based approach, by first estimating the most profitable prey sizes for both molluscs and by conducting a prey choice experiment, on which both prey were provided simultaneously to a crayfish under different size combinations.

Prey selection was dependent on prey size, handling time and crayfish size for both preys.

According to profitability estimation, snails should be more profitable than mussels in the size range 6.9-11.0 mm, but for larger sizes (>11.0 mm) the mussel should be more profitable.

The results of the prey choice experiment indicated that *D. polymorpha* size, *P. acuta* size and the difference in profitability between the offered individuals were all relevant for the choice of a species over the other by *P. clarkii.* However, the overall tendency was the choice of the smallest prey, regardless of its species.

Our study confirms that *D. polymorpha* represents a novel prey resource for *P. clarkii*, even in the presence of an alternative prey. *D. polymorpha* may be a preferred prey, especially the juvenile stages, or individuals of small size (5-10 mm). However the estimation of the profitability of a prey species is not always useful to predict prey choice when multiple types of prey are available.

***Key words**: Procambarus clarkii; Dreissena polymorpha; Physella acuta;* predation; prey choice; profitability

4.1 *INTRODUCTION*

Most predator species do not feed on prey in proportion to their abundance, but select for prey of particular sorts and sizes. Such selective predation pressure can have a profound effect on the structure of prey populations (Mascaró and Seed 2000; Rovero et al. 2000).

It is commonly hypothesized that the most consumed preys are the “preferred” by the predators (Jackson and Underwood 2007), but demonstrating a choice between, e.g, two prey species, requires that the predator exercise a non-random consumptive behaviour when simultaneously presented with individuals of the two types of prey. As the time necessary to find, consume, digest an item of type A is very probably different from those necessary for type B, predators usually eat different numbers of each type of prey in a given period, even if they are not exercising any preference (Underwood and Clarke 2005).

Understanding the behavioural mechanisms under the selection of prey by predators is therefore an essential component of unravelling the processes that rule predator-prey dynamics (Fryxell and Lundberg 1994). In particular, knowledge of predator-prey interactions involving alien invasive species has conservation and economic relevance because these species have the potential to interfere with ecosystem functions and human activities (Lodge and Shrader-Frechette 2003; Torres et al. 2012).

To better comprehend why predators prefer certain sizes of prey, prey-size-selection studies have frequently applied an optimality perspective (Charnov 1976). In 1966 MacArthur and Pianka proposed for the first time the optimal foraging theory, stating that an organism will show preference towards a prey that provides a high profitability. When selecting prey, predators should consider the costs (in terms of handling time) against the benefits (net energy gained) to maximize energy intake per unit handling time. This first version of the energy maximization theorem (Emlen 1966; MacArthur and Pianka 1966; Charnov 1976) is regularly used to predict optimal prey size for molluscivorus predators (Elner and Hughes 1978; Sanchez-Salazar et al. 1987; Mascaró and Seed 2000; Smallegange and Van Der Meer 2003; Smallegange et al. 2008) and prey profitability appears to be the most important factor affecting prey preference (Ebersole and Kennedy 1995).

Prejs et al. (1990) assumed that crushing resistance of the mollusc shell reflects the energy expended in crushing, i.e. as the shell strength increases, so does the energetic cost of crushing it. Therefore, for the evaluation of mollusc prey by predators, shell-crushing resistance can be also important, and shell size is expected to be a strong predictor of crushing resistance (Stein et al. 1984; Osenberg and Mittelbach 1989).

Invasive species exert several effects on native communities. In freshwaters, in particular, the extinction of indigenous species is becoming common as aggressive species invade degraded ecosystems (Richardson et al. 2000). The rate of invasion by freshwater alien species is increasing worldwide (Ricciardi 2006; Keller et al. 2009), with crustaceans and mollusks representing some of the most successful and high impact invaders (Van der Velde et al. 2000).

Alien crayfish have relevant ecological effects on native water bodies. Once established, these animals may reduce populations of favored food items such as gastropods, algae, and macrophytes, with possible cascading trophic effects in the entire ecosystem (Klocker and Strayer 2004).

Alien molluscs that suspension-feed on phytoplankton and seston, graze on periphyton, or browse on vascular plants are also noteworthy invaders. They can develop massive populations in freshwater bodies, consuming so much primary production that they substantially affect the amount and community composition of primary producers, negatively affecting every part of the ecosystem (Strayer 2010).

The red swamp crayfish *Procambarus clarkii* (Girard, 1852), native to the south-central United States (Louisiana) and north-eastern Mexico (Gherardi and Holdich 1999), is a highly diffused alien species with many established abundant populations in Italy (Aquiloni et al. 2010). This is a large, prolific, aggressive species and is well adapted to live in areas with drastic fluctuations of water level. It is widely recognized that its introduction yielded minimal economic benefits, having on the contrary negative consequences to the colonized habitats (Hobbs et al. 1989), where it caused biodiversity loss (Sala et al. 2000; Gherardi 2007a). Its feeding habits can lead to changes in food webs and even to the disappearance of some species, with potential heavy impacts on macroinvertebrates, macrophytes or amphibians (Cruz et al. 2006; Souty-Grosset et al. 2006; Gherardi 2007b; Dana and Ortega 2010).

Recently, populations of the zebra mussel, *Dreissena polymorpha* (Pallas, 1771), a well-known alien invader, considered one of the most invasive freshwater animals in the world (Casagrandi et al. 2007) have been found in Tuscany (Central Italy) (Lori and Cianfanelli 2006). The zebra mussel is a freshwater, filter-feeding bivalve, native to the rivers flowing into the Black and Caspian seas (Reynolds and Donohoe 2001; Karatayev et al. 2002). Its high reproductive output, filtering capability and seasonal production of free swimming veliger larvae have enabled this species to invade an unexploited niche in freshwater systems, ultimately causing severe ecological and economic damages around the world (Reynolds and Donohoe 2001), including the extirpation of native bivalves, decreased plankton biomass, increased water clarity and macrophyte biomass, and altered food web dynamics (MacIsaac 1996). Once established, zebra mussels is nearly impossible to eradicate. Monitoring this species is therefore important (Reynolds and Donohoe 2001), considering that *D. polymorpha* is continuously spreading in southwestern Europe (Lori and Cianfanelli 2006).

Many studies showed that molluscs are one of the favourite prey types of *P. clarkii* (Covish et al. 1980; Ilhéu and Bernardo 1993a; Montes et al. 1993) and laboratory studies showed that *P. clarkii* preferred these prey with respect to other food items (Gutiérrez-Yurrita 1997).

Several studies have investigated crayfish predation on zebra mussels (Piesik 1974; Love and Savino 1993; MacIsaac 1994; Martin and Corkum 1994; Perry et al. 1997; Schreiber et al. 1998; Reynolds and Donohoe 2001). Some authors have also shown that predation rates on zebra mussels decrease in the presence of an alternative prey species (Love and Savino 1993; MacIsaac 1994; Perry et al. 1997). Therefore, the impact of crayfish predation on zebra mussel populations in nature will depend on the relative preference for zebra mussels over other invertebrates, aquatic plants, detritus, and other food types (MacIssac1994).

Two unpublished works (Stevens 1992 in USA and Orioli 2012 in Italy) have already addressed *P. clarkii* predation in *D. poymorpha*, estimating the size of preferred mussels, the quantity of mussels consumed in a day (Stevens 1992) and the size of the more energetic and/ or preferred mussels (Orioli 2012). Orioli (2012) demonstrated the high ability of this species to prey on the smallest zebra mussel individuals that usually compose the major part of the druses (aggregates of living mussels (Botts et al. 1996)).

In this work, we attempted to estimate the zebra mussel size that is most profitable to *P. clarkii*. Furthermore, and in order to evaluate if prey profitability is an important driver of *P. clarkii*'s choice, we conducted experiments where crayfish were offered two different mollusc species, with different combinations of prey size, energy content and estimated profitability.

The gastropod *Physella acuta* (Draparnaud, 1805) was chosen as an alternative prey for this study because the ability of *P. clarkii* to capture and consume snails, and in particular this snail, is well documented (Renai and Gherardi 2004). This is one of the alien species found in the same habitats of both *D. polymorpha* and *P. clarkii* in Italy, like Lake Bilancino (Tuscany).

We tested the following hypotheses:

1) given the different types of shell and the different proportions of living matter consumable, we expected the energy content of these two groups to vary, as well as the easiness of breaking the shell;

2) given the differences between these two parameters (and handling) the optimum size of each prey (= sizes for which the energy obtained by unit of handling time is increased, i.e. the profitability of the prey is larger) must be different;

3) the profitability differences will be reflected in the choice of prey.

4.2 *MATERIALS AND METHODS*

4.2.1 *Prediction of optimal prey size*

We estimated the energy content, crushing resistance and theoretical profitability (benefit/cost) of the *D. polymorpha* and *P. acuta* individuals used on prey size selection experiments (see below), using shell length as estimator of body size. We also estimated the values for the maximum and minimum sizes of these prey in Tuscany (Cianfanelli 2009; Gonçalves *pers. com*; Orioli *pers. com*).

We followed Stein et al. (1984), Magoulick and Lewis (2002), Baumgartner and Rothhaupt (2003) to calculate the value of dry mass, and thus energy content for both species. The relationship between linear body dimensions and dry mass is usually described by the function:

$$\ln \mathrm{DM} = \ln a + b \ln \mathrm{L};$$

where DM is dry mass (mg), L is a linear body dimension (mm) and a and b are fitted regression constants (Burgherr and Meyer 1997). With this relationship and the regression constants for gastropods (ln a= -4.74; b= 3.21) and for *D. polymorpha* (ln a= -3.44;b= 2.30) determined by Baumgartner and Rothhaupt (2003), we calculated the dry mass of both preys. The linear body dimension used (L) was the shell length for both species; *D. polymorpha* was measured along its longest axis and *P. acuta* from the outer lip of aperture to apex, to the nearest 0.1 mm, with vernier callipers.

Magoulick and Lewis (2002) estimated *D. polymorpha* energy content using a Parr 1341 oxygen bomb calorimeter and standard methods (ASTM D240-87, Standard Test Method of Hydrocarbon Fuels) (Bryan et al. 1996 *in* Magoulick and Lewis 2002). Stein et al. (1984) measured energy per unit mass of snails across a range of snail sizes also through a calorimetric bomb. We derived the equation relating energy content (J) and shell length of zebra mussels considering the value of 20740 J/g of dry mass (Magoulick and Lewis 2002). To obtain the same equation for *P. acuta* we considered the value of 19770 J/g dry mass, determined by Stein et al. (1984) for the genus *Physella*.

To estimate the crushing resistance of the *D. polymorpha* and *P. acuta* shells, we also used already proposed equations. For *D. polymorpha* we followed Orioli (*unpublished data*: crushing resistance = $4.4546\ e^{0.1202 \text{ mussel length}}$), while for *P. acuta* Osenberg and Mittrebach (1989) (crushing resistance= 0.883 snail $\text{lenght}^{1.1}$). Orioli measured *D. polymorpha* crushing resistance with the texturometer, an instrument that applies a growing force on the sample up to cause it to break. Osenberg and Mittrebach (1989) measured snail shell strength by placing a snail on a small platform on the bottom of a plexiglass tube. A slightly smaller tube, also with a bottom, was placed inside the first and on top of the snail. Sand was slowly poured into the inner tube until the snail shell was crushed. The mass of the sand and tube was determined and converted to newtons. A wide range of snail sizes was used, and the relationship between crushing resistance and snail mass was estimated using an allometric relationship.

To calculate benefit/cost, energy values of both molluscs were divided by the estimates of shell crushing resistance.

4.2.2 *Experimental procedure*

4.2.2.1 Field collection and laboratory maintenance

The experiments were conducted in the laboratory at the Department of Biology (University of Florence), in July-August 2010, May-July 2011 and June-July 2013.

Individuals of *D. polymorpha* and of *P. acuta* were hand collected from the Lake Bilancino (Tuscany, Italy) (43°58'41''N, 11°16'54''E), 40 km from Florence (central Italy), while 350 individuals of *P. clarkii* were collected using baited traps from Lake Trasimeno (43°08′N 12°06′E) (Umbria, Italy).

Upon their arrival in the laboratory, crayfish were measured using a vernier calliper (cephalothorax length without rostrum, CL, averaging 35.06 mm ± 5.35; n=85 **- *prey size selection experiment***; 34.99 mm ± 3.31; n=64 – ***prey choice experiment***). Mature males were identified by the presence of prominent copulatory hooks and cornified gonopodia (Huner 2002), and possible mutilations were recorded. Only intermolt animals with intact chelae and walking legs were used in the experiments. Crayfish were maintained in individual plastic tanks (26.5x16.2x15 cm) containing 1 L of well water at a temperature ranging 14-25 °C (18.72±3.49 S.D.), under natural light/dark cycle regime, and fed *ad libitum* with larvae of *Caliphora* sp. Water was changed once per week. Prior to the experiments, crayfish were starved for 7 days. To avoid pseudo replications each crayfish was used only once in the following experiments.

4.2.2.2 Prey size selection

Experiments were conducted in glass aquaria (45x25x27 cm) containing 5 L of well water at the same conditions of maintenance aquaria. Initially the aquarium was divided in two sides by a removable, opaque plastic divider. Each test crayfish was introduced into one side, and one individual of either *D. polymorpha* or *P. acuta* was inserted into the other side.

After 15 min of acclimation, the divider was lifted and the observations started.

From previous observations of crayfish detaching mussels from druses, the adherence strength appeared to be variable and not directly correlated with the size of the mussel (Gonçalves *unpublished*). For this reason and because of practical problems in presenting mussels attached to substrate, detached mussels were measured and presented one by one.

Each trial was videotaped for one hour and analysed by an experienced observer; for each trial, we measured the time required by the crayfish to open the mollusc (breaking time) and to eat it (eating time) and the handling time (Elner and Hughes 1978; Hughes and Seed 1981; Eggleston 1990; Rovero et al. 2000; Mascaró and Seed 2001; Mistri 2004). Handling time included both the time crayfish spent breaking the shell and consuming mollusc tissue (Brown and Haight 1992).

The tested *D. polymorpha* belonged to one of three size classes of shell length*:* *small* (5-10 mm), *medium* (10-15 mm), and *large* (15-20 mm). The tested *P. acuta* belonged to one of two size classes of shell length: *small* (5-10 mm) and *medium* (10-15 mm). The sizes of *P. acuta* and *D. polymorhpa* used followed the size distributions of the species population in the field (table Annex 4.1). A total of 30 replicates were run for each combination crayfish-prey size class.

We only analysed the observations from the crayfish that handled the offered mollusc (i.e. the number of active encounters (Eggleston 1990)). We also calculated the attack success as the proportion of active encounters resulting in consumption ($E_{s/}E_t$), where E_s = successful encounter rate, the total number of encounters that resulted in consumption; E_t = number of active encounters (Eggleston 1990).

4.2.2.3 Prey Profitability

Profitability for both prey species was determined following Elner and Hughes (1978) and expressed as the net energy intake (calorific value of the prey item) per unit handling time (Dudas et al. 2005). Both linear and non-linear functions were tested for the relationship between prey length and profitability. The most reliable model was inferred by comparing the adjusted R-squared values.

4.2.2.4 Prey choice

The experiment was conducted in plastic aquaria (31 cm diameter) containing 2 L of well water. Water temperature varied between 21- 28ºC (21.9±2.71).

Each trial started by inserting one individual of *D. polymorpha* and one individual of *P. acuta* into the experimental aquarium after having acclimatised a crayfish for 15 min. Each prey was previously measured.

Preys were presented to crayfish in five size combinations, according to the suspected optimal sizes - those where prey profitability was estimated to be the highest for each species: *P. acuta* optimal size and very small *D. polymorpha*, clearly below their optimal size; *P. acuta* optimal size and approximately the approximate same size for *D. polymorpha*; both *P. acuta* and *D. polymorpha* optimal sizes; *D. polymorpha* optimal size and P. acuta above its optimal size; both prey smaller than P. acuta optimal size. Furthermore, some of these combinations included prey of very similar sizes.

A total of 64 replicates were conducted. Each trial was videotaped for one hour and analysed by an experienced observer, identifying the consumed prey.

4.2.2.5 Statistical analyses

We analyzed the existence of relations between several pairs of variables (see below) with Spearman correlation test.

The outcome of each interaction between crayfish and different-sized prey of each species was analyzed with a logistic regression, using prey consumption as the dependent variable and crayfish sex, crayfish size, handling time and prey size as independent variables.

To analyze prey choice in the prey choice experiment we calculated all the differences (in size, energy, profitability and crushing) as the value of *P. acuta* minus the value of *D. polymorpha*; therefore a positive value of the difference means that the values of *P. acuta* were higher and a negative value means that the values of *D. polymorpha* were higher. Then we first tested the correlations between the following variables: *D. polymorpha* size; *P. acuta* size; crayfish size; crayfish sex; *D. polymorpha* energy; *D. polymorpha* crushing resistance; *D. polymorpha* profitability; *P. acuta* energy; *P. acuta* crushing resistance; *P. acuta* profitability;

size difference, energy difference; profitability difference; crushing resistance difference. One variable from each pair of variables significantly correlated was eliminated.

To analyze prey choice, we firstly used a logistic regression, using the consumption of either species as a binary variable and the remaining variables (*D. polymorpha* size; *P. acuta* size; profitability difference; crayfish size; crayfish sex) as independent variables.

Then, also due to the small number of variables, we tested all the possible combinations of models and model selection was based on AIC values (Akaike's information criterion; $AIC = -\ln L + p$, where L is the likelihood for an estimated model with p parameters; ΔAIC—difference to the lowest AIC; *wi*, or AIC weight—the model's probability of being the best model for the data). In situations where different but closely related models had equivalent explanatory power ($\Delta AIC < 2$), we performed a model averaging procedure (using the dredge function of the "MuMIn" R package), which provides for a relatively more stabilized inference (Burnham and Anderson 2002).

All statistical analyses were performed with SPSS Statistics 20, except for the model selection routine and model averaging procedures, calculated with R (version 2.13.0).

4.3 *RESULTS*

4.3.1 *Prediction of optimal prey size*

The theoretical equations indicated that the energy content of both preys (*D. polymorpha* - energy content= 0.665 mussel length $^{2.30}$; *P. acuta* - energy content= 0.173 snail length$^{3.21}$) is quite different within the range of presented prey, and that, for the same size, *P. acuta* is always a more energetic prey (fig. 4.1).

Fig 4.2 shows the estimated crushing resistance for the different-size individuals of both species. For both species, crushing resistance increased with shell length, but for the same size it is more difficult to crush zebra mussels, and this difference is especially strong for the large sizes. For a range of sizes (7-11 mm) the difference between the values of crushing resistance of both species is minimal.

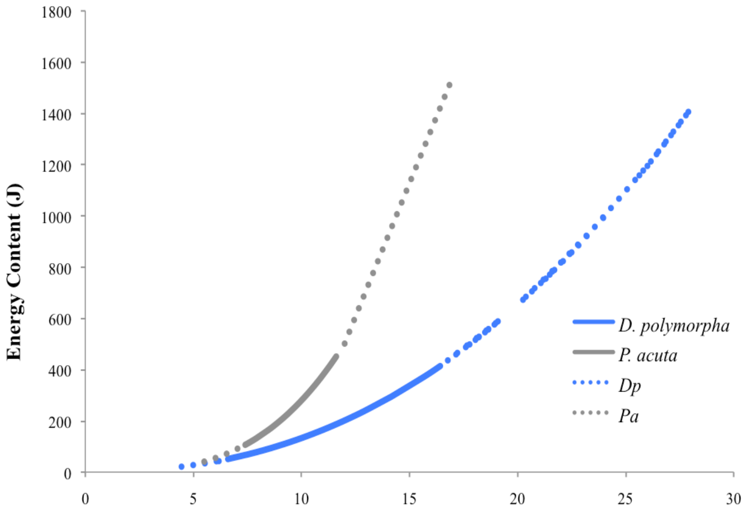


Figure 4.1. Energy content for both preys.
Energy content of *D. polymorpha* (blue filled line), estimated from Magoulick and Lewis (2002), and *P. acuta* (grey filled line), estimated from Stein et al. (1984). The dashed lines (*Dp* – *D. polymorpha*; *Pa* – *P. acuta*) represent the sizes used in other works, but not found in our study area.

The best equations relating benefit/cost for both preys were as follows: *D. polymorpha* ($\text{benefit/cost}=0.149\text{mussel length}^{2.30}/e^{0.1201\ \text{mussel length}}$) and *P. acuta* ($\text{benefit/cost}=0.195\text{snail length}^{2.06}$). The graphical representation of these equations (fig. 4.3) shows that this ratio is always higher for the snail. For *P. acuta*, the cost values (crushing) are similar to those of *D. polymorpha*, but there are large differences in the energy content, the snail being always more energetic.

In accordance with these equations, there is a theoretical optimum size only for the mussel (19.01 mm), but not for the snail, with a disproportionate increase in energy gained per unit increase of crushing resistance. This is the result of an exponential increase in prey energy with shell size (fig. 4.1), coupled with a simple linear increase in crushing resistance with shell size (fig. 4.2). Theoretically, *Physella* should thus be a more beneficial prey.

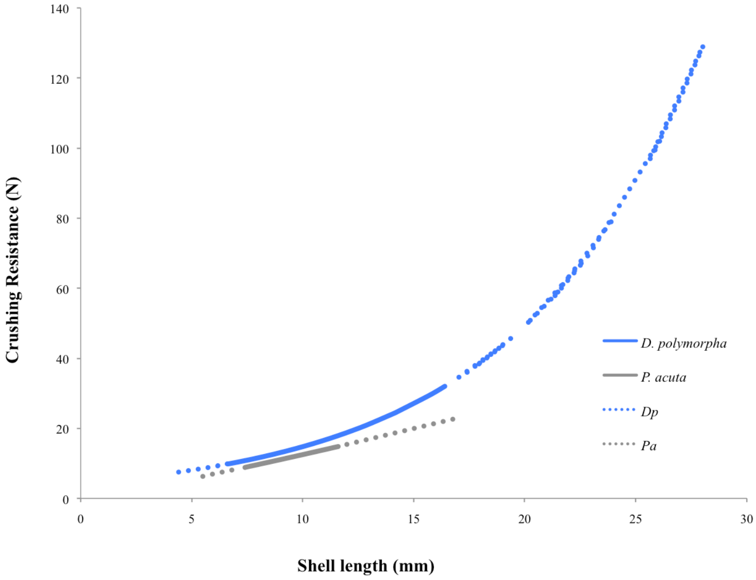


Figure 4.2. Estimated crushing resistance.
Crushing resistance of *D. polymorpha* (blue filled line), estimated by Orioli results (*unpublished data*), and *P. acuta* (grey filed line)**,** estimated from Osenberg and Mitterbach (1989). The dashed lines (*Dp – D. polymorpha*; *Pa – P. acuta*) represent the sizes used in other works, but not found in our study area.

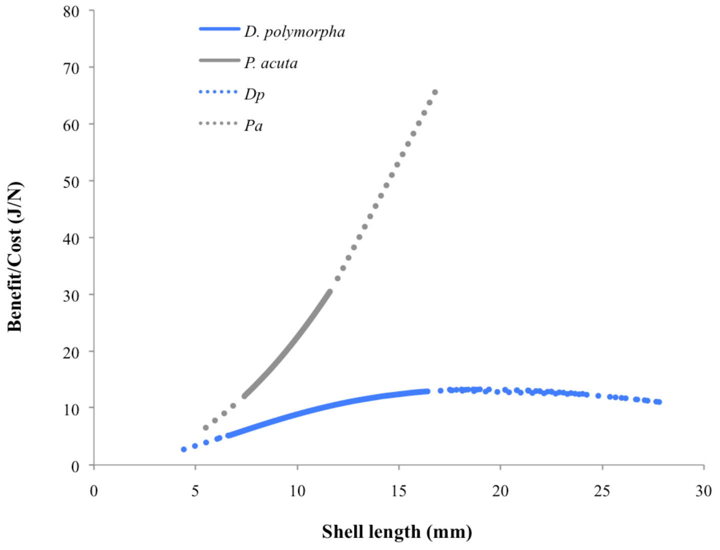


Figure 4.3. Benefit/cost (J/N) estimated for the consumed snails (grey filled line) and consumed mussels (blue filled line), as well as sizes not tested in this work (dashed lines; *Dp* – *D. polymorpha*; *Pa* – *P. acuta*).

4.3.2 *Experimental procedure*

4.3.2.1 Prey size selection

For *D. polymorpha*, prey selection was clearly dependent on prey size, handling time and crayfish size (p<0.05; table 4.1 and fig. 4.6), with no influence of crayfish sex (p>0.05; table 4.1). Consumption of *P. acuta* was also dependent on prey size and handling time. There was also a significant relation with crayfish size, but in this case the largest the crayfish, the less probable it would consume *P. acuta* (table 4.2).

The observed behaviours (breaking, eating and handling time) varied according to prey species (table 4.3) and prey size. Handling time ranged between 71-2319 seconds, for *D. polymorpha*, and 24-

2316 seconds for *P. acuta,* considered only the molluscs that were eaten.

Both prey exhibit a positive correlation between the size (*L*) and breaking time (*b*) – *D. polymorpha* (Spearman's rho= 0.393; *p*=0.011; n=41); *P. acuta* (Spearman's rho= 0.427; *p*=0.005; n=41) (fig. 4.4).

Table 4.1. Results of the logistic regression explaining *D. polymorpha* selection. Signal of the logistic regression coefficients (B) indicates the effect of each variable in the consumption of the species. S.E. – standard error of the estimate.

Variables	**B**	**S.E.**	**Wald**	**df**	**p**	**Exp(B)**
Crayfish size	1.778	0.882	4.063	1	0.044	5.917
Crayfish sex	-0.524	0.808	0.420	1	0.517	0.592
***D. polymorpha* size**	-5.382	1.459	13.603	1	0.000	0.005
Handling time	0.006	0.002	14.155	1	0.000	1.006
Constant	-1.433	2.842	0.254	1	0.614	0.239

Table 4.2. Results of the logistic regression explaining *P. acuta* selection. Signal of the logistic regression coefficients (B) indicates the effect of each variable in the consumption of the species. S.E. – standard error of the estimate.

Variables	B	S.E.	Wald	df	p	Exp(B)
Crayfish size	-2.928	1.195	6.004	1	0.014	0.053
Crayfish sex	0.376	0.859	0.192	1	0.662	1.456
***P. acuta* size**	-1.444	0.437	10.935	1	0.001	0.236
Handling time	0.003	0.001	6.450	1	0.011	1.003
Constant	23.318	6.877	11.497	1	0.001	$1.3E^{+10}$

Table 4.3. Descriptive statistics for the observed behaviours.

	Breaking time (s)	**Eating time (s)**	**Handling time (s)**
	mean± std. error	mean± std. error	mean± std. error
Dreissena polymorpha (n=43)	286.21±42.21	358.23±59.11	653.48±84.82
Physella acuta (n=42)	170.21±44.61	291.43±32.73	470.97±62.46

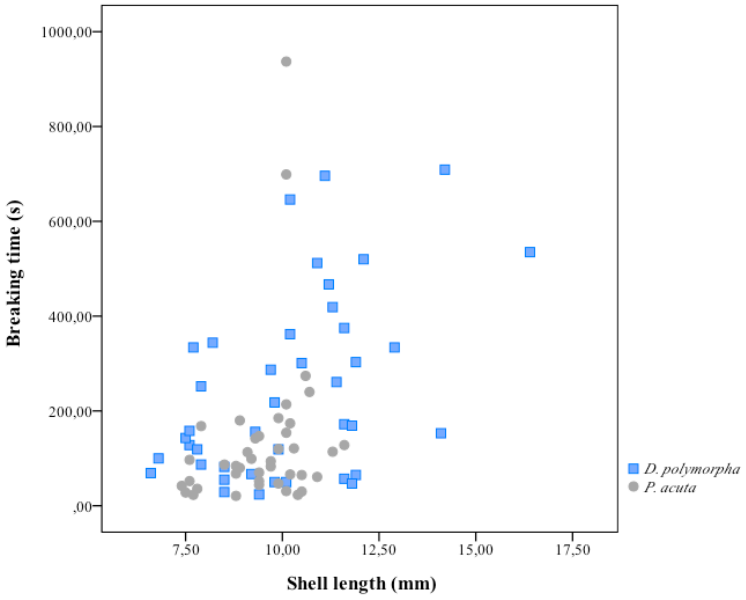


Figure 4.4. Breaking time for both preys: *D. polymorpha* (n=43) and *P. acuta* (n=42).

The length of the predator was negatively correlated with the breaking time for mussels (Spearman's rho= -0.370; *p*=0.017; n=41), but not with the breaking time for snails (Spearman's rho=-0.210; *p*=0.188; n=41).

There was a positive correlation between eating time and size of *D. polymorpha* (Spearman's rho= 0.357; *p*=0.02; n=42) but no correlation for *P. acuta* (Spearman's rho= 0.289; *p*=0.063; n=42).

The eating time presented for both preys was not related with the length of the predator - *D. polymorpha* (Spearman's rho= -0.206; *p*=0.190; n=42) and *P. acuta* (Spearman's rho= - 0.171; *p*=0.278; n=42) (fig.4.5).

When considering all prey that were handled, eaten and not eaten, there was a negative correlation between handling time (*h*) and *D. polymorpha* length (*L*) (Spearman's rho= -0.288; *p*=0.007; n=88) (fig.4.6a). When considering all snails that were handled (n=60) there was no correlation between the length of prey and handling time (Spearman's rho= 0.056; *p*=0.671) (fig. 4.6b).

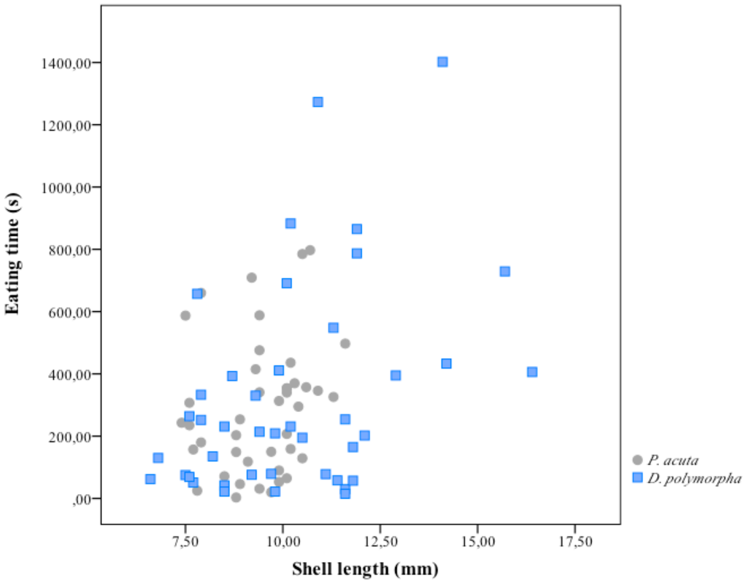


Figure 4.5. Eating time for both preys *D. polymorpha* (n=43) and *P. acuta* (n=42).

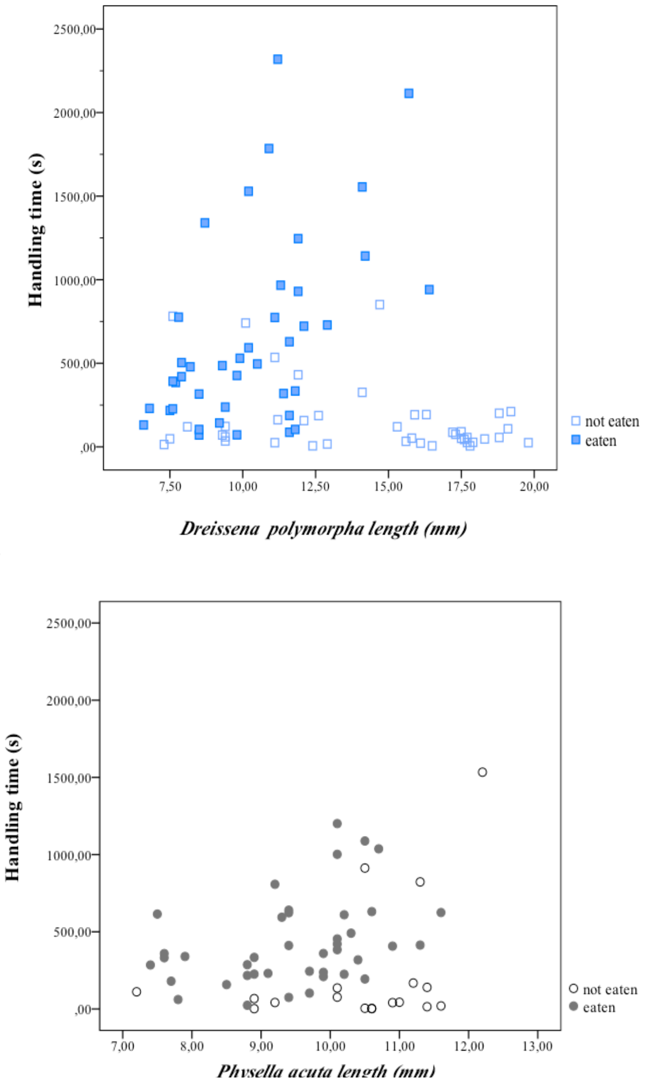
Considering only the eaten preys, there was a positive correlation between the length (*L*) of both preys and handling time (*h*) – *D. polymorpha* (Spearman's rho=0.483; *p*=0.001; *n*=42) and *P. acuta* (Spearman's rho= 0.427; *p*=0.005; n=41). For both species, larger prey required more time.

The length of crayfish was negatively correlated with snail handling time (Spearman's rho= -0.33; *p*=0.035; n=41), but not with mussel handling time (Spearman's rho=- 0.286; *p*=0.066; n=42).

The ratio between prey and predator sizes was positively correlated with handling time for both prey (fig. 4.7) - *D. polymorpha* (Spearman's rho=0.583; *p*<0.01; *n*=42) and *P. acuta* (Spearman's rho= 0.435; *p*=0.004; n=41).

4.3.2.2 Prey Profitability

Prey profitability (*Profit*) varied substantially with prey length (L) (fig. 4.8). The polynomial equations describing prey profitability had a poor fit for *D. polymorpha* ($Profit = -1.269+0.310L-0.013L^2$; $R^2=0.041$; $F=0.856$; $p=0.433$) and for *P. acuta* ($Profit = -14.162+3.323L-0.18L^2$; $R^2=0.036$; $F=0.718$; $p=0.494$). With this non-linear regression model we predicted the prey size with the highest profitability for mussels of 11.9 mm, and for snails of 9.2 mm. Snails should be more profitable than mussels in the size range 6.9-11.0 mm (fig. 4.8), but for larger sizes (>11.0 mm) the mussel should be more profitable. Despite the paucity of data, *D. polymorpha* may also be more profitable than *P. acuta* for very small sizes (<6.9 mm).



(a)

(b)

Figure 4.6. Handling time in successful (eaten) and unsuccessful encounters (not eaten), for both preys ((a) *D. polymorpha* n=88; (b) *P. acuta* n=59).

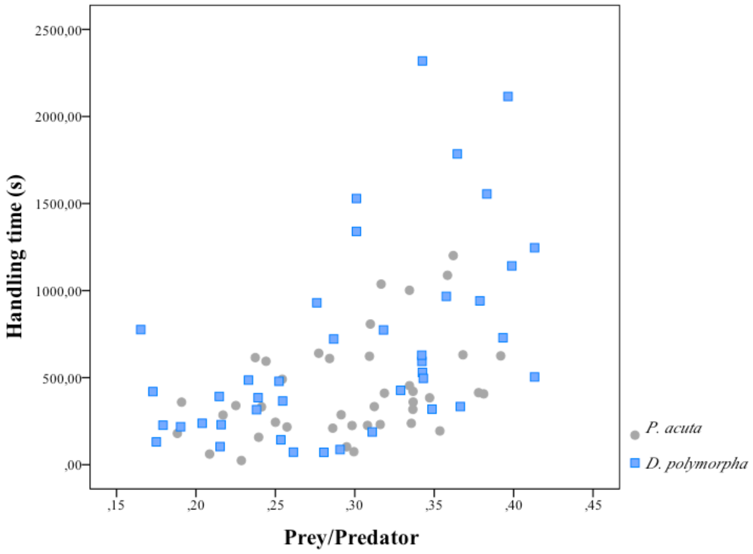


Figure 4.7. Handling time in successful encounters (eaten), depending on the relative size of prey and predator.

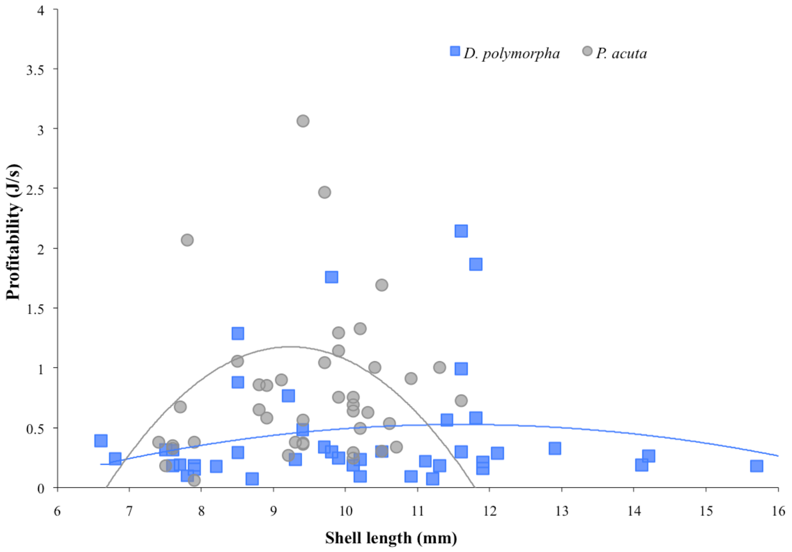


Figure 4.8. Profitability (J/s) curves for the snails (n=42) and mussels (n=43). Non-linear regression models (second-order polynomial) are plotted for both species.

4.3.2.3 Prey choice

The logistic regression indicated that *D. polymorpha* size, *P. acuta* size and the difference in profitability between the offered individuals were all relevant for the choice of a species over the other by *P. clarkii* (table 4.4).

The results of the model selection procedure indicated that four models had a delta AIC <2, preventing a clear discrimination of the best model (table 4.5). Model averaging indicated first *P. acuta* size, and then, with a similar importance, *D. polymorpha* size and difference in profitability as the variables that best explained prey choice (table 4.6) Relative variable importance was as follows: *P. acuta* size (1); profitability difference (0.73); *D. polymorpha* size (0.73); crayfish size (0.18); crayfish sex (0.15).

Analyzing graphically, the results of prey choice (fig. 4.9) for all prey size combinations, there was a preference for *D. polymorpha*, whenever its size was similar its predicted optimum of profitability, as well as in the combinations where the snail was at its optimum and *D. polymorpa* was in a slightly smaller size than its optimum. The choice was very similar when the size of both prey were similar to the optimal dimensions of *P. acuta*. There is a slight preference for *P. acuta* in the combinations where both prey were small, with shell sizes far below their optimum.

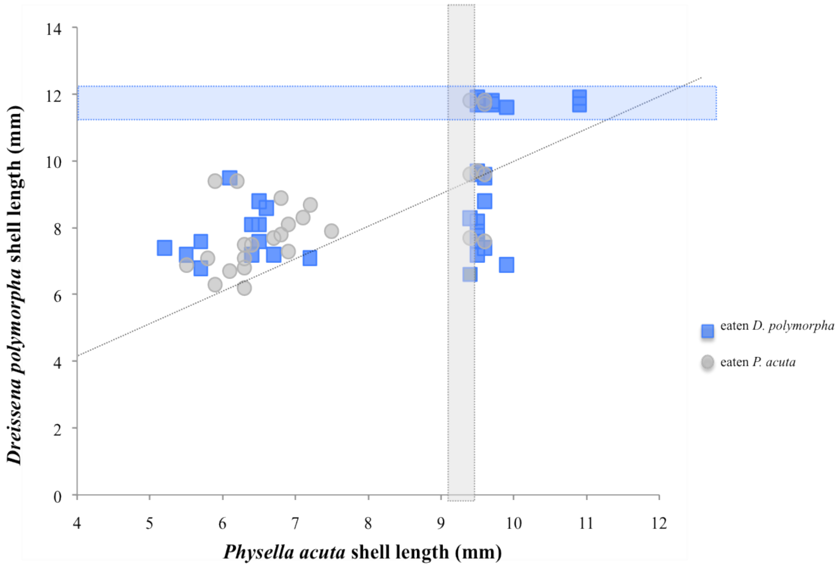


Figure 4.9. *Prey choice* - Outcome of each prey choice experiment, representing different size combinations. The gray bar represents the sizes of *P. acuta* with the estimated highest profitability and the blue bar represents the sizes of *D. polymorpha* with the estimated highest profitability. The diagonal line indictes same sized individuals (above it the *D. polymorpha* offered were larger than the *P. acuta*, while the area below the line corresponds to the opposite pattern).

Table 4.4. Results of the logistic regression explaining prey choice.

	ß	*Std. Error*	*z value*	*Pr (<|z|)*
(Intercept)	12.9659	6.4653	2.005	0.0449
Crayfish sex	-0.3197	0.6230	-0.513	0.6079
Crayfish size	-0.5724	0.7661	-0.747	0.4550
***D. polymorpha* size**	1.0950	0.5320	2.058	0.0396
***P. acuta* size**	-2.3713	0.9744	-2.434	0.0150
Profitability difference	6.1759	2.8359	2.178	0.0294

Table 4.5. Results of the model selection procedure. See "statistical analyses" for the explanation of the headings of the last four columns.

Model Rank	**(Intercept)**	**Profitability difference**	***D. polymorpha size***	***Crayfish sex***	***Crayfish size***	***P. acuta size***	***df***	***logLik***	***AICc***	***delta***	***weight***
1	8.0110	4.9770	0.907100	-	-	-1.9030	4	-38.443	85.6	0.00	0.189
2	2.6240	-	-	-	-	-0.3620	2	-41.061	86.3	0.76	0.130
3	12.800	6.2780	1.13800	-	-0.6581	-2.4090	5	-38.042	87.1	1.55	0.087
4	9.0840	5.0870	0.895100	-0.42430	-	-1.9490	5	-38.197	87.4	1.87	0.074

Table 4.6. Results of the model averaging procedure (first four models). See "statistical analyses" for more details.

	Estimate	***Std. Error***	***Adjusted SE***	***z value***	***Pr (<|z|)***
(Intercept)	7.5895	5.0860	5.1430	1.476	0.1400
Profitability difference	5.3229	2.5307	2.5808	2.062	0.0392*
***D. polymorpha* size**	0.9619	0.4796	0.4892	1.966	0.0492*
***P. acuta* size**	-1.5857	1.0380	1.0478	1.513	0.1302
Crayfish size	-0.6581	0.7494	0.7651	0.860	0.3897
Crayfish sex	-0.4243	0.6110	0.6238	0.680	0.4963

4.4 DISCUSSION

This study showed that two different approaches to estimate prey profitability were not useful for the correct prediction of prey choice by *P. clarkii*. An approach based on the relations already published among shell size, energy content and shell crushing resistance for the same species (*D. polymorpha*) or very similar ones (*P. acuta*) lead to a prediction for snail choice whichever the prey combination provided. The same prediction (but with important differences in the details) was obtained by balancing energy gain with handling time, measured in laboratory. However, in the prey choice experiments, there was a tendency for the consumption of *D. polymorpha*, especially when the crayfish chose between relatively large *P. acuta* (close or even at their estimated optimal size) and comparable sizes of the mussel. In fact, the overall tendency was the choice of the smallest prey, regardless of its species. Crayfish sex and size were less important for prey choice; however, the largest crayfish (and especially females) tended to choose the mussel over the snail, which can be related with a higher ability of large crayfish to crush the shell of large mussels.

Our study also confirms that the invasive zebra mussel, *D. polymorpha*, represents a novel prey resource for *P. clarkii*, even in the presence of an alternative prey. *D. polymorpha* may be a preferred prey of this crayfish, especially the juvenile stages, or small individuals.

Our results suggest that *P. clarkii* exhibits size-selective predation, as consumption levels decreased with increasing prey size. Laboratory trials indicated that other crayfishes (*Orconectes propinquus* and *O. limosus*) prefer small zebra mussels, with a positive correlation between predator and prey sizes (MacIsaac 1994; Pisek 1974). This pattern is common among decapod predators (Juanes 1992). There are many references reporting a selection of prey of highest profitability by crustaceans (crabs). This has been demonstrated for *Carcinus maenas* (Elner and Hughes 1918), *C. sapidus* (Hamilton I976) and *Ovalipes truncatus* (Chilton and Bull 1986). However, many other decapods, including *Cancer pagurus* (Lawton and Hughes 1985), *Ovalipes catharus* (Davidson 1986) and *Scylla sewata* (Hill 1979; Hughes and Seed 1981) tend not to select optimal-sized prey, but rather choose smaller "suboptimal" prey sizes. Other studies have shown that many crab species do not select the predicted optimal prey size, but rather choose prey which minimize the amount of time spent foraging (ap Rheinallt 1986; Davidson 1986; Blundon and Kennedy 1982; Lawton and Hughes 1985), which is normally explained by the higher vulnerability to predation of foraging animals.

Nyström (2002) stated that crayfish selective consumption of some macroinvertebrates seems to be related to handling times, encounter rates and to behavioural traits of the macroinvertebrates rather than to differences in their energetic content. Like crabs, crayfish may also prefer prey that require low handling times (Love and Savino 1993). For example, Alexander and Covich (1991) showed that the ingestion of snails (*Physella virgata*) by the crayfish *Procambarus simulans* decreased with increasing handling time, and Gherardi and Barbaresi (2007) showed that *P. clarkii* preferred *Urtica* sp. to earthworms, notwithstanding the higher nutritional value and assimilation efficiency of the latter, suggesting that feeding preferences of this species often depend on factors other than nutritional value and digestibility.

However, our results also show that when the zebra mussels provided are close to the theoretical optimum size, the crayfish tend to choose them over *P. acuta*, this being the only occasion when a preference for the largest prey is apparent (unfortunately the sample size is relatively small).

Theory predicts that the optimal diet depends only on the presence of high profitability prey (Hughes 1997). Several studies, but not our own, confirmed this theory, pinpointing that predator prefer prey sizes that are optimal, i.e. most profitable in terms of energy gained per unit handling time (Elner and Hughes 1978; Hughes and Seed 1995). Yet other research showed that predators preferably forage on prey of smaller sizes that are less profitable (Juanes 1992; Smallegange and Van Der Meer 2003), suggesting that other criteria may be adopted to select prey. One reason for this apparent selection of small (below optimal) sizes in crustacean predators is to minimise time spent feeding, which can also be an adaptation to reduce exposure times to predators and to other crayfish (Nyström and Pérez 1998). For crabs, it has been suggested that when the risk of predation or prey-robbing by conspecifics is high, minimising prey handling times may be more important than maximising the net rate of energy intake (Hughes and Seed 1981; Creswell and McLay 1990).

In preference experiments, *P. clarkii* prefered animal food to macrophytes (Ilhéu and Bernardo 1993b), whereas in the field, it mainly feeds on plant material and detritus (Feminella and Resh 1989; Gutiérrez-Yurrita et al. 1998). Preference for animal food was observed when aquatic macro-invertebrates (Odonata and Ephemeroptera larvae and Planorbidae snails) with weak escape reactions, or fish corpses were provided. The choice of feeding strategy may be interpreted in terms of cost-benefit analysis. In spite of the actual preference for animal food, the high costs involved in active predation may explain crayfish phyto/detritophagous feeding in most situations (Ilhéu and Bernardo 1993a).

Alcorlo et al. (2004) demonstrated that *P. clarkii* selected its animal prey - the absence of profitable prey in rice fields, like mayflies or water bugs, lead to increased selection of chironomid larvae. In addition, items rejected when prey diversity is high (ostracodes) were positively selected in habitats with poor prey diversity. This is an indication that this species has to meet part of its energetic demands by ingesting animal food and that it is doing so by preying on the most profitable prey items available (Alcorlo et al. 2004).

In experiments performed to determine the consumption and the behavioral responses of preys (Correia et al. 2005), the presence of *P. clarkii* significantly affected the abundance of *Chironomus riparius* and *P. acuta*, but not of *Corbicula fluminea* whether prey species were provided singly or simultaneously. The highest consumption of *C. riparius* was probably related to its sedentary habitats, whereas *P. acuta* avoided crayfish by crawling to the water surface. Failure in the consumption of the bivalve *C. fluminea* was probably due to the no co-evolution of *C. fluminea* and *P. clarkii* in its area of origin and to their recent co-occurrence in Europe. It is possible that *P. clarkii* populations have not yet developed the ability to prey *C. fluminea*.

Crehuet et al. (2007) studied the feeding preference of *P. clarkii* between chironomid larvae (Diptera, Chironomidae) and snails (*Physella acuta*) using juvenile crayfish. Even if there is a clear preference for chironomid larvae, their consumption decreases in the presence of snails. Crayfish can efficiently capture and feed both preys, but it maybe has a preference for gastropods only as secondary food source. Crehuet (2007) hypothesized that, in the presence of other preys, gastropods might be exploited not for metabolic energy, but mainly as sources of minerals (e.g. calcium carbonate) useful for crayfish growth.

In the wild, the spatial distribution of small mussels would be expected to influence the feeding pattern of the crayfish by adding a cost associated with the time required to find small mussels and avoid predators (Martin and Corkum 1994).

Zebra mussels at high density often form druses (a dense mass of mussels with intertwined byssus). The formation of a druse may impact crayfish size selectivity and feeding rate, because mussels are strongly attached within the three-dimensional structure of a druse and, therefore, potentially less accessible (Ermgassen and Aldridge 2011).

Furthermore, a druse will simply increase the handling time as the crayfish must initially handle the druse to remove individuals (and later handle those individuals). In laboratory observations, small druses (7.16±1.18 g) were handled on average for 810.88 s (±738.36) to be separated by zebra mussels, that then started manipulating the individual (Gonçalves et al. *unpublished*).

However, *D. polymorpha* juveniles (5-10 mm), that have a higher ability to detach their byssus from the druse, move using their foot, and find a new substrate (Toomey et al. 2002), are also more vulnerable to predation. Moreover, young zebra mussels are mostly in the periphery of the druse (*pers. obs.*), and consequently also more likely to be preyed.

The predation on the early life stages of zebra mussels should be higher than on adults because of reduced handling time (MacIsaac 1994; Martin and Corkum 1994; this study). MacIsaac (1994) found that crayfish (*Orconectes propinquus*) fed on zebra mussels of a range of sizes (3-14 mm), with a preference for mussels in the 3 to 5 mm size range. This size selective predation by *O. propinquus* on zebra mussel suggests that crayfish can alter the size structure of zebra mussel populations by preferring mussels ≤ 8mm, but discarding mussels larger than 20 mm (Martin and Corkum 1994).

The impacts of *P. clarkii* on *D. polymorpha* could thus be more relevant in places or during the periods when the juvenile stages of zebra mussels are most abundant.

Perry et al. (2000) had already suggested the potential impacts of predation on recruitment or on the early zebra mussel stages - at the beginning of an invasion the average zebra mussel size is small, and therefore more vulnerable to crayfish predation, thus leading to hypothesize that predation will have a significant impact on mussel recruitment.

From our study we can also state that zebra mussel will be a preferred prey by *P. clarkii* when alternative prey are scarce and when it is present in the juvenile phase. In the Italian ecosystems, this juvenile stage predominates between July-September (Annoni et al. 1978; Lancioni and Gaino 2006). During this period, *D. polymorpha* populations will be most vulnerable to predation by crayfish.

P. clarkii predation may not significantly reduce all the zebra mussel populations; however our results indicate that selective predation could potentially hamper initial zebra mussel colonisation. Although it is unlikely that crayfish will eliminate zebra mussels, it is possible that predation by crayfish may reduce the impact of zebra mussels on these systems.

Despite the many advantages of using laboratory experiments to assess predator–prey interactions, it is important to recognize potential limitations associated with them. For example, Dickey and McCarthy (2007) recognized that predator–prey interactions between crayfish and snails are dependent on the size of the experimental arena. In our study, the time spent by crayfish in searching for food was minimal, due to the size of the aquarium. Encounter rate and search time were not incorporated into this study, zebra mussel and snail being equally accessible to the crayfish. Factors affecting the selection of preys in the natural environment are much more complex than those of simple experiments.

Quantifying the ecological impacts of invasive species is a complex and challenging endeavor (Parker et al. 1999; Strayer et al. 2006) that becomes even more complex when studying the interaction between two invasive species. Future research should focus on rates of foraging of crayfish on *D. polymorpha*, and other available prey and/or vegetable material under field conditions.

4.5 *Acknowledgments*

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ANNEX 4.1

Table A4.1. Descriptive statistics of samples of *D. polymorpha* and *P. acuta,* from Lake Bilancino (Tuscany, Italy), representative of the animals that were provided as prey in the experiments.

Shell length (mm)	***Physella acuta*** (n=160)	***Dreissena polymorpha*** (n=246)
Mean	8.28	12.66
Std. Error of Mean	0.13	0.29
Median	8.25	11.8
Mode	7.40	8.50
Std. Deviation	1.68	4.5
Variance	2.8	20.3
Minimum	4.1	4.2
Maximum	12.2	27.1

CHAPTER 5

MODELLING THE PREDATION EFFECTS OF INVASIVE CRAYFISH (*PROCAMBARUS CLARKII*) ON INVASIVE ZEBRA MUSSELS (*DREISSENA POLYMORPHA*)

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(In preparation)

Abstract

The functional response of a predator is a key factor regulating the population dynamics of predator-prey systems. The goals of this study were to identify the consumption rates of *Dreissena polymorpha* juvenile stages by *Procambarus clarkii*, to determine the predator functional response, to evaluate the effects of predator satiation on the intensity of predation, and to model the impacts of predation by this crayfish on zebra mussel populations.

When presented with a range of mussel abundances, *P. clarkii* showed a functional response f the type II, where the mortality of prey increases with decreasing prey abundance. *P. clarkii* also exhibited a satiation effect, diminishing its consumption rate from 33 mussels /day to 6 mussels /day over a 6-day period.

The combined effect of the functional response and predator satiation allowed the production of a model that predicts the complete consumption of local populations of up to 150/160 juveniles by one single crayfish over the period of one month. This impact may be important in low-density or at equilibrium populations of *D. polymorpha*, and will be greater the larger the synchronization between the prey reproductive cycle and the activity period of the predator.

Keywords: functional response; predator-prey dynamics; *Dreissena polymorpha, Procambarus clarkii;* satiation

5.1 *INTRODUCTION*

Predation can exert a powerful influence in the abundance and population structure of prey (Sih et al. 1985; Hastings 1997). Frequently prey are most vulnerable during specific life stages (e.g., the juvenile or larval period), and the ability of a predator to effectively control their populations is dependent of the predation efficacy upon this stages.

However, predation may be circumvented if prey grow up to a size that protects the individuals from, e. g., gape-limited predators (Nilsson et al. 2003; Urban 2007).

The functional response of a predator is a key factor regulating the population dynamics of predator-prey systems. It describes the rate at which a predator kills its prey at different prey abundances and its estimation can thus help to determine the predator efficiency in regulating prey populations (Murdoch and Oaten 1975). The functional response is an essential component of predator-prey models (Oaten and Murdoch, 1975; Jeschke et al. 2002), but its estimation is rarely considered in an invasion context (Hooff and Bollens 2004; Radford et al. 2007), which is surprising, given the known impacts of introduced predators (Zaret and Paine 1973; Dick et al. 2002; Wanless et al. 2007). For instance, and assuming that a native predator abundance is independent of nonnative prey abundance during the stage of prey establishment, the predator's functional response can determine whether invasive prey are excluded from establishing, become established but do not proliferate and spread, or become over abundant and spread to new locations (Murdoch and Oaten 1975; Gascoigne and Lipcius 2004).

The shape of the functional response curve is dependent on several characteristics such as the encounter rate between prey and predator, capture efficiency and handling time (Holling 1965; Hassels 1978). These characteristics may differ within a species as a consequence of predator or prey size, interference among predators or presence of alternative prey (Spitze 1985; Chesson 1989; Safina 1990; Tripet and Perrin 1994; Elliott 2003).

In a basic type I functional response, prey consumption increases linearly with increasing prey abundance. In the type II response, the risk of mortality increases with decreasing prey abundance, whereas the type III response results from decreased predator efficiency in the capture of prey as prey abundance decreases. Consequently, while a type II response can be destabilizing, a type III response stabilizes predator-prey dynamics (Lipcius and Hines 1986; Eggleston et al. 1992). The distinction between Type II and Type III functional responses, and hence whether predators will likely de-stabilize or stabilize prey populations, relies on examining prey consumption rates at low prey abundances (Juliano 2001).

Limitation of predation rate at high prey abundances has usually been attributed either to the time it takes to handle each prey or to satiation (Jeschke et al. 2002), two important factors to determining the functional response type (Gotelli 1995). The exact nature of handling time, satiation and their relationship has been modeled in a variety of different ways and this has led to considerable confusion. The point is that handling prey is an active process whereas digestion is a background process. As a consequence, in contrast to handling prey, digestion does not directly prevent the predator from further searching or handling. Rather, digestion influences the predator's hunger level, which in turn influences the probability that the predator searches for new prey. It is thus necessary to discriminate satiation from handling in a functional response model (Jeschke et al. 2002).

A small number of authors have investigated the predatory functional response as a potential predictor of the predatory effects of an invader (Bollache et al. 2008; Hoff and Bolens 2004; Radford et al. 2007). This novel use for functional responses has important implications for invasion ecology: it helps to predict the predatory impacts of and invader, and conversely illuminates the role of native predators in resisting invasions by exotic prey. Furthermore, by quantifying invasion thresholds it may help managers predict the outcomes of species introductions and biocontrol efforts (Twardochleb et al. 2012).

The success of introduced species like the zebra mussel, *Dreissena polymorpha* (Pallas 1771), is due in part to ineffective predators in the newly colonized systems (Boles and Lipcius 1994). The magnitude of the impacts of zebra mussels, like those of other invading species, is strongly related to population size. Thus, identification of the factors that influence zebra mussel abundance can provide a basis for understanding the capacity of its populations to expand and to affect the ecology and economy of invaded aquatic ecosystems.

Zebra mussel densities commonly range from 2000 to 100.000 ind.m^{-2} (Nalepa and Schloesser 1993), though densities up to 340.000 ind.m^{-2} have been reported from western Lake Erie (Leach 1993). A reference of 1991 (Bij de Vaate) present zebra mussel densities near 3000 ind.m^{-2} for European lakes and large rivers; a newly established population of zebra mussels in the River Darent (Kent – England), contained zebra mussels in dense mats of up to 11.000 ind.m^{-2} (Bij de Vaate, 1991). This invasive has also colonized some Italian aquatic ecosystems (Lori and Cianfanelli 2006; Lancione and Gaino 2006; Gaino et al. 2009; Ciutti et al. 2011) and was found in highest values - maximum density ~200.000 ind. m^{-2} at Lake Trasimeno (Lancione and Gaino 2006); 756 ind. m^{-2} at Sirmione and 24.756 ind. m^{-2} at Bardolino, two Lake Garda localities, six years after invasion (Ciutti et al. 2011).

The life-history characteristics of this species, unique among freshwater bivalves and its extraordinarily high fecundity (Borcherding 1991) have facilitated its rapid spread (Carlton 1993; Johnson and Carlton 1996). Zebra mussels usually have an annual reproductive cycle (Juhel et al. 2003; Lancioni and Gaino 2006) and the life cycle includes a free-swimming larval stage (veliger) that, after spending several days in the water column, attaches to substratum by byssal threads and metamorphoses into a sessile adult. During the last part of the veliger stage, *D. polymorpha* individuals develop the byssum, the organ that allows attachment to a solid substrate (Casagrandi et al. 2007). The juvenile stage begins after settlement and ends when mussels become sexually mature and produce eggs and sperm (Casagrandi et al. 2007). Sexual maturity and first spawning seem to be correlated with a shell length of about 7-9 mm, independent of mussel age (Jantz and Neumann 1998).

The spawning period for zebra mussels is normally late spring or early summer, and a single colony of mussels is capable of producing large numbers of veligers through external fertilization (Timar and Phaneuf 2009). In the River Rhine (Germany), this species is able to undergo two spawning periods per year. The first one covered nearly 3 months (April to July) comprising several spawning events. The second shorter one occurred in August. Young mussels of the first spawning period settled at the end of May, and they were able to reproduce a few months later (end of August) when their shell length exceeded 9 mm. This first spawning period of the young-of-the-year coincided with the second spawning period of the older generations (Jantz and Neumann 1998).

The analysis of size-frequency distributions and the study of growth in experimental cages showed that zebra mussel's lifespan may vary from a minimum of 2 years to a maximum of 8 years (Karatayev et al. 2006). This lifespan is dependent on local conditions, being generally shorter in warmer waters (Stanczykowska 1977). In order to include in their model no more than four age classes, Casagrandi et al. (2007) grouped all the individuals aging 4 years or more in the oldest age-class.

Biologically, stage $\mathbf{n}_1$ corresponds to the juvenile population. Stages $\mathbf{n}_2$, $\mathbf{n}_3$, and $\mathbf{n}_{4+}$ reflect successively older and larger zebra mussel groupings (Davis 2010). Stanczykowska (1977) has also estimated these 4 size/age classes for the *D. plymorpha* specimens from Lake Garda according to the growth rings of the shell (0- 10 mm -1 year; 11-19 – 2 years; 17-23- 3 years; 21-26- 4 years) (Annoni et al. 1978).

McMahon (1991) proposed fishes as the most active predators of settled zebra mussels, but only a handful of studies have indicated the potential of fish predation to control invasion success and density of exotic mussels (Eggleton et al. 2004; Bartsch et al. 2005; Watzin et al. 2008). However, predation may not significantly reduce entire zebra mussel's populations. Magoulick and Lewis (2002) results indicate that predation could potentially suppress initial zebra mussel colonization and recolonisation of adult zebra mussels following temperature-dependent mortality, and the relative susceptibility of zebra mussels to predation can be influenced for several biological and ecological factors (Thorp et al. 1998). Perry et al. (2000) and Naddaffi et al. (2010) also suggested the potential impacts of predation on recruitment or on the early life history stages of zebra mussels, because during the early stages of an invasion the average zebra mussel size is small and therefore susceptible to predation and hypothesized that this would have a significant impact on mussel recruitment.

Crayfish are potentially significant predators of zebra mussels in inland waters and may have the potential to limit zebra mussel populations. Crayfish populations can attain high densities (≥ 18 adults/m2) and biomass (≤ 680 g/m2) (Lorman 1980; Mather and Stein 1993). Crayfish also prefer rocky habitats (Olsen et al. 1991), where zebra mussels are generally found, and can control the structure and function of benthic communities (Lodge et al. 1994; Charlebois and Lamberti 1996).

Several studies have investigated crayfish predation on zebra mussels (Piesik 1974; Love and Savino 1993; MacIsaac 1994; Martin and Corkum 1994; Perry et al. 1997; Schreiber et al. 1998; Reynolds and Donohoe 2001), including *P. clarkii* (Gonçalves et al. *submitted*). These two invasive species (*P. clarkii* and *D. poplymorpha*) coexist now in Italy and Gonçalves et al. (*submitted*) demonstrated that the stage $\mathbf{n_1}$ of the zebra mussel is the more vulnerable to predation.

The red swamp crayfish *Procambarus clarkii* (Girard 1852), native to the south-central United States (Louisiana) and north-eastern Mexico (Gherardi and Holdich 1999), is a highly diffused alien species with many established populations in Italy (Aquiloni et al. 2010), and is expanding, namely in Lake Trasimeno, an optimal habitat for this species (Dörr et al. 2006). Density estimates are still few; in 1998 this species had a peak density of 100 ind.m^{-2}, in a Tuscany Lake (Angori 1998). The highly aggressive behavior, potential for rapid population increase, and omnivorous feeding habits of *P. clarkii* have resulted in numerous ecological impacts manifested across entire lake food webs (Gherardi 2006; Gherardi and Acquistapace 2007).

In a first analysis of the interaction between *P. clarkii* and *D. polymorpha*, predation experiments were performed in order to know the preferred size of the prey, as well as to estimate the effects of the presence of an alternative prey (Gonçalves et al. *submitted*). This work showed that *P. clarkii* predation upon *D. polymorpha* is size-selective, predation being mainly concentrated in the $\mathbf{n_1}$ (juvenile) stage. Predation by *P. clarkii* upon individuals in this stage may have a profound influence in the zebra mussel population structure.

However, to forecast the impacts this crayfish may have on zebra mussels, it is necessary to know the maximum prey consumption as a function of prey abundance (i.e. the predator functional response), as well as the amount of mussels that will lead to predator satiation. Martin and Corkum (1994) showed that crayfish did not feed continually; instead they would stop feeding when they became satiated. Furthermore, crayfish were not limited by the handling time associated with consuming small mussels, and individual crayfish showed a functional response type II (Holling 1959) when presented with a range of mussel densities.

To properly assess the feeding rates of a predator, particularly crayfish, it is necessary to take into account the previous starvation time. To calculate a "normal" feeding rate, Covish et al (1980) considered the data obtained after the first two days of feeding, because the crayfish, in their experiment, had been starved for ca. 1 week. In various studies, the starvation time considered varies between 24 hours and 9 days (Martin and Corkum 1994; Reynolds and Donohoe 2001; Mistri 2004).

The goals of this work are to identify the consumption rates of *D. polymorpha* $\mathbf{n_1}$ stage by *P. clarkii*, to determine the predatory functional response, to evaluate the effects of predator satiation on the intensity of predation, and to model the predatory impact of the crayfish, with the final purpose of understanding whether this species can control, and in what conditions, the zebra mussel populations.

5.2 *METHODS*

5.2.1 ***Dreissena polymorpha* population dynamics - deterministic model**

The zebra mussel populations may be divided into four separate stages: n_i (t) represents the number of zebra mussels of stage *i* at time *t*, whereas

N (t) =$\mathbf{n}_1$ (t) + n_2 (t) + n_3 (t) + n_4 (t) is the total zebra mussel population at time *t*.

The deterministic model presented here was first presented by Casagrandi et al. (2007) and takes the following mathematical form:

$$\mathbf{n}_1(t+1)= \sigma_0 \exp[-\beta N(t)] \, [(f_2 n_2(t)/2) +(f_3 n_3(t)/2)+(f_4 n_4(t)/2)]$$

$$n_2(t+1)= \sigma_{11}(t)$$

$$n_3(t+1)= \sigma_2 n_2(t)$$

$$n_4(t+1)= \sigma_3 n_3(t)+ \sigma_4 n_4(t)$$

where the σ_i values (with $1 \leq i \leq 3$) represent the yearly individual survival from age *i* to age *i* + 1, σ_4 the yearly survival within the age class 4+ and the *f*i values (with $2 \leq i \leq 4$) are the numbers of eggs released by one adult female of age *i* (Casagrandi et al. 2007). As females do not reproduce during their first year and the sex ratio is typically balanced (Staczykowska 1977; Thorp et al. 1994), the yearly offspring production of the entire population amounts to $[(f_2 n_2(t)/2) +(f_3 n_3(t)/2)+(f_4 n_4(t)/2)]$.

The parameter σ_0 in the first equation is equal to σ_E x σ_V, where σ_E is the rate at which eggs are fertilized by adult males and σ_V is the survival rate of veligers in a low density of adult mussels. The exponential term in the same equation was adapted from the Ricker (1954) model, meaning that it accounts for the cannibalistic tendencies found in the system as a result of filter-feeding. The parameter ß is a filtration rate accounting for the intensity of veliger removal by adults.

The ranges of variation of fertility and survival in the first three adult age classes can be estimated from available data. The values to be attributed to the two remaining parameters σ_0 and σ_4 are much more uncertain than those of the other survival fractions (Casagrandi et al. 2007).

In their model, Casagrandi et al. (2007), mostly used the parameter values determined by Annoni et al. (1978) (table A5.1), which were determined from the study of the *D. polymorpha* population established in Lake Garda (Italy). The model presented by these authors explains the three different patterns of *D. polymorpha* population dynamics: chaotic, cyclic (with different periods), and stable.

Davis (2010), based on this model and parameters, recreated graphically the three possible population dynamics patterns, differentiating its effects on each of the four *D. polymorpha* life-cycle stages (fig. A5.1-A5.4). To display these charts, initial conditions were set by Davis (2010) such that all the life-cycle stages were equally abundant at the start of the model. This initial condition of equal importance of all the life-cycle stages is realistic only for a stable population. An analysis exploring the outbreak of an invasive population might have a large number of stage 1 mussels with little or no stage 2, 3, or 4+.

5.2.2 *Experimental procedure*

5.2.2.1 Field collection and laboratory maintenance

Individuals of *D. polymorpha* were hand collected, from the Lake Bilancino (43°58'41''N, 11°16'54''E) (Tuscany, Italy); *P. clarkii* were collected, using baited traps, in Lake Trasimeno (43°08′N 12°06′E) (Umbria, Italy).

Upon their arrival in the laboratory, crayfish were measured (CL – cephalotorax length without rostrum) using a vernier calliper. Mature males were identified by the presence of prominent copulatory hooks and cornified gonopodia (Huner 2002), and possible mutilations were recorded. Only intermolt animals with intact chelae and walking legs were used in the experiments. Crayfish were maintained in individual plastic tanks (26.5x16.2x15 cm) containing 1 L of well water at a temperature ranging 20-28°C (25± 1.49 SD), under natural light/dark cycle regime, and fed *ad libitum* with larvae of *Caliphora* sp. Water was changed once per week. To avoid pseudo replication each crayfish was used only once.

All laboratory experiments were run in plastic aquaria (31 cm of diameter/ an area of ~750 cm^2) without sediment or gravel at the bottom to prevent any constraints on searching, and containing 2 L of dechlorinated tap water (approximately 5 cm deep). Each animal was provided with one shelter (12 cm of gray plastic tubing with a 5 cm diameter) in order to minimize stress. These experiments were run between June and July of two years (2012 and 2013) in the laboratory of the Department of Biology (University of Florence).

5.2.2.2 Quantifying the functional response

Individual crayfish (both sexes, since our previous work showed that there were no sexual differences in *D. polymorpha* size preference) were supplied with *D. polymorpha* ranging in size from 5-11 mm shell length. These are the preferred sizes (Gonçalves et al. *submitted*) and correspond to the $\mathbf{n_1}$ stage (Annoni et al. 1978). Mussels were supplied at 4 different quantities (50, 100, 150, 200 individuals) under each of two crayfish starvation treatments (*crayfish starvation* – 24 hours and 1 week). The number of replicates of each combination varied from 5 to 7. The number of mussels remaining after 24 hours was then counted.

5.2.2.3 Predator satiation experiment

Satiation was determined by placing an individual crayfish (26 females /24 males =50 trials) that had been previously starved for 1 week in the aquarium with fifty zebra mussels ranging in size from 5-11 mm shell length (the preferred sizes (Gonçalves et al. *submitted*), corresponding to the $\mathbf{n_1}$ stage), and scattered over the bottom of the aquarium. Each crayfish was allowed to feed at will and the number of mussels remaining after 24 hours was then counted. The initial number of fifty zebra mussels was then replaced and the same procedure was repeated for 6 days, continuing until a relatively constant number of mussels were eaten each day. Individuals that did not consume zebra mussels or that stopped feeding during the six days were removed from the analysis.

5.2.2.4 Statistical methods

We used non-parametric Mann-Whitney U tests for the comparisons between lengths of crayfish used in the densities treatment as well as in the starvation treatments.

To estimate the functional response of crayfish we first calculated the proportional mortality rates (nº. of mussel eaten per mussel abundance per 24h) per abundance treatment (Mistri 2004).

The differences between the number of zebra mussel consumed by crayfish subjected to the two starvation levels were assessed using a General Linear Model that tested for the existence of interactions between prey density and the starvation level of the crayfish.

We used non-parametric Mann-Whitney U tests for the comparisons of maximum and minimum daily consumption between crayfish sexes, and tested for correlations between the crayfish length and these parameters.

For the satiation experiment we fitted the most significant curve to the average consumption of zebra mussel over the 6 days, and calculated the mean of the proportional decreases in the daily consumptions.

All statistical analyses were performed with SPSS Statistics 20.

5.2.3 *Modelling*

The functional response equation was associated with the satiation effect of the predator, displayed over time. Considering one month of predator activity, we then evaluated graphically the effect of the predator at different initial abundances of the *D. polymorpha* $\mathbf{n_1}$ stage.

Considering the estimated predatory impact of the crayfish over one month (see above) upon the $\mathbf{n_1}$ stage, we modelled its effect on the $\mathbf{n_2}$ stage in order to estimate the potential impacts of predation in the population structure of *D. polymorpha*.

5.3 *RESULTS*

5.3.1 *Experimental procedure*

The average size of the crayfishes used in both experiments was similar.

Quantifying functional response experiment: there were no differences in the CL size of the crayfishes that were starved for 1 week (36.23±0.64 cm; n=28) and starved for 24h (35.31± 0.92 cm; n=15); used in the different densities (*50 mussels*: U= 14; *p*=0.639; 100*:* U= 13; *p*=0.927*; 150 mussels:* U= 10.5; *p*=1*; 200 mussels:* U= 6; *p*=0.383*)*. Comparing the CL crayfish used among all treatments (consumed densities) no significant differences were observed -'starvation 1 week' ($F_{(3,24)}$=0.868; *p*=0.471); 'starvation 24h' (x^2=1.224 (Kruskal-Wallis Test); *p*=0.747).

Predatory satiation experiment: there were no significant differences in size between the sexes (U=80.50; *p*=0.421; CL males =34.05±3.62; n=14/CL females=34.71±6.73; n=14).

5.3.1.1 Quantifying the functional response

The results of the GLM indicated that the number of zebra mussels consumed by crayfish depends only on the initial abundance (F=4.314; *p*=0.011) and not of the starvation level of the predator (F=1.216; *p*=0.278), with no significant interaction between these two variables (F=0.475; *p*=0.702).

Grouping the results of both starvation treatments, we calculated the curve that best describes the behavior of crayfish: y=25.55 ln (x)-64.46 (R^2= 0.235; F=12.283; p=0.001; n=42; std. error of estimate= 25.65) (fig. 5.1). We found that *P. clarkii* is a predator with a functional response of the type II, characterized by significantly higher proportional mortality at low prey abundance than at high prey abundance (Hassel, 1978).

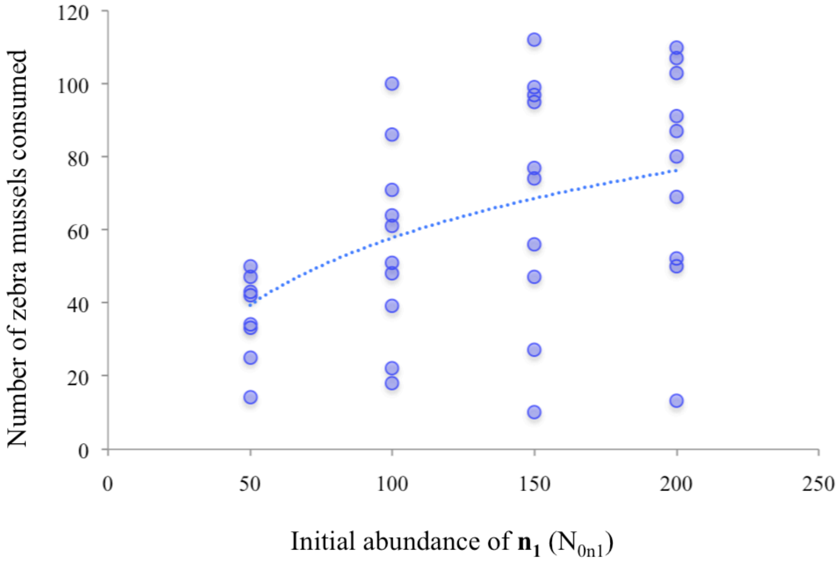

Figure 5.1. Number of zebra mussels consumed, in 24 hours, according to the available abundance of $\mathbf{n}_1$. The dotted line shows the function that best describes the predatory behaviour of crayfish faced with increasing numbers of prey (see text for the mathematical expression of this function).

5.3.1.2 Predator satiation experiment

There were no sexual differences in the maximum daily consumption (U=93.5; p=0.854, as well as in the minimum daily consumption (U=75; p=0.448) of *D. polymorpha*. Grouping the results of both sexes, there were positive correlations between crayfish size and both maximum (Spearman's rho=0.511; $p<0.001$; n=28) and minimum daily consumption (Spearman's rho=0.644; $p<0.001$; n=28).

Over the six days of observation there was a significant decrease in the consumption of *D. polymorpha* (F=882,123; $p<0.001$; n=28) (fig. 5.2). The quadratic function that best describes the results is as follows:

$y = 0.998x^2 - 12.18x + 43.55$; $R^2 = 0.998$

(y= nº of *D. polymorpha* consumed; x= time (days)).

In these experimental conditions, when crayfish were offered a constant prey abundance - $\mathbf{n_1}$= 50 – the amount of prey consumed decreased over time at a rate of 0.71/day (fig. 5.2), with a tendency for stabilization after 6 days at around 6 mussels/day (fig.5.2.).

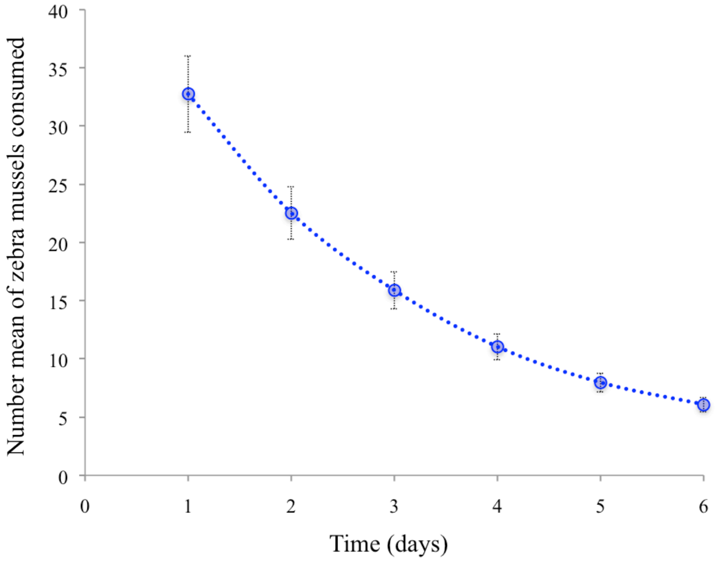

Figure 5.2. Number mean of zebra mussels consumed over time (6 days).

5.3.2 *Modelling*

Considering the equation of Holling (1959) to a functional response of the type II, the behavior of this predator could be represented as:

$$N_{\mathbf{n1}}=(aN_{0\mathbf{n1}})/(1+ahN_{0\mathbf{n1}})$$

($\mathbf{N_{n1}}$ is the number of prey consumed of the age class $\mathbf{n_1}$; $\mathbf{N_{0n1}}$ the number of initial prey; **a** the *attack success* and **h** the *handling* *time*). Our results (fig. 5.1) show a functional response of the type II, where the dependent variable (number of *D. polymorpha* consumed in one day) already includes the components "*attack success*" and "*handling time*". The study of this functional response on stage $\mathbf{n_1}$ already reflected the attack success (very high for this stage) and a low handling time, observed by Gonçalves et al. (*submitted*).

According to our results, the behavior exhibited by crayfish may be described by the equation:

$y=25.55 \ln(x) - 64.46$

So, this would be the mortality (M) of the $\mathbf{n_1}$ stage caused by one day of activity of one crayfish:

$M_{\mathbf{n1}}=25.55 \ln (N_{0n1}) - 64.46$

Combining the functional response with satiation of the predator, in order to approximate this to a more realistic situation:

$$M_{\mathbf{n1}} (t+1)= [(25.55 \ln (N_{0\mathbf{n1}}) - 64.46)*0.71^{(t+1)}] + M_{n1} (t)$$

Over time (in the example – one month activity of one crayfish) and according to different initial abundances of *D. polymorpha* stage $\mathbf{n_1}$ (the abundances used in the experiments) the proportional mortality caused by an individual crayfish in a *D. polymorpha* population can be plotted as in figure 5.3. As expected, crayfish will have a greater impact when the zebra mussel abundance is low.

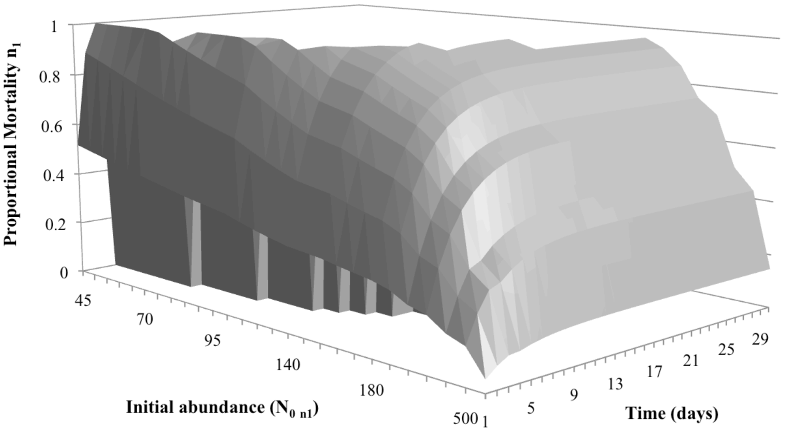

Figure 5.3. Variation of proportional mortality, caused by one crayfish over one month, with different initial zebra mussel abundances. Up to 150-60 individuals, crayfish predation will eventually consume the entire population (proportional mortality reaches "1"). When more than 500 zebra mussels are present proportional mortality will be below 0.1.

We used the values of $\mathbf{n_1}$, resulting from the effect of predation (fig. 5.3) and the survival parameters determined by Annoni et al. (1978) (table A5.1) to obtain the values of n2 (t+1) (fig. 5.4).

The difference between the number of zebra mussels that survive and pass to the stage n2, when the stage $\mathbf{n_1}$ does not suffer a effect of a month of predation or when predation occurs is obvious for all initial densities of $\mathbf{n_1}$, especially for low abundances, up to 150 mussels (fig.5.4). For increasing number of *D. polymorpha* provided, this effect becomes less and less important. For instance, for initial $\mathbf{n_1}$ abundances over 2000 individuals, one month of predation by one crayfish will decrease the abundance of $\mathbf{n_2}$ in the following year by only about 15% (Fig. 5.4).

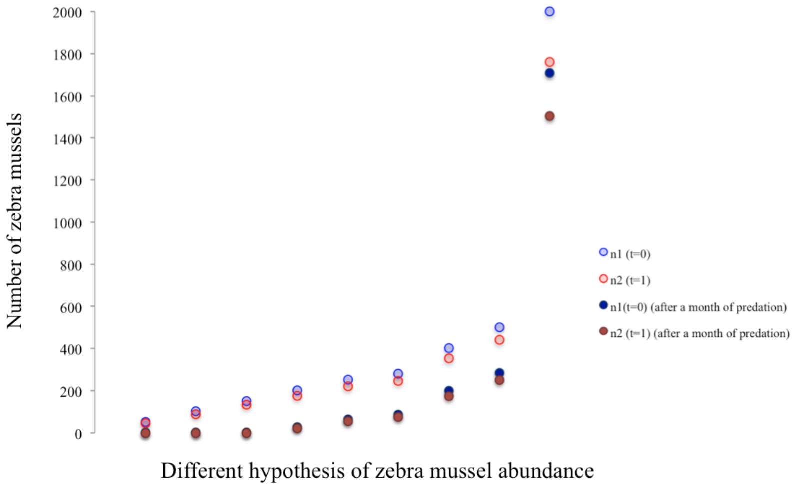

Figure 5.4. Abundances of $\mathbf{n_1}$ and $\mathbf{n_2}$ (in the following year) stages in the absence of predation (light colour circles) and after predation by one *P. clarkii* for one month (dark colour circles).

5.4 ***DISCUSSION***

With this study we estimated the maximum daily consumption of one crayfish on *D. polymorpha* $\mathbf{n_1}$ stages, and verified that this consumption has the potential to cause a strong decrease in the population of zebra mussels when the prey numbers are relatively low.

We also found that this maximum daily consumption depends on the abundance of available prey, according to a functional response type II (Holling 1959), which is characterized by a decrease in proportional prey mortality as prey abundance increases.

This type of predator functional response indicates that the real impact in established populations (usually with a high abundance in the case of zebra mussels) can be relatively low. Furthermore, the degree of satiation of the crayfish must also be taken into account.

Our results show that the daily consumption of one crayfish after being satiated can be only about 1/6 of the daily consumption of a starved individual.

Obviously this is the behavior of one crayfish; the higher the crayfish population, the greater will be the impact caused. Since this is a predator that only consumes the stage $\mathbf{n_1}$, the mortality effect in $\mathbf{n_1}$ will be transmitted to the other stages of the *D. polymorpha* populations in the following years. Thus, even a relatively small predation impact can bear relatively important consequences if it is all directed to one specific, more vulnerable stage.

The feeding rates of crayfish on small clams reported by Piesik (1974) were substantially higher than those we found. Mean consumption by *O. limosus* ranging from 47-90 mm (~23-45 mm carapace length) was ca. 36 small (4-5mm) *Dreissena* per day; one large female consumed an average of 110 clams per day for 5 days. *Procambarus clarkii* of similar size consumed on the average only about 10 small (4-6 mm) *Corbicula* per day, and maximally only 19 (Covish et al. 1980).

The functional response of *P. clarkii* to *D. polymorpha* was similar to that of other aquatic invertebrate predators given a single type of food (Thompson 1975; Walde and Davies 1984; Martin and Corkum 1994). The form of the functional response is dependent not only on prey density, but also on the relative distribution of predator and prey in time (Collins et al. 1981) and space (Kaiser 1983; de Lafontaine and Leggett 1987). It should also be noted that zebra mussels at high density in the wild often form druses (a dense mass of mussels with intertwined byssus). The formation of a druse may impact crayfish size selectivity and feeding rate, because mussels are strongly attached within the three dimensional structure of a druse and, therefore, potentially less accessible (Ermgassen and Aldridge 2011).

In agreement with our results Casagrandi et al. (2007) predicted that if the mortality of $\mathbf{n_1}$ is too high, it will have a significant impact in the population structure of *D. polymorpha*. The stages that influence population growth of *D. polymorpha* are $\mathbf{n_1}$ and $\mathbf{n_4}$. The population spikes are most sensitive to veliger survival and the retention of stage 4+ mussels (Davis 2010).

Thus, to estimate the real impact that *P. clarkii* may have on *D. polymorpha* it is necessary to take into account the season when the stage $\mathbf{n_1}$ is more abundant, as well as the seasonal activity of the predator. A coincidence of the phenology of both species will increase the potential impacts of the predator, while a desynchronization of predator activity and prey reproduction will render the impacts of predation almost null.

Unlike many other species, zebra mussels can have vastly different population behaviors based on the location of the colony, which has caused difficulty in modeling their local dynamics. This variance originates mostly from environmental conditions, such as salinity, pH, temperature fluctuations, and levels of different chemicals present in the water (McMahon 1996; Abaurre 2008). Considering Davis (2010) graphs, in a population with a chaotic cycle (fig. A5.1), the predator effect will be stronger during periods in which all stages (including $\mathbf{n_1}$) are present in very low densities. In cyclically fluctuating populations (fig. A5.2 e A5.3), the effect of the crayfish can be maximized if it is in accordance with the cyclical synchronization of the population. But where this predator can have a greater impact in a zebra mussel population is in equilibrium situations (fig. A5.4), which are characterized by relatively low densities of all life-cycle stages, including $\mathbf{n_1}$, with about 42% of a population in equilibrium composed of $\mathbf{n_1}$ individuals (Davis, 2010). Stage $\mathbf{n_1}$ is also found in lower densities when the population is characterized by a larger density of the stage $\mathbf{n_4}$, because when the adult population is large there is a higher cannibalization (by filtering) of larvae.

The abundance of $\mathbf{n_1}$ individuals is lower when the veliger larvae begin to fix to the substrate, i.e. after the intense reproduction period of warmer season (spring-summer) (Annoni et al. 1978; Lancioni and Gaino 2006). In Italian aquatic ecosystems, the reproduction period of *D. polymorpha* is between the months June-August, and the first colonization of substrate will be between August-November (Annoni et al. 1978; fig A5.5). In a review of population structure of mussels in another Italian ecosystem, Sebino (Lombardia) there was a similar population increase between the months of June - September, at different stations of the same lake (Roncaglio and Borsani 2005).

The period when *P. clarkii* is more active may or may not coincide with the time when the stage $\mathbf{n_1}$ is more abundant. Some studies have documented that *P. clarkii* activity levels peak during warmer months (Gherardi et al. 2000), so crayfish forage less actively during the winter. But, on Lake Trasimeno (Umbria, Italy) crayfish molted from the end of fall to the beginning of summer, with males being more active in winter than females (Dörr and Scalici 2013).

Considering this synchronization factor, predation by crayfish can be assumed to be probable after the zebra mussel reproduction period and the first colonization of the substrate, therefore coinciding partially (but not totally, as the colonization of the substrate by the veliger larvae may continue during the fall and winter) with the life-cycle of the mussel. We can thus predict that the impact of *P. clarkii* on *D. polymorpha* stage $\mathbf{n_1}$ will be a cyclical and seasonal impact, associated with the zebra mussel reproduction season.

One of the main objectives of the study of the interactions between these two invasive species is to understand whether predation by crayfish can somehow hinder the introduction (colonization) of *D. polymorpha*. Based on the results presented, it will be important to assess how different densities of crayfish may affect the populations of *D. polymorpha*, taking into account the possibility of interference competition between individual crayfishes, as well as the effects of the presence of alternative prey. The effects of satiation should also be studied further, as it is still unknown if crayfish may completely stop eating zebra mussels after a long period.

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ANNEX 5.1

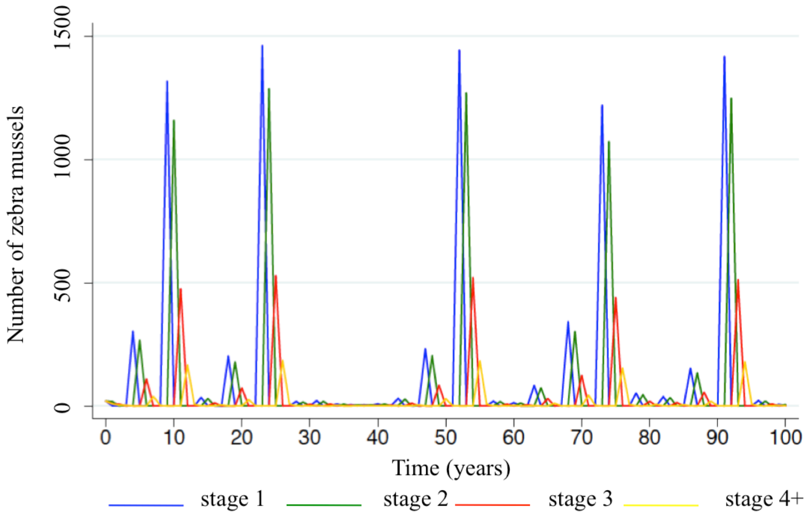

Figure A5.1. Population Breakdown – with chaotic regime; elaborate with Annoni et al. (1978) parameters (table A5.1.) (figure adapted of Davis 2010) (**n_1** (0) = n_2 (0) = n_3 (0) = n_4 (0) = 20. Thus, N (0) = 80 was the total abundance of zebra mussels at the start of the model).

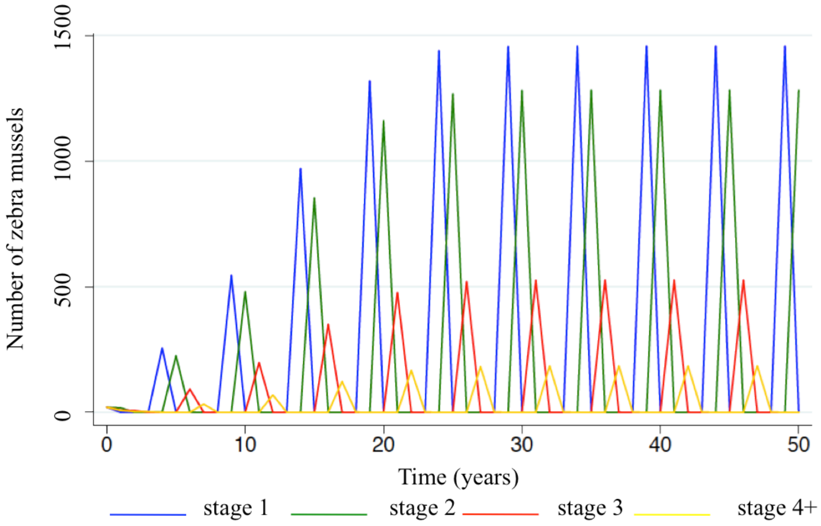

Figure A5.2. Population Breakdown - Cyclic One σ_4= 0.005 5-year cyclic pattern, increase to a constant peak height (figure adapted of Davis 2010) ($\mathbf{n_1}$ (0) = n_2 (0) = n_3 (0) = n_4 (0) = 20. Thus, N (0) = 80 was the total abundance of zebra mussels at the start of the model).

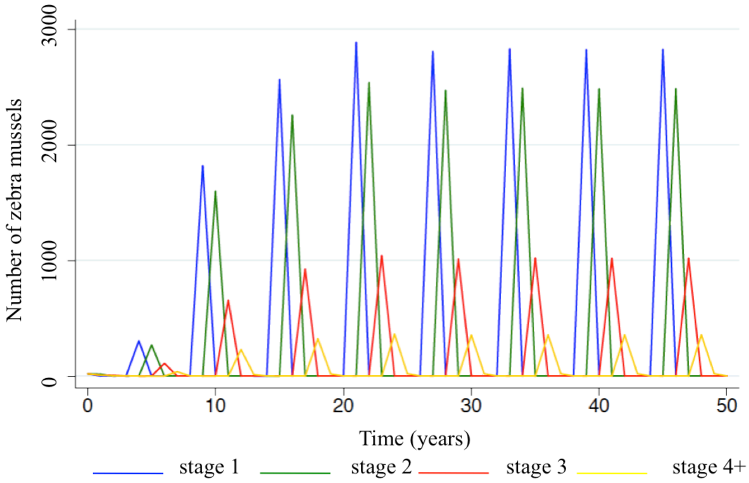

Figure A5.3. Population Breakdown - Cyclic Two ***σ₀***= 0.02 and ***σ₄***= 0.06. 6-year cyclic pattern, increase to a constant peak height (figure adapted of Davis 2010) (**n_1** (0) = n_2 (0) = n_3 (0) = n_4 (0) = 20. Thus, N (0) = 80 was the total abundance of zebra mussels at the start of the model).

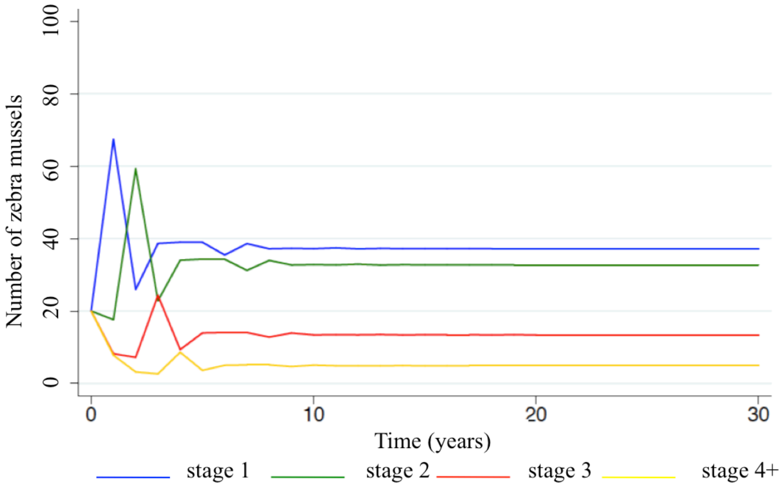

Figure A4. Population Breakdown - Equilibrium. $\boldsymbol{\sigma_0}$= 0.00001 and $\boldsymbol{\beta}$= 0.01. Stable pattern, levels out to a constant population (figure adapted of Davis 2010) ($\mathbf{n_1}$ (0) = n_2 (0) = n_3 (0) = n_4 (0) = 20. Thus, N (0) = 80 was the total abundance of zebra mussels at the start of the model).

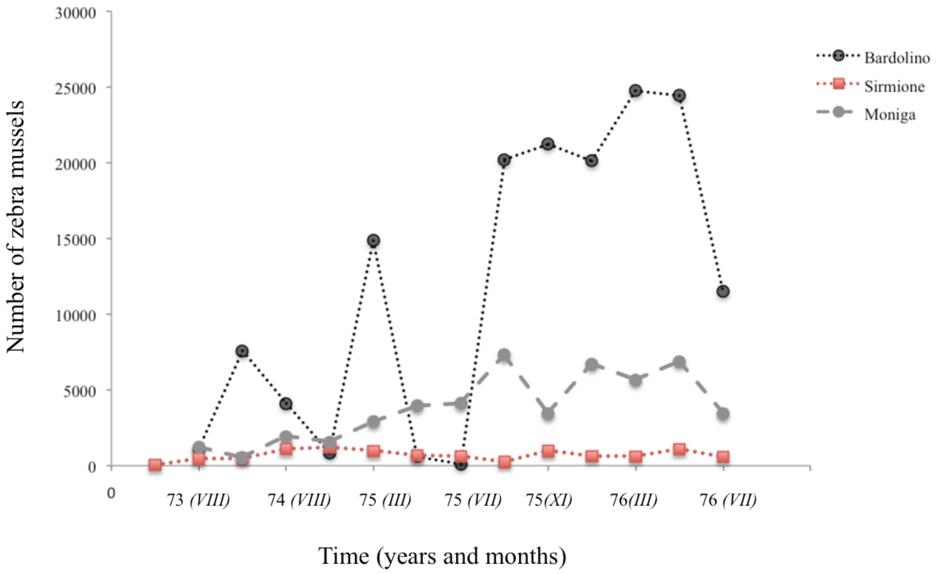

Figure A5.5. Population growth of *D. polymorpha*, over the years, in the three study sites of Annoni et al. (1978). The three study sites feature different behavioral patterns.

Table A5.1. The parameter set (adapted from Casagrandi et al., 2007).

Parameter	*Value*	*Description*
σ_0	0.01	combined rate of veliger survival and birth
σ_1	0.88	survival rate of stage 1mussels to stage 2
σ_2	0.41	survival rate of stage 2 mussels to stage 3
σ_3	0.35	survival rate of stage 3 mussels to stage 4+
σ_4	0.04	retention rate of stage 4+ mussels
f_2	0.24×10^6	fecundity of stage 2 female mussels
f_3	0.465×10^6	fecundity of stage 3 female mussels
f_4	0.795×10^6	fecundity of stage 4 female mussels

CHAPTER 6

GENERAL DISCUSSION

The findings of this thesis highlight the importance of quantifying the direct and indirect interactions among invasive species. The need for ecologists to address these issues is emphasized by the now commonplace occurrence of multiple invaders in many ecosystems. Identifying the multi-trophic impacts of biological invasions is a particularly urgent issue in freshwater ecosystems, which have suffered widespread invasions by non-indigenous species and exhibit high levels of extinction risk.

This study confirmed the existence of negative interactions between the two invasive species, *Procambarus clarkii* and *Dreissena polymorpha*, in the aquatic ecosystems of Central Italy, which developed within a short period of time, in less than a decade of coexistence.

We found that *P. clarkii* fouling by alien zebra mussels occurs in Lake Trasimeno and in the laboratory (***Chapter 2***). This is in agreement with previous studies reporting that zebra mussels can be found, occasionally, on most of the red swamp crayfish body, with a general preference for the ventral surface, and in our study case we found a particular preference for the pereopods.

Adults of both sexes were colonized equally. The occurrence of multiple zebra mussels on individual crayfish was unexpectedly high; this may be related to poor crayfish condition and may also explain the single colonization of an individual live crayfish recorded in the laboratory.

During the experimental observations concerning the study presented in ***Chapter 5***, where the crayfish had various quantities of zebra mussel available to feed upon, the fouling phenomenon was also observed in 3 individuals, and likewise with a preference for the ventral surface of the crayfishes.

Future studies should focus on other impacts of fouling, for instance, understanding how attached *D. polymorpha* may affect crayfish behavior or in what circumstances it can reduce crayfish fitness. It is also important to test the ability of *D. polymorpha* veliger larvae to colonize *P. clarkii* when compared with other substrates and/or when there is a strong limitation of *D. polymorpha* preferred hard substrate types. These experiments can be conducted in the laboratory, where the physiological requirements of zebra mussel colonization, like calcium, ph, temperature, water velocity and food supply, can be controlled. Finally, there is a possibility that zebra mussels may be transported upstream attached to crayfish, and the importance of this dispersal mechanism for *D. polymorpha* should be evaluated.

The most significant work of this thesis concerned the predation of zebra mussels by *P. clarkii* (***Chapters 3, 4 and 5***).

Our work demonstrated that the estimated profitability of a prey species cannot be used to predict the predation behavior in situations where several types of prey are available (***Chapter 4***). Different estimates of profitability indicated that the crayfish would prefer an alternative prey – a snail - instead of zebra mussels. However, the experiments regarding prey choice demonstrated that crayfish seems to prefer smaller preys, regardless of the species, even when more profitable alternative prey is offered simultaneously.

Crayfish sex and size were of reduced importance for prey choice; however, the largest crayfish (and especially females) tended to choose the mussel over the snail, which can be related with a higher ability of large crayfish to crush the shell of large mussels.

This study also confirmed that the invasive zebra mussel, *D. polymorpha*, represents a novel prey resource for *P. clarkii*, even in the presence of an alternative prey. Zebra mussels can be a preferred prey when present in their juvenile stages, or when of small dimensions.

Based on the results of the invasive predator preference for a particular life cycle stage of the zebra mussel (***Chapter 4***), another study (***Chapter 5***) allowed us to estimate the maximum daily consumption by one crayfish of the $\mathbf{n_1}$ (juvenile) stage of *D. polymorpha*. We found that this consumption may cause a strong decrease in the prey numbers. Moreover we found that this maximum daily consumption depended on the abundance of available prey, with a predator functional response of the type II, which is characterized by a higher predation the lower the initial abundance of available prey (Haddaway et al. 2012). Over time, predation will be also modified by predator satiation. A modelling approach showed that *P. clarkii* predation can influence the population dynamics of *D. polymorpha*, and that this impact will be greater the more synchronous the prey reproductive cycle and the activity period of the predator are.

All studies were based on laboratory work, however in the future it will be important to analyze the interaction of the two species in field experiments testing other factors that influence the whole process, specifically the presence of alternative prey, the level of predator activity and the effects of spatial heterogeneity.

One particular aspect of *D. polymorpha* is the formation of druses; these could affect densities of benthic invertebrates in soft substrate by providing increased physical structure or by providing increased resources through some biotic mechanisms (Botts et al. 1996), which could benefit the predator, *P. clarkii*, by increasing the available prey. It will be interesting to evaluate how zebra mussel mats affect crayfish distribution and foraging success.

The order of establishment of these two interacting species is also likely to be important in determining the nature of their interaction. Predation by an established crayfish population may confer resistance against zebra mussel invasion to a water body, even if just a relatively small number of small zebra mussel individuals are predated (Reynolds and Donohoe 2001). On the contrary, if a zebra mussel population is established first, it may facilitate crayfish invasion by providing an abundant food source directly, as well as by providing habitat for other macroinvertebrate prey (Stewart et al. 1998).

More long-term studies may help to better understand the interactions between these two important and invasive alien species. Although several factors may intervene to modify this relationship, only further studies, over time, will enable a comprehensive evaluation of the dynamics of both invasive species when the two coexist in the same ecosystem.

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