Water hardness influenced reproductive dynamics in two freshwater fish species; Poecilia reticulata and Betta splendens

Abarna Krishnakumar
University of Jaffna - Vavuniya Campus

Anton Patrick
University of Jaffna - Vavuniya Campus

Uthpala Jayawardena (✉ uajay@ou.ac.lk)
Open University of Sri Lanka https://orcid.org/0000-0001-9613-4385

Research note

Keywords: Water hardness, freshwater fish, fecundity, gonadosomatic index, growth performance

DOI: https://doi.org/10.21203/rs.3.rs-35557/v1

License: ☺ ☀ This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License
Abstract

Objective:

Hardness of water in the form of CaCO3 affect reproductive potential in various fish species, differently. The study evaluates the effect of water hardness on growth and reproduction of two aquarium fishes, *Poecilia reticulata* (Ovo-viviparous sp.) and *Betta splendens* (Oviparous sp.) by growing them under 150 (control), 320, 540 and 900 ppm CaCO3 levels in semi natural aquaria.

Results:

Growth increased with increasing water hardness, reporting a significant growth of *P. reticulata* (p = 0.005) at 900 ppm. Similarly, the reproductive potential of *P. reticulata* was improved significantly, recording the highest fecundity (16.22 ± 3.90) and Gonadosomatic Index (GSI-2.48 ± 0.597) at 900 ppm. However, in *B. splendens* water hardness adversely affected the reproduction by reporting a significantly low hatchability and disturbed bubble nests at 900 ppm, compared to the largest bubble nest formed at the control (108.58 ± 16.19 cm2). Thus, study revealed differential effects of water hardness on reproductive potential of the test species, by increasing the potential of *P. reticulata* while decreasing that of *B. splendens*. Though larval survival was affected in both species, larval growth was improvement significantly in *P. reticulata* at 900 ppm level. Understanding reproductive dynamics in natural waters are crucial for propagation and conservation perspectives of freshwater fishes.

Introduction

Water hardness is a crucial factor for the growth, reproduction and embryo development of fish [1]. It affects the growth and reproductive biology of oviparous and ovoviviparous fish species differently due to variations in their requirement of CaCO3 in the reproductive process [1–5]. Considering the importance of hardness in the hatchability, larval growth and survival of fish eggs, it is recommended to maintain the hardness levels of the fish culture ponds above 20 mg/L CaCO3 [6, 7].

Water hardness in some areas of the Dry Zone of Sri Lanka exceeds even 1000 mg/L. Vavuniya District reports remarkably high hardness values varying in between 100–1000 mg/L [8]. In Vavuniya, aquarium trade for ornamental fish keeping mainly depended on ground water sources. Thus, evaluating the effect of higher hardness on fish health is of prime economic and ecological importance.

Freshwater species, *Poecilia reticulata* (Guppy) and *Betta splendens* (Siamese fighting fish) were ovoviviparous and oviparous fishes, respectively and were popular verities in the aquarium fish trade. They can be easily reared and bred under laboratory conditions.

The study anticipates to evaluate the effect of water hardness on ovi-paraous & ovo-viviparaous fish biology in semi-natural aquaria by measuring the growth performance of adults and larvae, and reproductive performances in terms of gonadosomatic Index (GSI), fecundity, fertility, hatchability and
larval survival by using two aquarium fish species; *Poecilia reticulata* (Guppy) and *Betta splendens* (Siamese fighting fish) as model organisms.

## Methods

### Preparation of Aquaria

Glass aquaria of the size of 25x13x12 cm3 (24 tanks) were used for the exposure. Being an aggressive fish, male *Betta splendens* were planned to keep in 20 separate cubic aquaria (12x12x12 cm3) at a rate of each male per cube. The interior of aquaria was prepared as a simulation of natural pond using stones, aquatic plants and aeration.

The experiment composed of control (tap borne water 150 ppm CaCO3) and three treatment setups; 320, 540 and 900 ppm prepared by adding analytical grade CaCO3 to aged tap water. The hardness of the medium was determined by EDTA titrimetric method [9] and the treatment setups were monitored weekly basis to maintain the water hardness. Experiment setups were maintained at the Environmental Biology Laboratory (EBL) at the Vavuniya Campus in Sri Lanka. All the experiments were conducted as per the guidelines given by the research review panel of the Department of Bioscience, University of Jaffna.

### Introduction of fishes to Aquaria & Maintenance

Ovo-viviparous fish as *Poecilia reticulata* and oviparous fishes as *Betta splendens* were chosen for the experiment. The virgin fishes of both species (males and females) were purchased from a nearby aquarium in Vavuniya and were transferred to the EBL. Sexing was done by examining external morphology as both species showed sexual dimorphism. Initial weight and standard length were measured. Then the fishes were acclimatized to aquarium condition and introduced to the experimental setups (150 ppm (control), 320, 540 and 900 ppm CaCO3 and reared for 1½ month. In each experimental setup 15 females were introduced to 5 males of *B. splendens*, separately. To avoid aggression each males of *B. splendens* were kept separately in the smaller aquaria. For, *P. reticulata*, 25 females were introduced to 5 males. The exposure was conducted with three replicates.

### Determination of growth performance

Weight and length of adult fish were measured, and the length weight relationship analysis was conducted, using $W = a TL^b$: $(\log W = \log a + b \log TL)$ [10] to obtain the linear regression.

### Determination of reproductive potential
Two gravid females from each aquarium were randomly tested for fecundity (the number of ripening eggs found in the female just prior to spawning) and Gonadosomatic Index (GSI – the ratio of fish gonad weight to body weight). Euthanizing was conducted with 0.02% MS222 solution.

To estimate the bubble nest size of *B. splendens*, clean breeding aquarium (size-60x30x30 cm³) were prepared without artificial bottom stones and aeration. A floating plant leaf was put on the air water interface to facilitate the nest formation. After adding the male in to the breeding aquarium, a clean glass cube containing female fish was placed near it to stimulate the nest building. Then the bubble nests built by male *B. splendens* were measured (diameter) by a ruler [11].

Hatchability of *B. splendens* was estimated after allowing successful courtship with a gravid female inside the breeding aquarium. Repeated introductions of gravid females were required to obtain a successful breeding. Without disturbing the bubble nest, the number of eggs released was counted. At the end of the matting, female was removed, and male was allowed for 24 -48 hour for pre hatched parental care. Next, the hatched eggs were counted, and the hatchability was determined, as the number of larvae hatched over the total number eggs [12].

Fertility of *P. reticulata* was determined by counting the intra-follicular embryos inside the female fish by sacrificing few fishes at the 21st day after mating [13]. Breeding tank of *P. reticulata* was formed with the artificial aquarium stones and *Vallisneria* to provide hiding place for young ones. After female broods release young ones, they were separated from their parents and counted.

Larval survival rate was considered after one week of exposure to the water hardness treatments by counting the number of surviving one weeks-old larvae divided by the total number of hatched eggs / released young ones [12]. The larval growth performance was estimated in every 10 days interval by measuring the length gain.

**Statistical analyses**

One-way ANOVA and Tukey pairwise comparison were used to analysis the significant differences of weight, standard length, fecundity and Gonadosomatic Index, Laval growth (length) of the fishes. The linear regression analysis was used to find the length weight relationship (LWR) of the fish to know the growth pattern. In LWR linear regression analysis, the slope of regression lines explicit the exponent coefficient value ‘b’. Significant variation in the estimates of ‘b’ for the fish species was examined from the expected value (ideal value ‘b’ = 3) was tested by t-test [14,15]. Students t-test was employed by dividing the difference between ‘b’ and ‘3’ by standard error of ‘b’ [16].

**Results**

*Growth performance of P. reticulata and B. splendens in various water hardness levels*
Growth improved with the increasing water hardness by showing significantly high weight values, particularly (p<0.05) above 540 ppm treatments, reaching 80% and 40% weight increments, respectively for *P. reticulata* (N=30) and *B. splendens* (N=20) in the highest hardness level (900 ppm). Similarly, length of the fishes was also increased with higher hardness levels, though only 14-17% increments were recorded.

When the growth pattern was estimated *P. reticulata* showed isometric growth at 540 and 900 ppm levels while *B. splendens* showed isometric growth in all hardness levels. (Table 1).

Reproductive potential of *P. reticulata* and *B. splendens* in various water hardness levels

Reproductive potential of *P. reticulata* (live bearers/ovoviviparous) showed significant variations (p<0.05) in Gonadosomatic Index, fecundity, bubble nest surface area, hatchability, fertility, under varying water hardness levels. GSI of *P. reticulata* increased with the hardness reporting a significantly higher value (GSI=2.480 ± 0.597, p = 0.016) at 900 ppm. However, GSI of *B. splendens* (egg layers/oviparous) lowered though not significant with the treatment (GSI of 150 ppm =17.01, 900 ppm=15.833, p=0.731).

Fecundity of *P. reticulata* increased with the water hardness, reporting a significantly higher value at 900 ppm. (16.20 ± 3.90, p=0.016). Unlike the fecundity of *P. reticulata*, that of *B. splendens* declined considerably by hardness treatment, showing only a slight increment in 540 ppm (824 ± 175 eggs compared to 743 at the control, Figure 1).

Bubble nest surface area of *B. splendens* showed significant decline along the hardness series, reporting 108.58 cm² at the control and 26.5 cm² at 900 ppm. In the hard water environment 540 & 900 ppm, bubbles were blasted and male fish was unable to rebuild the nest.

Fertility of *P. reticulata*, given as the number of intrafollicular embryos, was increased along the hardness series, reporting 88% in 900 ppm compared to 74% at the control, though the increment was not significant (p>0.05). Hatchability of *B. splendens* declined significantly in higher hardness levels above 540 ppm reporting only 25% success at 900 ppm compared to 78% in the control (p = 0.006). The percentage of larval survival of both species declined gradually with the increasing hardness level, reporting significant decline above 320 ppm (p<0.0009) hardness. However, the larval growth of *P. reticulata* was improved by higher hardness levels reaching a significant growth at 900 ppm (p=0.006), though the effect on *B. splendens* larvae was insignificant.

Discussion

Water hardness in the form of CaCO3 had differential effects on *P. reticulata* and *B. splendens* growth and reproduction. Growth in the form of body weight and length, and reproduction by means of gonadosomatic index, fecundity, bubble nest diameter, fertility were improved in *P. reticulata* while those were degraded in *B. splendens*. These observations are compatible with previously reported studies for ovoviviparous and oviparous fishes, conducted elsewhere.
According to Shim and Ho [17], dissolved Calcium is essential for growth of live bearer (ovoviviparous) fish especially *P. reticulata*. They also found that rearing *P. reticulata* in extreme water hardness (2500ppm) showed 10 times higher body weight gain than in soft water (167 ppm). In the same way, James *et al.*, [18] found that *Xiphophorus helleri* (live bearer) reared in 1018ppm water hardness level exhibited better growth performance. Thus, higher water hardness positively favour the growth of *P. reticulata* in semi-natural aquarium. This observation is further reiterated by weight and length relationship, which revealed isometric growth occurred only above 540ppm hardness level. On the other hand, increasing water hardness showed no apparent effect on *B. splendens* resulting isometric growth in all hardness levels.

Reproduction of *P. reticulata* was enhanced showing faster sexual maturation, with higher hardness conditions in compliance with James [18] and Stratton [19], who reported higher and faster sexual maturation of *X. helleri*, in exceptionally high hardness medium. In line with this observation, Shim and Ho, [17] suggested that dissolved calcium is essential for sexual maturation of *P. reticulata*. As an oviparous fish, *B. splendens* showed retarded reproductive potential under higher hardness. This is reiterated by previous work, Ratinam [5] who found suppressed gonad development and maturity of *Pterophyllum scalare* and aborted maturation in *Barbus conchonius* and *Barbus letrazona* beyond 120 ppm hardness. Further it was reported that high calcium in hard water deposit on the surface of the fish eggs, blocking the water absorbed into the perivitelline [4, 20] leading to dehydration and shrinking of the eggs [3]. Thus, being an oviparous fish *B. splendens* is suitable to the soft water environment than the hard water environment.

Increasing water hardness caused high mortality in larvae of both species. Newborn are unable to tolerate the adverse environmental factors like extreme hard water [21] due to the stress condition in the physiology created by excess amount of calcium. Numerous studies carried on various fish species; *Clarias gariepinus*, Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), and brown trout (*Salvelinus fontinals*), *Rutilus frisii kutum* (kutum), reiterated this finding [3,4,22-24]. Thus, soft water is preferable for larval rearing for both *P. reticulata* and *B. splendens*.

Hence, it may conclude that *P. reticulata* requires more calcium for the growth and reproduction than *B. splendens* which grow and reproduce well in soft water environment.

**Limitations**

This study was not intended to describe mechanism/s of action of CaCO3 in mediating growth and reproductive alteration of *P. reticulata* and *B. splendens*.

**List Of Abbreviations**

ANOVA-Analysis of Variance, EBL- Environmental Biology Laboratory, EDTA- Ethylenediaminetetraacetic acid, GSI-Gonadosomatic Index, LWR-Length weight relationship
Declarations

- Ethics approval and consent to participate

Ethical approval was obtained from the Research Ethics panel at the Department of Bio Science, Vavuniya Campus, University of Jaffna, Sri Lanka. Authors declare that the experiments conducted are complied with the standard animal care guidelines and the current laws of Sri Lanka.

- Consent for publication

Not applicable

- Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

- Competing interests

The authors declare that they have no competing interests

- Funding

The study was conducted with the existing facilities of the Department of Bio Science, Faculty of Applied Science, Vavuniya Campus, and the Department’s allocations were utilized for purchasing animals and consumables. Thus, no any other form of funding involved in the study.

- Authors’ contributions

AK carried out the study under the guidance of PAES and UAJ. AK drafted the manuscript and UAJ and PAES reviewed it before the initial submission. All authors read and approved the final manuscript.

- Acknowledgements

The authors acknowledge the Department of Bio Science, Faculty of Applied Science, Vavuniya Campus for providing facilities and the assistance.

References

1. Wilkerling K. Platies for everyone. Trop fish hobby. 1992;41:8–21.
2. Lee CS, Hu F. Influences of Ca and Mg ions on the egg survival of grey mullet, Mugil cephalus L. Journal of Fish Biology. 1983 Jan;22(1):13–20.
3. Gonzal AC, Aralar EV, Pavico JM. The effects of water hardness on the hatching and viability of silver carp (Hypophthalmichthys molitrix) eggs. Aquaculture. 1987 Jul 1;64(2):111-8.
4. Ketola HG, Longacre D, Greulich A, Phetterplace L, Lashomb R. High calcium concentration in water increases mortality of salmon and trout eggs. The Progressive Fish-Culturist. 1988 Jul;50(3):129–35.
5. Rathinam K. 1993). Influence of certain environmental factors on growth and breeding of ornamental fishes. B.F.Sc., Thesis submitted to Tamil Nadu Veterinary and Animal Sciences University, Tuticorin. 1993 pp. 18–27.
6. Boyd CE, Tucker CS. Pond aquaculture water quality management. Springer Science & Business Media; 2012 Dec 6.
7. Brown DJ, Lynam S. The effect of sodium and calcium concentrations on the hatching of eggs and the survival of the yolk sac fry of brown trout, Salmo trutta L. at low pH. J Fish Biol. 1981 Aug;19(2):205–11.
8. Piyasiri S, Senanayake I. Status of ground water in Vavuniya City, Sri Lanka with special reference to fluoride and hardness.2016.
9. Apha A. *Standard methods for the examination of water and wastewater*, 2005; 21, 258–259.
10. Le Cren ED. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (Perca fluviatilis). The Journal of Animal Ecology. 1951 Nov;1:201–19.
11. Srikrishnan R, Hirimuthugoda N, Rajapakshe W. Evaluation of growth performance and breeding habits of fighting fish (Betta splendens) under 3 diets and shelters. Survey in Fisheries Sciences. 2017 Feb 15;3(2):50–65.
12. RALC. Integrated Fish Farming, Vol. 1. Regional Aquaculture Lead Center of China, Wuxi, People’s Republic of China. 1981; 411 pp.
13. Stolk A. Histo-endocrinological analysis of gestation phenomena in the cyprinodont *Lebistes reticulatus* Peters. I. Thyroid activity during pregnancy. In *Proc. Acad. Sci., Amsterdam*. 1951. Vol. 54, pp. 550 – 57.
14. Snedecor GW. The comparison of two groups. Statistical Methods, Chap. 4, The Iowa State College Press, USA, 1963; p. 534.
15. Jayaprakash AA. Length weight relationship and relative condition in *Cynoglossus macrostomus* Norman and *C. arel* (Schneider). Journal of the Marine Biological Association of India. 2001;43(1 & 2):148 – 54.
16. Zar JH. Biostatistical analysis. Pearson Education India; 1999.
17. Shim KF, Ho CS. Calcium and phosphorus requirements of guppy Poecilia reticulata. Bulletin of the Japanese Society of Scientific Fisheries (Japan). 1989.
18. James R, Sampath K. Effect of water hardness on growth and reproductive potential in *Xiphophorus helleri* and *Betta splendens*. Journal of aquaculture in the tropics. 2004.
19. Stratton RF. Secrets of the swordtail. Trop Fish Hobbyist. 1993;42:116–24.
20. Potts WT, Rudy PP. Water balance in the eggs of the Atlantic salmon Salmo salar. Journal of experimental Biology. 1969 Feb 1;50(1):223 – 37.
21. Chanu TI, Rawat KD, Sharma A, Das A, Devi BN. Effects of water hardness on egg hatchability and larval survival of aquarium fish, Danio rerio. Journal of Aquaculture. 2010;18:1–7.
22. Molokwu CN, Okpokwasili GC. Effect of water hardness on egg hatchability and larval viability of Clarias gariepinus. Aquaculture International. 2002 Jan 1;10(1):57–64.

23. Taghizadeh V, Imanpour M, Jahnbakhashi A. Effect of water hardness on growth, survival, hematocrit and some blood biochemical indices of kutum (Rutilus frisii kutum) fingerlings. Global Veterinaria. 2013;10(1):22–5.

Table

Table 1. Length weight relationship of *P. reticulata* and *B. splendens* in various water hardness levels

| LWR-Weight Relationship | Water hardness (CaCO3 ppm) | 150 (Control) | 320 | 540 | 900 |
|--------------------------|-----------------------------|---------------|-----|-----|-----|
| *Poecilia reticulata*    |                             |               |     |     |     |
| LWR                     | logW= 1.396+2.398 logL      | logW= -1.174+2.022 log L | logW=1.176+3.106 log L | logW= 1.704+3.019 logL |
| b value                 | 2.398                       | 2.022         | 3.106 | 3.53 |
| P value                 | 0.267                       | 0.171         | 0.002* | 0.003* |
| *Betta splendens*       |                             |               |     |     |     |
| LWR                     | logW=-1.424+2.844 logL      | logW=0.389+1.027 log L | logW= -1.631+3.143 log L | logW=-1.467+2.848 logL |
| b value                 | 2.844                       | 1.027         | 3.143 | 2.848 |
| P value                 | 0.038*                      | 0.003*        | 0.003* | 0.003* |

Figures
Figure 1

Logarithmic values of the fecundity of *P. reticulata* and *B. splendens* under varying water hardness treatments (150-900ppm). Error bars represents standard error of mean. *Significant alteration from the control (p<0.05).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- NC3RsARRIVEGuidelinesChecklist2014Abarnaetal.docx