



Salinity tolerance of wild *Poecilia reticulata* (guppy) under laboratory conditions

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ABSTRACT

The present study was carried out to investigate the salinity tolerance of *Poecilia reticulata* (guppy) occurring in Sri Jayewardenepura canal system, in order to assess its suitability to control brackishwater breeding mosquito species such as *Aedes* sp. Guppies were exposed to different salinity levels directly and gradually under laboratory conditions in glass tanks. To test the direct effect of salinity, 9 different salinities (ranging from 0ppt as a control to 35ppt) prepared in three replicates, each stocked with 10 fish were kept for a period of 12 weeks. To test the effect of gradual increase of salinity, three tanks containing freshwater were stocked with 10 fish in each and a gradual increase of salinity (by 5ppt once in 2 weeks up to 38ppt) was done. Mortality rates, growth (total length) and the breeding of fish were recorded in both experiments at regular time intervals. When the fish were exposed to different salinities directly, fish started dying at 10 ppt (10% mortality) after the fourth day of exposure and this mortality rate was observed in 10-20ppt salinity ranges within the 12 weeks (0% mortality was recorded in control tank). 50% survival was shown at 28ppt salinity level at the end of the 12th week. Beyond 28ppt, there was 100% mortality. Fish showed an average of 5-7 mm growth in salinity levels up to 28ppt but they were not significantly different ($p=0.886$). Breeding was seen in salinity levels up to 20ppt producing 7-15 fry/female and the fry also could tolerate the salinity levels they were bred into with a mortality level of 20-30% within the study period. Contrast to the above results, the fish when exposed to gradual increasing of salinity levels could survive up to 38ppt with only a 20% mortality rate. They showed a length increase between 1-5mm, in all salinity levels but they were not significantly different ($p=0.330$). In gradually increasing salinity levels, fish were seen breeding even at 35 ppt (7-10 fry/female) with 10-20 % mortality rate. The results show that the guppy can tolerate and survive in salinity levels up to 28ppt when they are directly introduced from the wild and they can tolerate up to a level of 38ppt salinity if the salinity levels increase gradually. They can grow under saline conditions and breed successfully and the fry can survive well in the saline environment. This makes wild caught *P. reticulata* (guppy) a potential candidate that can be used as a bio-control agent in the control of species such as *Aedes* sp-mosquito that breed in brackish water environments of Sri Lanka.

KEYWORDS: Guppy, Salinity, *Aedes* sp, Bio-control

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INTRODUCTION

Poecilia reticulata (guppy) was introduced in the 1930s from Central America to dry zone and Western province as a bio control agent for mosquito larvae (Silva and Kurukulasuriya, 2010). This species is now widely distributed across North Western (Weerasinghe, 2008), Western (Bambaradeniya, 2008) and Southern (Gunawardena, 2008) provinces, inhabiting a variety of aquatic habitats such as streams, marshes, paddy fields as well as ditches in urban areas and survive in polluted water and tolerate extreme environmental fluctuations. The guppy is considered as an euryhaline fish which has a potential for establishing many strains (Macaranas and Fujio, 1990). Brackish water and salt water marshes in the tropics are well known as mosquito breeding habitats where the water temperature does not drop below 15 °C (Chervinski, 1983). Although pre-imaginal development of *Aedes* sp mosquitoes have been known to exist in fresh water environmental conditions for many years in Sri Lanka, few recent findings have revealed the possibility of *Aedes* breeding and immature stage developments in brackish water conditions (Surendran *et al* 2007; Ramasamy *et al* 2011; Ramasamy and Surendran, 2012). This calls for the attention to find a suitable environment friendly control method to deal with brackish water breeding mosquito species. One of the control methods used to reduce mosquito larvae is stocking guppies in brackish water and salt water marshes as they are able to survive in different salinities (Chervinski 1983). However, as it has been demonstrated that the ability for

the guppy to survive in different salinities differs among strains (Shikano and Fujio, 1994; Shikano and Fujio, 1995), it is important to know about the salinity tolerance levels of the guppy occurring in the natural environment of Sri Lanka, if it is to be used for mosquito control in brackish water. The present study is thus an attempt to study the salinity tolerance of wild caught guppy under laboratory conditions.

MATERIALS AND METHODS

The guppies studied in these experiments were caught from wild populations in Attidiya, Bellanwilla and Rattanapitiya areas. Fish were maintained in glass tanks (75 cm x 30 cm x 45 cm) under laboratory conditions at the Department of Zoology, University of Sri Jayewardenepura and cleanliness of all tanks were ensured through regular cleaning and replenishment of water. They were fed with a commercial food pellet and during this period (two times a day at 5% body weight, Purified seawater (38ppt) was obtained from the Aquamarine Ornamental Fish Export Company at Ratmalana. Randomly selected guppies of 23-33 in mm total length (TL) were used in the experiments. The Temperature (T°C), Dissolved Oxygen (DO) and pH were maintained at 28-30 °C, 4-6 ppm and 8-9 respectively. A series of two experiments were conducted. Each experiment was carried out in three replicates. The study was carried out from June 2016 to November 2016.

First experiment was carried out to determine the effect of direct exposure of

guppies to different water salinities (i.e. 5ppt, 10ppt, 15ppt, 20ppt, 25ppt, 30ppt and 35ppt) while Experiment 2 was carried out to determine the effects of indirect exposure of guppies to different water salinities (i.e. from freshwater (0ppt) to sea water (38 ppt).

Experiments were conducted in 8 glass tanks (75 cm x30 cm x 45 cm) with 10 liters of aerated water. One tank had freshwater (0ppt), and the other 7 had mixed seawater and freshwater in proportions to produce the required salinities. (5ppt, 10ppt, 15ppt, 20ppt, 25ppt, 30ppt and 35ppt). Ten fish were transferred to each of these containers from the freshwater tanks. The mortalities (No. of dead fish) were recorded at frequent intervals for twelve weeks. The total lengths were measured once a month to determine their growth.

Second experiment was carried out in two glass tanks (75 cm x30 cm x 45 cm) each containing 10 fish in 10 liters of freshwater. The salinity was increased every 14 days adding seawater so that the fish remained 14 days in each salinity level (5ppt, 10ppt, 15ppt, 20ppt, 25ppt, 30ppt, 35ppt and 38ppt). The mortalities were recorded at each salinity level and experiment was

terminated after the fish had remained in 38ppt salinity level for 60 days.

Cleanliness of all tanks was ensured through regular cleaning and replenishment of water. They were fed with a commercial food pellet during this period (two times a day at 5% body weight), Basic water quality parameters such as pH, Salinity, Temperature and Dissolved Oxygen (DO) were measured using a multipara-meter (YSI ProPlus), Mean length values of *P.reticulata* and mortality rate were analyzed using MINITAB version 17 and Microsoft Excel 2013.

RESULTS

Table 1 show the percentage mortality of *P. reticulata* when directly exposed to different salinity levels. Fish started dying at 10 ppt (10% mortality) after the fourth day of exposure and this mortality rate was observed from 10-20ppt salinity ranges within the three months. 30% mortality was shown at 28ppt salinity level on the first day of stocking while the mortality rate increased to 50% both in 25ppt and 28ppt salinity levels on the second day of stocking. Beyond 28ppt, there was 100% mortality (Fig. 1).

Table 1: Percentage of mortality (given as an average of the three replicates) of *Poecilia reticulata* following direct exposure to different salinity levels

Salinity levels (ppt)	Mortality rate of <i>P.reticulata</i> (%) different time period											
	12 hr	1 day	2 day	3 day	4 day	5 day	6 day	7 day	2 week	4 week	8 week	12 week
0	No mortality											
5	No mortality											
10	No mortality					10 % mortality						
15	No mortality			10 % mortality								
20	No mortality		10 % mortality									
25	No mortality		50 % mortality									
28	No mortality	30 % mortality	50 % mortality									
30	No mortality	100 % mortality										
35	100 % mortality											

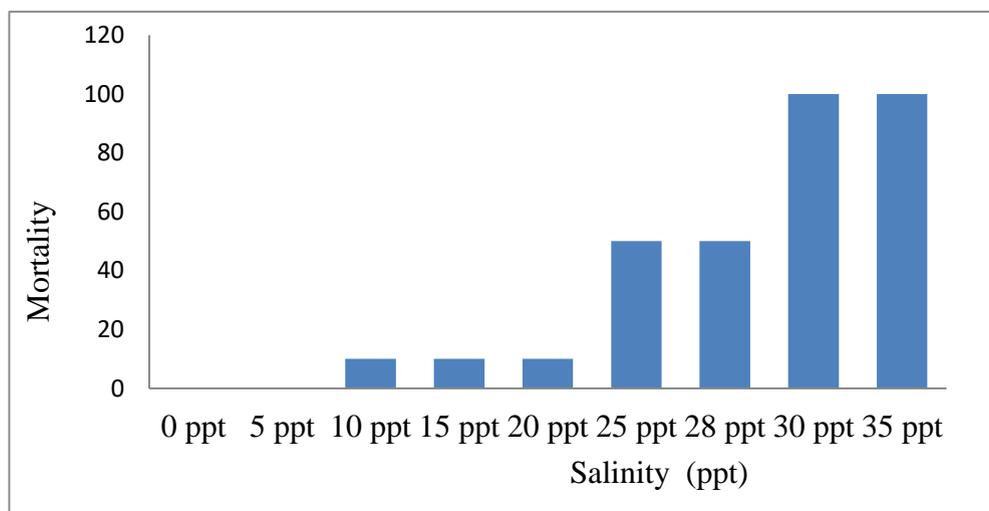


Figure 1: Mortality (%) of *Poecilia reticulata* after direct transfer from fresh water to various sea water concentrations.

Fish showed an average of about 3.2-7.3 mm growth in salinities upto 28ppt and was not significantly different for different salinity levels ($p=0.886$) (Table 2). Breeding of the fish was seen in salinities

upto 20ppt producing 7-15 fry/female and the fry also could tolerate the salinities they were bred into with a mortality level of 20-30% (Table 2) within the study period.

Table 2: The total lengths of fish measured at the end of each month ($P \leq 0.05$) and number of fry produced during direct exposure to different salinity levels.

Salinity of water (ppt)	Mean length of fish (mm)				Average growth (mm)	Number of fry produced during direct exposure		
	Initial (mm)	End of 4 th week (mm)	End of 8 th week (mm)	End of 12 th week (mm)		Average Length of fish(mm)	Number of fry	Mortality (%)
0	25.4±2.24	28.2±2.98	30.8±1.89	32.7±1.24	7.3	-	-	-
5	25.6±3.42	27.6±3.78	29.4±2.76	31.1±2.08	5.5	28.2	8	20
10	25.2±2.32	27.0±2.87	29.0±2.21	31.2±1.98	6.0	30.4	11	20
15	24.2±3.45	25.7±3.76	28.8±2.31	30.4±2.56	6.7	33.3	14	30
						32.6	15	30
20	25.7±1.87	27.2±2.45	28.9±1.67	30.7±2.34	5.0	20.4	7	30
25	23.6±3.27	23.3±2.64	24.8±2.22	26.8±2.45	3.2	-	-	-
28	26.3±2.84	27.8±3.34	29.8±2.36	31.8±2.23	5.5	-	-	-
30	26.3±2.59	-	-	-	-	-	-	-
35	26.3±3.65	-	-	-	-	-	-	-

In contrast to the above results, the fish when exposed to gradually increasing salinities, could survive upto 38ppt with only a 20% mortality rate (Table 3, Fig. 2), while, they showed a length increase between 1-6mm but the length increase of

fish at different salinity levels was not significantly different ($p=0.330$) (Table 3). In gradually increasing salinities, fish were seen breeding even at 35ppt levels (7-10 fry/female) with 10- 20% mortality rate (Table 3).

Table 3. Mortality rates and total lengths of fish measured at the end of each month following gradual exposure to different salinities ($P \leq 0.05$) and number of fry produced during gradual exposure to different salinity levels.

Salinity level and time period of exposure	Mortality (%)	Mean length of fish(mm)		Number of fry produced during gradual exposure		
				Length of fish	Number of fry	Mortality (%)
0 ppt(2 week)	0	Beginning of the experiment	23.5±2.47	-	-	-
5ppt(2week)	0	End of 4 th week	26.1±3.42	-	-	-
10ppt(2 week)	0					
15 ppt (2 week)	0	End of 8 th week	27.4±3.28	31.4	8	10
20 ppt (2 week)	0					
25 ppt (2 week)	0	End of 12 th week	28.2±2.87	33.2	10	10
30 ppt (2 week)	10					
35 ppt (2 week)	20	End of 16 ^{ed} week	29.5±2.54	30.8	7	20
38 ppt (2 week)	20	End of 18 ^{ed} week	31.2±4.22			

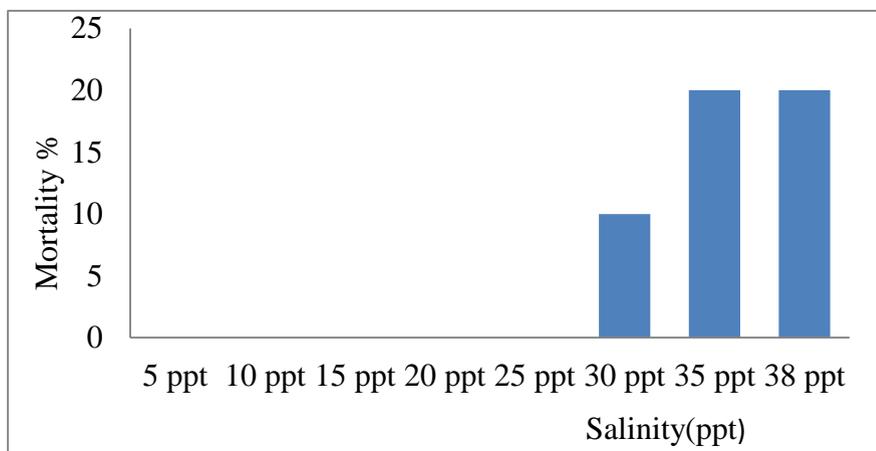


Figure 2: Mortality (%) of *Poecilia reticulata* after gradual transfer from freshwater to sea water.

DISCUSSION

The present study indicates that guppy taken from wild populations are able to tolerate a direct exposure of 28 ppt salinity with 50 % mortality but they show 100% mortality beyond this level, while they can tolerate salinities upto 38ppt if the salinity levels increase gradually showing only 20% mortality. Their growth or breeding does not get impaired under these salinity conditions and the fry are able to withstand the salinities they are born into. While the maximum tolerance level of the present study remains at 28ppt, another study on the salinity tolerance of different strains of guppy has recorded that no guppy survived in 35 ppt seawater upon direct transfer to different salinities (Shikano and Fujio,1994). Their study also has shown that there was no significant variation in relation to sexes and sizes of fish although sea water adaptability differed among strains.

The higher level of salinity tolerance (38ppt) with only 20% mortality observed when the fish were exposed to gradual increases in salinity in the present study has been observed in some other studies as well. Chervinski (1983) showed that guppy are able to tolerate a direct transfer to 50% sea water (19.5ppt) without any mortality while 80 % of the fish were able to tolerate gradual transfer to 100% (39ppt) and 150% (58.5ppt) sea water. Similar results were observed for *Gambusia affinis*, a close relative of the guppy (Chervinski, 1983). Such a high tolerance to a gradual exposure of high salinity levels can be explained by induced development of osmoregulatory functions such as brachial chloride cells in the guppy due to gradual acclimation to sea water (Pisam *et al.*, 1987).

According to the observations made in the present study wild guppy occurring in Sri Lankan aquatic environments is a potential candidate for controlling mosquito species occurring in brackish water and saltwater marshes as they can acclimatize to tolerate salinity levels as high as 38ppt.

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