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Significance of water pH and hardness on fish biological processes: A review

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Abstract
The success of any aquaculture endeavour broadly depends on water quality. Water quality determines to a great extent the success or failure of aquaculture operation. Optimum water quality is considered necessary for any aquaculture operation as it influences the productivity of production system. In the present review, the role of major abiotic factors such as water pH and hardness on the biological processes of fish like growth, survival, reproductive performance and embryology has been discussed.

Keywords: pH, Hardness, Growth, Reproductive Performance, Embryology

Introduction

Water quality is an integral part of any aquaculture system. It plays a major role in fish health and any deterioration in water quality causes stress to fish which ultimately brings about diseases (Arulampalam *et al.*, 1998) [1]. Physical and chemical characteristics such as suspended solids, temperature, dissolved gases, pH, nutrients and the potential danger of toxic elements must be considered for successful fish farming (Johnson, 1995) [2]. Each water quality factor interacts and influences the other parameters, sometimes in complex ways (Joseph *et al.*, 1993) [3]. Optimal water quality is considered prerequisite for the survival and growth as it influence the entire life processes in fish (Bolorunduro and Abdullah, 1996 [4]; Boeuf *et al.*, 1999) [5]. Among the various ecological factors, pH, hardness, temperature and salinity are considered as determining factors, which is perceived through receptors which may directly affect the growth in fishes (Makori et al. 2017) [6]. Limiting factors in the other hand like oxygen, ammonia, pH and hardness disturbs the growth performance if their levels are above or below an optimum levels (Boeuf and Payan, 2001 [7], Menni *et al.*, 1996 [8]; Mazerolle & Desrochers, 2005 [9]; Lacoul & Freedman, 2006) [10]. The objective of this paper is to survey the present knowledge of influence of water pH and hardness on fish.

Water hardness of the aquatic environment

Water hardness defined as the measure of all the divalent cations particularly calcium and magnesium. It is considered as a major aboitic factor influencing aquaculture. It is generally expressed as mg/L calcium carbonate ($CaCO_3$). Hardness of the aquatic medium increases due to leaching of sedimentary rock, containing sources of divalent cations, such as limestone and gypsum (Boyd 1979) [11]. Water hardness is often split into two categories; permanent and temporary. Temporary hardness is the part that is chemically associated with carbonate and bicarbonate of calcium and magnesium salts, such as $CaCO_3$ and permanent hardness is caused due to the sulphates and chlorides of calcium and magnesium (Boyd 1979) [11]. Water hardness has been shown to have a direct effect on the swelling of newly fertilized eggs, which is speculated as an important process during the early development of the teleost egg (Spade and Bristow, 1999) [12]. In general, calcium is of greater importance than magnesium in the management of water for aquaculture as it is required for the water hardening of newly fertilized freshwater fish egg and calcification of larval skeletal structure. Calcium also influences the membrane permeability which is essential for successful embryonic development (Whitaker, 2006) [13].

Acceptable range of hardness in aquaculture is 50-150 ppm. Tucker and Steeby, 1993 [14] reported that Channel cat fish (*Ictalurus punctatus*) larvae can be raised in a water having 10-100 ppm of hardness without any detrimental effects on growth. Copatti *et al.,* 2011 [15] reported that optimum level of hardness for the growth of juvenile catfish is 25-50 ppm.

Effect of water hardness on growth and survival

Water hardness in the range of 50-150 $mgl^{-1}$of $CaCO_3$, is considered desirable but the most preferable is above 100 mgl^{-1} of $CaCO_3$ (Swingle, 1997; Molokwu and Okpokwasili, 2002; Stone and Thomforde, 2004) [16, 17, 18]. Similar observations were made by Bhatnagar *et al.,* 2004 [19] who observed that a hardness of 75-150 mgl^{-1} is optimum for pisiculture, over >300 mgl^{-1} of $CaCO_3$ is lethal to the fishes and hardness under 20 mgl^{-1} causes stress to fishes due to unavailability of nutrients in water. Research carried by Milad and Seyed, 2011 [20] reported highest survival of *Pterophyllum scalare juveniles* at water hardness of 100 mgl^{-1} $CaCO_3$.Water hardness of 80 – 91 $mgl^{-1}$of $CaCO_3$ is considered optimal for rearing of *Clarias magur* (Surnar, 2018) [21]. Similar results were reported in *Rhamdia quelen* (Townsend, 2003) [22] and *Ictalurus punctatus* (Perschbacher, 1999) [23]. Kumawat, 2018 [24] observed that at 150 mgl^{-1} water hardness, highest fry survival (95.33%) was recorded for *Labeo rohita* fry and the lowest survival and growth was observed in 125 mgl^{-1} hardness.

Effect of water hardness on embryology

Environmental calcium is required for "water hardening" of newly fertilized freshwater fish eggs and calcification of larval skeletal structure. Calcium also influences membrane permeability and is regarded important for successful embryonic development, especially in water of low pH or low ionic strength (Alderdice, 1988) [25]. Although few data are available, it appears that egg and larval development of most freshwater fish are best if calcium concentrations are greater than about 5-10 mgl^{-1}. Hatching success of brown trout (*Salmo trutta*) eggs incubated at pH 4.5 was highest if the water contained at least 10 mgl^{-1} of calcium (a calcium hardness of 25 mgl^{-1} as $CaCO_3$). Chung *et al.,* 1980 [26] observed that soft water has been reported to have caused premature hatching of Chinese carp (silver and bighead carps) eggs and poor survival of larvae. The effect of water hardness on survival of fish egg and preference for water hardness varies in oviparous and ovo-viviparous aquarium fishes (Lee and Hu, 1983 [27]; Gonzal *et al.,* 1987 [28]; Ketola *et al.,* 1988 [29]; Wilkerling, 1992) [30]. Gonzal *et al.,*1987 [28] reported that water absorption at 100–200 mgl^{-1} $CaCO_3$ caused silver carp (*Hypophthalmichthys molitrix*) eggs to burst prematurely while successful hatching was obtained at a water hardness of 300–500 mgl^{-1} $CaCO_3$. Tucker and Steeby, 1993 [14] suggested that survival, development, and stress resistance of channel catfish yolk-sac fry were adversely affected at calcium concentrations below 5 mgl^{-1}. Molokwu and Okpokwasili, 2002 [17] reported that total hardness also interferes the incubation period in *Clarias batrachus* eggs. The incubation time increased from 19 hr at total hardness of 10 mgl^{-1} to 23 hr at total hardness of 200-700 mgl^{-1}. Drastic changes were also observed with respect to mean hatching rate, which was 42.31% and 64. 66% at a hardness of 10 mgl^{-1} and 200 mgl^{-1} $CaCO_3$ receptively. At higher water hardness, beyond 200 mgl^{-1} abnormalities in the *Clarias geriepinus* fish larvae were observed. Silva *et al.,* 2003 [31] suggested increase of water hardness to 70 mgl^{-1} $CaCO_3$ significantly improve the hatching rate of silver catfish (*Rhamdia quelen*) eggs. Researchers have suggested that relatively small quantities of suitable hardened water were required to markedly improve egg hatching of several fishes like Atlantic salmon (Peterson et. al., 1980) [32], rainbow trout (Whitehead et. al., 1978) [33]. This effect might be specific for Ca^{2+}, or due to bivalency, a characteristic that Ca2+ ions share with other elements, such as Mg^{2+} (Ketola *et. al.,*1988) [29]. Wurts and Stikney, 1989 [34]; Pursley and Wolter, 1994 [35], suggested that when red drum are grown in fresh water, the water should contain a minimum of 25 mgl^{-1} Ca^{2+} and levels of 50-100 mgl^{-1} or more are desirable for best survival, growth, and feed conversion efficiency. James and Sampath, 2004 [36] reported that when water hardness of culture media is increased (76, 316, 540 and 1018 mgl^{-1} $CaCO_3$, *Xiphophorus helleri* exhibited maximum growth parameter and reproductive performance is in highest water hardness of 1018 mgl^{-1} $CaCO_3$. On the contrarily *Betta splendens* elicited better growth, feeding parameters and fecundity in hardness of 316 mgl^{-1} $CaCO_3$. The ionic composition of ambient water is important for egg development (Vander velden *et al.,* 1991) [37]. Teleost can directly absorb Ca^{2+} from the water through the gills or by feeding but the main sites of absorption are gills (Hwang and Hirano, 1985 [38] and Hwang *et al.,* 1996) [39].

Effect of water hardness on reproductive performance

There is no direct evidence that demand for calcium from the ambient medium is heightened prior to and during, the breeding season. Plasma calcium concentration do increase over oogenesis period (e.g. Whitehead *et al.,* 1978 [33]; Scott *et al.,* 1980) [40], resulting from mobilization of yolk proteins as calcium complexes. It is possible that these temporary increase come about by redistribution between intracellular and extracellular compartments. However, uptake of calcium from the ambient medium may be of importance. Calcium uptake from the medium by freshwater fish probably occurs predominantly by extraintestinal active transport (Sayer *et al.,* 1991) [41]. Although calcium influx may be stimulated in low calcium media (Perry and Wood, 1985 [42]; Flik *et al.,* 1986 [43]), water of low pH (Hobe *et al.,* 1984 [44]; Reader and Morris, 1988) [45] with or without trace metals (Reader and Morris,1988 [45]; Sayer *et al.,*1991) [41], may inhibit calcium influx or stimulate efflux, eighter way resulting in calcium loss. At a stage when enhanced plasma calcium concentration are essential for successful reproduction, sustained branchial calcium loss must surely be detrimental. Calcium ions are important for keeping vitellogenin in solution (Whitehead *et al.,* 1978) [33] during its transport to ovary and its incorporation into developing oocytes (Mount *et al.,* 1988) [46]. Vitellogenin, a large phosphorylated protein, is the source for most of the exogenous yolk in mature eggs, so when plasma calcium concentration are depressed, reduced egg quality must be likely to happen.

pH of the aquatic environment

The pH value expresses the intensity of the acidic or basic character of water. It is defined as the negative logarithms of hydrogen ion concentration. The pH is expressed in the scale of 0 to 14. The conditions become more acidic as pH value decreases and more basic as value increases. Exposure of aquatic animal to extremes of pH is stressful or lethal, but the indirect effects of pH and interactions of pH with other variables are usually more important in aquaculture than direct toxic effects Doudoroff, 1956) [47]. The optimal pH for growth and well-being of most of the freshwater aquatic animals is in the range of 6.5 to 9.0 (Zaniboni-Filho *et al.*, 2002) [48]. Number of studies have illustrated the importance of water pH on early life stages, mortality and disease resistance of a fish and its influences on growth and reproduction, detrimental pH of rearing media may lead to mass mortality in fish culture (Doudoroff 1956 [47]; Kwain 1975 [49]; Jezierska and Witeska 1995 [50]; Zaniboni-Filho *et al.*, 2002 [48]; Scott *et al.*, 2005 [51]; Zaniboni-Filho *et al.*, 2009 [52]; Nchedo and Chijioke, 2012) [53]. The low inorganic solute content of water is typical of acid sensitive areas is characterized to have a low buffering capacity. When in equilibrium with atmospheric carbon dioxide they will generally have a pH value of approximately 5.6, if the concentration of humic substances is low and anthropogenic acid input is absent. In general terms, an acidified soft water system can be defined as having a pH lower than 5.6 (Haines, 1981) [54]. In lakes and larger rivers, the minimum pH value recorded is typically between 3.9 and 4.5. In terms of acidity, the threshold for survival of freshwater fishes will vary with species and life stages. However, fish species indigenous to soft, acid water will generally have thresholds of between pH 4.2 and pH 5.5 (Brown and Sadler, 1989) [55]. Njoku *et al.*, 2007 [56] revealed that hybrid cat fish (*Heterobranchus bidorsalis* x *Clarias gariepinus*) reared in pH of 7.0 and 7.5 shown significantly higher specific growth rate and feed conversion ratio than the 6.0 and 8.0 reared fishes. Iqbal *et al.*, 2012 [57] stated that increase in pH has positive effect on Nile tilapia (*O. niloticus*) growth which is not true in other fish species. pH range of 6 to 8 are generally preferred by fish species found in neotropical environment (Lopes *et al.,* 2001 [58]; Townsend and Baldisserotto, 2001 [59]; Baumgartner *et al.*, 2008) [60]. Although there is a marked difference between different species and their life cycle (Lloyd & Jordan, 1964 [61]; Laurence & Howell, 1981 [62]; Ferreira *et al.*, 2001 [63]; Parra and Baldisserotto, 2007) [64].

Effect of water pH on growth and survival

Various metabolic activities are control by water pH and when fish exposed to high acidic or high alkaline water, decrease in the ionic balance of gills is observed, which eventually resulted in last high mortality (Lloyd and Jordan, 1964 [61]; Alabaster and Lloyd, 1980 [65]; Freda and Mcdonald, 1988 [66]; Mcgeer and Eddy, 1998) [67]. At pH below below 6.0 or above 9.0, their is an substantial decrease in growth performance of most of the fish species (Parra and Baldisserotto, 2007) [64]. Any change in pH, above or below the optimal level may hamper the physiological or metabolic functions of the fish like growth performance, reproductive behaviour and ecological distribution (Boyd, 1998 [68]; Zweig *et al.*, 1999) [69]. In fish, exposure to low pH has been shown to either have detrimental effect on growth or have no effect (Mount 1973 [70]; Leivestad *et al*., 1976 [71]; Menendez 1976 [72]; Jacobsen 1977) [73]. At acidic pH of 5.5, the reduced growth was reported in various fishes (Menendez, 1976 [72]; Craig and Baksi, 1977 [74], Ndubuisi *et al.,* 2015) [75]. The brook trout exposed to low pH showed anoxia which can be attributed to loss of sodium from the body, subsequently resulting to mortality (Packer and Dunson, 1972) [76]. The innate immune response is also influenced by low pH as phagocytic activity of channel catfish neutrophils was reduced at these condition (Ainsworth *et al.*, 1991) [77]. The best pH range for survival and growth of larvae of silver catfish is 8.0-8.5 (Lopes *et al.*, 2001) [58] and at pH 5.5 or 9.0 juveniles growth is reduced as compared to pH 7.5 (Copatti *et al.*, 2005) [78]. Low pH condition hampers the homeostasis in fishes as it disturbs acid-base balance, thereby an increase in H^+ and NH_4^+ excretion in urine is noted (Wood, 2001 [79]; Bolner and Baldisserotto, 2007) [80]. One of the suggested causes of fish death in very acidic water is failure to regulate their internal ion concentration associated with a reduction in ion uptake rates (Laurent *et al.,* 2000) [81]. The growth in fishes during Its early stages is influenced by temperature and water pH of the rearing medium (Nwosu and Holzlohner, 2000 [82]; Morehead and Hart, 2003 [83]; Zaniboni-Filho *et al.*, 2002) [48]. Studies of Nchedo and Chijioke, 20125 [53] indicated that the optimum pH range for normal hatching and larval survival of *Clarias gariepinus* was pH 7.5-8.5. Studies carried out in *Cyprinus carpio* by Sapkale *et al.*, 2013 [84] reported that highest growth performance and survival at pH 7.5.

Effect of water pH on embryology

Unsuccessful fertilization of eggs along with increased mortality of fish embryo were observed at water pH lower than 4.0 for *Salmo salar* (Carrick, 1979 [85]; Daye and Glebe, 1984 [86]; Peterson *et al.,* 1980 [32]), *Oncorhynchus nerka (*Parker and McKeown, 1987 [87]; Zaniboni-Filho, 2000 [88]), *Salvelinus fontinalis* (Swarts et al., 1978) [89] and *Cyprinus carpio* (Korwin-Kossakonski, 1988) [90]. There is a profound decrease in the ability of the egg to resist deformations due to mechanical actions as low pH leads to disturbances in the osmotic activity of perivitelline colloids leading to reduced water uptake of eggs (Eddy and Talbot, 1983 [91]; Westernhagen, 1988) [92]. Additionally low pH also deters the activation of enzyme chorianase, which is responsible for hatching of eggs in fishes (Kelley, 1946 [93]; Westernhagen, 1988) [92]. Activities of hatching enzymes mainly depends on the fish species and pH of the water, highest chorianase activity is noted at a pH over 6.5 in rainbow trout eggs (Hagenmaier, 1974) [94], between pH 7.5 and 8 in *Oncorhynchus keta* (Bell et al., 1969) [95]. pH of the incubating medium also influences the developmental stages of the embryo, delayed development was noted in *Clupea pallasii* by Kelley, 1946 [93] and correspondingly accelerated development was observed at slightly higher pH in *Danio rerio* by Johansson *et al.,* 1973 [96]. According to Johansson and Kihlstrom (1975) [97] pH of the incubating water had a influence on the size of Northern pike (*Esox lucius*) eggs, those eggs incubated at lower pH (pH 4.2) larvae were smaller in size than those at neutral pH. Futhermore research conducted by Menendez, 1976 [72] states that lower pH of 5.0 lead to delayed absorption of the yolk sac in brook trout. Similar observation were also reported by Nelson, 1982 [98]. The perivitelline fluid tends to allow cations such as Na^+, K^+, Ca^{2+}, Mg^{2+}, and H^+ to penetrate under normal conditions of external and internal pH, where these conditions are optimal in freshwater with neutral pH, and most detrimental in acidic media (Alderdice, 1988) [25]. Moreover, the development of embryos in acidic waters could result in a decrease of ion accumulation and according to Westernhagen (1988) [92] acidic incubating pH delayed all developmental stage in fish egg.

Effect of water pH on reproductive performance

Embryos of many aquatic taxa develop in direct contact with the external environment and are highly influenced by environmental stressors. pH is considered as an important stressor which mediate negative effects via the disruption of ion balance and thereby affect the reproductive success and viability of natural populations (Stumpp *et al*., 2012 [99];

Parker *et al.*,2009) [100]. Sensitivity to extreme pH conditions varies according to fish species and age, with fish showing lower tolerance at the embryonic and larval stages (Lloyd and Jordan, 1964 [61]). Impaired oogenesis and spawning failure have been reported in fish in acidified lakes, albeit with high heavy metal concentration (Almer, 1972 [101]; Beamish *et al.*, 1975 [102]; Frenette and Dodson, 1984) [103]. Some species have reported to cease spawning at pH levels higher than those at which major fish kills have occurred (Beamish, 1976 [104]; Vuorinen *et al.*, 1992) [105]. Reproduction may fail owing to an inability to produce and/or release egg or sperm if viable gamete release is achieved leading to unsuccessful fertilization. Reproduction potential can be estimated by measuring the size of gonad prior to spawning. A crude indication of gonadal maturation in fish is given by the gonadosomatic index (GSI), which in essence represents the ratio of gonad to body weight. GSI measurements of female perch (*Perca fluviatilis*) taken over a full season were significantly lower in fish from an acid lake (pH 5.1-5.2) than in those from a circumneutral lake (pH 6.6) (Valtonen and Laitinen, 1988 [106]).This effect cannot be attributed to low pH alone, however, as there was considerable difference between the waterborne calcium concentration (53 and 106 µmol l^{-1} respectively). Conversely Vuorinen *et al.*,1992 [105] reported GSI values for male perch to be higher in acidified lakes (pH 4.3-4.8) than in circumneutral lakes (5.9-6.4) with only slight differences in water calcium concentration (10-40 and 60-70 µmol $^{-1}$ respectively). The drawback of GSI as an indicator of reproduction potential is that it gives no direct information about gamete numbers or condition. Furthermore, oocyte atresia has also been noted in brook trout (*Salvelinus fontanalis*, Salmonidae) exposed to pH 4.5, and also in relatively hard water pH 9.5 (Tam and Payson, 1986 [107]; Tam *et al.*, 1990) [113]. In a separate study on brook trout, female subjected to an artificial acid, soft-water medium with aluminium (pH 5.0; [Ca] 12.5 µmol l^{-1}; $[Al]_{total}$ 8.0 µmol l^{-1}) had higher GSI value after spawning than other animals which had been maintained in less deleterious treatment (Mount *et al.*,1988) [46]. The GSI was high because mature oocytes were still embedded in the ovary even after manual spawning, although with no evidence of inhibited oocyte growth or atresia. Because manual stripping was used, the unreleased oocytes may indicate delayed maturation in the acidic stressed fish. Oocytes in brook trout subjected to pH 4.2-4.8 in hard water developed at a similar rate to fish kept at circumneutral pH (Tam and Payson, 1986) [107]. However, ovulation was delayed in the acid water group. Delayed time to spawning was recorded for flagfish (*Jordanella floridae*, Cyprinodontidae; a species not found in acidic water) subjected to acid and heavy metal mixture, although egg production was unaffected (Hutchinson and Sprague, 1986) [108]. Delayed, but not inhibited, spawning of perch (Vuorinen *et al.*, 1992) [105] and ovulation in whitefish (*Coregonus wartmanni*, Salmonidae;Vuorinen *et al.* 1990) [109] was delayed in acid lakes. Although it has not been investigated experimentally, it is possible that delayed gonad maturation or gamete release may be an adaptation to unfavourable water conditions, although equally, it could be a pathological effect. The effect of water bourne calcium concentration on reproductive potential have been little studied under conditions relevant to acid waters. Wiener *et al.*,1985 [110] found serum calcium concentrations in female white sucker (*Catostomus commersoni*, Catostomidae) to be 19% lower in fish from lake pH ranging from 5.60 to 6.35 than in specimens from circumneutral lakes (pH 6.75-7.78) Harvey, 1980 [111]. Exposure to low pH has been shown to inhibit oocyte yolking and development in flag fish and brook trout (Ruby *et al.*, 1978 [112]; Frenette and Dobson, 1984 [103]). Brook trout exposed to pH 4.97 and 8.0 µmole l^{-1} total aluminum in low calcium water (12.5 µmole l^{1}) had reduced plasma calcium and vitellogenin concentrations, and low estradiol value (Mount *et al.*, 1988 [46]). Oestradiol is synthesized in and released from the ovary, and circulates to the liver where it stimulates the synthesis of vitellogenin. Tam *et al.*, 1990 [113] found no consistent effect of low pH (pH 4.5) on vitellogenin and estradiol levels in maturing brook trout and suggest that it is the poor physiological condition associated with acid stress which is responsible for reduced oocyte production. Egg production is related to impaired growth in female brook trout, is also corroborated by Tam and Payson, 1986 [107] and Mount *et al.*, 1988 [46], using relevant water conditions and analysis. Few studies have considered the effects of acidic conditions on spermatogenesis. Both Ruby *et al.*, 1978 [112] studying flag fish and Wiener, *et al.*, 1985 [114] who examined rainbow trout (*Onchohynchus mykiss*, Salmonidae) reported adverse effects on production and quality of sperm. It has become generally accepted that oogenesis is more sensitive to low pH than spermatogenesis. This assumption, however, appears to have arisen from the study of Ruby *et al.*, 1978 [112] alone and remains untested for species indigenous to acid waters. The quality and release of gametes may be irrelevant if successful fertilization is not possible in acidic condition. At the time of fertilization, mature fish egg is physiologically quiescent and in the stage of development was arrested. Resumption of development may be triggered by changes in pH and/or calcium levels in the egg cytoplasm (Hart, 1980) [115]. However the quality of the spawning environment affects the eggs or spermatozoan quality, or their ability to fuse, has not been subject of direct study in relation to freshwater acidification. Brown (1982) [116], however found no effect of fertilizing eggs of brown trout in low pH or calcium solutions. This conflits with the report of Carrick (1979) [85] and Parker and McKeown., 1987 [87] who found that acid fertilization of salmonid eggs reduced subsequent embro-larval survival. But in all of these studies, eggs were obtained from species not originating from acidic water. Indigenous adults, given the opportunity, avoid water of low pH when selecting spawning sites (Johnson and Webster, 1977) [117] suggesting that higher pH levels may be beneficial for successful fertilization.

Conclusion
It is evident from this brief discussion that the both pH as well as hardness plays an important role on the physiological as well as reproductive behaviour of the fish. Therefore much augmented research should be carried out in this field to have an proper understanding of these abiotic water quality parameters.

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