

## Nutrient load estimation in the waste of Nile tilapia *Oreochromis niloticus* (L.) reared in cages in tropical climate conditions

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### Abstract

We sought to estimate the nutrient load in the waste released into aquatic environments based on the feeding of Nile tilapia (*Oreochromis niloticus*, L.) reared in cages that were installed in artificial reservoirs. For the calculation, an analysis of the chemical composition of commercial feeds intended for this species in their various stages of production was conducted ( $N = 130$ ). We combined this information with a meta-analysis of published data from commercial producers in Brazil about expected feed intake, feed conversion and other animal production indices, and body composition. With these data, it was possible to estimate the load. We estimated that 18% of the feed given to the animals is not consumed and is lost in the aquatic environment. The calculated average digestibility was 71.97% for the organic matter in the diet, 84.06% for protein and 54.40% for phosphorus. The estimated nutrient deposition efficiency, with respect to what was actually consumed by the tilapia, was 26.39% for organic matter, 43.25% for protein and 34.07% for phosphorus. The total nutrient load in the waste per tonne of biomass of produced tilapias was estimated to be 1040.63 kg of organic matter, 44.95 kg of nitrogen and 14.26 kg of phosphorus, representing 78%, 65% and 72% of the respective nutrient amounts supplied by the feed. The information obtained in this study serves as a reference for predicting the potential impact of tilapia farming in reservoirs and to establish scientific parameters for the planning of this activity.

**Keywords:** digestibility, phosphorus, organic matter, nitrogen, balance

### Introduction

It is no longer possible to promote the development of commercial aquaculture projects without considering the relationship between the economic benefits, the animal production parameters associated with aquaculture ventures and the environmental costs involved. It should also be recognized that the relationships established between the several productive functions and the environment are not simply economic but also technological, environmental, biological and social (FAO 2011). However, many countries are unable to promote aquaculture in a sustainable manner. The environmental laws are either so strict that they prevent the development of aquaculture or so mild or poorly enforced that it is not possible to avoid the eventual adverse effects of aquaculture on environmental and ecosystem services and assets (El-Gayar & Leung 2000).

One of the problems that legislation seeks to mitigate is eutrophication, which is one of the main ways that humans alter aquatic environments (Salas & Martino 1991). Anthropogenic eutrophication is mainly caused by the discharges of domestic, agricultural and industrial effluents in bodies of water, by nutrient leaching caused by agricultural activities, and by the decomposition of organic matter from vegetation (Henry & Tundisi 1983). The intake of nutrients associated with these effluents and waste in the water bodies alters the productivity of the aquatic ecosystems

by creating conditions for the uncontrolled growth of photosynthetic microorganisms (Harper 1992; Smith, Tilman & Nokolac 1999). Such growth increases the photosynthesis and respiration rates, which in turn may cause great variations in pH and in the dissolved oxygen concentrations in the water. These effects can eventually cause major ecosystem changes and can even lead to death of the aquatic fauna (Conley, Paerl, Howarth, Boesch, Seitzinger, Havens, Lancelot & Likens 2009).

Nutrients, particularly nitrogen and phosphorus, are directly related to the eutrophication of waters. These nutrients are present in the main biological cycles, they are components of plants, animals and bacteria, and they are essential elements for organismal development. Consequently, they are also abundant in the feed used for aquaculture (Von Sperling & Chernicharo 2005).

Every ecosystem has a maximum assimilative capacity, which is determined by the maximum acceptable environmental impacts, according to previously defined technical, legal and scientific criteria, and by the regeneration capacity of the ecosystem itself (Samuel-Fitwia, Wuertza, Schroeder & Schulza 2012). Therefore, it is important to correctly estimate the loads of the nutrients that are deposited during the production of fish in cages and pens and to determine the ecological carrying capacity of the locations where aquaculture ventures will be implemented, especially in freshwater reservoirs (Byron & Costa-Pierce 2010).

Based only on the knowledge of real anthropic impacts on water bodies, it is possible to properly plan the occupation and use of these public spaces and to organize specific actions for the control and mitigation of the impacts (Koudstaal, Rijsberman & Savenije 1992). However, there is a dearth of studies and data in the literature regarding estimates of loads deposited by commercial feeds, especially for the main model of tropical fish farming used in Brazil, which involves the farming of tilapia in small-volume cages installed in large public reservoirs. Fish farms of this nature are spread across the country (Sarà 2007).

This study aims to estimate the nutrient loads released into aquatic ecosystems based on the production of Nile tilapia in cages installed in artificial reservoirs, using the techniques and conditions of the type of commercial production practised in Brazil.

## Materials and methods

### Nutritional content and digestibility of commercial feeds

Data were collected on the composition of all commercial tilapia feeds with active registrations at the Ministry of Agriculture, Livestock, and Supply (MAPA) in the state of Paraná, Brazil. For each brand of feed selected for the study, it was necessary that the records included its indication as feed for tilapia and indicated the production phase for which it was intended (fry, initial stage, growth stage or termination stage). Information about the chemical composition of ingredients was collected from the feed records. These compositions were recorded in appropriate software for the calculation of nutritional content (Optimix, version 4.1, Domit Ltd.).

The nutritional content and digestibility rates for several nutrient fractions of the selected feeds were calculated according to Furuya (2010), NRC (2011), and Rostagno (2011). Based on the digestibility rates and the calculated nutritional content, it was possible to determine the fraction of indigestible nutrients, which, in theory, would make up the excreta of animals that consume the feed.

### Tilapia production performance

The performance indices for each stage of caged tilapia production were determined from (a) a systematic review and subsequent meta-analysis of data available in the literature, according to the methodologies presented by Lovatto, Lehnem, Andretta, Carvalho and Hauschild (2007) and Sampaio and Mancini (2007) and (b) field data collection.

For the meta-analysis, we selected original scientific articles published between 2002 and 2012 in indexed journals with 'ad hoc' evaluation showing the indices of tilapia production in pens and cages in artificial reservoirs under tropical climate conditions. The search was performed in academic article search portals, using the keywords 'production', '*Oreochromis niloticus*', and 'cages', considering only data from papers that used harvest weight between 600 and 900 g. Using this methodology, 73 scientific articles were selected as sources.

We also collected results and technical information relating to the management practices for

commercial caged tilapia production in artificial reservoirs in the Paranapanema River channel, which is at the border of the states of Paraná and São Paulo in southern Brazil. This information was collected to compare, evaluate and validate the data observed in the literature. The secondary data obtained in the literature were added to the data obtained from commercial farms, and these combined data served as the basis for the calculation of pollutant load estimates.

### Body composition

The body composition of the animals was determined from the processing and analysis of 10 sexually reversed tilapia that were grown under commercial conditions and weighed between 600 and 900 g (similar to the final weight of the commercial fish in the region). The animals were removed alive from the growth site, anaesthetized, placed in a container with ice and transported to the laboratory. The full bodies (including viscera, blood, skin and scales) were homogenized, and the body composition was evaluated by proximate chemical analyses (moisture, protein, fat, ash, calcium and phosphorus), according to the Association of Official Analytical Chemists (AOAC 2012) by the Animal Nutrition Laboratory of the Federal University of Paraná (UFPR).

The body composition of Nile tilapia grown in cages in tropical regions was also investigated. We considered data for fish with a slaughter weight similar to the final average weight found by a literature search of animal performance data (600–900 g). These data made it possible to calculate nutrient retention in animals raised under the aforementioned conditions. The search was performed in academic scientific article portals, using the keywords ‘body composition’, ‘*Oreochromis niloticus*’ and ‘cages’. Using this methodology, 26 scientific articles were selected for the meta-analysis.

### Nutrient loads in waste

According to the methodology described by Dosdat (2001), Fernandes, Lauer, Cheshire and Angove (2007), and Azevedo, Podemski, Hesslein, Kasian, Findlay and Bureau (2011), the nutrient load present in the waste from the production of Nile tilapia grown in cages was estimated based on three contributions: (1) feeding losses, (2) the

indigestible fraction of the diet and (3) soluble excreta. The first fraction represents the amount of the feed given to the animals but not consumed, which was directly lost to the environment. The nutritional composition of this component is the same as the composition of the feed supplied. The second contribution comes from the consumed nutrients that were not absorbed by the animals due to the limits of dietary utilization. The last contribution considers the excretion of non-deposited digestible nutrients, i.e., nutrients that were effectively absorbed by the tilapia but were not retained as body tissues and were therefore excreted after being metabolized.

The contribution of nutrients present in the waste from losses generated during feeding was estimated from the difference between the results obtained in the literature for the apparent feed conversion (relationship between the feed input and biomass gain) and the true feed conversion (relationship between the actual feed intake and biomass gain) for each production stage. The difference between these indices enables the relatively accurate calculation of the amount of feed that was given to the animals but not consumed. The amount was estimated as the sum of the amount of feed lost during feeding for each stage, and it was multiplied by the chemical composition of the respective feed.

$$FL_n = \left\{ \sum_S^{Fry \rightarrow Ter} [(FP_S - FC_S) \cdot C_{tot}^n F_S] \right\}$$

$FL_n$  → Amount of nutrient ‘n’ per feeding loss, in grams of nutrient ‘n’ per kg of produced tilapia biomass;  $S$  → Production stage (Fry, Juvenile, Growth and Termination);  $FP_S$  → Amount of feed provided in production stage ‘S’, in grams of feed per kg of produced tilapia biomass;  $FC_S$  → Amount of feed consumed by the animals in production stage ‘S’, in grams of feed per kg of produced tilapia biomass;  $C_{tot}^n F_S$  → Total content of nutrient ‘n’ in the feed intended for stage ‘S’, in grams of nutrient ‘n’ per kg of feed.

The input of nutrients in the waste coming from the indigestible fraction was estimated from the sum of the chemical components of the faeces for each production stage, which was considered relative to the intake in the respective stages. This faecal composition, in turn, was calculated from the difference between the total amount of each

nutrient present in the diet and the digestible amount of these respective nutrients.

$$FC_n = \left\{ \sum_S^{Fry \rightarrow Ter} [(C_{tot}^n F_S - C_{dig}^n F_S) \cdot FC_S] \right\}$$

$FC_n \rightarrow$  Amount of nutrient 'n' in faeces composition, in grams of nutrient 'n' per kg of produced tilapia biomass;  $S \rightarrow$  Production stage (Fry, Juvenile, Growth and Termination);  $C_{tot}^n F_S \rightarrow$  Total content of nutrient 'n' in the feed intended for stage 'S', in grams of nutrient 'n' per kg of feed;  $C_{dig}^n F_S \rightarrow$  Digestible fraction of nutrient 'n' in the feed intended for stage 'S', in grams of nutrient 'n' per kg of feed;  $FC_S \rightarrow$  Amount of feed consumed by the animals in production stage 'S', in grams of feed per kg of tilapia biomass.

The fraction of soluble excretion was estimated based on the difference between the actual intake of each nutrient and the nutrient body composition. The nutrients absorbed and not deposited in the animal bodies were considered to be deposited in the environment as urine or as endogenous losses by tissue scaling and other excretions (mucus, enzymes, etc.).

$$SE_n = \left\{ \left[ \sum_S^{Fry \rightarrow Ter} (FC_S \cdot C_{dig}^n F_S) \right] - C_n C \right\}$$

$SE_n \rightarrow$  Amount of nutrient 'n' in soluble excretions and endogenous losses, in grams of nutrient 'n' per kg of produced tilapia biomass;  $S \rightarrow$  Production stage (Fry, Juvenile, Growth, and Termination);  $FC_S \rightarrow$  Amount of feed consumed by the animals in production stage 'S', in grams of feed per kg of tilapia biomass;  $C_{dig}^n F_S \rightarrow$  Digestible fraction of nutrient 'n' in the feed intended for stage 'n', in grams of nutrient 'n' per kg of feed;  $C_n C \rightarrow$  Content of nutrient 'n' in the entire tilapia body, in grams of nutrient 'n' per kg of tilapia biomass.

The total nutrient load in the waste from tilapia production deposited in the environment was calculated as the sum of the three fractions described above.

$$TLW_n = (FL_n + FC_n + SE_n)$$

$TLW_n \rightarrow$  Amount of nutrient 'n' deposited into the environment by the waste from tilapia production,

in grams of nutrient 'n' per kg of produced tilapia biomass;  $FL_n \rightarrow$  Amount of nutrient 'n' lost during feeding, in grams of nutrient 'n' per kg of produced tilapia biomass;  $FC_n \rightarrow$  Amount of nutrient 'n' in faeces, in grams of nutrient 'n' per kg of produced tilapia biomass;  $SE_n \rightarrow$  Amount of nutrient 'n' lost in soluble excretions and other endogenous losses, in grams of nutrient 'n' per kg of produced tilapia biomass.

### Data treatment and statistical analyses

To obtain an idea of the variability in the results, we calculated confidence intervals for the data. We determined the lower and upper limits of the confidence intervals ( $\alpha = 90\%$ ) for the indices and coefficients used in the estimate calculations. In the end, we obtained results that fell within a range that represents the most probable conditions of the contribution of waste in the aquatic environment.

Descriptive statistics for the chemical compositions of the commercial feeds and respective excreta, as well as the animal production parameters from the meta-analysis, were analysed using Statistica (version 8.0, Statsoft Inc.).

## Results

### Nutritional content and digestibility of commercial feeds

Table 1 shows the calculated nutritional compositions for the tilapia feeds registered at MAPA in the Paraná state. Altogether, 130 feeds met the methodological requirements to be used for the analysis, and these feeds were proportionally distributed among the four production stages (fry:  $N = 32$ ; juvenile:  $N = 30$ ; growth:  $N = 38$ ; termination:  $N = 30$ ).

Table 2 shows the fractions of indigestible nutrients in the analysed feeds. Among the evaluated nutrients, the one that showed the lowest average digestibility was phosphorus, at 54.41%. Protein was the nutrient with the highest digestibility, 84.06%.

### Tilapia production performance

Table 3 shows the results of the meta-analysis of performance indices for the production of tilapia in cages. The average initial weight of the fry was

**Table 1** Nutritional content calculated for the feeds intended for Nile tilapia (*Oreochromis niloticus*, L.) farming with active records in the Ministry of Agriculture, Livestock and Supply of the state of Paraná (N = 130)

Nutrients	Fry (n = 32)	Juvenile (n = 30)	Growth (n = 38)	Termination (n = 30)
Total dry matter (g/kg)	906.04 (±7.67)	904.72 (±6.36)	902.26 (±6.75)	902.18 (±7.16)
Digestible dry matter (g/kg)	628.65 (±32.49)	629.12 (±45.67)	608.50 (±37.79)	611.17 (±44.21)
Total organic matter (g/kg)	777.65 (±34.05)	790.42 (±22.24)	806.82 (±22.53)	803.59 (±27.32)
Digestible organic matter (g/kg)	577.32 (±41.88)	585.58 (±33.03)	578.92 (±35.81)	576.35 (±39.92)
Crude energy (kcal/kg)	4157.12 (±207.80)	4105.03 (±150.14)	4133.61 (±148.92)	4105.71 (±152.66)
Digestible energy (kcal/kg)	3092.57 (±280.90)	3048.40 (±237.85)	3017.11 (±220.42)	3051.20 (±241.44)
Crude protein (g/kg)	399.60 (±47.82)	352.28 (±49.79)	276.68 (±36.80)	240.78 (±38.00)
Digestible protein (g/kg)	346.96 (±34.27)	310.23 (±46.46)	236.76 (±32.26)	207.08 (±36.45)
Crude fat (g/kg)	54.82 (±19.37)	54.52 (±16.15)	47.31 (±13.63)	50.12 (±16.84)
Non-nitrogen compounds (g/kg)	289.77 (±75.37)	328.77 (±71.06)	439.98 (±54.67)	470.46 (±52.16)
Crude fibre (g/kg)	33.46 (±10.61)	39.45 (±14.14)	42.84 (±11.61)	42.24 (±10.68)
Ash (g/kg)	128.39 (±39.59)	114.30 (±25.64)	95.44 (±26.51)	98.59 (±31.29)
Total calcium (g/kg)	27.39 (±11.55)	24.53 (±8.42)	18.49 (±7.39)	19.61 (±10.47)
Digestible calcium (g/kg)	19.57 (±8.32)	16.80 (±6.11)	13.25 (±5.50)	14.53 (±9.37)
Total phosphorus (g/kg)	15.59 (±5.83)	15.03 (±4.28)	11.74 (±3.84)	11.48 (±4.06)
Digestible phosphorus (g/kg)	9.68 (±4.00)	8.93 (±2.83)	6.45 (±2.29)	6.09 (±2.39)

**Table 2** Indigestible contents calculated for the feeds intended for the farming of Nile tilapia (*Oreochromis niloticus*, L.) with active records in the Ministry of Agriculture, Livestock, and Supply of the state of Paraná (N = 130)

Indigestible fraction	Fry (n = 32)	Juvenile (n = 30)	Growth (n = 38)	Termination (n = 30)
Dry matter (g/kg)	277.39 (±32.42)	275.60 (±43.92)	293.76 (±37.81)	291.01 (±42.86)
Organic matter (g/kg)	200.33 (±29.33)	204.84 (±27.39)	227.90 (±24.30)	227.24 (±28.50)
Nitrogen (g/kg)	8.42 (±4.10)	6.73 (±1.90)	5.91 (±2.37)	6.79 (±1.91)
Calcium (g/kg)	7.82 (±4.28)	7.74 (±3.18)	5.24 (±2.59)	5.08 (±2.51)
Phosphorus (g/kg)	5.90 (±2.09)	6.10 (±1.63)	5.29 (±1.62)	5.39 (±1.75)

**Table 3** Performance results of Nile tilapia (*Oreochromis niloticus*, L.) reared in cages, obtained from meta-analysis (n = 73), and results from the commercial production in the artificial reservoirs of the Paranapanema River\* (n = 17)

Animal production index	Fry	Juveniles	Growth	Termination
Initial average weight (g)	8.65 (±4.01)	49.92 (±13.37)	117.51 (±41.69)	284.93 (±67.45)
Final average weight (g)	49.92 (±13.37)	117.51 (±41.69)	284.93 (±67.45)	791.36 (±95.18)
Apparent feed conversion (g:g)	1.47 (±0.23)	1.25 (±0.13)	1.59 (±0.17)	1.78 (±0.18)
True feed conversion (g:g)	1.00 (±0.15)	1.03 (±0.12)	1.31 (±0.17)	1.48 (±0.17)
Weight gain (g)	41.27 (±12.86)	67.59 (±40.28)	167.41 (±76.63)	506.43 (±105.38)
Feed input (g)	61.23 (±23.44)	83.06 (±47.60)	265.62 (±117.86)	901.11 (±199.36)
Real feed intake (g)	41.70 (±15.52)	69.91 (±43.27)	217.93 (±97.90)	748.31 (±178.55)
Uneaten feed (g)	19.53 (±12.16)	13.15 (±7.99)	47.69 (±35.59)	152.81 (±98.69)
Feeding losses (%)	30.82 (±11.76)	17.01 (±8.90)	17.64 (±9.18)	16.70 (±8.78)

\*Located on the border between the states of Paraná and São Paulo, southern Brazil.

8.65 g (±4.01), and the average slaughter weight was 791.36 g (±95.18). For the total production time, averages of 1.639 (±0.305) for apparent feed conversion and 1.345 (±0.272) for true feed conversion were obtained.

Feeding losses were 30.9% for fry, 17.0% for juveniles, 17.6% for growing and 16.7% at the

termination stage, with a final weighted average of 18.0%.

### Body composition

The chemical composition of the tilapia bodies and accompanying meta-analysis are shown in



Table 4. Between the methodologies used to estimate body composition (analytical and meta-analysis), significant differences were found for the ash content (21% difference between methods), crude protein and nitrogen (25% difference), calcium (41% difference) and phosphorus (39% difference). However, the average variability in the analyses conducted in the laboratory was lower ( $P < 0.05$ ) than the variability in the results obtained from the literature.

At the end of production, the estimated nutrient deposition efficiency, relative to what was actually consumed by the tilapia, was 26.39% for organic matter, 43.25% for protein, 36.96% for calcium and 34.07% for phosphorus. Considering the supplied feed, the estimates decreased to 21.70%, 35.41%, 30.30% and 27.94% respectively.

#### Nutrient load in waste

Tables 5, 6 and 7 show the estimates of nutrient load in waste (uneaten feed, faeces, and soluble excreta respectively) from tilapia production in cages. Pollution loads were also estimated for extreme scenarios (best and worst).

Table 8 shows the estimated nutrient balance between the portion that is released during tilapia production through feeding and the portion that ends up not being incorporated into the biomass produced and moving into the environment in the form of various wastes. We estimated that for every tonne of final tilapia biomass produced, a nutrient load of 1040.63 kg of organic matter, 44.95 kg of nitrogen and 14.26 kg of phosphorus is deposited into the environment, with an organic matter:

**Table 4** Meta-analysis ( $n = 26$ ) and laboratory analysis ( $n = 10$ ) of body composition of Nile tilapia (*Oreochromis niloticus*, L.) reared in cages, with live weight between 600 and 900 g

Content (g/kg, original matter)	Meta-analysis	Laboratory
Moisture	693.61 ( $\pm 38.75$ )	678.32 ( $\pm 26.57$ )
Ash*	40. ( $\pm 13.18$ )	33.32 ( $\pm 2.60$ )
Organic matter	305.82 ( $\pm 51.81$ )	288.36 ( $\pm 29.16$ )
Crude protein*	192.06 ( $\pm 31.17$ )	153.98 ( $\pm 6.06$ )
Nitrogen*	30.73 ( $\pm 4.99$ )	24.64 ( $\pm 0.97$ )
Calcium*	5.93 ( $\pm 0.19$ )	10.07 ( $\pm 1.23$ )
Phosphorus*	3.37 ( $\pm 0.10$ )	5.53 ( $\pm 0.71$ )

\*Marked contents showed significant differences ( $P < 0.05$ ) by Student's *t*-test between the two methods used.

**Table 5** Estimates of nutrient loads from the uneaten feed fraction of Nile tilapia (*Oreochromis niloticus*, L.). Mean values and 90% confidence intervals are given

Nutrients	Mean	Mean – CI (90%)*	Mean + CI (90%)
Dry matter (g)	265.97	240.33	293.31
Organic matter (g)	236.12	211.71	262.36
Nitrogen (g)	12.62	10.85	15.58
Calcium (g)	5.99	4.60	7.57
Phosphorus (g)	3.56	2.87	4.34

\*Confidence interval (CI), calculated with alpha of 0.10.

**Table 6** Estimates of nutrient loads from the faeces of Nile tilapia (*Oreochromis niloticus*, L.), formed by the indigestible fraction of the diet. Mean values and 90% confidence intervals are given

Nutrients	Mean	Mean – CI (90%)*	Mean + CI (90%)
Dry matter	395.04	333.51	463.58
Organic matter	306.29	260.42	357.11
Nitrogen	9.08	7.27	11.17
Calcium	7.34	5.48	9.54
Phosphorus	7.40	5.89	9.14

\*Confidence interval (CI), calculated with alpha of 0.10.

**Table 7** Estimates of soluble excretion of metabolized nutrients, per 1000 g of Nile tilapia (*Oreochromis niloticus*, L.) biomass

Nutrients	Mean	Mean – CI (90%)*	Mean + CI (90%)
Dry matter (g)	512.53	482.60	724.60
Organic matter (g)	498.22	404.59	597.49
Nitrogen (g)	23.25	15.13	32.17
Calcium (g)	9.83	3.99	16.49
Phosphorus (g)	3.30	0.87	6.04

\*Confidence interval (CI), calculated with alpha of 0.10.

nitrogen:phosphorus ratio of 72.98:3.15:1.00 in these loads. Of the total amount of nutrients deposited into the production system, 78% of organic matter, 65% of protein and 72% of phosphorus are not used by the tilapia (Figure 1).

#### Discussion

Brazil has extremely favourable conditions for fish farming in cages. There are more than five million hectares of freshwater in natural and artificial

**Table 8** Average estimate of nutrient balance (organic matter, nitrogen, and phosphorus) and total load in waste, per 1000 g of Nile tilapia (*Oreochromis niloticus*, L.) biomass

Nutrient balance (g)	Organic matter	Nitrogen	Phosphorus
Feed input	1328.99 (100%)*	69.59 (100%)	19.79 (100%)
Feeding losses	236.12 (18%)	12.62 (18%)	3.56 (18%)
Feed intake	1092.87 (82%)	56.97 (82%)	16.23 (82%)
Body deposition	288.36 (22%)	24.64 (35%)	5.53 (28%)
Indigestible fraction	306.29 (23%)	9.08 (13%)	7.40 (37%)
Soluble excretion	498.22 (37%)	23.25 (33%)	3.30 (17%)
Total waste load	1040.63 (78%)	44.95 (65%)	14.26 (72%)

\*Percentage in parentheses represents the share of each fraction, in relation to the input by feed input.

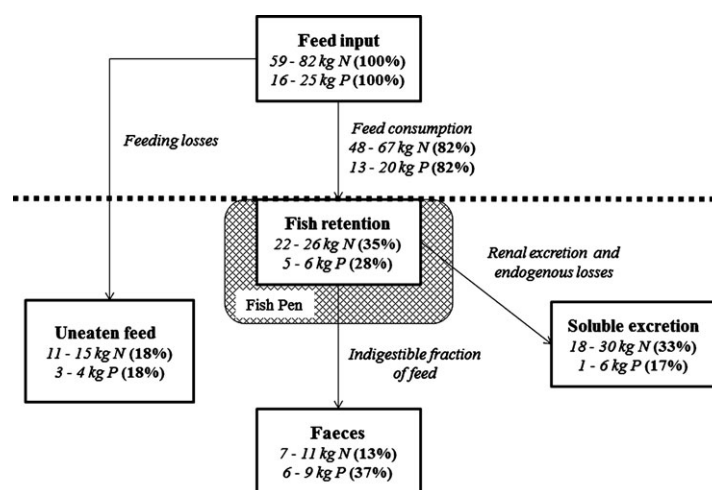
reservoirs that can be used for the production of aquatic organisms (Bueno, Marengoni, Gonçalves, Boscolo & Teixeira 2008). Production in cages and pens has become an important part of commercial fish production in Brazil, and the number of enterprises installed in hydroelectric reservoirs has been continuously increasing in recent years (Nunes 2012). If 1% of this area was used for the intensive production of fish with an average density of 100 kg.m<sup>-3</sup> and a 1.5-year cycle, there would be a potential production of more than 40 million tonnes per year. This value would make Brazil one of the largest aquaculture producers in the world, according to the Food and Agriculture Organization (FAO 2012).

Artificial reservoirs are environments with special hydrography and hydrology; these reservoirs are very different from the rivers that comprise them and very different from natural lakes (Simons 1980). According to Ahipathy and Puttaiah (2006), evaluating the characteristics of reservoirs

helps to understand their ability to receive pollutants, especially the discharges from industrial and domestic effluents, and to understand their capacity for self-purification and the transformation of the water body (Van Rijn 2013).

The dispersion of pollutants in these environments is directly related to hydrological features of the reservoir itself, such as current speeds, pollutant sources and loads and external factors, such as rainfall, winds and input from other water bodies (Wetzel 2001). Other factors have also been observed by several authors, such as soil type, climate and physical-chemical characteristics of the water resources, as well as the availability of light, the temperature and the micro-organisms that affect biological and photochemical reactions in the reservoir (Barbosa, Bicudo & Huszar 1995; Miller, Adamson & Hirst 2001; Ouyang, Nkedi-Kizza, Wu, Shinde & Huang 2006; Dellamano-Oliveira, Vieira, Rocha, Colombo & Sant'Anna 2008; Melo, Moreira & Bisinoti 2009).

**Figure 1** Estimation of environmental flow of nitrogen (N) and phosphorus (P) from the feeding of Nile tilapia (*Oreochromis niloticus*, L.) produced in cages. The values correspond to the confidence intervals of N and P load per 1000 kg of biomass produced.



Although it is not considered the most impactful activity in the aquatic environment compared to the pollution caused by agriculture, industry and especially domestic effluents, aquaculture can be a contributor to eutrophication (Beveridge & Phillips 1993; Paéz-Osuna, Guerrero-Galván & Ruiz-Fernández 1999). The impact from the loads generated by fish farming on the receiving water bodies depends on the differences in concentration of the quality parameters between the area where the aquaculture sites are located and the area of the receiving water body (Boaventura, Pedro, Coimbra & Lencastre 1997). Thus, the polluting potential of a certain production system can vary according to the site where the developments are implemented (Boyd & Tucker 1998).

Defining the amount of fish that can be farmed in a particular environment depends on the characteristics of each receiving water body. The environment needs be able to degrade and assimilate the pollutant load without suffering significant changes (Zhou, Jiang, Zhu, Wang, Hu, Cheng & Xie 2011), and in this case, the local currents are also an important pollutant dispersion factor.

A study conducted in a Chinese artificial reservoir revealed that the production units interfere with the environment at a distance of up to 50 m and that there is a decrease in the biological diversity of planktonic and benthic organisms, with a strong negative correlation ( $r = 0.936$ ) between the distance from the farms and the phytoplankton biomass (Guo & Li 2003). Buschmann, Riquelme, Hernández-González, Varela, Jiménez, Henríquez, Vergara, Guíñez and Filún (2006) suggested that the waste generated by fish farming conducted in cages could only disperse over an area of approximately 1.5–two times the area of the production units themselves.

Recent studies using stable isotope analyses have shown that in faster water current conditions, the waste can be dispersed over areas and distances much greater than those previously suggested (Sarà, Scilipoti, Mazzola & Modica 2004). Other studies have shown that sediment erosion does not occur in locations with a free-flow speed of less than 9 cm/s at the river bottom (Mitchener & Torfs 1996; Canal-Vergés, Vedel, Valdemarsen, Kristensen & Flindt 2010). That is, in locations with lower current speeds, there tends to be an organic enrichment of the sediments in areas immediately below the aquaculture structures or in an area of up to 10 m surrounding the structure. In this case, the

greatest impact of fish farming occurs in the centre of the exploration area (Findlay & Watling 1997; Rapp, Ramirez, Rivera, Carlo & Luciano 2007).

Alpaslanu and Pulatun (2008) found that 4 cm/s was enough to promote the dispersion of waste generated by rainbow trout farming (*Oncorhynchus mykiss*) in cages in a Turkish reservoir. However, the authors still detected certain localized impacts. Gondwe, Guildford and Hecky (2011) showed that average current speeds of 9.3 cm/s were enough to disperse the waste generated by fish farming in cages, but it was not possible to detect differences in the concentrations of nutrients and solid waste between the control area (without cages) and the fish farming areas.

The deposition of particulate organic matter at the bottom of the water bodies, immediately below the production units, causes a decrease in dissolved oxygen, and the oxygen concentration can reach values between 0 and 1.5 mg/l (WDF 1990). The qualitative and quantitative changes in the benthic fauna are well known, including an increase in populations resistant to the organic pollution (Levings 1994).

Among all the nutrients evaluated in the commercial feeds, nitrogen, phosphorus and organic matter are those that generate the most interest with regard to their eutrophying effects in aquatic environments (Schindler 1971; Bureau & Cho 1999). Some authors also cite other feed components (calcium, silicates, nutritional additives, growth promoters, etc.) that could be associated with environmental degradation, but the available information on these components is very variable, inconsistent and/or scarce (Levings 1994; Boyd & Massaut 1999; David, Maria, Siringan, Reotita, Zamora, Villanoy, Sombrito & Azanza 2009; Martinez 2009).

In this study, the relevant amounts of potentially eutrophying loads being deposited into the aquatic ecosystems were estimated based on the nutrients present in fish feeds. When examining the cumulative results in literature studies, Sarà (2007) also found that there are significant amounts of all eutrophication-inducing nutrients present in the waste loads.

Moreover, the same author also considers the number of studies that more accurately estimate these loads from aquaculture to be scarce. However, the characteristics of effluents generated by aquaculture activity are very similar to those of domestic effluents, showing high biochemical oxygen



demand and a high concentration of suspended solids, including nitrogen and phosphate compounds (Imai, Fukushima, Matsushige, Kim & Choi 2002). This similarity allows an analogy of the impacts caused by the farming, contributing to eutrophication of water bodies.

According to Vanni (2002), fish are able to influence the nutrient dynamics, either directly by the ingestion and excretion of phosphorus and nitrogen, or indirectly by changes in nutrient recycling rates of other components of the food chain. Several studies have been conducted to reduce the eutrophication of lakes through biomanipulation, which is an ecotechnological strategy that complements the control of external nutrient inputs in the water and sediments (Bueno *et al.* 2008).

These projections of phosphorus input by fish farming cannot be evaluated only from these sources. It is known that other biotic and abiotic factors are involved in the phosphorus dynamics of aquatic ecosystems. Furthermore, the feeding strategy used in commercial fish farming has an important impact on the water quality and, consequently, on the growth, health and survival of the animals, as well as the efficiency of dietary nutrient utilization, because feed efficiency of nutrient deposition decrease with increasing body size (Xie, Cui, Yang & Liu 1997a).

Bechara, Roux, Diaz, Quintana and De Meabe (2005), studying the digestibility and utilization of nutrients, demonstrated that high-quality feed showed less pollution potential and enabled an increase in the production per unit of area that, in general, is greater than the increase in associated production costs. Amirkolaie (2011) notes that the use of pro-nutrients, such as phytase, improves digestion and helps reduce the loss of nutrients to waste because the use of this enzyme in feeds enables increased performance, retention of minerals in bones, protein digestibility and availability of calcium and phosphorus, helping to reduce the excretion of these nutrients into the water environment. Xie, Cui, Yang and Liu (1997b) found a negative correlation between nutrients digestibility coefficients and ration size for fry Nile tilapias, emphasizing the importance of the feed processing and production on the environmental aspects.

Another way to control and manage the carrying capacity of the environment is by the monitoring and analysis of the sediments, as sediments can be considered the result of the integration of the whole process that occurs in an aquatic

ecosystem, including the biological, physical and chemical processes that influence the system metabolism (Wetzel 2001). The assessment of the digestibility and excretion of the feeds is also a useful tool to ensure the environmental safety of aquaculture. This issue not only involves environmental considerations but also directly reflects the viability of the activity, as feed usage is a major challenge for the practice of sustainable aquaculture (Pillay 2007).

In this study, approximately one-fifth of the nutrient load deposited into the water environment is derived from feeding losses. According to Pearson and Gowen (1990), typically approximately 20% of the feed for fish stocked in cages is lost before it is ingested. The need to minimize these losses is well known, in particular due to economic production issues (Talbot & Hole 1994). However, this solution is not as simple as it sounds because the producers do not tend to pay attention to the intake capacity of caged fish and do not necessarily consider the time required for the fish to consume the feed (Kolsäter 1995).

Most studies that have measured the nutrient loads in waste from fish production in cages have underestimated or have not considered these losses in feeding, especially when the evaluations were conducted in a controlled environment (Cho & Bureau 2001). According to Islam (2005), the feeding methods practiced in commercial fish farming can promote an amount of wasted feed up to 30 times greater than in a laboratory setting.

Other studies use fish species with more intake voracity (i.e., carnivores) and/or feeds with physical forms that promote intake (Toguyeni, Fauconneau, Boujard, Fostier, Kuhn, Mol & Baroiller 1997). Tilapia takes longer to eat than most farmed fish species, allowing the precipitation of a portion of the feed and its loss from the cages (Guerrero 1980; Jauncey 1998). This effect is notably more common if the feed provided is pelletized in high-density extruded granules that enable rapid water absorption (Xia, Yang, Li, Liu, Xu & Rajkumar 2013).

The soluble fraction of the uneaten feed, partly transformed into inorganic nutrients such as nitrates, nitrites, ammonia and orthophosphate, directly participates in the pollution of the aquatic environment (Xu, Lin, Lin, Yang & Wang 2007). It is estimated that this fraction represents one-fourth of the wasted feed and can be gradually increased as the solid components dissolve (Wu,

Shin, MacKay, Mollowney & Johnson 1999; Qian, Wu & Ni 2001).

According to Guo and Li (2003), the rate of deposition of dietary nutrients by fish farmed in cages is 15% for nitrogen and 11% for phosphorus. In this study, the estimate of deposition of these nutrients was more than double the values found by these authors. The high metabolic rates of these ectothermic fish not only account for high loads of nutrients in the environment but explain the partitioning between solid and dissolved residues. High rates of nutrient excretion due to endogenous losses largely explain the low nutrient retention in fish tissues, with high losses of dissolved residues in the water column and proportionally lower losses as faecal matter (Fernandes *et al.* 2007). However, the variability in tilapia body composition found in the literature was evident. Several factors could explain this variability, among them the slaughter age of the animals, the type and nutritional adequacy of the diets provided and the ambient temperature, among other factors. Xie, Zheng, Chen, Zhang, Zhu and Yang (2011) found that the nitrogen retention efficiency was highest at 28°C and lowest at 37°C. Faced with this wide variation in the published data, we opted to use results from the analysis performed in the laboratory. These results, in turn, were used to calculate the estimates.

Regarding the soluble excretion of organic matter input into the environment by the fish, most part comes from endogenous losses in the form of mucus, scales, exogenous enzymes, cellular renewal and others (Dosdat, Servais, Métailler, Huelvan & Desbruyères 1996). Another important part comes from the end products of the glycolysis of carbohydrates and the beta-oxidation of lipids, that are excreted through the gills in the form of CO<sub>2</sub> (achieving up to 50% of the carbon loss) and H<sub>2</sub>O, which generally have only a limited impact on the natural environment (Dosdat 2001). On the other hand, the high availability of CO<sub>2</sub> dissolved in the water is directly involved on photosynthesis, acting as a fuel for the eutrophication process (Schindler 1971).

Ammonia and urea are the two primary nitrogen molecules resulting from protein metabolism and waste production in fish (Cowey 1995; Wilkie 1997; Janis & Farmer 1999; Terjesen, Finn, Norberg & Rønnestad 2002). More than 80% of nitrogen excretion by fish is represented by ammonia (Tanaka & Kadowaki 1995; Paéz-Osuna *et al.*

1999). This compound is the most abundant form of farming waste, followed by nitrates and nitrites and, finally, phosphorus (Schneider, Sereti, Eding & Verreth 2005). However, different concentrations of these compounds are explained by the production stage, management, environmental conditions and metabolic origin of each nutrient (Hargreaves 1998). Ammonia is mainly derived from the excretions of organisms and, in shallow waters (shallow basins, lakes, estuaries), from sediments and the mineralization flow of organic matter from leftover feed and other sources (Qian *et al.* 2001). However, Xie *et al.* working with Nile tilapia reared at different water temperatures found that ammonia excretion were not significantly affected by water temperature, for the studied range (25–37°C).

The phosphorus is derived from the decomposition of organic matter, mainly the leftover feed and faeces, and from the metabolic losses of the animals. Phosphorus has been regarded as the most important nutrient in waste from aquaculture, followed by ammonia, and it is an important limiting factor for the primary productivity in most aquatic environments (Beveridge 2004). However, the eutrophying effects reported in the literature are usually more related to phosphorus than to the nitrogen compounds or to the organic matter (Sarà 2007).

According to a study by Ackefors and Enell (1994), the calculated loads from the production of salmonids in cages can reach 10 kg of phosphorus and 60 kg of nitrogen per tonne of produced fish biomass. The organic matter load in waste per produced tonne was estimated by the authors as 2500 kg, on a wet basis.

Studies indicate that only 32% of the phosphorus is used for the fish metabolism, and the remainder is transferred to the environment (Penczak, Galicka, Molinski, Kusto & Zalewski 1982) and may induce eutrophication (Wetzel 2001). Alves and Baccarin (2005) reported that 66% of the phosphorus deposited by intensive feeding goes to the sediment, 11% stays dissolved in the water and 23% is incorporated in fish farming. Fernandes *et al.* (2007) estimated that 88–93% of the nitrogen supplied by the feed is deposited into the environment as waste from tuna produced in cages, and the majority of it (59–64%) corresponds to soluble excretion. According to the authors, the load in the waste is between 75 and 90 kg of nitrogen per tonne of produced fish biomass. Hakanson (2005) estimated that the

production of 1 tonne of fish in cages brings between 10–20 kg of phosphorus, and 50–75 kg of nitrogen to the environment. These values match those estimated by this study.

The environmental losses of nitrogen and phosphorus per tonne of tilapia, using diets commercially available in Brazil, proved to be two- to tenfold greater than those reported in the literature for other types of farming, and even greater than results obtained in the laboratory (Alves & Baccarin 2005; Fernandes *et al.* 2007; Azevedo *et al.* 2011). The decreased loads of these nutrients could be achieved by the improvement of feeding strategies and associated technologies, such as by producing diets with an ideal ratio between protein and energy and an optimized balance of essential amino acids, by the use of more digestible ingredients and additive enhancers and by using formulations that meet the nutritional requirements for each production condition. However, it is known that these strategies are not enough to achieve drastic reductions in the nutrient loads, in comparison to what is observed in other production species, in particular poultry and swine, where these strategies have been and continue to be widely studied.

The estimated loads in this study are a primary assessment of losses of organic matter, nitrogen and phosphorus in the production of Nile tilapia in Brazil, considering the current management practices and literature data. Although the estimates are based on many simplifications, the level of uncertainty of the total loads is small because the calculations were based on data (from feeds and animals) provided by the industry. Thus, nutrient load estimates and flows presented in this study are suitable for environmental impact studies of Nile tilapia farming in cages, in the commercial conditions found in tropical regions.

Still, if the goal of a particular study is a more specific and accurate estimate of loads and prognostic evaluation of potential impacts in a specific reservoir, complementary information is essential. This information includes climate and hydrological data for the reservoir, information about the action of the fauna present in the reservoir on the farming waste and the associated feeding losses, and information about the segmentation of nutrient inputs in time scales throughout the tilapia production period. This greater level of detail will allow the systematic calculation of loads in a more appropriate manner and may even help to identify the periods when there is an increase in the level

of impact on the natural and operational processes occurring in the reservoirs.

The farming of tilapia in cages significantly contributes to the input of nutrients that can cause eutrophication in artificial reservoirs. The introduction of feeding management practices, as well as technology for better utilization of dietary nutrients, is a key point to reducing the input of these nutrients in the aquatic environment. The methodology for estimating the nutrient loads in waste presented in this study can be used as an essential part of the determination process of the pollutant potential of aquaculture activities in water bodies.

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