Evaluation of nitrogen uptake and excretion by *tilapia* in bio floc tanks, using ^{15}N tracing

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ABSTRACT

Uptake and excretion of ^{15}N by tilapia was evaluated in simulated bio floc technology (BFT) tanks. Bio floc suspension (suspended solids in the range of 200 mg l^{-1}) was enriched in respect to ^{15}N by adding tagged ammonium salt and starch, to ensure a complete immobilization of ammonium in the bio flocs. Fish were held for 14 days in tanks, when the only feed source was the flocs. Nitrogen species in the water, as well as ^{15}N enrichment in the suspended matter and in fish muscle were monitored at frequent intervals during this period.

The system under study underwent a series of processes, including the uptake by fish of suspended organic nitrogen, excretion of tagged and non tagged nitrogen by fish, microbial degradation of the bio flocs and the opposing process of new bio flocs production using the excreted nitrogen.

The daily net nitrogen uptake (i.e. nitrogen retained by the fish) was found to be 240 mg N kg fish^{-1}. This is equivalent to a daily uptake of about 1.6 g protein per kg fish, or about 25% of protein normally added with feed. Nitrogen excretion was found to be about twice as high as the net uptake, i.e. gross daily N uptake was about 700 mg N kg fish^{-1}. Bio floc residence time was estimated to be about 8 h, indicative of the high dynamics of the bio floc system. The results of this experiment were used to evaluate experimental and computational approaches to determining nitrogen fluxes.

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1. Introduction

Bio floc technology (BFT) is a new technique used in aquaculture systems. Dense and active aerobic microbial communities are manipulated so as to control water quality by the immobilization of ammonium into microbial protein and in order to recycle feed residues and raise feed efficiency (e.g. Avnimelech et al., 1989, 1992; Crab et al., 2007). Relatively high C/N ratio in feed (>15) leads to the immobilization of ammonium (Total ammonium nitrogen, TAN) in the microbial biomass and limits the accumulation of TAN in the water (Avnimelech, 1999). As found for shrimp and *tilapia*, the microbial flocs are harvested by the fish (in this paper, the term fish includes both fish and shrimp), digested and may replace a significant fraction of protein demand (Avnimelech 2007; Burford et al., 2003; McIntosh, 2000). To optimize this technology a quantitative evaluation of the microbial protein is required.

Pond experiments and the evaluation of fish responses to the presence of flocs are needed in order to obtain semi-quantitative information on the uptake and utilization of the microbial protein. However, this information is indirect and difficult to quantify. In addition, these types of experiments or farm observations demand a rather long time, usually a full growing season. Thus, it is difficult to refine the obtained information, e.g. to relate the uptake to fish age, floc size and other factors.

Several researchers followed the uptake of microbial protein through the use of ^{15}N enriched microbial biomass (Avnimelech, 2007; Burford et al., 2004; Epp et al., 2002; Preston et al., 1996). From the technical point of view, this method is well established. To obtain ^{15}N enriched microbial biomass, a portion of ^{15}N ammonium salt is added to the water. Burford et al. (2003) found that natural biota in shrimp pond water becomes enriched with ^{15}N tracer within 1 to 2 h. Avnimelech (1999, 2007) used starch addition to the water in order to enhance the ^{15}N ammonium immobilization in the microbial biomass and found negligible TAN residues 24 h after the addition of ammonium salt solution.

The determination of ^{15}N enrichment in the suspended microbial biomass and in the fish is straight forward, especially with modern automatic ^{15}N analyzers. Due to the high sensitivity of ^{15}N enrichment determinations, it is possible to run short termed experiments and to evaluate microbial protein uptake along short periods, and under variable sets of conditions.

However, the interpretation of the results is rather difficult. The system to be monitored is very complex and a series of processes affect the distribution of the tracer in the system. The added ^{15}N ammonium is readily introduced into the microbial biomass, apparently reaching an isotopic equilibrium within a relatively short

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time, due to the relative short residence time of the microbial cells in the system and the fast regeneration of new cells. However, the microbial biomass undergoes a constant process of degradation and if the whole system is not in a steady state, ammonium may be released to the solution with time. The tagged protein harvested by the fish is partially digested, and the rest is excreted as fecal material. In addition, metabolic processes in the fish lead to an excretion of ammonium (Hepher, 1988), including some of the digested tagged nitrogen. The measured enrichment of ^{15}N in the fish tissues is the difference between the gross uptake and the excretion processes, and thus represents the "net uptake", an important entity, but only part of the picture. It seems that the ratio of gross uptake to net uptake may differ under different conditions such as fish age, fish variety, environmental conditions, properties of substrate etc. Various kinds of metabolic chambers are used in studies related to nutrition of terrestrial animals (e.g. Harvard Bioscience, 2008). Using such chambers, it is possible to directly determine uptake of feed, the different routes of excretion and the accumulation of feed elements in the animal. This is not possible in nutritional studies with aquatic animals. Thus, the interpretation of the results and required computation are more complicated.

In addition to the conceptual problems, one is faced with practical experimental difficulties. Tagging with ^{15}N is quite costly, normally requiring experiments to be conducted in small enclosures (normally, <1 m^3). Biological variability, especially important in small population samples, and the need to simulate larger systems are inherent difficulties.

The goals of the present work were to further study the uptake and utilization of microbial protein by *tilapia*, using ^{15}N tagging, and to further develop the methodology of such experiments.

2. Materials and methods

2.1. Preparation of bio floc suspension

The bio floc suspension, used in the experiment was prepared in a 5 m^3 tank filled with water and stocked with 50 kg fish. The stocked fish were used just to provide relevant conditions for the experiment and were not used later on. Feed at a rate of 1.4 kg of 35% protein pellets plus 1.5 kg corn starch was added daily. Starch was added to minimize the level of mineral nitrogen species and to promote floc formation (Avnimelech and Kochba, 2006). Five hundred g clay (bentonite) was added to serve as seeds for fast bio floc formation. The tank was aerated using air lifts that had bottom and surface 90^0 elbows directing a circular water movement. Daily water exchange was limited to 10%.

Water was transferred to the experimental tanks (1 m^3 each) following 2 weeks floc suspension preparation period, when total suspended matter reached a level of about 250 mg l^{-1}.

The experimental system consisted of 5 tanks. Each tank was filled with 450 l floc suspension. Initial composition of the water in each tank is given in Table 1. Total suspended matter concentration was variable, due to separate pumping and transport of the water from the 5 m^3 tank. Two g [15]NH$_4$Cl (90% [15]N) were added to 4 tanks (# 17–20), amended with 20 g corn starch used to induce conversion of the ^{15}N to microbial protein (Avnimelech, 1999). The TAN concentrations were negligible (Table 1). Fish were stocked into four tanks, 2 days later. A fifth tank was used as a control treatment to monitor the degradation of the flocs. The "No fish" treatment was not replicated due to lack of more tanks and due to the assumption that experimental variability in this treatment is limited. This however is unfortunate and impairs the comparison with the blank treatment.

The goal of the first stage of the experiment was to evaluate the uptake, accumulation and excretion of the microbial protein when the bio flocs were the only source of feed. No external feed was added for a period of 13 days following fish stocking.

Table 1
Water characteristics during the experimental period

Tank #	DAY	Floc Volume	NH$_4$–N	NO$_2^+$– NO$_3$	Suspended organic nitrogen	Total suspended solids (TSS)	Suspended organic carbon
		ml/l	mg/l	mg/l	mg/l	mg/l	mg/l
17	1	120	0.00	0.4	12.4	338.0	167.8
	2	50	3.42	0.0	10.4	300.0	138.4
	3	45	7.20	0.5	8.0	333.7	127.9
	5	30	8.36	2.8	7.1	355.1	106.0
	7	57	0.07	6.6	5.4	370.0	126.9
	9	46	0.00	17.7	6.9	302.0	107.8
	11	23	0.14	16.5	6.2	252.0	90.3
	14	25	0.00	24.3	6.3	283.0	88.1
18	1	200	0.27	0.9	12.0	422.0	190.8
	2	70	4.15	0.0	11.6	428.8	183.5
	3	60	7.96	0.9	10.6	441.7	164.1
	5	58	6.45	8.2	5.5	437.0	131.5
	7	70	0.00	9.7	9.3	471.2	155.8
	9	43	0.02	18.5	7.4	388.0	120.2
	11	42	0.00	17.5	5.9	349.0	99.4
	14	35	0.00	29.1	6.5	386.0	103.2
19	1	55	0.00	0.9	8.2	248.4	120.4
	2	20	0.82	0.0	6.9	183.0	92.3
	3	10	3.50	0.5	6.4	153.8	75.1
	5	2.5	5.10	0.5	3.1	140.0	50.7
	7	6	0.00	0.7	4.0	162.0	76.8
	9	2	2.90	0.0	4.5	108.0	49.0
	11	2	2.73	6.1	3.2	108.3	45.2
	14	0.8	0.00	9.6	4.0	117.0	46.4
20	1	100	0.00	0.3	9.5	300.0	140.9
	2	40	2.60	0.0	8.5	322.0	141.4
	3	35	5.42	1.0	7.8	296.7	111.7
	5	37	6.81	2.9	4.6	282.0	88.4
	7	50	0.71	2.4	6.2	316.2	104.7
	9	25	0.07	11.6	6.4	255.0	87.7
	11	16	0.08	12.5	6.0	285.0	88.1
	14	18	0.00	14.2	5.3	239.0	69.6
No fish	1	200	0.00	0.4	10.7	210.0	106.4
	2	50	1.69	0.0	6.8	172.0	87.0
	3	80	3.81	0.6	5.9	318.3	69.9
	5	56	4.29	4.9	6.5	253.0	106.1
	7	40	0.00	0.0	8.3	337.0	147.7
	9	24	0.06	0.9	8.6	204.0	95.4
	11	10	0.53	5.8	2.7	100.5	39.7
	14	7.5	0.00	9.6	2.3	70.0	24.4

Water and fish were sampled during the first stage of the experiment every 2 days. Floc volume was evaluated on site using Imhoff cones (Eaton et al., 1995). A portion of the sampled water was filtered for the determination of soluble components and the rest used for the analysis of suspended matter. All samples were kept frozen.

Two fish were sacrificed at each sampling date. Fish were opened to separate the digestion tract, possibly containing suspended matter that is not necessarily digested.

2.2. Analytical methods

Floc volume (FV) was determined by sampling 1000 ml pond water into a series of Imhoff cones (Eaton et al., 1995). The volume of the floc plug accumulating on the bottom of the cone was determined 15 min following sampling. A period of 15–20 min was long enough to get a stable FV. Keeping the sample for longer periods of time led to the formation of gas bubbles in the floc plug and floatation phenomenon.

Filtered water samples were kept frozen till the colorimetric determination of TAN, NO$_2$ and NO$_3$, using an auto-analyzer, following standard methods (Eaton et al., 1995). Total suspended matter was collected over a GFA filter and dried at 105 °C and determined gravimetrically. Samples of homogenized suspension, filtered on 2.3 cm diameter GFA and pulverized samples of fish, both dried, were sent to UC Santa Barbara Marine Science Inst. Analytical Laboratory to
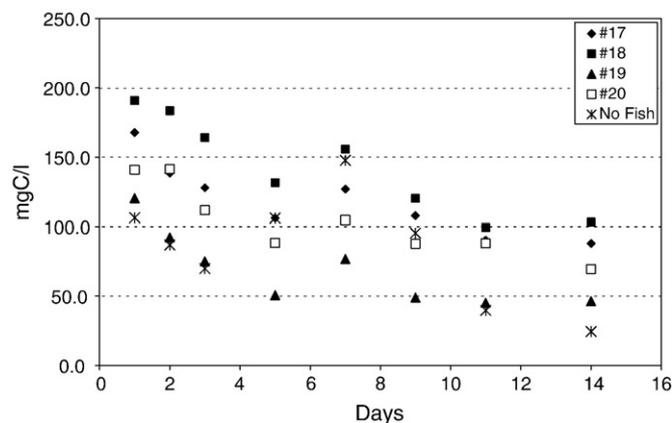
Fig. 1. Concentrations of suspended organic carbon in tank experiment. (Fish tanks No. 17–19 and blank "No fish" tank).

Fig. 3. Concentrations of suspended organic nitrogen in tank experiment (Fish tanks No. 17–19 and blank "No fish" tank).

determine total suspended carbon and nitrogen, using a CHN analyzer and ^{15}N enrichment.

3. Results and discussion

3.1. Water composition

Different levels of suspended matter (in the range of 200–400 mg l^{-1}) and suspended organic carbon (in the range of 100–200 mg C l^{-1}) were found in the different tanks at the start of the experimental period (Table 1). Since that day, when no feed was added, the organic fractions were affected by microbial degradation, harvesting by fish and a chain of reactions occurring following the excretion of feed materials by fish. Suspended organic carbon concentrations dropped steeply after one day, gradually decreasing down to stable levels following day 5 (Table 1, Fig. 1). Floc volume, as determined using Imhoff cones decreased rapidly (Table 1), at rates faster then those of TSS concentrations, leading to lower SVI (Sludge volume index, ml g^{-1}).

An increase of inorganic nitrogen species was found in all tanks (Fig. 2). The rise of inorganic nitrogen comprised an initial increase of TAN, total ammonium nitrogen, followed from day 5 onward by a drop in ammonium concentration and a subsequent rise of nitrite and nitrate (Table 1). Total inorganic nitrogen, the sum of TAN and NO$_x$–N concentrations rose linearly along the first 5 days, followed by a roughly stable, though variable concentration of total inorganic nitrogen. The changes of soluble inorganic nitrogen concentration may be affected by the processes of suspended organic nitrogen degradation and by the excretion of TAN by fish, both contributing to
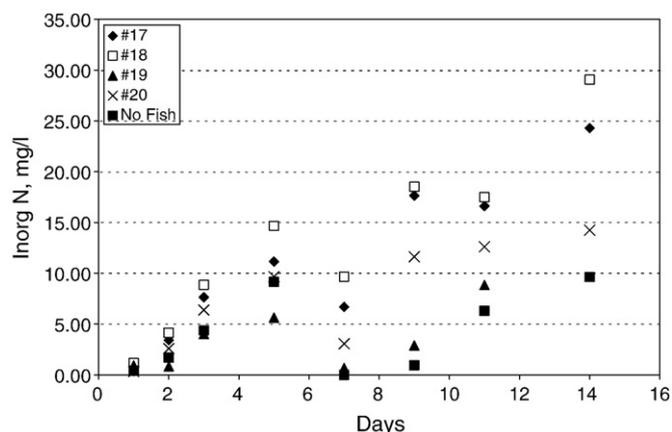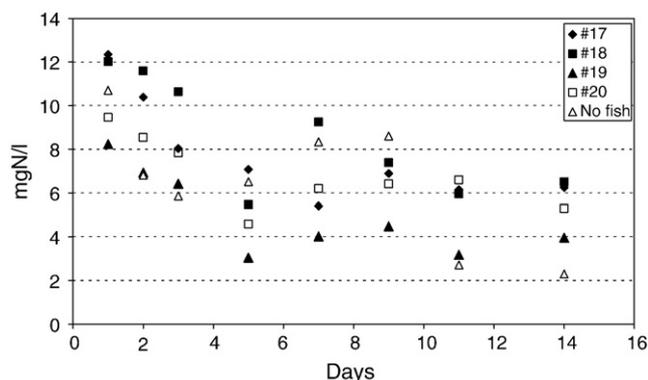
the inorganic nitrogen. The mobilization of inorganic nitrogen and possible denitrification processes, lead to the diminution of inorganic nitrogen. It seems that the two opposing fluxes reached a steady state after 5 days. Variability among the different tanks was observed following this day, probably due to the fact that the opposing processes were not identical in the different tanks.

The distribution between mineral N and suspended organic N changed with time, from the beginning of the starvation period at day 1 to day 5. While inorganic N rose, organic suspended N declined with time (Fig. 3). The rates of mineral N concentration increase (mg N l^{-1} day^{-1}) in the different tanks was linearly related to the average concentration of suspended matter in the tanks. The average rates of inorganic N rise and organic matter decline in the tanks with fish were 3.02 ± 1.04 and -1.14 ± 0.68, mg N l^{-1} day^{-1}, respectively. The rate of inorganic N production was almost 3 folds the rate of organic nitrogen degradation. The difference between the two rates can be explained by the presence of an additional source of inorganic nitrogen, namely the excretion of TAN by the fish. The corresponding changes in concentrations in the control tank without fish were 1.99 and -2.42 mg N l^{-1} day^{-1}, for inorganic N rise and organic N decline, respectively. The two rates are fairly close, as expected in this case when the rise of inorganic N is due only to the degradation of the suspended matter. A concentration rise of about 1.9 mg l^{-1} day^1 in the tanks with fish can be attributed to the excretion of nitrogen by fish. The amount of nitrogen excretion can be estimated by multiplying this concentration term by the water volume per tank (450 l) and dividing by the average weight of fish for the relevant period (2.7 kg). The estimate for that amount was found to be 310 mg N kg fish^{-1} day^{-1}.

The atom percentage of ^{15}N in the suspended matter dropped with time (Fig. 4), demonstrating a relatively fast and linear decrease along the first 5 days and slower decrease later on. This change is not trivial. Uptake of the tagged bio-flocs by fish is expected to be proportional to
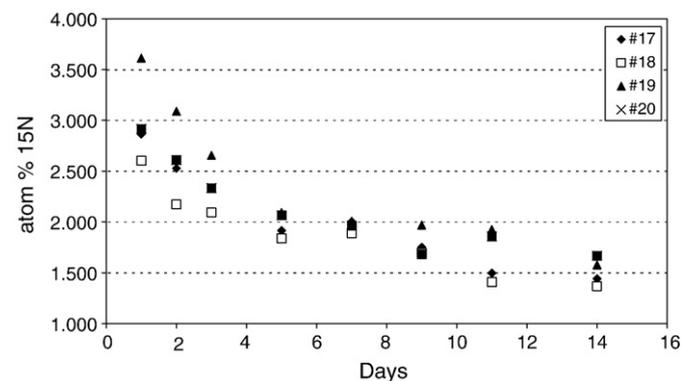

Fig. 2. Concentrations of inorganic nitrogen (TAN + NO$_x$) in tank experiment. (Fish tanks No. 17–19 and blank "No fish" tank).

Fig. 4. ^{15}N enrichment (% at ^{15}N) in suspended solids during tank experiment. (Fish tanks No. 17–19 and blank "No fish" tank).
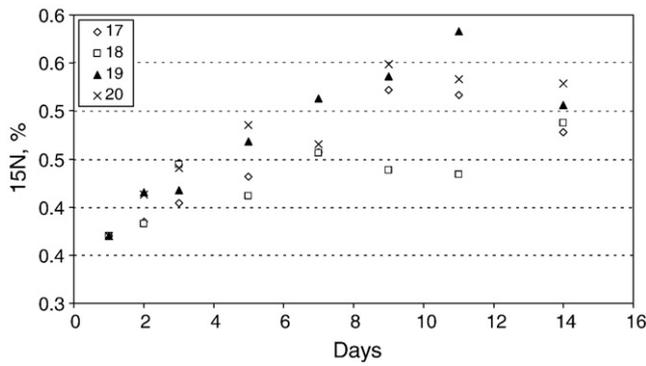
Fig. 5. ^{15}N enrichment in fish as a function of time (% ^{15}N) in the 4 fish tanks, No. 17–19.

the uptake on the non-tagged nitrogen, thus total nitrogen is expected to decline, but ^{15}N enrichment should not be affected. In a similar manner, the degradation of the suspended organic matter is not expected to significantly change the ^{15}N isotopic ratio. The decrease of ^{15}N enrichment can be attributed to the sequence of processes of ^{15}N uptake by the fish (Uptake of enriched bio flocs), the excretion of TAN by the fish, the specific enrichment of which is low, similar to the natural ^{15}N enrichment, and a subsequent immobilization of this nitrogen into fresh bio-flocs mass. The decline of the isotopic enrichment of the suspended nitrogen may enable to evaluate the dynamics and residence time of this biomass.

3.2. Uptake and excretion of nitrogen by fish

Tagged nitrogen enrichment in the fish, starting at 0.38% for the stocked fish, rose linearly to about 0.5% within a 3–7 days period (Fig. 5). Later, ^{15}N enrichment was stable or even dropped slightly. The amount of ^{15}N taken up (mg ^{15}N/kg fish) was calculated by multiplication of the percentage of ^{15}N by nitrogen contents in 1 kg fish (31.8 g).

The only sources of feed and ^{15}N uptake were the tagged bio flocs. Concentration of inorganic nitrogen in the solution was relatively low and transfer of soluble N into fish tissues is assumed to be negligible. The uptake of *total nitrogen* from the flocs can be thus calculated as ^{15}N uptake divided by the fraction of tagged nitrogen enrichment in the suspended matter (Fig. 6).

The uptake of nitrogen from the bio flocs by fish along a period of 9 days was found to follow the linear Eq. (1):

Nitrogen uptake (mg/kg fish per day) $= 87 + 242\ t$ $R^2 = 0.7314$ (1)

Where t is the time in days.

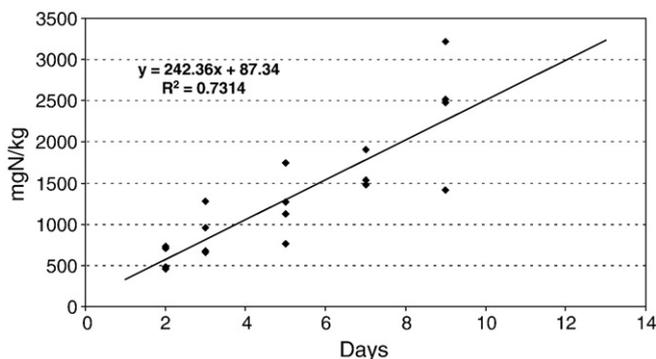**Fig. 6.** ^{15}N retained by fish (mg N kg fish^{-1}) as a function of time, averaged for the 4 replicates.

Average daily nitrogen uptake along a 9 day period, 242 mg kg^{-1}, equivalent to the daily uptake of 1.56 g protein, is about 25% of the normal protein ration given to *tilapia* (20 g of 30% protein feed per kg fish). It is interesting to note that though the ^{15}N enrichment in the fish is approaching a steady concentration at the 5 days period, total N uptake seems to continue linearly for up to 9 days. This apparent contradiction can be understood, since ^{15}N concentration in the suspended bio flocs decreases with time, and thus, more total nitrogen is taken up for any given amount of tagged nitrogen uptake.

3.3. Excretion of nitrogen by fish

Nitrogen uptake as described above represents net nitrogen uptake for a given period. Gross nitrogen uptake is higher, since a fraction of nitrogen taken up is excreted. Overall, only about 20–25% of the nitrogen applied as feed to fish is recovered in the fish upon harvest (Tabulated in Avnimelech and Ritvo, 2003). The rest is apparently excreted by the fish (Hepher, 1988).

A clear indication of the excretion of nitrogen with low ^{15}N enrichment in the present experiment is demonstrated by the decline with time of ^{15}N enrichment in the suspended particles.

Nitrogen excretion was evaluated using two models.

The first model was based upon nitrogen balance in the water. *Total nitrogen* (TN) in the water is the sum of soluble inorganic species and of nitrogen in the suspended particles. Total nitrogen in the water increased linearly with time (Table 2). The rise of TN concentrations in three of the tanks was consistently linear with relatively high R^2 values. The data for Tank #19 was not consistent. The TSS in this tank was appreciably lower than in the other tanks, probably due to some limitation of water mixing and resuspension. It is possible that suspended nitrogen settled down and was not represented in the water samplings. Change of TN with time in the tank with no fish was zero, as this tank did not have either nitrogen sources or sinks.

The nitrogen balance in the tanks is given by:

Daily TN change $=$ Daily Nitrogen excretion$-$Daily N Uptake   (2)

It has to be noted that feed was not added throughout this time, thus there is no feed addition term in Eq. (2). All fluxes in Eq. (2) are given as mg N/kg fish*day.

Daily TN changes and nitrogen uptake for each tank were inserted into Eq. (2) enabling the evaluation of the excretion rates.

Average daily *total nitrogen* (TN) increase in the water, as calculated from the slope of TN along the first 5 day period was 422 ± 195 mg N kg^{-1}, with R^2 of about 0.99, except for tank 19, where R^2 was only 0.727. Adding the daily uptake to these values, the calculated daily nitrogen excretion was 774 ± 231 mg N kg^{-1}. The computed excretion was found to be 2.2 times higher than the daily net uptake.

The second approach was based on the ^{15}N balance in the water.

Changes, with time, in the concentration (mg l^{-1}) of ^{15}N in the water are equal to:

$$\left(^{15}\text{N}\right)_t = \left(^{15}\text{N}\right)_{t-1} - ^{15}\text{N}_{\text{uptake}} + ^{15}\text{N}_{\text{excretion}} \qquad (3)$$

Where all quantities are normalized to the same concentration terms, mg l^{-1}.

Table 2
Regression parameters of total nitrogen (TN) concentrations in the water, with time (First 5 days)

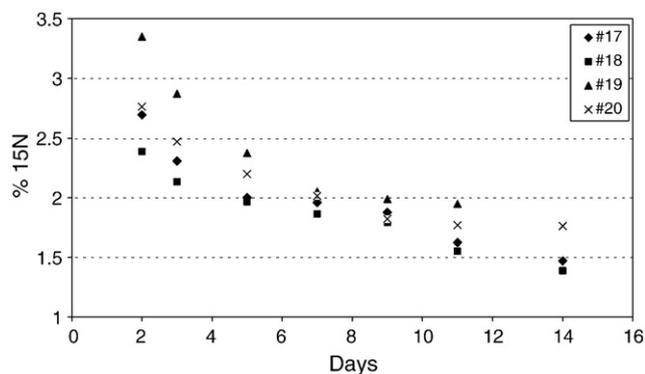| Tank #	Slope	Intercept	R^2
	Mg l^{-1} day^{-1}	mg l^{-1}	
17	1.246	10.70	0.7587
18	1.383	12.56	0.8429
19	0.284	7.38	0.218
20	0.728	9.71	0.646
No fish	−0.004	10.58	$6*10^{-6}$

Fig. 7. ^{15}N enrichment (% at ^{15}N) in nitrogen excreted by fish as a function of time in the 4 fish tanks, No. 17–19.

A conversion to fish weight base is obtained by multiplying these values by the water volume per tank (450 l) and division by the weight of fish at any given day.

We determined the percent enrichment of ^{15}N in the suspended matter, yet to calculate the terms in Eq. (3) one needs to know the ^{15}N enrichment in the *total nitrogen* fraction. To calculate this, it was assumed that the isotopic exchange among the soluble species and the suspended nitrogen species is fast enough to maintain an isotopic equilibrium at any given time. Thus, the percentage of ^{15}N in the suspended matter times the concentration of TN gives the concentration of ^{15}N in the aqueous phase. It is possible that the values obtained are higher than the true ones, due to incomplete equilibria. The ^{15}N uptake rates to be used to solve Eq. (3) are the appropriate uptake rate as described previously.

The computed daily excretion values for ^{15}N, based on the ^{15}N mass balance were highly variable. The average ratio of daily excreted ^{15}N to uptaken ^{15}N was found to be 1.1±4.25, similar to the 2.2 ratio found using the TN balances, yet with a 390% coefficient of variation as compared to 24% in the previous mode of computation. It may be concluded that the assumption of an isotopic equilibrium of nitrogen among the soluble and suspended fractions was an over simplified assumption and was not accurate enough for these computations.

Interestingly, the ^{15}N percent enrichment of the excreted nitrogen (i.e. dividing the ^{15}N excretion flux by the total nitrogen flux, both as mg l^{-1}) was found to be highly consistent and reproducible, all along the 13 days of the experiment (Fig. 7). The isotopic enrichment of the excretion fluxes is close to the isotopic enrichment of the bio flocs in equivalent dates. This seems to show that much of the excretion originated by the freshly harvested bio flocs.

3.4. Residence time of flocs

Bio flocs harvesting by fish is equivalent to the gross uptake of nitrogen, (i.e. equivalent to 3.2 times net uptake, 3.2×242 mg kg^{-1} per day), as normalized to water volume, equal to a daily harvest of 2.9 mg l^{-1} for the first 5 days of the experiment. Moreover, the computation given above did not take the microbial degradation of the bio flocs into account. The average decrease of suspended N in the tank without fish was −0.88 mg l^{-1} as compared with 1.34±0.21 in the tanks with fish. The decrease of suspended nitrogen disregarding microbial degradation can thus be approximated as equal to 0.5 mg N l^{-1}, i.e. about 17% of the suspended N harvested by the fish. All together, a daily decrease of bio flocs (given as suspended nitrogen equivalence) at a rate of 3.4 mg l^{-1} is expected.

The experimentally determined decrease of suspended nitrogen in the same period, averaged for the 4 tanks with fish is given by:

$$\text{Suspended N} = -1.34\,t + 11.34 \quad R^2 = 0.6423 \qquad (4)$$

i.e. average daily decrease of suspended N is only 1.34 mg l^{-1}, about one third of the expected decrease due to fish harvest and microbial degradation.

The meaning of this value is that though bio flocs were degraded and taken up by fish, new bio flocs were produced, using the excreted nitrogen as their nitrogen source.

These calculations enable the estimation of residence time and dynamics of the bio flocs. It was shown here that the concentrations of the bio flocs in the water are about 3 times higher than expected, considering the harvesting of bio flocs by fish and microbial degradation of the bio flocs. The percentage of young microbial cells produced and forming new bio flocs is about 70% of total bio flocs and the residence time of the bio flocs is around 8 h. This is an indication of the high dynamics of the system.

The above values are only a first estimate. A limitation in the above calculation is the fact that there was only one tank without fish. Moreover, it is quite possible that microbial degradation of bio flocs is affected by the presence of fish.

The bio floc nature changed with time, as bio flocs become denser with time, expressed by a lower sludge volume index (SVI, ml g). The nature of the newly formed flocs is probably affected by changes of nutrients in the system.

4. Conclusions

The present work is based on monitoring of changes in the distribution of nitrogen and ^{15}N in water, suspended matter and fish in a tank experiment, where bio flocs were the only source of feed to the fish. The objectives of this work were to get data on the dynamics of the bio floc system and to critically evaluate the available experimental procedures.

The evaluation of net uptake of the tagged material, through the determination of tagged nitrogen accumulation in the fish is relatively straight forward and yields consistent results. In the present study it was found that the daily net uptake of microbial protein by *tilapia* from a bio floc suspension of about 200 mg l^{-1}, amounted to 242 mg N kg^{-1}, equivalent to the daily uptake of 1.56 g protein, about 25% of the normal protein ration given to *tilapia*. This is a significant contribution to fish feeding in such systems. This result is a bit lower than that found in an earlier study (Avnimelech, 2007), but is similar to results obtained with shrimp, where 18–29% of protein retained by the shrimp was supplied by the natural biota (Burford et al., 2004).

The net uptake, though an important parameter, is just a part of the processes involved. Part of the protein taken up by fish is excreted, possibly at different rates under different conditions. Evaluating the gross uptake, or experimentally the amount of excreted nitrogen of tagged and total N is needed to evaluate the efficiency of different food sources. These can be evaluated based upon the mass balances of either total N or ^{15}N. In both cases, the amount of excreted nitrogen is obtained as the difference between measured values (total and up-taken fractions). However parameters obtained as a difference, especially as a difference between two large numbers suffer from high experimental error.

To minimize these effects, the present experiment was designed so as to minimize the concentrations of all soluble inorganic nitrogen species, by dosing a high starch diet during the preparatory part of the experiment. Due to the low background noise, changes in inorganic nitrogen were clearer. This approach is highly recommended for future studies. The evaluation of ^{15}N excretion was more complicated. It was assumed that an isotopic equilibrium exists among the organic particulate forms and the soluble N species. This assumption seemed to hold in the system under study, where flocs are in dynamic exchange with the solution, yet it was concluded that the time scale to get an isotopic equilibrium is too long for the validity of the above assumption in this case. It is possible, though not trivial, to overcome this problem by measuring ^{15}N enrichment in the soluble N pool in addition to the suspended pool.

It was found that nitrogen excretion is about twice as high as the nitrogen accumulating and detected in the fish tissues. This is in accordance with many data on the accumulation of feed protein in fish, about 25% of protein supplied in the feed (e.g. Tabulated in Avnimelech and Ritvo, 2003). Moreover, this result shows that actual bio floc harvesting by the fish was about 3 times higher than that estimated.

An interesting outcome of the present work is the estimate regarding residence time of bio flocs. Bio flocs are constantly harvested and degraded. Yet, new flocs are produced using the nitrogen excreted by the fish. Under the experimental conditions existing in the present experiment, it was estimated that the residence time of bio flocs was about 8 h, i.e. bio flocs were regenerated 3 times a day. This process could most probably be controlled through the addition of organic substrates (e.g. starch) to the pond.

It can be concluded that important information on bio floc dynamics in ponds, as well as other aspects of pond dynamics can be obtained if ^{15}N and untagged nitrogen fractions are monitored and carefully analyzed.

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