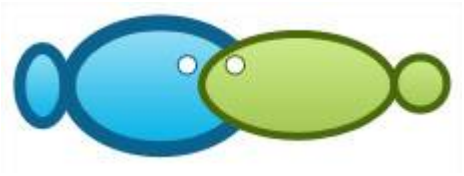

The effect of aquarium size/volume on the reproduction of the guppy fish *Poecilia reticulata* (Peters, 1859)

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Abstract. The main objective of this research was to determine a minimum size/volume required to obtain reproduction and offspring of the ornamental fish popularly known as guppy *Poecilia reticulata*, in order to contribute to a better management of the species in captivity, both at the level of mass production and maintain animal welfare standards. Although assumed that the space or volume of the aquarium is an important characteristic for the well-being and production of ornamental fish, this aspect has been little studied and few research articles on the subject were found. Three plastic aquaria with different sizes/dimensions were used: "large", 21.5 cm length x 11.5 cm width x 13 cm height, with a water volume of 2.5 liters; "medium", 14.5 cm length x 9.0 cm width x 9.5 cm height, with a water volume of 1.0 liters; "small", 8.6 cm length x 6.5 cm width x 7.0 cm height, with a water volume of 0.3 liters. The study lasted 6 months, in two experimental runs, that guaranteed two complete reproduction cycles and obtaining offspring. Three fishes (one male with two females) were placed in each tank. Guppies matured and produced young at the first maturation age (approximately 3 months) and sizes of 3.1-3.6 cm TL and 2.5-4.1 cm TL for males and females, respectively, at temperatures between 24 and 28°C. Live larvae were found only in the medium (1.0 L) and large (2.5 L) size aquaria. Those in the small aquarium (0.3 L) died (either male or female, or both). These findings demonstrated that mating, fertilization, embryonic development, and production of guppy fish larvae were possible in small spaces with low volumes of water. Thus, the minimum volume of the aquarium for breeding guppy and obtaining larvae was 1.0 liter. In other words, it can be concluded that a minimum volume for breeding guppy can be in aquariums of 1.0 liters or more. This is of great relevance in the mass production of guppies (or other ornamental fish) as it may be necessary as a tool to have a large number of containers to keep separate pairs/trios to achieve proper selection of desired traits of guppy colors and shapes.
Key Words: aquarium water volume, animal welfare, broodstock, culture conditions.

Introduction. A compilation of multiple survey results from the scientific and popular literature indicated that the guppy *Poecilia reticulata* (Peters, 1859) is among the most popular of the aquarium fish, where millions of individuals, generating millions of US dollars, are traded annually in the world's aquarium market (e.g., Conroy 1975; Chapman et al 1997; Livengood et al 2014; Kalous et al 2015; OEC 2023).

Although the native range for the guppy is from the coastal streams and rivers in western Venezuela, the Guianas, and nearby islands (Rosen & Bailey 1963; FishBase 2023), the species has been introduced and is widely distributed elsewhere around the world (Welcomme 1998; Kalous et al 2015). The guppy is now cultured primarily in countries in Southeast Asia, including Singapore, Malaysia, Indonesia, and Thailand; as well as countries in the Americas, like the Caribbean islands of Trinidad and Tobago, and Jamaica (Chapman et al 1997; Fernando & Phang 1994).

Guppies under cultivation are typically managed based on their genetic background, type of reproductive system, and the behavior both female and male exhibit during mating. Guppies are livebearing (ovoviviparous) fish that have an internal fertilization mechanism of reproduction in which the eggs are fertilized inside the female, and the embryos are nourished from the yolk of the eggs until hatched in the female (Turner 1940; Rosen & Bailey 1963; Liu & Lee 2014). When the young are fully developed, they are released to the outside environment of the female (Rosen & Bailey

1963; Meffe & Snelson 1989; Liu & Lee 2014). Guppies also have a mating system called polyandry where females can mate promiscuously with multiple males and can store the sperm from the different males for long periods of time (Meffe & Snelson 1989). Despite the species characteristics of the female reproduction process, in the aquarium hobby, male guppies are preferred purely from an aesthetic perspective because they have more vibrant colors and their tail patterns are more varied compared to those of females. In guppy, genes for body coloration are sex linked to the male Y-chromosome and the various tail forms are linked to both the male Y- and female X- chromosomes (Winge & Ditlevsen 1947; Khoo et al 1999; Nakajima & Taniguchi 2001). It has also been observed that guppy populations have been under strong domestication pressure, and have had a long-history of being cultured (e.g., spanning some 75 to 300 generations over 100 years of domestication, Fernando & Phang 1994; Nakajima & Taniguchi 2001; Teletchea 2016).

Guppies are raised primarily using artisanal methods, typically in concrete tanks (e.g., 2.18 m L x 0.76 m W x 0.46 m H) or net-cages (e.g., 2.76 m L x 0.93 m W x 0.93 m H) suspended in earthen ponds; at stocking densities of 115-180 guppies m^3 of water (Fernando & Phang 1994). Though the facilities vary greatly in size and scope (e.g., Chapman 2000), from small-scale integrated farms to organized monoculture facilities (e.g., Singapore, Hong Kong, U.S.A, Czech Republic). Broodstock fish are selected from growout tanks and placed into separate breeding aquariums at ratios of 1:2, 1:3, 1:4, or 1:5 males to females (Fernando & Phang 1994; Urueña & Mora 2007).

There is a wide variation in the sizes of tanks used for breeding purposes. However, in the scientific database FishBase (2023), the minimum recommended aquarium size for the guppy is "60 cm"; which in aquarium circles is commonly equivalent to a 60 cm x 30 cm x 30 cm tank, with a water capacity of about 54 litres. Although assumed that the size/volume of the aquarium is an important characteristic for the well-being and production of ornamental fish, this aspect has been little studied and few research articles specific to the subject were found after an extensive literature search using the databases Web of Science and PubMed, comprising information obtained from species both for human consumption and/or ornamental purposes; using search terms and combinations of key words as tank size or volume, aquarium, aquarium fish, ornamental fish, fish (e.g., Espmark et al 2017; Lee et al 2022; Shishis et al 2022). Therefore, the main objective of this study was to determine a minimum size/volume required to obtain reproduction and offspring of the ornamental fish popularly known as guppy *P. reticulata*, in order to contribute to a better management of the species in captivity, both at the level of mass production and maintain animal welfare standards.

Material and Method. The experiment was carried out in the municipality of Buenaventura, Valle del Cauca, Colombia and followed guidelines set by the AFS & ASIH for the use of fishes in research (AFS & ASIH 2014), and proper animal care used by investigators in our laboratory at the Universidad del Pacífico, Buenaventura, Colombia.

Guppies were approximately 1-2 months old and purchased from a local producer. At first sexual morphological differentiation, virgin females were separated from males. At the start of the experiment, guppies were first acclimatized for 1-week and fed daily, while in a 50-liter plastic container that had an average water temperature of 25^{o}C. After the acclimation period, individual fish were weighed, measured, and separated into trios of two females to a single male as recommended in the literature (Andrews 1986; Urueña & Mora 2007). The trios were randomly assigned and placed into 'small', 'medium', and 'large' aquariums with the following values: "large", 21.5 cm L x 11.5 cm W x 13 H, with a water volume of 2.5 liters; "medium", 14.5 cm L x 9.0 cm W x 9.5 cm H, with a water volume of 1.0 liters; "small", 8.6 cm L x 6.5 cm W x 7.0 cm H, with a water volume of 0.3 liters. The sides and bottom of the aquaria were slotted, and placed floating but submerged all in one same plastic tank (90 cm dia. x 30 cm H, 200 liters), to ensure that water quality remained the same for all aquaria. The slots on the aquarium also served as protection for the released larvae as they could swim out and away of the aquarium, and not be eaten by the parents. Additionally, and as a control group, a trio of guppies were placed in a close by, but separate plastic tank of the same dimensions as the treatment tank and life support system. In order to maintain water quality, a sponge

filter driven by a powerhead was placed in the middle of the tank, with aeration to guarantee the flow, oxygenation, and removal of nitrogenous compounds. There was also a weekly change of water of the volume of the main container (30%), as well as cleaning of the aquaria with a brush. Water temperature remained between 24 and 28°C (Inkbird-RTD), dissolved oxygen above 5 mg L^{-1} (YSI-DO200), and ammonia < 0.5 mg L^{-1} (Tetra-EasyStrips).

The guppies were exposed to natural light (approx. 12h light/12h dark), and observed daily with special attention to their behavior and state of health; such as normal or resisted swimming, rubbing against objects in the tank, presence of skin lesions, and food consumption. Dead fish were immediately removed and recorded. Guppies were fed ad libitum twice daily with a commercial diet for ornamental fish (White Crane-A.D.P., 200 to 500 microns). The experiment lasted 6 months, from September 2022 to February 2023; guaranteeing complete cycles of reproduction, development, and obtaining offspring. When hatchlings were observed, larvae/juveniles were removed, counted and recorded.

Results and Discussion. In this study it was demonstrated that mating, fertilization, embryonic development, and production of guppy larvae were possible in confined spaces and low volumes of water. Guppies matured and produced young at the first maturation age (approximately 3 months) and total length of 3.1-3.6 cm and 2.5-4.1 cm for males and females, respectively, at temperatures between 24 and 28°C. It is important to highlight that at the beginning of this study the females were virgins, so the previous presence of sacs storing sperm (spermatophores) was not a variable to consider in this study. The age and length were within the commonly reported reproductive sizes for the species at the given temperature range of 18 to 28°C (Andrews 1986; Meffe & Snelson 1989; FishBase 2023).

Guppies bred and hatchlings were found only in the medium (1.0 L) and large (2.5 L) size aquariums. Those in the small aquarium (0.3 L) died, first the male and then the females. In a second attempt with other trio, the result was the same, observing mortality in the aquarium, but first a female, then the male and finally the other female. In the medium size aquarium, 6 larvae were obtained in a first attempt and 10 larvae in a second repetition. In the large aquarium, 8 larvae were obtained in a first trial, and in the second trial 2 hatchlings were released. In both the medium and large size aquariums, 20 larvae and 19 larvae (respectively) were released again, seven days after the first group of larvae was obtained from each of the aquariums. Hatchlings were also obtained in the control group (plastic tank), with 4 and 12 larvae, respectively; equally separated by a seven days interval. The number of larvae obtained in this study were similar to other observations made with guppy by numerous hobbyyists, eg., up to 20 live hatchlings at a time (Andrews 1986; Fernando & Phang 1994; Urueña & Mora 2007; FishBase 2023). The number of larvae produced depends on many factors, such as the variety of the species, and the age of the female (Andrews 1986; Meffe & Snelson 1989). The absence of reproduction and mortality in the 0.3 L aquarium may be due to increased male-female interaction. Males of the species are recognized to be aggressive and when space tightness is minimal, they can get quite territorial towards one another and may even crash into the aquarium.

With aquarium keeping being primarily a hobby activity, there is a wide variety in the sizes of aquaria used, and it is obvious that these are based on the experiences of hundreds of thousands of hobbyists. Typical aquaria volumes for mating and breeding small ornamental fish such as guppies and swordtails (Poeciliidae), danios (Cyprinidae), and tetras (Characidae) are 15 to 37 liters (eg, Andrews 1986; Dabrowski & Miller 2018). Smaller aquaria were developed as intended for commercial establishments and for experimental use (eg, Rottmann & Campton 1989; Chapman et al 1998; Adey & Loveland 2007), to optimize mass production of fish, and greater use of space. Still there are no standards for the size of aquaria needed to breed ornamental fishes, including the zebrafish which is considered already a biomedical animal model (Lawrence & Mason 2012; Lee et al 2022).

The commercial industry commonly advertises aquaria between 1.0 and 3.6 liters for the maintenance and breeding of zebrafish to universities, hospitals, and R & D companies (e.g., Lawrence & Mason 2012; Zebrafish International Resource Center ZIRC; The Zebrafish Information Network ZFIN; AQUANEERING; ZEBCARE; SCANBUR). In this study, the minimum volume of the aquarium for reproduction and obtaining guppy larvae was 1.0 liters. The zebrafish is also used ornamentally and the size (4-6 cm) and weight (0.2-1 g), is similar to that of the guppy (3-6 cm; 0.1-1 g). Although changes in behavior (swimming and stamina) of zebrafish were observed in confinement in low-volume aquaria, no effects of stress (cortisol) were detected in individuals (Maierdiyali et al 2020).

Conclusions. In this investigation it was demonstrated that a minimum volume for reproduction and breeding in guppy fish could be in aquaria of 1.0 liters or more. This is of great relevance in the mass production of guppies (or other ornamental fish) as it may be necessary to have a large number of containers to keep separate pairs/trios and obtain the desired selection of colors and shapes in individuals.

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