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# Proposal of a dynamic model as a tool to simulate growth performance and nitrogen release in rainbow trout *Oncorhynchus mykiss* farming

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**Abstract**

In order to verify the possibility to apply of European Union methodology of calculating N balance in rainbow trout farms, a dynamic model was developed using Stella™ for the simulation of growth performance and nitrogen release (GPNR model). GPNR applied the thermal-unit growth coefficient to estimate biomass weight. Actual N release (ANR) was calculated as the difference between actual N allotted and N retained in biomass. Minimal N release (MinNR) and maximal N release (MaxNR) were estimated when actual N allotted was unknown. Datasets obtained by eight experiments of different feeding conditions and one field production were used to verify the suitability of the model on rainbow trout production. The results showed that GPNR can be used to simulate growth performance and N release at any time during the production cycle. ANR was within the range of MinNR and MaxNR when the ratio of digestible protein and digestible energy of the diet was less than 24.81 g/MJ. Daily maximal feed intake provides a yardstick for farmers in fish farming practice. GPNR model offers farmers and water authorities an useful and simple tool to maintain sustainable development of fish farming production and control N pollution in water.

**Keywords**: Rainbow trout farming, Dynamic model, Biomass weight, Growth performance, Nitrogen release

## Introduction

In recent years, most countries worldwide have tried to decrease nitrogen (N) excretion from animal productions because governments and the public opinion are paying ever more attention to N as a prevalent pollutant, owing to its potential damage to the environment (Costanza and Gottlieb, 1998; 2001; ERM/AB-DLO 1999; Boyd, 2003). Nitrate pollution of water has been particularly taken into consideration. The nitrate level in drinking water is limited to values no higher than 50 mg/L in EU (80/778/EEC) and the Water Framework Directive of EU (2000/60/EEC) intends to provide protection for ground, coastal, estuarine and surface waters. Among the many

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sources of water pollution, fish farming is important for N and phosphorous load even though their release is highly diluted (Roque d'Orbcastel et al. 2008). Sustainable fish farming relies on the reduction of pollution loads while maintaining optimal production. When fish farmers try to optimize production capacity and minimize production costs while maintaining product quality, fish farming strategies must meet or exceed environmental legislation (Færgemand 1995), and the best management practices must be adopted by farmers even if they tend to be reluctant (Boyd 2003). In fish farming production, N waste consists of excretory products ($NH_3$ and urea), N-containing faecal products and uneaten feed. $NH_3$ (that is rapidly converted to $NH_4^+$ in water) is excreted by the gills while the other compounds are found in different forms of wastes in faeces. Ammonia is always the dominating form of N excretion in salmonids often making up approximately 90% of the total (Frier et al. 1995) while 5-15% of total N is released as urea. Ammonia could be transformed by nitrification and denitrification or simply disappear through a black box (loss) (Lefebvre et al. 2001). Much research has been done concerning to either water output quality parameters including $NO_3$, $NO_2$, $NH_4^+$-N (Ziemann et al. 1992; Zoccarato et al. 1994) or total ammonia nitrogen (TAN) (Cheng et al. 2003; Webb and Gatlin 2003)

Problems concerning direct measurement of waste output from fish farms have been reported and several models have been developed to study N output (Frier et al. 1995; Lefebvre et al. 2001; Doglioli et al. 2004).

However, this research cannot indicate the exact amount of N release from fish farms because of the various aquaculture wastes (dissolved nutrients, faecal matter and feed wastes) and the complicated chemical changes of ammonia after excretion. In particular, N transformation by environmental bacteria is significantly different from farm to farm and very difficult to measure. This fact is one of variable in evaluating N release. Papatryphon and others (2005) pointed out that there are at least two main sources of potential measurement error: sampling error and analytical error. From an environmental management point of view, the concept of N release from fish farms could be compared to N excretion in terrestrial animal.

Comparing to on-farm measurements, the balance approach is more reliable and a rather inexpensive way to quantify fish farming wastes (Cho et al. 1991; Roque d'Orbcastel et al. 2008). In this way, the difficulty of measurement can be overcome considering N just as an element instead of its various forms following the principle established by ERM/AB-DLO (1999), i.e. N release equals N intake minus N retention in products.

According to what mentioned above, the opportunity to create an easy tool to estimate N release from fish farming using an effective model represents an interesting issue. Cho and co-workers first developed a bioenergetic model to estimate production and waste output in aquaculture (Cho et al. 1991; Cho and Bureau, 1998, Bureau et al. 2000). The nutritional approach proposed by Cho and co-workers required measurements of apparent digestibility coefficients, nutrient retention efficiencies and the quantity of feed waste. Conversely, the equation proposed by ERM/AB-DLO (1999) seems to make easier the assessment of nitrogen budget also in rainbow trout farms. Moreover, the availability of software package, such as Stella$^{TM}$ (High performance systems, Inc. Hanover New Hampshire), has become a useful tool for dynamic systems modelling in different fields of research (Chen et al. 1997; Montoya et al. 1999; Jamu and Piedrahita 2002a,b).

The aim of this paper is to develop a user-friendly dynamic model (GPNR model) to predict growth performance and N release (including all forms of N wastes such as N in faecal matter, excretion products and uneaten feed) in rainbow trout production using Stella$^{TM}$ according to the methodology indicated by ERM/AB-DLO (1999) and applied by Guo and co-workers in beef cattle production (Guo et al. 2004; Guo and Zoccarato 2005).

## Materials and methods

### *Model development*

#### *Theoretical bases*

In recent years, different models were set up to simulate the environmental impact of fish farming. The principle of these models involves a classical energy or mass balance reported by Færgemand (1995) and Doglioli et al. (2004). The general equation to calculate N release from rainbow trout can be the following.

$$NR = NA - N_{gain} \quad (1)$$

Where NR = N release during a certain period or production cycle, g;

NA = N allotted to fish during a certain period or production cycle, g;

$N_{gain}$ = N retention in fish during a certain period or production cycle, g.

In general, daily actual nitrogen allotted to fish (DANA, g/fish) is easily controlled by fish farmers whether feed restriction or voluntary feed intake is used, multiplying the quantity of daily actual feed allotted to fish (DAFA, g) by the N content of feed ($N_{diet}$, %).

$$DANA = DAFA \times N_{diet} \quad (2)$$

Then actual N allotted (ANA, g) for a certain period can be obtained by

$$ANA = \int DANA \quad (3)$$

If feed allotted to fish is unknown, then an estimation of the minimal NA (MinNA) to fish with certain initial biomass weight (ABW0) for a certain period is needed. To estimate MinNA, energy and protein requirements should be studied. Most research (Cho 1992; Cho and Bureau 1998; Bureau et al. 2000) used factorial analysis dividing digestible energy (DE, kJ/fish) requirement as recovered energy (RE, kJ/fish), maintenance energy or fasting at a given water temperature (HeE, kJ/fish), heat increment of feeding for maintenance and growth energy (HiE, kJ/fish) and non faecal energy losses (ZE + UE, kJ/fish). Presuming the ratio between digestible protein of feed (DPf) and digestible energy of feed (DEf) is in the range of 20-22 g/MJ, the following equations are used to predict DE and biomass weight (Cho and Bureau 1998):

$$DE = RE + HeE + HiE + ZE + UE \quad (4)$$

$$RE = GE1 - GE0 \quad (5)$$

$$GE = 8.6 \times ABW - 40 \quad (6)$$

$$HeE = (-1.04 + 3.26\ T - 0.05\ T^2)\ (ABW/1000)^{0.824} \times days \quad (7)$$

$$HiE = 0.17\ (RE + HeE) \quad (8)$$

$$ZE + UE = 0.09\ (RE + HeE + HiE) \quad (9)$$

$$TGC = (ABW1^{1/3} - ABW0^{1/3})/\sum(T \times days) \quad (10)$$

$$PBW = [ABW0^{1/3} + \sum(TGC \times T \times days)]^3 \quad (11)$$

where ABW is actual biomass weight of fish on a given day, g; ABW0 is actual biomass weight of fish at the beginning of a certain period or productive cycle, g; ABW1 is actual biomass weight of fish at the end of a certain period or productive cycle, g; GE is gross energy content of fish at a given ABW, kJ/fish; GE0 is gross energy content of fish at ABW0, kJ/fish; GE1 is gross energy content of fish at ABW1, kJ/fish; PBW is predicted biomass weight of fish at a given day, g; T is the average temperature of the period, °C; TGC is thermal-unit growth coefficient.

To make the calculation of HeE suitable for a wider range of ABW and more dynamic, equation (7) could be transformed as:

$$HeE = \int (-1.04 + 3.26\ T - 0.05\ T^2)\ (ABW/1000)^{0.824} \quad (12)$$

Combining the equations (4) with (8) and (9), DE can be calculated as:

$$DE = 1.2753\ (RE + HeE) \quad (13)$$

Based on equation (13) combining with equations (5), (6) and (12), MinNA can be calculated using predetermined digestible energy of feed (DEf) and N content in feed for a certain ABW and T.

$$MinNA = DE / DEf \times N_{diet}$$
$$= 1.2753(\int(8.6ABW) + (-1.04 + 3.26\ T - 0.05\ T^2) \int(ABW/1000)^{0.824})/DEf \times N_{diet} \quad (14)$$

To give a yardstick for fish farmer, daily maximum feed intake (DMaxFI, g/fish/d) of fish, which means maximal ration when the fish are fed each hour in the light period i.e. feeding level (f) = 1, need to be estimated. Rasmussen and From (1991) reported DMaxFI can be calculated using the equation of N balance.

$$DMaxFI = a \times \exp(bT) \times ABW^c \quad (15)$$

Where T is temperature, °C; a, b, c are calibration constants: a = 0.0390 ± 0.0021; b = 0.0759 ± 0.0092; c = 0.7246 ± 0.0316 (values and 95% confidence limits given for each value were from Rasmussen and From, 1991).

Successively, maximum N intake (MaxNI, g/fish) is:

$$MaxNI = \int DMaxFI \times N_{diet} \quad (16)$$

Theoretically, actual N allotted to fish (ANA, g/fish) should fall in the range between MinNA and MaxNI because the former is based on the requirement for a given ABW/PBW or ABW0 and TGC without considering uneaten feed, while MaxNI is maximal N intake capacity of fish beyond which the feeding practice will cause direct loss from both an economic and environmental point of view.

$N_{gain}$ can be calculated as the difference of N content between ABW1 and ABW0 during a certain period or productive cycle. Bureau and others (2000) summarized the previous research and concluded the crude protein of rainbow trout of various size fed practical diet with 20-22 g DPf per MJ DEf could be calculated as the following equation with high $R^2 = 0.999$.

$$CP = 0.169\ ABW - 0.07 \quad (17)$$

Where CP is crude protein content at a given ABW, g/fish.

$N_{gain}$ can be obtained as:

$$N_{gain} = 0.169\ (ABW1-ABW0) / 6.25 \quad (18)$$

At last, actual N release (ANR), minimal N release (MinNR) and maximal N release (MaxNR) can be calculated combining the equations (1) with (3), (14), (16) and (18).

$$ANR = \int DANA - 0.169\ (ABW1-ABW0) / 6.25 \quad (19)$$

$$MinNR = 1.2753(\int(8.6ABW)+ (-1.04 +3.26\ T – 0.05\ T^2) \int (ABW/1000)^{0.824}) / DEf \times N_{diet} - 0.169\ (ABW1-ABW0) / 6.25 \quad (20)$$

$$MaxNR = \int DMaxFI \times N_{diet} - 0.169\ (ABW1-ABW0) / 6.25 \quad (21)$$

***Model overview***

To facilitate the comprehension of the model, the conceptual development is shown in Figure 1. The inputs of GPNR are ABW0, TGC, T, DEf, $N_{diet}$ and DAFA when available. The outputs are MinNR and MaxNR estimated by GPNR and ANR as the difference between ANA and $N_{gain}$. The model can be considered dynamic because, using Stella™, MinNR, ANR, MaxNR can be easily estimated at any time of the productive cycle based on historical record of TGC, T and diet used (DEf and $N_{diet}$). Furthermore, GPNR can offer farmers a yardstick to distribute feed using daily maximal feed intake (DMaxFI) of fish and approximate PBW which would help farmers to decide harvest time. From this point of view, this model is more friendly for its utility than other models (Cho, 1992; Frier and others 1995; Cho and Bureau; 1998).

Since growth rate of fish are dependent on species, stock (genetics), nutrition, environment, husbandry and other factors, it is essential to calculate TGC for a given aquaculture condition using previous growth records or records obtained from similar stocks and husbandry. TGC is calculated using equation (10). Consequently, PBW is obtained by equation (11) as shown in Figure 1.

RE is obtained as result of the equations (5) and (6), while HeE is calculated using equation (12). Then DE and MinNA are the results of equation (13) and (14) respectively. On the other hand, DMaxFI is calculated using equation (15) and MaxNI is obtained by equation (16). At the same time, ANA is accumulated as the sum of DAFA multiplying by $N_{diet}$ (equation 3).

N retained in the body is calculated using equation (18) as the difference of N content between PBW and ABW0. In the last step, ANR, MinNR and MaxNR is calculated using equation (19, 20, 21).

***Model evaluation***

The inputs and outputs of T1, T2, T3 experimental datasets (Zoccarato et al. 1991; 1994; 1996), carried out in the experimental station of the Dipartimento di Scienze Zootecniche of Turin University, were used to verify the suitability of GPNR. Successively, the proposed model was applied on other five experimental datasets gleaned

from published sources (Cho et al. 1991; Brauge et al. 1995; Lanari et al. 1995; Sandi 1996; Zoccarato et al. 1995) and one field practices.

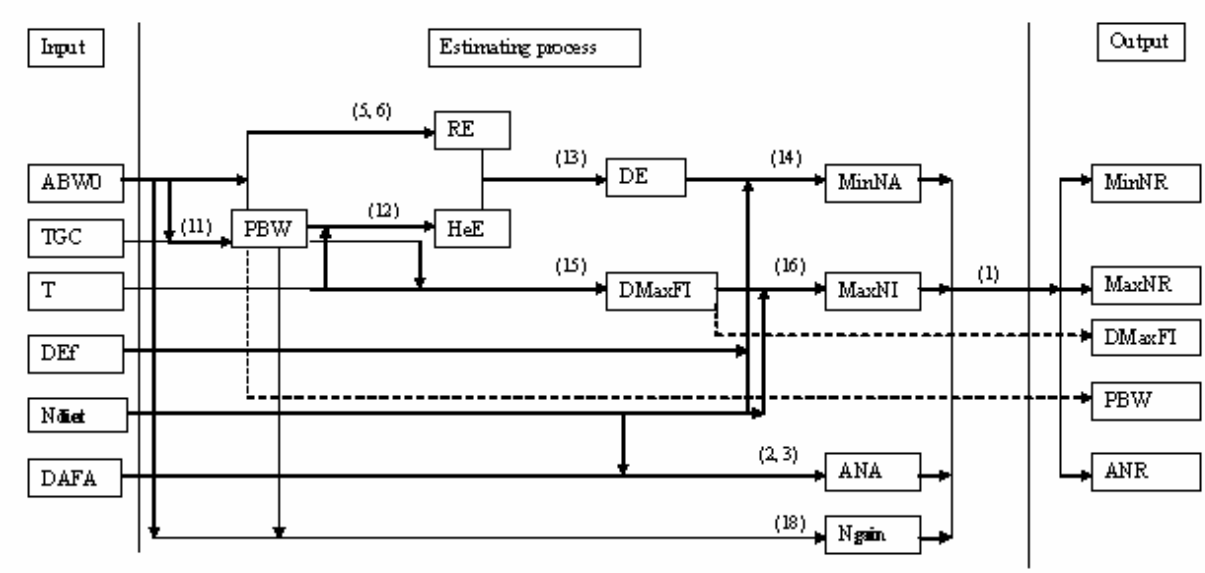


Fig. 1. ABW0: actual biomass weight of fish at the beginning of a certain period or productive cycle; ANA: actual nitrogen allotted; ANR: actual nitrogen release; DAFA: daily actual feed allotted; DE: digestible energy required; DEf: digestible energy contained in feed; DMaxFI: daily maximal feed intake; HeE: maintenance energy at a given water temperature; MinNR: minimal nitrogen release; MaxNR: maximal nitrogen release; $N_{diet}$: nitrogen content in the feed; $N_{gain}$: nitrogen retention in biomass; PBW: predicted biomass weight of fish at a given day; RE: recovered energy; T: the average temperature of the period; TGC: thermal-unit growth coefficient.

Table 1. Actual biomass N percentage (ABNP) and predicted biomass N percentage (PBNP) in different actual biomass weight (ABW) in three experiments (mean ± SEM)

| Trial | ABW (g) | ABNP (%) | PBNP (%)* |
|---|---|---|---|
| T1 9 a.m. | 328.0±51 | 2.69±0.14 | 2.70±6.16E-5 |
| T1 4 p.m. | 338.0±70 | 2.71±0.17 | 2.70±8.25E-5 |
| T1 1.4% | 326.0±61 | 2.68±0.13 | 2.70±5.56E-5 |
| T1 1.8% | 336.0±62 | 2.70±0.14 | 2.70±3.12E-5 |
| T2 LH (initial) | 82.8±1.7 | 2.69±0.05 | 2.69±2.79E-4 |
| T2 HH (initial) | 82.1±0.22 | 2.65±0.06 | 2.69±1.13E-4 |
| T2 LL (initial) | 82.1±0.55 | 2.66±0.04 | 2.69±3.77E-5 |
| T2 HL (initial) | 83.1±0.69 | 2.68±0.05 | 2.69±9.26E-5 |
| T2 LH (final) | 247.8±8.6 | 2.72±0.09 | 2.70±7.40E-5 |
| T2 HH (final) | 216.0±6.5 | 2.71±0.10 | 2.70±5.15E-5 |
| T2 LL (final) | 191.6±7.2 | 2.68±0.08 | 2.70±9.29E-5 |
| T2 HL (final) | 164.8±8.7 | 2.67±0.07 | 2.70±6.66E-5 |
| T3 PR | 277.8±11.9 | 2.68±0.09 | 2.70±5.61E-5 |
| T3 ER | 284.0±9-8 | 2.68±0.15 | 2.70±4.06E-5 |
| T3 PV | 290.4±4.1 | 2.70±0.11 | 2.70±5.24E-5 |
| T3 EV | 302.4±16.0 | 2.71±0.05 | 2.70±7.16E-5 |

* Calculated using equation: (0.169 × ABW – 0.07) / ABW / 6.25 × 100.

The eight datasets were chosen on the basis of different feeding conditions. T1, T2 and T3 were carried out in rectangular fibre glass tanks (1500 L each) with a flow rate of 30 L/m at constant spring water temperature 12-13 °C, oxygen content 7.8-7.9 mg/L.

T1 was designed to test the effects of meal timing and feeding level on growth performance. In all 800 rainbow trout (ABW0: 204.6 ± 2.0 g) were randomly divided, following the experimental design 2×2 with 2 replicates, into 8 groups and fed once per day at 9 a.m. or 4 p.m.; feeding level was 1.4 or 1.8% of the biomass weight. The commercial diet (crude protein 43.0% as feed bases, DPf/DEf: 22 g/MJ) was distributed as dry pellet (2.5 mm). To adjust feed supply, fish were counted and weighed in bulk on the 0, 21$^{st}$, 42$^{nd}$ and 63$^{rd}$ day. After 63 days of trials, 10 fish from each replicate were sampled for body composition analysis. The results were analysed as four groups (the effect of feeding time at 9 a.m. and 4 p.m. T1 9 a.m. and T1 4 p.m.; the effect of feeding level at 1.4% and 1.8%, T1 1.4% and T1 1.8%).

T2 was designed to test the effects of density and feeding level on growth performance. In all 2700 rainbow trout (ABW0: 82.5 ± 0.9 g) were randomly divided, following the experimental design 2×2 with 3 replicates, into 12 groups. The two density levels were 8 or 16 kg/m$^3$ of initial biomass; the two feeding levels were 1.3 or 2% of the biomass weight at the beginning decreasing to 1.1 or 1.8% at the end of the experiment. The commercial diet was the same as in T1. To adjust feed supply, fish were counted and weighed in bulk every two weeks. At the beginning and end of the 84-day-trial, 10 fish from each replicate were sampled for body composition analysis. The results were analysed as four groups (low density and high feeding level, T2 LH; high density and high feeding level, T2 HH; low density and low feeding level, T2 LL; high density and low feeding level, T2 HL).

In T3, two forms of diets and two feeding levels, restricted or satiation, were examined. In all 2160 rainbow trout (ABW0: 177.5 ± 8.2 g) were randomly distributed into 12 tanks following the experimental design 2×2 with 3 replicates. The trout were fed with two different diets: a pelletised diet (protein content: 48.6%; DPf/DEf: 24.9 g/MJ) and an extruded diet (protein content: 47.4%; DPf/DEf: 21.3 g/MJ). Feed was delivered according to 1.3% of biomass weight or satiation twice a day. To adjust feed supply, fish were counted and weighed in bulk on the 0, 21$^{st}$, 35$^{th}$ and 49$^{th}$ day. After 49 days of trials, 10 fish from each replicate were sampled for body composition analysis. The results were analysed as four groups (pelletised restricted diet, T3 PR; extruded restricted diet, T3 ER; pelletised satiation diet, T3 PV; extruded satiation diet, T3 EV).

The experimental condition of other 5 controlled experiments can be found in the referenced cited above. One ponds (15×15×2m$^3$) were also used for model validation. The pond was supplied with spring water which maintained a flow rate of 30 l/m at constant spring water temperature 12-13 °C, oxygen content 7.8-7.9 mg/L. The fish farming condition was similar with T2 HH which is usually practiced and TGC was assumed as $1.57\times10^{-3}$. Approximately, 90000 rainbow trout (80.00±2.58 g) were put into the pond. Ten fish were caught to control ABW and adjust feed distribution every two weeks except for the last weighing at the end of experiment the interval was just one week. The productive period was 105 days and the market weight was about 250 g/fish.

Results were used to: 1) calculate N content in the rainbow trout; 2) compare PBW and ABW; 3) compare MinNR and MaxNR with ANR using datasets obtained in T1, T2 and T3; 4) validate of the model with the other five controlled experiments and one field production.

***Statistics***

Differences between actual biomass N percentage (ABNP) and predicted biomass N percentage (PBNP) were analyzed with paired t test of SPSS 12.0 (SPSS Inc., Chicago, IL, USA). The same test was used to analyse the differences between ANR simulated by the model and the values reported in five sources datasets, between PBW and ABW and between simulated ANR (sANR) and real ANR (rANR) in the field condition.. Values of TGC from different groups were compared with ANOVA while the relation between PBW and ABW and ANR and ANR reported by some authors were analysed by correlation procedure of SPSS. Differences among MinNR, MaxNR and ANR in the three experiments were analysed by ANOVA and the univariate of SPSS.

## Results

***N content in the biomass weight***

The average harvested fish weight of different groups in T1, T2 and T3 ranged from 82.1 ± 0.6 g to 338.0 ± 70.1 g, while the weight of single fish ranged from 79.5 g to 509.0 g. The results of analysis of ABNP and PBNP of trout are shown in Table 1. There is no significant difference between ABNP and PBNP; the lack of difference allowed us

to use PBNP to estimate ABNP. This showed that the calculation of N content in biomass, which is the product of ABNP × ABW or PBNP × PBW, is correct. In this way, N retention in fish can be obtained as difference of N content between PBW (or ABW) and ABW0.

***Comparison of PBW and ABW***

During the experiments, each group was weighed and counted four times in T1 and T3, while seven times in T2. For each group, the single fish weight was obtained by the total biomass weight divided by the number of fish. Using ABW0 and ABW1 as parameters, TGC was calculated for each group (Table 2). There were significant differences among different groups for TGC when analysed with ANOVA. The analysis of covariance showed ABW0 had no significant influence on different value of TGC among groups, which means the calculation of TGC is suitable for all categories of ABW0. PBW were predicted using the dynamic model and compared with ABW (on the 21$^{st}$ and 42$^{nd}$ day of T1, on the 14$^{th}$, 28$^{th}$, 42$^{nd}$, 56$^{th}$ and 70$^{th}$ day of T2, on the 21$^{st}$ and 35$^{th}$ day of T3). The correlation between PBW and ABW was 0.996 and the fitness between them is shown in Figure 2, which demonstrate PBW can be used if ABW is unknown.

Table 1. Actual biomass N percentage (ABNP) and predicted biomass N percentage (PBNP) in different actual biomass weight (ABW) in three experiments (mean ± SEM)

| Trial | ABW (g) | ABNP (%) | PBNP (%)* |
|---|---|---|---|
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| T1 1.4% | 326.0±61 | 2.68±0.13 | 2.70±5.56E-5 |
| T1 1.8% | 336.0±62 | 2.70±0.14 | 2.70±3.12E-5 |
| T2 LH (initial) | 82.8±1.7 | 2.69±0.05 | 2.69±2.79E-4 |
| T2 HH (initial) | 82.1±0.22 | 2.65±0.06 | 2.69±1.13E-4 |
| T2 LL (initial) | 82.1±0.55 | 2.66±0.04 | 2.69±3.77E-5 |
| T2 HL (initial) | 83.1±0.69 | 2.68±0.05 | 2.69±9.26E-5 |
| T2 LH (final) | 247.8±8.6 | 2.72±0.09 | 2.70±7.40E-5 |
| T2 HH (final) | 216.0±6.5 | 2.71±0.10 | 2.70±5.15E-5 |
| T2 LL (final) | 191.6±7.2 | 2.68±0.08 | 2.70±9.29E-5 |
| T2 HL (final) | 164.8±8.7 | 2.67±0.07 | 2.70±6.66E-5 |
| T3 PR | 277.8±11.9 | 2.68±0.09 | 2.70±5.61E-5 |
| T3 ER | 284.0±9-8 | 2.68±0.15 | 2.70±4.06E-5 |
| T3 PV | 290.4±4.1 | 2.70±0.11 | 2.70±5.24E-5 |
| T3 EV | 302.4±16.0 | 2.71±0.05 | 2.70±7.16E-5 |

* Calculated using equation: (0.169 × ABW – 0.07) / ABW / 6.25 × 100.

***Comparison of MinNR, MaxNR and ANR***

The inputs and outputs of T1, T2 and T3 are summarized in Table 2. In T1, with similar ABW0, the four groups had no significant differences for ABW1, MinNR and MaxNR. Instead for ANR, there was significant difference between T1 1.8% and T1 1.4%, which confirmed high feeding level lead to high N release. In T2, with similar ABW0, the four groups didn't show significant differences for MaxNR, while significant differences were observed for ABW1, MinNR and ANR. T2 LH had the highest ABW1 while T2 HL had the lowest MinNR. T2 LL and T2 HL had lower ANR than the other two groups. The results of T3 showed no significant differences for ABW1 among the four groups, while it was evident that the feeding level at satiation caused more ANR than the restricted feeding level. Furthermore, the results showed pelletized diets would have higher biomass weight gain than extruded diets when the fish were fed at satiation level.

In general, ANR fell within the range from MinNR to MaxNR except in T3 PR group where DPf/DEf (24.9 g/MJ) was beyond the condition from which the model derived (DPf/Def as 20-22 g/MJ). MaxNR showed no significant differences when analysed using ABW0, duration and TGC as covariates. Using similar analytic procedure, the differences among ANR could not be eliminated.

***Validation of the model***

In Table 3, the results obtained in five sources of data, confirmed that ANR generally fell within the range of MinNR and MaxNR irrespectively to ABW0, diet, duration and water temperature; the proposed model showed high tolerance when DPf/DEf was lower than 20-22 g/MJ and low tolerance when DPf/DEf was higher than 20-22 g/MJ, in fact, ANR were less than MinNR when DPf/DEf was higher than 24.81 g/MJ.

The calculation of N release can be expressed as g per cycle of production or g per kg products. The transformation from ANR of a single fish to N release of a rainbow trout farm depends on the approximate number and weight of fish in the farm at the beginning and end of production cycles. ANR values obtained from the five datasets, expressed as g/kg of biomass weight gain, were also reported in Table 3. Moreover ANR and ANR reported by Cho et al. (1991) and Lanari et al.(1995) were well correlated ($R^2 = 0.88$).

There were no significant differences between PBW and ABW in field condition, so did between sANR and rANR (Table 4). rANR fell in the range between MinNR and MaxNR. The results in field condition showed that GPNR model can simulated the rainbow trout farming using the parameters of experimental records of similar condition.

Table 2. Comparison of MinNR, MaxNR with ANR of a single fish in the three experiments (mean ± SEM)

| Group | ABW0 (g) | ABW1 (g) | Duration (d) | N (%) | TGC ($\times 10^{-3}$) | MinNR (g) | ANR (g) | MaxNR (g) |
|---|---|---|---|---|---|---|---|---|
| T1 9 a.m. | 205.9±1.7$^{d*}$ | 328.1±3.5$^{ef}$ | 63 | 6.88 | 1.26±0.03$^{ab}$ | 5.21±0.06$^{de}$ | 8.84±2.12$^{bc}$ | 15.28±0.39$^{c}$ |
| T1 4 p.m. | 203.3±1.6$^{d}$ | 337.9±14.4$^{f}$ | 63 | 6.88 | 1.38±0.14$^{bc}$ | 5.42±0.29$^{de}$ | 8.31±1.76$^{bc}$ | 14.74±0.40$^{c}$ |
| T1 1.4% | 204.1±1.9$^{d}$ | 326.4±6.9$^{ef}$ | 63 | 6.88 | 1.27±0.08$^{ab}$ | 5.19±0.15$^{cde}$ | 6.92±0.40$^{ab}$ | 14.88±0.44$^{c}$ |
| T1 1.8% | 205.0±2.3$^{d}$ | 339.6±10.9$^{f}$ | 63 | 6.88 | 1.37±0.12$^{bc}$ | 5.44±0.23$^{de}$ | 10.24±0.51$^{c}$ | 15.14±0.52$^{c}$ |
| T2 LH | 82.8±1.7$^{a}$ | 247.8±5.7$^{f}$ | 84 | 6.88 | 1.83±0.06$^{f}$ | 5.37±0.13$^{de}$ | 8.58±0.13$^{bc}$ | 12.66±0.15$^{b}$ |
| T2 HH | 82.1±0.2$^{a}$ | 216.0±5.1$^{b}$ | 84 | 6.88 | 1.57±0.04$^{cde}$ | 4.69±0.11$^{c}$ | 8.07±0.15$^{bc}$ | 12.50±0.01$^{ab}$ |
| T2 LL | 82.1±0.6$^{a}$ | 191.6±7.2$^{b}$ | 84 | 6.88 | 1.35±0.05$^{bc}$ | 4.17±0.15$^{b}$ | 4.41±0.12$^{a}$ | 12.40±0.08$^{ab}$ |
| T2 HL | 83.1±0.7$^{a}$ | 164.8±7.7$^{a}$ | 84 | 6.88 | 1.06±0.08$^{a}$ | 3.58±0.17$^{a}$ | 4.42±0.41$^{a}$ | 12.31±0.07$^{ab}$ |
| T3 PR | 182.0±6.2$^{c}$ | 277.8±11.9$^{d}$ | 49 | 7.78 | 1.40±0.05$^{bc}$ | 5.03±0.23$^{cd}$ | 4.63±0.28$^{a}$ | 12.31±0.49$^{ab}$ |
| T3 ER | 179.9±6.8$^{bc}$ | 284.0±9.8$^{d}$ | 49 | 7.59 | 1.51±0.03$^{cd}$ | 3.69±0.11$^{ab}$ | 4.16±0.32$^{a}$ | 11.65±0.48$^{ab}$ |
| T3 PV | 169.1±9.9$^{b}$ | 290.4±4.1$^{d}$ | 49 | 7.78 | 1.79±0.13$^{def}$ | 5.62±0.06$^{e}$ | 8.20±0.92$^{bc}$ | 11.49±0.55$^{a}$ |
| T3 EV | 179.1±5.7$^{bc}$ | 302.4±16.0$^{de}$ | 49 | 7.59 | 1.75±0.11$^{ef}$ | 4.02±0.23$^{ab}$ | 6.93±0.85$^{ab}$ | 11.63±0.23$^{ab}$ |

$^{*}$ Means in the same column not sharing common superscript letters are significantly different: $P < 0.05$.
ABW0: initial biomass weight, g; ABW1: final biomass weight, g; TGC: thermal-unit growth coefficient; ANR: actual nitrogen release, g; MinNR: minimal N release, g; MaxNR: maximum nitrogen release, g.

## Discussion

***N retention in fish***

N retention in biomass weight, as an important parameter in the dynamic model, is difficult to define. Rasmussen (2001) reported that the estimation of body protein content is more difficult and less predictable than that of body

lipid because protein content has been observed either to increase until fingerling size is reached in trout or to increase continuously with size. Valente and others (2001) studied feed intake and growth of fast and slow growing strains of rainbow trout fed by automatic feeders or by self-feeders; the results indicated protein content of whole fish was not significantly influenced either by feeding system or by strain of fish. Kause and others (2002) examined whether selection for rapid growth rate in rainbow trout could potentially lead to correlated genetic responses in body composition; the results showed the differences in body composition could be mainly explained by the difference between sexes in body size. Brauge and others (1995) indicated protein retention was not affected by dietary carbohydrate / lipid ratio treatment at 8 or 18°C. In general, feeds based upon plant proteins did not result in considerable changes in fish body composition when compared with traditional feeds based mainly on fish meal and oil (Kim et al. 1998; Valente et al. 2001).

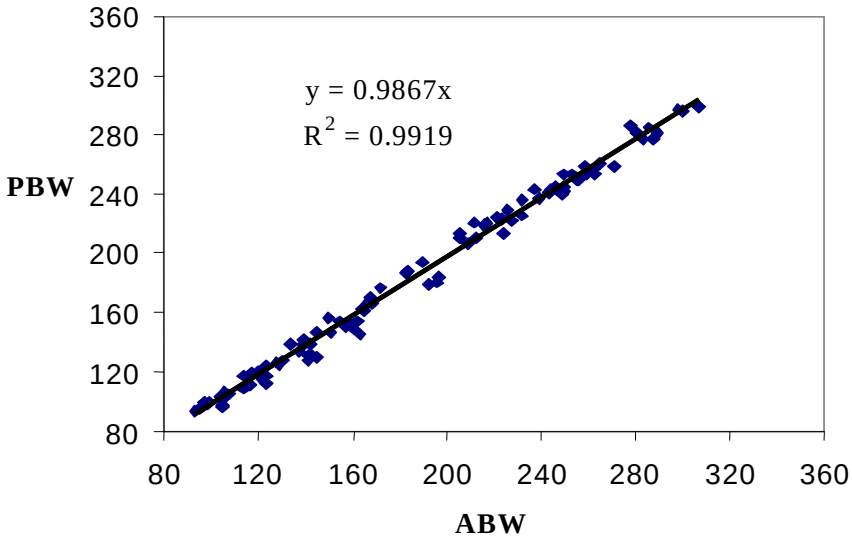


Fig. 2. Relationship between actual biomass weight (ABW) and predicted biomass weight (PBW)

N retention changes during the life stages of rainbow trout and different trends had been observed (Rasmussen 2001). On the contrary, after a detailed study of stage related body composition in brown trout, Jonsson and Jonsson (1998) indicated that protein concentration did not vary with age in immature fish. In Denmark, in order to evaluate feeding efficiency and to control the environmental impact of fish farming, N percentage of fish is conventionally fixed as 3.0% of biomass weight for the facility of calculation (Færgemand 1995), and the same value was used by Doglioli et al. (2004). While in some research the percentage of N retention is slightly lower than this value, about 2.68±0.25 % (Brauge et al. 1995; Færgemand 1995; Kim et al. 1998; Valente et al. 2001). As far as Italian rainbow trout production in concerned, Lanari et al. (1995) reported N retention resulted as 2.68±0.02 % for rainbow trout from 106 g to 269 g. Bureau et al. (2000) summarised various research and conclude the CP content of fish can be expressed in function of ABW. In this paper, the results of T1, T2 and T3 suited for the equation which resulted in the value 2.69-2.70% similar with the values mentioned above.

***Biomass growth estimation***

At present, the most commonly used parameter to express growth rate is specific growth rate (SGR) and TGC (Brett and Groves 1979; Satoh et al. 2003). However, Cho (1992) verified the use of the natural logarithm under-estimates the weight gain between ABW1 and ABW0 used in the calculation; the exponential form also grossly over-estimates predicted final biomass weight. In GPNR model, TGC formula was used and the results showed that growth prediction using TGC suits rather well with the examined datasets. The TGC value for commonly used commercial diet ranged from $1.06 \times 10^{-3}$ to $1.83 \times 10^{-3}$ depending on the density of fish, feeding level and farm

management. The range is in agreement with TGC values reported by Cho and Bureau (1998). It is essential to calculate TGC for a given aquaculture condition using previous growth records or records obtained from similar stocks and husbandry conditions as shown in field condition. The use of TGC, which can overcome the effect of temperature (Cho and Bureau 1998), allows GPNR model suitable for very wide feeding condition. In Table 2 and Table 3, it can be observed that with similar feeding condition, high TGC leads to low ANR and vice versa. In this model, the oxygen ($O_2$) level, which is also an important fish growth limiting factor, is not considered because critical dissolved oxygen level is 6 mg/l (Ruohonen and Mäkinen 1989), while in the examined experiments the oxygen content were higher than this value.

Table 3. Validation of the proposed model using different sources of data (mean )

| Sources | Group | ABW0 (g) | ABW1 (g) | $N_{diet}$ (%DM) | DPf/DEf | Duration (d) | T (°C) | FCR | TGC ($\times 10^{-3}$) | Min NR (g) | ANR (g) | Max NR (g) | ANR (g/kg fish) | ANR report (g/kg fish) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Zoccarato and others 1995[1] | A0 | 77.5 | 217.4 | 8.46 | 23.52 | 99 | 12.5 | 1.23 | 1.41 | 7.40 | 10.78 | 19.88 | 77.06 | |
| | A1 | 77.2 | 220.5 | 8.46 | 23.52 | 99 | 12.5 | 1.19 | 1.44 | 7.51 | 10.56 | 19.91 | 73.69 | |
| | A2 | 77.4 | 225.8 | 8.46 | 23.52 | 99 | 12.5 | 1.18 | 1.48 | 7.69 | 10.81 | 20.05 | 72.84 | |
| Sandi, 1996[2] | High | 40.0 | 263.3 | 8.26 | 23.00 | 128 | 12.5 | 0.95 | 1.87 | 8.85 | 11.50 | 21.75 | 51.50 | |
| | Medium | 39.1 | 229.2 | 6.85 | 21.00 | 128 | 12.5 | 1.12 | 1.7 | 6.10 | 9.44 | 16.24 | 49.66 | |
| | Low | 39.1 | 200.8 | 6.19 | 19.00 | 128 | 12.5 | 1.11 | 1.54 | 4.58 | 6.74 | 13.73 | 41.68 | |
| Brauge and others 1995[3] | HC | 56.0 | 120.4 | 7.06 | 26.47 | 84 | 8.0 | 1.10 | 1.65 | 3.28 | 3.26 | 6.63 | 50.62 | |
| | MC | 56.0 | 126.5 | 6.86 | 24.88 | 84 | 8.0 | 1.00 | 1.78 | 2.97 | 2.93 | 6.41 | 41.56 | |
| | LC | 56.0 | 123.3 | 6.85 | 24.81 | 84 | 8.0 | 1.00 | 1.71 | 2.95 | 2.79 | 6.39 | 41.46 | |
| | HC | 53.0 | 159.6 | 7.06 | 24.54 | 84 | 18.0 | 1.10 | 1.1 | 5.18 | 5.39 | 17.02 | 50.56 | |
| | MC | 53.0 | 166.2 | 6.86 | 22.28 | 84 | 18.0 | 1.10 | 1.15 | 4.52 | 5.49 | 16.65 | 48.45 | |
| | LC | 53.0 | 174.5 | 6.85 | 22.47 | 84 | 18.0 | 1.10 | 1.21 | 4.86 | 5.87 | 16.82 | 48.31 | |
| Lanari and others 1995[4] | V1 | 107.0 | 241.0 | 5.86 | 18.70 | 86 | 12.9 | 1.30 | 1.33 | 4.10 | 6.59 | 13.22 | 49.18 | 46.00<br>47.60 |
| | V2 | 106.0 | 249.0 | 6.24 | 19.90 | 86 | 12.9 | 1.30 | 1.4 | 4.90 | 7.73 | 14.27 | 54.06 | |
| | V3 | 106.0 | 269.0 | 6.88 | 21.30 | 86 | 12.9 | 1.20 | 1.55 | 5.77 | 9.05 | 16.26 | 55.52 | 49.70 |
| | V4 | 175.6 | 312.3 | 6.30 | 16.30 | 56 | 13.2 | 0.94 | 1.6 | 2.50 | 4.40 | 11.94 | 32.19 | 29.90 |
| | V5 | 175.9 | 321.0 | 6.72 | 17.20 | 56 | 13.2 | 0.90 | 1.68 | 2.89 | 4.85 | 12.95 | 33.43 | 29.80 |
| | V6 | 176.2 | 335.6 | 7.20 | 18.20 | 56 | 13.2 | 0.84 | 1.82 | 3.44 | 5.33 | 14.1 | 33.44 | 29.10 |
| Cho and others 1991[5] | Brown | 9.02 | 22.69 | 6.93 | 22.00 | 84 | 8.11 | 1.02 | 1.10 | 0.54 | 0.60 | 1.99 | 43.89 | 48.01 |
| | Rainbow | 1.00 | 101.0 | 7.04 | 22.00 | 140 | 15.00 | 0.83 | 1.74 | 2.41 | 3.16 | 7.93 | 31.60 | 38.55 |

Verification of the model using experiments with: [1]different levels of dietary avoparcin: A0, A1, A2, corresponding 0, 40, 80 mg/kg of DM respectively; [2] different levels of DPf/DEf; [3] different levels of carbohydrate and temperature; [4]different levels of vegetable ingredients: V1, V2, V3, V4, V5, V6 corresponding; 604, 563, 520, 474, 422, 355 g/kg of DM respectively; [5] different trout speciesandduratio.

***N release simulation***

The estimation of N release in the eight experiments showed GPNR was able to calculate MinNR, ANR and MaxNR for different feeding conditions in rainbow trout production. In this model, the N release value of single fish were calculated assuming that growth rates and feed allotted were constant within the period between two subsequent weighs. Generally, a rainbow trout farming cycle starts at 25 g and ends to a portion size of 250 g. GPNR model can dynamically indicate PBW and NR during farming cycle of rainbow trout. Generally, the ANR values in Table 3 were similar with those reported by the authors (Cho et al. 1991; Lanari et al. 1995) and the minimal differences between them, which were due to different methods of measurements and N retention values,

were not significant ($P < 0.05$) and $R^2$ between ANR and ANR reported was (0.88). This result was in agreement with Roque d'Orbcastel et al. (2008), who reported that the measured values of total nitrogen in the effluents of trout farming and the predicted values by nutrient balance were well correlated ($R^2$= 0.88). Nevertheless it should be noticed that there were large differences between MinNR and MaxNR, which means that the extra NR (ANR - MinNR) has a wide range and should be mitigated as small as possible. Even if it is difficult to reach MinNR, farmers or technicians should always pay more attention to feed allotted and growth performance of fish so as to approximate ANR to MinNR.

In agreement with ERM/AB-DLO (1999) concept, GPNR model simulates gross N release regardless ABW0, feed characteristics, duration and T. The simulated values were in the accordance with those reported by Roque d'Orbcastel et al. (2008). The utilization of GPNR model also allows us to neglect various forms of N and denitrification process. The advantage of GPNR is that it can simulate N release at any time of production cycles. Water authority can calculate MinNR and MaxNR of existing or future rainbow trout farms according to its previous record or record of similar condition to estimate environmental N load as well as to assure the quality of products. While fish farmers can use the indication of DMFI and decide on the quantity of feed allotted to fish based on predetermined ratio between DAFA and DMFI to estimate PBW and harvest time.

Even though the range of DPf/DEf (20-22 g/MJ), which was derived from Cho and Bureau (1998), was relatively narrow, verification results showed it has rather high tolerance; these make GPNR useful as a flexible inexpensive managerial tool to apply the best management practice in trout farming as suggested by Boyd (2003). However, further study should be made to extend the application of the model to a wider range of feeding condition, in particular to diets with DPf/DEf higher than 24.81 g/MJ.

The major inputs were ABW0, TGC, T, DEf, $N_{diet}$ and DAFA. In GPNR, ABW/PBW was obtained using ABW0 and TGC. The amount of N retention in biomass could be calculated as N content in biomass gain. MinNR was estimated based on energy requirement of fish with a given TGC and ABW0. ANR was calculated as the difference between N content in the feed allotted to fish and N retention in the body, while MaxNR was calculated assuming feeding level is 1.

The datasets of eight experiments and one field production were used to verify the dynamic model. Results showed that GPNR can simulate biomass weight and N release at any time of production cycle regardless of feeding condition. Furthermore, DMaxFI provides a yardstick for fish farmers in practice. Bearing in mind the large difference between MaxNR and MinNR, water authorities and farmers can greatly reduce N load to environment and approximate ANR to MinNR without influencing fish farming productivity and profitability.

In conclusion, even if the estimation of N balance in fish is, from a metabolic point of view, a complicate procedure due to the different N forms (N released by feed waste, faeces or gills), GPNR, which is based on a widely used nutritional balance in trout farming, provided a simple tool to estimate fish biomass weight and N release in rainbow trout production. GPNR will enable technicians to dynamically calculate N load to environment and apply the EU rules in order to protect the environment; it can also help farmers to evaluate optimal production and keep the development of fish farming production sustainable. Obviously, the accurate estimation of real N fate in environment requires further study in particular concerning the denitrification process.

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