E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 76.37
(GIF) Impact Factor: 0.549
IJFAS 2023; 11(5): 12-18

www.fisheriesjournal.com
Received: 16-06-2023
Accepted: 20-07-2023

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Trophic involvement of phytoplankton in the food bolus of three developmental stages of *Oreochromis niloticus* (Linnaeus, 1758) in aquaculture

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DOI: https://doi.org/10.22271/fish.2023.v11.i5a.2843

Abstract
The composition of stomach contents of three developmental stages of *Oreochromis niloticus* in intensive rearing was determined at the Research Station on Continental Fisheries and Aquaculture of Bouaké (SRPAC-Bouaké, Center of Côte d'Ivoire). The objective of this study was to know the phytoplankton consumed and its proportion ingested by this species in spite of the exogenous food intake. The phytoplankton composition of stomach contents was determined by analyzing the stomachs of 150 specimens including 50 fry, 50 fingerlings and 50 adults. The vacuity coefficient was 28%, 16% and 24% respectively following the same order. The relative importance index (IRI) of prey items allowed the identification of the most important taxa in the food bowls of *Oreochromis niloticus* and to evaluate their contribution. Thus, *Scenedesmus quadricauda, Phacus orbicularis* and *Pediastrum duplex* are the fry preferred prey, *Dictyosphaerium pulchellum* for fingerlings and *Phacus orbicularis* and *S. quadricauda* for adults. In addition, Cyanobacteria with toxinogenic potential such as *Cylindrospermopsis raciborskii, Anabaena circinalis, Anabaena* cf. *spiroides Anabaenopsis circularis, A. circularis* var. *javanica, A. arnoldii,* and *Microcystis aeruginosa* were found in the food bowls as incidental prey. The species *D. pulchellum* and *S. quadricauda* could be isolated and cultivated in the laboratory to evaluate their nutritional qualities.

Keywords: Food bowls, Prey relative importance index, SRPAC-Bouaké**,** Stomach contents, Tilapia

1. Introduction
Phytoplankton are very important in natural and artificial aquatic ecosystems for aquaculture use (Boyd, 2016) [3]. It participates in the expansion of aquaculture by serving as a trophic base and by stimulating the biomass of zooplankton essential to the larval and post-juvenile stages of shellfish and fish. In addition, it participates in the purification of the environment through its ability to absorb and eliminate excess dissolved effluents and also its great capacity to produce the oxygen necessary for the respiration of aquatic animals (Florescu *et al.*, 2022) [8]. Its trophic implication in intensive or semi-intensive fish farming seems not to be considered in Ivorian fish farms. Moreover, in Côte d'Ivoire, feed represents the highest cost of all expenses related to the fish production cycle up to the market stage (Siddhuraju and Becker, 2003) [18]. According to Dauda *et al.* (2015) [7], the cost of feed is a major constraint to the expansion of fish farming in developing countries. In recent years, the high cost of fish meal on the world market and of commercial feeds have led to the need to look for alternative, less expensive sources of protein for aquaculture needs (insect or maggot meal, micro-algae culture, agri-food by-products, etc.). The use of plant plankton as an additive in aquaculture has received much attention due to its positive effect on the zootechnical parameters of cultured organisms (Creswell, 2010.) [6]. This study therefore proposes to investigate the phytoplankton consumed by *Oreochromis niloticus* (the flagship species of Ivorian fish farming) in relation to the phytoplankton available in the fish ponds of the SRPAC of Bouaké. As a result, the isolation and cultivation of local algal species could contribute by solving feeding issue.

Materials and Methods

Study area

The Research Station of Fisheries and Continental Aquaculture (SRPAC) is located in central of Côte d'Ivoire, about 6 km from Bouaké's city (7º37'58.919"N and 5º2'34.051"W). It covers an area of 114 ha including 2.6 ha of water bodies. The farm is mainly composed of ponds (Figure 1), supplied with water by gravity from two intake canals coming from the Kan River dam located 1 km upstream and from an underground pipe coming from two boreholes through a pumping system. The study area is subject to a humid tropical climate. The study area is under the influence of two rainy seasons and two dry seasons with an annual temperature ranging between 25 and 38 ºC.

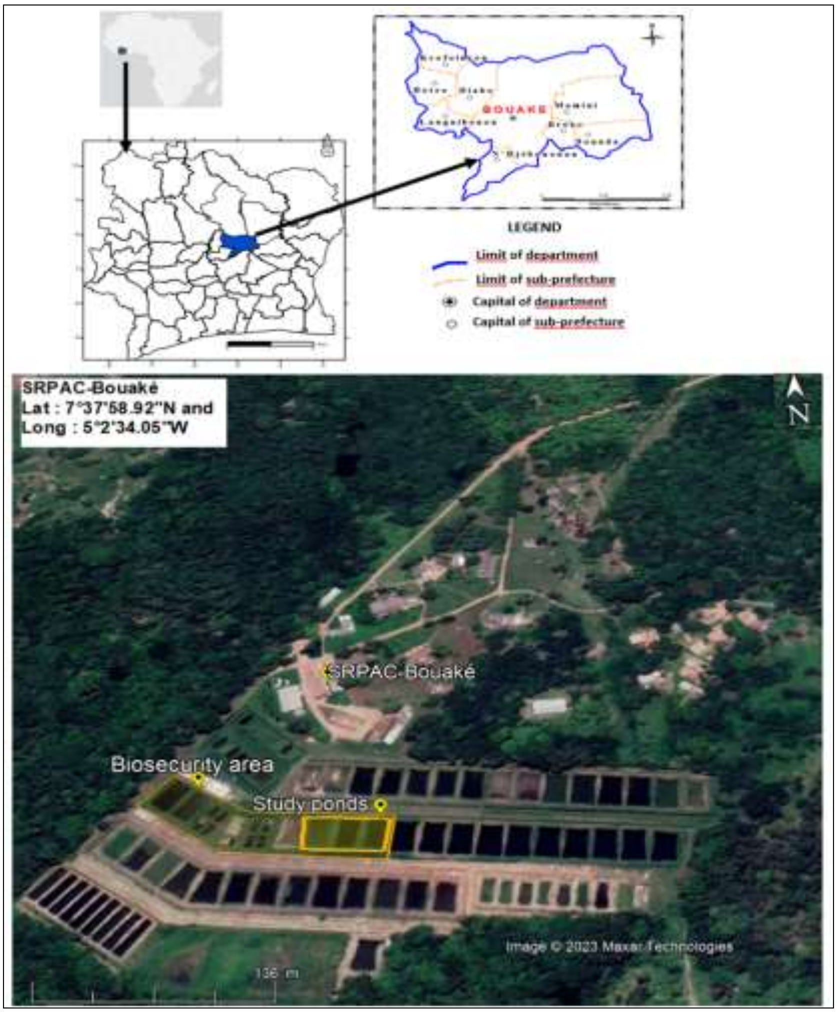

Fig 1: Rearing ponds at the Research Station (Google Maps modified)

Biological materials

The biological material consists of 150 specimens of *Oreochromis niloticus* (strain "Brazil") reared by the Research Station, usually fed with imported commercial food of the brand *RANAAN FISH BREED*. Specimens were captured March 2022, in three different ponds in the biosecure area (Figure 1) using a seine net (14 mm mesh), with 50 fry, 50 fingerlings and 50 adults. Each fish specimen was weighed and the size (standard length) determined with an ichthyometer before being dissected for stomach sampling.

Method of analysis of stomach contents

The stomach was collected and stored in a pillbox in which 5% formaldehyde was added. During the analysis, the state of stomach replenishment was noted. According to the method of Hyslop (1980) [10], stomachs filled to 1/4, 1/2, and 3/4 were considered full. Stomach contents were poured into a graduated cylinder containing 5 mL of distilled water to determine its volume by water displacement. The solution was adjusted to 10 mL and filtered through a 20 µm mesh filter and the filtrate collected in a pillbox to which two drops of formaldehyde were added for preservation.

In order to know the phytoplankton composition of the ponds from which the specimens were collected, a water sampling of the ponds was performed with a 20 micron mesh plankton net. Phytoplankton prey were observed using a bright field photonic microscope and counted on a Malassez cell (Niamien-Ebrottie *et al.*, 2017) [13]. Philipose (1982) [16]; Wołowski *et al*. (2013) [20], Conforti (1994) [4], Ling *et al*. (2007) [11] Mrutyunjay and Siba (2007) [12] and Patil *et al* (2018) [15] guides were used to identify phytoplankton taxa.

Determination of food indices

The biomass of phytoplankton ingested by fish was obtained from the average volumes of taxa (Niamien-Ebrottie *et al.*, 2017) [13]. Its expression consists in multiplying the number of taxa counted in the stomach contents by the average volume of the taxon. This result is expressed in biovolume and allows the biomass to be obtained by extrapolation, considering that 1 mm^3 of phytoplankton corresponds to 1 mg (Osman *et al.*, 2013) [14]. The intensity of feeding activity was evaluated by the stomacal vacuity coefficient (CV).

$$CV = (EV/NT) \times 100$$

Where *EV* is number of empty stomachs; *NT* is total number of stomachs analyzed; *CV* is stomach emptiness coefficient.

Determination of dietary indices

The phytoplankton composition of the food bolus of the three developmental stages was identified and the contribution of prey to the dietary profile was made on the basis of the calculation of dietary indices including:

The corrected percentage of occurrence (Poc)

It provides information on the fidelity of a population or a sub-population of fish to a given prey. It is the ratio between the number of stomachs containing a prey i (*n*) and the total number of stomachs examined (*N*) containing at least one prey (Hyslop, 1980; Cortés, 1997) [10, 5].

$$Poc = (Po \sum Po)$$

where Po = n×100 N

The numerical percentage (Pn)

It provides information on the abundance of a prey relative to others. It is the ratio between the number of individuals of a given prey (*ni*) and the total number of various prey (*Nt*) (Hyslop, 1980) [10].

$$Pn = ni \times 100/Nt$$

Percentage by weight (Pw) of prey

It represents the ratio between the weight of a prey *(Pi)* and the total weight (*Pt*) of all prey ingested by the fish (Hyslop, 1980) [10].

$$Pp = Pi \times 100/Pt$$

The relative importance index (IRI)

The most important prey items in the dietary profile were determined based on the relative importance index (*IRI*) values of Cortés (1997) [5]. This index combines corrected percent occurrence, numerical, and weight percent.

$$IRI = (Pn + Pp) \times Poc \text{ and}$$

$$\%\,IRI = (IRI/\sum IRI \times 100$$

The %IRI are arranged in descending order and added together in that order. Thus according to the values of %IRI, the following categories of prey are allowed: - Σ IRI = 50% or more: preferred prey.

- Σ IRI = 75 % or more: secondary preys.
- Σ IRI > 75%: accidental prey.

Statistical processing of the data

PAST 2.17c software was used to correlate the taxonomic composition of the environment with the relative abundance of ingested taxa to see the behavior of the fish towards phytoplankton prey.

Results and Discussion

Stomach emptiness coefficient

Analysis of the stomachs of 50 specimens of *Oreochromis niloticus* by developmental stage revealed that 14, 8, and 12 were empty in fry, fingerlings, and adults respectively. The emptiness coefficient established in the same order was 28%, 16%, and 24%. The number of full stomachs analyzed was 36, 42, and 38 in fry, fingerlings, and adults respectively.

Composition of stomach contents

Analysis of the stomach contents indicates that the different developmental stages of *Oreochromis niloticus,* ingested detritus and planktonic's preys.

Note that for the calculation of food indices, only phytoplankton taxa with a numerical frequency exceeding 0.10% were considered. The Diatom group is therefore excluded.

Quantitative analysis of phytoplankton prey ingested by fry showed that *Scenedesmus quadricauda* was the most consumed prey and was found in all stomachs containing at least one prey. The average amount of prey ingested by fry is estimated to be 39,456 preys for a corresponding biomass of 0.28 mg.

Table 1 indicates that among the prey consumed, the most important in the diet are *Scenedesmus quadricauda* (IRI = 27.59%), *Phacus orbicularis* (IRI = 20.27%), and *Pediastrum duplex* (IRI = 14.90%). These three prey alone constitute more than 70% of the ingested biomass (Cumulative weight = 78.96%) and are therefore considered preferential. Prey such as *Dictyosphaerium pulchellum* (IRI = 7.26%), *Tetradesmus dimorphus* (IRI = 4.37%), and *Desmodesmus bicaudatus* (IRI = 3.54%) are secondary. All other prey are incidental.

Table 1: Corrected percentages of occurrence (Poc), numerical (Pn), weight (wp) and relative importance index (%IRI) percentages of prey found in *Oreochromis niloticus* fry

Prey	Density of prey in ponds (Cells. mL^{-1})	Fries Aw = 14.7±4.7 g; Asl = 72.2±8.7 mm				
		Pn (%)	Poc (%)	wp (%)	% IRI	Prey category
Scenedesmus quadricauda	16,000	29.6	6.46	7.86	27.59	Preferential
Phacus orbicularis	5,000	3.98	3.05	54.31	20.27	Preferential
Pediastrum duplex	8,000	3.44	6.46	16.79	14.90	Preferential
Dictyosphaerium pulchellum	5,000	5.82	6.11	4.62	7.26	Secondary
Tetradesmus dimorphus	2,000	5.15	6.46	0.78	4.37	Secondary
Desmodesmus bicaudatus	3,000	4.41	6.46	0.4	3.54	Secondary
Tetraedron minimum	3,000	3.50	5.39	1.16	2.86	Accidental
Dictyosphaerium cf. *tetrachotomum*	1,000	3.24	4.31	1.87	2.51	Accidental
Willea apiculata	3,000	5.08	3.77	0.21	2.27	Accidental
Stauridium tetras	2,000	2.44	6.46	0.47	2.14	Accidental
Cylindrospermopsis raciborskii	1,000	2.94	3.59	0.73	1.50	Accidental
Microcystis aeruginosa	1,000	2.34	2.69	1.69	1.24	Accidental

Lepocinclis globulus	2,000	2.06	3.05	1.40	1.20	Accidental
Anabaenopsis circularis	1,000	1.62	3.95	0.90	1.13	Accidental
Crucigenia tetrapedia	1,000	3.38	2.69	0.19	1.10	Accidental
Anabaenopsis arnoldii	1,000	1.03	4.85	0.57	0.88	Accidental
Scenedesmus obtusus cf. *disciformis*	1,000	2.49	2.33	0.41	0.77	Accidental
Tetraedriella regularis	1,000	2.03	2.87	0.05	0.68	Accidental
Crucigeniella rectangularis	1,000	2.30	2.15	0.30	0.64	Accidental
Anabaena circinalis	1,000	1.76	2.15	0.30	0.64	Accidental
Monoraphidium arcuatum	1,000	1.90	2.69	0.01	0.59	Accidental
Acutodesmus acutiformis	1,000	1.76	1.08	3.01	0.58	Accidental
Raphidiopsis curvata	1,000	2.80	0.90	0.07	0.29	Accidental
Oocystis sp.	--	0.80	1.62	0.01	0.15	Accidental
Euglenaformis proxima	--	0.59	1.80	0.08	0.14	Accidental
Desmodesmus insignis	--	0.59	1.80	0.08	0.14	Accidental
Trachelomonas volvocina	--	0.90	0.72	0.14	0.09	Accidental
Scenedesmus pseudoquadricauda	--	0.44	1.62	0.05	0.09	Accidental
Anabaena cf. *spiroides*	--	0.86	0.72	0.12	0.08	Accidental
Anabaenopsis tanganyikae	--	0.44	1.08	0.24	0.08	Accidental

Note: Aw = Average weight; Asl = Average standard length.

In fingerlings, quantitative analysis of ingested phytoplankton prey revealed that stomachs contained an average of 148,410 preys for an estimated equivalent biomass of 0.78 mg. The ingested is mainly dominated by *Dictyosphaerium pulchellum* (IRI = 80.48%) which is the preferred prey and constitutes 72.87% of the ingested biomass. Other prey present in the food bowls are incidental (Table 2).

Table 2: Corrected percentages of occurrence (Poc), numerical (Pn), weight (Wp) and relative importance index (%IRI) percentages of prey found in fingerlings of *Oreochromis niloticus*

Proies	**Density of prey in ponds (Cells..mL^{-1})**	**Fingerlings Aw = 48.8±10.7 g; Asl = 110.8±8.1 mm**				
		Pn (%)	**Poc (%)**	**Wp (%)**	**% % IRI**	**Prey category**
Dictyosphaerium pulchellum	36,000	66.58	10.88	72.87	80.48	Preferential
Scenedesmus quadricauda	12,000	8.9	10.88	3.00	6.46	Accidental
Pediastrum duplex	1,000	1.67	7.51	11.24	5.14	Accidental
Dictyosphaerium cf. *tetrachotomum*	8,000	3.82	6.73	3.05	2.45	Accidental
Tetradesmus dimorphus	3,000	2.49	8.03	0.52	1.28	Accidental
Tetraedron minimum	1,000	1.80	5.70	0.82	0.79	Accidental
Desmodesmus bicaudatus	1,000	1.51	8.29	0.19	0.75	Accidental
Stauridium tetras	1,000	1.01	7.77	0.27	0.53	Accidental
Phacus orbicularis	--	0.24	2.07	4.52	0.52	Accidental
Microcystis aeruginosa	--	0.85	3.11	0.85	0.28	Accidental
Scenedesmus obtusus cf. *disciformis*	2,000	1.08	3.37	0.25	0.24	Accidental
Tetraedriella regularis	1,000	1.01	3.88	0.04	0.22	Accidental
Crucigeniella rectangularis	--	0.90	2.07	0.69	0.17	Accidental
Anabaenopsis circularis	--	0.11	1.29	0.08	0.08	Accidental
Monoraphidium arcuatum	--	0.31	3.63	0.03	0.06	Accidental
Lepocinclis globulus	--	0.30	2.07	0.28	0.06	Accidental
Acutodesmus acutiformis	--	0.37	1.55	0.06	0.04	Accidental
Euglena formis proxima	--	0.21	1.55	0.19	0.03	Accidental
Trachelomonas volvocina	--	0.50	1.04	0.11	0.03	Accidental
Scenedesmus pseudoquadricauda	--	0.21	2.07	0.03	0.03	Accidental
Willea apiculata	--	5.01	0.05	0.28	0.02	Accidental
Crucigenia tetrapedia	--	0.20	1.29	0.15	0.02	Accidental
Raphidiopsis curvata	--	0.20	0.52	0.01	0.01	Accidental
Anabaenopsis arnoldii	--	0.23	0.78	0.08	0.01	Accidental

Note: Aw = Average weight, ASL = Average standard length.

Similarly in adults, stomachs contain an average of 83,892 phytoplankton prey with a biomass of 0.43 mg. The ingested is composed of 2 preferential preys: *Phacus orbicularis* (IRI = 31.93 %) and *Scenedesmus quadricauda* (IRI = 25.33 %) representing more than 64 % of the biomass. In addition to these preys, the secondary preys *Pediastrum duplex, Dictyosphaerium. pulchellum* and *Willea apiculata* are also present. The other preys are incidental (Table 3).

In addition, potentially toxic species such as *Cylindrospermopsis raciborskii, Anabaena circinalis, Anabaenopsis circularis, A. arnoldii, A. circularis var. javanica*, *A. tanganyikae* and *Microcystis aeruginosa* were found in the food bolus of the different developmental stages as incidental prey.

Table 3: Corrected (Poc), numerical (Pn), weight (Wp) and relative importance index (%RI) percentages of prey found in adults of *Oreochromis niloticus*

Proies	Density of prey in ponds (Cells.mL^{-1})	(Adults: Aw = 70±10.8 g; Asl = 125.4±6.8 mm))				Prey category
		Pn (%)	Poc (%)	Wp (%)	% IRI	
Phacus orbicularis	1,000	2.74	6.08	52.88	31.93	Preferential
Scenedesmus quadricauda	12,000	3.39	6.42	11.41	25.33	Preferential
Pediastrum duplex	2,000	2.16	5.06	14.91	8.14	Secondary
Dictyosphaerium pulchellum	1,000	6.40	6.25	7.18	8.01	Secondary
Willea apiculata	2,000	8.58	5.91	0.32	4.97	Secondary
Desmodesmus bicaudatus	3,000	5.94	6.42	0.76	4.06	Accidental
Tetradesmus dimorphus	2,000	4.23	6.42	0.91	3.11	Accidental
Tetraedron minimum	1,000	3.78	4.90	1.77	2.57	Accidental
Stauridium tetras	1,000	2.74	6.42	0.75	2.11	Accidental
Crucigeniella rectangularis	1,000	5.60	2.37	1.03	1.48	Accidental
Monoraphidium arcuatum	3,000	4.23	2.87	0.03	1.15	Accidental
Acutodesmus acutiformis	1,000	2.63	3.21	0.43	0.93	Accidental
Anabaena sp.	2,000	2.02	3.38	0.40	0.77	Accidental
Tetraedriella regularis	1,000	1.55	4.90	0.06	0.74	Accidental
Crucigenia tetrapedia	1,000	2.22	3.04	0.18	0.69	Accidental
Microcystis aeruginosa	1,000	1.26	2.70	1.29	0.65	Accidental
Euglenaformis proxima	1,000	1.54	1.86	1.41	0.52	Accidental
Anabaena circinalis	--	1.04	2.70	0.78	0.46	Accidental
Trachelomonas volvocina	1,000	1.26	2.70	0.28	0.39	Accidental
Oocystis sp.	2,000	2.08	1.52	0.05	0.31	Accidental
Raphidiopsis curvata	1,000	1.70	1.52	0.10	0.26	Accidental
Scenedesmus pseudoquadricauda	--	0.78	2.53	0.12	0.21	Accidental
Dictyosphaerium cf. *tetrachotomum*	--	0.35	3.55	0.29	0.21	Accidental
Scenedesmus obtusus cf.*disciformis*	1,000	1.69	1.01	0.40	0.20	Accidental
Anabaenopsis arnoldii	--	0.70	1.18	0.55	0.14	Accidental
Lepocinclis globulus	--	0.80	0.84	0.77	0.12	Accidental
Anabaenopsis circularis	--	0.35	1.18	0.27	0.07	Accidental
Anabaenopsis tanganyikae	--	0.23	1.18	0.18	0.05	Accidental
Cylindrospermopsis raciborskii	--	0.34	1.01	0.12	0.04	Accidental
Desmodesmus insignis	--	0.34	0.51	0.06	0.20	Accidental

Note: Aw = Average weight; Asl = Average standard length.

To understand the feeding behavior of *O. niloticus* towards phytoplanktonic prey, a correlation was established between the density of prey found in the ponds and the quantities ingested of these prey. Indeed, the rearing structure of the fry has a density of 68,000 individuals/mL, that of the fingerlings of 83,000 individuals/mL and of 72,000 individuals/mL for the structure of the adults. The majority of the taxa are those found in the food bowls. A strong positive correlation was obtained for the three developmental stages for prey with a numerical frequency greater than or equal to 1%. This correlation is 0.90 for fry (Figure 2), 0.97 for fingerlings (Figure 3) and 0.95 for adults (Figure 4).

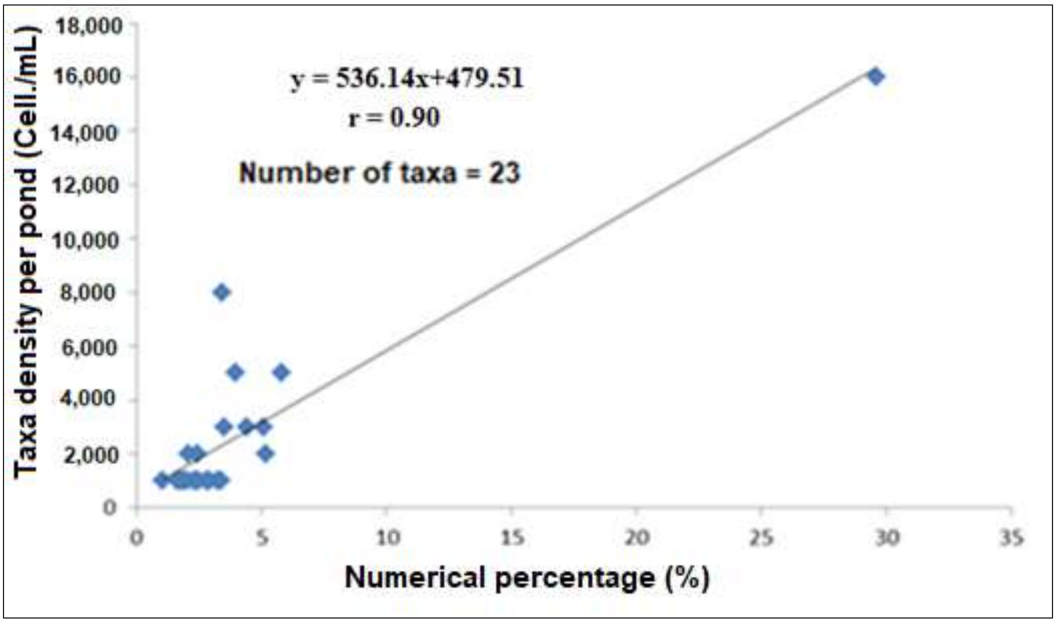

Fig 2: Correlation between densities of phytoplanktonic taxa in ponds and their proportion ingested by *Oreochromis niloticus* fry

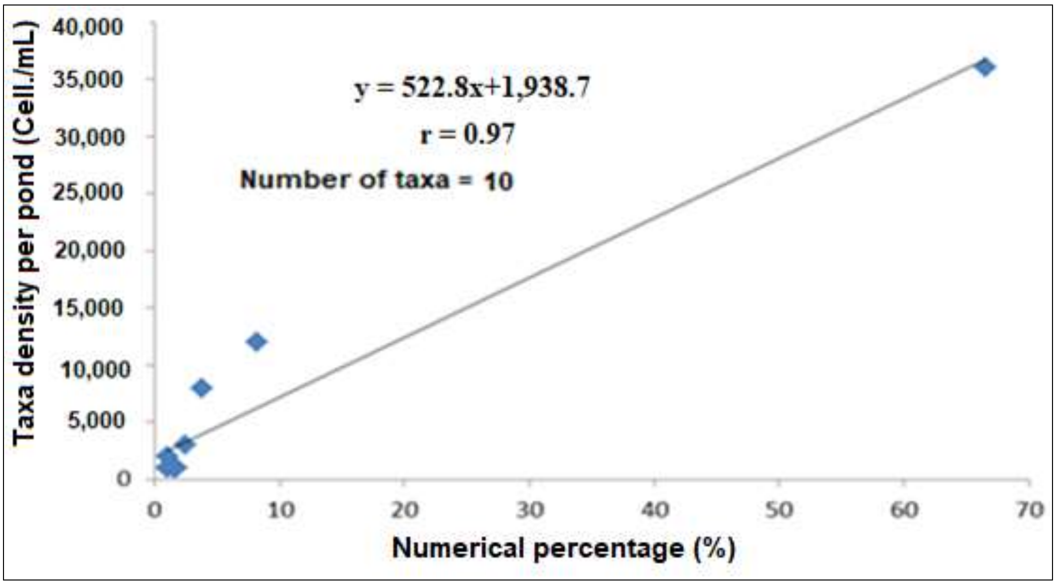

Fig 3: Correlation between densities of phytoplanktonic taxa in ponds and their proportion ingested by fingerlings of *Oreochromis niloticus.*

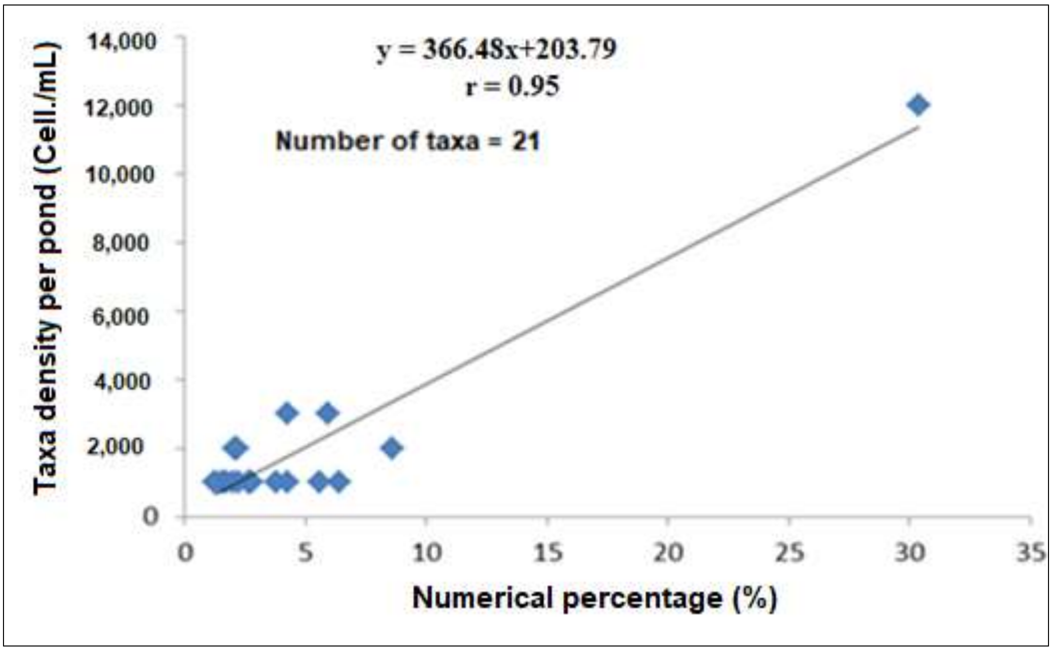

Fig 4: Correlation between densities of phytoplanktonic taxa in ponds and their proportion ingested by adults of *Oreochromis niloticus*.

Discussion

From the point of view of the feeding pattern, the vacuity coefficient is relatively low for the different developmental stages of *O. niloticus*: 28%, 16% and 24% respectively in fry, fingerlings and adults. This result could be justified by the fact that this species feeds according to a nycthemeral rhythm. Indeed, the work of Fortes-Silva *et al.* (2010) [9] carried out in a confined environment showed that the food intake of this fish was mainly done during the day during the light phase, the animal being at rest at night. In the Research Station fish pond, *O. niloticus* intensively consumed phytoplankton prior to the 9:00 am feedings. Also, analysis of stomach contents of different growth stages of *O. niloticus*, confirms the detritivore and microphagic regimes. This observation corroborates various works related to the diet of this same species (Temesgen *et al.*, 2022) [19]. For Abidemi-Iromini (2019) [1] and Temesgen *et al.* (2022) [19], phytoplankton was the primary consumed food item, which indicates the specialist feeding strategy of Nile tilapia in the lake. It is noted, however, that the biomass of phytoplanktonic prey ingested differs between developmental stages. Adults and fingerlings consumed more prey with about 2 and 3 times the biomass ingested by fry. This difference would be due to the nutritional requirements that vary for each developmental stage. These authors mentioned that the contribution of phytoplankton, zooplankton and insects were slightly highest in small-sized groups (<10 cm), whereas detritus, macrophytes and fish parts were highest in larger-size groups (>20 cm). So the proportions of natural food taken from the rearing environment are more important in adults and juveniles than in the fry of the species *O. niloticus*. Furthermore, the low amount of phytoplankton in the stomachs of fry could be attributed to the morphology of their digestive tract. Indeed, according to Bowen (1982) [2], tilapia fry prefers to consume small invertebrates, especially easily digestible and assimilable microcrustaceans, at the expense of phytoplankton.

In addition, the strong correlations between the density of prey in the pond and their proportion in the stomachs, show that the species is opportunistic. It passively consumes the prey in excess in the environment. This result was to the observations of Fortes-Silva *et al*. (2010) [9] according to which filter-feeding fishes do not select their prey visually but feed on prey available in their habitats. These authors also argue that fishes possess variable diets related to ontogenic changes. Analysis of the stomach contents of *O. niloticus* revealed a significant fraction of phytoplankton belonging to the Chlorophyte, Euglenophyte and Cyanobacteria groups of similar specific composition in both fry, fingerlings and adults. In conclusion tilapia self-feed at night (although locomotor activity was mostly diurnal) and chose plant-diets containing phytase, which should be taken into account when designing feeding strategies and practical diets for tilapia aquaculture.

Conclusion

The present work determined the dietary profile of *O. niloticus* in the Research Station fish ponds prior to the first feeding of the day. Analysis of stomach contents of the species showed a wide spectrum of phytoplankton resource utilization. The latter contributes to the diet of the species in spite of the exogenous supply of efficient food. The prey consumed at the different developmental stages are mainly Chlorophytes, Euglenophytes and Cyanobacteria. Due to their relatively small size adapted to the mouth opening of *O. niloticus*, and their appearance in colonies and coenobia, the species *Dictyosphaerium pulchellum* (5 µm diameter) and *Scenedesmus quadricauda* (15 µm length), can be cultured to reduce the conversion index. This will contribute to readjust the exogenous input of industrial feed to reduce the rearing costs.

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