Nutrient Retention by Fish in a Multispecies Recirculating Aquaculture Facility

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ABSTRACT

The nutrient content (nitrogen and phosphorus, N and P) of the dry weight gain of fish relative to N and P content of the dry weight of feed was used to determine nutrient retention in five species of fish that were reared in a commercial recirculating aquaculture facility. The culture system had five 39.2 m^3 dual-drain culture tanks, one tank each with largemouth bass (*Micropterus salmoides*), hybrid striped bass (aka sunshine bass, *Morone chrysops* x *Morone saxatilis*), and rainbow trout (*Oncorhyncus mykiss*), and two tanks with walleye (*Sander vitreus*). All fish were exposed to the same water temperature (15.8 - 24.1°C) and water quality. On the first day of the study, most rainbow trout (643 g) and walleye (497 g and 398 g) were at or near market size, whereas the largemouth bass (73 g) and hybrid striped bass (96 g) were fingerlings. Measured for a 56-d interval, the range in nutrient retention was 12.0 to 44.1% for N, and 14.8 to 53.8% for P. Nutrient retention was related to fish species and size; e.g., the larger size-group of walleye had nearly half the retention rates of the smaller size-group of walleye. Highly significant ($p \leq 0.01$) positive correlations occurred between retention of

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N and P, protein efficiency ratio, and net protein utilization, but nutrient retention was inversely related to food conversion ratio. Total ammonia nitrogen (g kg^{-1} feed fed) in the culture tank was inversely related to nitrogen retention. Values for TAN production ranged from 2.9 to 6.9% of daily feeding rate. This study demonstrated an interaction between nutrient retention with fish species, age or size, growth rates, temperature, feeding rates, nutrient content of the feed, and protein retention, all of which are factors that influence biofilter capacity to handle ammonia production and unit processes to reduce N and P content in the effluent.

INTRODUCTION

The nutrient composition of fish feed and its utilization by fish species in water reuse aquaculture systems has a major influence on effluent concentrations of nitrogen (N) and phosphorous (P), which may cause eutrophication in the aquatic environment (Cowey and Cho 1991, Tomasso 2002). Nutrient content of the water supply, endogenous loss from fish metabolism, fish feces, and uneaten feed are sources of nitrogen and phosphorus in effluents of aquaculture. Dietary protein supplies amino acids for energy and protein synthesis (i.e., growth), and serves as the major source of nitrogen in fish hatcheries and the effluents they discharge. Likewise, fish feeds with indigestible P (phytate in plant feed stuffs) or more P in the feed than needed for growth contribute P to the effluent, yet there is only limited information available on phosphorus retention in fish (Lall 1991). In recirculating aquaculture systems, indigestible feed ingredients also increase the solids in the discharge. Thus, optimized diet formulation and feeding practices are essential components of Best Management Practices for all types of fish culture operations, but are especially critical for water reuse aquaculture (WRA).

The engineering design of a WRA system depends on characteristics of the fish species: its size and growth, feeding rates and feed conversion, and stocking and harvesting strategies that are related to the production cycle of the fish as well as marketing issues. Realistic design guidelines must include biofilter capacity to handle ammonia production and unit processes to reduce the N and P content of the effluent. Controlling N and P in the effluent demands an understanding of nutrient retention (the amount of nutrients incorporated into fish flesh) relative to nutrients provided in the feed. Maximizing the retention of N and P by cultured fish can provide numerous benefits for operators. Poor nutrient retention may result from poor feed formulation (i.e., indigestible ingredients, inadequate protein/energy ratio), over-feeding, or reduced feeding activity that may be related to adverse environmental conditions or disease. From a production standpoint, fish that more efficiently utilize nutrients in feed require less feed to reach a marketable size, saving the operator money and increasing the profitability of the enterprise. From a regulatory and ecological standpoint, optimizing nutrient retention assists in the prevention of excessive nutrient loading in culture effluents (Jahan *et al*. 2003).

The objective of this study was to describe nutrient retention in an operating, small-scale commercial WRA facility that had a very low water consumption. The daily inflow of water was only 1.6% of total system volume. The entire system volume was exchanged with makeup water only once every 62 days (Summerfelt and Penne 2007). The operator simultaneously cultured largemouth bass (*Micropterus salmoides*), walleye (*Sander vitreus*), hybrid striped bass (aka sunshine bass, *Morone chrysops* x *Morone saxatilis*), and rainbow trout (*Oncorhynchus mykiss*) in separate tanks, but with a single integrated system with components used in common.

The nutrient content of the dry weight gain of fish relative to the nutrient content of the dry weight of feed added into each tank of fish was used to determine nutrient retention during a 56-d interval. This was not a study of fish nutrition and it was not carried out in a controlled laboratory environment using a single species of fish. Further, we did not control the feeding or stocking of the system; however, we had the advantage of having intensively studied the performance of the system (Summerfelt and Penne 2005, 2007). The findings of this study show the scope of nutrient retention values and suggest relationships to fish age or size, growth rates, temperature, feeding rates, and nutrient content of the feed, which are values useful for design and development of performance-based environmental standards for recirculating aquaculture operations.

MATERIALS AND METHODS

Recirculation system

The system had five 39.2 m^3 dual-drain culture tanks as described by Timmons *et al*. (1998) and Summerfelt *et al*. (2000). Each culture tank had a high-volume, low-solids effluent from a side-wall drain (78.7% of flow) and a low-volume, high-solids effluent from the center drain (21.3% of flow). Water leaving the five tanks through their sidewall drain flowed directly to the sump where two 7.5 hp (5.6 Kw) electric centrifugal pumps lifted water to a fluidized sand bed biofilter. From the biofilter, flow was sent through a multi-staged Low Head Oxygenator™ (PR Aqua, Nanaimo, BC, Canada) and then to a head tank before returning to the culture tanks. Flow from the center drain carried most of the suspended solids to an

Table 1. Mean of fish weight, total tank biomass (B, kg/tank), density and loading.

Tank	Species[1]	Fish weight (g)	Number of fish	Biomass kg	Biomass (%)	Density (kg m^{-3})	Loading[2] (kg m^{-3} min^{-1})
May 21, start of study							
1	LMB	73	9,946	726	(13.4)	18.5	926
2	WYE	497	351	174	(3.2)	4.4	222
3	HSB	96	9,865	947	(17.5)	24.2	1,208
4	WYE	398	3,586	1,427	(26.4)	36.4	1,820
5	RBT	643	3,324	2,137	(39.5)	54.5	2,726
		Totals	27,072	5,411	(100)	--	--
				Means		27.6	1,380
July 15, end of study							
1	LMB	92	4,305	396	(8.5)	10.1	529
2	WYE	552	329	182	(3.9)	4.6	243
3	HSB	120	7,404	888	(19.0)	22.7	1,187
4	WYE	441	3,058	1,349	(28.9)	34.4	1,803
5	RBT	660	2,809	1,854	(39.7)	47.3	2,479
		Totals	17,905	4,669	(100)	--	--
				Means		23.8	1,248

[1]Abbreviations: LMB, largemouth bass; WYE, walleye; HSB, hybrid striped bass; RBT, rainbow trout. [2]Loading expresses fish biomass (kg) relative to inflow of water to the culture tank (m^3 min^{-1}).

external triple standpipe (TSP) in which a greater part of the flow went to the DF (microscreen drum filter, Water Management Technologies, Inc., Baton Rouge, LA, USA) and a small intermittent flow of heavy solids was diverted to the septic tank. Water cleaned by the DF entered the sump while the backwash was discharged into a septic tank located exterior to the building. The culture tanks comprised 78.5% of the volume, while the plumbing and treatment components made up the balance (21.5%). Solids removal was accomplished by partitioning of solids into the culture tank's center drain flow and the subsequent capture in the quiescent zone of a TSP and by the 60 µm mesh of the DF. Recirculating flow to the culture tanks (0.78 m^3 d^{-1}) provided approximately 1.2 exchanges h^{-1}. During this study, daily inflow averaged 3.9 m^3 d^{-1} or 1.6% of total system volume.

Water quality

Water temperature, pH, and dissolved oxygen (DO) were measured daily with calibrated meters in each culture tank. Alkalinity (as $CaCO_3$), total ammonia nitrogen, NH_3-N (TAN), total phosphorus (TP), total dissolved solids (TDS), and total suspended solids (TSS) were measured in each culture tank at the start of the study and at bi-weekly intervals. Alkalinity measurements were performed using titrimetric methods with a colorimetric end point (APHA 1998). Biochemical oxygen demand (BOD) was determined by incubation at 20°C for 5 days (APHA 1998). Samples were analyzed for total dissolved solids (TDS) and total suspended solids (TSS). TAN was determined by the Nessler method and TP by the ascorbic acid method following manual digestion.

Fish stocks

The producer cultured four species of fish in five tanks (Table 1). Largemouth bass and walleye were marketed as stockers for fish enhancement or as food fish. Rainbow trout and hybrid striped bass were marketed exclusively as food fish. On the first day of the study, most of the rainbow trout (RBT, 643 g), and walleye in tank 2 (WYE, 497 g), were considered market size whereas the largemouth bass (LMB, 73 g) and hybrid striped bass (HSB, 96 g) were fingerlings.

Group and individual weights of samples of fish were obtained bi-weekly. Ten fish were collected from each tank in the first two samples, and 20 fish were collected in the last three samples.

Mean standing stock of fish in each tank varied substantially over the course of the study (Table 1). The average of initial and final stock was 5,040 kg, density 25.7 kg m^{-3}, and loading 1,314 kg m^{-3} min^{-1}. The mean standing stock consisted of 561 kg (11.1% of total) largemouth bass, 1,566 kg (31.1%) walleye, 918 kg (18.2%) hybrid striped bass, and 1,996 kg (39.0%) rainbow trout.

A proximate analysis of fish carcass and feed samples was performed by a commercial laboratory (Minnesota Valley Testing Laboratory, New Ulm, MN, USA).

Feed and Feeding

Feeds were commercial feeds, high in total phosphorus (TP) and total protein (TKN x 6.25) (Table 2). Feed types and sizes were selected by the farmer as appropriate for the fish size and species: largemouth bass and hybrid striped bass were fed Silver Cup™ brand (Silver Cup Fish Feeds, Murray, UT, USA) steelhead feed (502 g kg^{-1} protein, 16 g kg^{-1} P); walleye were fed Silver Cup™ brand salmon pellets (545 g kg^{-1} protein, 18 g kg^{-1} P); and rainbow trout were fed Silver Cup™ brand trout pellets (467 g kg^{-1} protein, 15 g kg^{-1} P). Feed samples were analyzed for moisture, total phosphorus (TP), Kjeldahl nitrogen (TKN), and total fat by the same commercial laboratory used for fish carcass analysis (Table 2). The non-protein nitrogen, which was 0.34%, was not added to the total nitrogen content of feed fed as it was not relevant to the measurement of net protein utilization (NPU).

Feed added to each culture tank was recorded by the owner-operator. The tabulated values were used to calculate the total quantity of feed fed. Overall, feeding rates (kg of feed fed per day as a percent of estimated tank biomass on the same day) ranged from 0.15 to 0.82 (Table 4). The lowest feeding rates were for rainbow trout (tank 5) and walleye (tank 4) and the highest rates were fed to hybrid striped bass (tank 3). Differences in feeding rates were related to fish size, market status, and water temperature; thus, rainbow trout (tank 5) and walleye in tank 4 were both at a harvestable size and required only maintenance levels of feeding;

Table 2. Nutrient content of fish feeds.

Tank	Species[1]	Crude Protein (Total N)	Total	Moisture	Lipid[2]	Ash
As-fed basis (%)						
1	LMB	46.0 (7.36)	1.47	8.43	15.1	9.0
2	WYE	48.8 (7.81)	1.61	10.50	12.6	9.1
3	HSB	46.0 (7.36)	1.47	8.43	15.1	9.0
4	WYE	48.8 (7.81)	1.61	10.50	12.6	9.1
5	RBT	42.1 (6.74)	1.36	9.81	12.4	9.0
Dry weight (%)						
1	LMB	50.2 (8.03)	1.61	--	16.5	16.5
2	WYE	54.5 (8.72)	1.80	--	14.1	10.2
3	HSB	50.2 (8.03)	1.61	--	16.5	16.5
4	WYE	54.5 (8.72)	1.80	--	14.1	10.2
5	RBT	46.7 (7.47)	1.51	--	13.8	10.0

[1]See Table 1 for species names.
[2]Ether extract

also, feeding rates for rainbow trout were reduced because of a high relative water temperature.

Nutrient Retention

Nutrient retention was based on analysis of the nutrient content of the dry weight of feed added and nutrient content of the dry weight of the biomass gain (growth, B_g) of the fish. With no mortality, or additions or removal of fish for marketing, biomass dry weight gain (B_g) in each tank of fish equals:

$$B_g = S_f\,(\overline{w}_f - \overline{w}_i) \qquad \text{(Equation 1)}$$

where: B_g = biomass (dry weight) gain during study

S_f = stock number at end of study

$\overline{w}_f$ = mean biomass (dry weight) of fish at end of study

$\overline{w}_i$ = mean biomass (dry weight) of fish at start of study

Table 3. Harvest and mortality of fish during 56-d study interval. Percent is of initial stock of each species.

Tank	Species	Harvest Number	(%)	Mortality Number	(%)
1	Largemouth bass	5,626	(56.6)	15	(0.2)
2	Walleye	20	(5.7)	2	(0.6)
3	Hybrid striped bass	2,452	(24.9)	9	(< 0.1)
4	Walleye	501	(13.9)	27	(0.8)
5	Rainbow trout	399	(12.0)	116	(3.5)
	Total	8,998	(33.3)	169	(0.6)

Fish population numbers changed as a result of harvest and mortality (Table 3), thus, the final stock (S_f) was always expected to be less than the number present at the start of the study. Overall, mortality was trivial; only 0.6% of initial stock. The major changes in fish stock were the result of harvest (33.3%), mainly from a large harvest of largemouth bass (5,626 fish) and hybrid striped bass (2,452 fish).

In most cases, fish that were harvested were weighed at that time, but fish that died were not weighed. To adjust for total biomass gain (dry weight) of fish that died or were harvested required an estimate of their weight on the day they died or were removed. The weight of the fish that died or were removed during the study was estimated from a regression equation of mean fish weight (Y-axis) against day of the study (X-axis).

The biomass gain of fish in each tank from the first day to the day the fish was removed was calculated using equation 2:

$$B_{gr} = N_{rt} (\overline{w}_i - \overline{w}_{rt}) \qquad \text{(Equation 2)}$$

where: N_{rt} = number of fish that were removed (r) on day (t)

$\overline{w}_{rt}$ = mean weight of fish that died or were removed on day (t)

Table 4. Protein efficiency, net protein utilization, feed conversion, and growth (means ± SE). Means in each row followed by different letters differ at the 0.05 probability level by Fisher's least-significant-difference procedure (Steel and Torrie 1980).

Tank	Species[1]	Total feed fed (kg)	FR[2] (%)	Total biomass gain[3]	PER[4]	NPU[5]	FCR[6]	SGR[7]
1	LMB	97	0.39	108.2	2.43^{a} ± 0.61	39.55^{a} ± 8.85	0.63^{c} ± 0.56	0.41^{a} ± 0.01
2	WYE	49	0.49	18.8	0.79^{b} ± 0.05	16.00^{b} ± 0.81	2.61^{b} ± 0.16	0.18^{b} ± 0.01
3	HSB	394	0.82	185.5	1.03^{b} ± 0.13	15.95^{b} ± 1.65	$2.14^{a,b,d}$ ± 0.27	0.39^{a} ± 0.03
4	WYE	181	0.24	140.6	1.60^{a} ± 0.12	36.35^{a} ± 7.55	$1.29^{c,d}$ ± 0.09	0.18^{b} ± 0.00
5	RBT	161	0.15	51.4	0.76^{b} ± 0.09	13.35^{b} ± 1.65	3.13^{a} ± 0.36	0.04^{c} ± 0.01
				***P*-value of ANOVA**	0.04	0.04	0.02	<0.01

[1]*See Table 1 for **species name***

[2]***FR**, feeding rate = (mean kg feed fed per day/mean kg fish biomass) x 100*

[3]***Wet weight** (kg)*

[4]***PER**, protein efficiency ratio = kg wet weight gain/kg protein in feed as fed.*

[5]***NPU**, net protein utilization = (kg dry weight protein gain by fish/kg dry weight protein fed) x 100.*

[6]***FCR**, feed conversion = kg feed fed in interval/kg gain.*

[7]***SGR**, specific growth rate (%/d) = $(lnw_f - lnw_i) / t \times 100$, where lnw_f = natural log mean fish weight at end of interval, where lnw_i = natural log mean fish weight at start of interval, and t = length of interval.*

Therefore, the adjusted biomass gain of the total tank population was equal to:

$$B_g = S_f (\overline{w}_i - \overline{w}_f) + B_{gr} \qquad \text{(Equation 3)}$$

Nutrient retention was calculated by dividing the kg of N and P gained (dry weight of fish) by the kg of N and P fed (dry weight of feed) and multiplying by 100. The amount of N and P (N_g) gained was derived by multiplying the dry weight of biomass gained by percent N and P in the fish carcass (equation 4):

$$\% \text{ Nutrient retention} = (N_g / N_f) \times 100; \qquad \text{(Equation 4)}$$

where: $N_g = B_g \times B_n$

$N_f = FF_t \times F_n$

N_g = nutrient (N or P) gain in dry weight (kg) of fish

N_f = dry weight (kg) of nutrients (N or P) fed

B_g = biomass gained (dry weight, kg)

B_n = nutrient content of fish (ratio of N or P content per 100 g of fish)

FF_t = total feed fed (dry weight, kg)

F_n = nutrient content of feed (ratio of N or P content per 100 g of feed)

Ammonia excreted by fish per kg of feed fed was calculated using equation 5 (Lawson 1995):

$$\text{TAN(g kg}^{-1}\text{ feed)} = \text{(1.0-NPU)(protein content of feed/6.25)(1000)}$$

$$\text{(Equation 5)}$$

Lawson (1995) defined NPU, net protein utilization, as the ratio = kg dry weight protein gain by fish kg^{-1} dry weight protein added in feed.

Table 5. Fish carcass composition: Values are means (± SE) of five samples collected on day 1, 15, 28, 41, and 55 (end of study).

		Fresh weight (%)				*Dry weight (%)*		
Tank	**Species[1]**	**Protein**	**TN[2]**	**TP[2]**	**Moisture**	**Protein**	**TN[2]**	**TP[2]**
1	LMB	16.20^{a} ± 0.46	2.59^{c} ± 0.07	0.56^{a} ± 0.10	69.79^{a} ± 0.60	53.61^{a} ± 0.92	8.58^{a} ± 0.15	$1.84^{a,c}$ ± 0.31
2	WYE	18.08^{b} ± 0.48	2.89^{b} ± 0.08	$0.76^{a,b}$ ± 0.06	72.48^{b} ± 0.54	65.72^{b} ± 1.27	10.53^{b} ± 0.21	$2.74^{a,b}$ ± 0.16
3	HSB	$15.54^{a,c}$ ± 0.31	$2.48^{c,d}$ ± 0.05	0.43^{a} ± 0.07	64.45c ± 0.51	43.74^{c} ± 1.00	6.99^{c} ± 0.16	1.20^{c} ± 0.19
4	WYE	18.48^{b} ± 0.53	2.96^{a} ± 0.08	1.13^{b} ± 0.23	71.94^{b} ± 0.57	65.97^{b} ± 2.39	10.57^{b} ± 0.39	4.08^{b} ± 0.89
5	RBT	$16.34^{a,c}$ ± 0.65	$2.62^{c,d}$ ± 0.10	0.71^{a} ± 0.12	69.24^{a} ± 1.14	53.25^{a} ± 1.97	8.52^{a} ± 0.32	$2.30^{a,c}$ ± 0.58
	***P-value*[3]**	< 0.01	0.01	< 0.03	< 0.01	< 0.01	< 0.01	< 0.01

[1]*See Table 1 for species name*

[2]*Total nitrogen (TN) and total phosphorus (TP)*

[3]*P-value from Analysis of variance (ANOVA). Column means that have a superscript letter in common are not significantly (≤ 0.05) different (Fisher's protected least significant difference multicomparison test).*

RESULTS

Feeding, feed conversion, and growth rates

Total quantity of feed fed to the four species during the study ranged from 49 to 394 kg (Table 4). Species differences in feeding rate were related to fish size and readiness for market; e.g., the owner reduced the feeding rate for large, market-size rainbow trout and walleye, which were held at a higher density. Larger fish had higher FCR values (i.e., poorer feed conversion); for example, largemouth bass had the lowest mean weight (83 g) and lowest FCR (0.63) and RBT the largest size and highest FCR (3.13). Walleye and HSB had similar FCR values that were between those of RBT and LMB (Table 4). There was also a statistically significant difference in protein efficiency (PER) among tanks of fish. The PER values ranged from 0.76 to 2.43 (Table 4). Largemouth bass and WYE tank 4 had significantly ($p < 0.05$) higher PER than all other tanks.

Table 6. Nitrogen and Phosphorus retention (NR and PR) of cultured fish as percent of N and P fed (kg values are dry weight).

		Nutrient Retention						
Tank	**Species[1]**	**Sum of N fed (kg)**	**Sum of N gained (kg)**	**NR (%)[2]**	**Sum of P fed (kg)**	**Sum of P gained (kg)**	**PR (%)[2]**	**TAN[3]**
1	LMB	7.17	3.16	44.07[a] ± 12.91	1.44	0.68	47.22[a,b] ± 18.47	28.6[a] ±14.71
2	WYE	3.61	0.49	13.57[b] ± 0.58	0.73	0.13	17.81[a] ± 1.12	69.2[b] ±1.71
3	HSB	28.94	4.55	15.72[b] ± 1.13	5.40	0.80	14.81[a] ± 1.64	50.6[b,c,d] ±5.03
4	WYE	14.13	4.07	28.80[a,b] ± 4.74	2.92	1.57	53.77[b] ± 22.41	47.3[a,d] ±10.90
5	RBT	10.85	1.30	11.98[b] ±1.27	2.19	0.35	15.98[a] ± 4.60	57.5[a,b,c] ±3.13
P-value of ANOVA				< 0.01			< 0.05	0.06

[1]See Table 1 for long form name of species.
[2]The standard errors (±) are of calculations for four intervals: 1-15 d, 16-28 d, 29-42, and 43-56.
[3]TAN (g kg^{-1} feed) calculated from NPU and protein content of feed with formula 5 in text.

Fingerling hybrid striped bass (HSB) and LMB had the fastest specific growth rates (SGR), and WYE (tanks 2 and 4) and RBT had the slowest growth rates (SGR, Table 4).

Carcass Composition

Moisture content among the cultured species ranged from 64.5 to 72.5% (Table 5). The moisture content of WYE was significantly higher than the other species, but on a dry weight basis, both tanks of WYE had higher levels of protein than the other species. HSB had a lower protein content than any other species (Table 5).

Nutrient retention

Nitrogen retention by LMB (44.1%) was substantially higher than any other species, although not significantly different from walleye in tank 4 (Table 6). Phosphorus retention for WYE tank 4 (53.8%) was not different from LMB, and both were greater than WYE in tank 2, RBT, and HSB.

*Table 7. Correlation coefficient (r-value) matrix (10 observed values) between nitrogen (NR) and phosphorus (PR) retention with total N and P content in feed fed, FCR, PER, NPU, TAN, RG and SGR[1]. Significance (p-value) of r was calculated with Fisher's r to Z; *p-value ≤ 0.05; **p-value ≤ 0.01.*

	Nitrogen retention	**Phosphorus retention**
Nitrogen fed	-0.22	-0.23
Phosphorus fed	-0.23	-0.23
Nitrogen retention (NR)		0.86**
Phosphorus retention (PR)	0.86**	
Feed conversion ratio (FCR)	-0.96**	-0.80**
Protein efficiency ratio (PER)	0.99**	0.79**
Net protein utilization (NPR)	0.96**	0.88**
Ammonia excretion TAN (g kg^{-1} feed)	-0.92**	-0.78**
Specific Growth Rate (SGR)	0.50	0.17

[1]*See text for definitions and formulas.*

		Feed as-fed		Fish		
Species	**Diet**	**N (%)**	**P (%)**	**N ret. (%)**	**P ret. (%)**	**Reference**
Largemouth bass	Commercial	7.4	1.5	44.1	47.2	Present study
Walleye (tank 2)	Commercial	7.8	1.6	13.6	17.8	
Hybrid striped bass	Commercial	7.4	1.5	15.7	14.8	
Walleye (tank 4)	Commercial	7.8	1.6	28.8	53.8	
Rainbow trout	Commercial	6.7	1.4	12.0	16.0	
Rainbow trout	HP 300 control	7.4	0.6	33.2	36.5	Vielma *et al*. 2002
	Soycomil P control	7.4	0.6	37.4	28.5	
Atlantic salmon	Fishmeal	8.1	1.8	45.9	27.7	Storebakken *et al*. 2000
	Soy protein	7.9	1.2	46.1	27.6	
Rainbow trout	Fishmeal	-	1.2	-	34.1	Johnson & Summerfelt 2000
	Fishmeal & spray-dried blood cells	-	0.9	-	42.0	
Turbot	Fishmeal	8.5	1.8	30.4	32.9	Burel *et al*. 2000
Tilapia hybrid	Commercial tilapia feed	5.4	0.8	21.4	18.8	Siddiqui and Al-Harbi 1999
European sea bass	Pelleted	7.8	2.1	24.7	20.1	Ballestrazzi *et al*. 1998
	Extruded	7.8	2.1	24.7	19.6	
Rainbow trout	Experimental diets	-	0.85-1.60	-	14-22	Ketola and Harland 1993

Table 8. Nitrogen and phosphorus retention levels from selected studies.

There was a highly significant ($p \leq 0.01$) positive correlation between nitrogen retention (NR) and phosphorus retention (PR), and both showed significant correlations (both + and -) with several other variables (Table 7). High positive correlations occurred between nutrient retention and protein efficiency ratio (PER), and nutrient retention and net protein utilization (NPU). There were also strong negative correlations with nutrient retention and both feed conversion ratio (FCR) and ammonia excretion (TAN). The correlations for either NR or PR with nitrogen and phosphorus fed, or either NR or PR with measures of growth were not significant ($p \leq 0.05$). There is obvious potential for autocorrelation between NR and PER and NR and NPU because of the relationship between N and protein content of the feed, as protein content is 6.25 times TKN. The relationship between NR and TAN reflects the inverse relationship between ammonia excretion and NR.

DISCUSSION

Published values for both N and P retention show substantial intra- and interspecies variation (Table 8). For example, three publications cited for RBT fed 0.6 to 1.6% dietary P had PR from 14 to 42% (Table 8), comparable to the results of the present study for RBT. It is likely that differences in bioavailability of phosphorus and phosphorus content of the feed in excess of requirements have a strong influence on relative retention. Nitrogen retention of pond-cultured channel catfish (*Ictalurus punctatus*) fed feeds manufactured from high quality ingredients ranged from 20 to 30% and phosphorus 25-35% of the N and P in the feed (Tucker *et al*. 2008).

Compared with other studies (Table 8), the N and P retention values for LMB were high, but about equal to values for Atlantic salmon (*Salmo salar*) (Storebakken *et al*. 2000). The LMB in the present study had an average PER value of 2.4% compared with a value of 1.7% reported by Portz *et al*. (2001). They found the best SGR values of 0.66 to 0.70 for a diet with 46.0 to 50.0% crude protein (CP), which compared with a mean SGR value of 0.41 in our study using a feed with 46% CP.

Brown *et al*. (1992) reported a PER value of 1.2% at 50% CP for small (2.7-11.4 g) HSB, similar to the 1.3% for HSB in the present study for fish with average weight of 108 g, fed a feed with 50.2% CP.

Juvenile RBT (98 to 216 g) fed a commercial diet 48.4% CP had nitrogen retention of 37.3% (Vielma *et al*. 2002), substantially greater than the value of 12.0% for the much-larger RBT (643 to 651 g) in the present study. Such large differences are likely related to differences in size and growth rates of the fish between the two studies. Vielma *et al*. (2002) reported 29.7% P retention for a commercial feed with 1.36% total P compared with the 14.8% P retention we calculated for commercial diet with 1.51% total P. Although the effects of age or size on digestibility of feed ingredients has not been well addressed in fish (Gallagher 1997), Ronsholdt (1995) noted that the phosphorus content of rainbow trout decreased with increasing weight. However, the association between fish size and nutrient retention is dependent on whether the comparison is for fish length or weight. The findings of Ketola (1991) show that the phosphorus requirement for bone mineralization (i.e., growth in length) is higher than that for body weight gain. The N and P retention values of LMB were comparable to values reported for Atlantic salmon, but substantially higher than that reported for RBT, hybrid tilapia or European sea bass (Table 8).

The range in water quality variables was always within a desirable range of values suitable for intensively cultured fish: DO 6.8, pH 7.2, alkalinity 61 mg/L, BOD 9.4 mg/L, TAN 0.65 mg/L, TP 24.5 mg/L, TDS 1,615 mg/L, and TSS 49.5 mg/L.

Although environmental temperature within the species' normal range for growth has little effect on digestibility (National Research Council 1993), Kibria *et al*. (1998) reported that environmental temperature closest to the optimal temperature produced significantly higher retentions of both P and N in silver perch (*Bidyanus bidyanus*). In the present study, water temperatures ranged from 15.8-24.1°C with a mean of 19.9°C. The four species cultured in our study represented cold-, cool- and warmwater categories, each with different optimal temperatures for growth. Largemouth bass, a warmwater species, have optimal growth at temperatures above 25°C (Stickney 1994). Walleye and HSB are considered coolwater species that have optimal growth between 15 and 25°C, which suggests that, all other things being equal, HSB and the smaller size group of WYE would exhibit the highest relative growth and nutrient retention because the water temperature stayed predominantly in the coolwater range. The smaller size group of WYE did show the highest P retention and second highest N retention of the 5 groups of fish studied. Although the temperature for LMB was less than their expected optimum, they nevertheless had faster growth, and displayed the second highest P retention and the highest N retention. The HSB had the lowest P retention and the median N retention of the fish cultured. In our study, growth (SGR) of RBT was significantly lower than that of the other species, which seems related to both their slow growth rate and the negative effect of relatively high water temperature (24.1°C), although less than the 28°C threshold for mortality of rainbow trout (Hardy *et al*. 2000), it was near the 25°C upper limit for growth (Hardy *et al*. 2000), and far above the optimum for growth, which has been reported to be between 10 and 16°C (Stickney 2000), or between 16.5-17.2°C (Jobling 1994).

There was a strong correlation between food conversion ratio (FCR), which is the inverse of feed efficiency, and nitrogen retention. Thus, when fish are overfed, the feed lacks palatability, or it has poor digestibility for the species, then a higher proportion of N in the feed will be excreted. High FCR values will lead to high ammonia concentrations in the tank effluent. Thus, when designing a new recycle aquaculture system, estimates of TAN production (g/kg feed) should be given consideration in order to avoid ammonia toxicity and excessive levels of ammonia in the effluent. Although it is useful to use a generalized value for ammonia excretion per unit of feed fed (i.e., 0.25-0.35 kg ammonia kg^{-1} of feed), the relationship varies with species (Table 8) and protein efficiency ratio (PER).

The equation by Tucker and Boyd (1985), cited by Lawson (1995), to estimate TAN production using values for NPU and protein content of the diet has TAN production rectilinearly related to protein content of the feed. A feed with a protein content of 42 to 49%, as used in the present study, has an estimated nitrogen content of 67 to 78 g/kg. For the NPU values calculated in our study, the estimated TAN production would range from 2.9 to 6.9% of daily feeding rate. Values of 2 to 3% were used by Westers (2001) to estimate TAN from feeding rates. Lawson (1995), Huguenin and Colt (1989), and Tucker and Robinson (1990) suggest TAN can be estimated by 3% of feeding rate.

Nutritional research on protein retention (i.e., N-retention) has demonstrated an interaction between fish age or size, growth rates, temperature, feeding rates, and protein concentration of the feed. Phosphorus retention is strongly affected by phosphorus content of the feed in excees of the fish's requirements, as well as the digestibility of plant products, because the the phosphorus in phytin (in plant tissues) substantially reduces P retention and the fiber content is largely indigestible. Unless a focus is placed on the size and species of fish as well as the characteristics of the feed, it will be difficult to prevent substantial error in estimates of nutrient content of effluents derived from nutrients in the feed.

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