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Importance of Optimum Water Quality Indices in Successful Frog Culture Practices

Cleber Fernando M. Mansano, Luiz Sérgio Vanzela,
Juliana Heloisa P. Américo-Pinheiro,
Beatrice I. Macente, Kifayat U. Khan,
João Batista K. Fernandes,
Danila Fernanda R. Frias and Marta V. De Stéfani

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.81747>

Abstract

The optimum quality of water indices is extremely important for successful frog culture. Frogs excrete their excreta and skin debris in water. Therefore, it is necessary to regularly renew the water and clean the tanks and bays of rearing systems. Such care is necessary for the prevention and prophylaxis of diseases, which may cause severe mortalities. Bullfrogs need water of good physical and chemical quality, and thus, water quality indices must be measured before starting a breeding and rearing program. Additionally, the producers should have a good knowledge about the water quality before establishing a rearing system. Aquatic ecosystems are dynamic and even in small rearing water tanks, physical and chemical parameters are interrelated. For example, any change in dissolved oxygen level depends on the water temperature and atmospheric pressure. The dissolved oxygen level is almost 9.08 mg L^{-1} near sea side at a temperature of 20°C , whereas its concentration rises up to 10.07 mg L^{-1} , if the temperature drops to 15°C , indicating that dissolved oxygen and water temperature are closely interrelated. Thus, physical and chemical parameters of water should be considered and analyzed together because all of these factors have a direct impact on the culture systems.

Keywords: aquatic ecosystems, *Lithobates catesbeianus*, raniculture effluent

1. Introduction

Frog culture consists of small- and medium-scale producers and is gaining the attention since several years. The improvements in production systems and research have contributed a lot to make it more viable and profitable for producers. Brazil is considered as the country that has the best technologies in the production of bullfrog, that is, the entire production cycle is completed under controlled conditions. However, the sector encounters some limitations such as fewer improvements in the areas of nutrition, reproduction and genetics, mismanagement of the sector, and little investment and actions by the public sector [1].

The frog's production has different markets including the domestic and foreign markets. The main product is fresh meat, represented mostly by the trade of frogs' thighs [2]. More than 90% of internationally traded frog meat comes from extractive hunting, but there is enormous pressure from environment protection agencies that are claiming the finishing of such practice. Data published by FAO [3] on world ranching showed Brazil as the second largest producer, the first being Taiwan.

In Brazil and China, raniculture is exclusively based on the production and rearing of bullfrog (*Lithobates catesbeianus*), an exotic species from North America that was introduced for captive breeding. When compared to native species, it presents higher productive performance in commercial productions [4]. The species presents extremely favorable zoo-technical characteristics for high-scale breeding, such as precocity, prolificacy, and rusticity, which greatly facilitate management [2].

As among all amphibian species, frogs are ectothermic animals, that is, the temperature of water and environment directly influences the animal's metabolism [5]. The temperature of water between 22 and 28°C is considered ideal for the excellent development of tadpoles [6] and may result in achieving better zoo-technical indices in the rearing phase [7].

Amphibians like bullfrog need water of specific physical and chemical quality. For producers, before starting a rearing system, it is extremely important to know and has a good knowledge about different water quality parameters and their role in successful frog culture. They should measure the pH, electrical conductivity, total alkalinity, total hardness, ammonia, nitrite, nitrate, phosphorus, chlorides, iron, and especially oxygen properly before starting breeding. These parameters are the most important indices that indicate the water quality.

2. Usage, water quality, and aquaculture effluents

Aquaculture has shown great development in the last decades and is competing with production systems of other aquatic animals due to water resource. Its development, however, presents risks of deteriorating the quality and quantity of water and in contributing declining the environmental, social, and economic quality. Technical, scientific, and representative links of Brazilian aquaculture have stated that frog culture does not consume but rather uses water, and this characteristic of nonconductivity could change approaches and strategies related to the management of water resources directed to aquatic productions, taking in consideration different from the industries [8].

The granting and charging due to the use of water resources by aquaculture become more relevant when it highlights the aspects of water quality used and release the water source at the expense of aspects that basically involve the use of large volumes of water. The usage of aquaculture water depends greatly on water quality [9].

Aquaculture uses water resources intensively, competing for water available to the population and other productive systems. However, unlike other production systems, aquaculture can collaborate with water quality control systems by constant monitoring while keeping in view its role in producing food for humans especially protein food [8].

An objective and consistent model of granting and charging due to the use of water for aquaculture refers to methods focused on quality differentials, which are possibly based on measurements of biochemical oxygen demand (BOD) and levels of nitrogen (N) and phosphorus (P) in the water collected and returned to its source or original course. Other factors, such as water surface area, chosen design, or management techniques, may interfere with higher actual water consumption and may also be taken into account in the models of granting and charging the use of water resources for aquaculture [8].

Evaluating water use in the last 100 years, Telles [10] stated that globally about 70% of the available water is destined for agricultural usage. According to the author, approximately 20% of the water is destined to the industry and less than 10% to population (hygiene and direct consumption). In response to increasing food demands, aquaculture has also advanced its production. In the last decade, world production has increased by 200% mainly searching for a healthy product and the next step is the search for an ecologically correct product.

For any production system to continue its growth, it must be economically and environmentally correct [11, 12]. In addition, it should be based on the concepts of food security and socioeconomic development [13]. According to Valenti et al. [13], modern aquaculture is based on three pillars: profitable production, social development, and preservation of the environment, having an intrinsic and interdependent relationship for a perpetual production.

Although aquaculture provides a number of social and economic benefits, it must seek new technologies to reduce impacts such as deforestation, diversion of watercourses, introduction of exotic species into the natural environment, and effluent emission into aquifers. Environmental pollution can be defined as any action or omission of man, which has a direct impact on water, soil, and air and causes a harmful imbalance on the environment as well [14]. The impacts caused by aquaculture result in reduced production, disease outbreaks in cultivated and wild populations, and in some cases may restrict aquaculture operations [15].

Environmental factors such as soil quality, water quality, risks of introducing exotic species/biodiversity, chemical and organic discharge in the natural environment, recycling and interaction with other neighboring fish farms have a great impact on fish farming [16]. Another type of impact is that which is caused by aquaculture on water quality is related to the accumulation of nutrients and organic residues at the bottom of tanks and nurseries and such impact is time dependent. This time is related to unused food, fertilizer usage, and the nutrients present in the sediment, which precipitate and then release into the water column [17].

The nurseries have high concentrations of nutrients, plankton, organic, and inorganic matter due to the food provided and the morphometry of the systems. The input and output of

constant water with short residence time and uncontrolled management of feed and water are some of the important factors that directly act on nutrient dynamics, accelerating or allowing greater availability in the water column [18].

The water inflow and outflow due to its great intensity remove excess nutrients and other material from the nursery, controlling the phosphorus dynamics in the medium in relation to its absorption in the sediment [19]. In times of high fish production, from November to April (increased water temperature), the addition of feed is more intense and climatic factors such as temperature and precipitation influence the dynamics of these systems [18].

The impacts of aquaculture can be classified as internal, local, and/or regional. Internal impacts refer to those that interfere with the breeding system itself, such as the depletion of dissolved oxygen in a fish farm. In general, local impacts extend 1 km downstream of the effluent discharge. Effects on aquatic environments with a spatial scale of several kilometers are considered regional impacts [20].

The effluents from aquaculture ponds have a high volume and low nutrient concentrations when compared to domestic effluents, which present little volume and high concentration of nitrogen and phosphorus [21]. Although the dilution value of these effluent discharges from aquaculture is considered high, its direct launch in the limnic environments can result in a chronic bioaccumulation and eutrophication, which can lead to an excessive increase of phytoplankton, causing dissolved oxygen deficit at night and possible death of local organisms [22]. Thus, the advancements in aquaculture simultaneously increase the concern of environmental agencies and societies with the environmental impact generated by it.

The main negative impact of effluents from aquaculture activities on aquatic ecosystems is the increase of nitrogen and phosphorus concentrations in the water column and accumulation of organic matter in the sediments [22]. In addition to effluent produced by natural processes, nutrient enrichment, feces, and unconsumed feed, chemical residues are also released, which are used in disinfection, pest and predator controls, disease treatments, hormones to induce reproduction, and sexual reversal beyond anesthetics for transport [23].

Not all breeding techniques have negative environmental consequences, since many of them are beneficial when environmental management is effective and socioeconomically sustainable [24]. As a positive impact of aquaculture, there are related consortiums between aquaculture and agriculture (irrigation and farming), rearing of tadpoles along with ornamental fish [25] or integrated systems of multiple uses such as recreation, gastronomy and rural tourism.

Another positive aspect is the maintenance of fish stocks in the sea and rivers, protecting and conserving endangered species, the use of industrial and domestic (treated) effluents in the enrichment of fish farms, or the coupling of a hydroponics system together with the residues of fish farming itself. It is also important to highlight the opportunities for new economic and working sources in the river basin [23] as a positive social and economic aspect.

The increasing growth of the animal industry has forced the reduction in the effects of intensive production systems on the environment. The concern with amino acids up to recent animal response considerations was restricted for maximizing production efficiency but little or no attention was given for reducing nitrogen excretion [26]. But now, there is a great concern about

modern aquaculture's response to intensive production systems—the sustainability and environmental impact or pollution caused by them. In these systems, the density of fish per volume of water is very high, needing high rations and large amount of feed consisting of ingredients of high digestibility and palatability in order to produce minimum residue from feed wastage and stop high excretion of phosphorus and nitrogen [27].

The development of nutritious and environmentally and economically viable rations depends on the knowledge regarding the alimentary habits and nutritional requirements of the species reared [27]. Being an alternative production in the agriculture sector, frog culture can be placed among aquaculture activities that are gaining importance in the natural scenario [28], as the natural populations of frogs in Asia are decreasing due to environmental contamination and uncontrolled capture [29].

3. Importance of water quality for raniculture

The quality and cleanliness of water used in production of aquatic organisms are essential factors for the success of these programs. The frogs leave their excreta, and skin remains in water due to constant changes. It is imperative to constantly renew the water and clean the tanks and bays, and such care is necessary to prevent diseases and mortalities.

Amphibians such as bullfrog need water with specific physical and chemical quality. Parameters such as pH, electrical conductivity, total alkalinity, total hardness, ammonia, nitrite, nitrate, phosphorus, chlorides, iron, and especially oxygen must be measured before starting a breeding. These parameters are the most important indexes that characterize the quality of water. For breeders who are starting the activity, it is of the utmost importance to understand these variables.

All animal or vegetable life forms breathe in inhaling oxygen and exhaling carbon dioxide. When an aquatic environment is polluted with organic matter, the consumption of O₂ (respiration) exceeds beyond the acceptable levels and a decrease occurs in its available concentration. If the imbalance persists under anaerobic conditions (without oxygen), fish and most other animals will be unable to exist and will die. Oxygen allows aerobic (oxygen-using) bacteria to be more efficient decomposers than anaerobic (non-oxygen) bacteria, reducing decomposing organic matter in the water without leaving harmful odors.

When large quantities of organic material are discharged into rivers, for example, a population explosion of decomposing bacteria occurs. By "breathing," oxygen depletion occurs and the water becomes anaerobic or septic. The aerobic bacteria then facilitate the anaerobic bacteria, which produce hydrogen sulfide gas that has an extremely unpleasant smell and affect the aquatic life.

Aquatic ecosystems are dynamic, and even in tanks with small volumes of water, the physical and chemical parameters interrelate and are dependent on one another. The level of dissolved oxygen in water varies with temperature and atmospheric pressure. The dissolved oxygen content is about 9.08 mg L⁻¹ at sea level and a temperature of 20°C, while this concentration rises up to 10.07 mg L⁻¹ of oxygen, if the temperature drops to 15°C, indicating them the two closely interrelated factors.

The behavior of several other parameters occurs in the same way. Thus, it is not enough to know only one parameter or strictly follow the literature. The physical and chemical evaluation of water must be analyzed together, taking into account all factors.

3.1. Variables of interest in water quality control

pH (hydrogenation potential): It is the ratio between concentrations of hydrogen ions (H^+) and hydroxyl ions (OH^-), that is, acidity or alkalinity. It has a scale of 0–14, with pH 7 being neutral, where H^+ and OH^- concentrations are the same. Below 7, the pH indicates acidity, and above 7, it indicates alkalinity. The most responsible for its variation is the carbonic acid, which is originating from the carbon dioxide produced by phytoplankton during photosynthesis, where in excess, it renders the pH acidic and vice versa.

Electric conductivity: It is determined by the presence of dissolved substances that dissociate into anions and cations. It is the ability of water to transmit electric current. Practically for aquatic organisms, the higher the conductivity the more charged the system will be.

Total alkalinity: It indicates the concentration of carbonate and bicarbonate salts in water. It has a function of water buffering, that is, it maintains the pH stable, besides participating in the carapace formation of some species of plankton. Carbonates and other salts react with carbonic acid, neutralizing their action.

Total hardness: It indicates the concentration of metallic ions mainly the ions of calcium (Ca^{2+}) and magnesium (Mg^{2+}) present in water. It is expressed in $CaCO_3$ equivalents. The values of total hardness are practically associated with alkalinity. It also potentiates the toxicity of various chemicals.

Ammonia, nitrite, and nitrate: Ammonia produced due to the excretions of aquatic organisms and bacterial decomposition of the organic material in water is divided into toxic ammonia (NH_3) and ammonium ion (NH_4^+). Through bacterial oxidation (nitrosomonas), the ammonia is transformed into nitrite. Then, nitrite is oxidized by bacteria of the genus *Nitrobacter* to nitrate. The denitrifying bacteria transform nitrate into nitrogen by completing the cycle. Generally, ammonia and nitrite are the toxic forms (depending on pH and temperature). Nitrate is not toxic.

Phosphorus: It is a nutrient with low concentration in water but is one with the highest concentration factor in phytoplankton, followed by nitrogen and carbon. Its compounds constitute an important component of the living cell, especially nucleoproteins, essential for cellular reproduction. It is also associated with respiratory and photosynthetic metabolism. They occur mainly in the form of soluble phosphates and phosphate rock. Organic wastes, especially domestic sewage, contribute to the enrichment of water with this element.

Iron: Among the physical and chemical parameters of water, iron is the one that most frequently makes impossible the implantation of a commercial raniculture. This metal when in high concentration causes tadpoles mortality due to its chemical toxicity. It is sometimes possible to remove the iron from water through its oxidation (Fe_3 – colloidal), in other words, introducing oxygen in the medium and aeration.

4. Water quality indices found in commercial farms for the cultivation of tadpoles

The excretion of the animals (feces and urine) results in ammonia-based compounds. Ammonia is extremely toxic when in large quantities and is converted into nitrite and nitrate by the action of nitrifying bacteria. Nitrite is also a toxic compound. It can oxidize hemoglobin in animals' blood; thus, converting it into methaemoglobin, a molecule incapable of carrying oxygen. This transformation process of ammonia (NH_3 —toxic) in nitrite (NO_2 —toxic) and then in nitrate (NO_3 —toxic only in high quantities) is called denitrification and occurs depending on the temperature, pH, and oxygen of water. This reaction is one of the most common causes of mortality in tadpole tanks but it can also be easily avoided by taking basic precautions such as controlling the amount of food offered, constant and efficient oxygenation, water renewal, and regular cleaning.

The changes in water are related to values that allow classifying water by its degree of contamination, origin or nature of the main pollutants, and their effects to characterize cases of loads or peaks of concentration of toxic substances and to evaluate the biochemical balance necessary for maintenance of aquatic life. In other words, the farmer observing the water of his tanks daily can infer and/or perceive its state. However, not even the experience gained over the years will spare the farmer from regular observing/monitoring the water of his tanks.

In the literature, we find little information available on the ideal water quality for raniculture. Many concepts and values come from other types of aquaculture animals. Thus, a gap occurs when the farmers apply this information in commercial ranks, which is a more practical activity. Another way to aid in the elucidation of this process is to conduct aquatic research regarding the impact of water quality on frog culture on large-scale laboratory tests in order to provide more accurate and practical information to the frog culturists.

Following are some data collected in the field from observations made in commercial ranks in Brazil (**Table 1**), which are demonstrated.

Analyzing the above mentioned variables, it is verified that there is a very great similarity between fish and amphibians with respect to the physical and chemical parameters of the water. It has been noted that bullfrog tadpoles require less oxygen than fish. This is one of the reasons why many amphibians are known as “homebodies,” that is, animals do not migrate from their place of origin when environmental conditions become adverse.

Normally, higher production of tadpoles is carried out in hot periods due to better adaptation and development of animals at an average temperature of 26°C [32]. The pH values did not show large changes among the frog farms, normally presenting average values of 7.0 for water supply and effluent, respectively. Thus, it remains within the standards recommended by the Brazilian resolution (pH between 6.0 and 9.0 for breeding waters of this species).

It is common to observe decrease in the dissolved oxygen content in the effluent than the water supply, but ideally, it is above the minimum required by the legislation (5.0 mg L^{-1}). As far as the ideal flow rate for spinning water is concerned, it is ideal that the total tank volume

Parameters	Desirable values	Values observed
pH	6.5–7.0	6.0–8.0
Oxygen	0.7–6.0 mg L ⁻¹	2.0–6.0 mg L ⁻¹
Ammonia (NH ₃)	Up to 0.5 mg L ⁻¹	Up to 0.7 mg L ⁻¹
Nitrite (NO ₂)	Up to 0.5 mg L ⁻¹	Up to 1.0 mg L ⁻¹
Nitrate (NO ₃)	Up to 1.0 mg L ⁻¹	—
Hardness	Up to 40 mg L ⁻¹	10–80 mg L ⁻¹ CaCO ₃ (most frequent)
Alkalinity	Up to 40 mg L ⁻¹	10–80 mg L ⁻¹ CaCO ₃ (most frequent)
Chloride (Cl ₂)	Up to 7 mg L ⁻¹	—
Chlorine (C ₁)	0.02 mg L ⁻¹	Up to 1 mg L ⁻¹
Fluoride (F ₂)	Less than 1 mg L ⁻¹	—
Iron	Up to 0.3 mg L ⁻¹	Up to 1 mg L ⁻¹
Orthophosphate (PO ₄)	Less than 0.3 mg L ⁻¹	—
Electrical conductivity	—	Less than 150 μS cm ⁻¹

Adapted Ferreira et al. [30] and Hailey et al. [31].

Table 1. Physico-chemical characteristics of water observed in commercial bullfrog tadpole (*Lithobates catesbeianus*) farms in Brazil.

is renewed at least once a day in small tanks. Some authors have already described some flow rates found in frogs: Borges et al. [33] found 0.11 L s⁻¹ and for the exit water, 0.08 L s⁻¹; and Pereira et al. [34] found the value of 0.064 L s⁻¹.

For electrical conductivity, there is no established standard for tadpoles. We find literature that demonstrates values of 30 to 200 μS cm⁻¹ for incoming water and runoff water [35–37]. Mercante et al. [38] working with sequential nurseries of semi-intensive fish production observed a mean variation between 46 and 113 μS cm⁻¹ in the nurseries (**Table 1**). The same author states that when the values are high, they indicate a high degree of decomposition and the inverse (reduced values) is intense primary production (phytoplankton).

The maximum limit for turbidity in effluents, according to Brazilian legislation, is 100 NTU (nephelometric turbidity units). According to Borges et al. [33], in a study with tadpoles, the maximum value obtained for incoming water was 20 NTU and for output was 26 NTU, thus remaining within the allowed range. Sipaúba-Tavares [39] argues that high levels of turbidity may be related to the presence of clays and dissolved or colloidal organic matter. In the same study by Borges et al. [33], ammonia and nitrate remained well below the limit established by legislation during the experimental period. However, from collection 2 (31 days of experiment), the concentrations were higher in the outlet water, with mean values of 0.40 and 0.49 mg L⁻¹, respectively, evidencing the organic matter decomposition and the rapid nitrification process due to the good oxygenation of the tanks.

5. Qualitative and microbiological characteristics of raniculture effluent

The main problem observed in raniculture as already observed in aquaculture is water enrichment (eutrophication) caused mainly by inadequate food management, which raises nutrient concentrations and modifies the environmental conditions of the farm [40]. The eutrophication process is a frequently observed problem and suggests the need for effluent studies and management techniques focused on ecological and specific aspects of these systems, favoring a lower impact of the effluents in the receiving water bodies [41].

In raniculture, commercial feed is usually used for carnivorous fish with average levels of 45% crude protein [42]. In addition to the increase in the amount of feed offered according to the growth of the animals, there is also a change in the use of this feed in each developmental stage of the animal [43]. The bullfrog produced in “anfigranja system” presents low protein efficiency of the commercial diet elaborated based on the requirements of carnivorous fish, which is used for feeding in most Brazilian ranicultures [44]. In addition, there is a significant loss of feed nutrients to the environment, resulting in the degradation of water quality [45].

A compound resulting from the catabolism of proteins is ammonia; therefore, the control of food quantity and quality, as well as adequate flow of water, is of fundamental importance for the maintenance of a good artificial breeding system [46]. In breeding systems for aquatic organisms, food introduced into the water and ammoniacal nitrogen fertilizers, such as ammonium sulfate, ammonium nitrate, phosphates, and urea, contribute to the increase of ammonia concentration in water [47]. The diet formulation should eliminate high levels of phosphorus and nondigestible components, testing the minimum necessity to grow a certain species [48]. Thus, the quality and quantity of food should be controlled for the sustainability of the harvesting system [49], since a relationship has been observed between commercial feed utilization and the water eutrophication process [50]. If the aquatic system is in imbalance, it becomes propitious to the development of diseases, compromising the sanitary sanity of that place.

Ectothermal animals such as bullfrog often serve as carriers for etiological agents by contact or as carriers when agents are ingested. When they are infected, it may result in an imbalance in the population of the pathogenic microorganisms in the environment, changes in the physical, chemical, and biological quality of water, immunological deficiency of these organisms, and unnecessary stresses [51].

The “Biological Institute of São Paulo, Brazil” has identified the presence of *Escherichia coli* bacteria in a very high load in diseased frogs (secondary and opportunistic infection) in a breeding system where the frogs fed larvae, and in the examination of feces of pigs used as substrate of larvae, an extremely high amount of this enterobacterium was reported [52].

E. coli is found in sewage, effluent, natural waters, and soils that have received recent fecal discharge, being inactivated. When ingested, it becomes active and pathogenic, but it is most used as indicator of fecal pollution in the environment and food, appearing in fresh and poor fish, frogs, mollusks, and shrimp [51].

Bacteria from the coliform group are also found in soil and vegetables, some with a certain ability to remain and even multiply in humid environments with high levels of organic and inorganic nutrients. Organisms in the coliform group may be introduced into water and food from nonfecal sources, such as plants and individual transporters, already polluted (lack of hygiene or sanity), such as contact with other animals or humans, even without release or direct contact with their excreta [51]. Gray et al. [53] suggest that frogs in metamorphosis or infected “froglets” may continue to release this pathogen, infecting other breeding members and eventually contaminating water sources. In addition, they can transport terrestrial pathogens to adjacent aquatic systems. It is possible that bullfrog adults may serve as suitable hosts for *E. coli* in stagnant aquatic systems. Another care that must be taken in raniculture is during the process of metamorphosis that is directly related to the reduced immunocompetence.

It has been reported that this microorganism can survive in fluvial waters for up to 27 days. Thus, persistence of *E. coli* in aquatic environments should be maintained through periodic contributions from primary reservoirs (homeothermic animals) or excreta contamination of wild host animals. The results of Borges et al. [33] suggest that bullfrogs can function as spill-over reservoirs of *E. coli* and thus contribute to its persistence in aquatic environments. In addition, since tadpoles in metamorphosis are capable of dispersion, they may play a role in the pathogen epidemiology [53]. **Table 2** shows the values of the biotic and abiotic variables of different types of aquatic cultures.

DO, dissolved oxygen; EC, electrical conductivity; BOD, biochemical oxygen demand; COD, chemical oxygen demand; MLN, most likely number.

Variables	Pisciculture	Carciniculture	Tadpole
	Macedo and Sipaúba-Tavares [54] ¹ Sipaúba-Tavares et al. [55] ² Macedo et al. [56] ³	Henry-Silva and Camargo [57] ¹ Pistori et al. [58] ² Keppeler [59] ³	Borges et al. [45]
EC ($\mu\text{S cm}$)	96 ²	70 ²	74
DO (mg L^{-1})	8.40 ²	4.63 ²	6.15
P-total (mg L^{-1})	0.25 ²	0.29 ¹	1.88
Nitrate (mg L^{-1})	0.13 ²	0.62 ³	0.68
Ammonia (mg L^{-1})	0.10 ²	0.13 ³	0.82
BOD (mg L^{-1})	7 ¹	7 ³	12
COD (mg L^{-1})	18 ¹	—	51
<i>Escherichia coli</i> (MLN/100 ml)	4×10^6 ³	—	1.3×10^3

Table 2. Comparison between the values of biotic and abiotic variables found in effluents from different aquaculture activities.

6. Water quality indices in commercial farms for terrestrial frog cultivation

Frogs are water-dependent organisms, thus, for the elimination of excreta, controlling their body posture, respiration, reproduction, protection and safety makes the quality of water extremely important in breeding times [60]. The quality of water used in the production of aquatic organisms is one of the essential factors for the success of these enterprises. In raniculture, postmetamorphic animals leave their excreta and skin remains from constant changes in water. Therefore, it is important to constantly renew the water and clean the tanks and bays. Such care is essential for the prevention and prophylaxis of diseases, because when a disease sets in, mortality is certain [30].

The water for use in commercial frog farms should be of good quality, without fecal coliforms, heavy metals, and iron, with neutral pH, being preferably of spring or artesian well. It is recommended to select places with higher ambient temperatures for its rearing, since the frogs are ectothermic animals, presenting a more accelerated growth in higher temperatures. To maintain the quality of the breeding place, the water used in the farm must come from its own source and protected, do not receive polluting load of any kind, and have its reservoirs protected and cleaned regularly [61].

Raniculture projects should include knowledge of local hydrography and concern for the rational use of water, mainly to reduce impacts on water resources. Particular attention should be paid to the construction of projects in ecologically sensitive areas of importance to environmental preservation, such as permanent preservation areas. According to the Brazilian Institute of Fisheries, the flow necessary for the installation of commercial frog farms of 500 m² is 0.5 L s⁻¹ ABRAPOA [62], resulting in an amount of 43,200 liters per day. However, this volume should not be discarded in the receiving water body without prior treatment.

The quality of the effluents must be periodically monitored, and the projects must provide for the installation of a treatment system for these effluents. Efforts should be made to increase the feed efficiency of the animals in order to ensure the reduction of the waste loads generated by the activity. The adoption of measures to reduce and eliminate the chances of diseases with preventive actions (sanitary management) and the maintenance of efficient and sustainable population densities is also important.

The main negative environmental impact observed is related to the degradation of the unconsumed feed, releasing to the water the nutrients and increasing the concentration of nitrogen and phosphorus. The excretion of animals (urea) is also released into the water and results in increased ammonia concentration. Remains of skin contribute to increase the amount of organic matter (total solids). Due to the nitrification processes that take place inside the bays, the amount of dissolved oxygen decreases drastically and reaches the anoxic levels.

The quality of the breeding place is based on the application of good animal breeding practices, in which the factors such as the technical knowledge about raniculture, ideal soil qualities (space), water in quality and quantity, constant, trained, and responsible workforce and projects that contemplate an economic planning should be properly addressed. The frog culture presents

Limnological variables	Bullfrog culture	Shrimp culture	Fish culture
	Borges et al. [45]	Henry-Silva and Camargo [57]	Sipaúba-Tavares et al. [55] ¹ Henry-Silva and Camargo [63] ²
Temperature (°C)	28.2	26.5	25.7 ¹
pH	7.2	8.1	7.2 ¹
Dissolved oxygen (mg L ⁻¹)	1.23	5.10	5.40 ¹
Conductivity (µS cm ⁻¹)	249	68	62 ¹
Turbidity (NTU)	66	62	26 ²
Total phosphorus (mg L ⁻¹)	6.09	0.23	0.20 ¹
Ammonia (mg L ⁻¹)	6.94	0.02	0.07 ¹
Nitrate (mg L ⁻¹)	2.37	0.16	0.16 ¹

Table 3. Comparison of the limnological characteristics of effluent from frog, shrimp, and fish cultures.

higher levels of dissolved nutrients, mainly concentrations of phosphorus, ammonia, and conductivity, as compared to other aquaculture activities, such as fish and shrimp cultures (**Table 3**).

Borges et al. [45] (2012) concluded that the management adopted in ponds of frog growth positively changed the quality of water. In contrast to other cultures of aquatic organisms (fish and shrimp), effluent from frog culture has a greater potential to cause eutrophication in receiving bodies of water. Best aquaculture practices (BAPs) should also be recommended for frog culture in order to avoid water pollution and contamination of animals (food biosecurity).

Mercante et al. [38] evaluated the mean concentrations of total phosphorus (TP) and flow in the water (inlet and outlet) and the load produced per day for bullfrogs and compare their results with other aquatic production systems (**Table 4**).

	Inlet			Outlet			
	Concentration (mg L ⁻¹)	Flow (L s ⁻¹)	Load (g day ⁻¹)	Concentration (mgL ⁻¹)	Flow (L s ⁻¹)	Load (g day ⁻¹)	
Bullfrog farming	0.03	0.06	0.21	0.19	0.06	14.3	Mercante et al. [38]
Bullfrog farming	0.07	0.03	0.18	6.09	0.02	11.57	Borges et al. [45]
Tilapia farming	0.42	2.76	9.7	2.45	2.76	49.1	Pereira et al. [64]
Trout farming	72.26	40.61	233.33	99.69	40.61	343.67	Moraes et al. [65]

Table 4. Mean concentrations of total phosphorus (TP) and flow in water (inlet and outlet) and the load produced per day in different systems of animal production.

Mercante et al. [38] concluded that a constant renewal of water in the breeding bay is necessary to avoid the toxic effects in bullfrogs. However, it can promote higher nutrient loads. In order to improve the effluent quality and to reduce the nutrient load, in addition to effluent treatment, management options such as (a) flow maintenance and density reduction of animals and (b) maintain flow and density storage with better control of food supply, quality, and digestibility are proposed.

7. Usage of “wetland” in effluent treatment

Effluent treatment systems generated in aquaculture farms can be deployed using wetlands constructed to remove nutrients and improve water quality [66]. The aquatic macrophytes that are used in biological treatment systems, such as constructed wetlands, function as a bio-filter, improving the environmental conditions of production nurseries [67].

The constructed wetlands are tools used for the treatment of waste in aquaculture whose physical, chemical, and biological processes together with the local climatic conditions can improve the effluent quality [57, 68]. The importance of the implementation of these systems for the treatment of fish farming effluents in Brazil is due to the fact that many producers release water directly into natural streams and rivers [69], and the organic load exceeds its capacity of support and resilience [70].

The disposal of the effluent in the soil together with the presence of microorganisms, aquatic macrophytes, and solar energy results in the production of biomass and chemical energy, removing the polluting load. This is a system artificially designed to use aquatic plants (macrophytes) on substrates (such as sand, soil, or gravel), where the occurrence of biofilms with diverse populations of microorganisms treats wastewater through biological, chemical, and physical processes [71].

The conversion of ammoniacal nitrogen into wetlands is mainly due to two basic factors: the assimilatory process of microorganisms and macrophytes present in the systems and nitrification due to the transfer of oxygen from atmospheric air through the leaves of the macrophytes that through the aerenchyma permits the distribution of oxygen to the rhizomes and roots of plants [72].

The phosphorus present in the wastewater is generally phosphate and its removal in wetlands is controlled by the biotic and abiotic processes. The removal occurs due to the use of phosphorus by plants, periphyton and microorganisms, sedimentation, adsorption, precipitation, and exchange processes between the substrate and the water layer that remain in the system [73].

According to Travaini-Lima and Sipaúba-Tavares [68], the removal of phosphate compounds is associated to the hydraulic flow of the system, retention time, and the macrophyte species used, and species such as *Cyperus giganteus*, *Typha domingensis*, and *Eichhornia crassipes* are resistant and highly effective plants for subtropical regions. Sipaúba-Tavares and Boyd [74] verified that the wetland installed in an aquaculture farm containing only the aquatic plant *E. crassipes* presented efficiency in the removal of nitrogenous and phosphate compounds, improving the water quality of the effluent and also confirming that the system of biofiltration can be applied in shallow water channels.

The reduction of thermotolerant and total coliforms occurs due to the combination of physical, chemical, and biological factors. Physical factors include filtration through the plants, biofilm fixation on the substrate and on the macrophytes, and sedimentation. The chemical factors involve oxidation, biocidal effect resulting from the material excreted by some macrophytes, and adsorption of the organic matter. The biological mechanism includes production and effusion of chemical substances in the environment, which prevent the development of other organisms (antibiosis) and predation by nematodes and parasites, bacterial lysis, and inactivation [75].

Effluent treatment in aquaculture is one of the main factors in systems for breeding aquatic organisms that improve the water quality, avoiding or minimizing eutrophication in the receiving body. In addition, it is important to note that the use of nitrogen and phosphorus as a source of nitrogen and phosphorus in the aquatic ecosystem is a very important factor.

The importance of using biofiltration in aquaculture in different locations around the world is to ensure the development of this enterprise. The production of aquatic organisms varies from country to country, directly influenced by climatic and edaphic factors, as well as the population habit of each site. The incorporation of effluent treatment mechanisms of aquatic organism breeding systems depends on the economic conditions of the enterprise, the degree of pollution of the effluent, the cultivated species, and the ecological management employed [76].

Technology transfer should be stimulated toward the use of water or even in the treatment systems for the production of aquatic organisms, as a way of minimizing the impacts caused by aquaculture, being located and identifiable as possibilities of techniques for the mitigation of waste [77].

Integrated management of the system for the establishment of built wetlands and organisms results in significant productive and environmental gains. The cost of constructing the wetland system is similar to the cost of building stabilization ponds. The advantage of using wetland is the quality of the effluent, which can be used in crop irrigation, and the macrophytes can be used as material for green fertilization in agriculture, since they contain the nutrients withdrawn from the water stored in their biomass [78].

7.1. Use of “wetland” in raniculture

Raniculture or frog culture, as well as any other aquaculture practice, requires a large volume of water and produces effluent with high organic load and can be a significant source of local environmental impact. The main characteristics of this type of effluent are the high concentrations of dissolved nutrients, mainly ammonia and phosphorus, high electrical conductivity, and low dissolved oxygen concentration, as compared to effluents from other aquaculture practices [45].

Due to the need to treat the effluent from aquaculture practices, the use of “wetlands” constructed in a growing way in the country has been studied to minimize the impacts produced by these aquatic organisms [63, 66, 68, 69]. The aquatic plants extract or take out nutrients and other substances from the surrounding water which are necessary for their development, besides requiring low capital, low operating cost and versatility in the removal mechanism than conventional treatments. Sipaúba-Tavares and Braga [69] are also important in the removal of nitrogen, phosphorus, biochemical oxygen demand, thermotolerant solids, and coliforms [79].

Studies on the treatment of raniculture effluents are still scarce, and however, the aim of the wetlands is to avoid the degradation of the water quality of receiving reservoirs. Keeping this in view, Borges and Sipaúba-Tavares [80] constructed a wetland for the treatment of effluent from bullfrog breeding and evaluated the efficiency of removal of nutrients and thermotolerant coliforms in two phases of the fattening period (post metamorphosis). The total area and volume of the “wetland” were 14.2 m² and 2.2 m³, respectively. It was 23 m long and composed of three boxes, the first with 0.51 m³, the second 0.72 m³, and the third 0.93 m³, connected by channels with surface water flow, according to **Figure 1**. The first box was used only for sedimentation of solid residues, the second one filled with *Eichhornia crassipes*, and the third with *Typha domingensis* and *Cyperus giganteus* planted at the density of 5 m² plants (**Figure 1**).

In the study by Borges and Sipaúba-Tavares [80], water harvesting occurred in two distinct phases, phase I corresponded to the period of highest stock biomass (IF) between July and October 2012, when the animals weighed on average 276 g and the other in phase II (FII) between December 2012 and March 2013, where the animals weighed on average 29 g. The total biomass of animals in the bays in phase I was 351 kg and in phase II 218 kg.

Borges and Sipaúba-Tavares [80] found the following values for phase I: limnological variables such as pH and dissolved oxygen (DO) were higher ($p < 0.05$) at the exit and turbidity, and NO₃, NO₂, total suspended solids (TSS), and thermotolerant coliforms (TC) were higher ($p < 0.05$) at the entrance to the wetland (**Table 1**). In phase II, the values of the limnological variables such as turbidity, NO₂, NO₃, SST, and TC were higher ($p < 0.05$) at the entrance and DO and NH₃ were higher ($p < 0.05$) at the wetland exit (**Table 1**). In both phases, it was possible to observe an increase of DO in the water leaving the wetland in relation to the input water (**Table 5**).

In phase I, the highest values of NH₃, PT, SST, and TC occurred at the entrance of the wetland, and in phase II, the highest values of NO₂, total solids dissolved (TSD), biochemical oxygen demand (BOD), and chlorophyll occurred. NO₂ was approximately twice as high at the water inlet in the wetland of stage II, and NH₃ and STS were also higher at the entrance in phase I (**Table 5**).

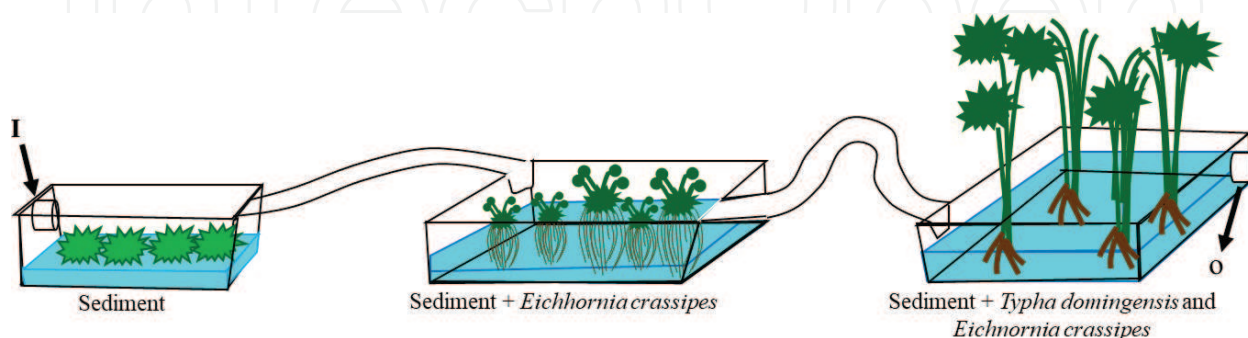


Figure 1. Layout of the wetland constructed in the frog breeding sector of the aquaculture center located at the State University of São Paulo (UNESP), Jaboticabal, SP, Brazil. I = entry point where the wastewater of the bullfrog production system entered the built marsh; O = the exit point where treated wastewater left the constructed wetland entering directly into a fish pond, adapted of Borges and Sipaúba-Tavares [80].

Variables	Phase I		Phase II	
	Entrance	Exit	Entrance	Exit
Temperature (°C)	28.0 ± 1.0	27.3 ± 1.0	27.9 ± 0.9	26.4 ± 0.9
pH	7.1 ± 0	7.3 ± 0.1	6.7 ± 0.3	6.9 ± 0.3
EC (µS cm ⁻¹)	182.9 ± 22.0	181.7 ± 19.9	162.6 ± 3.6	169.0 ± 5.4
Turbidity (NTU)	5.8 ± 1.6	2.9 ± 2.4	8.2 ± 3.4	2.3 ± 0.9
Alkalinity (mg L ⁻¹)	104.0 ± 2.3	108.4 ± 2.9	103.5 ± 1.6	104.9 ± 2.8
DO (mg L ⁻¹)	1.9 ± 0.3	2.6 ± 0.2	1.6 ± 0.4	2.9 ± 0.6
NO ₃ (µg L ⁻¹)	82.7 ± 45.7	37.2 ± 14.4	81.8 ± 12.6	22.9 ± 18.5
NO ₂ (µg L ⁻¹)	9.4 ± 3.6	5.3 ± 1.0	19.7 ± 3.1	11.6 ± 5.0
NH ₃ (µg L ⁻¹)	2.785 ± 1.219.6	3.590 ± 955.9	1.388 ± 217.2	1.863 ± 164.9
PO ₄ (µg L ⁻¹)	271.1 ± 132.6	309.9 ± 94.9	242.1 ± 52.2	265.4 ± 24.6
TP (µg L ⁻¹)	570.5 ± 285.5	480.4 ± 192.9	361.5 ± 102.2	283.4 ± 61.3
TSS (mg L ⁻¹)	21.0 ± 12.3	7.6 ± 6.8	9.0 ± 7.0	1.0 ± 0.8
STD (mg L ⁻¹)	135.7 ± 23.1	153.2 ± 31.7	194.5 ± 70.4	111.4 ± 9.5
BOD (mg L ⁻¹)	76.5 ± 13.6	71.6 ± 18.7	93.0 ± 3.8	85.6 ± 7.8
TC (NMP.100 m L ⁻¹)	50.750 ± 32.623	3.475 ± 2.284	25.250 ± 16.257	3.325 ± 1.362
Chlorophyll <i>a</i> (µg L ⁻¹)	4.5 ± 3.7	6.0 ± 4.8	7.2 ± 5.2	5.1 ± 3.4

EC, Electrical conductivity; DO, Dissolved oxygen; NO₃, Nitrate; NH₃, Nitrite; PO₄, Orthophosphate; TP, Total phosphorus; TSS, Total suspended solids; TSD, Total solids dissolved; BOD, Biochemical oxygen demand; TC, Thermotolerant coliforms.

Table 5. Mean, minimum, maximum, and standard deviation for the limnological variables of the water entering and exiting the wetland in phases I and II.

The authors concluded that there was a significant improvement in the quality of waste water of the bullfrog production system. Therefore, the treatment used was adequate and could be used in commercial farms, with only a few adjustments being made to improve the efficiency of nutrient removal.

8. Final considerations

Raniculture like other aquaculture practices is facing problems that affect the water regime, impacting natural water sources and directly water quality, which are responsible for the successful breeding and production systems. Although there are few studies on water quality in raniculture, aquaculture has already a number of studies but still there is a lack of understanding of how these ecosystems actually function and interact with biotic and abiotic factors. Any sudden disturbance rapidly changes the water quality and consequently causing certain damages to the commercially important species. Thus, future studies should focus on

the point to explain how different aquaculture systems function and how they interact with biotic and abiotic factors. These practices may provide excellent information to establish successful aquaculture systems with favorable environmental influences.

Acknowledgements

We thank the Brazil University (Univ. Brasil) for financial support.

Author details

Cleber Fernando M. Mansano^{1*}, Luiz Sérgio Vanzela¹, Juliana Heloisa P. Américo-Pinheiro¹, Beatrice I. Macente¹, Kifayat U. Khan², João Batista K. Fernandes², Danila Fernanda R. Frias¹ and Marta V. De Stéfani²

*Address all correspondence to: clebermansano@yahoo.com.br

1 Brazil University, Fernandópolis, SP, Brazil

2 Aquaculture Center of UNESP – Universidade Estadual Paulista, Jaboticabal, SP, Brazil

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