Augmentation of trematode parasite *Euhaplorchis californiensis* for conservation of coastal birds in California

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FISH 406 Parasite Ecology

December 8, 2017

Abstract

The trematode parasite *Euhaplorchis californiensis* is deeply integrated in the community structure of salt marshes in southern California. It utilizes California horn snails, California killifish, and shore birds for trophic transmission to complete its life cycle. It is known to increase conspicuous behavior in the killifish to increase the likelihood of predation by birds. The augmentation of the parasite in other parts of California may increase accessible food for shorebirds where majority of populations are declining due to habitat loss. Control and treatment experiment will be proposed using fecal samples containing trematode eggs of infected shorebirds to increase the abundance of *E. californiensis* and the conspicuous behavior of killifish. The use of *E. californiensis* to increase accessible prey for shorebirds may be an alternative to large scale saltmarsh restoration for conservation of bird populations in California.

Although parasites can be a threat to public health and other animals, some play an important ecological role in the community structure within an ecosystem. Parasite ecology is often times studied to find cause and method of control to reduce infection rates (Labaude et al. 2015). Parasites' connection to the community structure in the ecosystem is often disregarded (Lefèvre et al. 2009). Parasites depend on their host for survival and often depend on the host's behavior to infect another host (Lafferty and Kuris 2012). How much parasites utilize host manipulation methods and its impacts on host behavior are increasingly studied (Kuris 2005). Parasites with complex life cycles may use parasite-increased trophic transmission, where the parasite manipulates the host's behavior to increase the probability of predation by its higher trophic level host (Lefèvre et al. 2009). If the parasite has a high incidence of infection within the host population, manipulation can decrease abundance of prey and, to a lesser degree, increase its predator population (Lafferty 1992). Other organisms in close contact with hosts could be impacted by the shift in abundance and indirectly affect the food web in the ecosystem (Lafferty and Kuris 2012). There may be reason to augment parasites that have no known negative effects on humans to control host abundances and possibly become an alternative to large scale habitat restoration.

Euhaplorchis californiensis is a trematode parasite that has a complex life cycle (i.e., a life cycle in which more than one host species are used) ((Huspeni and Lafferty 2004). Distributed widely in Southern California, *E. californiensis* mainly inhabits estuaries, salt marshes and brackish water from Morro Bay in central California to southern Baja California, where the intermediate host, *Cerithidea californica* (the California horn snail), is found (Shaw et al. 2010). Like many trematodes, *E. californiensis* has a three-host life cycle, where intermediate hosts are used for asexual reproduction and growth, while the final definitive host is the destination for sexual reproduction (Lafferty and Morris 1996). The first intermediate host, California horn snail (*Cerithidea californica*), consumes parasite eggs, which hatch as miracidia (Lafferty and Morris 1996). Miracidia are small, ciliated stages that penetrate the gut wall to move to the digestive gland of the snail (Lafferty 1993). There, they develop into a mother sporocyst and begin asexual reproduction to create rediae, which are sac-like and absorb nutrients (Martin 1950). Rediae consume the snail host's gonads and sexually castrates the host (Lafferty and Morris 1996). The snail no longer can reproduce and houses the production of parasite rediae (Lafferty and Kuris 2009). The rediae create cercariae that are shed by the snail and is free swimming (Lafferty 1993). The cercariae are the infective stages of the parasite that actively swim and penetrates the skin of the second intermediate host, California killifish (*Fundulus parvipinnis*) (Shaw et al. 2010). There, they encyst as metacercariae mainly in the brain and wait for the second intermediate host to be eaten by the definitive host (Lafferty and Morris 1996). Once eaten, the parasite hatches from the cyst and reproduces sexually within the gut of shorebirds that consume killifishes (Shaw et al. 2010). The feces of the birds contain eggs, which are spread in the environment and continue the life cycle (Lafferty and Morris 1996).

E. californiensis is known to affect host behavior and host abundance which impacts community structure (Labaude et al. 2015). First, the abundance of its first intermediate host, California horn snail, is, in part, controlled by the parasite (Lafferty 1993). Population is reduced by reduction in eggs released and slow growth caused by the parasitic castration (Lafferty 1993). The trematode also manipulates the killifish while encysting in the brain, causing the fish to display conspicuous behaviors (Lafferty and Morris 1996): surfacing, flashing their sides, shimmying, and jerking. The increase in *E. californiensis* metacercariae on the fish brain lead to displaying four times more conspicuous behavior than uninfected fish (Lafferty and Morris

1996). This is thought to be a manipulation caused by the parasite to increase predation by birds and successfully transmit to the final host (Lafferty and Morris 1996). Infected fish are thirty times more likely to be eaten by birds (Lafferty and Morris 1996). Although the adult parasites live in the bird's intestine, the energy gained from the fish outweighs the small energy cost taken by the parasite (Lafferty 2008). The increase in number of easily accessible prey will benefit bird populations by increasing net energy flow (Labaude et al. 2015). The increase in prey items can even allow organisms to persist in areas where they usually cannot (Lafferty and Morris 1996).

Populations of shorebirds have been declining for the past few decades mainly due to loss and degradation of habitats (Dybala et al. 2017). California's marshlands are important foraging habitat and migratory pathways for wintering, migratory birds and other local shorebirds (Stralberg et al. 2011). So far California has lost over 90% of its historical wetlands due to agricultural and urban development (Stralberg et al. 2011). Coastal bird conservation becomes important because birds provide ecosystem services, such as pest control (Jedlicka et al. 2011) and reduction of snail and other lower trophic organisms from overcrowding (Moreira 1997). As a conservation method, saltmarsh restoration has successfully increased bird abundance and diversity within the location (Huspeni and Lafferty 2004). In order to reach conservation goals to match the abundance of coastal birds in California, large areas of agricultural land would need to be restored (Stralberg et al. 2011). Many of these areas are not recognized as conservation sites and it would be difficult to restore large areas of agricultural land (Stralberg et al. 2011). Usually, high priority areas are determined to lower costs and area of restoration (Stralberg et al. 2011).

Due to difficulties in large scale restoration projects, augmentation of *Euhaplorchis californiensis* will be proposed to instead increase food availability for shorebirds in California. As mentioned before, the trematode causes behavior changes in killifish to increase likelihood of predation by birds (Labaude et al. 2015). If trematodes can increase the likelihood of fish predation, it would increase the amount of easily accessible food and possibly decrease death of shorebirds caused by starvation. This will also allow more foraging opportunities for wintering birds. This requires increasing trematode parasites within individual killifish as majority of killifishes are infected with some metacercariae (Shaw et al. 2010) and behavioral changes were seen more in heavily infected killifish (Lafferty and Morris 1996). There is no worry of introducing new or invasive species because native host species will be used. *E. californiensis* is also highly host specific and do not infect many species other than its targeted hosts, California horn snail, California killifish, and piscivorous coastal birds (Fredensborg 2014).

A small scale control and treatment experiment will be conducted at the marshes in southern California to increase parasite abundance within one marsh and compare changes with the controlled marsh. Targeted increase in *Euhaplorchis californiensis* has not been conducted before and requires detailed environmental assessment of marshes to determine location best suited for the project and close monitoring of shifts in community. Three separate marshes will be assessed to ensure the presence of all hosts. Abundance of *E. californiensis* will be measured by sampling horn snails and dissecting them to quantify mean number of infections per snail (Hechinger and Lafferty 2005). Killifish will also be sampled within marshes to count the amount of metacercariae encysted on the brain (Shaw et al. 2010). Abundance of snails will be estimated by placing random transects within the marshes and counting snails within those transects (Huspeni and Lafferty 2004). Populations and diversity of coastal birds will be assessed by time-lapse videography to quantify birds at the sites (Hechinger and Lafferty 2005). The initial treatment and control site will have a low abundance of parasite, high abundance of snails, and low abundance of birds. Coastal bird fecal samples from a high parasite abundance and high bird abundance marsh will be sampled to estimate amount of eggs within it. Fecal samples with high numbers of trematode eggs will be placed in snail aggregation masses in one of the marshes with low parasite abundance but high snail abundance. Fecal samples will be placed in March to time with the cercariae shed by the snail in June (Fingerut et al. 2003). As a control, none will be placed in the second marsh. For the treatment, this will increase the number of infected snails and increase the overall cercariae that is shed into the water. This increase in cercariae in the water will likely increase the frequency of parasites that penetrates into the killifish and encyst on the brain. Predation is thought to increase with increase in number of killifish showing conspicuous behavior.

The control and experiment sites will be monitored for three years to assess changes in population of birds utilizing the marshes and shifts in community structure. All measurements will be conducted at both control and experiment marshes. As coastal birds and wintering birds have seasonal variation in distribution, measure of bird population and diversity will be conducted each season (Hechinger and Lafferty 2005). 50 killifish each will be sampled to compare average number of encysted metacercariae in the brain. This will determine whether increasing trematode eggs in the marsh lead to increase of metacercariae in killifish. 100 snails will be sampled to compare infection prevalence among the snails. Sites are monitored for three years for prevalence of trematodes to stabilize (Huspeni and Lafferty 2004). If higher number of birds and a higher number of killifish with high numbers of metacercariae are seen, it is likely that more fish is displaying behaviors leading to predation. Increased bird sightings could mean the location is more habitable than before. Although it is difficult to determine whether death from starvation is reduced, surveys of dead birds can be conducted near the coast and marshes to analyze the probable cause of death. A diet analysis of the birds in both marshes will be conducted to see the difference in diet. Results may not directly reflect the diet at the specific marsh site due to the birds' wide home range, but there may be a differences in how much killifish is consumed. Higher amount killifish in their diet may indicate increase in feeding at the marshes. Although this method cannot completely replace saltmarsh restoration, it may be used in conjunction with small scale restoration projects instead of restoring large areas of land.

Although this project utilizes native host species, there are challenges that may come with augmenting a parasite. First, all hosts must be present for the trematode to establish itself. Locations without horn snails and killifish naturally does not have *E. californiensis* (Shaw et al. 2010) and could not be used to increase available food for birds. This method could not increase shorebird population where environment has been degraded to where parasite's host species cannot survive. Habitable environment is another challenge associated with establishing trematodes. *E. californiensis* has a free swimming infective stage as cercariae and prefers shallow brackish waters that are relatively still (Lafferty and Morris 1996). The cercariae are shed from the snail around June and most are shed during September (Fingerut et al. 2003). *E. californiensis* cercariae are relatively resistant to high temperatures, salinity, and differing pH levels compared to other species of trematode cercariae found in the same snail host (Koprivnikar et al. 2010). Time used to find a host was a major factor in the survival of cercariae (Koprivnikar et al. 2010). Pollution may also be a limiting factor in establishment of *E. californiensis* (Pietrock and Marcogliese 2003). As the parasite spends most of its developmental stage in water inside its host, toxicity levels or type of pollution may impact its transmission and survival (Pietrock and Marcogliese 2003). Its intermediate hosts may also be affected in highly polluted waters and reduce survival of the parasite (Pietrock and Marcogliese 2003). Further study on the pollution tolerance of the cercariae may be needed to determine project location and method of redirecting pollution.

Another important issue with changing the abundance of a parasite is the possibility of a drastic community shift and unintended consequences to the organisms that are part of the food web. Increasing parasite abundance will likely reduce snail density and killifish within the area. However, the horn snail and killifish are some of the most common snail and fish in southern California estuaries and are in no danger of population decline (Shaw et al. 2010). Instead, increasing prevalence of *E. californiensis* may change trematode community structure by reducing other trematodes by competition. Another trematode, *Renicola buchanani*, is found infecting the liver of killifish that are infected with *E. californiensis* (Lafferty and Morris 1996). *R. buchanani* would likely increase in abundance with the help of *E. californiensis* due to needing a bird host to complete its life cycle (Lafferty and Morris 1996). *R. buchanani* is more pathogenic to the bird host, but less abundant in hosts relative to *E. californiensis* (Lafferty and Morris 1996). The benefit of attaining food may outweigh the costs of few pathogenic parasites. *E. californiensis* is one of the most abundant trematode in California and likely suppresses other competing species and may not minimize the ecological roles of other trematodes relative to if they were abundant (Lafferty et al. 1994). In addition, California killifish's main ecological importance is food for shorebirds (Fredensborg and Longoria 2012) and the impact of low numbers of killifish on other fishes may be minimal. Longnose mudsucker, a common fish alongside killifish, may feed on California killifish (Desmond et al. 2000), but further study on ecology and diet of predatory fish is required to understand the impacts on their prey selection.

The augmentation of *Euhaplorchis californiensis* in increasing predation of killifish may help shorebird conservation in southern California estuarine ecosystem. Increasing the prevalence of *Euhaplorchis californiensis* to ecologically assessed sites may benefit declining shorebird community by increasing availability of easy-to-catch prey and provide alternate feeding locations. Further study of this trematode and its interaction with its hosts may increase supporting evidence for benefits and efficacy in augmentation projects. Study will also yield important information about food webs and impacts that parasites have on salt marsh communities.

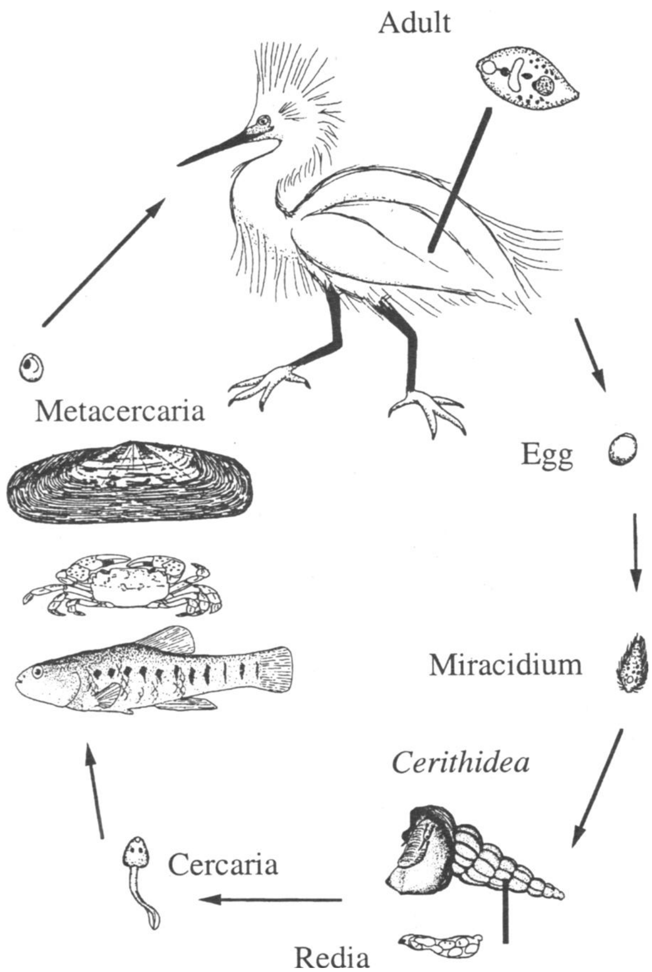

Figure 1. Life cycle of trematodes that use mud snails as first intermediate host. The second intermediate host is specific to the trematode species. (from (Huspeni and Lafferty 2004)

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