The Use of Barnacle Parasite *Sacculina carcini* to Eradicate or Control European Green Crab *Carcinus maenas*

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Abstract

The European green crab, *Carcinus maenas*, has invaded and threatens many ecosystems in the U.S. and different parts of the world, wreaking havoc on the habitat of native crabs. The damage to these ecosystems has negative economic impact on fishing industries, given the commercial importance of some native crabs, such as Dungeness Crab. One way to reduce the harm of detrimental pests such as *Carcinus maenas* is with biocontrol agents. I discuss the potential use of the parasite *Sacculina carcini* as a biological agent to control *Carcinus maenas.* with a summary of what we know about the parasite and what we still need in order to effectively utilize it. I give a summary of the life cycle of *S. carcini* from its larval free-living stage to its parasitic adult phase where it parasitically castrates its crab host, a function that makes it a potential biocontrol agent. I also give a summary of the experiment done by Goddard at al. 2005 that tested for the host specificity of *S. carcini*, an important factor in determining the possible consequences of applying the parasite in a marine environment. I conclude that there are circumstances where the application of *S. carcini* would be appropriate, such as when *C. maenas* is in low numbers directly after migrating to a new environment. Another circumstance would be when there are no other economically or biologically important crab species in the ecosystem where *C. maenas* is invasively present. More field data and information on the life history of the

parasite must be collected in the future to better understand the potential consequences of introducing *S. carcini*.

Introduction

Invasive species can damage ecosystems in several ways, including damaging habitats and reducing food supplies for native species, thereby upsetting the food webs, and destabilizing the ecosystem (Goddard et al. 2005). When the invaded ecosystem contains commercially important species, these negative impacts to the environment may also have a severe economic impact on the fishing industry (Goddard et al. 2005). For example, the European green crab, *C. maenas,* is an invasive species that causes damage to the ecosystems it invades (Bergshoeff et al. 2019). *C. maenas* has invasively established itself in Australia, Japan, South Africa, and North America (Thresher et al. 2000).

A natural enemy of *C. maenas* is *Sacculina carcini,* in the phylum Arthropoda, a castrating, parasitic barnacle, that has developed a sessile way of life and infects many species of crabs (Pasternak et al. 2005; Lützen 1984). How *S. carcini* infects crabs such as *C. maenas* can be broken up into three phases: 1) the infective phase; 2) the endoparasitic phase, meaning the phase where the parasite is developing inside the host; and 3) the reproductive phase (Lützen et al. 2018). As noted above, *S. carcini* is sessile, and as such, the adult form of the *S. carcini* cannot move when inside its host.

Therefore, in the first infective phase, the larval form, known as the cyprid, must find the next host to infect (Pasternak et al. 2005). The larvae are diecious, meaning they have separate sexes. There is a size difference between the male and female cyprids, with the male cyprid being larger than the females (Hoeg 1984). Also, each sex accomplishes different tasks. In particular, the female larvae attempt to find an uninfected host crab, either male or female, to infect (Pasternak et al. 2005). On the other hand, the male larvae try to find a host that has already been infected by the female larvae and that has not yet been inseminated by a male larva (Pasternak et al. 2005). The cyprid larvae accomplish this search by using their chemosensory organ, the lattice organ, which is located dorsally on the carapace and detects metabolites of its host in the water column (Pasternak et al. 2005).

The cyprid larvae also possess a distinct type of crustacean seta, known as aesthetascs, that have a cuticle thin enough for dissolved substances to permeate it, and very high amounts of ciliary branches per setae (Pasternak et al. 2005). This unique morphology leads to a hyper sensory ability of the larvae, which serves them well in the search for hosts (Pasternak et al. 2005). While both males and females have a fourth segmental aesthetasc that likely allows them to detect the host animal itself, the male cyprid has a male-specific aesthetasc that is most likely stimulated by the metabolites released by female juvenile parasites (Pasternak et al. 2005). This male specific feature of the chemosensory organs, combined with the fourth segmental aesthetasc, allows male cyprids to find a host that is infected with a female cyprid (Pasternak et al. 2005). In comparison, the female cyprid only needs to find an uninfected host, and therefore, only needs the fourth segmental aesthetasc to do so (Pasternak et al. 2005).

The second, endoparasitic phase to take place occurs when a *S. carcini* larvae finds a suitable host. While it is possible for multiple female cyprids to settle on a host crab, multiple cyprid infections are rare (Rees and Glenner 2014). When a successful female cyprid finds an uninfected, preferably small crab, it attaches to the crab and forms what is called a kentrogon, a dart larva stage which helps the female larvae inject a group of cells inside the crab (Walker 1985). Once inside, the cells will form the interna, an internal root system inside the hemolymph of the host, which receives nourishment from the surrounding tissues and eventually forms an abdominal nucleus which breaks through the ventral cuticle of the crab's abdomen to form a structure called the virgin externa (Walker 1985; Lützen et al. 2018). The virgin externa, once exposed to the exterior of the crab, will attract male cyprids and will not become sexually mature or grow until a male implants itself and becomes what is called a dwarf male (Rytter Jensen et al. 2019).

In the third, reproductive phase, the male cyprids attach themselves to a structure of the virgin externa called the mantle opening, and inject cypris cells into the mantle cavity of the externa, which eventually form spermatazoa, essentially sperm, which further develops the externa and allows the parasite's ovaries to mature (Walker 1985). The mature female parasite then releases nauplii, a planktonic larval stage, which develop and metamorphose into cypris larvae that then leave the crab to start the cycle again (Thresher et al. 2000). The larvae then look for their respective targets, either an already infected host for the male cyprids, or an uninfected host for the female cyprids (Thresher et al. 2000).

One consequence of an *S. carcini* infection is that the host is castrated and if a male is infected, it will take on feminine behavior and morphology (Mouritsen and Jensen 2007). More specifically, there is a re-division of the male crab's abdomen which broadens to a size comparable to that of a healthy female; the claws of the host crab shrink to a female's claw size; and the walking legs decrease in length, mimicking the female legs (Kristensen et al. 2015). In addition, by making the host morphologically feminine, it reprograms the male crab to behave as a female, meaning the host will protect the parasite's eggs as if it were its own, giving the parasite a higher fitness (Pardal et al. 2013). If a female is infected, the castration destroys the crab's ovaries, preventing the crab from reproducing. In this way, the ability to castrate the host, male and female, allows the parasite to direct energy that would have gone to the crab's reproduction to the reproduction and growth of the parasite (Pardal et al. 2013).

Another consequence of infection, is that the host crabs will forever stop moulting, leading to a higher abundance of epibionts, organisms that attach to other organisms, which can attach to the host permanently and potentially cause a decrease in fitness of the host (Mouritsen and Jensen 2007; Lyu et al. 2020). It has also been noted in a study by Glenner and Werner (1998), that *S. carcini* are more attracted to crabs that have recently moulted, meaning small crabs that have short intermoult intervals are more at risk of getting infected. This increased targeting may be because crabs that have recently moulted give off specific chemical and physical signals that attract more parasitic settlement (Glenner and Werner 1998).

Proposal

With the knowledge of *S. carcini's* effects and life cycle, I see the potential for using this crab hacking parasite as a possible way of controlling the invasive crab species *C. maenas,* that harms commercially important native crab species. I propose that funding should go to research that explores the possible consequences of introducing *S. carcini* in a natural environment. *C. maenas,* also known as European shore crab or European green crab, have been found on both the east and west coasts of North America, including in most of the estuaries between Morro Bay, California, and Vancouver Island in British Columbia, southern Australia, Tasmania, and South Africa (Goddard et al. 2005). *C. maenas* are problematic for mariculture by being generalist predators, preying on bivalves, gastropods and polychaetas (Goddard et al. 2005).

C. maenas have also been found to destroy eelgrass beds which serve as a habitat for species like cod, herring, and lobster (Bergshoeff et al. 2019). In addition, *C. maenas* has been found to possibly impact native predators such as shorebirds and Dungeness crab, *Cancer magister,* by over consuming native prey species and creating a food limitation (Goddard et al. 2005; Jamieson et al. 1998). This not only shifts the food webs of these ecosystems, possibly leading to unpredictable consequences, but it also affects commercially important species for the fishing industries (Goddard et al. 2005). For example, between 2012 – 2015, over 53.5 million pounds of Dungeness crab was harvested along the West Coast of the U.S., generating revenue of almost $200 million (Mao and Jardine 2020).

As expected, the potential economic losses due to this invasive species have led to many efforts to control *C. maenas* such as with poisoning, traps, and fences (Goddard et al. 2005). However, these control methods have several downsides, such as being labor intensive, expensive, and effecting non-targeted species (Goddard et al. 2005). The effort and consequences from these practices also do not work towards the goal of eradicating the invasive population, but instead, work to keep *C. maenas* in manageable numbers (Bergshoeff et al. 2019). This can then lead to the green crabs potentially adapting through natural selection to avoid or escape these control methods, making it harder to keep the invasive population under control (Morrison et al. 2007).

As such, the problem of invasive populations of *C. maenas* currently has no permanent solution and leaves the possibility of *C. maenas* to continue to invade and damage other ecosystems. However, a possible solution is to introduce *S. carcini* into environments with invasive populations of *C. maenas* in order to parasitically castrate the population and possibly eradicate or severely lower the number of invasive crabs in an ecosystem. *S. carcini* must be introduced through human intervention, because most invasive species come to a new environment without their natural parasite (Messing and Wright 2006). This would be a form of biological control, or biocontrol, i.e. "the reliance upon natural enemies to attack pest organisms that damage human interests" (Secord 2003; Bateman et al. 2017).

While there are different types of biocontrol, the one I propose is called classical biocontrol, which consists of introducing a natural enemy from the pest's natural range to the pest's new range (Secord 2003). The decisions of what to use and where to use a biocontrol agent are usually determined by host specificity and the ability to maximize damage to the pest (Secord 2003). The process of identifying and implementing a biocontrol greatly varies, depending on what country the ecosystem is in, the type of ecosystem the pest is in, what other organisms are found in the ecosystem, etc. (Secord 2003). However, the first step is usually host specificity testing, in order to determine the range of hosts that could be potentially infected (Secord 2003).

In a study to determine the host specificity of *S. carcini,* Goddard et al. 2005 exposed four crab species to *S. carcini,* where the four crabs were economically important and known to co-exist with green crabs (*C. maenas*) in different locations. Three California native crabs were exposed, *Hemigrapsus oregonensis*, *H. nudus*, and *Pachygrapsus crassipes* (Goddard et al. 2005). In addition, Dungeness crab (*Cancer magister*), was exposed (Goddard et al. 2005). The infected native crab species were then compared to infected non-native *C. maenas* in order to determine if the larvae of *S. carcini* could settle on the native crab species and if they could penetrate their cuticle (Goddard et al. 2005). In addition, the study was looking to see if *S. carcini* showed preference for native crabs vs. *C. maenas,* what effect the growth of the interna had on native crab species, and if *S. carcini* could develop to maturity in native species of crabs (Goddard et al. 2005).

Their results showed that *S. carcini* was able to settle on and infect all four native crab species, but with post-molt green crabs receiving more cyprid settlement (Goddard et al. 2005). This suggests that *S. carcini* prefers its co-evolved host, i.e. a host that has evolved defenses in response to the negative fitness effects of a parasite, and where the parasite has evolved ways to breach those defenses (Goddard et al. 2005; Ritchie and Hoeg 1981). If the amount of cyprids that settled were few, some of the native crab species were able to produce an effective immune response to the barnacle, including many *H. oregonensis* and few *C. magister* (Goddard et al. 2005). However, the green crabs overall were able to produce more of an innate resistance to the barnacle, possibly arising from the co-evolution of the two species (Goddard et al. 2005). If many cyprids did settle on a native species of crab, the parasitic process did not go normally, as the interna did not develop as it typically does in *C. maenas,* leading to no production of externa (Goddard et al. 2005). This resulted in damage to the crab's nervous system and increased mortality of the crab and parasite (Goddard et al. 2005).

The above study brings up the important implication that *S. carcini* does not develop enough to reproduce in native species of crab. This means that eventually, if there were no more invasive *C. maenas* populations in an ecosystem, *S. carcini* would eventually die out, unless it produced an adaptation that allowed it to reproduce in native crab species. However, as the population of *S. carcini* dies out along with the invasive *C. maenas,* it would cause an increase in the mortality of non-targeted crab species, due to the damage to the central nervous system of the crabs. So, even though the parasite would not be able to spread using the native crabs, it would still damage the population of native crabs, which could include crabs that are economically important, such as Dungeness crab, *C. magister.* Such a negative effect must be weighed against the possibility of saving the ecosystem from invasive green crab species, and saving the fishing industry of bivalves, gastropods, and polychaetas.

A possible solution to this problem would be to employ *S. carcini* when the population of *C. maenas* is in low numbers. As noted above, only the host green crabs can spread the parasites, as the parasitic process does not work correctly in native crabs. Therefore, although some number of the native crabs will also die, if there is only a small population of *C. maenas*, the parasite will die out with the green crabs faster than if there were many green crabs present. An example of when the invasive crab species might occur in low numbers would be when it recently moves to a new location. Another situation that might be appropriate would be when there is an invasive species in an ecosystem with little to no other crab species that are economically or biologically important. This would be appropriate because there would be little risk to the established ecosystem from introducing the invasive crab specific parasite.

In order to truly know the consequences of using this biocontrol agent though, more testing should investigate possible behavioral changes that could lead to non-targeted crab species becoming infected. For example, more testing should be done into the migration patterns of both the host and the parasite, specifically, how populations of green crabs are able to traverse easily into new environments. With this knowledge, measures might be available to stop an infestation before it happens. For example, if green crabs are somehow attaching themselves to fishing ships or gear that move between ecosystems, there can be inspections done to make sure there are no invasive stowaways before they enter a new environment.

Also, more testing should be done in order to determine how the host crab species moves in a given environment and how it would affect administering the parasite. For example, it was found in a study by Waser et al. (2016), that the movement and migration patterns of crabs change once the hosts are infected, e.g. they migrate towards deeper water. This could cause a problem, for example, if the migration of infected crabs causes them to move into other ecosystems, possibly spreading even more and infecting more native crabs. This would then be an unintentional negative impact of this biocontrol method, and more research should be done to look into the migration patterns of the invasive crab species and how they might change due to the parasite.

I also recommend that more research be done to collect field data on encounter rates of the cyprid larvae and on the life history of the parasitic barnacles (Goddard et al. 2005). Encounter rates would give us information on how much of the parasite we would need to introduce for a specific number of invasive crabs in an ecosystem. In addition, the life history would give us the knowledge of how the parasite evolved with the crab and vice versa, in order to predict what possible resistance the crab could develop to the parasite. We could gather this field data by fencing off areas where the green crabs have invaded an ecosystem with native crab species. In this way, we could administer the parasite and observe the effects without the parasite spreading uncontrolled.

Conclusion

I see the potential for using the crab hacking parasite *S. carcini* as a possible way of controlling the invasive crab species *C. maenas* that harms commercially important native crab species. The use of a biocontrol agent to control a pest species has risks, mainly because of the possibility of changing an ecosystem in a negative, irreversible way. However, with the right testing and the right procedures it might be used successfully to save an ecosystem and protect human industries. More particularly, I believe there are opportunities to use *S. carcini* to control the spread of *C. maenas,* either when there is a new low population of *C. maenas* in a new location or when there are no other important crab species located in the ecosystem. More testing and funding should be put into studying the migration patterns of both the host and the parasite and how behavior of *C. maenas* changes once infected. More field data should also be collected on encounter rates of the cyprid larvae and on the life history of the parasitic barnacles. With this information, we can achieve a better understanding of the potential of *S. carcini* as a biological agent and how to use it safely.

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