Environmental indicators in effluent assessment of rainbow trout (*Oncorhynchus mykiss*) reared in raceway system through phosphorus and nitrogen

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Received: May 6, 2015 – Accepted: July 7, 2015 – Distributed: November 30, 2016 (With 4 figures)

Abstract

The phosphorus and nitrogen discharge via effluent of intensive trout farming system was quantified through the use of environmental indicators. The nutrient loads, the mass balance, the estimated amount of nutrients in feed and the amount of nutrients converted in fish biomass were calculated based on the concentrations of phosphorus (P) and nitrogen (N) in the feed and in the water. Of the offered feed, 24.75 kg were available as P and 99.00 kg as N, of these, 9.32 kg P (38%) and 29.12 kg N (25%) were converted into fish biomass and 15.43 kg P (62%) and 69.88 kg N (75%) were exported via effluent. The loads and the mass balance show the excessive discharge of nutrients via effluent, corroborated by the feed conversion ratio (2.12:1) due to the low efficiency of feed utilization, therefore, it is proposed the use of this zootechnical parameter as environmental indicator. In addition, feed management practices are not adequate, highlighting the low frequency of feeding during the day, excessive amount and low quality of feed offered. These results demonstrate the need for adequate feed management and the need for careful monitoring of effluent.

Keywords: nutrients loads, mass balance, feed conversion ratio, eutrophication, Oncorhynchus mykiss (Walbaum).

Indicadores ambientais na avaliação de efluentes de truta arco-íris (Oncorhynchus mykiss) criados em sistema de raceway através do fósforo e nitrogênio

Resumo

A descarga de fósforo e nitrogênio via efluente do sistema intensivo de truticultura foi quantificada através da utilização de indicadores ambientais. As cargas de nutrientes, o balanço de massa, a quantidade estimada de nutrientes na ração e a quantidade de nutrientes convertidos em biomassa de peixes foram calculados com base nas concentrações de fósforo (P) e nitrogênio (N) na ração e na água. Da ração oferecida, 24,75 kg estavam disponíveis como P e 99,00 kg como N, destes, 9,32 kg de P (38%) e 29,12 kg de N (25%) foram convertidos em biomassa de peixe e 15,43 kg P (62%) e 69,88 kg N (75%) foram exportados via efluente. As cargas e o balanço de massa mostram a descarga excessiva de nutrientes via efluente, corroborado pela taxa de conversão alimentar (2,12:1), devido à baixa eficiência na utilização da ração, portanto, propõe-se a utilização deste parâmetro zootécnico como indicador ambiental. Além disso, as práticas de manejo alimentar não são adequadas, destacando a baixa frequência de alimentação durante o dia, quantidade excessiva e baixa qualidade da alimentação ofertada. Esses resultados demonstram a necessidade de manejo alimentar adequado e de monitoramento do efluente.

Palavras-chave: cargas de nutrientes, balanço de massa, taxa de conversão alimentar, eutrofização, Oncorhynchus mykiss (Walbaum).

1. Introduction

The annual export of nutrients to the environment arising from inland aquaculture in 2010 was approximately one million ton of phosphorus (P) and five million tons

of nitrogen (N) (Bouwman et al., 2013). These residues discharged via effluent, through the culture of aquatic organisms, may present high pollution potential of the

receiving water bodies (Koçer et al., 2013; Henares and Camargo, 2014).

The trout farming, if not well managed, shows potential for excessive discharge of nutrients which has its origin, mainly, in the feed introduced into the system, either directly by dispersing or the metabolic products generated by fish (Amirkolaie, 2011). This potential depends on the size of the production system, the amount of biomass, the nature and volume of water used, management practices and feed quality that is offered to the fish (Boaventura et al., 1997).

The feed is the most important parameter related to the environmental impact (Bureau et al., 2003; Wang et al., 2012), because of the total phosphorus and nitrogen supplied in the diet, about 30% will be present in the cultured biomass of the organisms and the remainder goes to the receiving water body via effluent (Baird et al., 1996), with capacity of causing the eutrophication process. Therefore, there may be greater or smaller losses of these nutrients, important in animal metabolism, to the environment depending on content and form that are in the diet, associated to the quality and amount of feed (Araripe et al., 2006).

Some industries have produced diets with low quality to be sold at lower prices, which can lead to the use of diets with low digestibility and inadequate levels of nutrients (Abimorad et al., 2012). This associated to the excessive feed offered impairs the production (reduced growth rate) and consequently increases the excretion of nutrients into the environment, causing the deterioration of water quality via the receiving water body enrichment by the nitrogenous and phosphate compounds (Noroozrajabi et al., 2013). Therefore, the allocated feed should be proportional to the consumption of organisms and be of high quality.

In countries as Denmark, Norway and the United States of America, where strict environmental regulations governing the operation of trout farming, the phosphorus content in the diet of trout and feed conversion ratio (FCR) cannot exceed 0.9-1.0% and 1.0, respectively (Bergheim and Brinker, 2003; MacMillan et al., 2003). However, in Brazil, there is no legislation that considers the nutrient content in the feed composition as a proposal to reduce the environmental impact. The National Environmental Council, federal collegiate of the Environment Ministry, provides for the classification of water bodies and environmental guidelines for its framework, as well as establishes the conditions and discharge standards of the effluent through the resolutions 357 of 2005 and 430 of 2011, i.e., regulates the limit allowed of nutrient concentrations in effluent discarded in accordance to the framework of the water body (Brasil, 2005, 2011).

Considering this scenario and the fact that the environmental impact of effluent from the aquaculture is an increasing issue of concern around the world, it is clear the importance of the environment conservation and the care that must be taken with the rational use of water resources (Gorlach-Lira et al., 2013; FAO, 2014). Therefore, it is necessary evaluation of the efficiency with which feed, nutrients and water are used in aquaculture. For this evaluation is suggested the application of environmental indicators in aquaculture systems in order to estimate the

environmental performance of the production system, assessing the efficiency with which resources are used (Boyd and Queiroz, 2001; Boyd et al., 2007).

Among the environmental indicators suggested by these authors, this study apply: a) nutrient load (relation between water flow and nutrient concentrations in affluent and effluent of the system); b) mass balance (quantitative description of all materials that enter, leave and accumulate in system with defined borders); c) input of nutrients via feed ($I_{\rm p}$); d) converted nutrients in fish biomass ($N_{\rm p}$).

Thus, the aim of this study was to use the environmental indicators as a method to quantify the discharge of phosphorus and nitrogen via effluent of intensive trout farming system.

2. Material and Methods

2.1. Study area

The present study was performed in intensive trout farming system, located in National Park of Serra da Bocaina, 35 km away from Bananal city, São Paulo State (SP), Brazil, in Hydrographic Basin Paraíba do Sul situated 1,160 meters above sea level (22°50'03.92"S and 44°25'46.33"W). Atlantic forest area covering large part of the springs that provide potable water to the population, located in tropical and subtropical region, in the mountainous regions where temperatures are close to 22 °C in the hottest month. The stream that supplies the trout farming system was classified as class I according to the current legislation (Brasil, 2005) with average flow of 72 L s⁻¹. During the study period, more than 50% of the stream flow was diverted into the fish farm.

2.2. Description of production system and feed management

The system used for the rearing of rainbow trout Oncorhynchus mykiss Walbaum (1792) was raceway characterized by intensive production with high continuous water flow. The trout farming system was populated with 56,000 fish at different stages of development distributed in twelve masonry tanks (a meter deep) and separated into three sectors of production, the first with 40,000 fingerlings (average weight of 2 g), distributed into four tanks 2 m³ (cubic meter) each; the second with 10,000 juveniles (average weight of 20 g), distributed into four tanks of 5 m³ each and the third with 6,000 adults (average weight of 200 g), distributed into four tanks of 19 m³ each (see Figure 1A). In all tanks the feeding was performed by the producer twice or three times daily with extruded ration containing 40% of crude protein (see Table 1), considering the stage of development of individuals and an estimated total biomass (fingerlings - from 5 to 7% of total biomass; juveniles and adults - from 3 to 5% of total biomass).

2.3. Sampling and limnological variables

The analysis were performed from 2 September to 25 November 2010 weekly in triplicate at six points (n = 13). The distribution of sampling points (see Figure 1B) followed the order: S1 = upstream production system; S2 = affluent; S3 = effluent; S4 = outlet after passing by the wetland (area

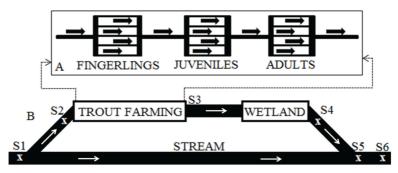


Figure 1. A = Representation of the organized trout farming system in three sectors: fingerlings, juveniles and adults; B = Schematic drawing of the trout farming system located in the Serra da Bocaina (SP), where: S1 = S upstream production system; S2 = S affluent; S3 = S under the receiving water body (stream), 60 meters away from the effluent. Adapted from Caramel et al. (2014).

Table 1. Characteristics of feed offered to fish in trout farming system.

	Assurance levels
Humidity (g kg ⁻¹ of feed)	100.00
Crude protein (g kg ⁻¹ of feed)	400.00
Ethereal extract (g kg ⁻¹ of feed)	120.00
Crude fiber (g kg ⁻¹ of feed)	45.00
Mineral matter (g kg ⁻¹ of feed)	130.00
Calcium (g kg ⁻¹ of feed)	20.00
Phosphorus (g kg ⁻¹ of feed)	10.00
Folic acid (g kg ⁻¹ of feed)	6.00

of 100 m^2 , depth of 2 m and with presence of *Thypha* sp and aquatic grass); S5 = mixing zone; S6 = in the receiving water body (stream), 60 meters away from the effluent.

Water samples were collected in the subsurface water column. Samples for analysis of the totals (phosphorus and nitrogen) were frozen. Samples for analysis of dissolved fraction were immediately filtered in vacuum pump using GFF Whatman filters with porosity of 0.47 μ m and after they were frozen. In the laboratory, the samples were analyzed for concentration of total phosphorus (μ g L⁻¹) and total nitrogen (μ g L⁻¹) according to the methodology described by Valderrama (1981), orthophosphate (μ g L⁻¹) according to the methodology described by Strickland and Parsons (1960) and ammonium (μ g L⁻¹) according to the methodology described by Apha (2005). To calculate the water flow (L s⁻¹) was used the float method proposed by Marques and Argento (1988).

2.4. Environmental indicators

Nutrient loads were determined by the product of the water flow values (L s⁻¹) and the nutrient concentrations (total phosphorus - TP, orthophosphate - PO₄-P, total nitrogen - TN and ammonium - NH₄-N) (μ g L⁻¹) in affluent (L_A) and in effluent (L_E) of trout farming system, through the following Equation 1:

$$L = [] \times W_{F} \tag{1}$$

where L = loads of TP, PO₄-P, TN and NH₄-N (kg d⁻¹), [] = nutrient concentrations (μ g L⁻¹), W_F = water flow (L s⁻¹).

Subsequently, to quantify the amount of waste generated by the activity of trout farming, mass balance was calculated (kg d⁻¹) of TP, PO₄-P, TN e NH₄-N through the nutrient load that leaves via effluent and subtracted from the water supply system, according to the Equation 2:

$$MB_{I} = L_{F} - L_{A} \tag{2}$$

where MBL = mass balance among loads of TP, PO₄-P, TN and NH₄-N (kg d⁻¹), L_E = the considered variable load in the effluent of the system (kg d⁻¹), L_A = the considered variable load in the affluent of the system (kg d⁻¹).

Throughout the study period (three months), at harvesting, the fish were counted and weighed to obtain the total productivity of trout. The feed conversion ratio of the production system was estimated thought the relation between the total of feed offered and the quantity of fish taken in each harvesting.

The nutrient load of production system via feeding was estimated by the amount of phosphorus and nitrogen contained in the total feed offered (Equation 3), of the levels of these nutrients that were converted into fish biomass (Equation 4) and of the lost via effluent (Equation 5) (Boyd and Queiroz, 2001; Boyd et al., 2007). It was assumed that the trout presents 25% (0.25 kg) of dry matter, whereas the dry matter containing 3.2% (0.032 kg) of phosphorus and 10% (0.10 kg) of nitrogen (Boyd and Queiroz, 2001; Boyd et al., 2007). The commercial feeds used in trout farming system contained 1.0% (0.010 kg) of phosphorus and 4.0% (0.040 kg) of nitrogen per kg of feed. For the calculations, it was assumed that 5% of the total feed offered were lost as food not eaten (Bureau et al., 2003). Whereas the production of fish and feed offered, were applied in the present study the following Equations 3 and 4:

$$I_{r} = F_{O} \times N_{r} \tag{3}$$

where $I_F = \text{input of nutrient via feed (kg period}^{-1} \text{ of P or N)},$ $F_O = \text{amount of feed offered (kg period}^{-1}), N_F = \text{amount of nutrient (kg) by kilogram of feed.}$

$$N_{B} = P \times DM \times N_{DM}$$
 (4)

where N_B = converted nutrient in fish biomass (kg of P or N), P = fish production (kg), DM = amount of dry matter by kilogram of fish (kg), N_{DM} = amount of nutrient (P or N) by kilogram of dry matter of fish (kg).

To calculate the amount of TP and TN of feed lost via the effluent in the production system during the study period (three months) was applied the following Equation 5:

$$MB_{F} = I_{F} - N_{R} \tag{5}$$

where $MB_F =$ mass balance between input of nutrients via feed and the amount of the converted nutrients in fish biomass (kg period⁻¹ of P or N), $I_F =$ input of nutrients via feed (kg period⁻¹ of P or N), $N_B =$ converted nutrients in fish biomass (kg of P or N).

2.5. Statistical analysis

The data of nutrients concentrations after log transformation (x+1) were analyzed using the Kruskal-Wallis test followed by the Student-Newman-Keuls (SNK) test to verify the spatial variations (Zar, 2010). The adopted significance level was 0.05.

3. Results

The trout farming studied used raceway system, receiving constant flow of water with average water flow of 38.26 L s⁻¹, maintaining residence time around 1 h 40 min throughout the system.

The total phosphorus (H = 154.67; P < 0.001), orthophosphate (H = 127.47; P < 0.001), total nitrogen

(H = 20.79; P = 0.001) and ammonium (H = 57.33; P < 0.001) demonstrated spatial variations through SNK test (see Figure 2).

Has occurred the formation of two distinct patterns [S1 and S2] and [S3, S4, S5 and S6] (p <0.05) related to the nutrients. After passage through the production system was increased from the upstream of production system (S1) to effluent of system (S3), without retaining effect on wetland (S4), with small dilution in concentrations when the effluent reached the receiving water body (S5 and S6).

When the nutrient loads were calculated, stood out the amount of total phosphorus, orthophosphate, total nitrogen and ammonium in effluent of the system (see Figure 3). The mass balance calculation showed that trout production exported in average 8.27 kg TP, 5.74 kg PO₄-P, 36.95 kg TN and 28.11 kg NH₄-N during the study period (3 months).

During the study period were offered 2,475 kg of feed with a production of 1,165 kg of fish. The average feed conversion of the system was 2.12:1. Of the offered feed during the study period, 24.75 kg (100%) were available as P and of these, 9.32 kg P (38%) were converted into fish biomass and 15.43 kg P (62%) were exported via effluent, being 1.24 kg P (5%) in non-feed intake and 14.19 kg P (57%) arising from the fish metabolism (see Figure 4a). Related to TN (see Figure 4b), 99.00 kg N (100%) were offered via feed throughout of study period, of these, 29.12 kg N (25%) were converted into fish biomass and 69.88 kg N (75%) were exported via effluent, being 5.94 kg N (5%) in non-feed intake and 63.94 kg N (70%) arising from the fish metabolism.

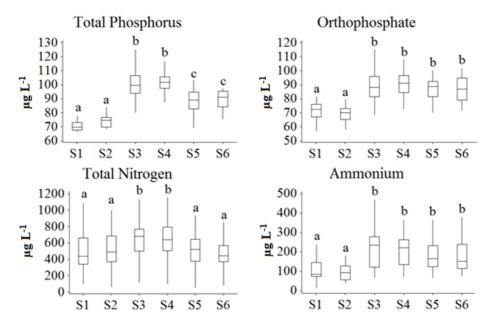


Figure 2. Nutrients analyzed during the study period in trout farming system in samplings sites S1 = upstream production system; S2 = affluent; S3 = effluent; S4 = outlet after passing by the wetland; S5 = mixing zone; S6 = in the receiving water body (stream), 60 meters away from the effluent, represented by Box-Whiskers Plot where the lower and upper limits of the Box correspond to the 25 and 75 percentiles, the whiskers correspond to the minimum and maximum, and the center line of distribution correspond to the median. Medians followed by the same letter do not differ by SNK test (0.05).

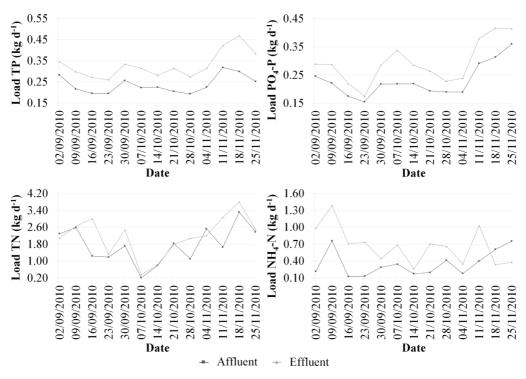


Figure 3. Nutrient loads, total phosphorus (TP), orthophosphate (PO₄-P), total nitrogen (TN) and ammonium (NH₄-N) (kg d⁻¹), in affluent (black) and effluent (gray) of the trout farming system throughout the study period.

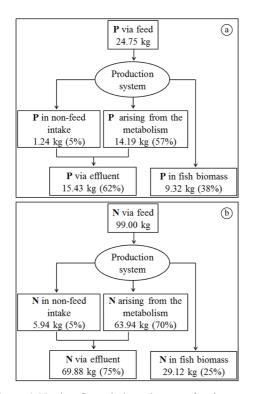


Figure 4. Nutrient fluxes in intensive trout farming system during the study period (three months) (kg period⁻¹): a = total phosphorus (TP); b = total nitrogen (TN). Adapted from Wang et al. (2012).

4. Discussion

Although the studied system is considered small due to the availability of water (production up to twenty tons of trout per year), this is the profile of 70% of intensive trout farming systems located in the Southeast and Southern of Brazil.

The increments of the nutrients after passage through the production system (S3) can be related to the feeding. On studies performed in tilapia farming (Araripe et al., 2006), in trout farming (Koçer et al., 2013; Amirkolaie, 2011) and in bullfrog farming (Mercante et al., 2014), the authors attributed the amount of nutrients to the wasted food and/or not used, use of unbalanced diet and fish metabolism.

After passing through the wetland (S4) there was no reduction in the concentrations of nutrients, indicating that the wetland was not efficient, which can be associated with the absence of management in this site. Among the different ways to improve this treatment system must consider its proportional scale to the production area and the residence time of the water (Caramel et al., 2014).

The trout farming's contribution related to nutrients concentrations on the receiving water body (S5 and S6) compared to upstream production system (S1) showed that the distance of 60 m was not enough to occur recovery of the receiving water body. This result might be related to the fact that more than half of the stream volume was directed to supply the trout farming system and that may have changed the stream metabolism.

Through the mass balance calculation the trout farming was characterized as an export system of nutrients due to high loads of nutrients in the effluent resulting from the relation between high water flow and higher nutrient concentration in the effluent of system. This relation of water flow in determining the loads of nutrients is more evident when comparing different production systems. The average concentration of phosphorus in effluent of the study trout farming (100.73 $\mu g \ L^{-1}$), when compared to the concentration of TP in effluent of bullfrog farming system (6,090.00 $\mu g \ L^{-1}$) (Borges et al., 2012) was lower, however, the trout farming showed the highest load of TP via effluent (0.33 kg TP d-1) due to the greater water flow (38.26 L s-1) when compared to the bullfrog farming (load of 0.01 kg TP d-1 and water flow of 0.02 L s-1).

In Brazil, studies performed in bullfrog farming (Mercante et al., 2014; Borges et al., 2012) and in polyculture system (tilapia and shrimps) (Araújo-Silva et al., 2014), showed that quantification of loads can be a useful tool for characterizing the impact, since it contemplates the flow and not only nutrient concentrations. This allows the comparison of different systems, methods of creation and evaluation of the effectiveness of management practices used in the production system (Boyd et al., 2007).

Regarding the P retained in the trout biomass (38%) (Figure 4a), the value was higher than those obtained by Wang et al. (2012) in salmon farming industry in Norway (30%) and Bartoli et al. (2007) in trout farming system in Italy (19%). Related to the N retained in trout biomass (25%) (Figure 4b), the values were lower than those obtained by Wang et al. (2012) (38%), Bartoli et al. (2007) (49%) and Noròi et al. (2011) in trout farming in the Faroe Islands (32%). These results can be explained by the difference in the composition of diets, feed management (quantity and quality of feed offered, time and feeding frequency throughout the day) and fish metabolism (Bureau et al., 2003; Coloso et al., 2001; Tello et al., 2010).

The amount of P and N exported via effluent (62% and 75%, respectively) indicates that the largest amount of the nutrients that entered via feed were discarded in the aquatic environment, because for each kilogram of feed offered, 6.23 g TP and 28.23 g TN were discharged via effluent. The production of waste arising from the aquaculture may be reduced based on nutritional strategies, as in the formulation of nutrients and the development of efficient feeding systems based on energy requirements of the species (Bouwman et al., 2013).

Such estimates can be corroborated by the result of feed conversion (2.12:1) that demonstrated excessive discharge of nutrients via effluent related to the leftover of the feed offered and to the low retention in fish biomass. The reduction of feed conversion ratio from 2.1:1 (similar to the present study) to 1.7:1 can lead to a 20% reduction in waste from aquaculture (Mungkung et al., 2013), factor related to environmental issues, since it is related to fish metabolism (absorption and excretion of nutrients), interfering with the concentrations of organic matter (Cyrino et al., 2010). Therefore, if the feed conversion of trout farming was 1.7:1,

the system would export 4.98 g TP and 22.58 g TN per kilogram of feed instead of 6.23 g TP and 28.23 g TN per kilogram of feed. From these results it is proposed from this study using the FCR, besides of production indicator, include it as an environmental indicator.

The loads and the mass balance show the excessive discharge of nutrients via effluent, corroborated by the feed conversion ratio (2.12:1) due to the low efficiency of feed utilization, therefore, it is proposed the use of this zootechnical parameter as environmental indicator. In addition, feed management practices are not adequate, highlighting the low frequency of feeding during the day, excessive amount and low quality of feed offered. Therefore, in order to reduce this excessive discharge in the receiving water body are recommended adjustments in management, as improvement in phosphorus and nitrogen assimilation considering the fish stage of development, amount control of the feed offered, use of appropriate stocking densities and effluent treatment.

Acknowledgements

We thank to Dr. Marcos Guilherme Rigolino, Scientific Researcher at the Experimental Station of Trout Farming, *in memoriam*, by participation in the analysis of results, to CAPES (Coordination of Higher Education Personnel Training) by the Masters scholarship, to CNPq by the research productivity scholarship to André Martins Vaz dos Santos (305403/2015-0) and to FAPESP (Research Support Foundation in São Paulo) by financial support (2010/07658-3).

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