Aquacultural Engineering
May 2000; 22(1-2) : 137-153
http://dx.doi.org/10.1016/S0144-8609(00)00037-6

A model for predicting the quantities of dissolved inorganic nitrogen released in effluents from a sea bass (Dicentrarchus labrax) recirculating water system

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Abstract:

Fish excretions and the transformation of nitrogen by bacteria in the nitrifying biofilter are two of the main sources of dissolved inorganic nitrogen (DIN) in fish farms that use recirculating water systems. In this study, the DIN concentration in an experimental *Dicentrarchus labrax* aquaculture system was calculated using empirical sub-models for fish growth, ingested food and water replacement. The specific growth rate (SGR) (% day^{-1}) and the daily feeding rate (DFR) (% day^{-1}) both depend on the average weight, W (g), of the fish: $Y=aW^b$, where Y may be SGR or DFR, and a and b are empirical constants. The DIN discharge rate, Γ_N (% of ingested nitrogen), in the experimental aquaculture system was expressed as a function of increasing replacement water flow rate, θ (day^{-1}): DIN$=c\theta^d$, where c and d are empirical constants. Only three variables (the number of fish, the initial fish weight and the replacement water flow rate) are required to run the general model, which was tested over a period of 12 months (June 1997–June 1998). This model, calibrated and validated on independent sets of data obtained from the same experimental system, accurately predicted the concentration of DIN in the effluent (r^2=0.92).

Keywords: Dissolved inorganic nitrogen; European sea bass; Fish farm effluent; Model; Nitrogen production; Recirculating water system

INTRODUCTION

The use of aquaculture to provide commercial species of fish continues to expand, but the pollution generated by these systems can have a serious impact on the environment and wild fauna (Brown *et al*., 1987; Rosenthal, 1994; Wu, 1995). Dissolved matter, fecal products and uneaten food are all major sources of nitrogen waste produced by aquaculture (Krom and Neori, 1989; Seymour and Bergheim, 1991). Teleost species such as *Dicentrarchus labrax* excrete nitrogen mainly in the form of ammonia released via the gills, and urea (Smith, 1929; Wood, 1958; Guérin-Ancey, 1976; Handy and Poxton, 1993). It is therefore important to estimate the concentration of nutrients released into the environment to prevent eutrophication.

In open farms, several studies have established a link between dissolved inorganic nitrogen (DIN) concentration and fish metabolism (Guérin-Ancey, 1976; From and Rasmussen, 1984; Lemarié *et al*., 1998). Total excreted ammonia (TAN) in effluent from open farms can be directly correlated with fish weight. Other studies suggest a relationship between the quantity of excreted dissolved inorganic nitrogen and the amount of nitrogen ingested in food (Savitz *et al*., 1977; Vitale-Lelong, 1989; Forsberg, 1996).

The use of recirculating water systems is one approach that is used to limit the impact of aquaculture on the environment. Although the total quantity of nutrients released is similar in flow through and recirculation systems, the small volumes of concentrated effluent that are produced by recirculation systems are easier to deal with (Lavenant *et al*., 1995). In these systems, a nitrifying biofilter transforms the excreted ammonia and urea into nitrate (Hagopian and Riley, 1998). No models have as yet been published that describe effluent nitrogen discharge in farms using recirculating water systems.

The aim of this study was to design a model to predict the amount of dissolved inorganic nitrogen discharge from a recirculating water system used for growing *Dicentrarchus labrax*. It is important to define and quantify the dissolved nutrients released into the environment in the effluent to estimate their potential impact on the environment and, if required, develop appropriate systems to address this problem.

The recirculating water system must be considered as an entity that is characterized by the water replacement flow rate and fish metabolism. The main characteristics of recirculating water systems are the constant water temperature and the concentration of nutrients in the effluent.

The three sub-models proposed in this study are: (1) fish growth rate, which takes into account the initial fish weight; (2) ingested feed, which takes into account fish weight, and (3) the influence of replacement water, expressed as the rate of nitrogen production within the system. The general model was calibrated and validated on independent sets of data.

MATERIALS AND METHODS

Experimental fish farm

The indoor aquaculture facilities consisted of two 10 m^3 self-cleaning tanks (tank A and B) connected to a recirculating water system (figure 1). The temperature and photoperiod were maintained constant at 22±1 °C and 16 hours of light per day, respectively. The pH was maintained at around 7.7 by the continuous injection of a sodium hydroxide solution. Pure oxygen was supplied to ensure a concentration of between 6 and 7 mg.L^{-1} within the tanks. These are considered to be the optimal values for this rearing system. In the recirculating system, water was filtered through a 50 µm mechanical mesh filter. Carbon dioxide produced by fish respiration was eliminated in a counter current air/water packed column, after which the water was passed through a pumping tank into which replacement water was added at a controlled flow rate (figure 2). The filtered and aerated water was pumped into a UV light disinfection unit. Finally, it was passed through a nitrifying biofilter filled with a microporous bed media composed of expanded and cocked clay (Biogrog) where the residual organic matter was transformed into mineral compounds (mainly nitrate). In this study, the tanks and the recirculating water circuit are considered to be a single unit. In the rearing system, the input comes from the replacement water and the ingested feed, the output comprises the water used to rinse the mechanical filter and the excess water from the rearing system (figure 3).

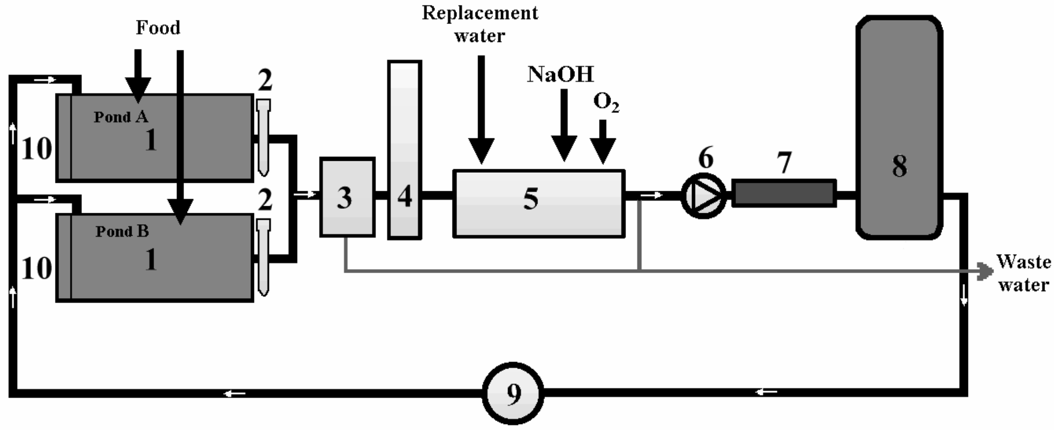

Figure 1: Recirculating rearing system diagram
1: rearing tank; 2: particulate separator; 3: mechanical filter; 4: CO2 stripping system; 5: pumping tank; 6: pumps; 7: UV lights; 8: nitrifying biofilter; 9: warm-cold exchanger; 10: oxygen supply system.

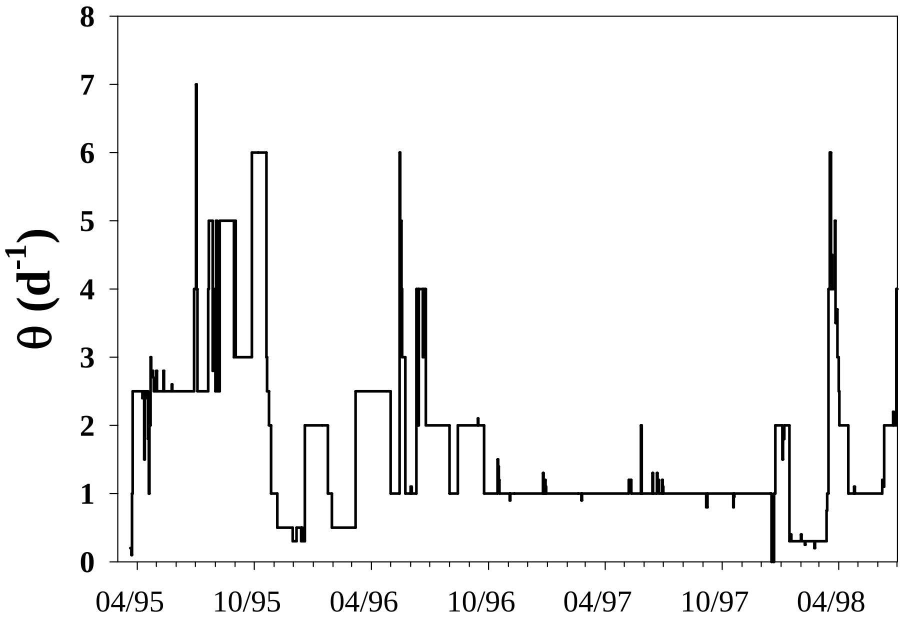

Figure 2: Temporal evolution of the replacement water flow rate

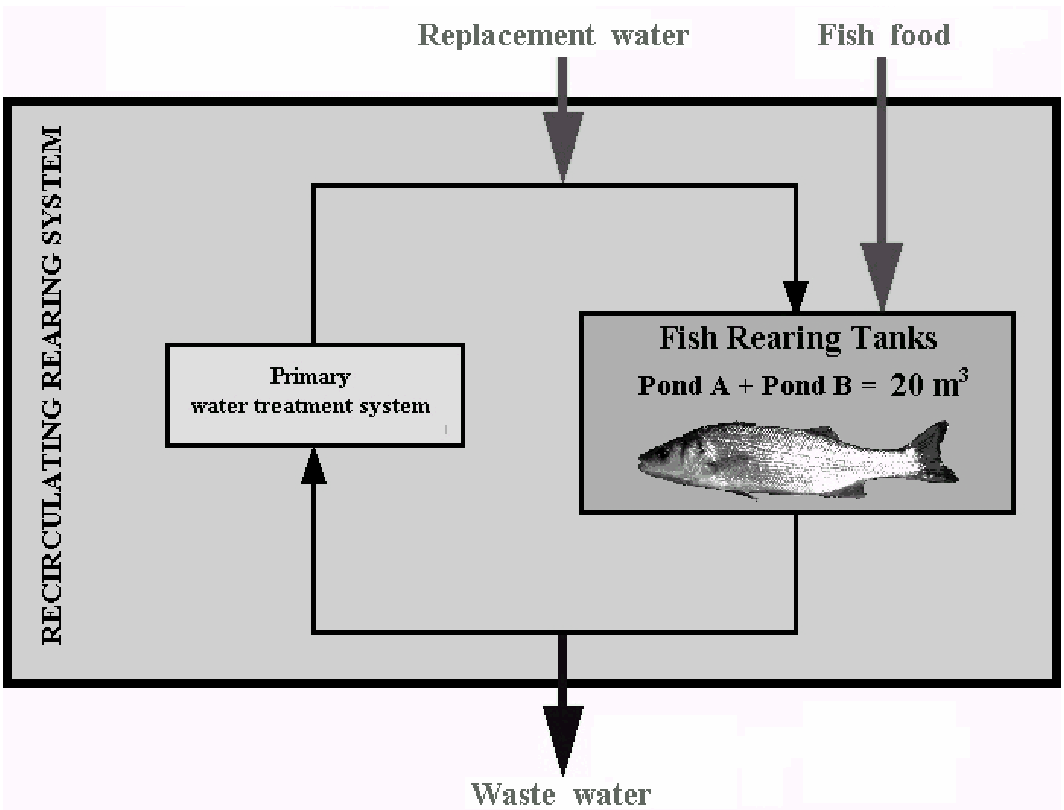

Figure 3: Fish rearing model

During the experiment, the fish (*Dicentrarchus labrax*) grew from 3 to approximately 1000 grams. The average fish weight and the standard deviation were determined on a sample of fifty fish taken from each rearing tank every 20 to 90 days. The fish biomass was carefully monitored to avoid exceeding an average density of 100 $kg.m^{-3}$ within the tanks; when the biomass reached this value, some fish were removed in order to decrease the biomass to around 80 $kg.m^{-3}$.
As described in Coves *et al*. (1998), fish were fed by self-feeders fitted with a trigger. When the fish activate the trigger, a fixed quantity of pellets is supplied. The same composition of feed was used throughout the experiment (table 1). The total quantity of feed consumed by the fish between two biomass sampling periods was measured by weighing daily the feed which was left in the self-feeder.

Table 1. Fish food composition of the diet fed to sea bass (manufacturer values)

Composition	**Values % of pellet weight**	**Digestibility %**
Crude protein	45.0	90
Crude fat	21.5	90
Ash	9.0	
Fibre	2.0	
total phosphorus	1.2	
Met. + Cys.	1.7	
energy	22.2 $MJ.kg^{-1}$	

Water sampling and analysis

Samples were taken twice a week at 14:00 directly from the recirculating rearing system outlet (tanks A and B) and were filtered on rinsed Wathman GF/C filters. Dissolved organic nitrogen (*DON*) was oxidized by potassium persulfate, as described by Solorzano and Sharp (1980). Total dissolved nitrogen (*TDN*), now present as nitrate, and dissolved inorganic nitrogen $(DIN = NH_4^+ + NO_2^- + NO_3^-)$ were measured with a Technicon® Autoanalyser II, as described in Treguer and Le Corre (1974).
These samples were compared to samples fed with a continuous flux of the pumping tank outflow and which were representative of the mean effluent of that day. A linear regression analysis showed no significant difference ($P<0.001$) between the *DIN* concentrations of samples taken using these two sampling methods.

Design of the model

The general model for the variation in concentration of *DIN* in the recirculating water system effluent was constructed using three empirical sub-models: (1) fish growth, (2) ingested food and (3) replacement water. All the symbols used in the model are described in table 2.

Table 2. Model parameters list

Symbol	**Description**	**unit**
CP	**Crude protein**	% of food weight
DFR	daily feeding rate	%.day^{-1}
DIN	concentration of dissolved inorganic nitrogen in the system effluent	mg N.L^{-1}
DON	Concentration of dissolved organic nitrogen in the system effluent	mg N.L^{-1}
IF	**Daily applied food**	g.day^{-1}
n	number of fish in tank	
Nf	nitrogen in food	7.2% of food weight
Q	replacement water flow rate	L.day^{-1}
SGR	**specific growth rate**	%.day^{-1}
t	time	day
TIF	total feed applied to a fish tank between two sampling period	g
V	total rearing volume	L
W_t	mean fish weight at sampling time *t*	g
Γ_N	nitrogen discharge rate	% of applied nitrogen
θ	replacement water flow rate ($\theta = Q/V$)	day^{-1}

Fish growth rate sub-model:

The average specific growth rate (*SGR*), expressed as the percentage of fresh fish weight per day, was calculated between two samplings of fish using equation 1.

$$SGR = 100 \times \ln(W_{t+\Delta t} / W_t) / \Delta t \qquad (1)$$

The specific fish growth rate depends on the average fish weight (*W*). The general form of the equations is $SGR = a\,W^{b}$, where *a* and *b* are constants; consequently $W_{t+1} = W_t \exp^{SGR/100}$. This general model was used to evaluate the average fish size on any day based on the average fish size on the previous day. The parameters *a* and *b* were determined by a logarithmic regression analysis between *SGR* and *W*.

Daily feeding rate sub-model:
The daily feeding rate (*DFR*) depends on the average fish weight (*W*). The general form of the equation is $DFR = c\,W^{d}$, where *c* and *d* are constants; *DFR* was calculated as:

$$DFR = 100 \times TIF / \left[n \times (W_t + W_{t+\Delta t}) / 2\right] / \Delta t \qquad (2)$$

The parameters *c* and *d* were determined by a logarithmic regression analysis between *DFR* and *W*.

Replacement water sub-model:
The rate of nitrogen production (Γ_N) in this experimental aquaculture system, expressed as the percentage of input nitrogen (in food) released from the recirculation system, was calculated using the following equation:

$$\Gamma_N = 100 \times \left[(1000 \times DIN) \times Q\right] / (Nf \times IF) \qquad (3)$$

Γ_N was determined for a replacement water flow rate ranging from 0.3 to 4 times the total daily rearing volume per day. The empirical model for Γ_N was obtained by logarithmic regression analysis as a function of the replacement water flow rate.

General model:
The *DIN* concentration in the aquaculture system effluent was calculated using equation 4.

$$DIN_{calc} = \left[(\Gamma_{N\,calc} / 100) \times N_{in}\right] / (Q \times 1000) \qquad (4)$$

with:

$$N_{in} = Nf \times IF_{calc} \qquad (5)$$

$$IF_{calc} = n \times W_{calc} \times (FR_{calc} / 100) \qquad (6)$$

where W_{calc}, FR_{calc} and Γ_{Ncalc} are respectively fish weight, feeding rate and the rate of nitrogen production calculated by the sub-models.

Calibration and validation procedures:
The procedure was divided into a calibration and a validation period. During the calibration period, we used a set of experimental data obtained from tank A

between March 1995 and June 1997. During the validation period, another set of data obtained from tank B between March 1995 and June 1996, and from tanks A and B between July 1997 and March 1998, were used.
In order to verify the validity of the models, the observed and calculated values were compared using a simple linear regression analysis, as described in Keller (1989), Summers *et al*. (1991) and Mesplé *et al*. (1996). The quality of the simulation was evaluated by the slope and the y-intercept of the regression line ($X_{observed} = aX_{simulated} + b$): a simulation reflecting the natural variability would have a value for *a* which would not significantly differ from 1, and a value for *b* which would not significantly differ from 0.

RESULTS

During this study, the DIN concentrations varied between 3.2 and 52.1 mg N.L^{-1}. The DON concentrations were always low, at around only 6% of the total dissolved nitrogen, and therefore were not taken into consideration in this model.

Fish growth sub-model

A significant correlation (P<0.001; n=21; r^2=0.92) was found between the specific growth rate of *Dicentrarchus labrax* and fish weight, when the temperature was maintained at 22°C in this recirculating rearing system (figure 4).

$$SGR = 13.90 \times W_t^{-0.61} \quad (7)$$

and $$W_{t+1} = W_t \exp^{\left[\left(13.9 W_t^{-0.61}\right)/100\right]} \quad (8)$$

During the calibration period, the mean deviation between the values calculated by the model and the mean weights observed in tank A was always below the coefficient of variation of the fish sample (9±3% and 26±1% respectively).
The characteristic parameters for the validation of the SGR model are shown in table 3. We verified that the variables used in this SGR sub-model had a normal distribution and that the residuals were randomly distributed. Consequently, the sub-model could be used in the general model.

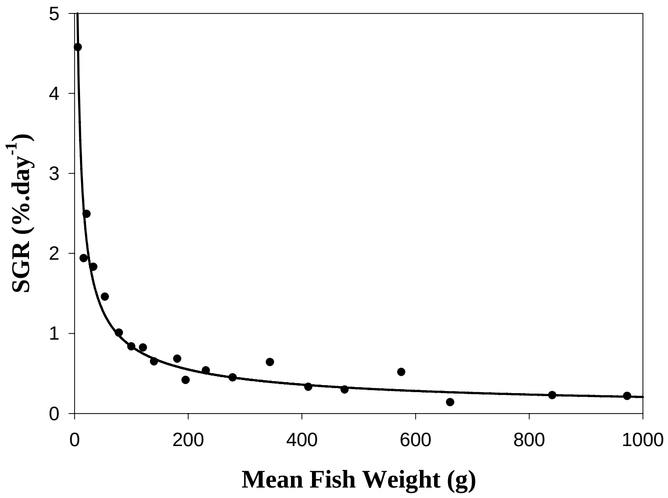

Figure 4: Specific Growth Rate sub-model

Table 3 : comparaison by linear regression between observed and calculated values

model	**n**	**SLOPE**			**Y-INTERCEPT**			**r^2**
		Slope values	**Different from 1**	**P**	**y-intercept values**	**Different from 0**	**P**	
SGR	28	0.95	no	0.09	8.14	no	0.23	0.98
DFR	28	0.90	yes	0.02	0.07	no	0.30	0.95
General	90	1.05	no	0.12	0.52	no	0.50	0.92

Ingested food sub-model

The daily feeding rate decreases as the average weight of the fish increases (figure 5), and may be calculated using equation 9:

$$DFR = 8.36\,W^{-0.41} \qquad (9)$$

($P<0.001$; $n=21$; $r^2=0.89$)

During the validation period, a comparison using a linear regression analysis with independent data gave the characteristic parameters presented in table 3. As with the SGR model, the normal distribution of the variables and the random distribution of the residuals indicated that the DFR sub-model could be used in the general model.

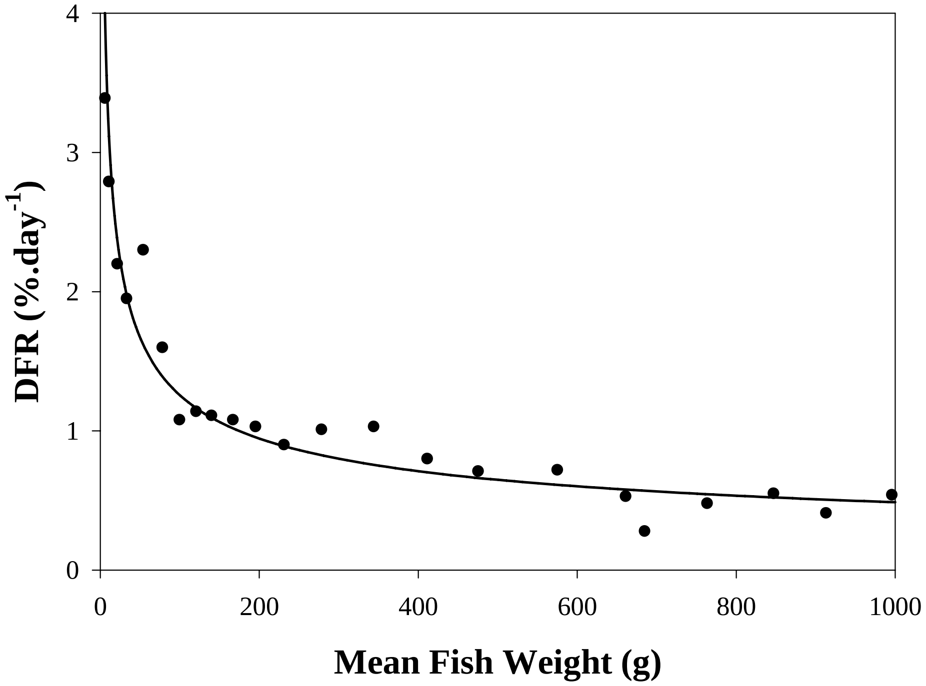

Figure 5: Daily Feeding Rate sub-model

Replacement water sub-model

When the quantity of replacement water increased from 0.3 to 4 times the daily fish rearing volume, the DIN discharge rate increased from 30 to 60% of the ingested nitrogen (equation 10):

$$\Gamma_N = 45.7 \times Q^{0.23} \qquad (10)$$

(P<0.01; n=5; r^2=0.97)

This relation is independent of fish size.

The more closed the system (i.e. the less replacement water used), the lower the amount of nitrogen discharged (figure 6). According to equation 10, when the replacement water volume was 10 times the daily rearing volume, nitrogen discharge was 75% of the total quantity of ingested nitrogen.

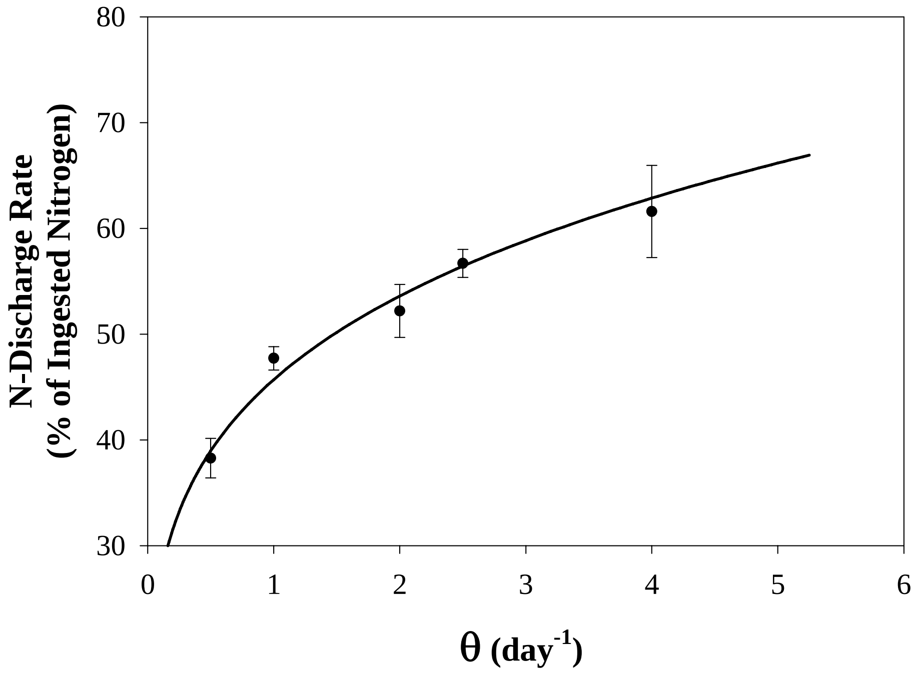

Figure 6: Representative evolution of DIN discharge rate with replacement water flow rate (mean values±SE)

General model

The prediction for the DIN concentration in the effluent between June 1997 and June 1998 is shown on figure 7. The comparison between the observed and the predicted values showed that the model was accurate, as is shown on table 3 and figure 8. The residuals were randomly distributed in this model.

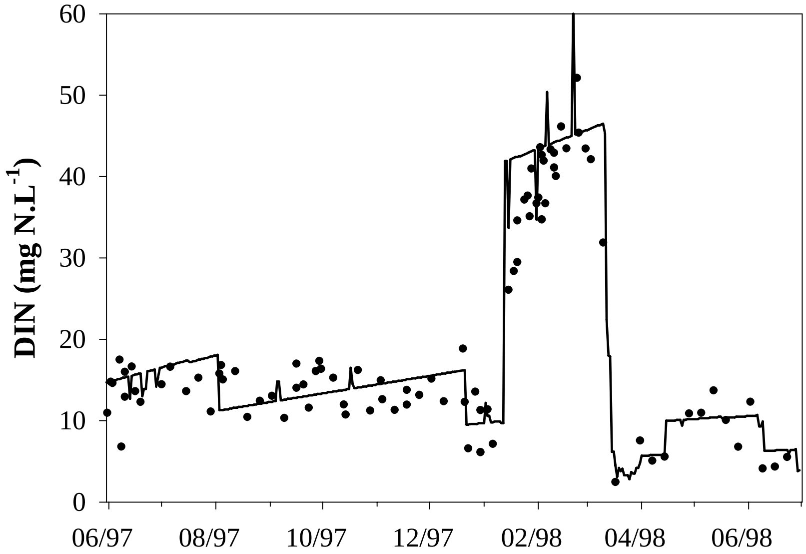

Figure 7: Predictive evolution of DIN concentration in the recirculating rearing effluent

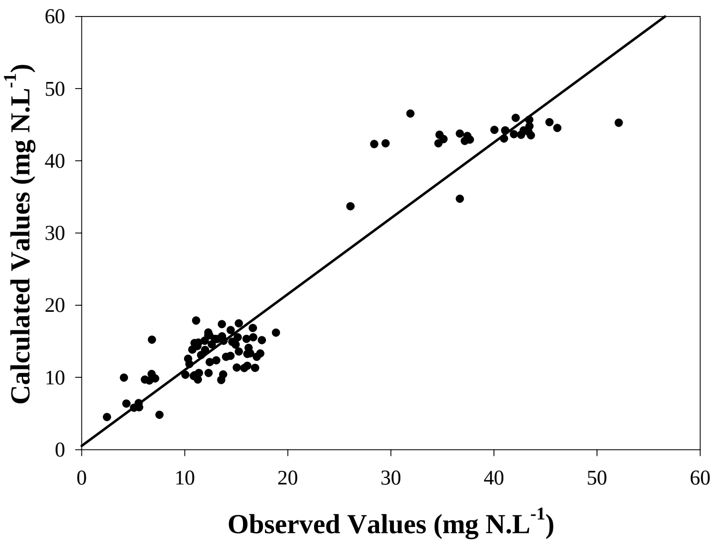

Figure 8: Validation of the general model

DISCUSSION

One of the main characteristics of recirculating rearing systems is that an optimal temperature can be maintained at a relatively low cost, which allows maximal fish growth (Maurel, 1984). In the case of *Dicentrarchus labrax*, the water temperature is generally maintained at between 22°C and 24°C (Lavenant *et al*., 1995; Pedersen, 1998).
In open rearing systems, different types of models are used to simulate fish growth. Some authors have used bioenergetics models, which take into account numerous variables such as temperature, oxygen and feed quality (From and Rasmussen, 1984; Cuenco *et al*., 1985a, 1985b, 1985c; Muller-Feuga 1990), while others have used empirical models (Tanguy et Le Grel, 1989; Koskela, 1992; Forsberg, 1996; Alanärä, 1998).
Nitrogen discharge in open fish farms has been estimated based on the linear relationship between excreted and ingested nitrogen (Table 4) or simply on excretion rates, including exogenous and endogenous excretion (Table 5). TAN excretion by sea bass was studied by Ballestrazzi *et al.* (1994, 1998) with 80 and 120 g fish reared at between 23 and 28°C. Dosdat et *al.* (1996) studied 10 to 100 g sea bass and Lemarié et *al.* (1998) studied the same fish weighing between 25 and 325 g, at between 16 and 19°C. These groups reported TAN excretion rates ranging from 30 to 58% of the total ingested nitrogen.
In the recirculating rearing system studied here, the use of our empirical model appeared to be well adapted to modeling nitrogen discharge in such a complex system, where rearing tank and various treatment units contribute to nitrogen discharge from the system.
For the fish growth sub-model, Tanguy and Le Grel (1989) determined that $SGR = 0.3\exp^{0.12\,T}\ W^{-0.34}$ for sea bass reared in cages. The use of this model, together with a constant temperature that is generally used in recirculating rearing systems (around 22°C), provided simulations that did not fit with our observations. In our system, fish growth was quicker below an average weight of 80 g and slower above this weight. A 5 g sea bass reared in our recirculating water system will attain a weight of 500 g one month later than predicted by the model of Tanguy and Le Grel (1989).
For the ingested feed sub-model, little data are available concerning the variation in the quantity of feed ingested daily by fish reared on farms because they are generally fed according to predetermined feeding tables that take into account fish size and temperature. Faure (1980), Koskela (1992) and Alanärä (1994) proposed feeding models that take into account these key variables, but using very different conditions with regard to fish species (*Onchorynchus mykiss*, *Coregonus lavaretus*), feed composition and temperature. With the temperatures used in a sea bass recirculating system, the DFR model presented is only dependent on fish weight.
As described by the replacement water sub-model, the dissolved inorganic nitrogen discharge in our system (fish excretion and nitrogen transformation by bacteria) decreased from 65% to 35% of ingested nitrogen when the replacement water volume was lowered from 5 times to 0.3 times the daily rearing water volume (figure 6). In a recirculating water system, nitrogen is almost entirely

present as nitrate as a result of the biofilter activity, which transforms the other dissolved nitrogen forms (DON, TAN and nitrite) into nitrate. Heinen *et al.* (1996) obtained similar results in a recirculating water system where trout were fed with pellets made of 42% protein and 21% lipid. They found a DIN discharge of 50% of ingested nitrogen when the daily replacement water volume was twice the tank water volume. In our case, the rate of nitrogen discharge by *Dicentrarchus labrax* fed with the same quality of food was estimated to be 52%. Two hypothesis could explain these nitrogen losses from the recirculation system. The first one is the occurrence of anoxic spot into the biofilter (denitrification and gazeous nitrogen production). The second one could be trapping nitrogen by the bacterial biomass (eliminated as suspended solids).

A good prediction of nitrogen discharge in our recirculating water system could be made with the general model, with a statistical error of less than 10%. The predicted values correspond to the variation seen with the observed values, but with a shorter reaction time. For instance, the culture system needed several days to stabilize after a quick change in the replacement flow rate, whereas the predicted values stabilized immediately.
The framework for this model could easily be adapted to other recirculating rearing systems by replacing the parameters developed for *Dicentrarchus labrax* with others defined for other farmed species.

Table 4 : Linear relationships between excreted and ingested nitrogen, EN and IN, expressed as g $N.kg^{-1}$ $FW.day^{-1}$.

Linear relationship g $N.kg^{-1}$ $FW.day^{-1}$	Species	Rearing conditions W g	T °C	CP / Energy % / $MJ.kg^{-1}$	Authors
EN=0.40×IN+0.19	*Micropterus salmoides*	46	21-23	Trash fish feed	Savitz *et al*. (1977)[a]
EN=0.25×IN+0.10	*Onchorynchus mykiss*	130	10	52.5 / 20	Kaushik (1980)[b]
EN=0.26×IN+0.13	*Onchorynchus mykiss*	130	18	52.5 / 20	
EN=0.31×IN+0.06	*Pleuronectes platessa*	35	10	trash fish feed	Jobling (1981)[b]
EN=0.53×IN+0.09	*Abramis brama*	80-100	18	tubifex feed	Tátrai (1981)[c]
EN=0.38×IN+0.01	*Onchorynchus mykiss*	380-425	10	34-49 / 18-21	Beamish and Thomas (1984)[c]
EN=0.49×IN+0.16	*Dicentrarchus labrax*	2-30	18	46-55	Vitale-Lelong (1989)[c]
EN=0.47×IN+0.19	*Dicentrarchus labrax*	2-30	23	46-55	
EN=0.14×IN+0.10	*Stizostedion vitreum*	2.6	21	41 / 17.5	Forsberg and Summerfelt (1992)[c]
EN=0.20×IN+0.06				61 / nc	
EN=0.31×IN+0.08	*Onchorynchus mykiss*	32-39	17	36-41 / 22	Médale *et al*. (1995)[b]
EN=0.34×IN+0.08	*Onchorynchus mykiss*			36-41 / 19	
EN=0.26×IN+0.04	*Salmo solar*	300-2000	4-10	40-45 / 18-19	Forsberg (1996)[c]

[a]: total nitrogen; [b]: TAN+urea; [c]:TAN; nc: not communicated

Table 5 : TAN excretion rate in some teleost fishes

Nitrogen Excretion rate % of ingested N	species	W g	T °C	rearing conditoion Feed	CP / E % / MJ.kg^{-1}	authors
29-44	*Onchorynchus mykiss*	50	18	Starch diert	41-43 / 21	Kaushik and Olivia Teles (1985)
30	*Sparus aurata*	3-90	24	Dry pellets	nc	Porter *et al.* (1987)
13	*Sparus aurata*	65	nc	Dry pellets	38 / nc	Krom and Neori (1989)
52-71	*Salvelinus namaycush*	215	12	Dry pellets	40-50 / 18-22	Jayaram and Beamish (1992)
19-23	*Scophthalmus maximus*	35-50	8-20	Dry pellets	nc	Burel *et al.* (1996)
35/38	*Dicentrarchus labrax*	10/100	16-20	Dry pellets	52 / 18	Dosdat *et al.* (1996)
33/35	*Sparus aurata*	10/100	16-20	Dry pellets	52 / 18	Dosdat *et al.* (1996)
21	*Scophthalmus maximus*	10/100	16-20	Dry pellets	52 / 18	Dosdat *et al.* (1996)
30/35	*Salmo trutta fario*	10/100	16	Dry pellets	52 / 18	Dosdat *et al.* (1996)
32/36	*Onchorynchus mykiss*	10/100	16	Dry pellets	52 / 18	Dosdat *et al.* (1996)
26-37	*Onchorynchus mykiss*	80	12	Dry pellets	52 / 18	Dosdat *et al*. (1997)
28-34	*Salmo trutta fario*	90	12	Dry pellets	52 / 18	Dosdat *et al*. (1997)

nc: not communicated

CONCLUSION

The proposed model was designed to enable a prediction to be made about dissolved nitrogen discharge in a fish farm functioning with a recirculating water system at a constant temperature, using three variables: fish weight, fish number and replacement water flow rate.

Using this model, an accurate prediction of dissolved nitrogen discharge was made. This model could represent an important tool to define: (1) replacement water management, and (2) the maximal fish biomass required to meet any future legislation on DIN-containing effluent.

This knowledge is of the utmost importance for designing a system for the treatment of effluent, which will be required if the calculated nitrogen load is likely to have a significant impact on the environment

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