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Controlling Dengue Spreading Aedes Mosquitoes (Diptera: Culicidae) Using Ecological Services by Frogs, Toads and Tadpoles (Anura) as Predators

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Abstract

Dengue is now the most important mosquitoes borne viral disease of humans in terms of both morbidity and mortality, and a lack of an effectual vaccine or treatment for the disease makes control of its vectors as a consideration of primary importance. Mosquito eggs can survive drought conditions while harboring pathogens and perpetuate diseases throughout breeding season. Biological control of mosquitoes using vertebrates mostly birds, mammals, reptiles, fishes and insect predators has been highlighted, but, sufficient scientific evidences through usage of amphibians as predators are few. This article explores the use of frogs, toads and tadpoles (immature frogs) as egg, larva and adult predators for control of dengue disease carrying Aedes vector mosquitoes. Frogs, toads and tadpoles are significant fractions of the ecosystem for their parts for predating on various life stages of insects including mosquitoes to fulfil vector's control task. Since the eggs of mosquitoes are minute, and in some species such as Aedes aegypti (Linnaeus) and Aedes albopictus (Skuse) (Diptera: Culicidae), only individual eggs are laid (as opposed to egg rafts in some other mosquito species), so the propensity of egg predation by predators is not apparent. Adult frogs and toads consume adult mosquitoes; however, tadpoles commonly consume mosquito larvae. Even when tadpoles are not interested to eat mosquito larvae, and as these grow up to become toads or frogs, can eat adult mosquitoes. With respect to available information from various studies, one frog or toad can consume about a hundred mosquitoes in a night. Still, the associations of different prey and predator relationships in the environment to assess the feasibility on the use of a species as biocontrol agent for vector control exist. However, frogs or toads cannot be used alone as an independent intervention for vector borne disease control in surroundings and more research is needed to use them effectively for mosquito control. Consequently, before deciding to raise frogs or toads make sure to understand state's laws on collecting wildlife, and prior to making a wild species as pet, it is also best to read and check with the legality of having a native species a pet as some amphibian types are very fragile and poisonous.

Keywords

Dengue, Mosquito, Vector Control, Predators, Frog, Toad, Tadpole, Amphibian

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1. Introduction

Dengue transmission in the community can only be reduced by controlling the *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) mosquito (Diptera: Culicidae) vectors. Among mosquito control methods, biological control is valued for its low ecological impacts and reduced side effects on humans (Sarwar, 2014 a; 2014 b; 2914 c). However, amongst vertebrates mostly fishes have been highlighted in biological control of mosquito larvae. But fishes, especially when introduced, could cause ecological damage by becoming a threat to native organisms including amphibians whose populations are often in decline. Furthermore, fishes

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need interconnected waterways to spread and are often not found in isolated pools, tree holes, rock pools, ponds and most ephemeral water bodies, which are ideal breeding grounds for mosquitoes. Amphibian predators, like frogs and toads that often survive in close association to humans, can move over on land and have great distributions, and also do not require interconnected waterways for movement. Often, tadpoles of many frogs and toads are in fact predators of mosquitoes and their impacts can be considerable. Adult of frogs devour adult stage of mosquitoes, but the immensity of their food is normally the bigger organisms. Immature frogs (tadpoles) are usually considered to predate on mosquito larvae, however tadpoles essentially feed on small organic debris and algae. Though the adult frogs do not consume enough adult mosquitoes to play a noteworthy check on mosquito populations, yet tadpoles can have an important predation role on mosquito larvae (Russell et al., 2001; Parthiban and David, 2007).

From a classification perspective, all frogs belong to the members of order Anura (tailess), while only the members of the family Bufonidae are termed as toads. The use of the term frog in common names usually refers to species that are aquatic or semi-aquatic and have smooth, moist skins, and the term toad generally refers to species that are terrestrial with dry, warty skins. However, there are numerous exceptions to this rule, as the European fire-bellied toad (*Bombina bombina*) has a slightly warty skin and prefers a watery habitat, whereas the Panamanian golden frog (*Atelopus zeteki*) is in the toad family Bufonidae and has a smooth skin (Ford and Cannatella, 1993).

Frogs have worldwide distribution and live in diverse habitats with wide diversity in tropical rainforests. Frogs are sensitive to environmental changes including human actions that have resulted in their rapid reduction and disappearance in many parts of the world. Globally frog populations have declined dramatically and habitat loss is a significant cause for the decline in their densities (Houlahan et al., 2000). Frogs and toads have come under a number of threats in recent years. Many of their usual breeding places are disappearing, such as ponds become filled in or polluted because of housing development or intensive agriculture. Their large numbers are dying on the roads, especially toads on the way to their breeding sites. Over collecting of offspring may be another factor for the decline of frog and toad populations.

The role of vertebrate predators of mosquito eggs has so far not been highlighted properly. Information on effectiveness of amphibians in mosquito control is not adequately highlighted (Raghavendra et al., 2008). The role of tadpoles in disrupting early life history stages of mosquitoes, especially the egg stage, remains poorly studied. This is of

utmost importance given that mosquito eggs with their ability to traverse dry periods are the primordial elements that determine the vector's density and hence there is difference between an epidemic and a low incidence of a disease in a given wet season. Mokany and Shine (2003 a) have suggested that frogs introduced into segregated mosquito larval breeding habitats such as ponds, puddles, tanks, etc., may prey on larvae and subsequently reduce vector population and vector born disease burden. However, nominal studies have revealed the fact that tadpoles feed on mosquito eggs. Competition between tadpoles and mosquito during early life history stages probably extends beyond egg predation by tadpoles. There is a substantial body of knowledge highlighting that competition between tadpoles and mosquito larvae results in poor growth or death in either the tadpoles, mosquito larvae or both (Mokany and Shine, 2003 b; Mokany, 2007; Hagman and Shine, 2007). They compete for food and change water quality (Mokany and Shine, 2002). There is a strong perception that the decline in amphibians leads to an increase in mosquito populations (Hagman and Shine, 2007).

Most adult frogs and tadpoles infrequently feed on mosquito larvae, however, mosquito larvae predation is mainly known for three species such as spade foot toad, green tree frog and giant tree frog. Mosquito A. aegypti tends to lay eggs in anthropogenic refuse like water filled discarded plastic containers, tires, etc., which however can be controlled by removing them from the environment with many anti-dengue campaigns usually aim to do across the world. However, mosquitoes also lay eggs in ecologically sensitive natural sites, which are also used by frogs for breeding like plant holes, wet areas, ponds and temporary pools, which cannot be removed from the environment (Weeraratne et al., 2013). During the experiment, it has been found that the tadpoles fed on eggs such laid and frogs often lay eggs in such habitats. Given the fact that these tadpoles do not directly predate on mosquito larvae, it is hypothesized that these tadpoles may be feeding on mosquito eggs or creating conditions that disrupt hatching. Profoundly, in the context of environmental management and biological control of mosquitoes, the importance of tadpoles in controlling mosquitoes may be very significant. Tadpoles are important for biological control of mosquitoes than given consideration previously. Experiment involving tadpoles of four species of randomly selected genera, Bufo, Ramanella, Euphlyctis and Hoplobatrachus has shown that mosquitoes have a preference to lay eggs in tadpole water and these tadpoles destroy such eggs laid. Direct observations substantiate that all tadpole species tested are mosquito egg predators. With about more than seven thousand frog species worldwide, living in a diversity of aquatic habitats where fish cannot reach, the role

of tadpoles in biological control of mosquitoes can be significant than currently understood. While raising tadpoles of *Polypedates cruciger* and *Taruga eques* for various experiments, it has been observed only a very few or no mosquito larvae in those tanks, although in tanks without tadpoles kept under similar laboratory conditions, there have been observed many mosquito larvae (Bowatte et al., 2013).

Since the eggs of mosquitoes are minute, and in some species such as *A. aegypti*, only individual eggs are laid (as opposed to egg rafts in some mosquito species), propensity of egg predation is not apparent. However, studies of mosquito eggs predators are important due to the ability of mosquito eggs to survive drought periods and hatch in the subsequent rainy season (Okogun et al., 2003; Rozilawati et al., 2007; Sarwar, 2014 d). It is also well known that transovarian transmission of dengue virus occurs from female mosquitoes to their eggs (Joshi et al., 2002). These eggs under natural conditions can survive several months of seasonal drought. Hence, dengue virus laden eggs that survive a drought can determine the starting mosquito population size and incidence of disease during the subsequent rainy season (Juliano, 2002).

In the light of the challenges for developing a dengue vaccine, an efficient mechanism of vector control is essential. Interest on studies for the role of amphibians in controlling mosquitoes is endearing, thus information on their effectiveness for mosquito control is lacking and the role of many species living throughout the world is needed to be considered. In this review, it is discussed the reports and available information from various studies undertaken on the feasibility of use of frogs in mosquito control.

2. Raising of Tadpoles

Of course, adding of fountains and other water features to the landscapes are beautiful and calming, but, if these are not managed properly, can add an unpleasant element to the landscape such as mosquitoes. Some of the information about frogs and toads regarding how these can help to prevent mosquito infestations are stated below:-

Increasing of tadpoles in the enclosed environment carries a heavy burden of responsibility and a number of risks, and do not endorse this activity unless comply with all of the below guidelines. Before deciding to raise tadpoles, make sure that persons concerned understand state's laws on collecting wildlife to protect their populations by preventing overcollections, accidental collections of threatened species, and disease transmission. Such frogs can carry pathogens and diseases (chytrid fungus) that may then be spread to other amphibians in an area because frogs are easily susceptible to diseases. Other diseases such as virus's infection could still be present and there is possibility of their spreading a long

enough. Because egg and tadpole identification is difficult, animals should be collected only by a biologist who has knowledge about amphibian identification and collection techniques. By raising and releasing tadpoles, persons not only get witness of their remarkable renovation, but also bring more frogs into the world that might eat bugs, gnats, flies, mosquitoes and much more.

2.1. Raising Tadpole Indoor

Growing of tadpoles indoor is tough as the young tadpoles are very delicate and conditions for their rearing must be near to perfect. Temperature for tadpoles should be kept high between 77-81°F and the pH may be between 7.4-8.0, and these must remain stable as any spike or drop can kill the tads. By raising tadpoles in captivity, more of them can reach metamorphosis to begin their lives as frogs; otherwise these are sensitive to changing conditions and weather patterns or may become food for other animals.

2.1.1. Collection of Eggs and Tadpoles

Sometimes frogs lay eggs in places like pool, flooded curbside or an ornamental container in a garden that is filled with water during a heavy rain and eggs are fertilized outside the body after these are laid. Frogs tend to lay eggs singly in masses, whereas toads usually lay eggs in long chains. Frog spawn is laid in clumps in shallow water, while toad spawn forms long strands winding around aquatic vegetation in deeper water. Eggs are very tiny for several days, but start to grow quickly, appear to be elongated between 24 and 48 hours after being laid and may leave their gelatinous eggshells about another day after that. After collection, put the eggs in the container of water, and it takes about three weeks for tadpoles to emerge and a further 12 weeks before they become mature froglets, while, 6-8 weeks for toadlets. The length of time a tadpole takes to develop really depends on what kind of frog it came from, some tadpoles can remain in their tadpole stage as long as 8 months, while others only take 6 to 9 weeks or between 6 to 12 weeks. One clump of spawn might produce 200 tadpoles, and in a confined space most would die of starvation or an oxygen shortage, while kept in overcrowded conditions eventually begin to eat one another.

There are many places to find tadpoles such as a nearby stream, swamp, drainage canal, dam, pond, lake or billabong, sometimes in deep puddles, and the most obvious option is the areas with old ponds having frog populations. Spawn should only be collected from garden ponds that are really overcrowded with these and it is important to take only a small numbers. It is needed to hike on down there with a dip net and bucket, slosh through the water and surely quite a few tads are captured into net. Avoid touching tadpoles or

froglets if possible and always wash hands before and after if touching is unavoidable. Tadpoles should be reared in isolation from other animals especially other amphibians or fishes. It is important to have a proper container for raising tadpoles, for instance, fishbowls, fish tanks or aquariums, plastic bucket or a wading pool, and change the tap water being used at least once a week, and for raising these water creatures use de-chlorinated water or add de-chlorinating drops to the water. Tadpoles feed such things as lettuce and boiled spinach and be sure to boil the lettuce leaves for 10 to 20 minutes and then chop the leaves up to very fine parts.

2.1.2. Housing the Tadpoles

Suitable and the best housing containers for rearing tadpoles include a large bowl container that should be shorter and wider to have sufficient oxygen availability as opposed to taller and narrower. Lay a suitable base on the bottom of the container using gravel for shelter and put small weeds and grass with the roots attached, in the water so the tadpoles can hang onto these and eat the roots. Make sure that there has not been a recent application of pesticides on any plant life added, as this can kill the tadpoles within a day. Sand on the bottom of the chosen container can be very useful for tadpoles or use of aquarium gravel is heavy enough to keep it in fixed place and then fill container with water to the top and after that add tadpoles. Keep about 5-10 tadpoles per liter of water, a container that holds 20 liters of water (about 4 gallons) should only have 20 or 30 full grown tadpoles in it, otherwise keeping more individuals may die faster or become carnivorous.

2.1.3. Water Quality

Tadpoles have gills so these need really clean water and dechlorinated water. Bottled or distilled water is ideal, but if the water is from the tap let it sit in a container for 24 hours or it is good to use water from where found the tadpoles. Do not use tap water too full of chemicals that can harm tadpoles or let tap water to sit uncovered for 24 hours, to allow the chlorine to dissipate. Try to only change half the water at a time to keep the pH of the water balanced, remove debris that gathers at the bottom of the container, and disturb the tadpoles as little as possible while cleanings.

2.1.4. Feeding the Tadpoles

Tadpoles can eat the bits of boiled lettuce or spinach, however, only use softer leaves, and boil the leaves for 10 to 15 minutes to make soft and squishy, drain, cut into little pieces for their tiny mouths and feed every day. Tadpoles love protein when these can get it, and it has been found that in the froglet or young frog stage, aphids are a good food source as these are easily found, so just snip off a stem or leaf of plant infested with aphids and place it in the container. But

the tadpoles can also feed normal flake fish food or food pellets sold in pet shops for rabbits or hamsters. For 100 tadpoles about 5 large pellets should be given every 3-4 days. As soon as the hind legs begin to appear, the tadpoles must be given small pieces of raw red meat, but be sure to remove any pieces of uneaten food before it begins to rot.

2.1.5. Development of the Tadpoles

A tadpole usually develops from egg to tadpole in 6 to 12 weeks, but in winter the tadpoles can grow more slowly and the ideal temperature is between 65-75 degrees Fahrenheit or around 20-25 degrees Celsius. When the front legs (arms) appear on tadpoles, these are fast approaching the delicate stage of turning into a frog, gills stop functioning and lungs start to work, intestinal track changes from the long intestine of a plant eater to the short intestine of a protein eater (insects are almost entirely protein and fiber), the skin changes from the smooth, slimy skin of an underwater dweller to porous skin which allows air and water to pass through, and the tail muscle and fin deteriorate and are reabsorbed by the body. When new frogs leave the water, these might still have a full tail but they can jump and the tail will shrink and be gone in one or two days. When tadpoles develop legs, there is need of a container with dirt for them to crawl up onto, or these can be drown. Do not feed the tadpoles when these have sprouted arms, at this time the tadpole can be using its tail as food to become an adult frog. Provide more food after metamorphosis, trap some tiny flies (such as vinegar or fruit flies, housefly larvae or maggots are also enjoyed by metamorphs) and put these in the tank. The tank or housing for these must be kept clean daily, otherwise bacteria may spread rapidly and can kill these. It can also dirty the waterleading to highly possible water infection. Be careful not to get sunscreen, soap, lotion or other things of that nature in the water as these can kill the tadpoles. At all costs, never allow pesticides to get into the water. Avoid putting the tadpoles in direct sun, but indirect sun is alright, and always try to provide three quarters shade.

2.2. Raising Tadpoles in Pond or Keeping Outdoor in Garden

Whichever method is chosen to raise tadpoles in ponds or garden, it is important to check the arena periodically that it is going on effectively. Some of the information far easier to practice about raising tadpoles in ponds and keeping outdoor in gardens is as given under:-

Fill the pond with water and introduce native pond plants to the pond to infuse the water with oxygen. Plant an assortment of flowering and non-flowering plants in the yard or garden to provide a diversity of the insects for the frogs to eat. Keep a compost heap and mulch in the beds of garden that are attracting to bugs to have a great food for them. Do not remove leaf litter from some areas in the yard to provide shelter for frogs and some of their prey also prefers leaf litter. It may take a few years for frogs to populate a garden, even after there have created a suitable environment for them. If frogs take longer time to populate a garden, then it can be try for seeding the pond with native tadpoles in the spring season. The dead tadpoles appear gray if these are of black color, these float at the top of the water and can be easily removed, otherwise it can also dirty the water leading to highly possible water infection. Keep a lookout for any signs of frog disease or with open sores and damaged limbs, which seems to have been caused by an attack by other wildlife, and then it is necessary to contact with a local biologist.

If there is an area where mosquito-borne diseases are a problem, make sure that outdoor enclosure does not become a breeding ground for mosquitoes. A suitable outdoor containers include may be a large tank or a small pool in an outside environment. In general, most garden fans like to keep their tadpoles in a pond that serves as a sort of incubator for these future frogs. Thoroughly clean and disinfect tanks and rinse well prior to use to reduce the risk of contamination from animals previously housed within them. Make sure to keep tadpole tank filled with lots of clean water since health problems can develop in these wee frogs if their water tank remains dirty for long periods of time. The spawn or tadpoles should be put into a small aquarium tank filled with rain or pond water and the water should be changed whenever it becomes cloudy or dirty.

Garden ponds are increasingly important habitats for frogs and it has been estimated that nearly 50% of frogs live in garden ponds. This can be good news for the gardener, as frogs eat a number of insect pests. Frog colonies tend to be fairly small, so they can exist quite easily in gardens, but toads in field ponds frequently form colonies of over a thousand. An ideal pond for amphibians should have shallow areas for spawning, and it should not contain a high density of predators such as fishes, newts, ducks or moorhens, and look for a site that matches that description. Frogs hibernate at the bottom of ponds, under old logs, in stone walls or in compost heaps. Toads hibernate on land under stones or logs, or in a hole in the ground and rarely hibernate in water. Try to provide such habitations for amphibians in or around pond to prevent killing from time to time. Sometimes there is fear that too many frogs have come to the pond to spawn and that there can be an overrun with frogs. It is important to bear in mind that only a tiny percentage (between 1 and 5%) of the eggs laid can reach to adulthood. Frog spawn, tadpoles, froglets and adult frogs are all predated upon by other creatures so only a very small numbers can reach to maturity. Do not try to move frogs or frog spawn from garden ponds

into the countryside as frogs and toads usually return to the same pond every year.

By avoiding excessive use of chemicals, it is easier to create a variety of habitats in which a range of helpful frog species can thrive. Log piles, rockeries, bog gardens and even compost heaps all can help to provide food and shelter for frogs and other wildlife. If a pond is build, make sure it is safe for children by building a fence around it or by installing strong wire mesh just above the surface of the water. It is also important to make sure that frogs and other wildlife have an easy way out of the water. This can be achieved by using gently sloping pond sides or by placing planters at the edge of the pond to which animals can use to climb out. Any area of a pond that has shallow or still water can be a breeding ground for frogs and toads. While rearing the tadpoles outside, keep the garden well watered and vegetated, and there should have been refreshing of the water at least once a week. Young frogs need a lot of ground cover to hide and there is not much point in rearing of frogs in a totally hostile environment. Frog ponds kept year-round may establish into a permanent breeding pond.

3. Release of Tadpoles

Try to create a natural balance in garden by employing the services of wildlife to help control vector species. The best time to release the froglets is when the hind legs are large, but before the front legs start to appear. If kept any longer, the froglets might escape from the container or can drown. Newly metamorphosed frogs are fragile, and might drown very easily. Froglets should be released in shallow water at the site from which the spawn has been taken. Generally, releasing frogs back into the wild is not recommended; however, if it is choosen to release captive reared frogs, it should be done so only in the location at which these have been collected. Under the current regulations, once tadpoles have metamorphed (this means when these leave the water, not when they grow legs), these must be released back to where these have been collected from or close to it within seven days of metamorphosis. Introducing animals into new environments can introduce disease, even if the frogs are not sick. If any of frogs do appear sick, do not release any of these back into the wild. If it is wanted to be sure that these did not have chytrid fungus, for example, it is need to keep the juveniles for at least one month before release. If the tadpoles are rescued from a swimming pool or flooded curb or from a stream that has since been polluted or developed over, then it is needed to find someplace else nearby to release the frogs. If it is going to put these in an outdoor pond, be sure to get only native species of frogs. The best time to release tadpoles is on cloudy or rainy days or late afternoons

so that the sun is not too strong, the temperature is starting to cool but there is still enough light for the tadpoles to move around and choose hiding spots. Always ask to local nature center or biologist for assistance in assessing and releasing froglets.

4. Monitoring Success of Frogs or Toads for Mosquito Control

This can be done by scooping pond or garden water into a white cup or bowl and any mosquito larvae present should be visible against the white surface. If there is seen any wriggling little objects, it is needed to consider an additional method of mosquito control. If there is found many larvae in pond, it is possible that predators are feeding on other diet too much. Then try to reduce the amount of any other offered food for a couple of days and then it can be seen that the larvae population is decreasing.

5. Conclusion

Here, it has been highlighted the role of amphibians in reducing mosquito populations through destruction of mosquito eggs, larvae, pupae and adults. Adult frogs consume adult mosquitoes while tadpoles can reduce mosquito population by preying on mosquito larvae. The introduction of frogs and toads into mosquito larval breeding habitats such as ponds, puddles, tanks, etc., can prey on larvae and subsequently reduce vector population and vector borne disease burden. On the other hand, selective removal of predators in the habitat by the use of pesticides or by other means might possibly lead to increase in vector populations and disease burden. The amphibians having different life histories and different breeding sites can predate on mosquito eggs laid in a variety of isolated habitats that have the potential to pass through dry periods. Since most frog species lay eggs with the start of the rainy season, and mosquitoes also breed during the rainy season, the interaction between tadpoles and mosquitoes can occur as soon as the mosquitoes start breeding. Available information suggests the existence of many direct and indirect factors affecting the growth and survival of both prey and predators. Other biocontrol agent species that have influence on this relationship also show considerable effect on both prey and predators. As a result, when decisions on environmental management policy are made, such as application of biocides or introduction of predatory fishes and other insect predators like dragonfly larvae and aquatic beetles, the role of amphibians in control of mosquito populations should be considered. consequence, there is a further need to generate quantitative evidence to ascertain the practicable role of frogs for management of dengue and malaria vectors and to control disease burden. Always learn to appreciate the role that all creatures play in the natural cycle of life and encourage peoples to learn more about wildlife by studying the fascinating development of frogs from spawn through to adulthood. Of course any introductions of predatory organisms beyond its natural range must be done with the utmost reluctance and caution.

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