Study on digestive tract contents of fish: Preliminary step for identification of indigenous species in mosquito larval control

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Abstract: The present study is based on screening of food preference by the indigenous fish under its natural condition. The gastrointestinal contents as an indicative of effective bioregulatory activity envisage the selection of a larvivorous fish. As many as 32 species of fishes were collected from their natural habitats at Imphal and Bishenpur districts in Manipur State between August 2007 to February 2008. Faecal drops of active fishes revealed remains of larvae / pupae of mosquitoes. However, the food types in 26 species of fish comprised larvae / pupae of mosquito, algae, weeds, tadpole fishes, crustaceans, insects, gastropods, worms and detritus / debris. On the basis of food preference Aplocheilus panchax, Polyacanthus fasciatus and Puntius manipurensis have been considered as most potent larvivorous fish of the study area.

Keywords: Larvivorous fish, Mosquito control, Digestive tract, Manipur

INTRODUCTION

The biological control of mosquito vectors using larvivorous fish plays an increasingly important role in integrated management strategies, particularly in urban and peri-urban areas (Gratz and Pal, 1988). It is usually based on establishing a predator population in the mosquito-infested water body. This method effectively restricts the use of fish to permanent water bodies of relatively high water quality which enables the natural propagation of the predator population. For several decades, different species of fish have been used in biological control of mosquito larvae, especially in natural breeding sites (Gerberich and Laird, 1968; Bence, 1988; Nelson and Keenan, 1992; Fletcher et al., 1993; Torrente et al., 1993; Lee, 2000; Hurst et al., 2004 and Rehage et al., 2005).

The larvivorosity of a fish can be tested depending on the consumption of mosquito larvae alone or along with alternative food under natural conditions. In fact, prior to investigating the feeding potential of a particular fish species towards mosquito immature, it is necessary to identify its feeding habits in natural conditions. This leads to food preferences by the selected species of fish. Hence, the gastrointestinal contents of the fish are indicative of its effective bioregulatory activity and on this basis the fish is to be considered as larvivorous.

Manna et al. (2008) experimentally shown that the regulation of the mosquito immature by the natural predators is affected by the presence of alternative prey; however, the prey consumption ability of the larvivorous fish increases with the body size in Peocilia reticulata as evidenced by their studies showing low preference for the Cx. quinquefasciatus larvae as compared to the chironomid larvae and tubificid worms, when all the three prey types were present. Earlier, Rojas et al. (2005) presented characteristics and selection of a larvivorous fish based on the contents of digestive tract and a conclusive interpretation towards an effective bioregulatory activity of the fish under natural conditions. In India, though a considerable amount of work on larvivorous fish and their larvivorosity tests has been carried out (Sharma and Ghosh, 1989; Das and Prasad, 1991; Jauhari et al., 1996; Haq and Yadav, 2003; Ghosh et al., 2006; Chandra et al., 2008) but still there are scattered reports on the effective bioregulatory activity of fish to be considered it as potent larvivore. Moreover, our knowledge on such aspects is meager in general and in particular from Manipur state and henceforth, an investigation is being made to identify an indigenous species of fish that could witness its role in larval mosquito control.

MATERIALS AND METHODS

Study site: For Ichthyo-faunal diversity in Manipur state, at the initial stage the water bodies existing in the vicinity of the following 2 districts viz., Imphal and...
Bishenpur were surveyed during August 2007 to February 2008. In the district Imphal, the fishes were sampled from rivers and ponds while from Bishenpur district, it was from rice fields, water fed low lying areas, rocky streams and Loktak lake.

**Collection of fish and analysis of gastrointestinal contents**: Fish were collected from their natural habitats by using locally employed fishing nets and then transported to the laboratory in a plastic bucket (10 l capacity) containing water from the collection site and aerated by a battery-operated portable air pump. Collected fishes were grouped into two categories - more active and less one. The active fishes were transferred to aquaria for analyzing their faecal drops in subsequent days. The faecal matter was collected by pipette and placed on a slide. Excess water was removed with filter paper, then added a drop of glycerine and, thereafter, examined under a zoom stereo trinocular for presence of mosquito larval remains *viz.*, head, hair, cuticles, *etc.*

Fishes were identified using relevant literature (Vishwanath *et al.*, 2007) and total length of each individual was measured. Selected fish was eviscerated and the stomach contents were scooped out. Food items were identified as much as possible. Whenever required the collected items were preserved in 4% formaldehyde solution for taxonomical identification.

**RESULTS**

The fish faunal diversity of selected areas of Manipur state during the study period revealed 32 species of

| Sl. No. | Name of species | Habitations |
|---------|-----------------|-------------|
| River | Lake | Pond | Rice field | Stream |
| 1 | *Barilius bendelisis* (Hamilton) | + | + | + | - | - |
| 2 | *B. ngawa* Vishwanath & Manojkumar | + | + | + | - | - |
| 3 | *Esomus danricus* (Hamilton) | + | + | + | - | - |
| 4 | *Danio rerio* (Hamilton) | - | + | - | + | + |
| 5 | *Rasbora daniconius* (Hamilton) | + | + | - | + | + |
| 6 | *Amblypbyranegodon mola* (Hamilton) | + | + | - | + | + |
| 7 | *Puntius chola* (Hamilton) | + | + | + | - | + |
| 8 | *P. sophore* (Hamilton) | + | + | + | - | - |
| 9 | *P. ticto* (Hamilton) | + | + | + | + | - |
| 10 | *P. manipuren* Menon, Rema Devi & Vishwanath | + | + | - | + | - |
| 11 | *P. Jayarami* Vishwanath & Tombi | + | + | - | + | - |
| 12 | *Mystus cavarius* (Hamilton) | + | + | + | - | + |
| 13 | *M. vittatus* (Bloch) | + | + | + | - | + |
| 14 | *Clarias batrachus* (Linnaeus) | + | + | - | + | - |
| 15 | *C. gariepinus* (Burchell) | + | + | - | - | + |
| 16 | *Heteropnuestes fossilis* (Bloch) | + | + | - | + | - |
| 17 | *Aplocheilus panchax* (Hamilton) | + | + | - | - | + |
| 18 | *Chanda nama* Hamilton | + | + | + | - | + |
| 19 | *Glossogobius giuris* (Hamilton) | + | + | + | - | - |
| 20 | *Anabas testudineus* (Bloch) | + | + | + | - | + |
| 21 | *Chaunia striata* (Bloch) | + | + | + | - | + |
| 22 | *Clarias punctatus* (Bloch) | + | + | - | - | + |
| 23 | *C. gachua* (Hamilton) | + | + | - | - | - |
| 24 | *Polyacanthus fasciatus* (Bloch & Schneider) | + | + | - | - | + |
| 25 | *P. sota* (Hamilton) | + | + | + | - | + |
| 26 | *Labeo rohita* (Hamilton) | + | + | + | - | - |
| 27 | *L. calbasu* (Hamilton) | + | + | + | - | - |
| 28 | *Carla calla* (Hamilton) | + | + | + | - | - |
| 29 | *Cirrhinus mirgala* (Hamilton) | + | + | + | - | - |
| 30 | *C. reba* (Hamilton) | + | + | + | - | - |
| 31 | *Osteobrama cotio* (Hamilton) | + | + | - | - | + |
| 32 | *Wallogo atta* (Schneider) | - | + | - | - | + |

**Note:** + = Present; - = Absent
fishes, representing 11 families (Table 1). Out of these, 26 species were common while remaining 06 species were rare. In all, 575 specimens of fishes were collected and grouped into 3 categories based on vertical distribution viz., typical surface feeder (Aplocheilus panchax); sub-surface feeder (Amphlypharyngodon mola, Danio rerio, Rasbora daniconius and Esomus danricus) and column feeder (Puntius chola, P. sophore, P. ticto, P. manipurensis, P. jayarami, Polyacanthus fasciatus, P. sota, Chanda nama, Barilius bendelisis, B. ngava and Anabas testudineus; fry of craps - Labeo rohita, L. calbasu, Catla catla, Cirrhinus mirgala and C. reba).

Table 2. Details of food contents* in the gut of selected fishes collected from water bodies of district Imphal and Bishenpur in Manipur state.

| Food Type               | Algae | Weeds | Tadpole fish | Larvae/Pupae | Crustacean | Insects | Gastropods | Worms | Others |
|-------------------------|-------|-------|--------------|--------------|------------|---------|------------|-------|--------|
| Barilius bendelisis     | 33.3  | 50.0  | 0            | 41.6         | 0          | 16.6    | 0          | 8.3   | 33.3   |
| Esomus danricus         | 28.0  | 20.0  | 0            | 58.0         | 0          | 20.0    | 0          | 32.0  | 12.0   |
| Rasbora daniconius      | 30.0  | 40.0  | 0            | 60.0         | 20.0       | 30.0    | 0          | 20.0  | 20.0   |
| Amblypharyngodon mola   | 11.1  | 0     | 0            | 55.5         | 0          | 44.4    | 0          | 29.6  | 0      |
| Puntius chola           | 8.5   | 12.5  | 0            | 47.8         | 2.7        | 53.2    | 0          | 17.2  | 3.5    |
| P. sophore              | 12.5  | 21.3  | 0            | 40.5         | 0          | 48.7    | 0          | 22.1  | 5.7    |
| P. ticto                | 13.7  | 15.2  | 5.3          | 57.9         | 5.3        | 51.9    | 2.2        | 15.7  | 1.5    |
| P. manipurensis         | 10.3  | 11.7  | 0            | 62.3         | 7.3        | 42.8    | 0          | 32.5  | 5.6    |
| Mystus cavasius         | 12.5  | 20.0  | 17.5         | 27.5         | 37.5       | 67.5    | 42.5       | 52.5  | 92.5   |
| M. vittatus             | 10.5  | 15.3  | 5.7          | 20.3         | 11.7       | 37.4    | 20.3       | 42.8  | 53.2   |
| Clarius batrachus       | 10.3  | 5.7   | 12.8         | 43.5         | 11.8       | 23.7    | 15.8       | 52.7  | 47.5   |
| C. gariepinus           | 20.7  | 10.2  | 21.7         | 40.7         | 15.8       | 27.9    | 20.3       | 48.5  | 68.5   |
| Heteropneustes fossilis | 5.2   | 8.7   | 10.3         | 15.7         | 7.2        | 37.4    | 11.3       | 42.7  | 57.2   |
| Aplocheilus panchax     | 14.5  | 8.1   | 0            | 76.3         | 24.5       | 44.2    | 0          | 45.9  | 0      |
| Chanda nama             | 8.1   | 18.9  | 0            | 21.6         | 5.4        | 32.4    | 0          | 29.7  | 8.1    |
| Glossogobius giuris     | 22.7  | 20.5  | 0            | 32.5         | 7.2        | 43.8    | 0          | 77.2  | 23.8   |
| Anabas testudineus      | 9.5   | 9.5   | 14.2         | 14.2         | 9.5        | 9.5     | 23.8       | 71.1  | 28.5   |
| Channa striata          | 16.6  | 19.0  | 47.6         | 35.7         | 30.9       | 52.3    | 35.7       | 64.2  | 11.9   |
| C. punctatus            | 18.5  | 22.9  | 62.8         | 29.7         | 40.2       | 33.9    | 20.8       | 37.4  | 15.2   |
| Polyacanthus fasciatus  | 11.7  | 10.5  | 2.8          | 67.3         | 13.7       | 39.7    | 15.7       | 48.2  | 18.3   |
| P. sota                 | 13.1  | 7.8   | 5.2          | 65.4         | 28.9       | 26.3    | 13.1       | 31.5  | 5.2    |
| Labeo rohita            | 18.7  | 6.25  | 0            | 50.2         | 0          | 43.7    | 0          | 31.2  | 0      |
| L. calbasu              | 24.5  | 12.2  | 0            | 47.2         | 0          | 36.7    | 0          | 22.3  | 0      |
| Catla catla             | 22.2  | 10.5  | 0            | 36.7         | 0          | 33.6    | 0          | 21.2  | 5.7    |
| Cirrhinus mirgala       | 10.5  | 20.5  | 0            | 47.7         | 0          | 53.2    | 0          | 11.5  | 5.5    |
| C. reba                 | 1.7   | 6.5   | 0            | 51.2         | 0          | 74.7    | 0          | 34.2  | 0      |

* The figure for each food type indicates the percentage of fish specimens harbouring that particular food.
punctatus and *Clarias batrachus* composed of mostly algae, tadpole fishes, debris and worms. Table 2 represents the details about the food types examined under the microscope in all the 26 species of fishes, categorised into 2 groups. The first group was of active fishes comprising 14 species as marked with an asterisk while remaining fishes were inactive i.e., the live specimens could not be obtained. The food items as available in the digestive tract of the dissected fishes, comprised the larvae and pupae of mosquitoes, algae, weeds, tadpole fishes, crustaceans, insects, gastropods, worms and detritus/debris.

It is recorded that the larvae/pupae of mosquito constituted major part of food items in *Aplocheilus panchax*, *Polyacanthus fasciatus*, *Polyacanthus sota*, *Puntius manipurensis*, *Rashora daniconius* and *Esomus danricus* while major portion of vegetation part was recovered in specimens of *Barilius bendelisis*, *Rasbora daniconius* and *Clarias batrachus* and *Mystus cavasius* the major part of food items belonged to worms. The detritus was present in most of the specimens of *Mystus cavasius*, *Clarias gariepinus* and *Heteropneustus fossilis*, followed by insects and worms remains. The percentage of gastropod snail was recorded more in the active fish than the inactive ones. Further, maximum specimens of *Channa punctatus*, *C. striata* and *Clarias gariepinus* consisted tadpole fish in their stomach contents as the major part of food.

Based on the dietary food items as recorded in the stomach of the fish, the following 8 species of fish viz. *A. panchax*, *Polyacanthus fasciatus*, *P. sota*, *Puntius ticto*, *P. manipurensis*, *Amblyparyngodon mola*, *E. danricus* and *Rasbora daniconius* were taken into consideration for larvivorous purpose. Gastro-intestinal analysis of all the selected fishes showing remains of larval/pupae of mosquitoes as compared to other items have been depicted in Fig. 1. In almost all the 8 selected fishes, the larvae/ pupae of mosquito along with alternative food constituted major part of diet. However, in *A. panchax* the gastrointestinal contents reveal high preference of larvae/pupae of mosquito with other alternative food as compared to only larvae/pupae or other elements. In the fishes like, *Puntius manipurensis*, *P. ticto*, *Polyacanthus fasciatus*, *P. sota* and *Esomus danricus* there was high preference towards mosquito larva/pupa along with other alternative food. As compared to only other elements i.e., debris/detritus, the fish *Amblyparyngodon mola* was on the first rank and next to it there were *Polyacanthus sota*, *E. danricus* and *Polyacanthus fasciatus* in succession.

**DISCUSSION**

The findings of the present study reveal a difference in the percentage of food items collected as a result of evisceration of stomach. This study exhibit that most of the selected fishes feed more on mosquito larvae in presence of alternative food. Comparatively, there was less percentage of fishes where mosquito larvae and pupae were present alone. According to Reddy and Shakuntala (1979) when mosquito larvae alone were offered in terms of numbers, the fish *Gambusia affinis* predated more; however, its preference to feed on larvae is reduced when larvae are supplemented with *Tubifex tubifex*. Further, feeding on mosquito larvae by *G. affinis* and *Poecilia reticulata* is reduced in presence of oligochaete worms.

As per views of Jacob and Nair (2006), the dipteran larvae are preferred items of diet of *Aplocheilus lineatus* in all size groups, depending upon habitat / environmental factors, seasons and stage of maturity. Comparing these findings with our results, it is to mention here that most of the specimens of selected fishes predated more on mosquito immature when supplemented with alternative food.

Fragments of aquatic macrophytes have not been recorded in the stomach contents of *Anabas testudineus* and *M. vittatus* (Wijeyaratne and Perera, 2001) while in the present study both algae and weeds have been collected from the stomach of these fishes. The major food items of *R. daniconius* was found to be detritus (Wijeyaratne and Perera, 2001) while in *Anabas testudineus*, the major food item was animal matter, contributing more than 70% of the diet. In their findings, the fish *M. vittatus*, the detritus / animal matter was main food item depending upon localities. The findings of present study are just contrary to this. As per results of Wijeyaratne and Perera, (2001) *A. testudineus*, although omnivore, fed mainly on animal matter but in our study worms are recorded as the most preferred diet of this fish. Jauhari *et al.* (1996) worked out the larvivorous potential of 9 indigenous species of fish based on the food specimens in their gastrointestinal contents and by providing different stages of *Anopheles culicifacies* larvae. As per their studies the food spectrum in the gut contents includes crustaceans, insects, algae, diatoms, detritus, digested matter and others. The findings of the present study resemble slightly considering the kind of food present but differ from them in having weeds, tadpole fish, mosquito larvae/pupae, gastropods and worms in the gut contents.

Kumar *et al.* (2008) observed that larval removal rate decreases with increasing larval size and instar stage. This is in accordance to our studies as the remains of 2nd and
3rd larval instar were obtained in the stomach contents in all the 8 selected fishes. With regard to the assumption that the predatory potential of larvivorous fish can be affected by the presence of alternative preys, the present findings are close to Manna et al. (2008). In the findings of Rojas et al. (2005) in respect of digestive contents of fishes and their implications as bioregulators of mosquito larvae, all the selected fishes preferred larvae and pupae of mosquitoes combined with other food. This is close to our studies; the only difference is with the species of fish. There is also a bit resemblance of the present study with the findings of Jacob and Nair (2006).

The possible explanation about the preference diet of larvivorous fish follows the views of Windell (1967) who found that in a fish the food intake is dependent on the gastric evacuation. Further, the soft food organisms such as worms and dipterans larvae are found to be digested more rapidly than the heavily chitinised forms (Nikolsky, 1963). Besides this, Oligochaete worms have been reported to be highly nutritive (Galinat, 1960) and easily digested by fish (Mann, 1935).

Conclusively, it is recommended that it is a must to observe an effective bioregulatory activity of fishes under natural condition to select them as biocontrol agent.

ACKNOWLEDGEMENTS

Financial assistance rendered to one of us (NPD) from DST, New Delhi in the form of WOS-B fellowship is highly acknowledged. Thanks are also due to the Principal D.A.V.(P.G.) College, Dehradun for providing adequate laboratory facilities and to the State Fisheries Department, Govt. of Manipur, Imphal for cooperation.

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