

## Comparison of brown trout (*Salmo trutta*) reared in fresh water and sea water to freshwater rainbow trout (*Oncorhynchus mykiss*): I. Growth and nitrogen balance

Antoine Dosdat <sup>(1)\*</sup>, Robert Métailler <sup>(2)</sup>,  
Elisabeth Desbruyères <sup>(1)</sup> and Christine Huelvan <sup>(2)</sup>

<sup>(1)</sup> Laboratoire de Physiologie des Poissons, IFREMER, BP 70, 29280 Plouzané, France.

<sup>(2)</sup> Laboratoire mixte de Nutrition des Poissons, IFREMER-INRA, BP 70, 29280 Plouzané, France.

\* Present address: IFREMER, Station de Palavas, Chemin de Maguelone, 34250 Palavas-les-Flots, France.

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### Abstract

Brown trout and rainbow trout (average weight 100 g) were reared in fresh water at 12°C under the same conditions before transfer of brown trout to sea water, in order to compare nitrogen utilisation in the two species. Apparent protein digestibility (ADC), nitrogen (ammonia and urea) excretion, protein productive value (PPV) and actual observed nitrogen mass balance were determined. Rainbow trout raised in fresh water had a higher growth rate (1.1 vs 0.8%.d<sup>-1</sup>), better food conversion ratio (0.7 vs 1.0), better ADC (91 vs 85%) and PPV (45 vs 35%) and lower ammonia excretion rates than brown trout reared in fresh water. Transferring brown trout to sea water induced lower PPV (30%) and ammonia and urea excretion. Salinity did not modify metabolic efficiency in brown trout. Fat content was higher in brown trout (7.7-8.9% ww) than in rainbow trout (5.7-7.6% ww). Nitrogen mass balance indicated that compounds other than ammonia and urea were produced in higher quantities by seawater brown trout. Behaviour, less domestication and specific ability to utilise protein could explain the differences between the two species.

**Keywords:** Rainbow trout, brown trout, excretion, ammonia, urea, digestibility, growth, protein productive value.

*Comparaison de la truite commune (Salmo trutta) élevée en eau douce et en eau de mer avec la truite arc-en-ciel d'eau douce (Oncorhynchus mykiss): I. Croissance et bilan azoté.*

### Résumé

Des truites communes (fario) et des truites arc-en-ciel de 100 g de poids moyen ont été élevées à 12°C dans des conditions strictement identiques, avant le transfert des truites communes en eau de mer, dans le but de comparer l'utilisation de l'azote chez les deux espèces. La digestibilité apparente des protéines (ADC), l'excrétion ammoniacale et uréique, et l'accrétion protéique (PPV) des poissons ont été estimées. Les bilans de masse ont été construits avec ces données. Les truites arc-en-ciel élevées en eau douce ont présenté un meilleur taux de croissance (1.1 vs 0.8%.j<sup>-1</sup>), un meilleur coefficient de transformation de l'aliment (0.7 vs 1.0), de meilleurs ADC (91 vs 85%) et PPV (45 vs 35%), ainsi qu'une excrétion ammoniacale plus faible que les truites communes élevées en eau douce. Après transfert en mer, l'excrétion ammoniacale et l'excrétion uréique ont diminué chez la truite commune, ainsi que la rétention protéique (30%). La salinité n'a pas modifié l'efficacité métabolique de la truite commune. Le contenu lipidique de la truite commune (7.7-8.9% poids frais) était plus élevé que celui de la truite arc-en-ciel (5.7-7.6% poids frais). Le bilan de masse de l'azote a indiqué que les autres molécules que l'ammoniaque et l'urée étaient excrétées en plus grandes quantités par la truite commune en eau de mer. Le comportement, une

domestication moins avancée et une capacité distincte d'utilisation des protéines pourraient expliquer les différences entre les deux espèces.

**Mots-clés :** Truite commune, truite arc-en-ciel, excrétion, ammoniacque, urée, digestibilité, croissance, rétention protéique.

## INTRODUCTION

Freshwater rainbow trout (*Oncorhynchus mykiss*) is the leading fish production in France, nearing 60 000 t in 1995. The first attempts to raise salmonids in seawater in France date from the mid 1970s (Boeuf and Harache, 1984b). Until the late 1980s, rainbow trout was the only species concerned. Nevertheless, the high temperatures and salinity prevailing in the French marine environment during the July to September period correspond to the biological limits of this species (Harache, 1985). They induce high mortality, which is unacceptable for fish farmers. Conversely, brown trout (*Salmo trutta*) withstands this critical period better than other salmonids (Boeuf and Harache, 1984a) and can be more easily adapted to seawater (Hogstrand and Haux, 1985). Thus it appears to be a promising species for aquaculture (Quillet *et al.*, 1991; Krieg *et al.*, 1992). Due to these particularities, over the last five years, it has been developed in seawater in France (Paquotte, pers. comm.). At the same time, genetic improvement (Chevassus *et al.*, 1991), linked to the development of production in seawater, has led to a production in freshwater, mainly for restocking purposes.

Nutritional requirements are far less known in brown trout than in rainbow trout (Gabaudan *et al.*, 1989; Arzel *et al.*, 1991). Very little information is available concerning nitrogen and phosphorus metabolism in brown trout (Cho *et al.*, 1991). There is also a lack of comparative studies concerning its place amongst salmonids (Kaushik and Cowey, 1991; Elliott, 1994). However, rainbow trout is one of the best known fish, and numerous publications have been devoted to this species for many years concerning nutrition (Nose, 1960; Cho and Kaushik, 1990), protein metabolism (Atherton and Aitken, 1970; Morales *et al.*, 1994), nitrogen excretion (Smith, 1947; Kaushik, 1980; Lanari *et al.*, 1993) and environmental impact (Willoughby *et al.*, 1972; Stirling and Dey, 1990).

### The aims of the study were

– To quantify the data (nitrogen and phosphorus accretion, nitrogen and phosphorus losses through faeces, ammonia, urea and phosphate excretion) required to enable sound impact studies when fish farms are foreseeing brown trout cultivation. These data were processed in order to build actual observed mass balances for nitrogen and phosphorus, the two main driving elements in both seawater and freshwater eutrophication processes (Stirling and Dey, 1990).

– To compare brown trout reared in freshwater or in seawater to freshwater rainbow trout (considered as a reference) in order to estimate the discrepancies between these two species, and to analyse the suitability of existing data on rainbow trout in order to assess the impact induced by brown trout farming.

Results on growth and nitrogen balance are presented here. Those concerning phosphorus will be presented in a forthcoming paper.

## MATERIALS AND METHODS

### Experimental protocol

The experiment was organised in four consecutive phases. Phase 1 compared the growth and nitrogenous excretion of brown trout and rainbow trout reared in freshwater. Phase 2 examined brown trout during their adaptation to seawater. Seawater adapted brown trout were then compared to the rainbow trout and brown trout kept in freshwater (Phase 3). The last phase determined hourly excretion rate and ADC in the same water quality conditions (Phase 4).

### Experimental design and rearing conditions

Rainbow trout (*Oncorhynchus mykiss*, "Cornec" strain) and brown trout (*Salmo trutta fario*, "Hardy DC87" strain) were obtained from the IFREMER-INRA Experimental Station in Sizun. The fish were transported to the IFREMER facilities in Plouzané and put in outdoor 4 m<sup>2</sup> rearing tanks supplied with freshwater. Three weeks later, the fish were sorted and dispatched into twelve indoor 500 litre tanks (1×1×0.5 m), supplied with desaturated freshwater from the same origin in a flow-through system. Eight tanks were filled with brown trout (BT.FW, average weight 90 g, 80 fish per tank) and 4 tanks with rainbow trout (RT.FW, average weight 80 g, 90 fish per tank). Water was treated through a lamellar decanter and subsequently supplied by gravity to the tanks. Fish were hand fed twice daily at 10:00 and 16:00 h with dry floating expanded pellets (Table 1). During the whole experiment (including acclimatization) particular attention was given to feed intake that was recorded at each meal in every tank. Water flows were set up in order to maintain dissolved oxygen levels above 80 % saturation in the outlet. Water temperature was not controlled during acclimatization (Fig. 1). Photoperiod was maintained on a 12L:12D (08:00-20:00 h) cycle. Tanks were half covered using a wooden lid.

Table 1. – Experimental diet composition.

Ingredients	(g/100 g mixture)
Norwegian fish meal (Norseamink <sup>®</sup> )	32.0
Soluble fish protein concentrate	14.9
Greaves meal	15.0
Lactic yeast	5.2
Cooked potatoes starch	12.0
Soy lecithin	1.0
Cod liver oil	11.9
Inorganic bulk agent (zeolite) <sup>1</sup>	5.1
Mineral premix <sup>2</sup>	1.0
Vitamin premix <sup>3</sup>	1.0
Choline chloride 50%	0.6
Ascorbic acid polyphosphate 25%	0.3
<i>Composition</i>	
Dry matter (%)	94.9 ± 0.1
Lipid content (% DM)	20.06 ± 0.33
Nitrogen content (% DM)	8.28 ± 0.03.
Digestible Energy (kJ g <sup>-1</sup> DM) <sup>4</sup>	17.54

<sup>1</sup> During the fourth phase, 1% zeolite was replaced by 1% chromic oxide.

<sup>2</sup> Mineral premix contained the following ingredients (g.kg<sup>-1</sup> mix): calcium carbonate, 215; magnesium carbonate, 124; KCl, 90; KI, 0.04; calcium hydrogen phosphate (CaHPO<sub>4</sub>, 2H<sub>2</sub>O), 500; NaCl, 40; sodium fluoride, 1; copper sulfate, 3; zinc sulfate, 4; cobalt sulfate, 0.02; ferric sulfate, 20; manganese sulfate, 3.

<sup>3</sup> Vitamin premix contained the following ingredients (g.kg<sup>-1</sup> mix, or IU when mentioned): vitamin A acetate, 1 000 000 IU; vitamin D<sub>3</sub>, 100 000 IU; -tocopherol (vitamin E) acetate, 4; vitamin K<sub>3</sub>, 0.1; thiamin, 1; riboflavin, 2.5; calcium d-pantothenate, 5; pyridoxin, 1; vitamin B<sub>12</sub>, 0.006; niacin, 10; folic acid, 0.5; biotin, 0.1; meso-inositol, 100.

<sup>4</sup> Estimated according to Guillaume (1991).

After two weeks acclimatization to rearing conditions, and an evaluation of spontaneous ingestion rate, the experiment began on November 28 (day 0). Fish global biomass was determined for each tank. The first phase of the experiment lasted 46 days until January 13 in order to take into account sufficient growth. Fish were weighed on day 0, 21 and 46. The

feeding level was fixed after every weighing at 1% of the biomass, and uneaten pellets were recorded. Temperature (averaging 12°C) was not controlled.

The second phase began on January 14 when brown trout in 4 of the 8 tanks were transferred to seawater (BT.SW) progressively over two days by adding thermoregulated (12°C) seawater (34 g.l<sup>-1</sup>), filtered through a high pressure sand filter, and decreasing the freshwater supply. This adaptation phase lasted 20 days during which plasma criteria linked to environmental modifications (osmotic pressure, Cl<sup>-</sup>, thyroid hormones) and branchial (Na<sup>+</sup>, K<sup>+</sup>)ATPase activity were monitored according to Boeuf and Prunet (1985). Two fish were sampled in each seawater tank at day 46, 48, 50, 53 and 60. Dead fish were recorded and weighed every day. At the end of the phase (day 66), the fish were weighed and re-allocated in order to balance the biomass in the tanks, which had previously been altered by differential mortality and growth rates. In each tank, the fish were sorted in order to reduce heterogeneity among the replicates. The environmental conditions, except salinity were similar. During the last days of Phase 2 (acclimatization to seawater), it was decided to reduce feeding levels in freshwater tanks in order to limit future differences in individual weight between fish raised in seawater and freshwater.

The third phase lasted 50 days. Both seawater and freshwater were thermoregulated at 12 °C. Thus, the fish were kept at the same temperature (close to the thermal *preferendum* for both species) during the entire experiment. The fish were weighed at day 98 and at the end of the phase (day 116). During this phase, ration was fixed at 0.9% of the biomass for the three conditions, taking into consideration increasing amounts of uneaten food.

The fourth phase took place when the fish were transferred into 3 cylindroconical tanks (1 m<sup>3</sup>) in order to determine feed digestibility and to monitor hourly excretion rates. This phase lasted 40 days, including acclimatization to the new tanks. Faeces and water were sampled during the last five days of the phase. In

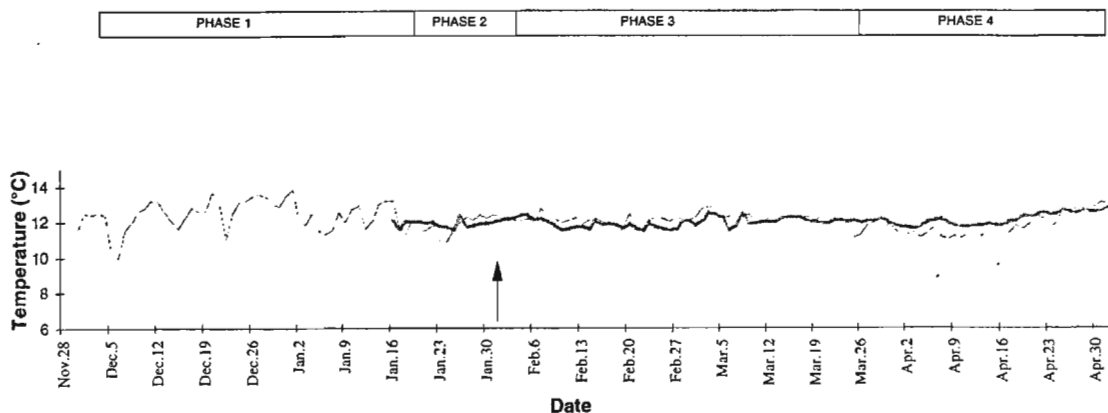


Figure 1. – Water temperatures during the experiments. Arrow indicates the beginning of the freshwater thermoregulation. Thick line represents seawater temperature, dotted line represents freshwater temperature.

order to achieve a satisfactory digestibility evaluation, the fish were fed once a day on a diet containing 1% chromic oxide (Table 1), and tanks cleaned every day.

### Sampling of wastes in effluent water

During the first three phases, outflowing water quality was monitored in each tank, including tanks with no fish as reference, using the method described in Dosdat *et al.* (1994). The sampling runs lasted the 5 consecutive days before each weighing: two runs occurred during Phase 1, one run during Phase 2 and two runs during Phase 3. Water was sampled through a peristaltic pump and pooled into polypropylene bottles with chloroform as a preservative. On these aliquots, Total Ammonia Nitrogen (TAN) was analysed by the modified indophenol blue method described by Tréguer and Le Corre (1975) and Urea Nitrogen (Urea-N) by the acetyl-monoxime method (Aminot and Kérouel, 1982) using a Technicon<sup>®</sup> Autoanalyser II. Average TAN and Urea-N contents of the water were thus evaluated on a daily basis. For each tank, the freshwater flow rate was measured once a day, and seawater flow twice a day before and after every filter cleaning operation.

During the fourth phase, digestibility of the feed was evaluated using a decantation bottle and chromic oxide as a marker (Cho *et al.*, 1982). Faeces were collected with 1 000 ml of supernatant water, over 5 consecutive days and then frozen ( $-20^{\circ}\text{C}$ ). Pooled samples were centrifuged (15 min,  $4^{\circ}\text{C}$ , 4 000 rpm) and the nitrogen content of the supernatant and the bottom analysed separately.

Patterns of TAN and Urea-N excretion were monitored using the methodology proposed by Dosdat *et al.* (1994). Water was sampled over 24 hours through a high pressure peristaltic pump to an automated apparatus enabling one sample to be analysed, for both TAN and Urea-N content, every 150 s.

### Sampling and chemical analyses of feed, fish and faeces

Rainbow trout and brown trout were sampled after one day of fasting in two sets at day 0 (5 fish per tank, pooled). On day 46, 66 and 116, 10 fish per tank were sampled and analysed separately. The fish were chosen close to the mean of each replicate and frozen ( $-20^{\circ}\text{C}$ ) before grinding. Feed was sampled three times during Phase 1 and 3 (pooled) and Phase 4.

Analyses of feed and fish were performed using conventional methods: dry matter after drying 24 h at  $105^{\circ}\text{C}$ , crude proteins by the Dumas method (Fison<sup>®</sup> NA 2000), crude lipid by dichloromethane extraction with an automatic Soxhlet<sup>®</sup> apparatus. Nitrogen in the faeces was analysed using the Dumas method for both particulate and soluble phases, and chromic oxide in faeces and feed by the method of Bolin *et al.* (1952).

### Data processing and statistical analysis

In all calculations, ingested feed is given in dry matter. The following key parameters were utilised:

Initial average wet weight (g):  $W_0$

Final average wet weight (g):  $W_f$

Biomass wet weight (g): B; Feed intake (g): F;

Water flow rate ( $\text{l}\cdot\text{h}^{-1}$ ): Q; Number of days: n.

Basic data were processed to determine:

– Specific growth rate ( $\%\cdot\text{day}^{-1}$ ):

$$\text{SGR} = 100 [I_n(W_f) - I_n(W_0)]/n$$

– Feeding level ( $\%\cdot\text{day}^{-1}$ ):

$$\text{FL} = F / [(B_0 + B_f) \times \frac{n}{2}]$$

– Feed gain ratio:

$$\text{FGR} = F / [(B_f + \text{dead fish } B) - B_0]$$

– Apparent digestibility coefficient of Nitrogen (%):

$$100 - [(100 \text{ Cr.diet} / \text{Cr.faeces}) \times (\text{N.faeces} / \text{N.diet})]$$

– Protein productive value (%):  $\text{PPV} = 100 \times (\text{Final nitrogen fish content} - \text{Initial nitrogen fish content}) / \text{Nitrogen intake}$

– Daily excretion rate ( $\text{mg N}\cdot\text{kg}^{-1} \text{ ww}\cdot\text{day}^{-1}$ ):  $(\text{Average outflow concentration} - \text{Average outflow concentration of empty tank}) \times Q \times 24 / B$

– Hourly excretion rate ( $\text{mg N}\cdot\text{kg}^{-1} \text{ ww}\cdot\text{h}^{-1}$ ), defined in Dosdat *et al.* (1994) as:

$$\left( \frac{Q}{1 - e^{-QT/V}} \right) \times (C_{t+1} - C_i - (C_t - C_i) e^{-QT/V}) \times \frac{1}{B}$$

where V is tank volume,  $C_t$  ( $C_i$ ) outflowing (empty tank) concentrations and T time interval.

– Nitrogen recovery rate (%):  $100 \times (\text{Final nitrogen fish content} + \text{Nitrogen faeces content} + \text{Excreted TAN} + \text{Excreted Urea-N}) / (\text{Initial nitrogen fish content} + \text{Nitrogen intake})$

Where two conditions were analysed, a comparison of the means was carried out using the Student test ( $t$ ), after testing the homogeneity of the variance in the case of an unbalanced number of tanks (Phase 1). One way ANOVA was used in balanced experiments involving three conditions (Phase 2 and Phase 3), followed by a Newman-Keuls test for a *posteriori* classification. When percentage values were tested, the arcsin  $\sqrt{\quad}$  transformation was employed.

## RESULTS

## Mortality, growth and diet utilisation

The feeding behaviour of rainbow trout and brown trout proved to be totally opposite in nature. Rainbow trout had a positive movement towards the person who fed them, whereas brown trout showed fear. The consequence was noticeable as regards meal duration: it lasted a few minutes for rainbow trout whereas it was 15-30 min in brown trout. This had no effect on feed quality, due to the high water stability of the expanded pellets. Between two consecutive fish weighings, feeding levels decreased along with the growth of the fish, due to the fixed ration.

Rainbow trout showed a significantly higher growth ( $p < 0.001$ ) than brown trout kept in freshwater, during each of the three phases (Table 2). Final average weight was 282 g in RT.FW and 229 g in BT.FW (Fig. 2). There were significant differences after 112 days in freshwater ( $p < 0.001$ ). Transferring brown trout to seawater decreased their growth rate during the first 20 days, which was associated with a large decrease in feed intake (Fig. 2). Afterwards (Phase 3), BT.FW and BT.SW growth rates were not different (Table 2). At the end of Phase 3, BT.SW were significantly lighter (208 g) than BT.FW (229 g).

Survival rate fell dramatically in brown trout when they were transferred to seawater (Fig. 2), especially during the second week when the mortality level reached 10%. Survival stabilised 35 days after transfer of fish. Blood plasma parameters, *i.e.* osmotic pressure and chlorine concentration, showed typical profiles: they increased significantly from  $303 \pm 7.0$  mOsmol.l<sup>-1</sup> and  $123 \pm 4.6$  mmol.l<sup>-1</sup> respectively at day 0 to  $364 \pm 18.4$  mOsmol.l<sup>-1</sup> and  $152 \pm 14.1$  mmol.l<sup>-1</sup> at day 4, before reaching the initial levels at day 14 ( $314 \pm 5.0$  mOsmol.l<sup>-1</sup> and  $130 \pm 5.4$  mmol.l<sup>-1</sup> respectively). (Na<sup>+</sup>,K<sup>+</sup>)ATPases activity levels were normal, averaging  $7.4 \pm 2.5$  ( $\mu$ mol. P<sub>i</sub>.mg<sup>-1</sup> protein.h<sup>-1</sup>) at day 0.

**Table 2.** – Comparison of growth rates and food conversion ratios, for rainbow trout reared in freshwater (RT.FW), brown trout reared in freshwater (BT.FW) and seawater (BT.SW), during the three phases of the growth experiment. SGR represents Specific growth rate and FGR Feed gain ratio. Total biomass gain includes dead fish. For each phase, values in the same column not sharing a common superscript are different at the level  $p < 0.05$ . Significance of Student *t* and ANOVA (F) tests are given at: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.005$ .

	Type	Nb Tank	Average temperature (°C)	Biomass gain (g)	SGR (% d <sup>-1</sup> )	<i>t</i> test or ANOVA	FGR (g.g <sup>-1</sup> )	<i>t</i> test or ANOVA
PHASE 1 Nov. 28 → Jan. 13	RT.FW	4	12.4	4 621	1.06 ± 0.02	<i>t</i> = 10.84	0.73 ± 0.01	<i>t</i> = 9.71
	BT.FW	8	12.4	3 100	0.80 ± 0.05	***	0.99 ± 0.05	***
PHASE 2 Jan. 13 → Feb. 2	RT.FW	4	11.9	1 830	0.75 ± 0.06 <sup>c</sup>	<i>F</i> = 31.19	0.95 ± 0.06 <sup>a</sup>	<i>F</i> = 7.06
	BT.FW	4	11.9	1 104	0.60 ± 0.07 <sup>b</sup>	***	1.32 ± 0.18 <sup>a</sup>	*
PHASE 3 Feb. 2 → Mr. 24	BT.SW	4	11.9	183	0.31 ± 0.09 <sup>a</sup>		5.49 ± 3.28 <sup>b</sup>	
	RT.FW	4	12.2	4 159	0.92 ± 0.06 <sup>b</sup>	<i>F</i> = 28.54	0.82 ± 0.05 <sup>a</sup>	<i>F</i> = 21.63
	BT.FW	4	12.2	3 147	0.76 ± 0.04 <sup>a</sup>	***	1.02 ± 0.05 <sup>b</sup>	***
	BT.SW	4	12.0	2 856	0.73 ± 0.01 <sup>a</sup>		1.12 ± 0.09 <sup>b</sup>	

This high mortality rate during Phase 2 was accompanied by a decline in the FGR (Table 2). During this phase, no difference was statistically apparent between RT.FW and BT.FW: the great variability in BT.SW decreased significance in the Newman-Keuls test. Feed conversion ratios were significantly different among RT.FW and BT.FW during Phase 1 and Phase 3. No difference was noted in FGR among BT.FW and BT.SW after the fish were acclimatised (Phase 3).

## Total ammonia nitrogen and Urea-N excretion

Total ammonia nitrogen (TAN) excretion rates (in mg N. kg<sup>-1</sup> ww.d<sup>-1</sup>) were different for every class of fish during the first three phases (Table 3). In freshwater, rainbow trout excretion was systematically lower than brown trout, with a tendency to increase relatively to BT.FW from Phase 1 to Phase 3. TAN excretion rates in brown trout kept in seawater were lower ( $p < 0.001$ ) than those kept in freshwater. Plotted against ingested nitrogen, TAN excretion was systematically lower in RT.FW than in BT.FW, and lower in acclimatised RT.SW, than in BT.FW (Table 5).

Urea-N excretion rate (in mg N. kg<sup>-1</sup> ww.d<sup>-1</sup>) was significantly lower in BT.SW. It was not different between RT.FW and BT.FW. The same trends were noted when Urea-N excretion was plotted against ingested nitrogen.

Excretion profiles confirmed the data obtained by the pooling method (Table 3). TAN and Urea-N excretion rates were systematically lower in BT.SW (Fig. 3) than in the other two cases. TAN excretion peaked 6-7 hours after feeding, amounting to 10 to 14 mg N. kg<sup>-1</sup> ww.d<sup>-1</sup>. TAN average daily excretion rates evaluated from excretion profiles were similar in RT.FW and BT.FW, respectively 180 and 181 mg N. kg<sup>-1</sup> ww.d<sup>-1</sup>. Urea-N excretion profiles showed no apparent trend (Fig. 3).

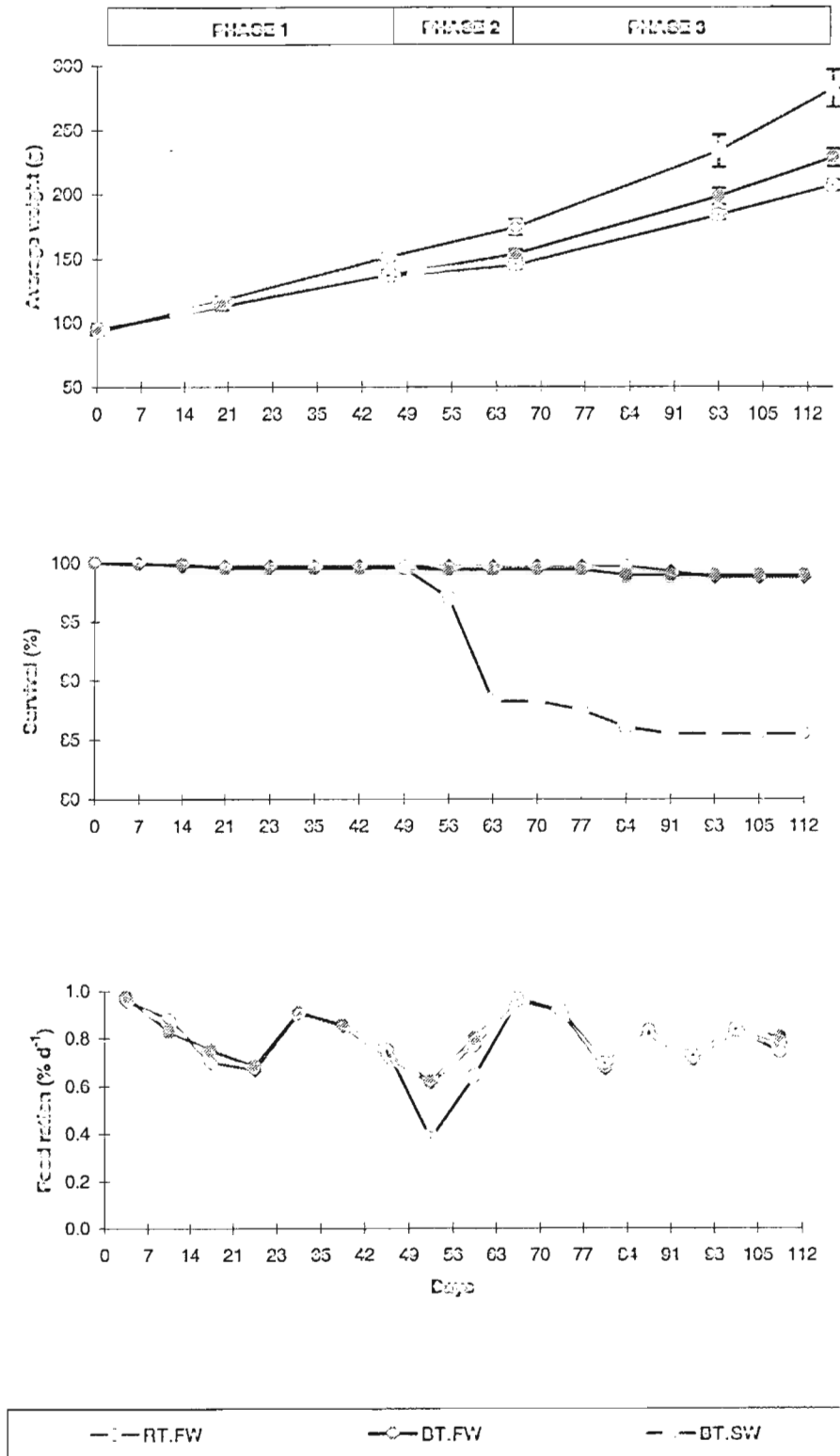


Figure 2. – Growth, survival rate and feed intake for rainbow trout reared in freshwater (RT.FW), brown trout reared in freshwater (BT.FW) and seawater (BT.SW). Vertical bars represent standard deviation.

**Table 3.** – Comparison of Total ammonia nitrogen (TAN) and Urea nitrogen (Urea-N) excretion during the 4 phases of the experiment. Type represents rainbow trout reared in freshwater (RT.GW), brown trout reared in freshwater (BT.FW) and seawater (BT.SW). Ingested and excreted nitrogen are given in (mg N.kg<sup>-1</sup>.d<sup>-1</sup>). Values for excretion are recalculated from hourly excretion rates. For each run, values in the same column not sharing a common superscript are different at the level  $p < 0.05$ . Significance of Student *t* and ANOVA (F) tests are given at: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.005$ .

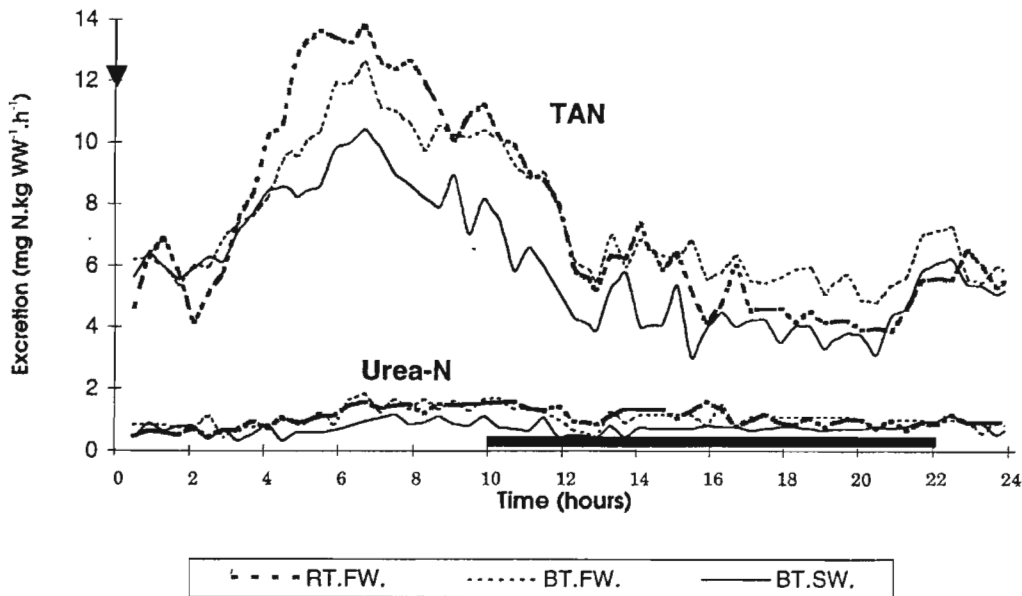
		Fish	Nb Tank	Average weight (g)	Nitrogen intake	Excreted TAN	<i>t</i> test or ANOVA	Excreted Urea-N	<i>t</i> test or ANOVA
PHASE 1	RUN 1	RT.FW	4	112.5	666.9	179.3 ± 4.9	<i>t</i> = 10.6	30.3 ± 0.86	<i>t</i> = 0.5
	Dec.12 → Dec. 16	BT.FW	8	108.9	704.6	224.4 ± 7.6	***	29.6 ± 2.7	N.S.
PHASE 1	RUN 2	RT.FW	4	151.1	620.9	164.8 ± 9.4	<i>t</i> = 4.9	27.2 ± 1.9	<i>t</i> = 0.1
	Jan. 9 → Jan. 13	BT.FW	8	136.8	640.3	203.0 ± 13.8	***	27.1 ± 1.5	N.S.
PHASE 2	RUN 1	RT.FW	4	158.7	752.0	199.3 ± 3.4 <sup>b</sup>	<i>F</i> = 1358.5	29.2 ± 2.8	<i>F</i> = 104.5
		BT.FW	4	142.8	782.5	236.3 ± 4.3 <sup>c</sup>		31.3 ± 2.6	
		BT.SW	4	139.1	319.9	95.4 ± 4.1 <sup>a</sup>		9.7 ± 1.2	
PHASE 3	RUN 1	RT.FW	4	227.7	669.2	195.0 ± 4.6 <sup>b</sup>	<i>F</i> = 82.9	33.1 ± 0.9	<i>F</i> = 103.4
		BT.FW	4	193.5	682.3	214.6 ± 3.9 <sup>c</sup>		29.7 ± 1.4	
		BT.SW	4	179.4	546.5	155.1 ± 9.8 <sup>a</sup>		18.3 ± 2.1	
		RT.FW	4	282.5	608.7	195.3 ± 8.9 <sup>b</sup>		28.3 ± 2.3	
PHASE 3	RUN 2	BT.FW	4	229.3	624.1	210.8 ± 3.4 <sup>c</sup>	<i>F</i> = 37.4	27.8 ± 1.6	<i>F</i> = 4.6
		BT.SW	4	207.8	604.8	159.2 ± 11.5 <sup>a</sup>		23.9 ± 2.7	
PHASE 4	RUN 1	RT.FW	1	380	541	180		25.6	
		BT.FW	1	308	554	181		25.7	
		May 1 → May 2	BT.SW	1	323	534	146		18.2

**Lipid and protein accretion (PPV)**

Lipid content (Table 4) was higher in BT.FW than in RT.FW at the end of Phase 2 ( $p < 0.001$ ) and at the end of Phase 3 ( $p < 0.05$ ). It was at an intermediary level in BT.SW. During Phase 3, lipid gain and deposition were higher in RT.FW than in BT.FW; it was the

opposite during Phase 1. During Phase 2, transferring brown trout to seawater induced a stabilisation in body lipid level and a decrease in global lipid gain (0.08 compared to 0.81 g.kg<sup>-1</sup> ww.d<sup>-1</sup> in BT.FW).

PPV was higher in RT.FW than in brown trout during every phase (Table 5). Over the 3 Phases,



**Figure 3.** – Total ammonia nitrogen (TAN) and urea nitrogen (Urea-N) excretion profiles for rainbow trout reared in freshwater (RT.FW), brown trout reared in freshwater (BT.FW) and seawater (BT.SW). Arrow indicates feeding. Black line represents the night period.

**Table 4.** – Fat content and lipid gain during the three phases of the experiment. Type represents rainbow trout reared in freshwater (RT.FW), brown trout reared in freshwater (BT.FW) and seawater (BT.SW). For each phase, values in the same column not sharing a common superscript are different at the level  $p < 0.05$ . Significance of Student *t* and ANOVA (*F*) tests are given at: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.005$ .

	Fish	Nb Tank	Whole body lipid (% ww)	<i>t</i> test or ANOVA	Lipid gain (g.kg <sup>-1</sup> .d <sup>-1</sup> )	<i>t</i> test or ANOVA
INITIAL	RT.FW		5.33			
	BT.FW		5.53			
END PHASE 1	RT.FW	4	5.66 ± 0.43	<i>t</i> = 6.72	0.620 ± 0.090	<i>t</i> = 4.02
	BT.FW	4	7.68 ± 0.42	**	0.875 ± 0.900	**
END PHASE 2	RT.FW	4	5.78 ± 0.57 <sup>a</sup>	<i>F</i> = 29.91	0.472 ± 0.125 <sup>b</sup>	<i>F</i> = 20.85
	BT.FW	4	8.44 ± 0.58 <sup>b</sup>	***	0.805 ± 0.138 <sup>c</sup>	***
	BT.SW	4	7.71 ± 0.32 <sup>b</sup>		0.080 ± 0.203 <sup>a</sup>	
END PHASE 3	RT.FW	4	7.57 ± 0.41 <sup>a</sup>	<i>F</i> = 8.44	0.958 ± 0.087 <sup>b</sup>	<i>F</i> = 9.13
	BT.FW	4	8.90 ± 0.50 <sup>b</sup>	**	0.740 ± 0.135 <sup>a</sup>	**
	BT.SW	4	8.12 ± 0.45 <sup>ab</sup>		0.625 ± 0.108 <sup>a</sup>	

**Table 5.** – Nitrogen mass balance. All values, except recovery rate are given in proportion of feed intake. Type represents rainbow trout reared in freshwater (RT.FW), brown trout reared in freshwater (BT.FW) and seawater (BT.SW). ADC represents Apparent digestibility coefficient of protein; TAN, Total ammonia nitrogen; Urea-N, Urea nitrogen. Recovery rate is calculated as given in Materials and Methods. For each phase, values in the same column not sharing a common superscript are different at the level  $p < 0.05$ . Significance of Student *t* and ANOVA (*F*) tests are given at: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.005$ .

	Type	Nb Tank	Retained Nitrogen (=PPV)	<i>t</i> test or ANOVA	N fecal loss (=100-ADC)	TAN loss	<i>t</i> test or ANOVA	Urea-N loss	<i>t</i> test or ANOVA	N recovery rate	<i>t</i> test or ANOVA
PHASE 1	RT.FW	4	44.9 ± 0.9	<i>t</i> = 10.5	8.93	26.8 ± 0.7	<i>t</i> = 4.79	4.5 ± 0.1	<i>t</i> = 1.75	91.5 ± 0.7	<i>t</i> = 0.06
	BT.FW	4	34.5 ± 1.8	***	14.66	31.2 ± 1.7	**	4.2 ± 0.2	N.S.	91.4 ± 1.4	N.S.
PHASE 2	RT.FW	4	34.4 ± 2.5 <sup>c</sup>	<i>F</i> = 87.08	8.93	36.6 ± 0.5 <sup>a</sup>	<i>F</i> = 6.04	3.7 ± 0.6 <sup>ab</sup>	<i>F</i> = 4.51	92.7 ± 0.8 <sup>b</sup>	<i>F</i> = 13.95
	BT.FW	4	26.2 ± 3.3 <sup>b</sup>	***	14.66	30.2 ± 0.4 <sup>b</sup>	*	4.0 ± 0.3 <sup>b</sup>	*	91.8 ± 0.9 <sup>b</sup>	***
	BT.SW	4	7.1 ± 3.1 <sup>a</sup>		12.98	29.9 ± 2.8 <sup>b</sup>		3.1 ± 0.4 <sup>a</sup>		89.9 ± 0.5 <sup>a</sup>	
PHASE 3	RT.FW	4	39.4 ± 1.8 <sup>c</sup>	<i>F</i> = 23.29	8.93	30.3 ± 1.7 <sup>ab</sup>	<i>F</i> = 5.62	4.9 ± 0.3 <sup>b</sup>	<i>F</i> = 12.18	90.4 ± 0.5 <sup>b</sup>	<i>F</i> = 16.11
	BT.FW	4	33.6 ± 1.5 <sup>b</sup>	***	14.66	32.3 ± 1.7 <sup>b</sup>	*	4.4 ± 0.3 <sup>b</sup>	***	91.4 ± 1.7 <sup>b</sup>	***
	BT.SW	4	29.8 ± 2.1 <sup>a</sup>		12.98	28.2 ± 1.7 <sup>a</sup>		3.6 ± 0.4 <sup>a</sup>		85.5 ± 2.0 <sup>a</sup>	

RT.FW had a better PPV than BT.FW,  $40.39 \pm 1.05$  and  $32.54 \pm 0.87$  respectively ( $t = 11.44$ ,  $p < 0.001$ ). PPV was also higher in BT.FW than in BT.SW during Phase 3 ( $p < 0.001$ ). During Phase 2, BT.SW retained very little protein ( $0.169$  vs  $0.875$  g.kg<sup>-1</sup> ww.d<sup>-1</sup> in BT.FW).

### Mass balance evaluation

Monitored output parameters, *i.e.* faeces nitrogen, TAN, Urea-N and final whole body nitrogen, represented roughly 90% of total nitrogen input from feed and initial whole body. They were significantly lower in BT.SW ( $p < 0.001$ ), only representing 85% in acclimatised fish (Table 5). Protein digestibility (Table 5) in the form of faecal losses, was higher in RT.FW than in brown trout. Nitrogenous soluble compounds from the faeces represented 35-40% of the whole faeces nitrogen content.

### DISCUSSION

Differential growth between rainbow trout and brown trout has been reported previously (Gjedrem and Gunnes, 1978; Quillet *et al.*, 1991; Krieg *et al.*, 1992), though it was not based on strict comparison protocols. Our experiment was carried out under the same conditions for both species, especially concerning temperature. The present study shows that brown trout, kept in seawater and freshwater, have a lower growth potential than rainbow trout. It also shows that salinity *per se* has no noticeable effect on growth. This low growth may be due to lesser domestication in brown trout as expressed by its feeding behaviour in small tanks. Better growth (SGR = 0.98) has been recorded in the same brown trout strain by Arzel *et al.* (1991) in 27 m<sup>3</sup> sea cages, using an equivalent diet (containing 52% protein and 18% lipid) at a higher feeding level (1% by day), where fish were probably less stressed. Our experiment



also confirms that diet yield is better in rainbow trout than in brown trout, where FGR is systematically higher. The feed used in the experiment, containing 52% protein and 20% lipid, was closer to brown trout requirements as expressed by Arzel *et al.* (1992). Energy intake per g of fish, 39.4 cal. g<sup>-1</sup>.d<sup>-1</sup>, was close to the optimum described by Elliott (1994), between 35 and 40 cal. g<sup>-1</sup>.d<sup>-1</sup>. This strengthens the observed differences as regards FGR. In BT.SW, we observed a better food conversion ratio than Gabaudan *et al.* (1989) and comparable to Arzel *et al.* (1992). Their experiments were carried out in sea cages, and there was no estimation of ingested feed. Thus no positive effect of seawater as such was noticeable on FGR. This is in contrast with the work of Quillet *et al.* (1986). These latter observations can be attributed to differential water temperatures between freshwater and seawater on-growing facilities.

The standard indicators evaluating fish ability to be transferred to seawater were used. They were consistent with the literature (Boeuf and Prunet, 1985). They demonstrated that fish were theoretically able to withstand the osmotic shock linked to seawater transfer. Nevertheless, high mortalities, accompanied by large decreases in FL, FGR and SGR, occurred during the three weeks following transfer. Although these indicators are necessary to enable correct seawater transfer, they are probably not sufficient to predict success in all cases. The actual reason for the high mortalities could not be determined.

The study also produced comparative data on nitrogenous wastes and nitrogen metabolism in two salmonids reared under identical conditions. Protein digestibility was higher in rainbow trout. This observation has already been made by Dosdat *et al.* (1996) who compared digestibility in five teleosts. Cho *et al.* (1991) encountered such differences in estimating nitrogen releases from rainbow trout and brown trout. Moreover, genetic influence on apparent digestibility was demonstrated by Austreng and Refstie (1979) in rainbow trout. They showed that ADC varied with protein content and family origin. Therefore particular attention must be given to diet formulation. It must be well adapted to species requirements in order to minimise nitrogenous wastes in the form of settleable solids. No difference was noticeable between BT.SW and BT.FW, indicating that salt presence in the water had little or no effect on digestibility. This is in agreement with Smith and Thorpe (1976) as regards rainbow trout adapted to freshwater and seawater.

PPV, above 35%, was very good in rainbow trout. PPV up to 55% has been frequently reported in recent studies (Davies, 1989; Lanari *et al.*, 1993; Morales *et al.*, 1994). The highest value we obtained was 44.5 % during the first phase. The combination of the feeding level and diet quality was probably best suited to 100 g rather than 250 g rainbow trout, where PPV dropped to 39%. PPV recorded in brown trout was similar to those obtained by Gabaudan *et al.* (1989) and Arzel

*et al.* (1992) who estimated PPV at 31-33% in fish of the same size fed a similar diet. This value seems to be a good evaluation of the species potential between 100 and 300 g. In brown trout too, PPV tended to decrease as the average weight increased. PPV was systematically better in rainbow trout than in brown trout kept in freshwater. This metabolic superiority of rainbow trout could also be due to a lower energetic expenditure linked to stress. When raised in seawater, brown trout expressed an even lower PPV that could be interpreted as an increased adaptation cost. Maxime *et al.* (1986) established that oxygen consumption increased in brown trout transferred to seawater, assuming that a higher energetic expenditure was required for hydromineral balance maintenance. The same effect of salinity was observed by Shaw *et al.* (1975) in Atlantic salmon (*Salmo salar*).

Lower protein accretion and a higher lipid accretion were observed in 100 g brown trout. In rainbow trout, lipid gain and composition increased with increasing size. Increasing fat content has been observed in every reared fish during its life (Shearer, 1994). Lipid contents were higher in brown trout than in rainbow trout, showing a worse energy intake utilisation and an accumulation of fat as body reserves, linked to a lower protein growth potential. It could be interpreted as a derivation of amino-acid catabolism towards acetyl-CoA, a precursor of fatty acids. This is confirmed by the higher ammonia excretion rate in brown trout, pointing to a higher amino-acid catabolism. This could be due to better nutrient balance of the diet and feeding level for rainbow trout. Nevertheless, final lipid levels encountered in BT.FW were in the range of those observed by Arzel *et al.* (1992). During adaptation to seawater (Phase 2), lipid accretion was low (10% of average value) when protein accretion represented 20% of previously recorded accretion rate. Brown trout utilised principally lipids to meet this additional energetic requirement. In fasting fish, liver lipids are the primary fuel, before red muscle protein involvement (Black and Love, 1986). No additional ammonia excretion was noted during adaptation to seawater, demonstrating that amino-acid catabolism was not increased at that time.

TAN excretion was systematically lower in rainbow trout than in brown trout raised in freshwater. It is consistent with the higher PPV found in rainbow trout. Ammonia production is related to protein catabolism through a deamination process. The values obtained here are in agreement with the data obtained by Dosdat *et al.* (1996) who observed a daily excretion rate of 152 mg N. kg<sup>-1</sup> ww.d<sup>-1</sup> in rainbow trout fed 430 mg N.kg<sup>-1</sup> ww.d<sup>-1</sup> using the same type of feed. Concerning brown trout, our observations are consistent with data from Elliott (1975, 1976) who mentioned TAN losses representing 33% of nitrogen intake. The excretion profiles pointed out that the maximal ammonia excretion rates occurred 6-7 hours after feeding. This is in agreement with the observations made by Kaushik (1980). No differences in the kinetics of ammonia

and urea excretion rates were noticeable among the species and water quality, highlighting the same metabolic processes. Urea excretion profile showed no trend. Daily urea excretion rates were fairly equal in every case, except for brown trout acclimatized to seawater where they were lower than BT.FW. The same tendency was noticeable in ammonia excretion as observed by Dosdat *et al.* (1996). As PPV was lower in BT.SW, these lower TAN and Urea-N excretion rates cannot be explained by a better protein anabolism of the fish.

The analysis of nitrogen mass balance raises another question. Due to the method chosen for mass balance calculation (avoiding extrapolation), it does not appear to be well balanced in every case. Only ammonia and urea, that represent the bulk of excretory products in fish (Forster and Golstein, 1969; Kaushik, 1980; Dosdat *et al.*, 1996) were analysed. Other nitrogenous catabolites are known to occur within fish excretory products. Water soluble molecules (tri-methyl-amine, tri-methyl-amine-oxide, creatine, creatinine, uric acid, etc.) are mainly voided through the gills and the kidney (Forster and Goldstein, 1969). Other soluble compounds (biliary cholic acids, bilirubin, undigested amino-acids) may also be voided through the digestive tract (Urich, 1994), some of which may be found

with the faeces in the supernatant fraction. Other secretions may enter the mass balance, such as mucus glycoproteins (mucin) as well as desquamating cells. Mass balance was remarkably similar in rainbow trout and brown trout in freshwater, amounting to 90%. It was significantly lower in brown trout adapted to seawater. A larger amount of undetected compounds may have been wasted in seawater, instead of ammonia and urea that were significantly lower.

BT.FW exhibits a worse nitrogen yield than RT.FW, expressed by a lower protein productive value and digestibility, and higher ammonia losses. Consequently, brown trout raised in fresh water tends to induce a higher environmental impact on both particulate matter (faeces production) and soluble compounds (ammonia and urea excretion). For brown trout raised in sea water, such a statement is more difficult to prove, due to unrecovered nitrogen losses in seawater. Nevertheless, the low protein productive value observed implies higher nitrogenous wastes. Thus, using the rainbow trout as a model to predict nitrogen output from brown trout fish farms could induce systematic errors, thus underestimating their environmental impact. More investigations concerning larger fish are required to validate this statement.

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