

Habitat heterogeneity and prey selection of *Aplocheilus panchax*: an indigenous larvivorous fish

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ABSTRACT

Objective: The ability of the native larvivorous fish *Aplocheilus panchax* (Hamilton, 1822) (Cyprinodontiformes: Aplocheilidae) as predator of mosquito larvae was assessed under laboratory conditions using multiple prey and habitat conditions.

Methods: The consumption of larvae of the mosquito *Culex quinquefasciatus* Say 1823 (Diptera: Culicidae) by *A. panchax* was evaluated in the presence of tubificid worms and chironomid larvae under complex and simple habitat conditions. The number of mosquito larvae consumed in comparison to other prey types was noted and an index of prey selectivity was used to evaluate the preference for mosquito larvae.

Results: *Aplocheilus panchax* consumed 53 to 65 mosquito larvae in a three hour feeding bout contrast to 29–38 tubificid worms and 43–62 chironomid larvae depending on the habitat conditions. The prey consumption differed significantly between the habitats and the prey type. The index of prey selectivity was positive for *Cx. quinquefasciatus* larvae over other alternative prey in all the habitat conditions.

Conclusion: It is apparent from the study that the larvivorous fish *A. panchax* can be employed for biological regulation of mosquitoes in rice-fields and similar wetlands where the multiple prey choices are available under complex habitat conditions. However, field studies including other prey species will be required to substantiate this finding.

Key words *Aplocheilus panchax*; biological control; complex habitat; mosquito larvae

INTRODUCTION

Biological control of vector mosquitoes using predators and pathogens is a feasible alternative alone or as a part of integrated vector management programme to combat the mosquito menace. Among the predators of larval stages of mosquitoes, different species of fishes are known to be effective in regulating the population of mosquitoes. Empirical evidences from diverse larval habitats from different parts of the globe substantiate this. However, till date the most widely used fish species for regulation of mosquito population appears to be *Poecilia reticulata* and *Gambusia affinis* Baird & Girard (Cyprinodontiformes: Poeciliidae) in varied larval habitats both in the urban and rural environments. For instance, in the sewage drains, guppies have been effective in regulating mosquitoes and were successful in establishing their populations^{1–5}. While the ability of these fishes to adapt to diverse situations can be of advantage, the invasive nature of these fishes poses a serious problem of environmental degradation by altering the species interactions at the community levels^{6–11}. For instance, the invasive mosquito fish *G. affinis* reduced the load of water bugs that contributed to the population

control of mosquito immatures in rice-fields^{12,13}, apart from augmenting development of *Culex tarsalis* Coquillett (Diptera: Culicidae) mosquitoes¹⁰. In the presence of competitors, the fat-head minnows *Pimephales promelas* Rafinesque (Cypriniformes: Cyprinidae) or predators, the small mouth bass *Micropterus dolomieu* Lacep (Perciformes: Centrarchidae), the mosquito fishes *Gambusia* spp foraged more efficiently indicating their invasive nature¹¹. The indigenous larvivorous fishes coexisting in the mosquito larval habitat, naturally, offer an alternative in this regard. This is elaborated by the studies on the indigenous fishes from different parts of the world^{12–14}. In Indian context, several indigenous fishes have been documented as potential biological control agents of mosquitoes^{15–17} substantiated through empirical evidences^{18–20}.

The indigenous larvivorous fishes occur naturally in the larger mosquito larval habitats like rice-fields and temporary pools and other similar wetlands. The diet of these fishes consists of several food resources including mosquito larvae. Therefore, it is pertinent to test whether an indigenous larvivorous fish bears the ability to select mosquito larvae from varieties of food resources available.

To endure a successful regulation of mosquito population, a fish as a potential biocontrol agent should exhibit the positive preference for mosquito larvae in the presence of an alternative prey. The prey predator interactions in aquatic communities are influenced by the presence of vegetations and associated elements that render heterogeneity of the available space. This is evident in case of insects²¹ and fishes^{22–24}, where the predatory efficacy against dipterans and other prey vary owing to the vegetations that act as prey refuge. The presence of alternative prey makes the interaction more complex often leading to a context dependent predation pattern²⁵. Since most of the mosquito larval habitats are heterogeneous in nature, the predatory ability of the fishes needs to be tested in the presence of structural complexity of the habitats that influences the outcome of prey-predator interactions.

These complexities of prey predator interactions are relevant in determining success of mosquito control using natural predators like indigenous fishes and predatory insects. In the present study, this hypothesis is being tested to infer about the suitability of the killifish *Aphlocheilichthys panchax* (Cyprinodontiformes: Aplocheilichthyidae), commonly known as ‘panchax minnow’, a surface feeder and used as potent biocontrol agent in several aquatic habitats^{16,17}. Since species like chironomid larvae and tubificid worms that are controphic to mosquito immatures affect the predation pattern of fishes²⁶, these prey were used to evaluate the prey preference of *A. panchax*. This would also provide a basis to judge the hypothesis concerning the ‘effects of alternative prey that share the similar trophic levels to mosquito larvae^{27,28}. The results are expected to highlight the use of alternative fishes to *Poecilia* spp (guppies) and *Gambusia* spp (*Gambusia*, mosquito fish) that affects the native aquatic community as invasive species^{6,11} and fails to yield satisfactory results in many instances^{8–11, 14}.

MATERIAL & METHODS

Collection and maintenance of fishes and prey organisms

Aphlocheilichthys panchax fishes were collected from East Calcutta Wetlands, Kolkata, adjacent to Ruby Fish Farm, using local made nylon nets attached with a long handle. The fishes were (5.2–5.5 cm in total length and 1.25–1.32 g in weight) kept in the laboratory in the aquaria (38 × 30 × 30 cm) containing 27 L of water (pond: sewage:: 1:1) in a temperature of 25 to 30°C for seven days and were fed with fish food (Tokyu®, Tokyo Corp., Japan) *ad libitum*, for a period of seven days before using them in experiments. In all the experiments, individual fishes were starved

for a period of 24 h before introduction into the experimental aquaria.

The larvae of *Culex quinquefasciatus* Say 1823 (Diptera: Culicidae) were collected using plankton net (200 µm mesh size) from sewage drains in and around Ballygunge Science College, Kolkata. The collected larvae were segregated as small and large through repeated separation using pipettes and were placed in separate containers. The smaller larvae were fed with fish food (Tokyu®) and reared to IV instar stages (~6–7 mm in length and 1.2–1.4 mg in weight), which were used in the experiments. The chironomid larvae (Diptera: Chironomidae: Chironominae) and the tubificid worms (Oligochaeta: Tubificidae) were collected through dredging sewage drains from the same location. From the collection containing chironomid larvae of different sizes, the ‘large’ size (25–20 mm in length; 1.6–2.1 mg in weight; corresponding to IV instar stage) larvae were segregated and reared in plastic trays containing sediments. These larvae were used in experiments. The tubificid worms (15–20 mm in length and 1.8–2.3 mg in weight) were mostly *Tubifex* spp and *Branchiodrilus* sp, separated from the clumped heterogeneous population and were maintained in the laboratory in plastic trays placed under running tap water. All the prey animals were maintained in the laboratory under optimal conditions of temperature 25–30°C, and 7.5–7.9 pH of the water (1:1 v/v:: pond: tap water).

Habitat conditions

The laboratory microcosms were constructed in glass aquaria (size 38 × 30 × 30 cm) using pebbles and vegetations as elements of complex habitat. Laboratory experiments were carried out to determine the feeding preference of *A. panchax* under different habitat conditions. The pebbles approximately round and of different colours, were obtained from a local aquarium fish shop. The aquatic weeds—*Lemna minor* (Araceae: Lemnoideae) (common name duck weed), *Pistia stratiotes* (Araceae: Aroideae) (common name water lettuce), *Hydrilla verticillata* (Hydrocharitaceae) (common name Hydrilla), and *Echinodorus palaefolius* var. *latifolius* (Alismataceae) (common name: Amazon sword plants) were used as vegetations. The aquatic weeds were collected from the pond inside Ballygunge Science College campus maintained by the Department of Botany (University of Calcutta) and *E. palaefolius* was purchased from the local aquarium fish shop. In each aquarium, 150 *L. minor* (all two leaf size), 3 each of *P. stratiotes* (nine leaf size) and *H. verticillata* (three branches ~30 cm long), and 3 *E. palaefolius* (seven leaf size) were placed as vegetation. The weeds have been attached in such a way that the upper surface of the

aquarium looked like bushy stagnant water body with full vegetation.

The pond water (pH 7–7.9) was sieved through plankton net (200 µm mesh size) to avoid entry of plankton. However, nano-plankton or plankton propagules could not be eliminated from the system. The sewage water was similarly sieved through metal grid followed by plankton net sieving to obtain water containing micro-aggregates of suspended organic matter that rendered the water dark grey colour (pH 7.9–8.2). Sieved tap water was also used for the purpose having pH between 7.17 and 7.3.

The experiment

In the glass aquaria microcosms, 27 L of water, either pond, tap or sewage origin was used containing pebbles, vegetations or a combination of both. A control microcosm, without these elements of habitat complexity was also set. For each set nine replicates were made. In each of these microcosms, 100 each of *Cx. quinquefasciatus* larvae, chironomid larvae and tubificid worms were added and were allowed to settle for 24 h. This was followed by addition of one *A. panchax* to each of the microcosms to observe its predatory efficacy. After 1 h of feeding bout, from each aquarium the fishes were removed and the number of prey alive was counted. This value was subtracted from the number of prey provided to obtain the number of prey consumed by the fishes.

The data on prey consumption were subjected to two way factorial ANOVA to justify the effects of complex habitat and prey types on the selection of mosquito larvae by *A. panchax*²⁹. A prey preference analysis was made using the following formula of Ivlev and Jacob³⁰.

$$\text{IPE}_i = (r_i - p_i)/(r_i + p_i) - 2r_i p_i$$

Where, IPE_i —Index of prey electivity for i th prey species, (r_i —Proportion of i th prey consumed; and p_i —Proportion of i th prey available). Here, i prey types are mosquito larvae, chironomid, and tubificid worms. The IPE-value can range between –1 (negative electivity) and +1 (positive electivity). The higher the value of IP, higher is the electivity for the prey species. A significant positive or negative electivity was tested using a t -test for deviation from zero.

RESULTS

The prey types *Cx. quinquefasciatus* larvae, chironomid larvae and tubificid worms showed clumped orientation in space with varying numbers in each cluster (for mosquitoes 2–18 individuals; for chironomid larvae 2–4

individuals, and for tubificid worms 3–12 individuals initially prior to addition of the predators ($N = 10$ observations per prey type on pond water conditions), irrespective of their occurrence on the surface (mosquito larvae) or at the bottom (chironomid larvae and tubificid worms) of the aquaria. Contrast to these in the complex habitat conditions, the tubificid worms and the chironomid larvae used the pebbles as refuge and oriented in the spaces between the pebbles. The clumping pattern of mosquito larvae differed from simple habitat conditions in that the average number of mosquito larvae in the clumps ranged between 30 and 40 with few single individuals oriented in the open spaces. For all prey types, movement of individuals from one patch to another was noticed with most frequent for the mosquito larvae and least for the tubificid worms.

The foraging pattern of *A. panchax* differed between the habitat conditions. In the course of experiment, it was observed that *A. panchax* searched for the prey mostly on the water surface with irregular exploration in the mid column of the aquarium in simple habitat (pond water) conditions. The encounter for the tubificid worms and the chironomid larvae increased with this movement. Similar activity was seen in case of sewage. Contrast to this, the effective space for movement was restricted in complex habitat and the fishes remained for more time in the mid-column of the space.

The number of prey consumed by *A. panchax* varied with different habitat conditions. On an average in the simple habitat conditions, *A. panchax* consumed 136.67 (± 3.28 S.E.) numbers of prey. Compared to this, in complex habitat the consumption was 205.89 (± 3.95 S.E.) and differed significantly ($t_{(2)} = 13.48$; $df = 16$; $p < 0.001$). However, comparison of prey consumption in complex habitat with the tap water habitat (average prey consumption 156.56 ± 4.37 S.E.) showed significant difference ($t_{(2)} = 8.373$; $df = 16$; $p < 0.05$) but not with sewage water habitats (average prey consumption 146.56 ± 4.86 S.E.; $t_{(2)} = 1.686$; $df = 16$; $p = 0.113$, not significant). The prey consumption by *A. panchax* was significantly ($t_{(2)} = 3.637$; $df = 16$; $p < 0.002$) higher in tap water conditions than the simple habitat (pond water). The relative numbers of different prey consumed (Fig. 1) varied between the habitat conditions. Two way factorial ANOVA revealed that the significant differences in the consumption of prey among the habitat conditions (For habitat: $F_{(1) 3, 96} = 7.531$; $p < 0.001$) and prey types (For prey types: $F_{(1) 2, 96} = 127.27$; $p < 0.001$) as well. Significant difference was noted for the interaction between the prey type and habitat condition (For habitat prey type interaction: $F_{(1) 3, 96} = 9.696$; $p < 0.001$) indicating that the vulnerability of a particular

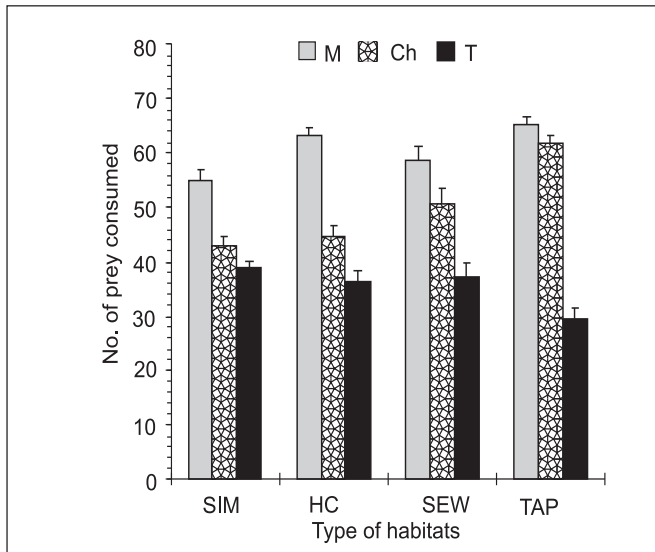


Fig. 1: The mean numbers of prey consumed in different habitat conditions, by *A. panchax* in a 3 h feeding bout. TAP— Tap water; SEW — Sewage water; HC — Vegetation and pebbles in pond water, complex habitat; SIM— Pond water, simple habitat; M—Mosquito larvae, Ch—Chironomid larvae, T— Tubificid worms.

prey or preference by the fish *A. panchax* varied with combinations of the habitat conditions and prey types. The post-hoc test (Tukey test) revealed significant differences ($p < 0.001$) in relative consumption of all the three prey types (between mosquito and chironomid larvae $|q| = 8.25$, between tubificid and chironomid larvae $|q| = 14.61$, between mosquito and tubificid $|q| = 22.86$; $|q|$ = Studentized value; S.E. = 1.45; df = 96, 2). Comparison of habitat types through post-hoc test (Tukey test) revealed significant differences ($p < 0.05$) between selected habitat types

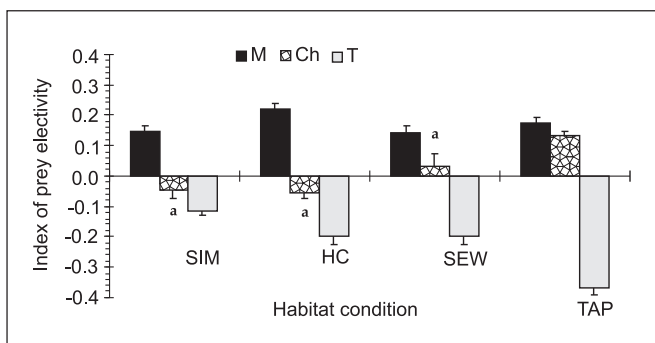


Fig. 2: The values of index of preference (IP) of *A. panchax* for mosquito larvae (M), chironomid larvae (Ch) and tubificid worms (T) prey, under different habitat conditions. Under all conditions, mosquito was selected over other controphic species and differed significantly ($p < 0.05$, t -test). Bars sharing common letters did not vary significantly from 0, indicative of unbiased electivity, for chironomid larvae. Positive electivity value was noted for mosquito (*Cx. quinquefasciatus*) larvae and negative electivity value for tubificid worms.

($|q| = 6.925$, between complex habitat and tap water; $|q| = 6.629$; between simple and tap water; $|q|$ = Studentized value; S.E. = 1.676; df = 96, 3). The index of prey electivity differed among prey types and between simple and complex habitats. The index of prey electivity of *A. panchax* showed significant deviation from zero for *Cx. quinquefasciatus* larvae as prey under all conditions. A negative electivity was noted for tubificid worms in all instances, while for chironomid larvae the electivity remained unbiased except for tap water conditions where a positive electivity was noted. The trend of electivity for mosquito larvae remained higher than other controphic species (Fig. 2).

DISCUSSION

From the results it is apparent that *A. panchax* fish consumed substantial amount of prey in all the habitats considered with variations in the absolute and relative number of prey types. While the mosquitoes were consumed at higher numbers in the complex habitat conditions, in sewage drain water and tap water. These were consumed at a higher rate than simple habitat conditions. Possibly, under simple habitat conditions the benthic macroinvertebrates tubificid worms and the chironomid larvae were more vulnerable than complex habitats thus making the fishes satiated with other prey. However, in all instances the mosquito larvae were preferred over the other two prey species, evident from the index of predation. This supports that mosquito larvae will be preferred over alternative prey under situation when multiple preys will be available. The ability to consume prey under sewage water and tap water conditions shows that *A. panchax* can be employed in these habitats, though the establishment of these fishes in wastewater conditions and storage water tanks need to be evaluated.

Earlier studies^{31,32} reported the consumption of mosquito larvae by *A. panchax*, in the rice-fields, pools and marshes. In the present study, it was observed that the mosquito larvae are preferred over alternative prey suggesting that these fishes can be used in the biological regulation of the mosquitoes in the habitats where the fishes will have a wide range of prey to predate upon. The exotic fish *P. reticulata* exhibited low preference of mosquitoes in presence of alternative prey²⁶. The mosquito fish *G. affinis* were also found to consume alternative prey over mosquitoes⁶⁻¹¹. Under such situation as an indirect effect of consumption of alternative prey the mosquito larvae grew faster and the control rendered by other predatory insects was also affected^{10,11}. Habitat conditions and alternative prey influence predation by larvivorous fishes,

native or exotic, evident from several studies^{11,14,17,26,27}. In case of insect predators too, alternative prey induces an effect on target prey selection²¹. Since the genera list insect and larvivorous predators have a wide range of prey choice, the presence of alternative prey can affect the target prey consumption. In addition to the alternative prey, habitat complexity can facilitate or reduce predation²³⁻²⁵. From the observations of the present study it can be concluded that *A. panchax* can efficiently prey upon mosquito larvae in the presence of alternative prey and in complex habitats. The bioecology of this fish indicates its natural occurrence with mosquito larvae and other insects²⁷⁻²⁸. Under natural conditions in lakes and ponds, these fishes were found to feed on a wide range of plankton and insect species including mosquito³³, as revealed through gut analysis³¹. Therefore, it needs to be explored further whether the predation of *A. panchax* is affected by the presence of predatory insects³⁴ and plankton as alternative prey. This would justify their ability to be effective as bio-control agents under local ecological conditions^{16,17,31}. Nonetheless, the results of the present study support their use as biological resource against mosquito larvae in the rice-fields and allied wetlands, as well as sewage drains, compared to the known exotic fish species *P. reticulata* and *G. affinis*.

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