The Benefish Consortium reports on

Deviation from expected feed intake in relation to farm management at turbot, sole, trout, salmon, seabass farms

Oliver Schneider. Edward Schram. Chris Noble. Hilde Toften. Bjoern Steinar Saethar, Iain Berrill, James Turnbull, Gilles Lemarie, Marie Laure Bégout, Arnaud Campéas, Marie Eichinger, Mitsuo Tabata, Felicity A. Huntingford, Jean Paul Blancheton and Jeannine Person

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Institute for Marine Resources and Ecosystem Studies

Wageningen IMARES

Client: Dr. Sunil Kadri
Partner, TNC Partners EEIG
34 Lawrence St 1/L
Glasgow G11 5HD
Scotland, UK

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1 Introduction

Commercial fish farmers often state that their fish are doing just fine and have a good welfare as long as fish feed intake or realized feeding level meets their expectations (Vis, et al. in press). However, fish feed intake is not necessary stable and might vary due to different reasons. A number of commercial aquaculture species exhibit daily feeding rhythms in food intake and appetite (Noble et al., 2005) and there is variability in daily feed intake between days and groups. Feed intake can be defined as the amount of food an animal actually consumes. Feed intake can be affected by a number of abiotic variables including, but not limited to: changes in the daily light/dark cycle (Boujard and Leatherland, 1992); temperature (Fraser et al., 1993); light intensity (Noble et al., 2005); oxygen levels (Thetmeyer et al., 1999); ammonium concentrations (Beamish and Tandler, 1990); wave action (Bégout Anras, 1995); wind speed, rainfall (Bégout and Lagardère, 1993) and turbidity (Ang and Petrell, 1997). Biotic factors might include: gastric emptying time (Ruohonen et al., 1997); disease or increased parasite loads (Bloch and Larsen, 1993); group size (Boujard, 1995) and intraspecific competition (Brännäs and Alanärä, 1997). Other management variables that might impact feed intake can include water refreshment rate of the system, handling, disturbance and system cleaning (Strand et al, 2007 and references therein). These changes in feed intake can either be structural, so a deviation from expected feed intake can be observed over several days or be incidental, as during a meal or during a short period of time. Based on these considerations, it is of scientific and commercial interest to investigate the hypothesis that welfare affects feed intake. If this can be validated for aquaculture species a simple but effective operational welfare indicator becomes available and the -above named- empirical observations by fish farmers are confirmed. To prove that hypothesis it is necessary not only to investigate data that might be obtained in rather artificial situations in laboratories but as well under commercial conditions. The objective of the present study is therefore to relate deviations in expected feed intake to farm management practices for various species and culture systems.

2 Individual datasets

In total 21 commercial and experimental datasets have been subjected to this evaluation (Table 1). They were collected by different international partners: Nofima (Norway), University of Glasgow (Scotland), University of Stirling (Scotland), Ifremer (France) and IMARES (The Netherlands). In several cases it was necessary to assume that feed load would equal feed intake, as otherwise no operational data would have been available. Especially in commercially operated farms it is hardly possible to measure feed intake on large scale. Therefore several obtained results on feed intake might be confounded by feed spillage, which was not accounted for.

Table 1: Overview of the evaluated datasets. Commercial (1) or experimental dataset (2)

No	Data set ID	Partner	Species	System type
1	ba-lab-T°	IFREMER	Juvenile seabass	Tanks[2]
2	BC1/ Heritabolum	IFREMER	Juvenile seabass	Tanks[2]
3	ETHIQUAL 1&2	IFREMER	Juvenile seabass	Tanks[2]
4	FASTIFSH 1	IFREMER	Juvenile seabass	Tanks[2]
5	FASTIFSH 2	IFREMER	Juvenile seabass	Tanks[2]
6	Density 1	IFREMER	Juvenile & Adult Seabass	Tanks[2]
7	Density 2	IFREMER	Junvenile Seabass	Tanks[2]
8	Hypercarbox	IFREMER	Juvenile seabass	Tanks[2]
9	Cortisol	IFREMER	Juvenile seabass	Tanks[2]
10	DEB_SOLE	IFREMER	Dover sole	Aquaria[2]
11	FT Benefish EXP	IMARES/IFREMER	Turbot	Different Flow through and RAS[2]
12	ZLV Benefish	IMARES/ZLV	juvenile/adult turbot	RAS[1]
13	Solea	IMARES/Solea	juvenile/adult Dover Sole	RAS[1]
14	GFI0	UGLA	juvenile 0+ Atlantic salmon	3 x 12x12x4m Freshwater production cages[2]
15	GFI1	UGLA	juvenile Atlantic salmon	3 x 12x12x4m freshwater production cages [2]
16	LEFI	UGLA	Atlantic salmon post-smolts	5x5x4m marine cage[2]
17	RTFI	UGLA	juvenile rainbow trout	3x 200 l RAS tanks[2]
18	cagesalmon	NOFIMA	Adult atlantic Salmon	Cage system[1]
19	IPN2002	NOFIMA	Juvenile atlantic Salmon	Flow thorugh tank system[2]
20	AW1205	USTIR	juvenile/adult rainbow trout	Freshwater ponds, raceways, tanks and cages[1]
21	RTGE	USTIR	juvenile/adult rainbow trout	Freshwater ponds, raceways, tanks and cages[1]

Each evaluated dataset was obtained during experimental work or from commercial farms under specific conditions. These conditions varied based on species, culture system, experimental layout, farm management and various other parameters. The single conditions in relations to the datasets are therefore described separately in the next sections. The dataset are grouped by the analysed factors, such as temperature, water quality, system management and other.

2.1 Experimental Effects

2.1.1 Effect of the interaction between genetic x environment, and effects of stress on feed intake in juvenile seabass

2.1.1.1 BC1 / HERITABOLUM

2.1.1.1.1 Experimental design

The Heritabolum experiment was carried out to study the interaction between genetic x environment using family and individual variabilty (253 families) for SGR, morphometric traits (25), muscular fat content, fillet & carcass yields (7), weight of body compartments (5), sex. Fours groups of 1750 fish were followed during 2 years. They were hand-fed to apparent satiation and FI was calculated as feed provided minus feed waste (collected in a waste trap), (Vandeputte et al. 2009).

2.1.1.2 ETHIQUAL 1, ETHIQUAL 2

2.1.1.2.1 Experimental design

a) Fish were raised in four experimental tanks, during 217 days, with 50 fish per tank. Each fish was tagged (with PIT-tag) and the fish were fed by self-feeder. The fish that activate the self-feeder was identified thanks to an antenna which red its PIT-tag. During the whole experiment, some fish were periodically removed from the tanks, killed and measured. FI was calculated for each day as feed provided minus feed waste (collected in a waste trap). Morphological and physiological measurement were performed for each fish, at the end of the experiment, all remaining fish were killed and measured. (Millot et al., 2008)

b) Fish were raised in three experimental tanks, during 84 days, with 50 fish per tank. Each fish was tagged (with PIT-tag) and the individual intake was monitored 4 times during the experiment with Ballotini glass bead method. FI was calculated for each day as feed provided minus feed waste (collected in a waste trap). Hence, individual growth and individual feeding have been recorded, which allowed to calculate an individual deviation of feed intake for each fish (Di Poï 2008, Di Poï et al., submitted).

2.1.1.3 FASTFISH 1, FASTFISH 2

2.1.1.3.1 Experimental design

c) In fastfish 1 the 2 tested populations have been hatched and reared at the experimental research station of Ifremer in Palavas-les-Flots (France). The experiment was carried in Ifremer L'Houmeau on juveniles issued from either wild brood fish or a strain selected for growth. The effects on feed demand and feed intake of a standardized acute stress (tank drained and fish out of the water during 1 min) applied 2 times over 112 experimental days were monitored. The experiment was carried out testing each condition with a duplicate per strain (4x60 fish). The 4 tanks (450 l each) were supplied with recirculated seawater. Water temperature was maintained at 20.2 ± 1.5°C, oxygenation above 80 % of saturation in the water-outlet, and salinity was 22.3 ± 3.3 g l-1. At the beginning of the study, fish were 14 months-old, Wild fish weighted an average of 106 ± 3 g and Selected fish an average of 129 ± 4 g. Fish were placed under self-feeding conditions and food access was possible all day (24 h). Apparent feed tank consumption (feed amount dispensed minus wasted pellets counted on the bottom of the tank and in the sediment trap) was monitored daily. Triggering activity recordings were done continuously for 112 days except before (24 h) and during fish handling (8 days off in total). Growth measurements were taken every 3 weeks (Millot 2008, Millot et al., in prep).

In fastfish 2 the 4 tested populations have been hatched and reared at the experimental research station of Ifremer in Palavas-les-Flots (France). The experiment was carried out in Ifremer Palavas with a triplicate per strain (issued from wild brood, domesticated and 2 strains selected for growth). After a first control period, the fish were submitted from day 35 and during 56 days to a chronic stress treatment including frequent and random application of 4 acute stressors (pursuing fish with a net during 1 min, switching off the light for 2s during the day or, conversely, switching on the light for 2 s during the night, and overflying a bird predator silhouette above the tank during 30 s). The 12 tanks (1m3 each) were supplied with semi-recirculated seawater. For each tank, the flow rate was 4 m3 h-1 and the water renewal, 30 % per day. Water temperature was maintained at 20.3 ± 1.1°C, oxygenation above 90 % of saturation in the water-outlet, and salinity was 36.3 ± 1.5. The experiment was realized over 91 days with 600 fish (50 fish per tank, 150 fish per strain). At the beginning of the study, fish were 24 months-old, *Wild* fish weighted an average of 468 ± 7 g , *Domestic* fish an average of 443 ± 6 g, *Massal* fish an average of 530 ± 8 g and *Prosper* fish an average of 523 ± 10 g. Fish were again weighted every 3 weeks. Fish were placed under self-feeding conditions. Apparent feed consumption within each tank (feed amount dispensed minus wasted pellets in the sediment trap) was monitored daily. Triggering activity recordings were done continuously for 77 days except 24 hrs before and during fish handling (8 days off in total). (Millot 2008, Millot et al., in prep).

2.1.1.3.2 Statistical analysis

The main analytical method is to develop a mechanistic growth model of sea bass and sole, based on dynamic energy budget (DEB) concepts, to describe the rates at which a fish assimilates and utilizes energy for maintenance, growth and reproduction, as a function of the state of the organism and of its environment. Such model can be later used to evaluate discrepancies between normal and abnormal situation where environmental and /or welfare status are altered.

2.1.1.3.3 Results

The above listed data set (BC1/HERITABOLUM, ETHIQUAL 1&2, FASTFISH 1 &2) were used to build a growth model: we applied the Dynamic Energy Budget (DEB) theory to the sea bass and estimated a set of DEB parameters for this species by using different data sets and from the literature. We developed a methodological approach to calibrate accurately parameters using minimization of the Least Squared Error. To overcome some biases due to auto-correlation of the repeated measures and to heterogeneity in the fish size, the data were weighed. We also developed a method for quantifying the variability in the estimated DEB parameters considering the data used for calibration. This innovative method allowed us to evaluate the precision of estimation for each parameter, but also the covariance between each couple of parameters. This information could be of great interest to determine the reliability of the estimation of each parameter, but also to evaluate the quality of the data used for estimating parameters. We obtained a set of reliable DEB parameters and we showed that the estimation of the maintenance costs [p_M] was quite imprecise because of its minor influence on growth, and so was the estimation of the influence of temperature on this parameter (T_A). The evaluation of kappa (κ) was much more precise, though we worked with immature fish implying that κ represents only the maturation. Finally, we proposed a measurement of the energetic reserve E using calorimetric data. From the set of parameters we estimated, we obtained a bio-energetic model which allowed us to study the difference of growth under different rearing conditions (Campeas et al., in prep.).

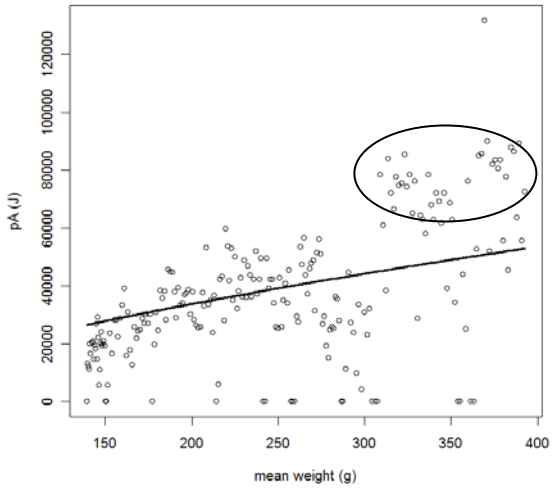

Figure 1: Estimating feed energy requirement (pA) in sea bass in relation to weight {pAm}= 980 J.cm^{-2}.day^{-1} (T=22°C). Encircled data evoked hyperphagia in sea bass which might be related to environmental perturbation and not allocated to growth.

2.1.1.3.4 Conclusion

By fitting the model to the data, we obtained parameters that indicate the amount of energy assimilated, but also the proportion used for maturation and the cost of maintenance. From these parameters we can estimate the relative importance of each energy flux and calculate some production indicator such as feed efficiency. As a first conclusion, it appeared that the most variable parameter was the assimilation rate {pAm} which indicated that the major difference in growth obtained by selection is due to the difference in ingestion and/or assimilation. It appears also that the main effect of stress is on ingestion. These results suggest that the selection goal should be adjusted to better target new criteria of sustainability such as better feed efficiency (Campeas et al., 2009).

2.1.2 Effect of induced cortisol levels on feed intake in juvenile seabass

2.1.2.1 Cortisol

2.1.2.1.1 Experimental design

Immature sea bass, which were vaccinated against vibriosis and furonculosis, were supplied from a land based farm at the Ifremer Research Station in Palavas. The fish were stored in a 5m3 tank during 18 days, then graded under anaesthetic conditions (350 ppm phenoxy-2-ethanol) within the weight range 70-200g and were randomly distributed in 6 experimental tanks (135-140 fish per tank corresponding to a 17.7-17.9 kg of fish biomass). The fish were then acclimated for 21 days prior to the experiment. The 6 experimental tanks were $1m^3$ each, black coated, U-shaped and located in a isolated room. Each tank was covered with a black plastic sheet to protect fish against external visual stress. Light intensity at the water surface was 500 lux using an incandescent lamp (OSRAM Decor Silver E27-75W) at 1 m above the water surface, and the photoperiod was maintained at 16h light - 8h dark, including a 30 min artificial dawn and dusk. The open flow-through tanks were fed by gravity from a $4m^3$ header tank with sea water which previously had been sand-filtered at 15 µm, UV sterilised, heated and degassed in a packed column. Water was enriched in oxygen (159% saturation) by bubbling an adequate flow (2 $L.mn^{-1}$) of gaseous oxygen through a porous stone located at the bottom of the header tank. The water quality was maintained in the $1m^3$ tanks at an optimum level with a water renewal about 3.5 times more than necessary (Lemarié, 2003) and water parameters were checked daily either twice a week. As a result, the waterflow rate was set at 1±0.1 $m^3.h^{-1}$ per tank (accuracy of the flow-meter : ± 0,05 $m^3.h^{-1}$) in order to maintain permanently an oxygen saturation between 90 and 100% saturation in the outflow water and to provide an efficient self-cleaning into the tank. The water temperature was 20.0±0.5°C. The salinity was 36.7±1.4 ppt. and the TA-N concentration was 0.17± 0.05 $mg.L^{-1}$, determined by the indophenol method (Bower and Holm-Hansen, 1980) using a Technicon Analyser®. The pH was 7.6±0.1 and the carbon dioxide content was 4.1±1.1 $mg.L^{-1}$ using a LI-COR® analyser . The concentrations of the others nutrients (P-PO4, N-Urea) were closed to their detection limit. The fish were fed expanded pellets (43% protein, 20% crude fat, 10% humidity, Neogrower marin 4mm, Le Gouessant producer), from the same batch of production throughout the experiment, once a day ad libitum during a 45 to 60 mn meal starting at 9h00 using a semi-manuel system. The feeders were poured the day before to avoid any stress of the fish. A switch located outside each tank was actuated by the operator who can observe the feeding behaviour of the fish through a small window in the plastic sheet. At each actuation of the switch, the electric feeder released at the water surface a predetermined amount of feed (1.7 to 1.9 g) in 3 equal doses in 8 seconds. The switches were activated successively several times until apparent satiety determined visually by 3 accompanying indicators: i) indifference of the fish towards the food, ii) observation of uneaten pellets in the bottom of the tank and iii) presence of some pellets in the particle trap. 30mn after the end of the meal, the number of pellets in the traps was counted. The implants without cortisol were prepared *ex temporane* by mixing at 40°C in a double boiler 640 ml shortening and 160 ml oil. The shortening is a commercial mixture containing water (80%), and various saturated, mono and polyunsaturated fat (20%). The implant with cortisol was prepared by mixing carefully 400 ml of the former mix with 8g powder hydroxycortisone at 40°C. Both of the implants were solid at ambiant temperature. Before injection, the implants were getting warmer at 30-35°C in a double boiler in order to be liquid when injected in fish. The injection was done in the intraperitoneal cavity of the fish with a 1ml syringe (graduated in 100 parts) at a rate of 3.75 µl of $implant.g^{-1}$ BW, corresponding to a quantity of 75µg of $cortisol.g^{-1}$ body weight. The experimental design was based on the comparison of effects in fish of three different treatments in duplicate tanks:
fish non injected (control or T1).

fish injected with implant without cortisol (0 µg hydroxycortisone /g body weight) or T2.

fish injected with cortisol implant (75 µg hydroxycortisone /g body weight) or T3.

Two groups of 135-140 fish each were subjected at random at the same treatment in 2 different tanks and were maintained in satisfactory rearing conditions (see above) during 42 days. The total number of experimental tanks was 6. Mortality and behaviour were controlled every day. Feed in feeders were weighted at the nearest g for each tank before and after each meal. The weight of uneaten and trapped pellets was daily calculated by multiplying the number of pellets by the mean weight of one pellet calculated from a sample of 100 pellets. Oxygen concentrations in inlet and outlet waters of tanks were taken specifically 3 times a week before feeding in order to calculate apparent oxygen uptake. Flowrates were measured sharply at the start of the experiment

and were maintained at a constant level throughout the experiment. At d1 and d42, weight (at the nearest 0.1g) and fork length (at the nearest 0.1cm) of each fish were measured after anesthetization at 400 ppm phenoxy-2-ethanol and enrichment of seawater at 150% O2 saturation by bubbling gaseous oxygen in the tank. At d44, fin status was observed in 90 fish per tank. At d7, d21, d35 and d40, blood samplings were taken in respectively 7 fish per tank after a 24h fasting period. The i-STAT® 1 hand-held system of ABBOTT was used, utilizing self-contained test cartridges (ref EC8+). For this blood analyser, two drops of freshly taken blood were necessary. Corrections for temperature were done on the results when needed.

2.1.2.1.2 Calculations and statistics

The mean wet weight S±D and the mean length ±SD were calculated as the arithmetic mean from the fish. At the end of the experiment, the specific growth rate (SGR) was determined as:

$$SGR = [(Ln\ Wf - Ln\ Wi) * 100] / T$$

where Wf is the mean wet weight in grams at the end of the experiment , Wi is the initial mean wet weight and T the duration of the period in days. Variation in fish size within the tank was described by means of a comparison of coefficients of variation (CV) using the following equation:

$$CV = 100 * SD \text{ of the mean weight} / \text{mean weight}$$

The distributed food was calculated daily per tank by difference of the weight of the poured and left pellets in the feeder. The daily amount of ingested food (DAIF) is calculated as the difference of the distributed food and uneaten food. The uneaten food is the number of pellets counted in the particule trap multiplied by the mean weight of one pellet. When needed mobile means can be calculated as the sum of the data of several following days (3 here) divided by the number of the days and expressed as a % of the control fish. The mean daily Feed Intake (FI, % of initial fish biomass) was calculated as follows:

$$FI\% = (\text{Ingested feed} / Wi * \text{fish number}) * 1/T * 100$$

T is the number of days. This simplified FI, using only Wi, can be calculated because the fish samples were done similarly in the 6 tanks and they made the calculation of FI with initial and final biomass per period too complex. When needed, a mobile mean is calculated by using the mean of 3 following FI. The food conversion ratio (FCR) was calculated as :

$$FCR = \text{Ingested feed} / \text{Weight gain of fish biomass}$$

The condition index (CI) was calculated as:

$$CI = Wf.\ Lf\text{-}3 * 100$$

where Lf is fork length at the end of the experiment. The experimental data were processed by a one-way analysis of variance (ANOVA), and when necessary by an analysis of co-variance (ANCOVA). Differences among the means were determined by a Student-Newman-Keuls (SNK) test that was applied to ranks when normality was not established. Linear regressions (LR) were performed to analyse correlations between factors. The comparisons between CV were performed by means of a sign-test. The SigmaStat package was used to process the data.

2.1.2.1.3 Results

No mortality after injection was observed from d1 to d18 in all treatments. At d19, 1 and 6 dead fish respectively were removed from the 2 tanks submitted at the cortisol treatment (T3), 5 hours after a failure in the lightning system. From d20 to the end of the experiment, no mortality was observed. The Table 2 below shows the global FI for each tank. The feed intake per tank was significantly 21% lower ($P<0.05$) in treated fish with cortisol (T3) than in control (T1) and T2 conditions. The daily amount of ingested food (DAIF) in g per day per tank showed larges variations due to external and internal factors and their complex combinations leading to unclear graphs. When mobile means (3 following data) of DAIF were expressed in % of control fish and were plotted against time, clear trends appeared over the experiment (Figure 1). The fish injected without cortisol (T2) showed a feed intake 85% lower than control fish in the very first days of the experiment, but they recovered a similar feed level within 7 days. The fish injected with cortisol (T3) exhibited a DAIF corresponding to 70% of the control one during the 15 first days, then there was a progressive increase from d16 to d30 to recover a level closed to the control fish T1. The global SGR is deeply and significantly affected in T3 (Table 2) where fish were injected with cortisol implants and represents only 61% of the growth observed in control fish. No difference was found between control fish and those which received an implant without cortisol. As a result of a lower feed intake and a reduced growth rate, the FCR of the fish in T3 was significantly higher by 143% than control and T2 (Table 2). The Coefficient of Variation of weight and the Condition Index were not significantly different in any treatment, even if the mathematical values were slightly unfavourable in the T3 condition (Table 2) compared to control. No

statistical difference were found in Na, K, TCO2, Glucose, Hematocrit, pH, PCO2, HCO3 and Hemoglobin between treatments (Table 3). The apparent oxygen uptake was significantly different in T3 than in the other treatments and was 115% higher than the control fish (Table 2).

Table 2: Performance indicators in seabass observed during the 44 days experiment:

Treatment	T1 : Control	T2 : implants without cortisol	T3 : implants with cortisol 75µg/g bw
Mortality %	0a	0.35 ± 0.5a	2.65 ± 2.68a
FI % bw/day	1.16 ± 0.03a	1.14 ± 0.03a	0.92 ± 0.02b
SGR % bw/day	0.61 ± 0.03a	0.68 ± 0.05a	0.37 ± 0.04b
FCR	1.70 ± 0.08a	1.49 ± 0.05a	2.43 ± 0.09b
VC weight %	22.1 ± 1.85a	21.3 ± 0.40a	23.7 ± 3.13a
CI g/cm3	1.33 ± 0.02a	1.33 ± 0.03a	1.29 ± 0.02a
O2 Uptake mg/h/kg bw	229 ± 30a	223 ±33a	263 ± 29b

FI: Feed Intake, SGR: Specific Growth Rate in % of the body weight per day. FCR: Feed Conversio ratio. VC weight: Variation Coefficient on weight in %. CI: Condition Index in g/cm3. O2 Uptake: Apparent Oxygen Uptake in mg/h/kg of body weight. Means in the same line not sharing a common following letter were significantly different ($p<0.05$).

Table 3: Blood parameters at day 43.

Treatment	T1 (control) N=	T2 implants without cortisol N=	T3 implants with cortisol (75µg/g bw) N=
Na mmol/L	153.3 ± 6.4	154.6 ± 5.5	154.1 ± 6.7
K mmol/L	5.1 ± 0.7	4.9 ± 0.5	5.9 ± 0.7
TCO2 mmol/L	6.5 ± 0.6	6.5 ± 0.5	6.6 ± 1.0
Glucose mg/dL	108.3 ± 21.6	114.4 ± 34.8	112.6 ± 32.6
Hematocrit % pcv	21.6 ± 3.3	20.8 ± 3.5	19.9 ± 3.6
PH	7.24 ± 0.07	7.22 ± 0.04	7.22 ± 0.09
PCO2 mmHg	14.2 ± 2.0	14.3 ± 2.4	14.4 ± 1.2
HCO3 mmol/L	6.1 ± 0.6	5.8 ± 0.7	5.8 ± 0.9
Hemoglobin g/dL	7.3 ± 1.1	7.0 ± 1.2	6.8 ± 1.2

Means in the same line not sharing a common following letter were significantly different ($p<0.05$).

2.1.2.1.4 Conclusion

This study investigated the response of exogenous cortisol to mimic chronic stress conditions. Swimming activity, global feed intake, specific growth rate, feed conversion ratio and apparent oxygen uptake were significantly impaired when cortisol was injected in fish.

2.2 Husbandry Effects

2.2.1 Effect of temperature on feed intake in junveile seabass

2.2.1.1 ba-lab-T

2.2.1.1.1 Experimental design

The temperature experiment was carried out using duplicate groups of 84 fish each (initial weight 82g) held at constant temperature: 13, 16, 19, 22, 25, 29°C for 84 days (water quality was optimal). They were hand-fed twice a day to apparent satiation and FI was estimated for each meal and per day as feed provided minus feed waste (collected in a waste trap). Daily FI was calculated every 14 days by taking into account the average fish mass per (t2-t1) period and expressed as% of fish mass (g feed,100g fish).

2.2.1.1.2 Statistical analysis

An Anova was used to test the effects of temperature on days 0-84 FI. The main analytical method is to develop a mechanistic growth model of sea bass and sole, based on dynamic energy budget (DEB) concepts, to describe the rates at which a fish assimilates and utilizes energy for maintenance, growth and reproduction, as a function of the state of the organism and of its environment. Such model can be later used to evaluate discrepancies between normal and abnormal situation where environmental and /or welfare status are altered

2.2.1.1.3 Results

In the sea bass temperature experiment survival was 100% in all groups. Specific growth rates (SGR), feed intake (FI) and feed efficiency (FE) versus temperature are reported for the period of day 0-84 (Figure 2). Calculated temperature for maximum SGR,FI and FE were 26.1, 27.5 and 23.9°C respectively. (Person Le Ruyet et al. 2004)

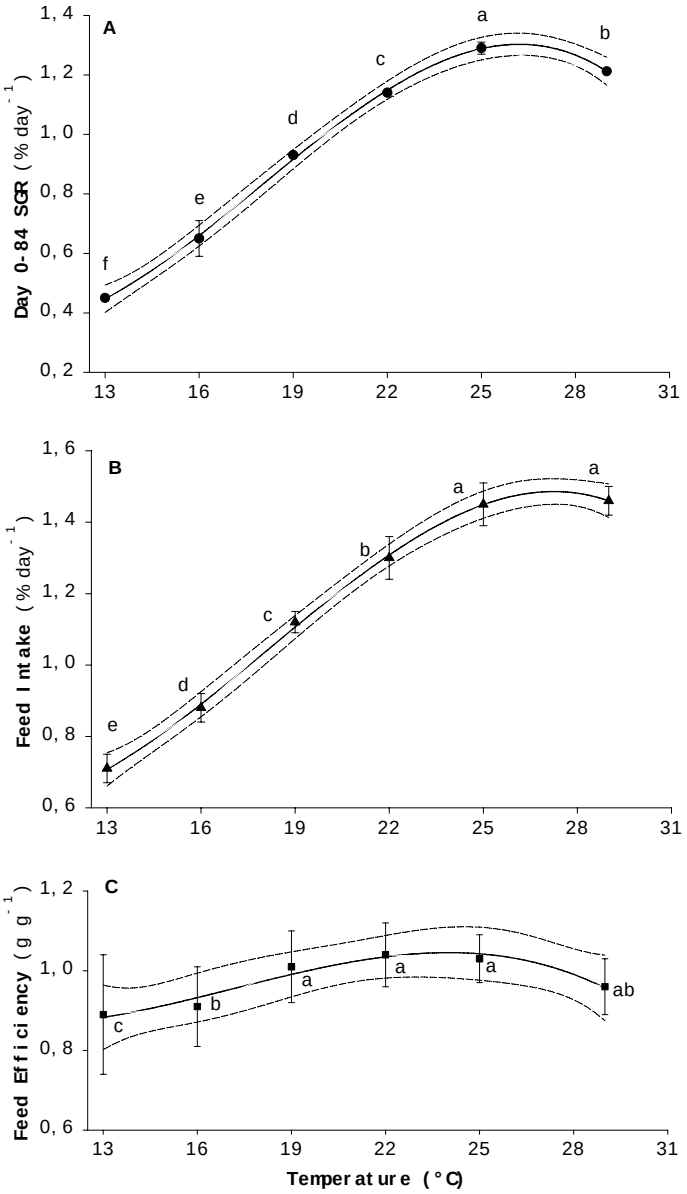

Figure 2: Days 0-84 specific growth rate (A), feed intake (B) and feed efficiency ration (C) of sea bass juvenile in relation to temperature.Means are given with standard errors (n=2 replicates) and dashed lines represent confidence intervals.

2.2.1.1.4 Conclusion

FI increased with temperature up to a maximum at 25-29°C (NS differences between 25 and 29°C). It was maximum at the upper temperature but could not compensate for the decrease in growth partly explained by a lower feed efficiency. As long as temperature is below the maximum for growth, any observed deviation of FI could be explained by a change in ambient temperature. Conversely when temperature comes over the maximum for growth there is no marked adjustment of FI and when it is excessive FI declines sharply.

2.2.2 Effect of density on feed intake in juvenile seabass

2.2.2.1 Density 1, density 2

2.2.2.1.1 Experimental design

800 kg of fish were raised in three experimental tanks, during 4 months. 5 modalities of density were tested (20, 40, 70 and 100 kg/m^3) with 3 replicates, which lead to 15 experimental units. Every 3 weeks, some fish were removed to keep the density constant. At the end of the experiment, the fish were killed and biometric and physiologic measurements were performed. The experiment was done twice : first in a flow through system and second in a semi-closed circuit.

2.2.2.1.2 Statistical analysis

The main analytical method is to develop a mechanistic growth model of sea bass and sole, based on dynamic energy budget (DEB) concepts, to describe the rates at which a fish assimilates and utilizes energy for maintenance, growth and reproduction, as a function of the state of the organism and of its environment. Such model can be later used to evaluate discrepancies between normal and abnormal situation where environmental and /or welfare status are altered.

2.2.2.1.3 Results

This experiment explained the influence of density on feed intake. It revealed relations between the stocking density and the inner state of fish. Potential welfare improving actions are therefore changes in the rearing density (cf. EU WEALTH final report). The data sets were also used for the modeling action cited above.

2.2.2.1.4 Conclusion

With non limiting water quality, there was no significant difference on fish performance (mortality, DFI, SGR and FCR), blood parameters and nodavirus resistance capacity up to 70 kg m-3. At 100 kg m-3, the DFI was slightly decreased with a correlated SGR decrease.

2.2.3 Effect of feed feeding level on feed intake in juvenile seabass

2.2.3.1 DEB_SOLE

2.2.3.1.1 Experimental design

For this experiment fish (8-10 cm in length) were individually housed and under two feeding ration (1% or 2%, in 16 aquaria for each treatment). Individual feed intake was followed for 90 days by counting uneaten pellets every day (4 hrs after meal distribution). Another batch of fish was left unfed for the same duration. Growth in weight and length was measured every 20 days. Using additional experimental growth data from an ongoing French research project, ANR-VMC-SOLEBEMOL, a DEB model was constructed.

2.2.3.1.2 Statistical analysis

The main analytical method is to develop a mechanistic growth model of sea bass and sole, based on dynamic energy budget (DEB) concepts, to describe the rates at which a fish assimilates and utilizes energy for maintenance, growth and reproduction, as a function of the state of the organism and of its environment. Such model can be later used to evaluate discrepancies between normal and abnormal situation where environmental and /or welfare status are altered.

2.2.3.1.3 Results

WeUnder controlled and constant conditions, individual daily feed intake showed large day to day variation (Figure 3). Individuals showed homogeneous weight between treatments for both initial mass (mean 5.2 g ± SD 0.79)

and final mass (8.6 ± SD 2.57). We noted a larger variation in weight in treatment ration 2% and also greater daily feed intake variability (Figure 3).

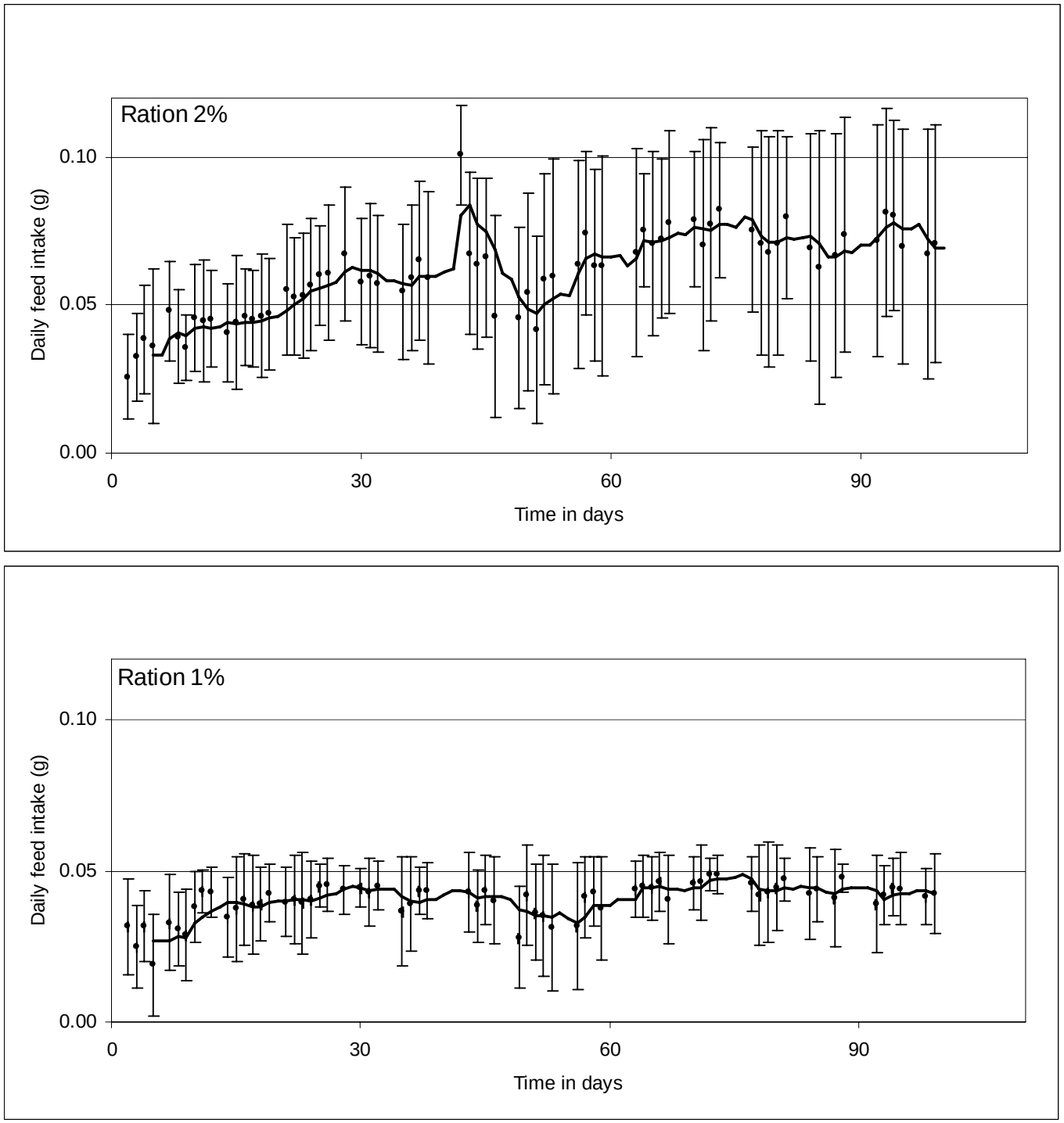

Figure 3: Mean individual daily feed intake (N=16 soles) and standard deviation over the experiment duration for each treatment (initial feed ration 2% (=0.1g) or 1% (0.05g)).

We have also used the dynamic energy budget (DEB) theory to model the growth of common sole (*Solea solea* L., 1758; Figure 4). The model has been calibrated and validated on data sets on juveniles based on both *in situ* measurements and on experiments gathered under controlled conditions.

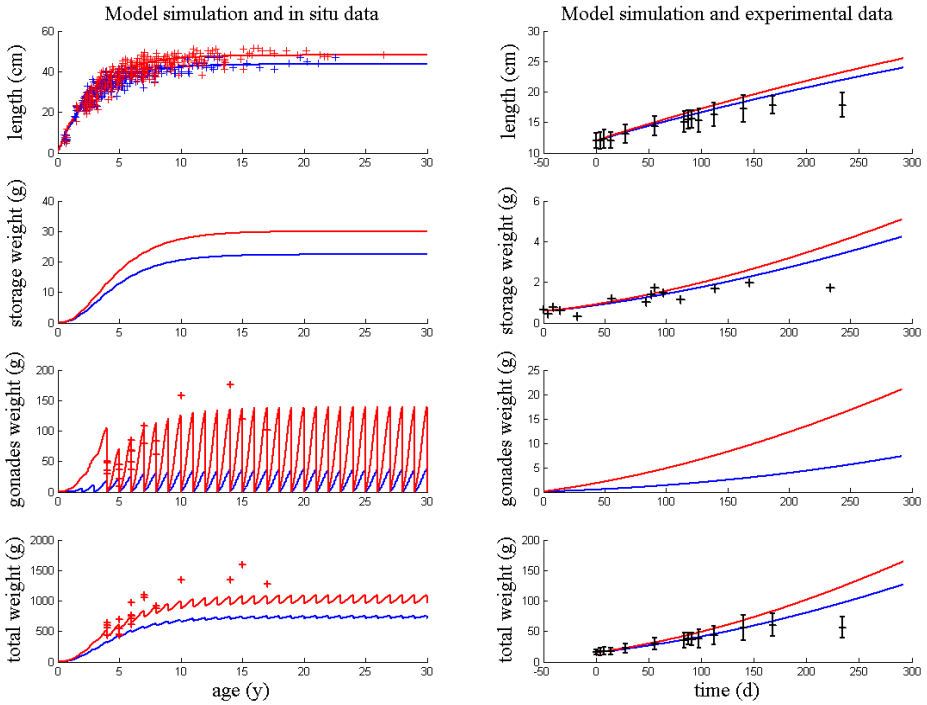

Figure 4: Left part: comparison between DEB model outputs (red lines for female and blue lines for male) and in situ data from the Douarnenez Bay (Deniel 1981) for length, storage weight (i.e. reserve compartment),, gonad and total wet weight. Right part: comparison between DEB model outputs (red lines for female and blue lines for male) and experimental data based on juvenile soles (black crosses ± sd) for length, storage, gonad and total wet weight. Parameter values are exactly the same for both simulations.

2.2.3.1.4 Conclusion

The above model is being used to compare fish growth potential under different conditions (normal vs. stress conditions). In particular, chemical stressors are being considered by Eichinger et al. (2009).

2.2.4 Effect of system water refreshment rate on feed intake in turbot

2.2.4.1 FT Benefish EXP

2.2.4.1.1 Experimental design

As there might be effects of system water refreshment rates on feed intake in fish, a experiment was set-up using turbot as example species, for which an impact of system water refreshment rate has been reported by several commercial farmers. For this experiment three systems were used. Recircualtion aquaculture system (RAS) 1%, RAS 5% and one flow through system. Each system comprised six culture tanks. The two RAS were designed similarly. The main difference between the two RAS is the refreshment rate of makeup water. Both systems consist of six fish tanks, a drum filter, pumps, a bio filter, six U.V. lights, a oxygenating column per tank and a ceramic oxygen diffuser per tank (Figure)

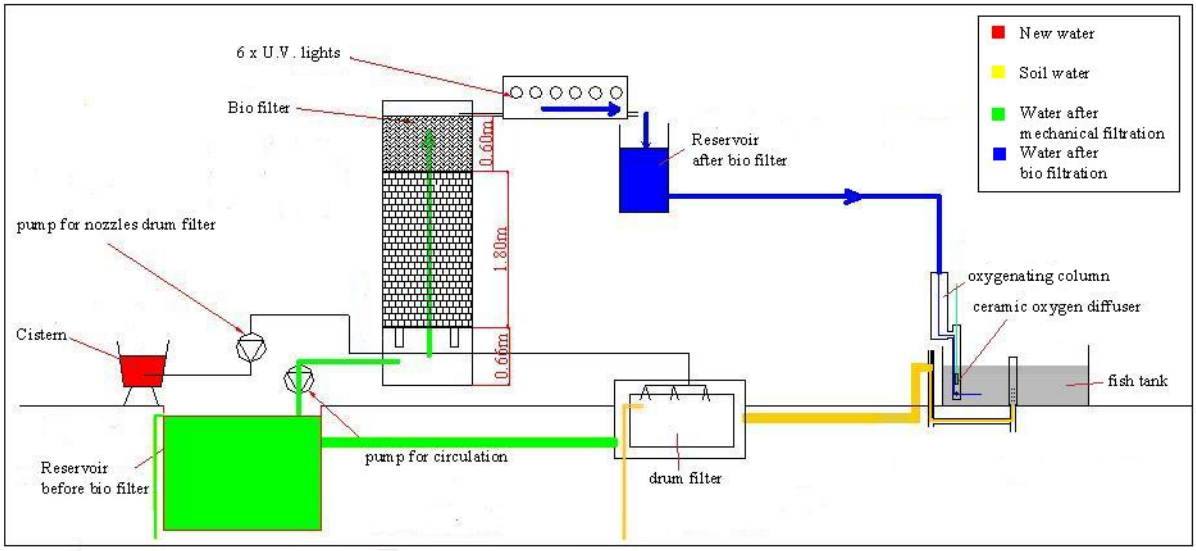

Figure 5: Scheme RAS 1 & 2.

More specific information on system design of the RAS systems and the flow through systems is provided in Table 4. In spring 2008 the water refreshment rates have been lowered to increase the potential effect of low water refreshment rates as result on feed intake and fish growth.

Table 4: system dimensions and characteristics of the three used cultured systems, 2 RAS and one flow through system

	RAS 1%	RAS 5%	Flow through
Total system volume (m^3)	25.69	24.53	16.8
Tank volume (m^3)	2.75	3.00	2.80
Tank surface area (m^2)	5.3	5.3	5.3
Tank flow rate (m^3/h)	2.27	3.00	2.24
Tank hydraulic retention time (tankvolume/h)	1	1	0.8
Averaged system refreshment rate (m^3/kg feed)	1.4	5.0	71
Averaged system refreshment rate (% of total volume/h)	0.9	3.8	80
Volume biofilter (m^3)	3.87	3.87	-
Drum filter mesh size (µm)	30	30	-
U.V. (W)	450	450	-

The flow through system had a water refreshment rate of 71 m^3/kg feed. This means no water was re-used, but all water was discharged after passing the tanks. This system lacks logically all water purifying components. Similar to the RAS the six tanks of the flow through system had their own oxygenating column and ceramic oxygen diffuser. In the present experiment, turbot, *Psetta maxima,* was stocked in the culture systems. There were two fish size groups stocked for the experiment: smaller and bigger fish. The small fish had an average weight of 400g (+/- 145g) and the large fish an average weight of 900g (+/- 217g). In all three systems there were three tanks with bigger fish and three tanks with smaller fish. The bigger and the smaller fish were divided into three groups; small, medium and large. Fish were fed with Turbot Label Rouge (floating) from Le Gouessant. The fish were fed by hand by meals (ad libitum).

2.2.4.1.2 Statistical analysis

Growth and feed intake data was analyzed using GLS Models in R (2008). Whereby parameters without significant influence were deleted from the model, before the model analysis was repeated.

2.2.4.1.3 Results

The obtained data was analyzed for two periods before and after the RAS with the lowest refreshment rate was closed even further. Data is shown for the lightest and heaviest fish cohort (Figure and Figure 4).

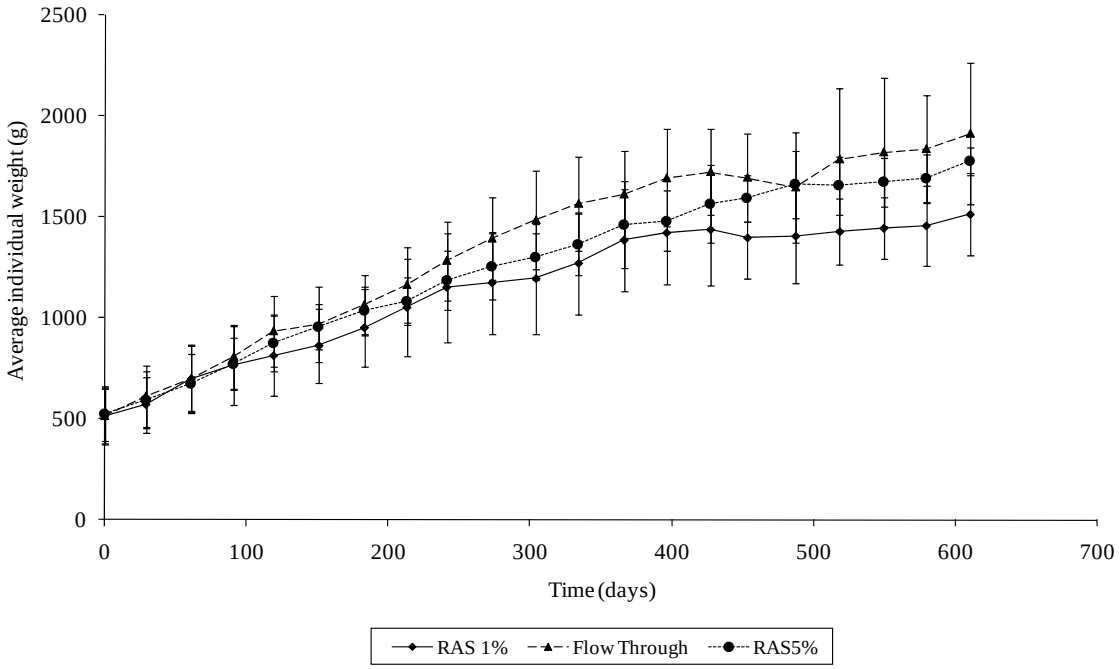

Figure 6: averaged weight development of the smallest fish cohort in the flow through system (1) and the two RAS systems (2=lowest water refreshment and 3= highest water refreshment rates), bars are indicating standard deviations

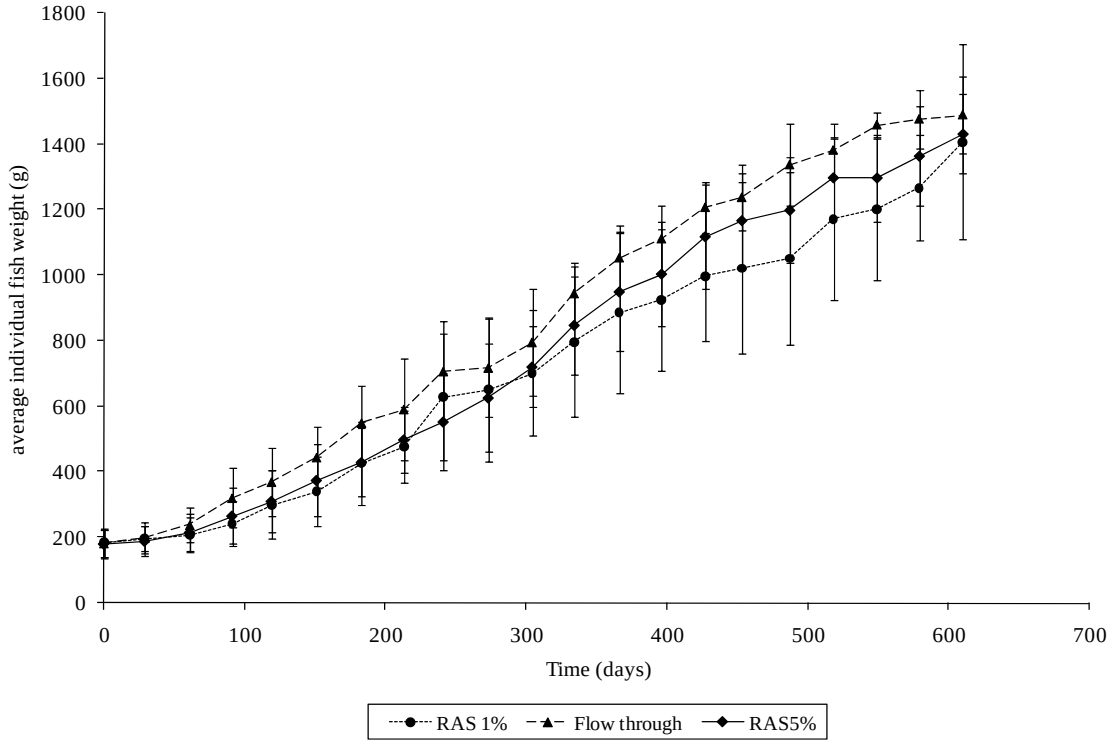

Figure 4: averaged weight development of the heaviest fish cohort in the flow through system (1) and the two RAS systems (2=lowest water refreshment and 3= highest water refreshment rates), bars are indicating standard deviations.

While weight development showed no significant effect of system type (water refreshment rate), feed intake was affected by total system water refreshment rates (
Figure *5*,
Figure *6* and Table 5).

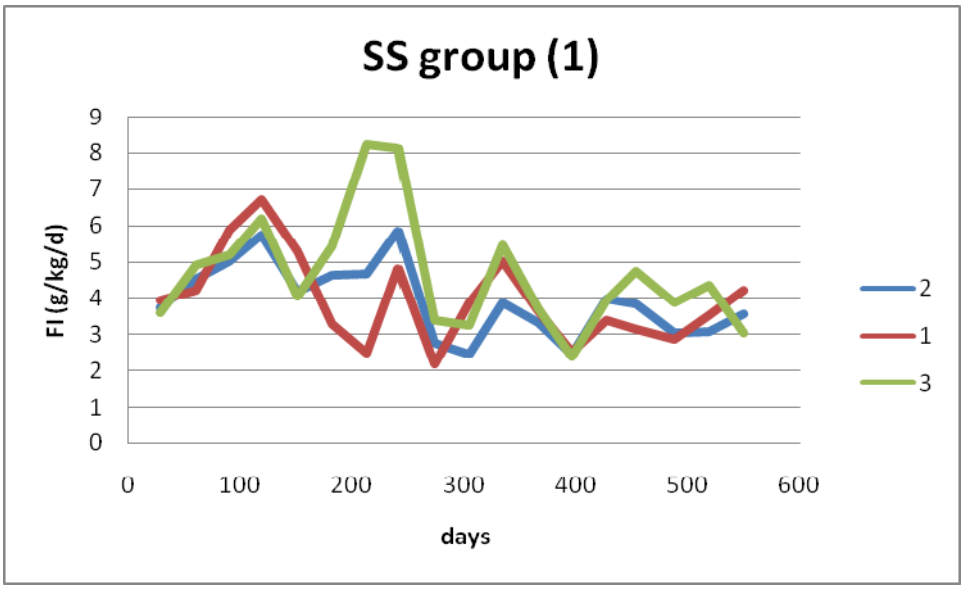

Figure 5: Feed intake development of the smallest fish cohort in the flow through system (1) and the two RAS systems (2=lowest water refreshment and 3= highest water refreshment rates)

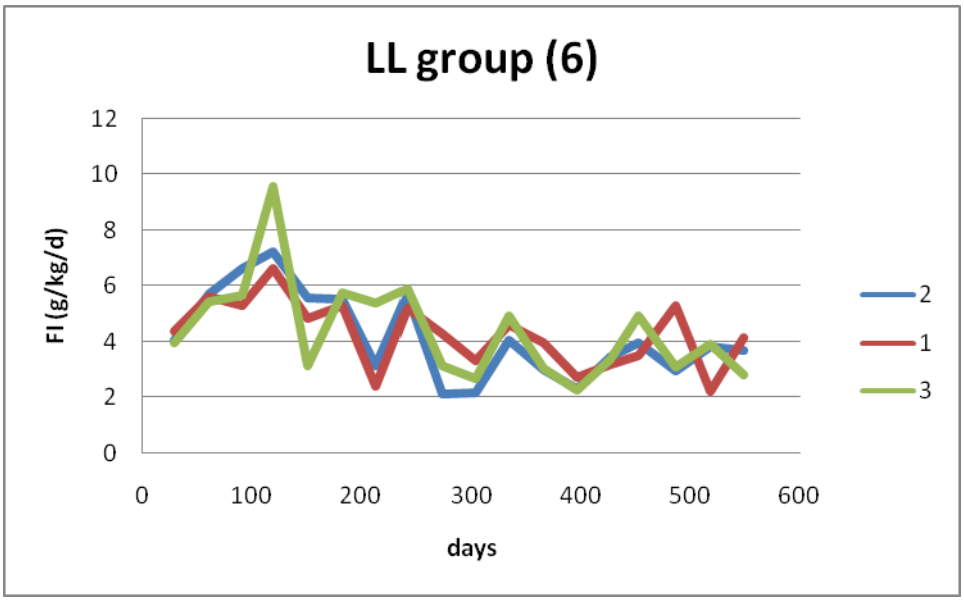

Figure 6: Feed intake development of the heaviest fish cohort in the flow through system (1) and the two RAS systems (2=lowest water refreshment and 3= highest water refreshment rates)

Based on the obtained results, it can be concluded that system water refreshment rate as handled at the moment in commercial recirculation aquaculture system has no negative impact on fish growth when compared to flow through system (Table 5). As stated by several authors (Losordo, Eding, Schneider per comm.) it was confirmed

that feed intake of fish in recirculation systems is lower than in flow through systems while maintaining fish growth. Thus feed conversion ratios are improved in RAS. That improvement might be related to different aspects as the more stable environment not only for abiotic factors, but as well for biotic factors such as the surrounding microbial matrix.

Table 5: p values derived from the gls model testing overall weight and feed load development accounting for size classes (day 0-549, day 0-396 and day 396-549) and for the different size classes using RRkg as system water refreshment rate (l/kg feed), temp as temperature(average per month) and the system time interaction.

	Intercept	RAS 1%	RAS 5%	days	RRkg	temp	RAS 1%*d	RAS 5%*d
Evaluated dataset	p-value	p-value	p-value	p-value	p-value	p-value	p-value	p-value
Day 0-549	0.000	0.489	0.232	0.000	n.a.	0.000	0.000	0.056
Day 0-396	0.018	0.855	0.962	0.000	n.a.	n.a.	0.000	0.000
Day 396-549	0.000	0.729	0.402	0.000	n.a.	n.a.	0.065	0.874
SS	0.174	0.848	0.130	0.000	n.a.	n.a.	0.000	0.000
SM	0.000	0.824	0.660	0.000	n.a.	0.000	0.000	0.003
SL	0.000	0.035	0.006	0.000	n.a.	0.001	0.639	0.612
LS	0.240	0.100	0.028	0.000	n.a.	0.000	0.000	0.435
LM	0.000	0.945	0.751	0.000	n.a.	n.a.	0.000	0.000
LL	0.000	0.079	0.083	0.000	n.a.	n.a.	0.000	0.002
Day 0-549	0.000	0.000	0.000	0.000	0.000	0.000	0.373	0.945
Day 0-396	0.004	0.000	0.000	0.008	0.000	0.000	0.715	0.434
Day 396-549	0.013	0.032	0.084	0.440	0.033	n.a.	0.080	0.255
SS	0.002	0.006	0.018	0.017	0.001	0.006	0.947	0.994
SM	0.229	0.050	0.050	0.027	0.040	0.000	0.104	0.700
SL	0.396	0.071	0.406	0.094	n.a.	0.011	0.778	0.432
LS	0.609	0.001	0.325	0.224	n.a.	0.010	0.003	0.029
LM	0.000	0.006	0.001	0.001	0.003	0.038	0.717	0.192
LL	0.013	0.028	0.031	0.011	0.005	0.000	0.437	0.505

2.2.4.1.4 Conclusion

It can be concluded based on this elaborated experiment that the hypothesis that changes in feed intake can be associated with changed fish welfare status in fish, using turbot as model species cannot be supported if closed RAS system were assumed to impair fish welfare status. In contrary feed intake in RAS 1% and RAS 5% was significantly influenced by system and lower than in the flow through system. The second hypothesis that changed fish welfare status is caused by different system water refreshment rates and fish and system management, leading to lower growth rates and less feed intake in more closed RAS, can therefore neither be supported. All the obtained differences in feed intake however are not reflected in the physiological or behavioral data hinting in the direction that fish welfare is not impaired in either system type.

2.2.5 Effect of water quality on feed intake in juvenile seabass, turbot, Dover sole and salmon

2.2.5.1 Hypercarbox

This experiment investigates chronic effects of 3 levels (control, medium and high) of hyperoxic associated to hypercapnic water conditions on growth performances and physiological responses in seabass.

Experimental design

Immature sea bass were supplied from a land based farm at the Ifremer Research Station in Palavas in early October 2005. The fish were stored in a 5m3 tank during 8 days, then graded under anaesthetic conditions (350 ppm phenoxy-2-ethanol) within the weight range 35-110g and were randomly distributed in 9 experimental tanks at approximately 8 kg fish biomass per tank. The fish were then acclimated for 21 days prior to the experiment. The 9 experimental tanks were 1m^3 each, black coated, U-shaped and located in a isolated room. Each tank was covered with a black plastic sheet to protect fish against external visual stress. Light intensity at the water surface was 500 lux using an incandescent lamp (OSRAM Decor Silver E27-75W) at 1 m above the water surface,

and the photoperiod was maintained at 16h light - 8h dark, including a 30 min artificial dawn and dusk. The fish were submitted during 63 days at three levels of hyperoxia conditions associated to hypercapnia (control, medium and high) which were tested in triplicate tanks. These 3 experimental treatments were obtained by using separately (control and high conditions) or after mixing in half (medium condition) 2 different gas content waters (n°1 and n°2) which were prepared in separate header tanks and then fed by gravity to the tanks. The sea water used in the header tanks was previously sand-filtered at 15 µm, UV sterilised, heated and degassed in a packed column. Water n°1 was enriched with oxygen at a concentration of 0.9 mg.l-1 (150% saturation) by bubbling an adequate flow of gaseous oxygen through a porous tube located at the bottom of the header tank under a 150 cm water column. Water n°2 in the other header tank was enriched both with oxygen (21.6 mg.l-1, 298% saturation) and with carbon dioxide (48.8 mg.l-1) by bubbling adequate flows of gaseous oxygen and gaseous carbon dioxide through 2 separate porous tubes located at the bottom of the header tank under a 70 cm water column. The mean concentrations in oxygen and carbon dioxide measured in the 3 experimental treatments (T1, T2, T3) during 63 days are summarized in Table 6.

Table 6: Oxygen and Carbon dioxide concentrations, pH in the 3 experimental conditions.

	O_2 (mg/l)	O_2 (%)	CO_2 (mg/l)	Induced pH
T1 control 100% water Nr. 1	7.1 ± 0.6	98	7.7± 2.6	7.5± 0.3
T2 medium	12.0 ±0.9	164	28.5 ±3.5	6.7 ±0.1
T3 high 100% water Nr. 2	17.1 ±1.0	233	53.0 ±4.0	6.4 ±0.1

The total flowrate was 700L.h-1 per tank, sufficient to provide an efficient self-cleaning into the tank and to maintain the other water parameters at an optimum level: temperature = 20.7 ± 0.5°C, salinity = 34.9 ± 1.9 g.L^{-1}, ammonia= 0.3 ± 0.2 mg.L^{-1} N-(NH3+NH4+), urea = 0.1 ± 0.1 mg.L^{-1} N-Urea, nitrite < 0.1 mg.L^{-1} N-NO2, nitrate <0.3 ± 0.1 mg.L^{-1} N-NO3 and phosphate <0.1 mg.L^{-1} P-PO4. The fish were fed expanded pellets (43% protein, 20% crude fat, 10% humidity, Neogrower marin 4mm, Le Gouessant producer), from the same batch of production troughout the experiment, once a day ad libitum during a 45 to 60 mn meal starting at 9h00 using a semi-manuel system. The feeders were poured the day before to avoid any stress of the fish. A switch located outside each tank was actuated by the operator who can observe the feeding behaviour of the fish through a small window in the plastic sheet. At each actuation of the switch, the electric feeder released at the water surface a predetermined amount of feed (1.7 to 1.9 g) in 3 equal doses within 8 seconds. The switches were activated successively several times until apparent satiety determined by 3 accompanying visual indicators: i) indifference of the fish towards the food, ii) observation of uneaten pellets in the bottom of the tank and iii) presence of some pellets in the particule trap 30mn after the end of the meal, the number of pellets in the traps were counted. Water flows were checked once a day from flow meters. Temperature was measured once a day in the outflow water of one tank using a Checktemp 1 thermometer. Dissolved O_2 was measured daily in the outflow water of each tank and in the inflow water for each treatment using a YSI 550A oxymeter. pH was measured once a day in the outlet of each tank using an Ecoscan pH meter. Dissolved CO_2 was measured twice a week in each tank using an Oxyguard CO_2 analyser and once a week using a Li-cor CO_2 analyser. Total gas pressure was measured once a week in the outflow water of each tank with an Alpha tensionometer 300E. The probe was calibrated to 0 in air before use. Salinity was measured twice a week with an ATAGO hand refractometer (S/Mills-E). Catabolites concentrations: ammonia, nitrite, nitrate, phosphate and urea were measured once a week using a Technicon Analyser®. The TA-N concentration was determined by the indophenol method (Bower and Holm-Hansen, 1980). The water samples were taken in the inlet and outlet of each tank during the postprandial period when their production by the fish was maximal and stored until the analysis in appropriate conditions. Analyses were performed in the lab of the station the same day or the day after (the samples were preserved with chloroform or frozen before analysis. Mortality and behaviour were controlled every day. Feed in feeders were weighted daily at the nearest g for each tank before and after each meal. The weight of uneaten and trapped pellets was daily calculated by multiplying the number of pellets by the mean weight of one pellet calculated from a sample of 100 pellets. At d1 and d63, weight (at the nearest 0.1g) and fork length (at the nearest 0.1cm) of each fish were measured after anesthetization at 400 ppm phenoxy-2-ethanol. At d1 and d63, blood samplings were taken in 10 fish per tank after a 24h fasting period. The fish were caught in each tank in one netting then put in a bucket with 10L of seawater at 3-4°C for a 3mn period. The temperature was obtained and maintained at this low level by adding large ice cubes in seawater. Blood was taken in the

caudal vein with a 2 ml heparinised syringe and shared in 2 eppendorf tubes taken in ice. The blood samples were analysed by a I-Stat analyser (ABBOTT) using EC8+ cassettes. PH and PCO2 were adjusted according the temperature using respectively the equations of Heisler, 1984 and Boutillier et al, 1984.

2.2.5.1.1 Calculations and statistics

The mean wet weight ± SD and the mean length ± SD were calculated as the arithmetic mean from the fish. At the end of the experiment, the specific growth rate (SGR) was determined as:

$$SGR = [(Ln\ Wf - Ln\ Wi) \times 100] / T$$

where Wf is the mean wet weight in grams at the end of the experiment , Wi is the initial mean wet weight and T the duration of the period in days. Variation in fish size within the tank were described by means of a comparison of coefficients of variation (CV) using the following equation :

$$CV = 100 \times SD \text{ of the mean weight} / \text{mean weight}$$

The distributed food was calculated daily per tank by difference of the weight of the poured and left pellets in the feeder. The daily amount of ingested food (DAIF) is calculated as the difference of the distributed food and uneaten food. The uneaten food is the number of pellets counted in the particule trap multiplied by the mean weight of one pellet. When needed mobile means can be calculated as the sum of the data of several following days (3 here) divided by the number of the days and expressed as a % of the control fish. The mean daily Feed Intake (FI, % of initial fish biomass) was calculated as follows:

$$FI = \text{Ingested feed} / (Wi \times \text{fish number}) \times 1/T$$

This simplified FI, using only Wi, can be calculated because the fish samples were done similarly in the 6 tanks and they made the calculation of FI with initial and final biomass per period too complex. When needed, a mobile mean is calculated by using the mean of 3 following FI. The food conversion ratio (FCR) was calculated as:

FCR = Ingested feed / Weight gain of fish biomass

The condition index (CI) was calculated as:

CI = Wf. Lf-3*100

where Lf is fork length at the end of the experiment. The experimental data were processed by a one-way analysis of variance (ANOVA), and when necessary by an analysis of co-variance (ANCOVA). Differences among the means were determined by a Student-Newman-Keuls (SNK) test that was applied to ranks when normality was not established. Linear regressions (LR) were performed to analyse correlations between factors. The comparisons between CV were performed by means of a sign-test. The SigmaStat package was used to process the data.

2.2.5.1.2 Results

Table 7: Main rearing index performances in seabass in the 3 experimental conditions (63d).

	Mortality (%)	FI (%/d)	SGR (%/d)	FCR	O_2 Up, $mg.h^{-1}.kg^{-1}$
T1 control 100% water Nr. 1	0	1.43±0.08 a	1.18± c	1.32±0.11 e	298±6 f
T2 medium	0	1.35±0.02 a	1.10± c	1.33±0.04 e	357±15 g
T3 high 100% water Nr. 2	0	1.21±0.02 b	0.91± d	1.30±0.05 e	394±13 h

No mortality was observed from d1 to d63 in all treatments (Table 8). The daily amount of ingested food (DAIF) per tank showed larges variations due to external and internal factors and their complex combinations leading most of the time to unclear graphs. When mobile means (3 following data) of DAI F were expressed in % of control fish and were plotted against time, more clear trends appeared over the experiment. From the first day of the experiment to the end, the fish subjected to T3 had a significantly lower feed intake than control fish (Figure *7*). The average feed intake in T3 is 84% of the control T1. The feed intake of T2 exhibited the same trend, but slightly and not significantly lower than control (94%).

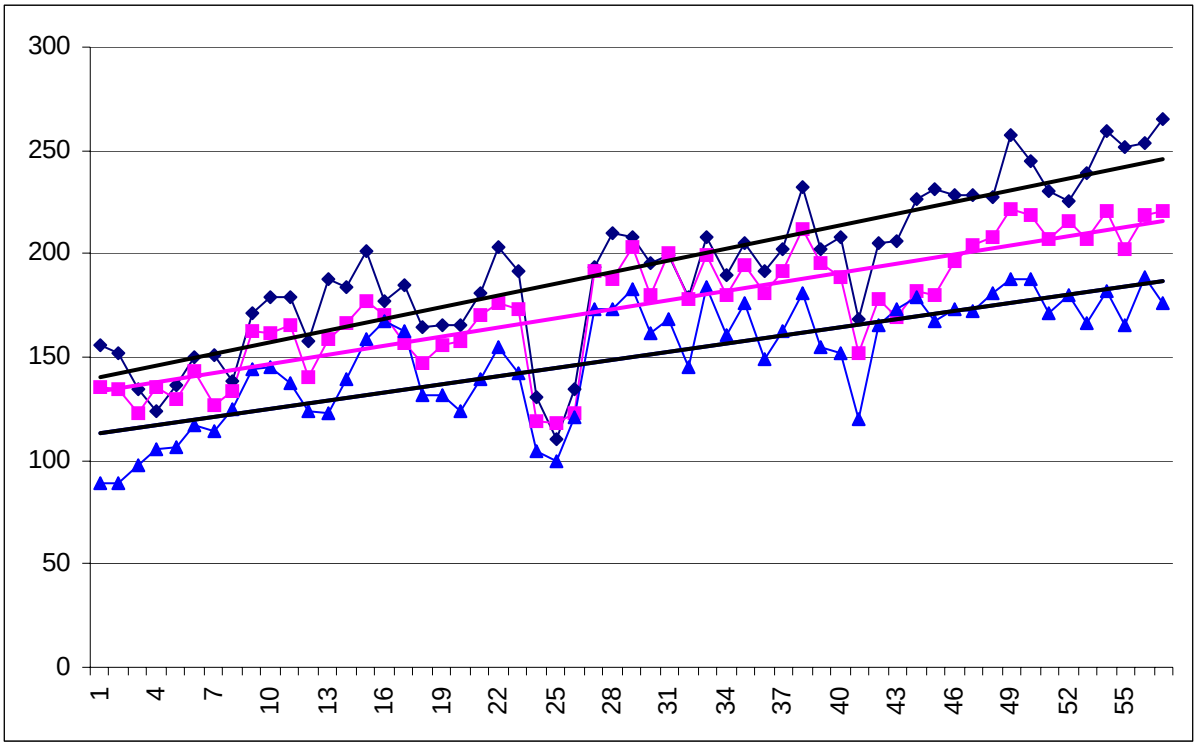

Figure 7: Daily ingested Food expressed in % of control fish. Dots are mobile mean of the daily amount of ingested feed for 3 days running. Means of the 3 tanks per treatment

The global SGR was deeply and significantly affected in T3 where fish were subjected to high hyperoxia and hypercapnia conditions and represented 84.5% of the growth observed in control fish (Table 8). In T2 conditions, fish exhibited an intermediary growth which was 93% of the control fish but not significantly different. The FCR was similar in the 3 treatments, as the predicted result of a reduced feed intake and a reduced growth in the same percentage (Table 8). No statistical difference were found in Na, K, Glucose, and pH. TCO2, PCO2 and HCO3 increased dramatically in relation with increasing hypercapnia and hyperoxia. Hematocrit and hemoblobin content deacreased when increasing hyperoxia (Table 9). The apparent oxygen uptake was significantly higher in T2 (120%) and T3 (132%) compared to the control fish T1(100%) (Table 8).

Table 8: Blood parameters at day 63.

Treatment	T1 (control) N=10	T2 medium N=10	T3 high N=10
Na mmol/L	162.5 ± 5.4 a	157.7 ± 5.4 a	158.5 ± 7.5 a
K mmol/L	5.4 ± 0.9 a	4.9 ± 0.5 a	5.9 ± 0.7 a
TCO2 mmol/L	10.0 ± 1.1 a	22.2 ± 2.0 b	33.5 ± 4.0 c
Glucose mg/dL	86.1 ± 21.2 a	102.1 ± 18 a	106.0 ± 33.4 a
Hematocrit % pcv	20.5 ± 4.3 a	18.2 ± 3.9 b	15.5 ± 2.5 c
PH	7.1 ± 0.1 a	7.2 ± 0.1 a	7.3 ± 0.1 a
PCO2 mmHg	26.9 ± 5.1 a	48.6 ± 7.7 b	58.5 ± 10.6 c
HCO3 mmol/L	9.1 ± 1.0 a	21.1 ± 1.7 b	31.8 ± 3.8 c
Hemoglobin g/dL	7.0 ± 1.4 a	6.0 ± 1.3 a	5.4 ± 1.0 b

2.2.5.1.3 Conclusion

When fish were subjected to severe hyperoxia and hypercapnia conditions, global feed intake, specific growth rate, apparent oxygen uptake and blood parameters in relation with increasing hypercapnia and hyperoxia were significantly impaired. Some other blood parameters and feed conversion ratio were similar in the 3 treatments, as a reduced feed intake led a reduced growth in the same percentage.

2.2.5.2 ZLV Benefish

2.2.5.2.1 Database

Growth and feed load data from a commercial turbot farm in The Netherlands were evaluated for deviations from expected feed intake. This farm has collected several data on fish growth, water quality and incidents over a period of several years.

2.2.5.2.2 Statistical analysis

Deviations were detected in relation to a customized base line. This base line was generated from the overall farm performance. Recorded Incidents were correlated with the growth data of ten turbot families present in this farm during several years. Data availability was limited to weighing moments, that means feed loads and initial and final weight between those moments could be distilled from the management paper archive. The data was regressed to obtain the farm base line and outliners with +/- 2 std. residuals were identified.

2.2.5.2.3 Results

The turbot farmer encountered several difficulties during operation (diseases and technical etc) on structural or incidental base. In general, all families had experienced at least one incident during its farm live. Geometric average weight (X Axis in g) plotted versus feed load in (g/g/d, Y Axis) for all 10 evaluated families shows several outliers and therefore deviations from expected feed intake could be identified (
Figure 8).

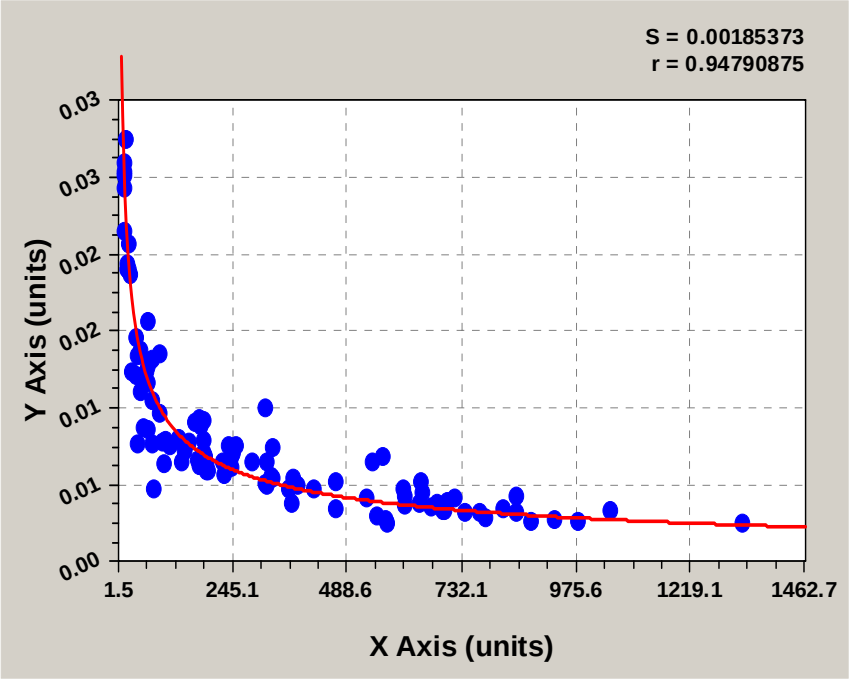

Figure 8: Geometric average weight (g, X Axis) of turbot at the commercial farm versus feed load in g/g/d (Y Axis) for 10 families followed during the life cycle on the farm.

Considering these outliers, in six cases Std. residuals were bigger than +/- 2. These outliers or better cases were correlated with incidents on farm level (water quality changes, diseases etc). One case related to bad water quality in general, one case related to high NO_2^- values and one case to disease outbreaks with subsequent formalin treatment. Furthermore all data was evaluated further and feed efficiency in relation to growth response was evaluated and therefore specific growth rate (%) and feed load in g/g/d were linearly regressed (
Figure 9).

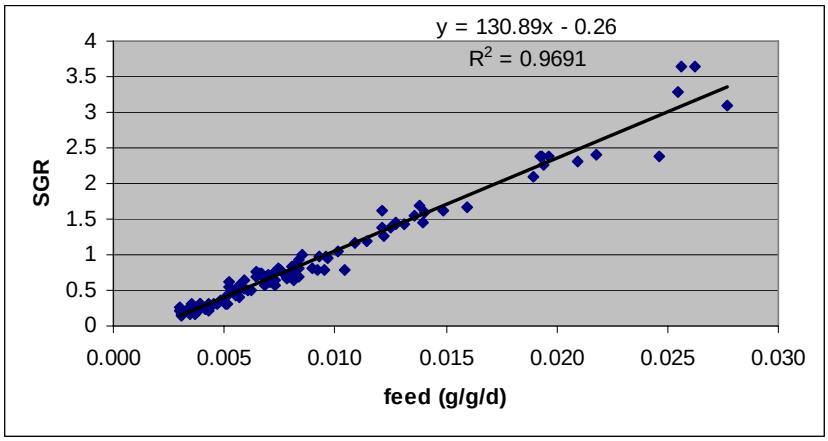

Figure 9: Linear regression of feed load in g/g/d and SGR (%) for turbot held in a commercial production facility.

In five cases Stud. Residuals bigger than +/- 2 were obtained and therefore ideintified as outliers. Only two of them were related to NO_2-N values. The other three could not be related to any incidents.

2.2.5.2.4 Conclusions

Inappropriate water quality or outbreaks of diseases might, as explained, impact on feed intake. Those have therefore to be avoided. Implementation of better management practices of farm level, such as appropriate water quality management and hygiene management including health monitoring and avoidance of contaminations either through intake water or newly stocked fish. Feed intake can therefore be related with suboptimal farming practices in turbot.

2.2.5.3 Solea

2.2.5.3.1 Database

Data from a commercial Dover sole farm in The Netherlands was evaluated based on datasets derived from their farm management base. Due to commercial sensitivities the access to the dataset was limited. Data was evaluated on tank level. Different time series over 1-3 years on weekly basis have been made available by the farm management. These data include information on number of fish, mean weights, feed load and management intervention (sorting, add on grading, harvest, etc.).

2.2.5.3.2 Statistical analysis

Deviations from expected feed intake were based on farm's expectation and realized feed load in the system or tanks. A part of the data is presented in the figure below. The data itself is commercially highly sensitive and their presentation possibilities therefore limited.

2.2.5.3.3 Results

The commercial farm data was evaluated on tank level. Different time series over 1-3 years on weekly basis have been made available by the farm management. These data include information on number of fish, mean weights, feed load and management intervention (sorting, add on grading, harvest, etc.). Based on data from literature and the expert judgment of the farmer an expected feed load was established. This feed load could then be compared to the realized feed load (Figure 10).

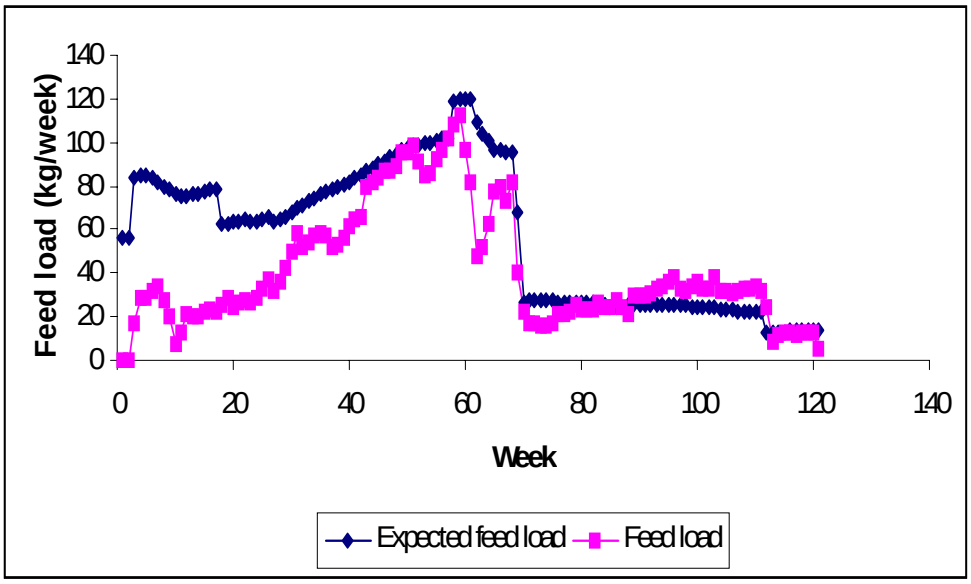

Figure 10: Expected and realized feed load of commercial sole farm in The Netherlands

These data were then subsequent translated into deviations from expected feed intake. These deviations were then correlated with different parameters that were measured and recorded by the farmer (Figure 11).

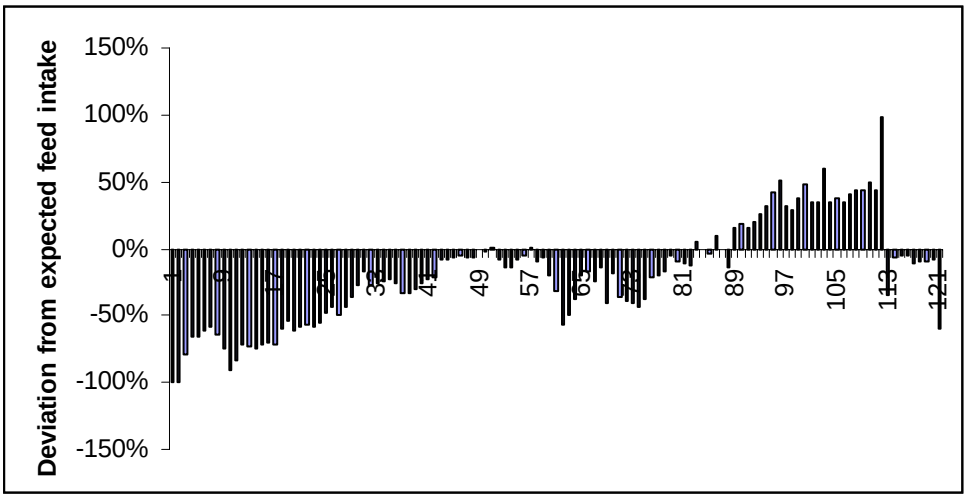

Figure 11: Deviations from expected feed intake in % over time in days for sole in a commercial facility.

Several (water) parameters were related with the observed deviations, such as temperature, pH, turbidity, mortality. Furthermore the influence of grading was investigated (Figure 12, Figure 13, Figure 14 and Figure 15).

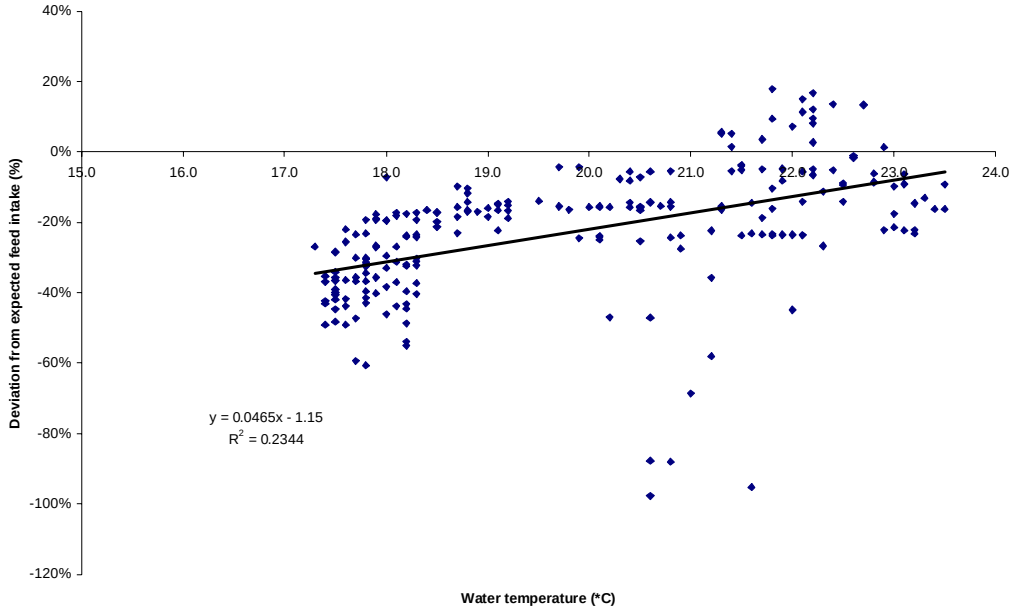

Figure 12: Effect of water temperature on expected feed intake expressed as deviation.

A decrease in water temperature seemed to result in lower feed intake. This is not surprising as water temperature influences feed intake in fish due to its influence on fish metabolism. Furthermore is the expected feed intake based on higher temperatures (based on experiments) than realized on the farm (20-23C). In sole feed intake might therefore be related to fish metabolic state. If actually this is as well impacting fish welfare remains open. The widely used hypothesis that feed intake is related to fish welfare cannot be supported based on this result.

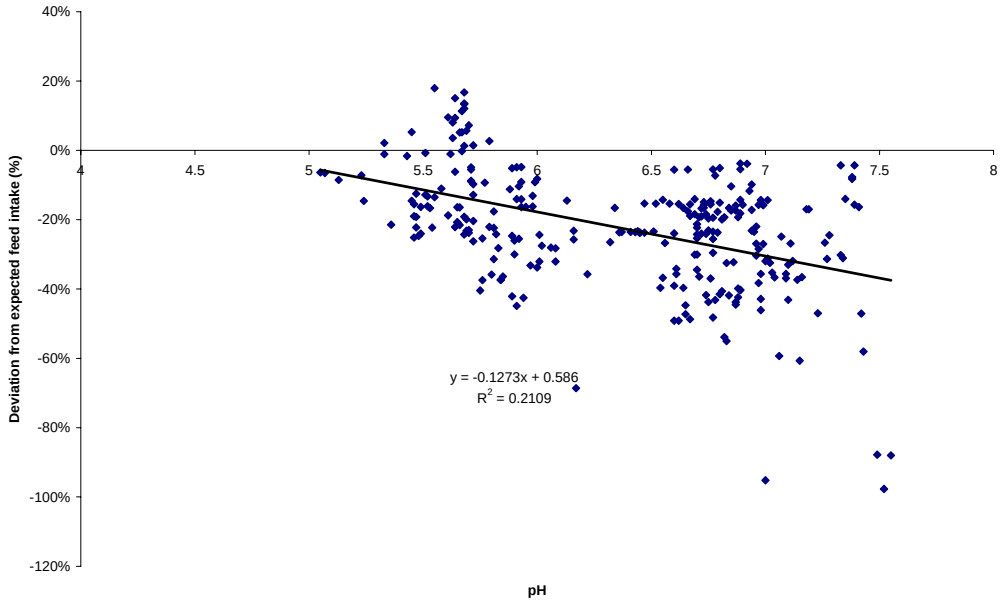

Figure 13: Effect of pH on expected feed intake expressed as deviation

It was observed that feed intake increased with decreasing pH. Low pH seems therefore by definition of farmers not a welfare issue as the deviation from expected feed intake decrease. This might be supported by the fact that

several parasitical species have a narrow pH tolerance. A lower pH might therefore lower the disease pressure on the animal and increase their appetite.

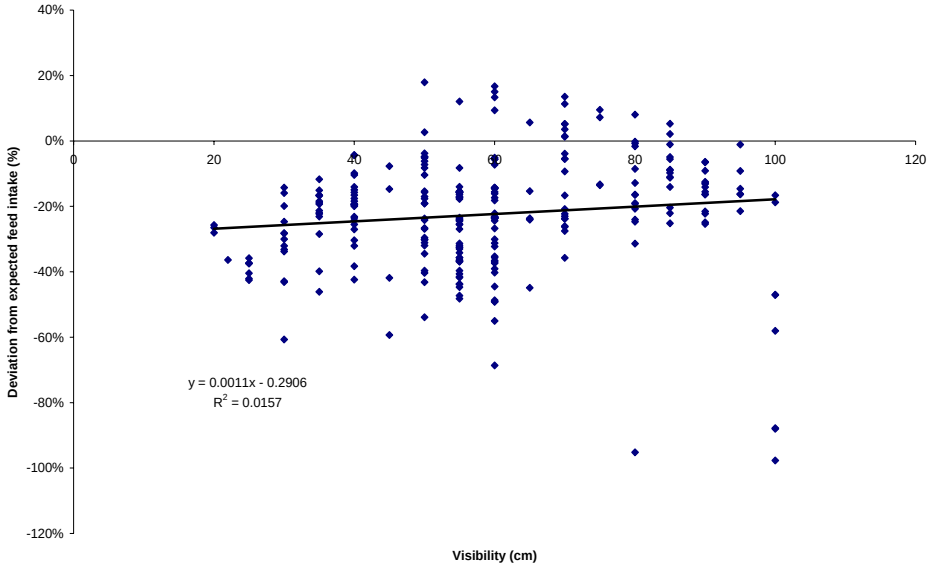

Figure 14: Effect of visibility on expected feed intake expressed as deviation

Particle load in the water is likely to increase with decreasing visibility. This higher particle load is in RAS often related with increased populations of heterotrophic bacteria utilizing the particular load. This might impair fish health due to oxygen depletion from the water and by the general interaction of a high bacterial load including pathogenic bacteria and the fish. Here realized feed intake might be related with fish welfare, supporting general argumentations of fish farmers.

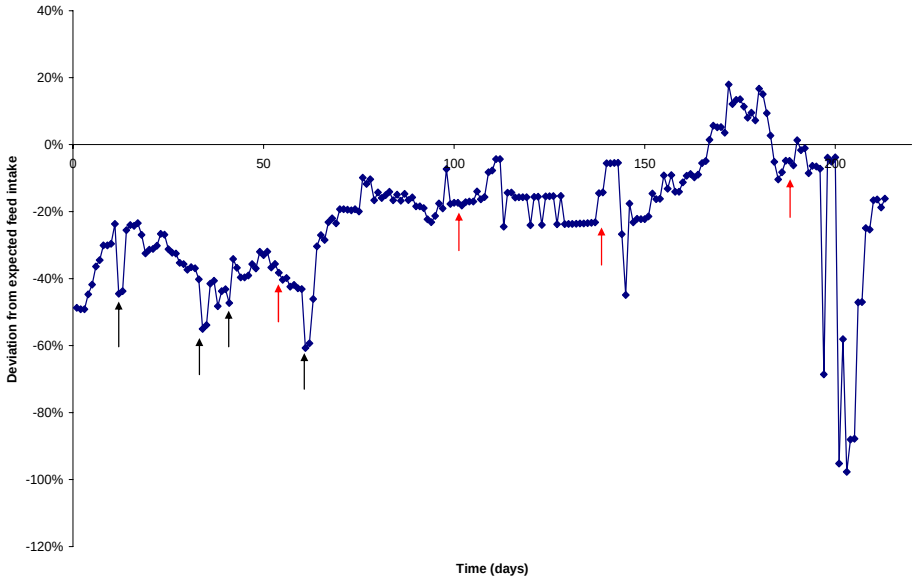

Figure 15: Effect of grading on expected feed intake expressed as deviation

No negative effect of grading on fish feed intake was apparent from the deviation of expected feed intake over time (Figure 15)

2.2.5.3.4 Conclusions

Based on the present data, it is concluded that decreased water quality relates to lower feed intake and that this might serve as an indication of impaired fish welfare. Grading does not influence feed intake. This was confirmed in various experimental studies, were sole was hand-fed and normal feeding levels could be reached only hours after fish grading (van der Heul, pers. Comm., Ende et al, 2008).

IPN2002

2.2.5.3.5 Experimental design

Dataset IPN2002 investigated the effect of three different levels of CO_2 upon the growth, mortality, feed intake and seawater performance of tank-held Atlantic salmon pre-smolts during the parr-smolt transformation. Additional water quality and physiological variables were also measured. 12 groups of 0+ pre-smolts (n = 320 fish $tank^{-1}$), were held in 500 l tanks at a stocking density of ca. 33-37 kg m^{-3} and specific water flow was high (1.3 l $kg^{-1}min^{-1}$), similar amongst all tanks. Different levels of CO_2 were tested in a 4 x 3 experimental design and treatments were subject to either 1) control: 5.4 mg l^{-1} CO_2, pH 6.63; 2) 12.7 mg l^{-1} CO_2, pH 6.21; 3) 17.6 mg l^{-1} CO_2, pH 6.04; 4) 27.3 mg l^{-1} CO_2, pH 5.84. Growth performance (SGR) was measured on days 21, 42 and 84. Mortality was also tracked throughout the study and feed intake was recorded every third day throughout the experimental period.

2.2.5.3.6 Statistical analysis

Only initial parametric comparisons have been undertaken.

2.2.5.3.7 Results

Fish held at the elevated CO_2 concentrations had significantly lower feed intake as compared to control fish, they also showed lower growth than the control group during the freshwater period. However, fish held under the 2 lower CO_2 concentrations had significantly higher SGR's than controls following seawater transfer. Elevated CO_2 had no significant effect upon mortality during the freshwater period. Elevated CO_2 levels did not affect mortality.

2.2.5.3.8 Conclusions

Subjecting Atlantic salmon pre-smolts to elevated levels of CO_2 had a significant and detrimental impact upon their freshwater growth performance and feed intake. Based on these observations, a potential welfare action would be to reduce the negative impact of CO_2 upon growth and feed intake is to utilize CO_2 stripping technology.

2.2.6 Effect of the interaction of affected fish health status and water quality on feed intake in rainbow trout

AW1205

2.2.6.1.1 Experimental design

Dataset AW1205 was collected as part of a large epidemiological study investigating the effects of water quality on the welfare of farmed rainbow trout in the UK. The study involved collecting water quality, biological (morphological and physiological measures from fish) and farm (including feed - FCR) data from a large number of rainbow trout farms during both the summer and winter. The farms visited represented a significant proportion of the UK trout farming industry.

2.2.6.1.2 Statistical analysis

Relationships between water quality, biological and husbandry parameters (which include potential welfare risk factors) were analysed using a statistical approach incorporating multilevel modelling techniques (using MLwiN).

2.2.6.1.3 Results

Following a detailed review, datasets AW1205 was not considered suitable for analysis to address the feed intake hypothesis. The data were deemed insufficient for two main reasons: 1) the data provides an assessment of feed intake on commercial farms using feed conversion ratios (FCR) but it does not provide an assessment of what normal feed intake is or by how much feed intake deviates from that normal level, 2) the data were gained from commercial farm records and the calculation of FCR cannot be considered sufficiently accurate or reliable to perform robust analyses.

2.2.6.1.4 Conclusions

Whilst dataset AW1205 did not provide inputs to address the feed intake hypothesis, it has highlighted some of the issues that surround the collection, assessment and interpretation of feed intake data that is collected from commercial farms.

2.2.6.2 RTGE

2.2.6.2.1 Experimental design

Dataset RTGE was collected as part of a study investigating rainbow trout gastroenteritis (RTGE) in the UK. Biological data was collected from a large number of UK trout farms with husbandry and management (including feed) data collated from farm records and downloaded from farm management software (i.e. FarmControl and Djournal).

2.2.6.2.2 Statistical analysis

Both univariate and multivariate techniques were used to investigate risk factors associated with RTGE.

2.2.6.2.3 Results

Following a detailed review, datasets RTGE was not considered suitable for analysis to address the feed intake hypothesis. The data were deemed insufficient for two main reasons: 1) the data provides an assessment of feed intake on commercial farms using feed conversion ratios (FCR) but it does not provide an assessment of what normal feed intake is or by how much feed intake deviates from that normal level, 2) the data were gained from commercial farm records and the calculation of FCR cannot be considered sufficiently accurate or reliable to perform robust analyses.

2.2.6.2.4 Conclusions

Whilst datasets RTGE did not provide inputs to address the feed intake hypothesis, it has highlighted some of the issues that surround the collection, assessment and interpretation of feed intake data that is collected from commercial farms.

2.2.7 Effect of management intervention and biotic and abiotic factors on feed intake in salmon

2.2.7.1 GFI0

2.2.7.1.1 Experimental design

Dataset GFI0 investigated the impact of environmental variables upon deviations from expected feed intake in Atlantic salmon parr, using year class 0+ fish subjected to 24h light during the final stage of their freshwater phase. Three cages of parr (n = 61847 ± 2620 fish group^{-1}) were held in 12 x 12 x 4m production cages for 64 days. Fish were fed on-demand throughout the light phase using commercial AQ1 on-demand feeders. Potential factors affecting feed intake included: husbandry interventions such as weight sampling during Day 1-4 (n = 300 fish sample^{-1}), Day 37-39 (n = 400 fish sample^{-1}) and Day 63-64 (n = 500 fish sample^{-1}). Days with additional husbandry interventions (such as disease treatment), and the introduction of 24h light (day 55) were also noted. Environmental variables including daily water temperature, clarity, average daily windspeed (used as a proxy for wind driven water currents at the site), daylength, change in daylength were also measured.

2.2.7.1.2 Statistical analysis

Deviations from expected feed intake can be obtained by plotting expected feed intake against regression residuals, creating an index of deviation from expected feed intake. When using existing data on daily feed intake, expected feed intake can be defined using either 1) Moving averages 2) Polynomial regression lines, or 3) Spline piecewise polynomials, to create a smoothed line of best fit. When using moving averages to generate a best fit line, a number of moving average lengths can be used (e.g. a 3-, 4-, or 5-day moving average, etc). An unbiased selection criterion is to choose the moving average length that contains no autocorrelation. This procedure can be repeated for expected feed intake lines generated by Polynomial and Spline Polynomials. Once an expected feed intake line has been generated, incidental (short-term) deviations from this line can be examined using regression residuals. Once residuals have been generated, a simple GLM model can be used to determine whether deviations from expected feed intake are related to a number of measured variables (e.g. abiotic, biotic or management factors). For the GFI0 dataset both 3-day Moving averages and polynomial regression lacked

autocorrelation. Once residuals were generated, simple GLM models were used to determine whether deviations from expected feed intake were related to abiotic, biotic or management variables.

2.2.7.1.3 Results

Changes in daily feed intake between cages and days within a cages are shown in Figure 16. Residuals from these lines are shown in Figure 17. Using 3-day moving average residuals from cage 1 as an example, Figure 18 shows days with husbandry interventions imposed onto the residual plot. GLM analyses showed no clear significant predictor of daily deviation from expected feed intake for any cage, irrespective of the length of time lag (Table). Further, when analysis was carried out using moving average residuals with a 2+ day time lag, water clarity was a significant predictor of deviations from expected feed intake irrespective of the choice of day length parameter used.

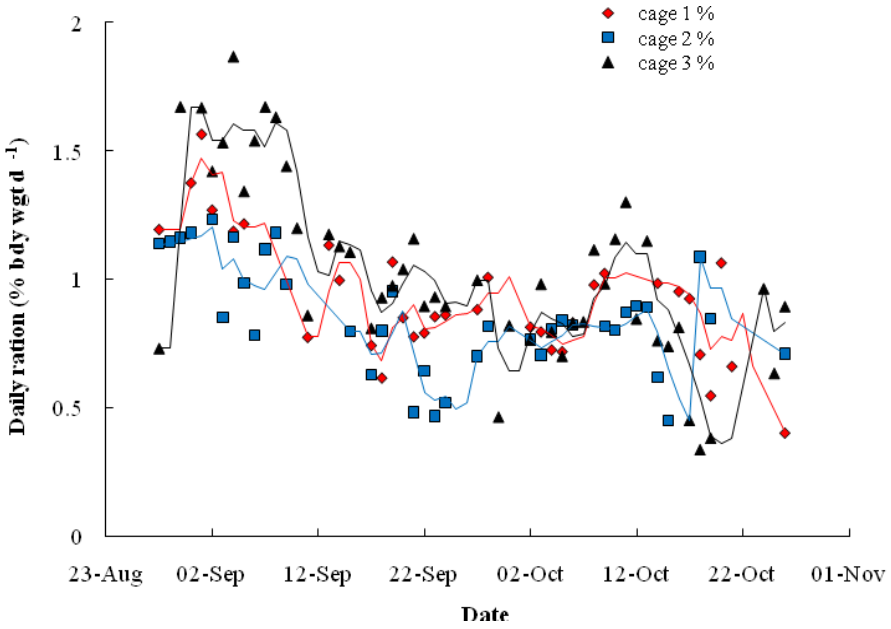

Figure 16: Changes in the daily feed intake of cage-held Atlantic salmon 0+ pre-smolts between days and groups. Trendlines represent 3-day moving averages for each cage.

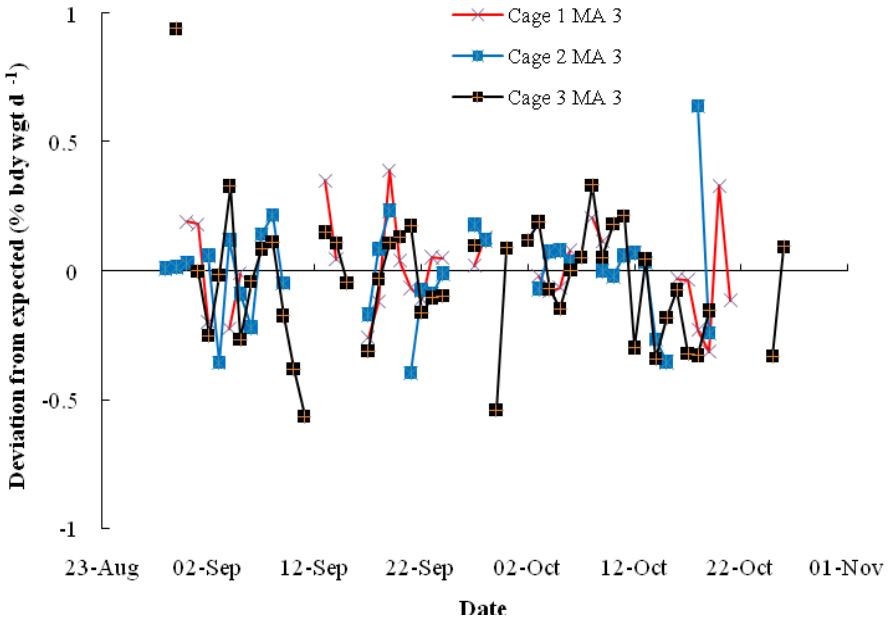

Figure 17: Deviations from expected feed intake for each cage based on 3-day moving averages.

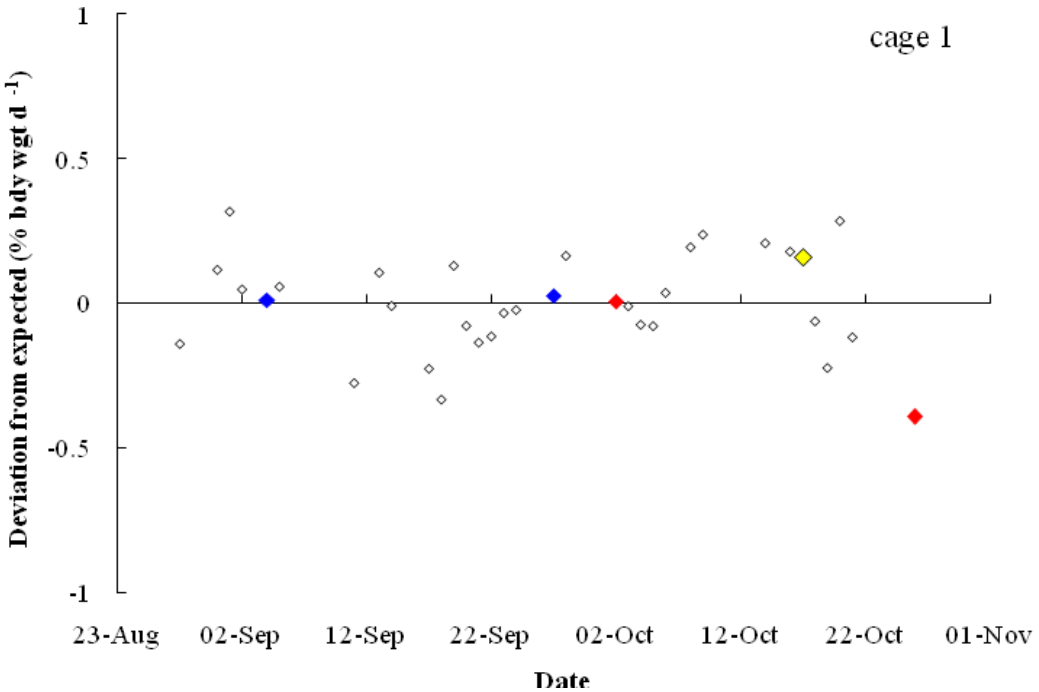

Figure 18: Daily deviations from expected feed intake in relation to water quality and husbandry interventions. Red points indicate sampling events. Blue represents a disease treatment. Yellow represents the onset of 24h light.

Table 9: Predictors of deviation of expected feed intake for cage-held 0+ Atlantic salmon pre-smolts. Factors included in every model: husbandry interventions; water clarity; water temperature; average daily wind speed.

	Additional factors included in model			
	Ambient Daylength	Ambient Daylength inc. 24h light	CiAD	CiA+24D
MA 3	-	-	CiAD 0.021	-
MA 3 +1	-	-	-	-
MA 3 +2	Clarity 0.039	Clarity 0.016	Clarity 0.036, Temp 0.043, CiA+24D 0.012	Clarity 0.006
MA 3 +3	-	-	-	Husbandry Intervention 0.044
Polynomial	-	-	-	-
Poly +1	-	-	-	-
Poly +2	-	-	CiAD 0.043	-
Poly +3	-	-	-	Clarity 0.041

2.2.7.1.4 Conclusion

Dataset GFI0 suggests that if cage-held Atlantic salmon 0+ pre-smolts are fed on-demand, they generally have the opportunity to catch up and compensate for any periods of sub-optimal feed intake, and husbandry interventions have no significant effect upon this.

2.2.7.2 GFI1

2.2.7.2.1 Experimental design

Dataset GFI1 on cage-held juvenile Atlantic salmon fed on-demand was evaluated for deviations from expected feed intake over a period of ca. 9 months.

2.2.7.2.2 Statistical analysis

All feed intake residual data shows a high degree of autocorrelation and the data was deemed unsuitable for WP4.

2.2.7.2.3 Results

For the GFI1 dataset all feed intake residual data shows a high degree of autocorrelation and the data was deemed unsuitable for WP4.

2.2.7.2.4 Conclusions

Nothing could be concluded from this dataset due to suitability problems associated with the data.

2.2.7.3 LEFI

2.2.7.3.1 Experimental design

Dataset LEFI investigated the relationship between episodes of sub-optimal feed intake and husbandry incidents/interventions, biotic and abiotic factors in cage-held (n =1) Atlantic salmon post-smolts fed on-demand for 88 days. Potential factors affecting feed intake included: husbandry interventions such as weight sampling the fish on days 29, 57 and 88 (n = 100 fish sample^{-1}). Environmental variables including daily water temperature, clarity, average daily windspeed (used as a proxy for wind driven water currents at the site), rainfall, salinity, tidal range, daylength, change in daylength were also measured.

2.2.7.3.2 Statistical analysis

For the LEFI dataset quadratic polynomial regression lacked autocorrelation. Once residuals were generated, simple GLM models were used to determine whether deviations from expected feed intake were related to abiotic, biotic or management variables.

2.2.7.3.3 Results

There was no significant predictor of daily deviation from expected feed intake irrespective of the length of time lag.

2.2.7.3.4 Conclusions

Dataset LEFI suggests that if cage-held Atlantic salmon post-smolts are fed on-demand, they generally have the opportunity to catch up and compensate for any periods of sub-optimal feed intake, and husbandry interventions have no significant effect upon this.

2.2.7.4 RTFI

2.2.7.4.1 Experimental design

Dataset RTFI investigated the relationship between episodes of sub-optimal feed intake and husbandry incidents/interventions, biotic and abiotic factors in tank-held rainbow trout (n = 15 fish tank^{-1}) fed on-demand using self-feeding systems for 62 days. Potential factors affecting feed intake included husbandry interventions such as weight sampling the fish on days 1, 30 and 62. The days where dead fish were removed from tanks by netting were also noted. Water quality parameters (nitrite, ammonia, pH and temperature) were monitored daily at 10.00h. An additional husbandry intervention (a 1ppm 8h Cu_2SO_4 treatment for an outbreak of white-spot) was also carried out on day 22.

2.2.7.4.2 Statistical analysis

For RTFI the only method that lacked autocorrelation was a 3-day Moving average. Once residuals were generated, a simple GLM model was used to determine whether deviations from expected feed intake were to abiotic, biotic or management variables.

2.2.7.4.3 Results

Changes in daily feed intake between tanks and days within a tank are shown in Figure 19. Residuals from these lines are shown in Figure 20. Using 3-day moving average residuals from Tank 1 as an example, Figure 21 shows days with husbandry interventions imposed onto the residual plot. GLM analysis revealed no significant predictor of daily deviation from expected feed intake for any tank, irrespective of the length of time lag. This suggests that if rainbow trout are self-fed, they have the opportunity to catch up and compensate for any periods of sub-optimal feed intake.

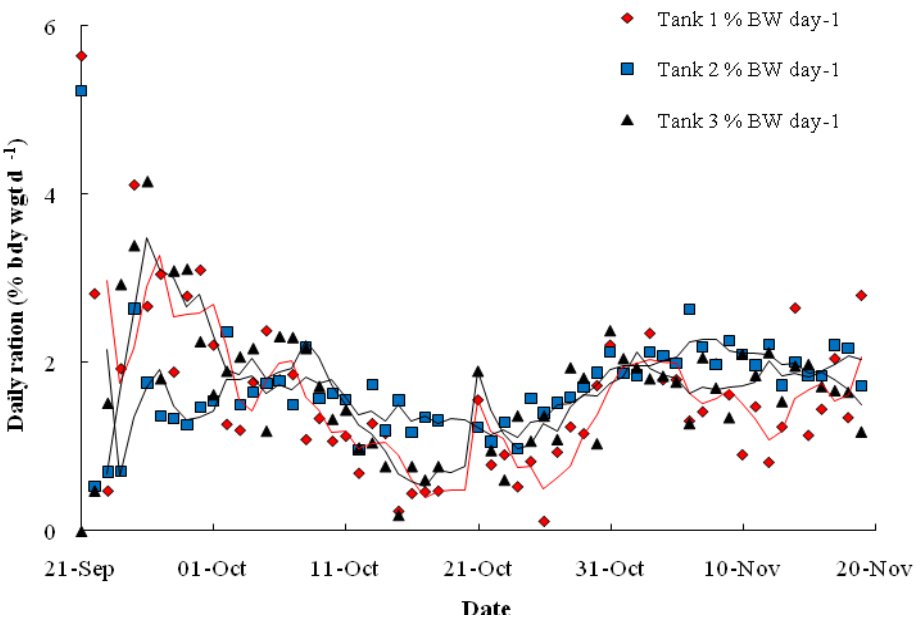

Figure 19: Changes in the daily feed intake of self-fed juvenile tank-held rainbow trout between days and groups. Trendlines represent 3-day moving averages for each tank.

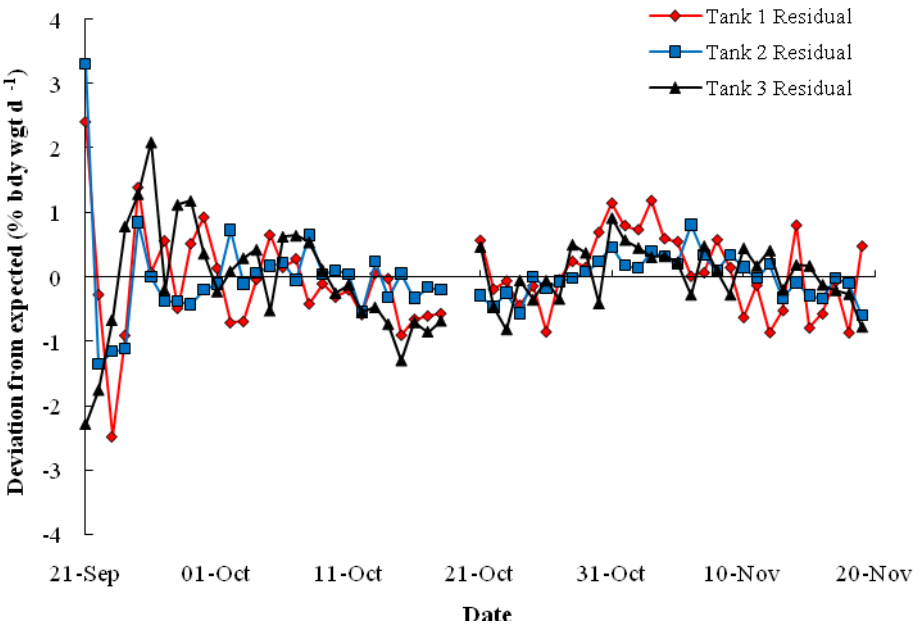

Figure 20: Deviations from expected feed intake for each tank.

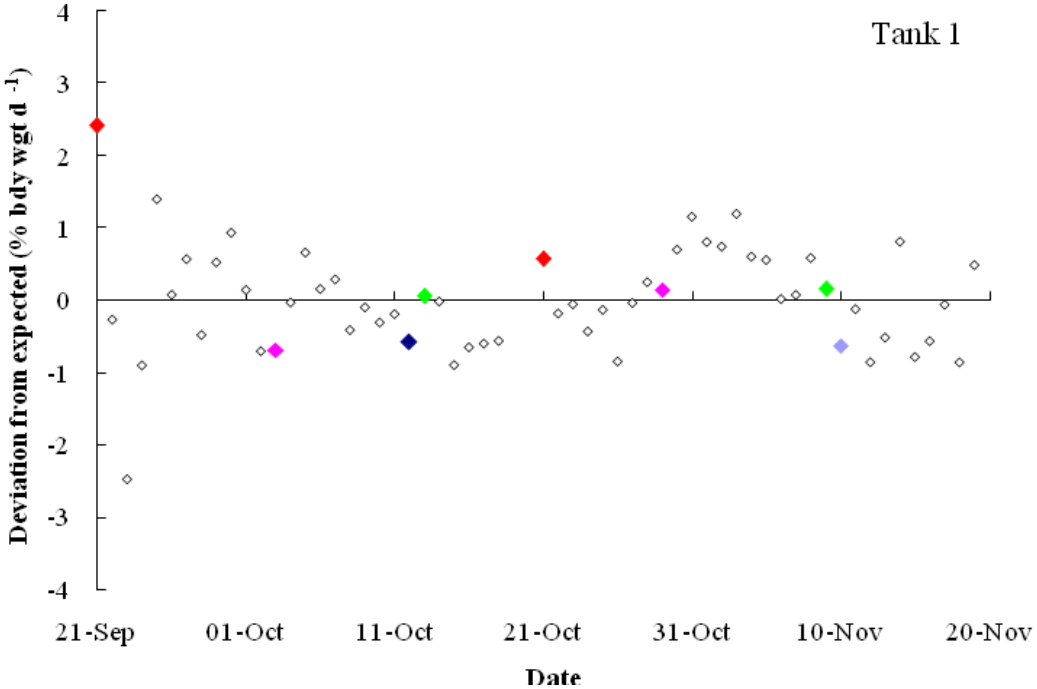

Figure 21: Daily deviations from expected feed intake in relation to water quality and husbandry interventions for Tank 1. Red points indicate sampling events. Pink represents a husbandry intervention e.g. netting of dead fish. Blue represents a disease treatment. Green represents a water quality improvement intervention. Lavender represents a reduced water quality incident.

2.2.7.4.4 Conclusions

In Dataset RTFI neither water quality, disease treatment nor sampling event had any significant effect upon deviations from expected feed intake. Dataset RTFI suggests that when rainbow trout are given the opportunity to

obtain feed from self-feeders, they have the opportunity to quickly recover from any episodes of sub-optimal feed intake, and husbandry interventions have no significant effect upon feed intake over short periods.

2.2.7.5 Cagesalmon

2.2.7.5.1 Dataset

The cages used in this study were 29 metres in diameter and 20 metres deep. Individually moored cages (e.g. not compact type of farm). The farm is located in Northern-Norway, in a relatively narrow sound, with dominating south-west water current direction. Surface water was more wind driven and the north-east direction dominates. The typical water current speed was between 5 and 10 cm sek^{-1}, thus not high but very homogenous the upper 45 metres. A small vertical water current of approximately 2 cm sek^{-1} also contribute to the water exchange. Despite that the location had a peak load of more than 3000 tonnes of salmon during the summer in question; there was no sign of aggregation of waste under the cages (surveyed by independent company). Water temperature declined from 10ºC (August) to7ºC (November), and the oxygen levels in the cages fell from 100% saturation (July) to 85% saturation (August) during the trial. The environmental conditions (water temperature, salinity, oxygen level and water currents) were regarded as good throughout the whole period. The site is regarded as relatively good for cage salmon farming. The fish were divided into two replicate groups (2 cages holding 48000 salmon each). One group was fed according to appetite between 7 am and 4 pm (cage 2 and 4; farm protocol), whereas the other was fed according to appetite during light hours (cage 1 and 3). Appetite was assessed using submerged cameras and the stop signal for feeding was according to the farms protocol in both groups. The fish were size graded and weighed (subsample) at the start of the experiment. The experiment was terminated at slaughter, providing a full record of individual fish sizes (slaughter weight, gutted head on). In addition, subsamples of 20 fish per cage were collected to check for round weight and weight loss during slaughter. Changes in body weight were assessed on a daily basis using biomass estimators. These calculate fish weight based on fish length and volume measurements (Storvik Biomass Estimators). Feed data was collected routinely by the farm operators, and the project had access to their data.

2.2.7.5.2 Statistical analysis

Regression analysis was used integrating dummy variables.

2.2.7.5.3 Results

The results revealed a limited but significant effect of feeding regimes on growth and feed utilisation; fish fed during light hours performed the better (
Figure *22* and Figure 23). During the experiment, two of the cages (3 and 4) showed reduced appetite as compared to the others (1 and 2). This difference crossed the treatments, but as the fish were fed according to appetite and the same stop signals results is still comparable. After one month, the appetite in cage 3 and 4 was reduced and consistently lower as compared to cage 1 and 2, resulting in a significantly lower amount of feed delivered. The pattern of change was similar to what might be expected in fish groups held under sub-optimal farming conditions, development of disease or other conditions compromising welfare. As such, if feed intake was used as a welfare indicator, the reduced appetite should alarm the operators accordingly. However, as the weight increment was measured on a daily basis and there was no sign of any drop in growth, the operators decided not to take any action to these apparently underperforming cages. There was no difference in growth rate between the cages, despite the difference in appetite. The feed conversion was improved in the cages delivered less feed, and based on feed efficiency they outperformed the fish with highest appetite. There was nothing in the data describing the environment and water quality that offer any explanation to this difference. All fish had comparable conditions throughout the trial. Thus, feed intake does not seem to be a good candidate for welfare indicator under these circumstances, as the fish that were thought to under-perform in the end was the fish that performed best according to feed utilisation.

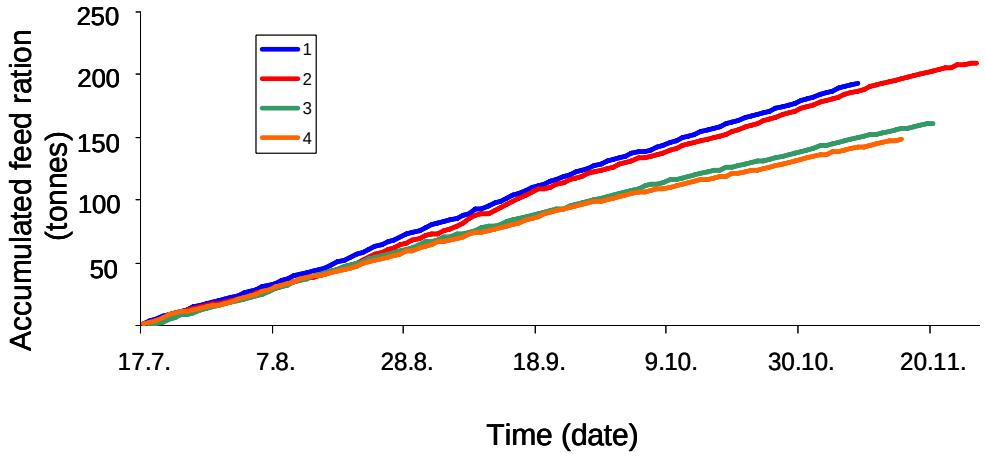

Figure 22: Accumulated feeding rations to salmon held in cages fed according to appetite. Fish in cage 3 and 4 got significant lower feeding rations than fish in cage 1 and 2.

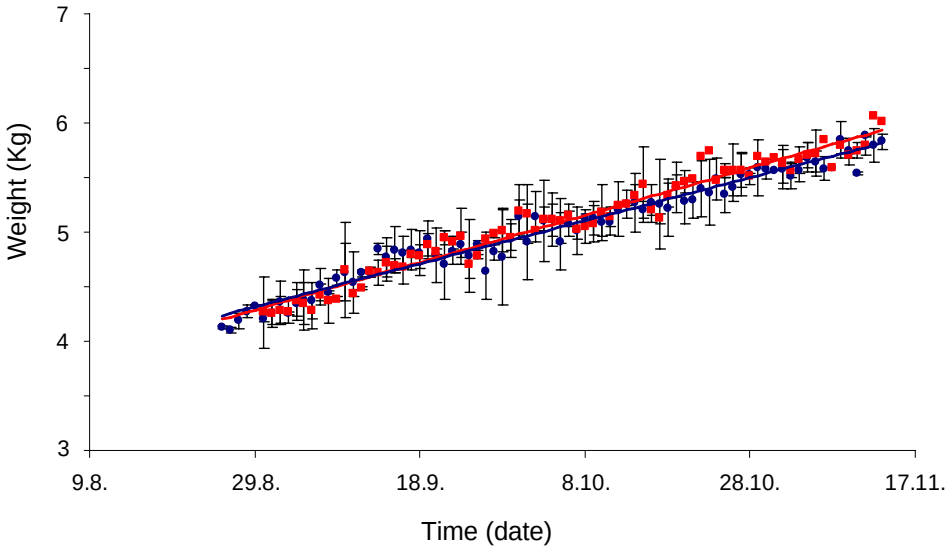

Figure 23: Growth in two groups of salmon held in cages. Although one group, consisting of cage 3 and 4; red), were fed significantly lower accumulated rations as compared to fish in cage 1 and 2 (blue), no difference in growth occurred.

2.2.7.5.4 Conclusions

Use of feed intake as a welfare indicator requires that appetite is correlated to the general well being and status of the fish. There is a general conception amongst fish culturists that this is also the case. The present results demonstrate that feed intake alone may not always be a good indicator, and that if used it should be combined with recording of growth. When based on feed intake alone, cage three and four seemed to under-perform as compared to the other two, when in fact they had at least similar growth rate and therefore were the better performing fish in terms of feed efficiency. The data are from a commercial farm and the trial ran under full scale commercial conditions. This means that accuracy of the data is not as good as under more controlled experimental conditions, but this does not explain the final results. The cages were treated equally in terms of feeding according to appetite, and were fed by operators that focus on feed utilisation and growth. Feed intake does not seem a reliable welfare indicator when used alone. It should be combined with some other measure of

performance, e.g. growth. Fish welfare in this experiment was not compromised, at least not in the group that showed lowest appetite.

3 Discussion

Several datasets have shown that the overall appetite and feed intake of fish can be correlated with abiotic factors such as changes temperature (Fraser et al., 1993); light intensity (Noble et al., 2005); water quality (Thetmeyer et al., 1999, Beamish and Tandler, 1990 and Ang and Petrell, 1997, Person Le Ruyet et al. 2002). Important biotic factors that could be correlated here with feed intake are disease or increased parasite loads (Bloch and Larsen, 1993). Other management variables that might impact upon feed intake (decreased water refreshment rate of the system, handling, disturbance and system cleaning (Strand et al, 2007 and references therein) could not be confirmed as negative for feed intake and fish welfare. The commonly among farmers accepted observations that feed intake can be correlated with fish welfare is therefore supported by this study. However, several datasets were missing there thoughtful evaluations of the welfare status of the fish as such. Impaired fish welfare was assumed indirectly based on literature hypothesis that certain factors affect fish welfare and as well fish feed intake.

4 Conclusion

Fish feed intake and within limits realized feed load can be related to expected feed intake and therefore translated to deviation of expected feed intake. This can within limits be related to fish welfare, when fish welfare data are measured or established to the observed conditions a-priori. Feed intake might therefore serve as operational welfare indicator on fish farms under certain conditions. It has to be remarked that several datasets which are related back to fish welfare do this based on literature data and circumstantial evidence.

5 Literature

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6 Justification

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The scientific quality of this report has been peer reviewed by the a colleague scientist and the head of the department of Wageningen IMARES.

Approved: Henk van der Mheen
Head Department of Aquaculture

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