

REGULATORY FACTORS AFFECTING LARVAL MOSQUITO POPULATIONS IN CONTAINER AND POOL HABITATS: IMPLICATIONS FOR BIOLOGICAL CONTROL

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ABSTRACT. Empirical studies have shown that populations of larval mosquitoes developing in ground pools are subjected to different mortality factors than populations developing in water-filled containers. In general, larvae in ground pools are limited by natural enemies whereas those in containers are limited by resource availability. Containers also are typically smaller than ground pools and lack appreciable internal primary productivity. These physical and biological features have significant implications for successful implementation of biological control agents. Island biogeography theory suggests that container habitats will support smaller populations of fewer species compared to ground pools, implying that it may be more difficult to establish natural enemies in container habitats. The lack of primary productivity within containers may limit the number of trophic levels and reduce the likelihood of establishing and maintaining predator populations. Most importantly, larval mosquito populations in containers are regulated by competitive interactions, and mortality from natural enemies is likely to be compensatory. These habitat and population characteristics, combined with difficulties in locating and treating containers, suggest that successful control of ground pool mosquitoes using biological control agents is more feasible.

INTRODUCTION

Larval mosquitoes develop in a wide variety of fresh and brackish waters that traditionally have been classified into as many as 20 major habitat types. Recently, Laird (1988) in "The Natural History of Larval Mosquito Habitats" provided a detailed inventory of these aquatic environments and the mosquitoes that inhabit them. Laird's classification divides all the various types of habitats (pools, puddles, containers, etc.) into 9 above-ground types of water and 2 subterranean waters. In contrast, some other workers simply divide these mosquito sources into the 2 broad categories of ground-water habitats and containers. Although this dichotomy oversimplifies what is actually a broad and complex spectrum of aquatic habitats, I think that artificial and natural containers comprise a distinct group with unique ecological properties that are relevant to the fate of biological control strategies targeted at reducing the larval mosquito populations they support.

There are at least 3 principles from the ecological literature that I believe bear directly on the potential effectiveness for using biological agents for mosquito control in the 2 broad habitat groupings of ground-water habitats and containers. These themes address different structural and organizational elements of populations and communities and the habitats in which they are found; specifically, these are the ecological consequences of: 1) habitat size on species number, population levels, and extinction rates, 2) sources of productivity and their relationship to food web organization, and 3) the variable impact of parasites and predators on host and prey popu-

lations in a natural context where other factors are operative.

HABITAT SIZE

The vast majority of artificial and natural container habitats are significantly smaller in physical size (e.g., water volume) than the pools, ponds, or swamps where mosquitoes develop. Instead of being aggregated into a few relatively large pools at ground level, populations of container-developing culicids are spatially arrayed in 3 dimensions into a series of compartmentalized aquatic pockets that are highly variable with regard to physical, chemical, and biological attributes. Most workers involved in the practical business of controlling mosquitoes have experienced first hand the logistical problems associated with locating and treating these widely dispersed and often cryptic sources; such habitats are very different from the larger, and I would argue more homogenous, ground-water habitats. Ecologists have long recognized that habitat size has important inherent consequences on the organization, size, and persistence of resident biological communities. Some of these observations were formalized into a conceptual framework nearly 30 years ago with the publication of "The Theory of Island Biogeography" (MacArthur and Wilson 1967). This text introduced a theoretical model for predicting the species composition of oceanic islands as a consequence of island age, size, and distance to mainland sources of colonizing species. Three important and perhaps intuitive predictions of island biogeography theory are that smaller islands: 1) will contain

fewer species, 2) will support smaller populations, and 3) will exhibit higher rates of extinction compared to larger islands. These and other predictions of this model were validated by careful analyses of island biota, but application to many other biological systems also has been fruitful. Since its original publication, tenets of the theory have been applied to explain a spectrum of natural phenomena of size and scale such as the number of herbivorous insects found on plants of different sizes and the total number of species parasitizing hosts that have different geographic areas (e.g., Cornell and Washburn 1979). For example, the number of ectoparasitic mite species found associated with individual species of rodents in North America is proportional to the geographic size of each rodent's range (Dritschilo et al. 1975). Thus, the size of an individual, the range of a species, and the area of a habitat are all ecologically analogous. This illustrates that there are certain inherent and predictive consequences of size in biological systems, and that size-related processes operate at the individual, population, and community levels.

The tenets of this theory predict that aquatic container habitats will support fewer species with smaller population sizes than larger pools and that extinction rates, including those of natural enemies, will be higher regardless of whether these agents are naturally occurring or introduced. Although faunal inventories are rarely complete, Laird (1988) reports species diversity data from 10 larval mosquito habitats; among these, 8 different ground pool habitats supported approximately 100–275 species, an intermittent puddle supported 62 species, and a container habitat only 45 species. Similarly, we have found the aquatic immatures of more than 20 insect species inhabiting several small woodland vernal pools in northern California, whereas a survey of more than 50 nearby tree holes yielded only 6 species. Interestingly, no culicids were represented among the 20 vernal pool species, whereas 2 were found in water-filled tree holes.

I am not aware of any survey data that compare the long-term stability of natural enemy complexes associated with mosquito populations in pool and container habitats. During 9 years of monitoring the natural enemy complexes within numerous tree holes inhabited by larvae of the western tree hole mosquito, *Aedes sierrensis* (Ludlow), we have found colonization and extinction rates of less than 10% per year for the 2 major mosquito parasites, the mermithid nematode *Octomermis troglodytis*, and the ciliated protozoan *Lambornella clarki*; whether such rates are "high" or "low" needs to be evaluated with comparative natural enemy data from pool habitats. The positive correlation between the

size of habitats and the populations they support is clearly true for water-filled containers. For example, maximum water volume is the single best predictor of the number of *Aedes sierrensis* adults that emerge from tree holes in California, and it accounts for 50% of the variation in production among holes (Washburn et al. 1988a). No other single physical, chemical, or biological factor we have investigated to date has been predictive (Washburn and Hartmann, unpublished data). Thus, I believe that these ecological correlates of habitat size will affect specifically the outcome of biological control introductions into container habitats because they may set limits on the number of natural enemy species, the size of their populations, and their extinction rates.

FOOD WEBS

The second ecological theme concerns the structure of food chains, or more precisely food webs, as trophic interactions among species within the communities where mosquito larvae develop are rarely strictly linear. Primary productivity is the basis for all life and is defined as the amount of carbon dioxide incorporated or "fixed" into organic molecules by the living organisms in a specified area over a certain period of time. With few exceptions, nearly all primary productivity can be attributed directly to the photosynthetic activities of green plants. For all habitats, the amount of primary productivity is a powerful determinant for the number of resident species and their population sizes because of inherent limits on conversion and utilization efficiencies at the various trophic levels (Begon et al. 1986); thus, primary productivity can set the upper limit on the number of trophic levels a community can support.

Ground pool habitats exhibit variable levels of internal productivity from aquatic plants, emergent vegetation, algal mats, and plankton, whereas most container habitats have almost no internal productivity. In the absence of measurable primary productivity, the food webs of water-filled containers are based on the decomposition of dead organic matter, most often primary productivity that falls into the habitat in the form of leaf litter and other detritus (e.g., Carpenter 1983). Decomposition of this detritus by microorganisms such as bacteria and fungi slowly releases nutrients, which are then harvested by the first level of consumers. Because of this nutrient base, food webs within container communities tend to be broad rather than long, with larvae of pest mosquitoes at or near the top; in contrast the trophic structure of ground pools and ponds incorporates more levels and supports a greater number of species, usually including one or more

mosquito predators. Such structural features suggest that predation, either natural or manipulated, may be intrinsically more important for larval mosquito population dynamics in pools. The relative paucity of naturally occurring mosquito predators in water-filled containers such as tree holes and tank bromeliads is probably a consequence of both small habitat size and detritus-based food web structure.

REGULATORS OF MOSQUITO LARVAL POPULATIONS

Finally, and foremost, is the issue of what factors actually regulate larval mosquito populations developing in pools and containers. Are they controlled from the bottom up by resource availability, internally by density-dependent mechanisms, or from the top down by predators? At the root of all biological control efforts is the notion that the ultimate impact of natural enemies is to numerically reduce target populations. In reality, of course, natural enemies do not function in an ecological vacuum; the performance and effects of both biological and chemical mortality agents are modified by other elements in the environment. Both theoretical and empirical studies have shown that the activities of natural enemies, overlaid on the template of these other factors, can produce 3 basic effects on the numerical abundance of target populations such as those of larval mosquitoes.

First, predators and parasites may reduce the number of prey or hosts that successfully complete development; when this occurs, the mortality they cause during the larval stages is termed *additive*. That is, mortality from the natural enemies is added to the mortality caused by other factors, and as a result, habitats with natural enemy populations produce fewer pests than those without them. This kind of response by target populations is the fundamental assumption and goal of all biological control strategies. A second possibility is that natural enemies may have no impact on the numbers of hosts completing development. This effect is termed *compensatory*, referring to the fact that these agents eliminate individuals that would have died from other mortality factors anyway. When this occurs, habitats with and without natural enemies realize the same rates of mosquito emergence success, and the target population is not controlled. In the third case, populations with natural enemies actually realize higher rates of success than those without. This response is termed *depensatory* or overcompensation, and although rarely demonstrated in natural populations, this response

should be considered for programs based on biological control agents.

The potential for these kinds of responses by larval mosquito populations has been raised repeatedly in the literature by various authors (e.g., Service 1983). Despite the inherent need for careful studies that confirm additive mortality and the regulatory potential of each biological control agent *in situ*, few empirical investigations have addressed the issue directly. Part of this may be due to bias within the biological control community, where there appears to be an underlying assumption that all natural enemies cause additive mortality, and that the more species that are introduced into the habitat the better the chances of successfully controlling pests. I think part of this philosophy is derived from the agricultural settings where many biological control programs are implemented. I would argue that these are artificial ecological constructs that differ in very significant ways from most sources of mosquito production, with the possible exception of tire yards. Crop systems represent very large and relatively homogeneous resource pools for agricultural pests. Selective crop breeding over the past several decades has enhanced the quality of these food resources both for us and for the pests that infest them with a concomitant reduction in naturally evolved defenses of plants such as secondary plant compounds, which can function as antifeedants and toxins, and physical structures such as trichomes, which can damage the tissues of herbivores. In the artificial environments of modern agriculture with their abundant high-quality resources, it may well be true that more and diverse kinds of biological agents actually do result in better pest control. My own personal experiences with biological control programs for both plant protection and mosquito suppression suggest to me that the latter task is a more complex and challenging issue. With mosquitoes, however, we have the important advantage that we are not concerned with larval feeding damage.

LAMBORNELLA: A POTENTIAL BIOLOGICAL CONTROL AGENT

During the last 11 years, a major research area of our laboratory at Berkeley has been to evaluate the biological control potential of endoparasitic ciliates in the genus *Lambornella*. Most of our efforts have focused on *Lambornella clarki*, and its natural host, *Ae. sierrensis*. Both the larval host and its parasite are widely distributed in California in water-filled tree holes in forested habitats of the coastal range and the Sierra Ne-

vada. *Lambornella* initiates chronic and fatal infections in its larval hosts and has many desirable attributes of a promising biological control agent. The ciliate is highly specific to mosquitoes and does not infect other invertebrates; it is completely harmless to vertebrates. It has a free-living bactivorous form that is easily cultured in the laboratory and may persist for years in natural tree holes in the absence of mosquito hosts. This ciliate possesses a unique induction response in which the free-living trophont stage transforms into a parasitic stage in response to substances produced by larval mosquitoes; thus the parasite is very responsive to the presence of target populations (Washburn et al. 1988b). Desiccation-resistant cysts enable *Lambornella* to survive during periods of habitat drying, and infected adult mosquitoes disperse the parasite to new habitats. Infected females do not seek blood meals, and they are parasitically castrated so they contribute neither to the biting population nor to the next generation (Egarter et al. 1986, Egarter and Anderson 1989). Finally, *Lambornella* tolerates a wide range of aquatic conditions typically found in container habitats, produces high infection levels in target populations even when ciliates are at low densities, and causes natural epizootics and eliminates larval mosquitoes from about 10–15% of the tree holes in which it naturally occurs.

Assessing the natural regulatory role of this parasite proved to be a formidable task because of factors such as multiple parasite cycles during the host's larval tenure and continual larval recruitment within tree hole populations. Control of mosquito populations could not be assessed by parasite incidence rates alone. We therefore embarked on a series of laboratory and field experiments in which we manipulated mosquito density, larval food resources, and the introduction of the parasite. In total, we have studied the emergence success (i.e., adult number and size) of tens of thousands of 1st-instar larvae of *Ae. sierrensis* developing in hundreds of laboratory microcosms and natural tree holes. Results from these experiments unequivocally demonstrated that the mortality caused by *Lambornella* could be additive, compensatory, or depensatory depending on the conditions under which larval populations developed (Washburn et al. 1991). Specifically, when larval resource levels were low, introducing the parasite resulted in depensatory mortality, and we consistently observed the production of more and larger adult mosquitoes. In other populations, and specifically those where resource levels were high, mortality from *Lambornella* was additive and we observed a significant decline in the number, but generally not the size, of emerging adults. It appears that under

the imposed experimental conditions, parasite effects on larval mortality were modulated by resource availability and intraspecific competition among larvae. How do we interpret these results in the context of naturally occurring mosquito populations? Our findings are encouraging to the extent that they show that *L. clarki* controlled *Ae. sierrensis* in container habitats that had high resource levels and were potentially the most productive. But how frequently are these conditions met in nature?

A large body of evidence suggests that larval resource limitation is probably normal for many, if not most, populations of container-developing species. First, there are convincing data from empirical studies documenting high larval attrition rates in these habitats in the absence of significant mortality from natural enemies. Second, adult mosquitoes emerging from containers exhibit much higher variation in size compared to the often nearly uniform size of many species developing in pools (Fish 1985), and size is a plastic phenotypic trait that is often a direct measure of resource availability during the larval stages. Finally, the high incidence of competitive mechanisms (such as compounds that retard development of conspecifics and larvae of other species) demonstrates how important resource limitation has been as a selective force in the evolution of life histories of mosquito species that utilize containers for larval development. Thus, compensatory and depensatory responses may be more frequent in water-filled containers compared to pools.

CONCLUSIONS

In summary, I believe that biological control does have a future in our ongoing efforts to control pest mosquitoes from both pools and containers, but ecological theory and empirical evidence suggest that larval populations developing in containers are intrinsically more difficult targets to control. Effective biocontrol agents face a challenging diversity of physical, chemical, and biological conditions in these container habitats, and performance is therefore likely to be more variable. Careful analysis of the population effects of each agent is going to be essential if rational and effective strategies are to be realized. Augmentive and inundative releases of self-dispersing natural enemies, coupled with source reduction of artificial habitats, are fruitful avenues to pursue. Clearly, biological control within water-filled containers is possible. During the past decade, we have witnessed one of the most successful and complete cases of control of a container-developing culicid. Specifically, the near elimination of *Aedes aegypti* (Linn.) from the

southeastern USA by *Aedes albopictus* (Skuse) documents that a "natural enemy" can effectively eliminate larval populations and reduce adult mosquitoes; unfortunately, in this case the agent is as bad, or worse, a pest than the mosquito it has eliminated.

The outlook for successful biological control of mosquitoes is probably brighter for noncontainer-breeding species because of the ecological characteristics of their habitats, the logistics of application, and because environmental sensitivity is restricting more traditional methods, especially those based on chemicals.

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