

ANALYSIS OF THE FORAGING BEHAVIOR OF GUPPY (*Poecilia reticulata*) IN RELATION TO ITS USE AS A BIOLOGICAL METHOD FOR THE ENVIRONMENTAL CONTROL OF MOSQUITO LARVAE

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ABSTRACT

Guppy has been introduced widely for mosquito control and little is known about its foraging behavior and efficacy in relation to the control of mosquito larvae. This study investigated the foraging behavior of guppy in relation to its use as a biological control method of mosquito larvae under varying conditions of prey types, the physicochemical condition of the habitat and also the social context in which they are foraging. Guppies were exposed to mosquito larvae of *Culex* and *Anopheles* under varying conditions which included foraging alone, female focal singly and male focal singly, alongside male and/or female conspecifics in laboratory and field water samples. The study showed that female guppy fed significantly more than the male guppy both in the presence and absence of companions, the guppies' higher preference for *Culex* to *Anopheles* larvae was significant at $p < 0.05$ and their foraging behavior was better in the laboratory water as compared to their habitat water. These results suggest that the preference and the foraging behavior of the guppies varied with social and environmental conditions which may be as a result of varying social conditions and the relative differences in the physicochemical quality of the media. It is apparent that the sex of the forager, the social environment, habitat quality and complexity are each capable of affecting foraging behavior and prey use, therefore these factors should be integrated when introducing guppies as biological agent for mosquito control.

Keywords: *Mosquito larvae, Foraging behaviour, Guppy, Habitat quality, Environmental control*

1. INTRODUCTION

Guppy is a small, ovoviviparous fish of the family Poeciliidae as described by Magurran [1] and is an ideal species for investigating the characters contributing to invasive success. There is an extensive body of research relating to its evolutionary ecology, as well as to its basic biology, behavior, life history and ecology as described by Courtenay and Meffe [2]; Reznick *et al.* [3]; and Magurran [1]. Wild studies are carried out with relative ease in the shallow, clear habitats by Magurran and Seghers [4] and likewise in outdoor mesocosms when a semi-naturalistic approach is required as described by van Magurran *et al.* [5]. Their small size, hardiness and the ease with which they reproduce means that guppies are also extremely easily maintained in the laboratory for more controlled studies as described by Magurran and Seghers [6].

Guppy is a small member of the family *Poeciliidae* (Female 4-6 cm long and male 2.5 – 3.5 cm long and like all other members of the family is live bearing described by Magurran and Seghers [7]). Guppy has been widely introduced as a biocontrol agent throughout the tropics. Despite this, surprisingly little is known about its foraging behavior and efficacy in relation to the control of mosquito larvae. There are several variables that might affect the interactions between larvivorous fish and their prey, each of which would be discussed. These include the food preferences of the fish in the presence of two prey types, the physicochemical condition of the habitat and also the social context in which they are foraging. Furthermore, given the considerable sexual dimorphism in *Poecilia reticulata*, and documented differences in feeding niches of males and females as described by Magurran [8], there may be sex differences in other aspects of their foraging behavior.

Guppies are omnivorous in nature, and their diet may include algae, insects, small crustaceans, tubificid worms, fish eggs and larvae, and almost anything else that happens to fall into their habitat as described by Arthington [9]. In the laboratory, most live prey items are consumed extremely readily, but it is unclear what might happen in a multi-prey system when there is a choice of more than one attractive prey source. This has particular relevance to their use as biological control agents in India, where there are several species of mosquito present, belonging to three distinct genera which are *Anopheles*, *Aedes* and *Culex*. In Nigeria, only a subspecies within the *Anopheles* genus appears to be responsible for the vast majority of the malaria incidence.

Most natural habitats will have some form of vegetation or equivalent cover which provides prey with refuge; this can reduce visual encounter rates of prey by a predator when compared with a non-vegetated area as described by Priyadarshana *et al.* [10]. Furthermore, different prey species may utilise cover in different ways, and many will have evolved to actively seek it out as described by Laegdsgaard and Johnson [11], therefore in a multi-prey system the extent of habitat structure might affect prey selection as described by Anderson [12]. The larvae of different genera of mosquito display differences in feeding behavior, habitat preferences as investigated by Merrit *et al.* [13] and escape abilities described by Sih [14], therefore it is possible that some species might be better at utilising cover for refuge from guppy predation. Such differences could also affect the impact that guppy predators have on each species in such a scenario. The larvae of the two genera of mosquito that will be examined here each have distinct morphology, posture in the water and coloration. *Anopheles* tends to be paler, and rests horizontally immediately below the water surface. *Culex* is generally darker and rests diagonally with a pronounced siphon touching the surface.

Sex differences in guppy foraging behavior have often been ignored, despite considerable sexual dimorphism as described by Dussault and Kramer [15]. For example, Murdoch *et al.* [16] neglect to specify which sex they tested in their widely cited and influential ‘prey switching’ paper. Male guppies exhibit determinate growth; after sexual maturity, growth slows considerably or stops completely. Females, conversely, continue to grow throughout their lives, and as a result mean female size is always greater than mean male size in guppy populations described by Magurran [8]. Dussault and Kramer [15] said wild females tend to have longer feeding bouts than males and spend a greater proportion of time foraging as described by Magurran and Seghers [17]. There is also evidence of sex differences in feeding niches; female guppies tend to prefer benthic foraging, whilst males are more often found feeding in the water column described by Magurran [8].

Guppies are highly social fish. Shoaling tendency is present from birth described by Magurran and Seghers [18] and is central to predator avoidance strategies in most wild populations. Shoaling has been shown to increase foraging efficacy and facilitate social transmission of foraging information and described by Laland and Williams [19]; social learning also affects both mate choice described by Dugatkin and Godin [20] and antipredator behavior in the guppy described by Kelley and Magurran [21].

Furthermore, the presence of conspecifics is likely to encourage intraspecific competition as investigated by Bertram [22], and persistent sneaky mating and courtship behaviors displayed by male guppies have been shown to be costly to harassed females by reducing their foraging time as investigated by Magurran and Seghers, 1994 [17] and fecundity described by Ojanguren and Magurran [23]. Such costs could lead to harassment avoidance strategies such as increased female boldness as described by Piyapong *et al.* [24] and habitat segregation of the sexes described by Darden and Croft [25].

The guppy, *Poecilia reticulata*, has been introduced to water bodies in many tropical countries as a biological control agent of mosquitoes, often in keeping with government policy described by Dash [26]. Despite this widespread practice, the success of such introductions is largely undocumented and the few reports that are available present conflicting results as described by Seng *et al.* [27].

The introduction of guppies for mosquito control is based on the assumption that they will consume sufficient numbers of water-borne larvae to substantially reduce mosquito populations, and ultimately reduce the incidence of malaria in humans. However, there is surprisingly little literature concerning guppy feeding behavior, and even less relating specifically to their use in biological control. One of the few studies with relevance to biological control was conducted by Murdoch *et al.* [16]. Murdoch and colleagues presented evidence for the existence of ‘prey-switching’ in guppies; a behavior defined as consuming more than is proportional of the most abundant prey in a multi-prey system – switching to an alternative prey type if this becomes most abundant. Switching behavior can be viewed as an application of optimal foraging theory, and as such is a means by which an organism might most efficiently exploit the food resources in a habitat. By employing switching behavior, guppies may be more likely to control invertebrate prey populations and thus be deemed a suitable biocontrol agent as described by Murdoch and Oaten [28].

However, the ecological validity of findings from simple laboratory experiments is questionable, particularly given the social nature of guppy populations; shoaling behavior can encourage intraspecific competition described by Bertram [22], and persistent sneaky mating and courtship behaviors displayed by male guppies have been shown to be costly to harassed females by reducing their foraging time and consequently their fecundity as described by

Magurran and Seghers [17]. Recently, Darden and Croft [25] demonstrated that female habitat preference is affected by the presence of males, with females preferring shallow, safer areas when in an all-female environment and only venturing into deeper, high-predation risk areas when the shallower habitat is occupied by males. This suggests that the presence of conspecifics could also have an important bearing on foraging behaviors such as prey-switching, particularly when two prey patches are spatially distinct.

It is important to improve our knowledge of guppy foraging behavior given that this species is now established in at least 70 countries outside its native range of Trinidad and north-eastern South America. In approximately 60% of these countries, the presence of guppies is wholly or partly due to introduction for mosquito control, yet there is growing evidence that introduced guppies have a negative impact on native species and invaded ecosystems as described by Valero *et al.*[29]. Until more is known about the foraging behavior of the guppy, we cannot evaluate their efficacy as mosquito control agents and therefore cannot justify their introduction to natural habitats for this purpose as described by Simberloff and Stiling [30].

Bleakley [31] suggested that previous studies which involved situations where man-made containers were the main breeding ground for mosquitoes, rather than natural water bodies may be crucial in determining the success of guppies as a biocontrol tool. There is huge gap in the knowledge of the efficiency of guppies as biological control agents of mosquito larvae under varying social conditions relative to the physico-chemical quality of their environment, hence this study.

The specific objectives of this study are to determine the feeding preference of guppies among two species of mosquito larva; investigate the differences in the consumption and prey preference between male and female guppies; determine the effects of the presence of conspecifics on the consumption and prey preference in male and female guppies; and determine the effect of physico-chemical quality of test media on prey consumption and prey preference in male and female guppies.

2. MATERIALS AND METHODS

2.1. Sources and Collection of Guppies and Mosquito Larvae

A total of 120 guppies (*Poecilia reticulata*) were collected from the drains at the second gate of the University of Lagos, Lagos State and stored in experimental tank until required. The geo reference coordinate of point of collection are within longitude 6° 30.674' N and latitude 3° 23.282' E. Newly bred and sorted species of mosquito larvae (*Anopheles spp* and *Culex spp*) were collected from National Institute of Medical Research (NIMR), Yaba, Nigeria. The larvae were selected to be approximately the same size and all were late 3rd instar or early 4th instar. Subsamples of 50 larvae of each species were photographed in Petri dishes before experiments.

2.2. Collection of Water Samples

Surface water sample was collected from the fish habitat by dipping plastic containers (1L capacity) about 2 – 5 cm below the surface film of the water body. Water samples collected were fixed with 10 ml of 10 N Nitric acid, stored in iced cold containers and transported to the laboratory for heavy metal analysis.

2.3. *In situ* Measurements of Physico-chemical Parameters

Physico-chemical parameters which included temperature, pH, electrical conductivity (EC), turbidity, dissolved oxygen (DO), total dissolved solids (TDS) and salinity were measured *in situ* using a multisampling portable meter (Horiban U50 multisampler).

2.4. Digestion of Water Samples for Atomic Absorption Spectrometry (AAS)

Surface water samples from experimental sites were digested according to the methods of APHA/AWWA/WPCF [32]. Samples were mixed and a suitable volume (50ml) was transferred to a beaker. 5ml of conc. HNO₃ was added. It was brought to a slow boil and evaporated on a hot plate to the lowest volume possible (about 15 to 20 ml) before precipitation or salting-out occurred. 5 ml of conc. HNO₃ was added, covered with a watch glass and heated to obtain a gentle refluxing action. Heating was continued and addition of conc. HNO₃ as necessary until digestion was completed as shown by a light coloured, clear solution. 1 to 2 ml conc. HNO₃ was added and warmed slightly to dissolve any remaining residue. Filtrate was transferred to a 100 ml volumetric flask with two 5-ml portions of water and rinses were added to the volumetric flask. It was then cooled, diluted to mark and mixed thoroughly. Portion of this solution was taken for required heavy metal analysis.

2.5. Determination of Heavy Metals in Water Samples.

The digested extracts were filtered through Whatman No.1 filter paper and made up to the mark in appropriate volumetric flasks (50cm³ for water samples), the heavy metal content of the sample was then determined by comparing their absorbance's with those of standard AAS solution using an Alpha-4 Cathode on Atomic Absorption Spectrophotometer.

2.6. Bioassay Technique

2.6.1. Acclimatization and Selection of Test Guppy for Bioassay

The test fishes were kept in the two (2) experimental tanks containing dechlorinated water (one for the female guppies and the other for the male guppies) in order to create enough space for the guppies to acclimatize to laboratory conditions (28 ± 2°C, R.H 70 ± 2%) for a period of seven days before they were used in the bioassays. During the acclimatization, the guppies were fed on 0.1 – 0.5mm Coppens feed (5g per 100 animals). Although, the water in the experimental tanks was aerated, the water was changed once every two days to prevent accumulation of wastes and decaying of uneaten food particles. Feeding was discontinued 24 hours prior to commencement of bio-assays.

2.6.2. Identification and Segregation of the Male and Female Guppies

The male guppies usually reach 2 inches in length while the female guppies are usually a bit bigger at about two and the half inches in length. The males were colourful for attracting a mate. The male has larger dorsal and caudal fins. The female guppies are larger around the abdominal area and have a gravid spot directly above the vent. The male has a gonopodium, an anal fin that is modified into an intromittent organ used for reproduction with the female. The female has brown or black spot near its anus. The male guppies have colours on their body, fins, and tail while the females have stocky bodies.

2.6.3. Identification of *Culex* and *Anopheles* Larvae

The Culicine larva has breathing tube (siphon which it uses to hang down from the water surface while the Anopheline larva has no siphon and therefore rests parallel to and immediately below the water surface.

2.6.4. Bioassay

Trials took place in transparent plastic containers (15 x 8 x 8 cm) filled with dechlorinated water and guppies were introduced into the containers at least 3 hours, and in most cases more than 12 hours (overnight), before the experiment in order for them to settle. Each guppy was only used once as a focal fish, but some were re-used as companion fish in other trials. Ten larvae of each species were placed into a glass prior to each trial and introduced to the plastic by pouring the tube into the front right corner. The number of mosquito larvae consumed in every 10 minutes was recorded. In the trials where companion fish were present, the number of larvae of each species remaining after 10 minutes was also recorded.

2.7. Social Experiment

In this experiment, focal fish were assigned to one of four conditions: single female, single male, female with two female companions and female with two male companions, male with two female companions and male with two male companions.

2.7.1. Habitat Complexity Experiment

This experimental design included the adult female and male guppies. Fishes (male focal and female focal) were tested in one of three social conditions: 1) alone; 2) with two female companions; 3) with two male companions. Covers made from a square of green leaves (6cm x 6cm) with 'fronds' were provided for each experiment cut from the edge to the center to serve as shelter in order to create a natural environment for the larvae. The leaves were held together with a piece of string.

The leaf bundle then floated on the water surface, with a few fronds dangling down into the water column. It covered a 2-dimensional area of approximately 30cm² and was positioned on the right side of the tank, immediately behind where the larvae were introduced at the start of each trial. This provided an opportunity for larvae to actively seek refuge in the cover but they were not actually placed there initially. These experimental design were carried out separately in field and laboratory water.

2.8. Statistical Methods

Data collected were subjected to descriptive and inferential statistical analysis such as mean, standard deviation, bar graphs, paired sample t-test, One way analysis of variation (ANOVA) and Post-hoc multiple comparison were used to determine the significant difference in the variation of the mean feeding rate of the guppies under varying social conditions. Also, One way analysis of variation (ANOVA) was used to analyze the significant differences in the mean concentrations of the water quality parameters between the field and laboratory water.

3. RESULTS

4.1 Foraging Behavior of Guppies Under Varying Social Conditions in both Field and Laboratory Water.

Foraging Behavior of Guppies (Adult female and male singly) Under Varying Social Conditions in Field water (Table 1) and Laboratory water (Table 2). Single female guppy had a higher feeding rate on mixed *Anopheles* and *Culex* larvae (12.00 ± 2.83 in field water, 17.00 ± 1.41 in laboratory water) compared to the males (3.00 ± 1.41 in field water, 4.00 ± 1.41 in laboratory water). The difference observed in the feeding rate was statistically significant at $p < 0.05$ (Table 1). The feeding rate of the guppies (both the male and the female guppy) on mixed *Anopheles* and *Culex* larvae were higher when they had female companions in contrast to male companions in both the field water and laboratory water. The difference observed in the feeding rate among the varying social conditions was statistically significant at $p < 0.05$ (Table 2).

Based on preference to *Culex* or *Anopheles*, the guppies showed a higher feeding rate for *Culex* in the field water and in the laboratory water for the varying social conditions while the only exception to this higher preference for *Culex* was the male with two male companions which had a higher preference for *Anopheles* in field water. The single female guppy showed a higher feeding rate with preference to *Culex* larvae (9.00 ± 1.41 field water, 11.50 ± 2.12 laboratory water) than to *Anopheles* (3.00 ± 1.11 field water, 5.50 ± 0.71 laboratory water) and so is the preference of single male guppy for *Culex* (2.00 ± 1.41 field water, 3.00 ± 1.41 laboratory water) than to *Anopheles* (1.00 in both field and laboratory water) as shown in Tables 2 and 3 respectively. In the field water, paired sample t-test showed a significant higher preference ($t = 3.095$, $p < 0.05$) of the Guppies' for *Culex* than *Anopheles* when the guppies were exposed to a mixture of *Culex* and *Anopheles* (Table 1). In the laboratory water, paired sample t-test showed a significant higher preference of the guppies for *Culex* than *Anopheles* ($t = 5.318$, $p < 0.05$) when the guppies were exposed to a mixture of *Culex* and *Anopheles* (Table 2)

On exposure to *Culex* larvae only, the guppies fed more in laboratory water both singly and under varying social conditions than in field water. The single female guppy had higher feeding rate (9.00 ± 1.41 field water, 13.00 ± 1.41 laboratory water) compared to the male (1.41 ± 1.00 field water, 6.00 ± 1.41 laboratory water). The feeding rate of the female guppy with male and female companion was the same in laboratory water but differ in field water as it was higher for female with female companions than female with male companions. The feeding rate of the male guppy was higher with female companions than with male companions. The difference observed in the feeding rate for *Culex* larvae only among the varying social conditions was statistically significant at $p < 0.05$ only in the field water

On exposure to *Anopheles* larvae only, the guppies fed more in laboratory water both singly and under varying social conditions than in field water as shown in Table 2. The single female guppy had higher feeding rate (7.00 ± 1.41 field water, 10.00 ± 2.83 laboratory water) compared to the male (0.71 ± 0.50 field water, 4.00 ± 0.00 laboratory water). The feeding rate of the guppies was higher when they had female companions in contrast to male companions. The difference observed in the feeding rate among the varying social conditions was statistically significant at $p < 0.05$ in field water while it was not significant in laboratory water.

Table 1. Foraging Behavior of Guppies (Adult female and male singly) Under Varying Social Conditions in Field water.

Social status	Culex + Anopheles	Preference to Culex	Preference to Anopheles	Culex only	Anopheles only
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F	12.00 ± 2.83 ^a	9.00 ± 1.41 ^a	3.00 ± 1.41 ^a	9.00 ± 1.41 ^a	7.00 ± 1.41 ^a
M	3.00 ± 1.41 ^b	2.00 ± 1.41 ^b	1.00 ± 0.00 ^a	1.41 ± 1.00 ^b	0.71 ± 0.50 ^b
F + 2F	9.00 ± 1.41 ^{ab}	7.00 ± 1.41 ^a	2.00 ± 0.00 ^a	7.00 ± 1.41 ^a	6.00 ± 0.00 ^a
F + 2M	8.00 ± 0.00 ^{ab}	7.00 ± 1.41 ^a	1.41 ± 1.00 ^a	5.00 ± 1.41 ^a	4.00 ± 2.83 ^a
M + 2F	7.00 ± 1.41 ^{ab}	4.00 ± 2.83 ^b	3.00 ± 1.41 ^a	4.00 ± 2.83 ^{ab}	3.00 ± 1.41 ^a
M + 2M	5.00 ± 1.41 ^b	2.00 ± 1.41 ^b	3.00 ± 0.00 ^a	2.00 ± 0.00 ^b	1.41 ± 1.00 ^b

Variables within the same column with different superscript are significantly different at $p < 0.05$

Table 2. Foraging Behavior of Guppies (Adult female and male singly) Under Varying Social Conditions in Laboratory Water.

TEST	<i>Culex</i> + <i>Anopheles</i>	Preference to <i>Culex</i>	Preference to <i>Anopheles</i>	<i>Culex</i> only	<i>Anopheles</i> only
F	17.00 ± 1.41 ^a	11.50 ± 2.12 ^a	5.50 ± 0.71 ^a	13.00 ± 1.41 ^a	10.00 ± 2.83 ^a
M	4.00 ± 1.41 ^b	3.00 ± 1.41 ^a	1.00 ± 0.00 ^b	6.00 ± 1.41 ^a	4.00 ± 0.00 ^a
F + 2F	13.00 ± 1.41 ^{ac}	7.00 ± 0.00 ^a	6.00 ± 1.41 ^a	12.00 ± 1.41 ^a	9.00 ± 1.41 ^a
F + 2M	11.00 ± 1.41 ^c	7.00 ± 0.00 ^a	4.00 ± 1.41 ^a	12.00 ± 1.41 ^a	8.00 ± 1.41 ^a
M + 2F	11.00 ± 1.41 ^c	7.00 ± 0.00 ^a	4.00 ± 1.41 ^a	12.00 ± 1.41 ^a	8.00 ± 1.41 ^a
M + 2M	8.00 ± 1.41 ^{bc}	5.50 ± 0.71 ^a	2.50 ± 0.71 ^b	8.00 ± 1.41 ^a	7.00 ± 1.41 ^a

Variables within the same column with different superscript are significantly different at $p < 0.05$

KEY: F – Female, M – Male, F + 2F – Female + 2 Female companion, F + 2M – Female + 2 Male companion, M + 2F – Male + 2 Female companion, M + 2M – Male + 2 Male companion.

3.2. Variation in the Physicochemical Quality of the Exposure Media (Field and Laboratory Water).

The physicochemical properties and concentration of heavy metals (Mean ± S.D) of the exposure media (field and laboratory water samples) assessed are presented in Tables 3 and 4 respectively.

3.2.1. Variation in the Physicochemical Parameters of the Field and Laboratory Water Samples.

As shown in table 4a, pH was found to be lower in laboratory water sample with a pH value of 7.35 ± 0.21 than in the field water sample with a pH value of 7.70 ± 0.2 . Conductivity was higher in the field water sample with a value of 271.30 ± 9.48 and lower in laboratory water sample with a value 141.10 ± 9.05 . Salinity was lower in laboratory water sample with a value of 0.03 ± 0.01 and higher in field water sample with a value of 0.07 ± 0.01 . Turbidity was higher in the field water sample with a value of 23.35 ± 2.33 and was not detected in laboratory water sample. Alkalinity was found to be lower in the laboratory water sample with a value of 27.25 ± 3.89 and higher in the field water sample with a value of 36.90 ± 4.38 . Total suspended salt (TSS) was detected in the field water sample to be 12.80 ± 1.70 but it was not detected in the laboratory water sample.

Table 3. The Physico-chemical Properties of Field and Laboratory Water Samples used as Medium of Exposures to the Guppy Fishes.

S/N	PARAMETERS	LEVEL DETECTED IN FIELD WATER	LEVEL DETECTED IN LAB WATER	FME/WHO Permissible Limit
1	pH	7.70 ± 0.28	7.35 ± 0.21	6.5 – 8.5
2	Colour (Pt-Co)	11.50 ± 2.12	ND	≤15
3	Conductivity (μS/cm)	271.30 ± 9.48*	141.10 ± 9.05	500
4	Salinity (‰)	0.07 ± 0.01	0.03 ± 0.01	0.2
5	Turbidity (NTU)	23.35 ± 2.33*	ND	≤5
6	Appearance	ND	ND	≤15
7	Odour	ND	ND	NS
8	Chloride Cl ⁻ (mg/l)	33.45 ± 3.61*	10.90 ± 1.56	600
9	COD (mg/l)	86.75 ± 4.60*	1.80 ± 0.28	≤7
10	BOD ₅ ²⁰	36.00 ± 5.66*	1.70 ± 0.42	≤4
11	TSS (mg/l)	12.80 ± 1.70*	ND	≤500
12	TDS (mg/l)	192.25 ± 6.01*	93.85 ± 5.59	≤200
13	NO ₃ ⁻ (mg/l)	2.68 ± 0.19*	1.14 ± 0.01	≤45
14	PO ₄ ³⁻ (mg/l)	0.19 ± 0.03*	0.03 ± 0.01	≤100
15	SO ₄ ²⁻ (mg/l)	8.45 ± 0.64*	4.35 ± 0.49	≤100
16	D.O (mg/l)	2.55 ± 0.21	6.05 ± 0.35*	≥5
17	NH ₄ ⁺ (mg/l)	0.06 ± 0.01*	ND	-
18	S ²⁻ (mg/l)	0.03 ± 0.01*	ND	0.2
19	Phenols (mg/l)	ND	ND	0.2
20	Oil & Gas (mg/l)	0.02 ± 0.01	ND	10
21	Alkalinity (mg/l)	36.90 ± 4.38	27.25 ± 3.89	NS
22	CN ⁻ (mg/l)	ND	ND	0.1
23	THC (mg/l)	ND	ND	10
24	Detergent (mg/l)	0.37 ± 0.03*	ND	15

*-Variables across the rows are significantly different at $p < 0.05$

Table 4. The Concentration of Heavy Metals in Field and Laboratory Water Samples used as Medium of Exposures to the Guppy Fishes.

S/N	PARAMETERS	LEVEL	LEVEL	FME/WHO
		DETECTED IN FIELD WATER	DETECTED IN LAB WATER	Permissible Limit
1	Ba (mg/l)	0.06 ± 0.01*	ND	NS
2	Cd (mg/l)	0.04 ± 0.01*	ND	≤0.01
3	Cu (mg/l)	0.13 ± 0.04	0.02 ± 0.01	≤0.50
4	Fe (mg/l)	2.29 ± 0.04	0.06 ± 0.01	≤0.30
5	Mn (mg/l)	0.06 ± 0.01	0.02 ± 0.00	≤0.50
6	Ni (mg/l)	ND	ND	NS
7	Pb (mg/l)	0.09 ± 0.02*	ND	≤0.05
8	V (mg/l)	ND	ND	NS
9	Zn (mg/l)	4.30 ± 0.05*	1.19 ± 0.03	≤5.00

*-Variables across the rows are significantly different at $p < 0.05$

KEY: ND – Not Detected.

Total dissolved salt (TDS) was found to be higher in field water sample with a value of 192.25 ± 6.01 and lower in the laboratory water sample with a value of 93.85 ± 5.59 . The variation in the concentrations of physicochemical between the field and laboratory water was significant for EC, Turbidity, TSS and TDS at $p < 0.05$. The variation in these physical parameters for the laboratory and field water was all within the FME/WHO permissible limit.

Chloride (Cl^-) ion was lower in the laboratory water sample with a value of 10.90 ± 1.56 and higher in the field water sample with a value of 33.45 ± 3.61 . Nitrate (NO_3^-) ion was found to be lower in laboratory water sample with a value of 1.14 ± 0.01 and higher in the field water sample with a value of 2.68 ± 0.19 . Phosphate (PO_4^{3-}) ion was found to be higher in the field water sample with a value of 0.19 ± 0.03 and lower in the laboratory water sample with a value of 0.03 ± 0.01 . Sulphate ion (SO_4^{2-}) was lower in the laboratory water sample with a value of 4.35 ± 0.49 and higher in the field water sample with a value of 8.45 ± 0.64 . Ammonium ion (NH_4^+) was detected in the field water sample with a value of 0.06 ± 0.01 but was not detected in the laboratory field water. Sulphide (S^{2-}) was found in the field water sample with a value of 0.03 ± 0.01 but not detected in the laboratory water sample. Manganese (Mn^{2+}) was found to be lower in the laboratory water sample with a value of 0.02 ± 0.00 and higher in the field water sample with a value of 0.06 ± 0.01 . The variation in the concentrations of ions between the field and laboratory water was significant for Cl^- , NO_3^- , PO_4^{3-} , SO_4^{2-} , NH_4^+ , and S^{2-} at $p < 0.05$. The variation in these Ions (anions and cations) for the laboratory and field water was within the FME/WHO permissible limit (Table 3).

Oil and gas was detected in the field water sample with a value of 0.02 ± 0.01 but it was not detected in the laboratory water sample. Detergent was also detected in the field water sample with a value of 0.37 ± 0.03 but it was not detected in the laboratory water sample. Chemical oxygen demand (COD) was found to be lower in the laboratory water sample with a value of 1.80 ± 0.28 and higher in the field water sample with a value of 86.75 ± 4.60 . The Biochemical oxygen demand (BOD) was found to be higher in the field water sample with a value of 36.00 ± 5.66 and lower in 1.70 ± 0.42 . The Dissolved oxygen was found to be lower in the field water sample with a value of 2.55 ± 0.21 and higher in the laboratory water sample with a value of 6.05 ± 0.35 . The variation in the concentrations of oxygen parameters between the field and laboratory water was significant for Detergent, COD, BOD and DO at $p < 0.05$. The variation in COD, BOD, and DO in laboratory water was within the FME/WHO permissible limit while the variation in COD BOD and DO in field water were beyond the FME/WHO permissible limit.

3.2.2. Variation in the Concentration of the Heavy Metals in the Field and Laboratory Water Samples.

Barium (Ba) was found in the field water sample to be 0.07 ± 0.01 but it was not detected in the laboratory water sample. Cadmium (Cd) was found in the field water sample with a value of 0.04 ± 0.01 but not found in the laboratory water

sample. Copper (Cu) was found to be lower in the laboratory water sample with a value of 0.02 ± 0.01 and higher in the field water sample with a value of 0.13 ± 0.014 . Iron (Fe) was found to be higher in the field water sample with a value of 2.29 ± 0.04 and lower in the laboratory water sample with a value of 0.06 ± 0.01 . Magnesium (Mn) was found to be lower in the laboratory water sample with a value of 0.02 ± 0.00 and higher in the field water sample with a value of 0.06 ± 0.00 . Lead (Pb) was detected in the field water sample with a value of 0.09 ± 0.02 but it was not detected in the laboratory water sample. Zinc (Zn) was found to be lower in the laboratory water sample with a value of 1.19 ± 0.03 and higher in the field water sample with a value of 4.30 ± 0.05 . The variation in the concentrations of heavy metals was significant for Ba, Cd, Pb and Zn at $p < 0.05$ between the field and laboratory water. The variation in the concentration of the heavy metals in the laboratory water was within the FME/WHO permissible limit while the variation in the concentration of Cd, Fe, and Pb in field water were beyond the FME/WHO permissible limit (Table 4).

4. DISCUSSION

The total number of mixed *Culex* and *Anopheles* fed on by female guppies was higher than male guppies under the varying social conditions in both field and laboratory media. Guppies of both sexes were capable of consuming 3rd and 4th instar *Culex* and *Anopheles* larvae, and did so eagerly. As might be expected due to their sizes, females were more efficient foragers under the varying social conditions of exposure to *Culex* and *Anopheles* than males, consuming significantly more larvae. When male was in the presence of female companions the difference in number of larvae consumed compared with the single male forager was very evident. The trend in the feeding potential of the guppies in relation to social conditions in this study is in line with the earlier work carried out by Amy [33] who investigated the foraging behavior of guppy in relation to its use in the biological control of mosquito larvae. There was a marked preference for *Culex*, when fish of either sex were feeding alone. However, this is in contrast with the findings of Anyaele and Obembe [34] who carried out a comparative studies of the feeding capacity and preference of *Aphyosemion gularis* on some aquatic macro invertebrates. Elias *et al.* [35] also found that female guppies consumed nearly double the quantity of *Culex* larvae consumed by males. It was observed that males sometimes struggled with the *Culex* larvae, due to the protruding siphon present in this species but this did not usually stop the eventual consumption of the prey. There are a number of possible explanations for a social effect on prey preference. It is possible that in the presence of two females, increased competition may lead to reduced discrimination between prey species by the focal female. Alternatively, a greater number of fish in the arena may mean that the larvae were more active either as a behavioral response to predators or simply because there was an increased predator-prey encounter rate. The latter seems more likely as larvae of both species tended to remain still until the made contact, at which point they would move potentially attracting the attention of all three predators in cline with earlier description by Sih [14]. Whereas the diagonal resting position of *Culex* larvae means that this species is likely to be relatively more conspicuous when still, *Anopheles* tends to remain cryptic until it is prompted to move.

There was no evidence that prey species were using the cover more than the other, but it is possible that any difference may have been undetectable given the experimental design. The variation in the feeding potential of the guppies was found to be higher in the laboratory water than in the field water. This variation may be as a result of the relative differences in the physico-chemical quality of the media such as pH, conductivity, TSS, TDS, detergent, oil and gas, chloride which were found to be higher in field water than laboratory water but are still within the FME/WHO permissible limit. Some oxygen parameters such as Turbidity, COD, BOD were found to be higher in field water than in the laboratory water, they were also higher than the FME/WHO permissible limit. Some of the heavy metals detected in the field water which includes copper, zinc, lead, magnesium, cadmium and iron were above the FME/WHO permissible limit. However there were no heavy metals detected in the laboratory water. This difference in the water quality might be responsible for the high foraging behavior of the guppies in laboratory water than in field water. Recent explorations of heavy metals in fresh water habitats reveal that they can modify chemical communication between individuals resulting in info-disruption that can impact ecological relationships within species thereby affecting animal behavior and social structure in line with earlier discovery by Manna *et al.*[36]. Therefore, previously neglected factors such as social context, physicochemical quality of guppies' environment and habitat complexity which has shown to increase the ecological validity of this study, should be integrated when introducing guppies as biological agent for mosquito control.

5. CONCLUSION

The findings of this study highlight the potential effects that a multi-prey system might have on the effectiveness of biological control measures using guppies. One effect might be that guppy populations are able to persist for longer even once the target prey has been successfully eradicated or reduced as described by Manna *et al.*[36], which could

be beneficial from a biological control perspective as it would mean that fish would not have to be frequently replenished. However, it might simultaneously render the fish less likely to bring the target prey population under control if they are also feeding on other prey species, depending upon their relative preference for the target prey. It was apparent that the sex of the forager, the social environment, habitat quality and complexity are each capable of affecting foraging behavior and prey use. Although single fish display a preference for *Culex*, the non-preferred larvae were nevertheless readily consumed, supporting the continued investigation of guppies in malaria control.

7. REFERENCES

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