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Biological Control of Invasive Green Crabs: a New Rapid and Reliable
Safety Test of a Proposed Control Agent

Final Report for Project R/CZ-162

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Introduced marine species are a major environmental and economic problem. The rate of these biological invasions has substantially increased in recent years due to the globalization of the world's economies. The damage caused by invasive species is often a result of the higher densities and larger sizes they attain compared to where they are native. A prominent hypothesis explaining the success of introduced species is that they are relatively free of the effects of natural enemies. Most notably, they may encounter fewer parasites in their introduced range compared to their native range. Parasites are ubiquitous and pervasive in marine systems, yet their role in marine invasions is relatively unexplored. Although data on parasites of marine organisms exist, the extent to which parasites can mediate marine invasions, or the extent to which invasive

parasites and pathogens are responsible for infecting or potentially decimating native marine species have not been examined.

Our project had four main objectives. 1) Establish general guidelines and methodologies to assess the safety of natural enemies as biological control agent against introduced marine pests. 2) Develop a rapid, reliable and conservative safety test for *Sacculina carcini* as a biological control agent against the European green crab, *Carcinus maenas*. 3) Develop methods to efficiently infect large numbers of green crabs. 4) Determine the mechanisms of host specificity with respect to stages in the life cycle of *S. carcini*.

We developed a theoretical framework to model invasion success and examine the evidence for a relationship between parasite presence and the success of introduced marine species (Torchin et al. 2002). For this, we compared the prevalence and species richness of parasites in several introduced populations of marine species with populations where they are native.

Introduced species often seem to perform better than do populations of conspecifics in their native range. This is apparent in the high densities they may achieve or the larger individual sizes they attain. To test the hypothesis that introduced green crabs are released from the pressure of its native parasites where it has been introduced, we conducted a global assessment of the effect of parasitism and predation on the ecological performance of European green crab

populations (Torchin et al 2001). In Europe, where the green crab is native, crab body size and biomass were negatively associated with the prevalence of parasitic castrators. When we compared native crab populations with those from introduced regions, limb loss (an estimator of predation) was not significantly lower in introduced regions, parasites infected introduced populations substantially less and crabs in introduced regions were larger and exhibited a greater biomass. Our results are consistent with the general prediction that introduced species suffer less from parasites compared to populations where they are native. This may partly explain why the green crab is such a successful invader and, subsequently, why it is a pest in so many places.

The European green crab, *Carcinus maenas*, is an invasive marine predator spreading along the west coast of North America. Its adverse impact on native animals has been demonstrated and further costs are anticipated as these pests become more widespread and abundant (Grosholz and Ruiz 2000). We conducted experiments on the host specificity of a natural enemy of the green crab, the parasitic barnacle, *Sacculina carcini*, to provide information on the safety of its use as a possible biological control agent of this introduced pest. Four species of non-target, native crabs (*Hemigrapsus oregonensis*, *H. nudus*, *Pachygrapsus crassipes* and *Cancer magister*) were exposed to infective larvae of *S. carcini*. In simultaneous exposure tests, using juvenile green crabs, and either *C. magister* or *H. oregonensis*, cyprid larvae of *S. carcini* settled on a higher proportion of green crabs. They also settled in higher numbers on

individual green crabs than they did on individuals of either native species. In all exposure trials combined, settlement by *S. carcini* on the four native species ranged from 33 to 53% of the crabs exposed, compared to 79% of all the green crabs exposed. A majority of both native and green crabs settled on by *S. carcini* became infected by the parasite, especially when settled on by more than three cyprids. Infected native crabs suffered damage to their nervous systems and died more quickly than did infected green crabs. The parasite only completed its development to emergence of the external reproductive sac (externa) in infected green crabs. The higher mortality rates of the native crabs than green crabs, as well as the rapid growth of the parasite and gross damage to the host nervous system in the former indicate that *S. carcini* will kill the native crabs before maturing in them. Up to 29% of the native crabs, and possibly a few green crabs, arrested early infections by melanizing the rootlets of the parasite. However, more than one third of the latter did not develop infections, some even after settlement by high numbers of cyprids. Taken together, these results suggest that some green crabs and fewer individuals of the native California species may be resistant to infection to *S. carcini*, and that the parasite is better adapted to locate and settle on green crabs.

Most importantly, *S. carcini* can only complete its development in green crabs. No parasite ever matured in a California native crab. Hence, for *S. carcini* to have any non-target impact it must become established in green crab populations. The higher the prevalence in green crabs, the more likely *S. carcini* is to be an effective biological control agent of the pest species. So, we can now

demonstrate an unexpected positive relationship between the effectiveness of *S. carcini* as a green crab biological control agent, and its safety risk to non-target native crabs. A cost-benefit analysis is now simplified and feasible. Since green crabs have been shown to have a very strong negative impact on some California native crabs (Grosholz et al. 2000), we can now model these two possible impacts. The direct damage due to *C. maenas* on these native crabs can be compared with the potential spillover of *S. carcini* to these native crabs, as it reduces the abundance (and thus the impact) of *C. maenas*.

These experiments called for several lines of further research. There are European crabs, closely related to our native crabs (congeneric) that have never been reported as parasitized by *S. carcini*, even though these crabs may be found under the same rock as a sacculinized green crab. One such species is *Pachygrapsus marmoratus*; which is abundant along the southwest coast of Europe. We exposed these crabs to *S. carcini* under the experimental conditions used in the host specificity tests of the California native crabs above (Kuris et al in prep). The cyprids of *S. carcini* readily attached to *P. marmoratus*. But, unlike the exposures to either the green crab or to the California native crabs, all early interna stages of *S. carcini* in *P. marmoratus* elicited a melanization response and died in the thoracic ganglion of the host. This strongly suggested that these European crabs, perhaps under a long period of attack by this potential natural enemy, have evolved an effective cellular host defensive response.

We were then able to use the melanization host to early interna stages in the thoracic ganglion of *P. marmoratus* as a technique to, for the first time, determine if in fact a “wrong” host, *P. marmoratus* is attacked by *S. carcini* in nature and to estimate the rate of attack to a non-target host. To do this we went to the Mira River estuary in Portugal, where both *C. maenas* and *P. marmoratus* are abundant, have considerable habitat overlap, and where *C. maenas* is heavily parasitized by *S. carcini* (over 50%, Torchin et al 2001). We examined the thoracic ganglia of *P. marmoratus* for the characteristic melanized lesions associated with its effective host response to *S. carcini*. Since any foreign body can induce a melanized lesion in a crab, we examined outer coast *P. marmoratus* as controls. No green crabs are found within 30 km of our control site. There was no significant difference in the abundance of lesions at the two sites and very few lesions detected at either site. The control site had more lesions than did the site with abundant *S. carcini* being transmitted to green crabs. Hence, we conclude that the major cause of specificity for *S. carcini* is behavioral/ecological. An abundant host in the same habitat, which is susceptible to cyprid attachment, is rarely if ever attacked, despite high prevalence of *S. carcini* in its green crab host.

Our experiments to initiate study of host specificity (described above) were designed to maximize the conditions for infection of crabs by cyprids. Our experiments to examine the ecological basis for the host specificity evident in the limited range of hosts used by *S. carcini* in Europe, and in our *P. marmoratus*

studies described above, were less successful. We conducted exposure trials of green crabs and California native crabs at different volumes. *Cancer magister* was settled on more heavily compared to green crabs and *Hemigrapsus oregonensis* (Torchin et al in prep). Effect of container volume was not significant ($P > 0.05$). The overwhelming effect was whether the crab molted during the experiment ($p < 0.0001$), and there were also significant differences between crab species. ($P < 0.0017$). There was a significant interaction between species and molting status of the experimental crabs ($P < 0.05$).

Cyprids were able to find and settle on all crab species at all treatment volumes. *Cancer magister* may have been more readily parasitized because it was actively molting a greater percentage of the time compared to the other crab species. (To control for host size, we used early instar *C. magister*, but older, more slowly growing, individuals of the other species). The number of female cyprids put into the experimental containers also had a strong effect on cyprid settlement. This is an indicator of brood health and suggests that although we are able to get reliable results under our present experimental protocol, there is still room for significant improvement in the quality of cyprids reared in the laboratory for experimentation.

The experimental studies on host specificity, while still indicating that *S. carcini* may be an effective and relatively safe biological control agent (its safety being tied to its efficacy as a natural enemy), also show that it in no sense is

“absolutely’ safe. Under some conditions it clearly can infect native crabs and kill them even though it cannot complete its development in them. Accordingly, we reviewed the literature on the parasites of green crabs and our extensive parasitological investigations in Europe. We recognized that an interesting and potentially important natural enemy had been overlooked (Kuris et al in review). This is the fecampiid flatworm, *Fecampia erythrocephala*.

The flatworm, *Fecampia erythrocephala*, infects small shore crabs. Part of its life cycle is spent in the hemocoel of its host. At maturity it emerges from the crab. The adult worm attaches to the underside of a rock where it secretes a cocoon and produces eggs. Although *F. erythrocephala* was reported to be common in areas of England and France, most of the reports on its biology predate the 1950’s. We provided an ecological assessment of *F. erythrocephala*, reporting its habitat distribution, abundance, host specificity, size-specific prevalence, frequency distribution among hosts, effect on host growth, and its site specificity within these hosts (Kuris et al in review). At the Isle of Man and near Plymouth, *Fecampia erythrocephala* cocoons were generally abundant on the undersides of rocks in the knotted wrack and serrated focus zones. Infected crabs were also most common in these habitats. Both *Carcinus maenas* and *Cancer pagurus* were parasitized at similar prevalences, however the former crabs were relatively much more common in the habitats where the worm cocoons were abundant. We did not find *F. erythrocephala* in crabs larger than 11 mm carapace width and prevalence decreased significantly with crab size.

Prevalences reached 11% in areas where cocoons were abundant. Together with the large size of these worms relative to the size of the host crabs and the observations on worm emergence, these life history features indicate that *F. erythrocephala* is a parasitoid of young shore crabs. Parasitoids are intimate consumers, common in terrestrial systems, in which a single infection invariably causes death of the host. *Fecampia erythrocephala* cocoon abundance is often high in localized areas and size prevalence information suggests that worms mature rapidly in these crabs. This suggests that *F. erythrocephala* is an important contributor to crab mortality and to the ecology of shore crabs at these sites.

To summarize, we have developed effective protocols to expose and infect crabs with the rhizocephalan barnacle, *S. carcini* at a scale sufficient to conduct ecological experiments. We can readily infect several non-target native California species with *S. carcini*, but development of the parasites in those unnatural hosts is impaired, highly neurotropic, and all such infected hosts die before the parasite can mature. This leads to the conclusion that safety and efficacy, formerly considered to be separate considerations, are inextricably linked. There is only risk of collateral damage to non-target crabs if the pest crab can become heavily parasitized by *S. carcini* in nature. Modeling should be able to parameterize and assess this risk.

A European crab, related to one of the native California crabs, can also be infected in the laboratory, but the parasite always dies and is melanized by host cellular defensive response. No evidence of such lesions can be detected at a location in Europe where that crab co-occurs with *C. maenas* and where *C. maenas* is heavily parasitized by *S. carcini*. This leads to the conclusion that a major element of host specificity for *S. carcini* is behavioral or ecological. A wrong host, readily settled upon by cyprids under our laboratory conditions is rarely, if ever, attacked by cyprids in nature. Ecological aspects of host specificity have been little studied and should be critically investigated.

Another natural enemy, the flatworm, *F. erythrocephala*, is a parasitoid and a major mortality source for juvenile green crabs in certain habitats. Its host specificity and its impact on host populations merits further study to evaluate it as a natural enemy for biological control of introduced populations of the European green crab.

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