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Three Anuran Tadpoles from Odisha do not help in mosquito biocontrol

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Abstract
All the mosquitoes and amphibian species share the aquatic habitat for reproduction, growth and survival of respective larvae. The amphibian tadpoles and different instars of mosquito larvae interact with each other in several ways: both negative and positive. The trophic relationship between the tadpoles of three common anuran species of Odisha, India (*Duttaphrynus melanostictus*, *Polypedates maculatus* and *Microhyla ornata*) and various instars of *Anopheles vagus* larvae was examined. Initial and late feeding stages (varying sizes and feeding abilities) of tadpoles in starved/well-fed conditions were kept for up to 48 hours in different bowls containing *Anopheles vagus* larvae (both tadpoles and mosquito larvae in differential densities). There was no evidence of active predation of all three species on larvae of *Anopheles vagus*. Size, feeding state and density did not evoke any feeding relationship among the larvae. However, conspecific cannibalism was observed among the tadpoles but not among the mosquito larvae. The study concludes that these tadpoles cannot function as suitable mosquito biocontrol agents. Larvae of both the groups might be involved in other types of interactions which needs further investigation.

Keywords: Anuran tadpoles, mosquito larvae, *Duttaphrynus melanostictus*, *Polypedates maculatus*, *Microhyla ornata*, biocontrol

1. Introduction

Mosquito related diseases are endemic to most tropical and subtropical countries. However, till date among vertebrates only fish have been highlighted as a potential biological controller of mosquitoes. Few genera of mosquitoes are major vectors of human diseases such as malaria, filariasis and viral diseases like Japanese encephalitis, dengue, dengue haemorrhagic fever, yellow fever, chikungunya, etc. Mosquitoes breed in varied habitats such as ponds, marshes, ditches, pools, drains, water containers and other similar water collections. Different genera have shown specific breeding preferences. Among six dominant mosquito vector species involved in malaria transmission in India [1], *Anopheles vagus* is the major vector species in Odisha and responsible for high rise of *Plasmodium vivax* infections leading to attributable death cases [2]. *Anopheles* sp. are associated with fresh water habitats whereas *Culex* sp. and *Mansonia* sp. may also be found in polluted waters, including septic tanks; and *Aedes* breeds in domestic, peri-domestic and other small water collection including desert coolers.

Amphibians exhibit a biphasic lifecycle including an aquatic larval stage. Among amphibians, anuran tadpoles have been little accepted as a biological regulatory agent of mosquitoes [3]. Predatory activity is a well-known phenomenon in the animal kingdom. It is also well documented among several anuran tadpoles which demonstrate predatory interactions [4, 5, 6, 3, 7, 8]. Predation on mosquito larvae by the tadpoles of *Bufo viridis* [9], *Hyla cinerea* [3], *Hyla septentrionalis* [5], *Lechriodus fletcheri* [10] and *Rana tigrina* [11] has been documented; though there is little evidence that mosquito larvae can effectively be controlled by the use of tadpoles alone. Bowatte *et al*. [12] explained the role of amphibians (*Polypedates cruciger*, *Bufo melanostictus*, *Ramanella obscura*, *Euphlyctis cyanophlyctis* and *Hoplobatrachus crassus*) in reducing mosquito populations through destruction of mosquito eggs in Srilanka. On the contrary, certain tadpoles are also reported to be exploited as food by the larvae of some mosquito species [13].

Tadpoles are frequently, but falsely, promoted as efficient predators of mosquito larvae, but majority of them are generally herbivorous in nature and suspension feeders, filtering their food and browsing [14]. Most tadpoles and mosquito larvae feed on detritus and affect each other strongly by reducing each other's growth irrespective of the presence of resources; it is more likely to be competition than predation [13, 15]. Information on effectiveness of amphibians in mosquito control is not adequately highlighted [16].

Predatory activity is common among anuran tadpoles that develop in arid regions. There are 36 anurans known to exhibit intra-specific predation whereas 11 species were reported to show tadpole-tadpole cannibalism [17, 8], but few studies have been done on tadpole-mosquito interactions. Although majority of anuran tadpoles do not prey on mosquito larvae, considering the large amphibian diversity, it remains a great curiosity to recognize the species that might be involved in predatory behaviour. Such amphibian species may carry out an important role in bio-control of mosquito related diseases. The role of tadpoles in the reduction of early life history stages of mosquitos is almost non-existent in this part of India. This study addresses whether the tadpoles of *Duttaphrynus melanostictus, Polypedates maculatus and Microhyla ornata* (the three common anuran species in the region) prey on *Anopheles vagus* mosquito larvae under experimental conditions. It was hypothesized that predation is dependent on the availability of food resources and also on predator size.

2. Materials and methods

The three different anuran species such as *P. maculatus*, *D. melanostictus*, and *M. ornata* were opted for the present study due to their wide distribution in Odisha. They were frequently found in urban areas where mosquito vector elimination is largely essential because of the higher disease occurrence. Long strings of egg mass of *D. melanostictus*, foam nests of *P. maculatus* and small packets of small granular eggs of *M. ornata* were collected from different water bodies in an urban area in and around Bhubaneswar (20.2961^{0}N, 85.8245^{0}E), Odisha during July, 2017. The eggs were kept separately in the laboratory in rounded plastic containers (40 cm diameter, 16 cm height) with 5 litres of water where the eggs hatched. Tadpoles were fed with boiled spinach and algae collected from the pond. All tadpoles used in the experiments were at least of Gosner stage 25 [18].

Tadpoles of each species were divided into two developmental batches: initial feeding stages (Gosner stages 25-29 and late feeding states (Gosner stages 35-39). Two groups of tadpoles, one starved (prior to 48 hours of experiment) and the other well-fed, were maintained and the experiment was repeated with both the developmental and starved/well-fed tadpoles. Tadpoles from each batch and each group were taken in three densities (N: 10, two from each stage; N: 15, three from each stage; and N: 20, four from each stage) from each species in rounded plastic bowls (16 cm diameter, 7 cm height, filled with 1.5 litre of aerated water). Each set of tadpoles was maintained in triplicate to which 10, 20 and 30 *A. vagus* mosquito larvae of mixed instars in almost equal proportions collected from the vicinity of Utkal University, Bhubaneswar were added to observe the prey-predator relationship. The mosquito larvae were identified following Rattanarithikul *et al*. [19]. A total of 36 bowls (two developmental batches, two feeding conditions, each in triplicate) were maintained per species and the observations were made after 24, 36 and 48 hours of release of the mosquito larvae into the bowls. The bowls were exposed to indirect sunlight and ambient temperatures ranged between 25º C during the night and 34º C during the day time.

3. Results

None of the conditions, either developmental stage/age or starved-fed, made the tadpoles of any of the species studied to predate/feed on the mosquito larvae. Hours of exposure also did not have any effect on the feeding behaviour of tadpoles. However, conspecific cannibalism observed among the starved group of tadpoles after 36 hours. The relatively weak tadpoles were attacked first and by the end of 48 hours, about 20% of tadpoles were consumed either fully or partially. Since the mosquito larvae were not attacked at all, none of the statistical calculations could be made. Besides, conspecific predation among the mosquito larvae was not observed. Almost all the tadpoles started feeding actively when boiled spinach was provided after 48 hours of the experiment.

4. Discussion

The results of the present study suggest that tadpoles prefer alternate food resources rather than mosquito larvae, and thus the tadpoles are unlikely to be used as biological control agents. It is probably possible that mosquito larvae are also not a food resource for tadpoles in natural ecosystems. There are some basic requirements of an organism that make them to be a successful biological regulator of mosquitoes [20, 21]. Fish and certain predaceous aquatic macro-invertebrates have the ability to actively predate the mosquito larvae; however, tadpoles lack this prey searching ability which makes them unsuitable biological controller. Thus, our results confirmed that tadpoles of *D. melanostictus*, *P. maculatus* and *M. ornata* do not prey on *Anopheles vagus* larvae. Although sometimes tadpoles are assumed to be the predators of larval mosquito, but in reality; most of the tadpoles do not consume mosquito larvae [22]. Some mosquito larvae are also predacious; they could feed on tadpoles [13]. Neither conspecific predation among mosquito larvae nor on tadpoles was observed during the present study.

Oral morphology of predatory tadpoles shows that they often have enlarged mouthparts and serrated beaks that used as assets in the shredding of large foods into small pieces but most of the tadpoles have mouthparts that cannot manipulate food [23], and therefore mosquito larval predation is suspicious. For example; the oral opening of *M. ornata* does not possesses keratinized jaw sheath with denticles but only has a U- shaped fold on the lower labium that possibly aid in the injection of food, which confirms its surface feeding nature (personal observation). However, *Ramanella obscura* tadpoles predate on *Aedes aegypti* larvae [12], although they only have a keratinized ridge on the lower lip but no keratinized oral structure [24]. Similarly, *D. melanostictus* and *Polypedates cruciger* larvae have been shown to feed on *A. aegypti* eggs [12], but not *A. vagus* (present study) larvae in spite of the presence of well developed keratinized oral structures in both. Weterings [25] also reported the inability of the *Bufo melanostictus* tadpoles to predate on *Culex quinquefasciatus* larvae.

As the experimented tadpoles did not show mosquito larvae predation, they cannot be enlisted as effective biological control agents although Table 1 lists the tadpoles that feed on some mosquito larvae. However, there are other possible mechanisms that might trigger this type of behavioural activities such as competition or contest against oviposition site selection [13, 26, 27].

Amphibian decline in many parts of the world [28, 29] indicates that studies on competition in between tadpoles and other groups of animals deserve urgent attention. Especially, our study suggests that tadpoles to some extent may reduce the viability of mosquitoes either by sharing of the resources or by competition. However, whether the decline of amphibian populations can raise the disease frequency needs sufficient scientific verification.

Table 1: Predation of anuran tadpoles on mosquito larvae of various species.

Anuran species	Mosquito species	Reference
Polypedates cruciger *Bufo melanostictus* *Ramanella obscura* *Euphlyctis cyanophlyctis* *Hoplobatrachus crassus*	*Aedes aegypti*	Bowatte *et al*. 2013 [12]
Scaphiopus hammondii	*Anopheles pseudopunctipennis Aedes dorsalis*	Barber, 1927 [4]
Bufo marinus	Australian Mosquitoes	Maltis Hagman & Richard Shine, 2007
Hyla septentrionalis	*Culex pipiens*	Spielman & Sullivan, 1974 [5]
Rana tigrina	*Culex fatigans*	Marian *et al*. 1983 [11]
Hyla cinerea	*Culex nigripalpus*	Ritchie, 1982 [3]

5. Conclusions

Future research should focus on the species-specific role of tadpoles in the control of mosquito populations. Ecological investigations may provide a door for future research and should incorporate studies on the interactions and prey-predator associations among amphibians, mosquitoes and other related species. Thus, there is a need to expand our understanding to generate adequate evidence on the possible role of amphibians for disease vector control and management.

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