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

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Research note

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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 900ppm. 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 900ppm. However, in *B. splendens* water hardness adversely affected the reproduction by reporting a significantly low hatchability and disturbed bubble nests at 900ppm, 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 improved significantly in *P. reticulata* at 900ppm level. Understanding reproductive potential of aquarium fishes in natural waters is crucial for their propagation purposes.

Introduction

Water hardness i.e. a measure of Calcium (Ca2+), Magnesium (Mg2+) and/or Iron (Fe2+) in water, is crucial for the growth, reproduction and embryo development of fish [1-4]. It affects the growth and reproduction of oviparous and ovoviviparous fishes differently due to variations in their requirement of CaCO3 in the reproduction [1, 5-8]. Considering the importance of hardness in the early life stage processes such as hatchability, larval growth and survival of eggs, it is recommended to maintain the water hardness above 20 mg/L CaCO3 [9,10].

Water hardness in Vavuniya District, of the Dry Zone of Sri Lanka reports remarkably high values, 100 – 1000 mg/L [11]. In Vavuniya, ornamental fish trade is mainly depended on ground water. 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. *Preticulita* produces eggs that are hatched within the body (ovoviviparous, live bearers) and the hatchlings are born alive while *B. splendens* lay eggs in a foam nest (oviparous, egg layers) for external fertilization. Both species, can be easily reared and bred under laboratory conditions.
The study anticipates to evaluate the effect of water hardness on two aquarium fish species; *P. reticulata* (Guppy) and *B. splendens* (Siamese fighting fish) as representatives for ovo-viviparous & oviparous fishes by measuring the growth performance of adults and larvae, and reproductive potential in terms of gonadosomatic Index (GSI), fecundity, fertility, hatchability and larval survival under semi-natural aquaria conditions.

**Methods**

**Preparation of Aquaria**

Glass aquaria of the size of 25x13x12 cm$^3$ (24 tanks), simulating natural pond environment were used for the exposure. Being an aggressive fish, male *B. splendens* were planned to keep in 20 separate cubic aquaria (12x12x12 cm$^3$) at a rate of each male per cube.

The experiment composed of control (tap borne water 150 ppm CaCO$_3$) and three treatment setups; 320, 540 and 900 ppm prepared by adding analytical grade CaCO$_3$ to aged tap water. The hardness was determined by EDTA titration [12] and the treatment setups were screened weekly to maintain the hardness. 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 *P. reticulata* and oviparous fishes as *B. 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 laboratory. Sexing was done by examining external morphology, where male fishes of both species possessed narrow and bright colored bodies and colorful caudal fins compared to round bellies and short caudal fins of the female fishes, displaying sexual dimorphism. Initial weight and standard length were measured, reporting 0.48753 ± 0.00833 g/ 2.9844 ± 0.0522 cm for *P. reticulata* and 1.2667 ± 0.0721 g/ 3.4969 ± 0.0120 cm for *B. splendens*. Then the fishes were acclimatized to aquarium condition (20 min. in each setup) and introduced to the experimental setups, 150 ppm (control), 320, 540 and 900 ppm CaCO$_3$ and reared for 1½ month. This series was selected to cover the hardness range, 100-1000 ppm in Vavuniya [8]. In each experimental setup 15 females were introduced to 5 males of *B. splendens*, separately. To avoid aggression, each male of *B. splendens* was kept separately in the smaller aquaria. For, *P. reticulata*, 25 females were introduced to 5 males. The exposure was conducted with three replicates.

Feeding (5% of the total body weight) were done twice a day at *ad libitum* with commercial fish pellets. The aquaria were maintained to keep the temperature 25-27 ºC and pH 6.5-7.5 by replacing the media with newly prepared hard water on weekly basis. Debris was siphoned out. Mild aeration level was maintained as the fishes are air breathers.

**Determination of growth performance**
Weight and length of adult fish were measured, and the length weight relationship was analyzed, using 
$W = a TL^b$: (Log $W = \log a + b \log TL$) [13] to obtain the linear regression.

_Determination of reproductive potential_

Two gravid females from each setup (N= 12 per each species) were randomly tested for fecundity (the 
number of ripening eggs found in the female just prior to spawning) and GSI (the ratio of fish gonad 
weight to body weight). Euthanizing was conducted with 0.02% MS222 solution.

_Reproductive potential of B.splendens_

To estimate the bubble nest size of _B.splendens_, three sets of clean breeding aquaria (size-60x30x30 
cm$^3$) 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 into 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 [14].

Hatchability of _B.splendens_ was estimated after a successful courtship with a gravid female where 
repeated introductions of the females were required. Without disturbing the bubble nest, the number of 
eggs released was counted. After mating female was removed, and male was allowed for 24 -48 hour for 
pre hatched parental care. Next, the hatched larvae were counted, and the hatchability was determined, as 
the number of larvae hatched over the total number eggs [15].

_Reproductive potential of P. reticulata_

Fertility of _P. reticulata_ was determined by counting the intra-follicular embryos inside the female by 
sacrificing few fishes (N=6) at the 21$^{st}$ day after mating [16]. 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 hardness treatments by counting 
the number of surviving one weeks-old larvae divided by the total number of hatched larvae / released 
young ones [15]. The larval growth performance (N=12) was estimated in every 10 days interval by 
measuring the length gain.

_Statistical analyses_

Normality of the data set was tested before applying the statistics with SPSS 20.0 (IBM, USA). One-way 
ANOVA and Tukey pairwise comparison were used to analyse the 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). 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 [17,18].
Students t-test was applied to analyze the variation i.e. derived by dividing the difference between ‘b’ and ‘3’ by standard error of ‘b’ [19].

Results

Growth performance of exposed fishes

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

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

Reproductive potential of exposed fishes

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

Fecundity of *Pretticulata* increased with the water hardness, reporting a significantly higher value at 900ppm. (16.20 ± 3.90, p=0.016). Unlike the fecundity of *Pretticulata*, that of *B. splendens* declined slightly by hardness treatment, showing only a slight increment in 540ppm (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, 540 & 900ppm, bubbles were blasted and male fish was unable to rebuild the nest.

Fertility of *Pretticulata*, given as the number of intrafollicular embryos, was increased along the hardness series, reporting 88% in 900ppm 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 540ppm 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). However, the larval growth of *Pretticulata* 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

*Preticulata* and *B. splendens* displayed varying growth and reproductive potentials under different water hardness conditions. Growth (body weight and length) and the reproductive potential (gonadosomatic index, fecundity, bubble nest diameter, fertility) of *Preticulata* were improved while most of those parameters were lowered in *B. splendens* with increasing hardness levels. These observations are compatible with previously reported studies for ovoviviparous and oviparous fishes, conducted elsewhere [20,21].

According to Shim and Ho [20], dissolved Calcium is essential for growth of live bearer (ovoviviparous) fish especially *Preticulata* as they are native to hard waters. They also found that rearing *Preticulata* in extreme water hardness (2500ppm) showed 10 times higher weight gain than in soft water (167ppm). In the same way, James and Sampath [21] found that *Xiphophorus helleri* (live bearer) reared in 1018ppm hardness level exhibited better growth performance compared to 76ppm level. Water hardness influenced growth performances of *Preticulata* were further reiterated by weight and length relationship, which revealed isometric growth occurred only above 540ppm level. On the other hand, increasing water hardness showed no apparent effect on *B. splendens* resulting isometric growth in all hardness levels. Similarly, common snook *Centropomus undecimalis* and largemouth bass *Micropterus salmoides* exposed to higher hardness showed no apparent growth performances compared to their counter parts [3,4].

Reproduction of *Preticulata* was enhanced showing faster sexual maturation, with higher hardness conditions in compliance with James and Sampath [21] and Stratton [22], who reported higher and faster sexual maturation of *X. helleri*, in exceptionally high hardness medium. In line with this observation, Shim and Ho, [20] suggested that dissolved calcium is essential for sexual maturation of *Preticulata*. As an oviparous fish, *B. splendens* showed slightly lowered reproductive potential under higher hardness. This is reiterated by previous work, Ratinam [8] who found suppressed gonadal development and maturity of *Pterophyllum scalare* and aborted maturation in *Barbus conchonius* and *Barbus letrazona* beyond 120 ppm hardness. Further, in compliance with previous studies, it was observed that high calcium in hard water deposit on the surface of the eggs of *B. splendens*, blocking the water absorbed into the perivitelline [7, 23] leading to dehydration and shrinking of the eggs [6]. Thus, being an oviparous fish *B. splendens* is suitable to the soft water than the hard water environment.

Increasing hardness caused high mortality in larvae of both species. Newborn are unable to tolerate the adverse environmental factors like extreme hard water [24] 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 fontinalis*), *Rutilus frisii kutum* (kutum), reiterated this finding [6,7,25,26]. Thus, soft water is preferable for larval rearing for both *Preticulata* and *B. splendens*.

Hence, it may conclude that *Preticulata* 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, 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.

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Table
|                           | 150 ppm | 320 ppm | 540 ppm | 900 ppm |
|---------------------------|---------|---------|---------|---------|
| **Growth performance**    |         |         |         |         |
| Weight Gain               |         |         |         |         |
| *P. reticulata* (N=30)    | 0.685\(b\) ± 0.058 | 0.742\(b\) ± 0.075 | 0.772\(a,b\) ± 0.091 | 0.887\(a\) ± 0.107 |
| *B. splendens* (N=20)     | 1.730\(a\) ± 0.124 | 1.702\(a\) ± 0.049 | 1.796\(a\) ± 0.066 | 1.774\(a\) ± 0.167 |
| Length Gain               |         |         |         |         |
| *P. reticulata* (N=30)    | 3.250\(b\) ± 0.035 | 3.313\(b\) ± 0.042 | 3.313\(b\) ± 0.055 | 3.488\(a\) ± 0.024 |
| *B. splendens* (N=20)     | 3.770\(a\) ± 0.204 | 3.930\(a\) ± 0.315 | 3.920\(a\) ± 0.239 | 4.010\(a\) ± 0.315 |
| Larval length gain        |         |         |         |         |
| *P. reticulata* (N=12)    | 16.667\(b\) ± 0.0764 | 18.167\(b\) ± 0.0577 | 19.333\(b\) ± 0.0289 | 21.833\(a\) ± 0.0289 |
| *B. splendens* (N=12)     | 7.6670\(a\) ± 0.577 | 9.6670\(a\) ± 0.577 | 7.3330\(a\) ± 0.577 | 6.3330\(a\) ± 0.5770 |
| Reproductive dynamics     |         |         |         |         |
| GSI                       |         |         |         |         |
| *P. reticulata* (N=12)    | 1.347\(b\) ± 0.396 | 1.378\(b\) ± 0.541 | 1.50\(b\) ± 0.662 | 2.48\(a\) ± 0.597 |
| *B. splendens* (N=12)     | 17.01\(a\) ± 1.150 | 15.03\(a\) ± 2.61 | 17.250\(a\) ± 4.41 | 15.833\(a\) ± 1.330 |
| Fecundity                 |         |         |         |         |
| *P. reticulata* (N=12)    | 8.800\(b\) ± 2.59 | 9.00\(b\) ± 3.54 | 9.80\(a,b\) ± 4.32 | 16.20\(a\) ± 3.90 |
| *B. splendens* (N=12)     | 743.3\(a\) ± 83.3 | 605\(a\) ± 179 | 824\(a\) ± 175 | 694\(a\) ± 272 |
| Bubble nest               |         |         |         |         |
|                | B. splendens (N=5) |                |                |                |
|----------------|-------------------|----------------|----------------|----------------|
|                | 108.58 ± 16.19    | 74.3 ± 24.5    | 36.8 ± 19      | 26.5 ± 22.2    |
| Hatchability   |                   |                |                |                |
| B. splendens   | 78.1 ± 17.6       | 85.72 ± 3.52   | 29.02 ± 10.32  | 24.89 ± 3.42   |
| (N=12)         |                   |                |                |                |
| Fertility      |                   |                |                |                |
| P. reticulata  | 73.89 ± 7.70      | 80.56 ± 5.78   | 83.77 ± 3.13   | 87.77 ± 4.75   |
| (N=12)         |                   |                |                |                |

Table 1: Growth performance and reproductive potential of *P. reticulata* and *B. splendens* under varying hard water treatment

(N values represent the number of fishes in each dose, ^a^ & ^b^ Value denoted by the same alphabetic superscript were not significantly differed from each other)

**Figures**

![Graph showing growth performance and reproductive potential of *P. reticulata* and *B. splendens* under varying hard water treatment. The graph displays the log fecundity values against water hardness levels (in ppm of CaCO3). The x-axis represents the water hardness levels ranging from 150 ppm (Control) to 900 ppm, while the y-axis shows the log fecundity values. The bars for each water hardness level are labeled with asterisks (*) to indicate significant differences. The graph compares the performance of *P. reticulata* and *B. splendens* across different water hardness conditions.]
Figure 1

Logarithmic values of the fecundity of P. reticulata and B. splendens under varying water hardness treatments (150-900ppm). [N= 12 per each species were used for the analysis]. Error bars represents standard error of mean. *Significant alteration from the control (p<0.05).