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Effect of Water Hardness on Fertilisation and Hatching Success of *Clarias gariepinus* (Burchell, 1822) and *Heterobranchus longifilis* Valenciennes, 1840 Eggs Fertilised with *C. gariepinus* Sperm

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Abstract

The effect of water hardness on fertilisation and hatching of eggs of *Heterobranchus longifilis* Valenciennes, 1840 and *Clarias gariepinus* (Burchell, 1822) was studied. Eggs were obtained from five artificially-induced females of each species. Milt from five male *C. gariepinus* was suspended in 5 mL physiological saline and mixed with 100 g eggs from a homogenised egg mass from five individuals of each species. Two mL aliquots containing 294±4 and 274±8 *C. gariepinus* and *H. longifilis* eggs respectively were incubated at 27 ^{o}C in groups of quadruplicate troughs containing water of 20, 80, 160, 300 mg·L^{-1} $CaCO_3$, and control dilution medium of 8 mg·L^{-1} $CaCO_3$ total hardness. Fertilisation and hatching rates were compared between species using a t-test. Effect of hardness on the variables was determined using the ANOVA. Water hardness level did not significantly affect fertilisation rate of *C. gariepinus* eggs ($p>0.05$), while *H. longifilis* eggs were significantly affected ($p<0.0008$), showing significantly higher rates in control medium (68±4%) ($p<0.05$). Water hardness significantly affected hatching of *C. gariepinus* ($p<0.0017$) and *H. longifilis* ($p<0.0006$) eggs. Hatching of all eggs was lowest in media of 300 mg·L^{-1} $CaCO_3$ total hardness. *Clarias gariepinus* eggs showed a higher tolerance for hardness than *H. longifilis* eggs, which showed preference for soft water.

Introduction

Temperature, dissolved oxygen, and pH are the more frequently investigated factors affecting the fertilisation and hatching success of fish species. Members of the family Clariidae are highly fecund (Clay 1979; Nwadukwe and Ayinla 1993). The insufficient supply and relatively high cost of fingerlings of *Clarias gariepinus* (Burchell, 1822) and *Heterobranchus longifilis* Valenciennes, 1840 (Ofor 2007), resulting from low output per breeding attempt, indicates the need to widen the scope of factors affecting the low output. Water hardness influences development of juvenile fathead minnows (Blanksma et al. 2009), eggs (Townsend et al. 2003) and larvae (Silva et al. 2003) of *Rhamdia quelen* (Quoy and Gaimard, 1824) and tilapia (Guerrero 1982), and *Hypophthalmichthys molitrix* (Valenciennes, 1844) (Rach et al. 2010). Water hardness has been shown to affect the toxicity of silver to early life stages of *Oncorhynchus mykiss* (Walbaum 1792) (Morgan et al. 2005), as well as the toxicity of some common aquaculture chemicals used in protecting fish eggs from fungus infection (Adhikari 2003). There is a need to create awareness of the potential roles played by water hardness in the

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breeding of *C. gariepinus* and *H. longifilis*. One of the few attempts in this regard was by Molokwu and Okpokwasili (2002).

The objective of this study is to determine and compare the effect of water hardness on a wider range of early life history stages between the two main culturable catfish species *C. gariepinus* and *H. longifilis*. This is with a view to providing information, from which a breeding procedure incorporating water hardness as a factor can be developed, for the systematic and successful breeding of the two species.

Materials and Methods

Experimental fish

The experiment was conducted in the laboratory of the Department of Fisheries and Aquatic Resources Management, Michael Okpara University of Agriculture, Umudike, Nigeria. The fish used were farm-raised and fed a diet of commercial, dry pellets of 45% crude protein content during grow-out.

Preparation of incubating media

Incubating media were prepared from calcium chloride and magnesium carbonate, according to Molokwu and Okpokwasili (2002) to obtain water of total hardness level 20, 80, 160 and 300 $mg{\cdot}L^{-1}$ $CaCO_3$. The diluted water had a hardness of 8 $mg{\cdot}L^{-1}$ $CaCO_3$ and a pH of 7. The levels of total hardness tested approximate the hardness levels that can be encountered in the various sources of water used by fish culture facilities, and that can develop in water re-circulation system.

Induced spawning and fertilisation

Virgin, 16-month old female *H. longifilis* (mean weight 1300±50 g) and *C. gariepinus* (mean weight 1400±80 g) were induced with Ovaprim® following standard procedures. After a latency period of 10 hr following inducement, five male *C. gariepinus* were sacrificed to yield milt used for fertilisation of eggs. The milt was pooled and suspended in 5 mL physiological saline solution and kept until used. Physiological saline minimised pre-egg contact motility of sperm. The females were stripped of eggs. The eggs were pooled. One half of the milt was mixed with 100 g from the pooled *H. longifilis* egg mass. The other was mixed with 100 g from the pooled *C. gariepinus* egg mass. Eggs and milt were homogenised by stirring with a feather. Two mL aliquots of the egg-sperm mix of each species were taken in quadruplicate. The mean number of eggs was calculated. Two mL volume of eggs was then placed in a 4 L plastic container. Groups of four such units containing water of 20, 80, 160 or 300 $mg{\cdot}L^{-1}$ $CaCO_3$ total hardness were used for incubation and hatching of each species. Incubation was without aeration. Dilution water of 8 $mg{\cdot}L^{-1}CaCO_3$ total hardness was used as control. The eggs in the various media were monitored for fertilisation and hatching. Temperature was 27±2 ^{0}C.

Data collection

Data were collected on egg fertilisation rate, incubation period, and hatching rate. All eggs (dark brown in the case of *C. gariepinus* and green in *H. longifilis*), 8 hr after placement, were considered fertilised, and counted (Hogendoorn 1979). The incubation period lasted from placement to the attainment of ≥3% hatching success. Hatching period started from placement of eggs in media and ended when eggs stopped hatching. Eggs were said to have stopped hatching when similar hatching rates were measured in two consecutive 3-hr periods. Fertilisation rate was calculated by expressing the number of fertilised eggs as a percentage of the total number of eggs in each container. The number of eggs hatched was enumerated at three-hourly intervals, and was expressed as a percentage of total number of eggs fertilised. Water samples were taken from the Imo River and reservoir of the National Root Crops Research Institute, Umudike. These are two local water bodies within the areas of natural occurrence of the species (Teugels 1990). The water samples were analysed for hardness. Hardness in this study is defined and determined according to Boyd and Tucker (1992). Dissolved oxygen, pH and temperature were measured using a Hanna Instruments portable waterproof dissolved oxygen meter (Model HI 9142) and portable microprocessor printing and logging pH/ORP meter (Model HI 98240) (Hanna Instruments Inc. Woonsocket, RI, USA).

Data analysis

Data were arc-sine transformed and analysed to detect significant differences using t-test and ANOVA. Means were separated using the Fischer's Least Significant Difference (LSD) at 5% level.

Results

Each 2 mL aliquot of eggs of *C. gariepinus* and *H. longifilis* contained 294±4 and 274±8 eggs respectively. Incubation period of *C. gariepinus* eggs was 23 hr at 20, 80, 160 $mg \cdot L^{-1}$ $CaCO_3$ water hardness, and 26 hr at 300 $mg \cdot L^{-1}$ at 27 ^oC water temperature, while for *H. longifilis* eggs it was 25 hr at 20 and 80 $mg \cdot L^{-1}$ $CaCO_3$, and 28 hr at 160 and 300 $mg \cdot L^{-1}$ $CaCO_3$. The t-test comparison of fertilisation rates between *C. gariepinus* and *H. longifilis* at different water hardness levels is shown in Table 1. Fertilisation was significantly higher in *C. gariepinus* than *H. longifilis* at all hardness levels ($p<0.02$) except control. *Clarias gariepinus* hatched at significantly higher rates than *H. longifilis* only in 80 $mg \cdot L^{-1} CaCO_3$($p<0.01$) and 160 ($p<0.05$) $mg \cdot L^{-1} CaCO_3$ hardness (Table 2). Hatching of *H. longifilis* eggs was significantly affected by hardness ($p<0.0006$) and decreased with increasing levels of hardness. Hardness level had no significant effect on *C. gariepinus* fertilisation success ($p>0.05$) (Table 3). Fertilisation of *H. longifilis* eggs was significantly affected by hardness ($p<0.0008$), decreasing with increase in hardness. *Clarias gariepinus* eggs commenced hatching 23 hr after fertilisation and attained the peak first in $80 \cdot mg \cdot L^{-1}$ $CaCO_3$ hardness at 29 hr. *Heterobranchus longifilis* eggs commenced hatching 25 hr after fertilisation in the control medium and 28 hr in the test media except for 160 $mg \cdot L^{-1} CaCO_3$ and 300 $mg \cdot L^{-1} CaCO_3$ hardness levels. Hatching of *C. gariepinus* eggs was significantly affected by hardness level ($p<0.0017$), being highest in 80 $mg \cdot L^{-1}$ $CaCO_3$ water

hardness. Hardness and dissolved oxygen levels of the Imo River were 90 $mg{\cdot}L^{-1}$ $CaCO_3$ and 4.8 $mg{\cdot}L^{-1}$ respectively, while the levels of the reservoir were 65 $mg{\cdot}L^{-1}CaCO_3$ and 6.8 $mg{\cdot}L^{-1}$ dissolved oxygen. Dissolved oxygen and pH levels did not significantly differ among the hardness levels ($p>0.05$). Dissolved oxygen levels had a mean of 3.7±0.06 $mg{\cdot}L^{-1}$. pH ranged from 7 in the control to 8.2 in 300 $mg{\cdot}L^{-1}CaCO_3$ hardness level. Despite the high fertilisation success of eggs of *C. gariepinus* in the higher hardness levels very few of the fertilised eggs (<10%) hatched.

Table 1. Fertilisation rates (% of 294±4 and 274±8 eggs in 2 mL aliquots of *C. gariepinus* and *H. longifilis* egg masses respectively) incubated in media of different levels of water hardness. Values are means (±SD) of four replicates. Control= 8 ($mg{\cdot}L^{-1}$ $CaCO_3$) hardness. ****- $p<0.0001$, ***-$p<0.001$, **-$p<0.01$, *-$p<0.05$.

Variable	Hardness level ($mg{\cdot}L^{-1}$ $CaCO_3$)				
Fertilisation Species	20	80	160	300	Control
C. gariepinus	52±2[a]	58±2[a]	60±6[a]	60[a]	50±3[b]
H. longifilis	45±3[b]	40[b]	25±3[b]	25±2[b]	68±4[a]
Significance	**	*	**	***	**

Table 2. Hatching rates (% fertilised eggs) of *C. gariepinus* and *H. longifilis* eggs incubated in water of different levels of hardness. Values are means (±SD) of four replicates. Control= 8 ($mg{\cdot}L^{-1}$ $CaCO_3$) hardness. ****-$p<0.0001$, ***-$p<0.001$, **-$p<0.01$, *-$p<0.05$.

Variable	Hatching (%)		
	C. gariepinus	*H. longifilis*	Significance
Hardness level ($mg{\cdot}L^{-1}$ $CaCO_3$)			
20	67±9	47±3	NS
80	70±6	37±3	**
160	53±7	27±7	*
300	8±6	3	NS
Control	20±2	76±5	**

Table 3. Effect of hardness level ($mg \cdot L^{-1}$ $CaCO_3$) on fertilisation (% of 294±4 and 274±8 eggs in 2 mL aliquots of *C. gariepinus* and *H. longifilis* egg masses respectively) and hatching rates (% fertilised eggs) of *C. gariepinus* and *H. longifilis* incubated in water of different levels of hardness. Values are means (±SD) of four replicates. Control= 8 $mg \cdot L^{-1}$ $CaCO_3$ hardness. Means in the same row with different superscript letters are significantly different ($p<0.05$) (Fischer's LSD).****- $p<0.0001$, ***-$p<0.001$, **-$p<0.01$, *-$p<0.05$****- $p<0.0001$, ***-$p<0.001$, **-$p<0.01$, *-$p<0.05$.

Hardness ($mg \cdot L^{-1} CaCO_3$)	20	80	160	300	Significance	Control
	C. gariepinus					
Fertilisation	52 ± 2^{a}	58 ± 2^{a}	60 ± 6^{a}	60^{a}	NS	50 ± 3^{a}
Hatching	67 ± 9^{a}	70 ± 6^{a}	53 ± 7^{b}	8 ± 6^{c}	**	20 ± 2^{b}
	H. longifilis					
Fertilisation	45 ± 3^{b}	40^{b}	25 ± 3^{c}	25 ± 2^{c}	***	68 ± 4^{a}
Hatching	47 ± 3^{b}	37 ± 3^{bc}	27 ± 7^{c}	3	***	76 ± 5^{a}

Discussion

The American Society of Engineers classifies water of 0-60, 61-120, 121-180, and above 180 $mg \cdot L^{-1}$ $CaCO_3$ total hardness as soft, moderately hard, hard, and very hard respectively. In this study, these categorisations were represented by the control and 20 $mg \cdot L^{-1}$ $CaCO_3$, 80, 160, and 300 $mg \cdot L^{-1}$ $CaCO_3$ hardness levels respectively. Results indicate that water hardness had a species-specific effect on fertilisation and hatching success of *C. gariepinus* and *H. longifilis* eggs. *Heterobranchus longifilis* eggs were more sensitive to hardness, with a high level of preference for soft water, while *C. gariepinus* eggs were tolerant of both soft and hard water. The results may also be explained by the differences in the effect of water hardness on sperm motility parameters between the species.

Accounts of effect of water hardness on egg fertilisation and hatching are varied. Small et al. (2004) reported a calcium-critical period in embryo development and low hatching of Channel catfish within the first 24 hr after fertilisation in low Ca hardness (4.7 $mg \cdot L^{-1}$ $CaCO_3$) water. Moderately hard water had a positive influence, while hard water had a negative influence on hatching rate of eggs of *R. quelen* (Silva et al. 2003). Such influence was not found in striped bass, *Morone saxatilis* (Walbaum 1792) (Spade and Bristow 1999) and silver carp (*H. molitrix*) eggs (Uphoff 1989; Gonzal et al. 1987). The ability of eggs of common carp to be fertilised increased in a hypotonic medium (Plouidy and Billard 1982; Saad and Billard 1987). However, water hardness had no effect on the fertilisation success of eggs of the Arctic Grayling, *Thymallus arcticus* (Pallas 1776) and Dolly Varden, *Salvelinus malma* (Walbaum 1792) (Brix et al. 2010). In this study, the control medium may have had the effect of increasing the ability of *H. longifilis* eggs to be fertilised, while having no such influence on eggs of *C. gariepinus*.

Though sperm motility was not investigated in this study, the influence of hardness on egg fertilisation may have been achieved through the effect of hardness on sperm motility

characteristics. Sperm motility indicates sperm fertilising ability (Alavi and Cosson 2005) and is strongly positively correlated with hatching percentage ($p<0.001$) (Mansour et al. 2005). Calcium chloride and magnesium carbonate were dissolved in water to produce the various media hardness. The resulting Ca^{2+} and Mg^{2+} influence sperm activation and motility duration in some fish species (Billard and Cosson 1992; Hamamah and Gatti 1998; Alavi and Cosson 2006; Mansour et al. 2005). According to Mansour et al. (2002) the addition of calcium to motility-activating medium had no effect, while addition of magnesium slightly increased, *C. gariepinus* sperm motility rate. The changes in Cl^- concentration associated with changes in hardness may have caused osmotic changes in the media. Osmotic shock is known to trigger motility of sperm in some freshwater and marine fish species (Alavi and Cosson 2006). Osmotic shock has been known to increase sperm motility duration in cyprinids (Billard et al. 1995), and zebrafish (Takai and Morisawa 1995; Wilson-Leedy et al. 2009). Mansour et al. (2002) suggested that motility period of sperm of *C. gariepinus* does not depend on osmotic sensitivity. According to Mansour et al. (2002), the pH range recorded in the various hardness levels have no effect on *C. gariepinus* sperm motility characteristics. Thus *C. gariepinus* sperm in the present study caused similar fertilisation rates in soft, hard, and very hard water, being insensitive or mildly sensitive to the physico-chemical changes associated with changes in water hardness. In contrast, *H. longifilis* had high fertilisation rates only in the control due probably to sensitivity of *H. longifilis* sperm to increased osmotic strength, Ca^{2+} and Mg^{2+} concentration, and pH; likely to have occurred with increase in hardness of media.

The results of the present study on hatching of *C. gariepinus* eggs agree with Molokwu and Okpokwasili (2002) that total hardness level of 30-60 $mg{\cdot}L^{-1}CaCO_3$ was optimal for the hatching of *C. gariepinus* eggs. The total hardness level in nearby water bodies are above the optimal levels for fertilisation and hatching of *H. longifilis* eggs reported in this study, but lie within the optimal range for fertilisation and hatching of *C. gariepinus* eggs. These results suggest that *C. gariepinus* may experience greater fertilisation success in nature than *H. longifilis*. *Clarias gariepinus* and *H. longifilis* are preferred aquaculture candidates in Nigeria, with *H. longifilis* having much higher preference due to its superior growth rate (Legendre et al. 1992) and market acceptance. The widespread culture of *H. longifilis* is hampered by a chronic shortage of its fingerlings. *Clarias gariepinus* fingerlings are not available in adequate quantities. Consequently, catfish seed cost accounts for a high proportion of the production cost or market price (Ofor 2007; Miller and Atanda 2011).This is more acutely felt in the case of *H. longifilis*, where demand for fingerlings is much higher than supply. It is therefore important to develop a hatchery fingerling production protocol, taking the effect of water hardness on early life history of the species into account.

Conclusion

Water hardness is an underestimated and under-reported water quality parameter whose effect on the spawning of catfish species has been shown in this study. The effect of total water hardness on fertilisation and hatching varies between the two most preferred aquaculture candidates in Nigeria. *Heterobranchus longifilis* and *C. gariepinus* eggs seem to have opposing water hardness requirements for fertilisation and hatching. Early life stages of *H. longifilis* are

more vulnerable to influence of water hardness than *C. gariepinus*. *Heterobranchus longifilis* spawns successfully in the lower hardness range of soft water, while *C. gariepinus* spawns successfully from the upper hardness range of soft, to hard water. Hatchery practices in the fingerling production of the two species should take into account the effect of water hardness on their early life stages.

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