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Presence and abundance of phytoplankton and zooplankton in a Biofloc production system using two carbon sources: 1) Molasses and 2) Molasses + rice powder, culturing *Oreochromis niloticus*.

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ABSTRACT

Biofloc technology consists in propitiate formation of flocs in a culture of *Oreochromis niloticus*, having as carbon source molasses and rice powder. It was used a conical container of 450 L, where 30 tilapias were introduced with an average length of 5.0 ± 0.95 cm and weight of 4.2 ± 1.08 g. To guarantee formation of flocs and development of phytoplankton and zooplankton communities, it was maintained a relation C/N=20:1. With molasses it was obtained six genders of phytoplankton, being 35% of total; ciliates with 12 genders corresponding to 60% and only one genus of rotifers (5%). With molasses mixed with rice powder, it was obtained three genders of phytoplankton, being the 27.27% of total; five genders of ciliates corresponding to 45.45% and three genders of rotifers (27.27%). The results of this research provide insight into changes in communities of microalgae, ciliates and rotifers during the experimentation period and the used carbon source, which allows to know the contribution of Biofloc as source of *in situ* natural food, this is very important in fish and crustaceans diet of commercial importance, mainly in larval stages of ornamental fish.

Key words: Phytoplankton, zooplankton, Biofloc, tilapia.

RESUMEN

La tecnología Biofloc consiste en propiciar la formación de flóculos en un cultivo de *Oreochromis niloticus*. teniendo como fuente de carbono melaza y polvo de arroz. Se utilizó un recipiente cónico de 450 L, se introdujeron 30 tilapias de una longitud 5.0 ± 0.95 cm y un peso promedio de 4.2 ± 1.08 g. Para garantizar la formación de flóculos y el desarrollo de las comunidades de fitoplancton y zooplancton, se buscó mantener una relación C/N=20:1. Con la melaza se obtuvo siete géneros de fitoplancton, correspondiendo al 35% del total; los ciliados con 12 géneros correspondiendo al 60% y tan solo un género de rotífero (5%). Con melaza+pulido de arroz se obtuvo tres géneros de fitoplancton, correspondiendo al 27.27% del total; los ciliados con cinco géneros correspondiendo al 45.45% y para el grupo de rotíferos el 27.27%. Los resultados de esta investigación aportan conocimiento sobre los cambios en las comunidades de microalgas, ciliados y rotíferos a lo largo del periodo de experimentación y la fuente de carbono empleada, lo que permite conocer la contribución del Biofloc como fuente de alimento natural *in situ*, que es tan importante en la dieta de peces como de crustáceos de importancia comercial sobretodo en estadios larvarios y de peces ornamentales.

Palabras clave: Fitoplancton, zooplancton, Biofloc, tilapia.

INTRODUCCIÓN

Biofloc technology consists in propitiate flocs formation, which are constituted of organic

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matter by a heterogeneous mix of microorganisms (fungus, bacteria, microalgae, protozoans, and rotifers among others) (Avinmelech 2009), also it contains around 30 to 40% of organic materials as colloids, organic polymers, and dead cells, which can be consumed by other organisms and reintegrated in productive chains (Avnimelech and Kochba 2009).

Biofloc acts as a nutrient retention trap in ponds and containers of fish and crustaceans culture. When pond sediments are suspended, it allows that compounds as phosphorus, carbon and nitrogen mainly, favor microorganisms development present in water and their biodiversity, which will be lodged in aggregates of organic matter or flocs and will depend on microbiota found in water (Ray et al. 2010).

Flocs can provide important nutrients such as proteins (Azim and Little 2008; Emerenciano et al. 2011), lipids (Wasielesky et al. 2006; Emerenciano et al. 2012), aminoacids (Ju et al. 2008) and fatty acids (Azim and Little, 2008; Ekasari et al. 2010). Because this technology has been focus of research in aquaculture nutrition field by being an alternative as food source (Moriarty 1997; Emerenciano et al. 2013, 2017).

Regarding to plankton composition as a Biofloc product, Monroy et al. (2013), identified heterotrophic bacterial communities of genus *Sphingomonas*, *Pseudomonas*, *Bacillus*, *Nitrospira*, *Nitrobacter* and yeast *Rhodotorula* sp that favor water quality and physiological wellbeing of cultured organisms. Also, they describe diverse species of microalgae and different species of ciliates and flagellate protozoans, rotifers and crustaceans and a specie of nematode, using molasses as carbon source. The composition of phytoplankton and zooplankton of flocs varies per Biofloc maturity time, this means that a recent Biofloc will be constituted mainly by heterotrophic bacteria, while an old one will be constituted by fungus.

The aim of this study was to describe the species that integrate plankton developed in Biofloc having as a carbon source molasses and rice powder culturing *Oreochromis niloticus*.

MATERIAL AND METHODS

Experimental design and culture conditions

For experiment, it was used a conical container of 450 L with an air diffuser in the middle to guarantee continuous movement and homogeneous suspension of particles (Fig.1). Thirty tilapias were introduced with an average length of 5.0 ± 0.95 cm and weight of 4.2 ± 1.08 g. They were fed daily with commercial food (Alimentos del Pedregal®, Toluca, Estado de México, México) with a protein content of 45% and a particle size of 0.6-0.8 mm. The diet was provided at 10% of their body weight and amount of food was adjusted every 15 days.

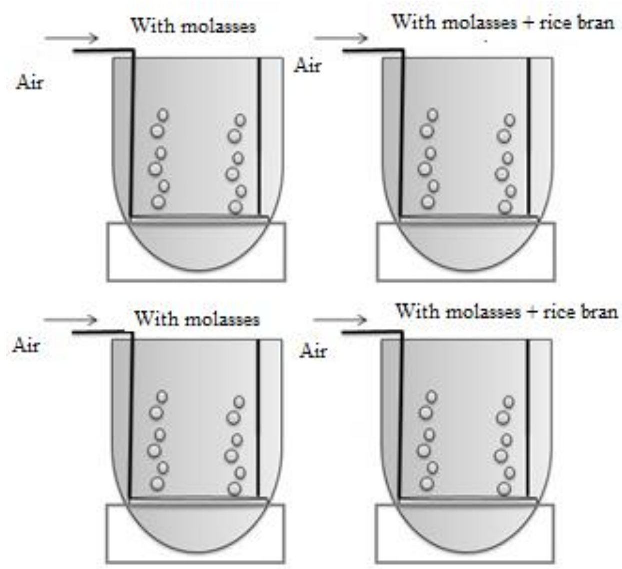

Fig. 1.- Biofloc culture system with *Oreochromis niloticus.*

To guarantee flocs formation and development of microbial communities in culture

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system, it was maintained a relation C/N=20:1 (Emerenciano et al. 2012), through controlled input of an external carbon source. The treatment 1 was with molasses and treatment 2 with a mix of molasses and rice powder. It was used nitrogen from commercial food. With these two variables, the requirement calculations were made (Emerenciano, 2011).

Identification of phytoplankton and microfauna associated to Biofloc

For observation and quantification of associated organisms to microbial flocs formed in macrocosm section, water samples (10 mL) were took weekly during a period of 12 weeks. With microalgae, it was transferred 1 mL of sample to a Sedgewick-Rafter camera (Azim and Little 2008), and through optical microscopy (microscope ZSX50 Olymlus®), it was observed microalgae with an objective of 100X and with an image program, it was proceeded to count four fields chosen randomly from count chamber.

In case of microfauna (ciliates, rotifers, nematodes) three samples of 10 mL were took from water of culture container. Were fixed with formalin at 5% for observation and quantification in a direct way. The microscope was connected to an image program (Image® Pro Plus 7.0). Finally, the taxonomic identification of observed groups was made at genus level with specialized literature (Aladro-Lubel 2009).

2.3 Information processing

The values and phytoplankton groups, of micro and macro fauna were introduced to a database in Excel 2010, to determine its descriptive statistic (mean ±S.D.).

2.4 Statistical analysis

It was made a variance analysis (ANDEVA) per phytoplankton group, micro and macro fauna, to determine significant differences ($P<0.05$) per week between groups and treatments. By finding significant differences, it was proceeded to make a test of multiple means by Tukey technique. Also, it was made a two-way significance test, with variables of group of organism, weeks of culture and treatment, to determine the percentage of that significance (%).

RESULTS

Biofloc with molasses

In Table 1 it is presented the information about phytoplankton abundance (org mL^{-1}) with seven genders, corresponding to 35% of total, while 12 genders of ciliates were identified (60%) and only one genus of rotifers (5%).

Regarding to microalgae, the genus that obtained highest density was *Chlorella* sp. with 320 org mL^{-1} in Week 12 of culture and it reached a total of 660 org mL^{-1}. For ciliates *Peranema* sp and *Chrococcus* sp. genders obtained highest density with 110 org mL^{-1} in week 6 and week 12 respectively. The only genus of rotifer was *Lecane* sp. with a density of 100 org mL^{-1} in week 9.

Both groups of microalgae and ciliates showed at week 3 of culture until end of it. The microalgae with an increase of its density and ciliates with a decrease. The rotifer shows at week 5 until end, except in week 11.

In Table 2 it is observed average value (±S.D.) of abundance by group (phytoplankton, ciliates and rotifers), and obtained their significance during 12 weeks of culture (Fig. 2). The observed significance ($P<0.05$) in this treatment points out that week variable shows 44.17% of that significance; variable planktonic group has 18.94% and interaction between both variables has 36.15% of that significance.

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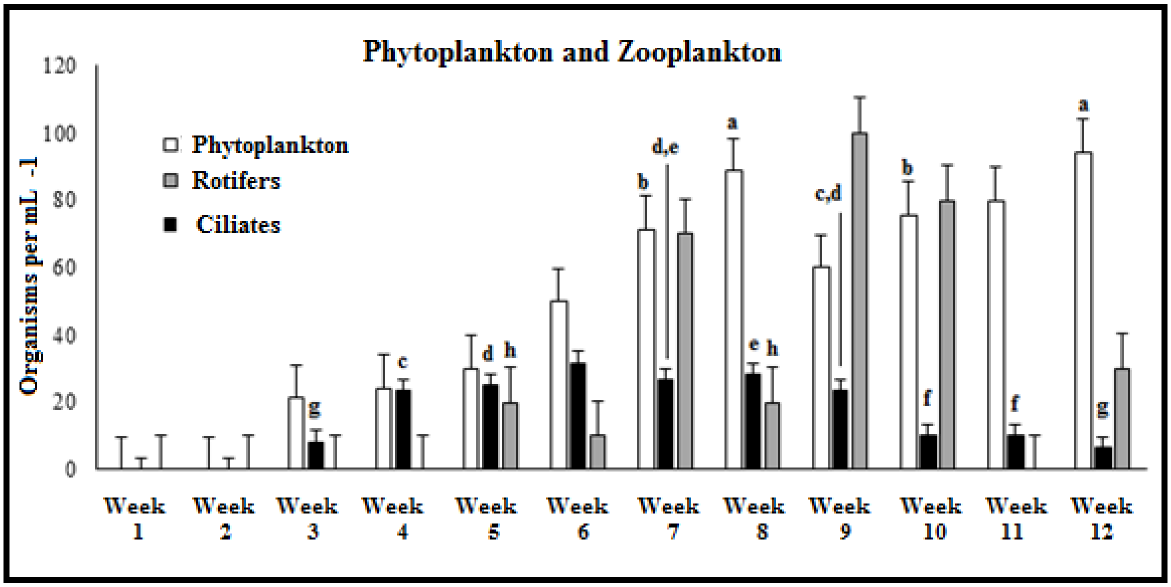

Fig. 2.- ANDEVA of phytoplankton, ciliates and rotifers abundance in Biofloc with molasses in week 12 culture with *Oreochromis niloticus*

Same letters indicate no significant difference (P>0.05). Columns without letters per planktonic group indicate significant differences (P<0.05).

Biofloc with molasses + rice powder

In Table 3 it is presented information about abundance (org mL^{-1}) of phytoplankton with three genders, corresponding to 27.27% of total. The ciliates with five genders corresponding to 45.45% and for rotifer group 27.27%.

Regarding to microalgae group, diatoms obtained highest density with 900 org mL^{-1} in week 10 and 12 of culture and for total of microalgae corresponded to 1,580 and 1,550 org mL^{-1} respectively. For ciliates, *Paramecium* sp. with 102 org mL^{-1} in week 5 and for total of ciliates it was of 177 org mL^{-1}. For rotifer group, the *Philodina* sp. genus with 196 org mL^{-1} shows maximum density in week 7 and total organisms was 287 org mL^{-1}.

All groups (microalgae, ciliates, and rotifers), showed up from week 3 of culture until end of it.

In Table 4 it is shown the mean value (±S.D.) per group (phytoplankton, ciliates and rotifers), and variance analysis (ANDEVA) during 12 weeks of culture (Fig. 3). The observed significance percentage (P<0.05) in this treatment, points out that planktonic group variable has 50.13% of significance; week variable 20.66% and interaction between both variables has 28.88%.

It is noted that as populations of phytoplankton grow, in turn, populations of protozoa and rotifers increase.

Statistical analysis

Significant differences were observed in ANDEVA between treatments per each of weeks (P<0.001). While two-way analysis points out that planktonic group present highest percentage of significance with 55.0%, followed by week variable

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Table 1. Organisms abundance (organisms mL^{-1}) of phytoplankton, ciliates and rotifers in Biofloc with molasses in a 12 week culture with *Oreochromis niloticus.*

PLANKTON	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
PHYTOPLANKTON												
Chlorella	-	-	100	140	110	240	190	210	180	200	230	320
Chlamydomona	-	-	50	30	60	20	70	20	10	-	-	-
Coleastrum	-	-	-	-	40	40	20	20	50	70	30	40
Cyclotela	-	-	-	-	-	-	20	60	40	60	30	60
Navicula	-	-	-	-	-	-	80	150	60	120	170	100
Oocystis	-	-	-	-	-	40	80	80	20	50	30	80
Scenendesmus	-	-	-	-	-	10	40	80	60	30	70	60
Total			150	170	210	350	500	620	420	530	490	660
PROTOZOANS												
Acanthocystis	-	-	-	-	-	-	-	10	10	10	30	40
Ameba desnuda	-	-	-	20	20	10	10	10	20	-	-	-
Anisonema	-	-	20	30	70	50	40	70	30	50	10	-
Arcella	-	-	-	-	-	20	10	30	30	20	10	10
Chrococcus	-	-	20	80	90	40	40	30	20	40	70	110
Entosiphon	-	-	-	-	30	30	30	80	50	20	30	20
Euglypha	-	-	10	40	50	40	30	50	20	10	30	10
Paramecium	-	-	10	30	10	20	50	30	60	10	20	10
Peranema	-	-	30	70	90	110	50	30	20	10	10	20
Podophrya	-	-	-	-	-	-	20	10	10	-	-	-
Prorodon	-	-	-	-	-	-	-	20	10	20	-	-
Vorticela	-	-	-	-	-	20	10	30	20	10	-	-
Total			80	270	360	320	290	400	300	200	210	230
ROTIFERS												
Lecane	-	-	-	-	20	10	70	20	100	80	-	30

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Table 2. Organisms abundance of phytoplankton, ciliates, and rotifers in Biofloc with molasses in week 12 culture with *Oreochromis niloticus.*

Plankton	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
Phytoplankton	-	-	21 ±4	24 ±5	30 ±4	50 ±8	71 ±6	89 ±7	60 ±6	76 ±7	80 ±9	94 ±10
Ciliates	-	-	8 ±1	23 ±3	25 ±4	32 ±4	27 2	28 ±1	23 ±2	10 ±1	10 ±1	7 ±1
Rotifers	-	-	-	-	20 ±2	10 ±1	70 ±7	20 ±2	100 ±10	80 ±8	-	30 ±3

Table 3. Organisms abundance (organisms mL^{-1}) of phytoplankton, ciliates and rotifers in Biofloc with molasses and rice powder in week 12 culture with *Oreochromis niloticus.*

Plankton	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
PHYTOPLANKTON												
Clorofitas	--	--	130	145	200	450	650	680	740	680	600	650
Diatomeas	--	--	--	200	280	400	640	780	890	900	850	900
Cianobacterias	--	--	--	--	--	--	50	80	85	--	--	--
Total			**130**	**345**	**480**	**850**	**1,340**	**1,540**	**1,715**	**1,580**	**1,450**	**1,550**
CILIATES												
Paramecium	--	--	50	80	102	89	75	62	45	32	25	17
Stylonychia	--	--	20	32	23	10	4	--	--	--	--	--
Colpidium	--	--	10	23	18	45	40	34	23	43	56	34
Vorticellas	--	--	--	--	34	45	56	23	12	--	--	--
Halteria	--	--	--	--	--	--	--	20	38	56	45	63
Total			**80**	**135**	**177**	**189**	**175**	**139**	**118**	**131**	**126**	**114**
ROTIFERS												
Philodina	--	--	5	11	45	67	196	189	120	115	106	122
Keratella	--	--	--	--	--	45	67	85	70	23	--	--
Lecane	--	--	80	95	105	50	24	--	--	--	--	--
Total			**85**	**116**	**150**	**162**	**287**	**274**	**190**	**138**	**106**	**122**

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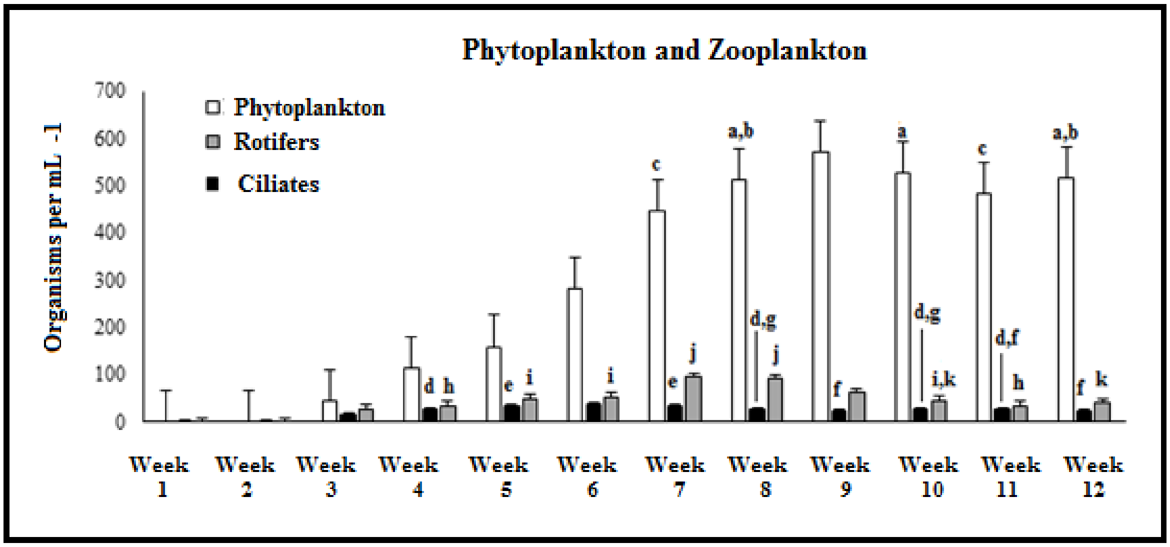

Fig. 3.- ANDEVA of phytoplankton, ciliates and rotifers abundance in Biofloc with molasses plus rice powder in week 12 culture with *Oreochromis niloticus*.

Same letters indicate no significant difference (P>0.05). Columns without letters per planktonic group indicate significant differences (P<0.05).

with 30.93%. In Table 5 it is shown those treatments that did not presented significant differences.

DISCUSSION

In a Biofloc system, microorganisms that grow are closely related with particulate organic matter which is maintained in suspension by continuous aeration. The phytoplankton and bacteria that are part of this complex of living organisms found in Biofloc, metabolize nitrogenous wastes from intensive fish and crustacean culture (Green et al. 2014). Even though, phytoplankton is present in Biofloc production systems in ponds that are found outdoors, with direct sun light, there are only registered three studies that discuss about composition of phytoplankton. Schrader et al. (2011) mentions that phytoplanktonic composition in an outdoor freshwater pond of catfish, was dominated by green algae (Chlorophytes), diatoms (bacillariophyta) and cyanobacteria (cyanophytes), or by chlorophytes and cyanophytes in brackish water ponds (Ray et al. 2010) or marine in culture of *Litepenaeus vannamei* (Vinatea et al. 2010). Becerra-Dórame et al. (2011) had a concentration of $2.1x10^4$ cells mL^{-1} in a heterotrophic culture of Biofloc, in comparison to an autotrophic culture with $3.3x10^6$ cells per mL^{-1}. In this experiment, it was obtained 662 cells mL^{-1}, dominated by *Chlorella* with 320 cells mL^{-1} in week 12 with treatment of molasses. With rice powder, value of phytoplankton rises to 1,550 cells mL^{-1} in week 12.

Nutrients are not a limitation for phytoplankton in a Biofloc system, but phytoplankton is exposed to light intensities that vary constantly because of continuous mix of medium by aeration, that favors quick growth of diatoms and chlorophytes over growth of cyanobacteria (Reynolds 1984; Green

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Table 4. Organisms abundance of phytoplankton, ciliates, and rotifers in Biofloc with molasses and rice powder in week 12 culture with *Oreochromis niloticus*.

Plankton	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
Phytoplankton	-	-	43	115	160	283	447	513	572	527	483	517
			±7	±10	±14	±25	±34	±38	±43	±47	±44	±46
Ciliates	-	-	16	27	35	38	35	28	24	26	25	23
			±2	±3	±4	±3	±3	±2	±2	±2	±3	±3
Rotifers	-	-	28	35	50	54	96	91	63	46	35	41
			±4	±5	±5	±1	±9	±9	±6	±6	±6	±7

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et al. 2014). Planktonic algae that dominate at beginning of Biofloc systems, decrease and disappear per development of Biofloc until diatoms are main group in and related to organic matter in Biofloc system (Monroy et al. 2013). Which was observed in this experiment, where the group of diatoms remain until end and cyanobacteria disappeared.

Regarding to ciliates, Ballester et al. (2010) in a culture of *Farfantepenaeus paulensis* postlarvae in a Biofloc system, obtained concentrations of 39 to 169 individuals mL^{-1}. Maicá et al. (2012) reports mean concentrations of these protozoa of 164, 64 and 29 ciliates per mL^{-1}, in salinities of 2, 4 and 15% respectively. In this experiment, protozoans reached values from 80 individuals mL^{-1} in week 3 and up to 400 mL^{-1} individuals in week 8 with molasses treatment.

Published information regarding rotifers present in Biofloc cultures, mention concentrations of 4.6 to 151 organisms mL^{-1} in seawater (Balllester et al. 2010). On other hand, Monroy et al. (2013) report concentrations between 28 and 96 organisms' mL^{-1} in Biofloc cultures in fresh water. In this work, in molasses+rice powder Biofloc, rotifer concentrations where higher than previous investigations, because it was obtained values of 85 organisms mL^{-1} in week 3 up to 287 organisms mL^{-1} in week 7. It is possible that additional carbon source of rice powder gives better conditions for higher abundance of phytoplankton community and it increase rotifers species and number of individuals.

The results of this investigations provide knowledge about changes in microalgae, ciliates and rotifers communities during experimentation period and used source of carbon, which allows to know the contribution of Biofloc as natural food source *in situ*, which is important in diet of fish and crustaceans of commercial importance mainly in larvae stages and ornamental fish.

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