14(6):01-05(2020)
Journal of fisheriessciences.com
E-ISSN 1307-234X
@2020 www.fisheriessciences.com
Research Article

Acceptance of Thermal and pH Shock on Red Belly Pacu (*Piaractus brachypomus*) in Adverse Rapid Environmental Conditions

Aadil Yahiya, Saeed Mohammed and Jithu Paul Jacob*

School of Industrial Fisheries, Cochin University of Science and Technology, Kerala, India

Abstract

Uncertainty of environmental parameters in an aquatic ecosystem may occur within matter of time leads to sudden fluctuations. It may happen anywhere at any time due to both natural and anthropogenic sources. The survival of the aquatic organisms in these ecosystems may directly or indirectly depends on the tolerance limit of that particular organism against the sudden change and the physiological immune response of the species. Here, an attempt has been created with an experimental run where sudden temperature and pH change were monitored. Red Bellied Pacu has shown a greater survival towards rapid fluctuations in temperature with variety of behavioral response. But when it comes to the case of rapid change in pH the fish has shown mortality with different point of pH value and got higher death rate in higher pH value. This results shows that pH has a more significance in tolerance and survival of Red Bellied Pacu when compared to temperature in different environments especially in more alkaline waters.

Keywords: Tolerance limit; Red Belly Pacu; Rapid fluctuation; Temperature; pH

*Correspondence to: **Jithu Paul Jacob**, School of Industrial Fisheries, Cochin University of Science and Technology, Kerala, India, E-mail: jithupaul007@gmail.com

Introduction

Rapid fluctuations in environmental condition are a growing concern in both natural aquatic ecosystem and confined condition. When a fish has exposed to a rapid change, stress will occur resulting in a cascade of physiological and behavioral response and in many cases, death. These changes may infer different response in fish which differ among varied species to species and in different age group. Environmental shocks in aquatic environments may occur from natural (storm, rain events etc), anthropogenic sources (industrial drainages, pollution) and sudden changes in environmental conditions like disaster (floods, erosion etc). The tolerance level of different species to these changes in aquatic organisms helps them to survive these extreme conditions. The effect of mortality level and tolerance rate related to specific organism can vary based on their nature of its innate immunity system to the specific ecosystem. An invasive (introductory) species if showed a high tolerance towards an environmental stress possess a greater threat to the ecosystem. It is entirely different in the case of indigenous or commercially cultured species. In case of aquarium fishes tolerance towards rapid environmental changes is a positive sign where there is a high risk of rapid change in environmental conditions like temperature, pH etc. The environmental shock tolerance of important freshwater fish species offer opportunities to develop many conservative initiatives on inland resource management policies and aquaculture industries.

Yet to date, studies on shock tolerance has been largely unfocused, few studies attempts have been done on the tolerance level of freshwater species like rainbow trout (O. mykiss) on rapid change in pH [1]. Temperature and pH levels can be considered as two important environmental parameters which can be fluctuated easily in an environmental shock that gains stimulated immune response of a fish species. The two major environmental shocks viz. Temperature and pH, and its tolerance level determination, leading to behavioral changes and ultimately mortality were focused in this study. Here an attempt was made to find out the consequence on a single species study of particular species *Piaractus brachypomus* (Red belly pacu). Red belly pacu is an important species in many parts of the world and considered as a commercially important aquaculture species.

Rapid Change in Water Temperature

Thermal shock has a massive impact on physiology behavioral and distribution pattern of aquatic organisms in an ecosystem. It is an a biotic master factor as defined by Brett JR [2], most fishes exhibit a direct relationship between temperature and metabolic rates at a variation of 10°C from the ambient temperature. Respiratory rates are assumed to represent the physiological state of a fish however, different activities like swimming, resting, feeding etc. could be affected differently by change in temperature and can often fatal to fish. Stress and strains of large magnitude occurs when the heat flux and component temperature gradient changes. The tolerance to survive this stress varies with species and age group. Fries and juveniles of most freshwater fish species are less tolerance to rapid change in temperature which makes it a great concern in aquarium industry, aquaculture industry and natural environment. This could be because of natural causes or human influence.

Rapid change in water pH

Shock due to pH fluctuation is a condition that can kill or seriously harm any aquatic animal. The role of pH in fish and other aquatic organisms to regulate basic life sustaining process, like exchange of respiratory gases and salts with water are least known. Failure of this process can result in numerous sub-lethal effect and mortality when the range of pH shock exceeds the tolerance level of the organisms. There is no definite pH range within which all freshwater fishes is unharmed. These should be a gradual determination in acceptability as pH values become further removed from the normal range [3-5]. Tolerance level against rapid change in pH depends on numerous factors including prior pH acclimatization, temperature, DO, ammonium level, concentration and ratio of various cautions and anions [6].

Although it was once believed that fish could not tolerate sudden pH changes, studies conducted by Brown HW, Jewell ME and Wiebe AH [7,8] showed that certain fish species could tolerate such rapid changes within normal pH range. The amount osudden change of pH is tolerated differently by different species and causes can also be different.

Materials and Methods

Juvenile red bellied pacu were purchased from a supplier in Moovatupuzha, Ernakulum district, Kerala, India. Since it is an invasive species, source data and origin cannot be disclosed. Fish seeds had a standard length of approximately 3 cm and a total of 100 numbers were purchased. They were randomly divided into 8 groups of 10 each for acclimatization and one group of 20. The 8 groups of 10 were maintained in a glass tank of 30 × 30 × 15 cm size with 10 liters of water. Glass tanks were named T1, T2, T3, T4 respectively for temperature treatment and P1, P2, P3, P4 for pH treatment. All groups of fishes were maintained in the same place with equal photoperiod and surrounding environmental conditions. De chlorinated tap water (hardness in terms of CaCo3 between 115 ppm and 125 ppm; pH above 7; Ammonia 1 ppm; Nitrite-0; Nitrate-0; Residual chlorine 0.2

ppm) was filled in glass tanks and was given proper aeration. Before releasing in to the tanks they were acclimatized to the temperature of water in the tank (28°C). When released to the tank most fishes had shown a sign of discomfort for attaining equilibrium. After few hours they seemed acclimatized to the water condition and started swimming actively. They were not fed for next 12 hours. There were no signs of abnormality. The fishes were then fed with formulated fish feed Varsha, 34% [protein content product of CMFRI (Central Marine Fisheries Research Institution), India) and peeled shrimp throughout acclimation period of 10 days. Feeding continued till the 9th day that is 24 hours before the environmental shock trials. Glass tanks were then divided into 2 groups for temperature and pH and each group contained 4 tanks (T1,T2,T3 and T4) for temperature and (P1,P2,P3,P4) for pH. One group of tanks for trails with rapid change in temperature, placed as two aquarium for low temperature (T1,T2) and two for high temperature (T3,T4). The other group of tanks with rapid change in pH were placed same, two for low pH value (P1,P2) and two for high pH values (P3,P4).

On the 10th day everything was ready to conduct the shock tolerance with respective environmental conditions of temperature and pH. Before trails the water conditions were verified.

Thermal shock treatment

For the determination of temperature tolerance level of red bellied pacu, 4 glass tanks containing 10 fishes each were equipped and was acclimatized for 10 days. At the time of trials the temperature of water contained in the tank was approximately 28°C. Standard aquarium thermometers were used for determining the water temperature and temperature fluctuations in the trials. Fishes from the four tanks (T1,T2,T3 and T4) were transferred temporarily into plastic containers with the same water as they were inhabited [used] in the glass tanks. This process is made for creating a shock in the same tank used to inhabit the fish. The treatment glass tanks were roughly cleaned using water sprayers and filled with warm and cold tap water accordingly for creating the temperature shock manually. Temperature of two tanks (T1,T2) which was assigned for low temperature shock, were lowered to 18°C (T1) and 23°C (T2) respectively that is -10°C and -5°C difference from the temperature of water were the fish first immersed before treatment. The next two tanks (T3 and T4) for high temperature shock, warm water was added and the temperature was raised to 33°C (T3) and 38°C (T4) respectively that is a difference of +5°C and +10°C from the temperature of water were the fish first immersed before treatment. Fishes were suddenly transferred to the glass tank and kept under observation (12 hours). Regular feed was provided at 5% body weight of the fish.

pH shock treatment

For determination of pH tolerance level of red bellied pacu 4 glass tanks (P1,P2,P3,P4) containing 10 fishes, each were acclimatized for 10 days. At the time of trials the pH value of water contained in the tank was approximately 8. Universal pH indicator solution from nice chemicals was used for sudden pH check and further clarification on pH level was done using electronic pH meter. Fishes from the four tanks (P1,P2,P3,P4) were transferred temporarily into plastic containers with the same water as they were inhabited (used) in the glass tanks. This process is made for creating a shock treatment in the same tank in which the fishes were placed initially. The glass tanks were then roughly cleaned using water sprayers and filled with de chlorinated tap water which was regularly used in the whole experiment. The pH value of 1st two tanks (P1 and P2) which was assigned for low pH shock. The pH was lowered to a neutral value of 7 (P2) and slightly acidic value of 6 (P1) at a lowering value of -1 and -2 points from the initial pH value. The adjustments for obtaining the lower pH were done using synthetic vinegar [prepared from synthetic acetic acid] for dietary purpose. The other group of two tanks (P3 and P4) was assigned for high pH values. The pH level was raised to +1 and +2 points that is up to pH of 9 (P3) and 10 (P4), respectively. The high pH value was obtained using sodium hydroxide (NaOH) pellets diluted in distilled water. After the adjustment of pH the fishes was suddenly transferred to the glass tanks and kept for observation.

Feeding trials

Fishes were starved for 24 hours before trial. Fishes are then fed with formulated feed (Varsha) after the trials of corresponding stress given to each tanks. Feeding rates in each treatment tank was observed. Feeding rates as a behavioral parameter was determined to denote the level of stress in fishes within an observation period of 12 hours from the time of the trial.

Estimation procedure

Trail conditions were observed using video visual record system. Recordings were monitored back and forth and pausing mode in each tank before and after the treatment. Behavioral changes was compared and verified from video recordings as a factor of fish movement and gill movement. Differences in the treated and untreated tanks were considered as the physiological and behavioral variations for a particular shock condition. The sudden change was monitored and mortality of individuals in the respected tanks was considered as end point. Behavioral differences such as fish movements, gill movements, feeding behavior and feeding rate was observed and tabulated against the treated conditions. The mortality rate from each individual tank was also recorded. The obtained data is quantified to find the relationship between the respected stimulated environmental shocks so as to extrapolate similar changes in natural ecosystem.

Results

Effect of thermal shock

When a shock was provided by shifting the temperature suddenly from 28°C to about 18°C and 23°C, the fishes in both tanks T1 and T2 showed similar behavioral changes with no mortality. All fishes started aggregating towards the corner of the glass tank and that continued up to 1 hour (Figure 1). They have also shown signs of stress as represented in the Table 1. There was no consumption of feed even after 3 hours after the shock was applied. There was no mortality in 10 hours and 24 hours of observation period but fishes started showing normal behavior after 12 hours. The fishes went back to normal stage when acclimatization occurs with the temperature. In case of fishes of T3 and T4 tanks with high temperature of 33°C and 38°C soon after introduction to the glass tank fishes have showed a loss of equilibrium (Figure 2). Most fishes in both tanks lay on the bottom of the tank this behavior only extended up to a period of 15 min. An individual fishes started moving actively across the tank was observed from the T4 provided with a shock of 38°C. It was a sudden response of active swimming in shock to escape from the situation. Feed consumption was about 40% when fed with formulated feed after 3 hours. Signs of stress were observed in the time period of 10 hours. No further mortality was observed in 24 hours.

Figure 1. *Fish aggregating owards corner.*

Figure 2. *Loss of equilibrium in fish.*

Effect of pH Shock

To determine the direct effect of pH shock on the fish they were provided with 4 different pH values in a range of lower and higher values of 1 and 2 points difference from the initial pH value. There was variation in response for each tank P1,P2,P3, and P4 and were showed in Table 2. In P1 (pH6) fishes were suddenly aggregated towards the corner of the tank, since the pH was slightly acidic. This behavior extended for more than 3 hours. There was no feed consumption observed after 3 hours. Fishes were heavily stressed in P1 with 20% mortality occurred within 5 hours. In P2 were the pH was neutral there was no extreme behavioral changes and no mortality were occurred in 12 hours of total observations period, except a slight distress at the time of transfer. Feed consumption was up to 90%. After 3 hours, however in the case of tank (P3) with high pH value which was within the tolerated limit of 9, fishes show rapid movement without schooling, rapid gill movements shows the distress in normal respiration of the fishes in P3 (Figure 3). Two fishes loss equilibrium within the 1st 10 min after the trail. Behavioral changes went back to normal after 3 hours but the feeding rate was lowered to 20%. 40% mortality was occurred within 10 hours of observation. In P4 with an extreme pH level which was out of the tolerance limit, fishes start trying to jump out of water with high mucus secretion from the body. Acute mortality of 100% was observed with a time period less than 15 min after the treatment.

Table 1. *Observations temperature treatment.*

Characteristics		T1	T2	T3	T4
Temp		18°C	23°C	33°C	38°C
Fish	10 min	Aggregate Towards Corner	Aggregate Towards Corner	Loss of Equilibrium	Loss Of Equilibrium
Movement	3 hrs	Slow movement, shown schooling behaviour	Slow movement, shown schooling behaviour	Active movement	Active movement
(In Three Time Gaps After Treatment)	12 hrs	Normal	Normal	Normal	Normal
Gill	10 min	Fast	Fast	Fast	Fast
Movement	3 hrs	Fast	Fast	Normal	Normal

(In Three Time Gaps after Treatment	12 hrs	Normal	Normal	Normal	Normal
Feeding Behaviour		No feed in take	No feed in takec	Slow feeding on bottom sinked pellets	Slow feeding on bottom sinked pellets
(Feeding was Conducted 3 hours after the trial)		0%	0%	40%	40%
Feeding Rate (After 3 Hours)		0%	0%	40%	40%
Mortality (%)	10 min	0%	0%	0%	0%
(In Three Time Gaps after Treatment	3 hrs	0%	0%	0%	10%
	12 hrs	0%	0%	0%	10%
Remarks		Shown signs of stress and was aggregated to corner of the glass tank up-to 1 hour	shown signs of stress and was aggregated to corner of the glass tank up-to 1 hour	Loss of equilibrium was extended up-to 15 minutes and started active random movements	Equilibrium was lost to 10% of fishes and mortality occurred after 6 hours. Rest 90%shown active random movements

Table 2. *Observation [pH treatment].*

CHARACTERISTICS		**P1**	**P2**	**P3**	**P4**
pH		6	7	9	10
Fish	10 min	Aggregate towards corner and loss of equilibrium	Aggregate towards corner	Rapid movement without schooling	Fishes started breching out of water surface
Movement	3 hrs	Aggregate towards corner and loss of equilibrium	Active movement	20% fish lost equilibrium, rest actively moving	100% mortality
(In Three Time Gaps after Treatment)	12 hrs	Slow movement	Normal	Normal	100% mortality
Gill	10 min	Fast	Fast	Very fast	100% mortality
Movement	3 hrs	Fast	Normal	Normal	100% mortality
(In Three Time Gaps after Treatment	12 hrs	Fast	Normal	Normal	100% mortality
Feeding Behaviour (Feeding was Conducted 3 Hours after the Trial)		No feed in take	Active feeding take from floating feed	Slow feeding on bottom sinked pellets	Complete mortality within 10 minutes after the trial
Feeding Rate (after 3 Hours)		0%	90%	20%	100% Mortality
Mortality (%)	10 min	0%	0%	0%	100%
(In Three Time Gaps after Treatment	3 hrs	0%	0%	0%	100%
	12 hrs	20%	0%	40%	100%
Remarks	Fishes was heavily stressed and mortality occurred at 5 hours after the treatment	slight stress during the time of transfer and recovered within 1 hour	Shown rapid movement and body was coated with white slime like secretion	Heavy mucous secretion from fish body and 100% mortality occurred after 15 minutes from the time of treatment	0%

Discussion

Rapid fluctuation in environmental condition is a growing concern in both natural and confined aquatic ecosystem. Stress is the primary effect that occurs in a fish when exposed to this change. The level of stress that occurs in the fish varied from species and in different age group which may leads to lethal and sub lethal effect. The changes in aquatic ecosystem may occur from both natural and anthropogenic sources. Humans in a great extent may have a direct or indirect effect from these occurrences in natural and confined ecosystem as they rely on aquatic organisms as a source of food and aesthetic purposes. Also questions the existence of the ecosystem. This effect could be positive or negative based on the scenario. When considering an invasive fish Red Bellied Pacu which is important as both food species and aquarium species makes it easy to display both the positive and negative side arises from the level of stress occurred in the fish due to rapid shock in environmental conditions. The tolerance of the hardy species against this environmental shock could be considered as a positive sign in the field of aquaculture and aquarium industry. But considering the natural environment, high tolerance of an exotic fish species may be a great threat. This alien species was brought top India via Bangladesh for aquaculture purpose and successfully bred and cultured because of its favorable nature as an aquaculture candidate [9]. They were 1st introduced into Kerala for aquarium trade. There were reports of many occasions when accidently found Red Bellied Pacu in natural water bodies of Kerala. The introduced population of Amazonian pacu in tropical waters of Chalakkudy river system is also established and reproducing well and acclimatization of this species to a wide range of pH and temperature is clearly understood. But their tolerance against the rapid change in the environmental conditions has to be clarified to consider this species as a good aquaculture candidate to protect and conserve the natural ecosystem from their invasions.

Rapid Temperature Change

Temperature is an abiotic factor as defined by Brett JR [2]. Sudden temperature change in an aquatic ecosystem may be from variety of sources like a water change in an aquarium sudden climatic change and water inflow to aquaculture ponds during rain and other anthropogenic sources like flow of industrial waste water to natural water bodies. In our studies fish exhibits abnormality in their behavior when exposed to a sudden temperature change of four different ranges. However, the result of behavioral or physiological responses also shows similar response to shock as reported by Schreck CB [10]. When two groups T1 and T2 were brought to the lower temperature points 18°C and 23°C (difference of -5 and -10 units). They started aggregating towards the corner of the glass tank and show less activity even if it reached to normal temperature. In our studies there was a vast dissimilarity in the behavior to the results of Friedlander et al. Where he examined the behavioral and cerebellar function of gold fish (Carassius auratus) by providing extreme low temperature even up to 2°C which was far below physiologically acceptable level of the fish for an extended period up to 50 minutes. The feeding trails shows; there was no consumption of feed. In other hands, two groups T3 and T4 were brought towards the higher temperature points of 33°C and 38°C (difference of 5 and 10 units). According to the studies of Wayne (1997) at a lower temperature of 15°C fish remained tightly schooled and displayed a marked reduction in general activity and were more active and aggressive with increasing water temperature. He also noticed there was a less food consumption in the group acclimatized to the lower temperature when compared to the groups acclimatized to the higher temperature. Similar observation shown in our studies in which most fish shows loss of equilibrium followed with active movements there may be an increased metabolic activity due to the active movements which results in a feed consumption of 40% during the feeding trials. Mortality of 10% occurred in the tank T4 cannot be considered as a serious end point.

Without any further extent of time the altered temperature may be returned back to normal. The tolerance towards the rapid change was well displayed by the survival rates at the end point. Releasing of the fish to the environment where the temperature was altered and allowed to change freely based on external environmental conditions was the best possible way to replicate the situation of an environmental shock in an aquatic ecosystem. The absence of an accurate temperature monitoring system was one of the limitations that should be considered.

Rapid pH change

When it comes to the case of pH of an aquatic ecosystem there is no definite range in which all the fishes is unharmed and outside where there an adverse impact occurs. The acceptance range may depend on many factors based on the fish species. A pH range of 6.5 to 9 are satisfactory for fishes and other fresh water aquatic life as the information provided in technical memorandum prepared by Shreck CB, Sanchez WC et al., Bennett WA [10,11]. The pH range that is not directly lethal to the fresh water fish is in the range of 5 to 9 (5). Sudden pH change in aquatic environment may be from variety of sources, starting from photosynthesis, waste flow to natural water bodies, water changes and waste deposition in aquaculture systems and water inflows due to rain into aquaculture ponds. There may be other reasons for this rapid change. In our study fishes exhibits a wide range of abnormalities when treated at four different points of pH from the acclimatized pH of 8. At a slightly acidic pH of 6 (2 units below the acclimatized pH), the group exhibits aggregating behavior towards the corner, equilibrium loss, sluggish movement and fast gill movement compared to normal behavior displayed before treatment. Fishes may be heavily stressed in the given condition and the mortality rate was 20%. The second group of fishes treated with the neutral pH of 7(1 unit below the acclimatized pH) shows least behavioral changes active feed intake of 90% and no mortality. Studies conducted by Brown HW, Jewell ME and Wiebe AH [7] [8] showed that certain fish species could tolerate such rapid changes. Coming to the third group which was treated with a high pH of 9 (1 unit above the acclimatized pH) they have soon exhibited an abnormality in mucus secretion with a white milky coated body. They have also shown loss of equilibrium and rapid movement without schooling. Few fishes had consumed 20% feed at feeding trials and there was a mortality of 40%. The fourth group with extreme pH of 10[2 units above the acclimatized pH] had shown behavior such as jumping out of water and heavy mucus secretion with a drastic hike of 100% mortality within next fifteen minutes of treatment. Similar results were observed when; Rainbow Trout transferred from water of pH 7.2 to water of pH 8.5, 9.0, 9.5 and 10. Survival after 48 hours was 100% for fish transferred to water of pH 8.5, 88% for those transferred to 9.0, 66% for pH 9.5 and complete mortality occurring for fish transferred to pH 10 [1]. Survival rate considered as the end point and it displays that the tolerance of Red Bellied Pacu against rapid change in pH within physiologically acceptable range shown significant behavioral effects. Our studies have also shown similarities of results of Wiebe AH [8] that sun fish and gold fish survived rapid changes from pH 7.2 to 9.6 (2.4 units), Largemouth bass from 6.1 to 9.6(3.5 units) and Smallmouth bass from 6.6 to 9.3 (2.7 units). In concurrent to these results the tolerance level of Red Belly Pacu was also comparable with a similar change in units. The addition of chemical substance to the treatment water and continuous checking of pH using pH indicator solution as the method for pH alteration and quantification was a limiting factor and could be questioned for the accuracy of result.

Practical limitations that could affect the findings of the study include fish size, where the environmental shock tolerance of Red Bellied Pacu may alter significantly by fish age and size. It is difficult to entirely rule out the size as a limiting factor to the shifts that may have occurred in the tolerance level of this particular species. The tank size and the conditions provided for the experiment is also a concern as there may be natural and anthropogenic alteration in different environmental factors which was not considered for this particular experiment. Likewise, the points of environmental conditions when used in different patterns may have shown different results in the behavioral effects. But within the board perspective of survival rate recommended by related literatures.

The work demonstrated that the fish species tolerated rapid pH changes of 1 to 2 units of lower limits and 1 unit of upper limit from the acclimatized pH when these change occurred within the physiologically tolerable pH range. The fish also tolerated rapid temperature change of 5 to 10 unit of lower and 5 to 10 units of upper limits from acclimatized temperature.

It is concluded that the Red Bellied Pacu has shown an above average level of tolerance and adaptability without acute mortality and chronic sub-lethal effects against rapid environmental changes within physiologically acceptable range. Conversely these studies also suggest that a small change could have adverse impact when the values fall outside the physiologically acceptable range for the given species.

Based on the above discussion this study is of a serious significance in hatchery stocking programs and freshwater aquarium industry where it is common to move fish from one water body to another that may differ in environmental conditions mentioned. The result also displays a clear overview that the tolerance of Red Bellied Pacu towards rapid environmental shock could also be a serious threat to natural water bodies where they will survive and reproduce.

Conclusion

The application of environmental shock to aquatic organisms is an ongoing and exciting direction of study, Many studies have investigated the survival of fishes against the acute changes occurring in the environmental factors. A gap still exists in understanding the relation between the ecological and physiological effects in aquatic organisms which could alter change in factors from both sides. These relations could be a fundamental factor in managing anthropogenic sources of environmental shocks. In conclusion, this study points out that over all behavioral changes and survival rate may not be accurate indicators of the tolerance of Red Bellied Pacu and other freshwater fishes against rapid fluctuations in environmental parameters. Other key physiological responses and ecological interactions with in a wide spectrum of environmental parameters in respect with different aquatic organisms should be analyzed.

Authors Contribution Statement

Aadil Yahiya-Experimental work done; Saeed Muhammed-Analytical work; Jithu Paul Jacob-Compilation data and literature writing

Statement Confirming Animal Ethics

All the work was done under the guidelines of animal ethic committee.

Statement Confirmation

The work was approved by the animal ethical committee of

Journal abbrevitaion: **J fisheriesci.com**

Research department of fisheries and aquaculture, St. Albert's College (Autonomous)

References

1. Witschi WA, Ziebell CD (1979) Evaluation of pH shock on hatchery-reared rainbow trout. Prog Fish-Cult 41: 3-5.
2. Brett JR (1971) Energetic responses of salmon to temperature, A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (Oncorhynchu nerka). Amer Zool 11: 99-113.
3. EIFAC(European Inland Fisheries Advisory Commission) (1969) Water quality criteria for European freshwater fish, Report on extreme pH values and inland fisheries, Prepared by EIFAC Working Party on Water Quality Criteria for European Freshwater Fish, Water Research. 3: 593-611.
4. Alabaster JS, Lloyd RS (2013) Water quality criteria for freshwater fish. Elsevier.
5. McKee JE, Wolf HW (1963) Water quality criteria (second edition) State. Water Quality Control Board Pu 340-548.
6. Robert TV, Yates BM, Thomas EA, Carlos FM, Rosemarie RC (1979) A review of the EPA Red Book: Quality criteria for water. American Fisheries Society 305.
7. Brown HW, Jewell ME (1926) Further studies on the fishes of an acid lake. Trans Amer Microsc Soc 45: 20-34.
8. Wiebe AH (1931) Note on the exposure of several species of pond fishes to sudden changes in pH. Trans. Amer Microsc Soc 50: 380-383.
9. Chatterjee NR, Mazumdar B (2009) Induced breeding of pacu (*Piaractus brachypomus*) in captivity with pituitary extract. Aquac. Asia 14: 2-23.
10. Schreck CB, Sanchez WC, Fitzpatrick MS (2001) Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture 197: 3-24.
11. Bennett WA, Currie RJ, Wanger PF, Beitinger TL (2011) Cold Tolerance and Potential Overwintering of the Red-Bellied Piranha Pygocentrus nattereri in the United States, Transactions of the American Fisheries Society 126: 113-125.