AQUA EXCEL 2020

AQUAculture infrastructures for EXCELlence in European fish research towards 2020 — AQUAEXCEL2020

Deliverable D5.6

Final model for growth, feed consumption and waste production simulation

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Executive Summary

Objectives:

The objective of the AquaFishDEB model is to capture the effects of feed quality, feeding schedule, and water characteristics on growth, feed consumption and waste production (faecal and non-faecal nitrogen loss, faecal dry matter, CO_2) as well as oxygen consumption for three aquaculture species (Atlantic salmon, gilthead seabream and rainbow trout). This report describes the development and validation of the AquaFishDEB model.

The AquaFishDEB is one of the main components in the AQUAEXCEL^{2020} virtual laboratory, which is developed in WP5: "Virtual laboratories and modelling tools for designing experiments in aquaculture research facilities". The main components of the virtual laboratory are:

- Growth, nutrition and waste production models for different fish species
- Water quality and water treatment modelling
- Modelling of hydrodynamic flow fields in tanks and cages

Rationale:

The AquaFishDEB model is based on the Dynamic Energy Budget (DEB) theory for metabolic organization, a theory that provides the conceptual and quantitative framework to study the whole life cycle of an individual while making explicit use of energy and mass balances (Kooijman, 2010). Its ability to model the bioenergetics of organisms as a function of temperature and food quantity and quality throughout their life cycle renders DEB appropriate for studying fish metabolism in aquaculture. We used this framework to develop a model explicitly tied with feed (quantity and quality) and temperature that can accommodate different feeding strategies (e.g., *ad libitum* or restricted, feeding frequency, adaptive feeding) and feed compositions.

Main Results:

The AquaFishDEB model was developed and validated against data from literature and AQUAEXCEL^{2020} partners for the three species. The AquaFishDEB model is the end product of a two-step modeling procedure. The first step involves the development and parameterisation of the DEB model that describe the dynamics of an individual fish of a given species. In the second step, the DEB parameters obtained from the first step feed the AquaFishDEB model that simulates the dynamics for a group of fish exposed to specified rearing conditions. The inputs of the model include physicochemical parameters of the tank water (temperature, oxygen, salinity, and pH) as well as feeding schedule, feed composition and group characteristics. Temperature affects the physiological rates, while oxygen concentration, salinity and pH act as red flags when their values fall outside a pre-specified range. The model output includes growth (e.g., weight-at-time) and feeding characteristics (e.g., feed intake, feed conversion ratio) as well as waste production (faecal and non-faecal nitrogenous loss) and gaseous exchange (O_2 consumption and CO_2 production).

Parameterization of the DEB models resulted in acceptable goodness of fit (symmetric mean squared errors ranging from 0.11 to 0.21, for all species). Validation using data on growth, O_2 consumption, CO_2 production, and nitrogenous excretions showed that the AquaFishDEB model performed well and was able to capture the diverse nature of the outputs of the validation datasets. Particularly for growth, the level of agreement between predictions and observations was very high across species, while deviations were higher for gaseous exchange and nitrogenous waste. Finally, the sensitivity of the model outputs on changes of the input parameters was performed while the limitations of the model and its interconnection with the water treatment model were also discussed.

Authors/Teams involved:

The authors of this Deliverable are from the HCMR team (Konstadia Lika, Orestis Stavrakidis-Zachou, Nikos Papandroulakis). The NOFIMA group contributed with providing the data for Atlantic salmon, the INRA group with providing growth data for rainbow trout, and the WU team with providing nutrition data for rainbow trout and advices for the nutrient utilization module of the model.

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1 Background

This document is part of the AQUAEXCEL2020, WP5/Joint Research Activity 1 – Virtual laboratories and modelling tools for designing experiments in aquaculture research facilities.

One of the main research activities in AQUAEXCEL2020 is to develop a virtual laboratory system that enables virtual experiments in aquaculture research facilities. This system features a framework (see Bjørnson et al., 2019) that allows the integration of mathematical models of different subsystems in common simulations, replicating the system operation of research laboratories. The overall system architecture is shown in **Erreur ! Source du renvoi introuvable.**.

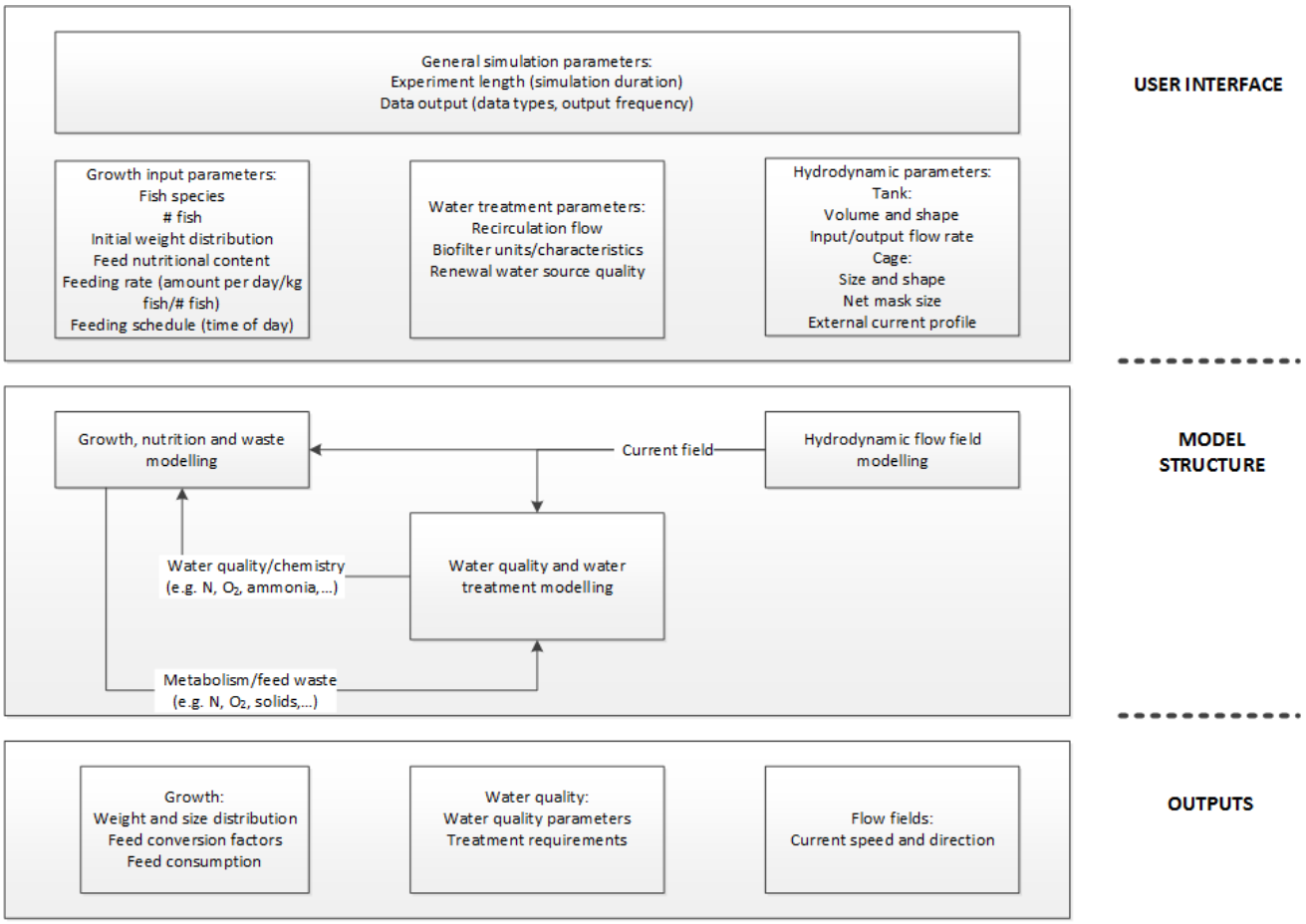

Figure 1: Virtual laboratory system architecture.

This document describes the modelling framework that is used to develop the AquaFishDEB model that captures the effects of feed quality, feeding schedule and water characteristics on individual growth, feed consumption, waste production (faecal and non-faecal nitrogen loss, faecal dry matter, CO_2) as well as oxygen consumption for different species. The model was developed and validated for three species (Atlantic salmon, gilthead sea bream and rainbow trout).

The AquaFishDEB model is based on the Dynamic Energy Budget theory (DEB), a qualitative and quantitative framework to study individual metabolism throughout the entire life cycle of an organism making explicit use of energy and mass balances (Kooijman, 2010). Its ability to model the bioenergetics of organisms as a function of temperature and food quantity and quality throughout their life cycle has established the DEB theory as a widely applicable approach to study fish metabolism on both wild populations and farmed fish (e.g., Pecquerie *et al.*, 2009; Serpa *et al.*, 2013; Fore *et al.*, 2016). DEB theory describes the interconnections

among the processes of assimilation, maintenance, development, growth and reproduction of an organism throughout all stages of its life cycle, and in a dynamic environment.

This document describes the final version of the AquaFishDEB model. It builds on the report D5.2 (Lika et al., 2018) which described the prototype growth model. For completeness, this report repeats the description of the DEB model, on which the AquaFishDEB is based (Tables A1 and A2) and the input/outputs of the model (section 2.2). This report adds the description of the new module (section A2), which models the assimilation-digestion of food, the methodology to estimate and calculate parameters (sections 2.1 and A.3), and discussion of the integration with the other components of the Virtual Laboratory (section 2.3). Additionally, the parameterization of the AquaFishDEB model for the three species is given (sections 3.1 and A4), as well as the performance (section 3.2), the validation (section 3.3) and the sensitivity (section 3.4) of the model. In Appendix A5 we demonstrate the configuration and use of AquaFishDEB model in the Virtual Laboratory.

2 Model description

The AquaFishDEB model is the end product of a two-step modeling procedure (Figure 2). The first step involves the development and parameterisation of the DEB model that describe the dynamics of an individual fish of a given species. In the second step, the DEB parameters obtained from the first step feed the AquaFishDEB model that simulates the dynamics for a group of fish exposed to specified rearing conditions. The model output includes growth (e.g., weight-at-time), feeding characteristics (e.g., feed intake, feed conversion ratio) as well as waste production (faecal and non-faecal nitrogenous loss) and gaseous exchange (O_2 consumption and CO_2 production).

Figure 2 (top) shows the main metabolic processes as defined by the DEB theory. An individual fish converts food to reserves (a process called assimilation) and allocates mobilized reserve to somatic and maturity maintenance, growth (i.e., increase in structural body mass) and maturation/reproduction. Food uptake depends on food availability and fish size. Food uptake is converted into reserves with a constant efficiency, which is specific to feed quality. A fixed fraction κ of the mobilized energy is used for somatic functions, such as somatic maintenance and growth, while the remaining $1\text{-}\kappa$ fraction is allocated to maturation/reproduction, after subtraction of maturity maintenance costs.

The AquaFishDEB model is an extension of the standard DEB model and assumes three life stages (larvae, juvenile and adult) as well as metabolic accelerated development for early stages which is an established practice for studying fish species in the DEB context (Lika *et al.,* 2014; Kooijman, 2014). The most important transitions include birth, which is marked by the start of exogenous feeding, metamorphosis as the completeness of metamorphosis, and puberty, denoted by developmental completeness and the start of allocation to reproduction. This approach allows following individual fish metabolism through all the stages that are relevant for aquaculture, which may not completely overlap with the aforementioned DEB life stages. For instance, the on-growing stage of production usually contains fish that transition from the juvenile to adult stages before reaching harvest size. Life stage transitions occur when the cumulative investment into maturation reaches certain thresholds.

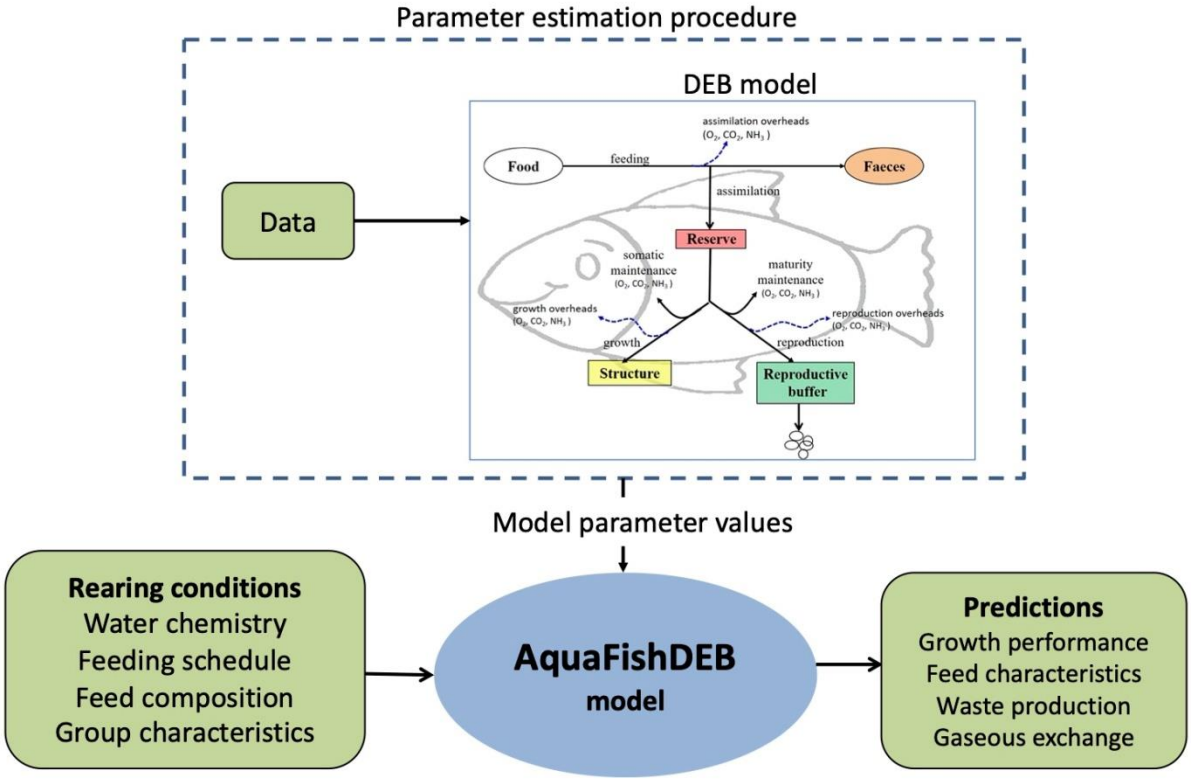

Figure 2: Schematic representation of the two-step procedure of the AquaFishDEB model. The top describes the main metabolic processes as defined by the DEB theory. Boxes represent state variables and arrows energy fluxes. DEB parameter values feed the AquaFishDEB.

The state of an individual fish is described by four variables: volume of structural mass V, energy reserve E, energy invested to maturation E_H, and energy invested to reproduction E_R (for adults). The state variables, the energy fluxes and the dynamics of an individual fish are summarized in Table A1 in the Appendix A1. For a more comprehensive description of the DEB theory and a full list of the equations and the nomenclature used see Kooijman (2010) and Stavrakidis-Zachou (2019). A digestion-assimilation module is incorporated in the AquaFishDEB to model the food, M_X, dynamics in the gut and the process of assimilation of the food substrates from the gut wall. The derivation of the equations is given in Appendix A2.

One of the core assumptions of DEB theory is that all organic compounds, food, faeces, structural mass and reserves (the latter two comprise the individual biomass), consist of a mixture of polymers such as proteins, lipids and carbohydrates which form generalized compounds of constant chemical composition. The composition of a generalized compound is expressed as the relative abundance of hydrogen (H), oxygen (O) and nitrogen (N) to carbon (C). Thus, for example, a molecule of reserve has the formula $CH_{n_{HE}}O_{n_{OE}}N_{n_{NE}}$, where n_{*E} are the chemical indices, e.g. n_{NE} represents the molar N:C ratio of reserve. Each generalized compound has specified chemical potential ($\mu*$), specific density ($d*$), and molecular weight ($w*$). Therefore, identification of the chemical indices of the mineral and organic compounds found in the food, the structure, and the reserves, allows for the quantitative and qualitative assessment of metabolic waste output under various experimental scenarios. All DEB models for animals assume one food, one reserve and one structure. In this work, we extend the model to include “two types” of food of different composition. In particular, we separate protein from lipids and carbohydrates by assuming that a fraction of food is protein and the remaining are lipids and carbohydrates. Appendix A3 gives the derivation of the chemical indices for organic compounds.

The abstract state variables of reserves and structure can be linked to commonly measured quantities, which in our case are: weight-at-time, feed intake rate, feed conversion ratio, faeces production, faecal and non-faecal nitrogenous loss, O_2 consumption and CO_2 production. Mass fluxes of organic (food, faeces, reserves and structure) and mineral (O_2, CO_2, nitrogenous waste) compounds can be written as weighted sum of three basic fluxes: assimilation ($\dot{p}_A$), growth ($\dot{p}_G$), and dissipation ($\dot{p}_D$) (metabolic work that converts reserve into mineral products in ways that do not lead to the production of new biological material) (Kooijman, 2010, Stavrakidis-Zachou, 2019). Model equations that produce the output are presented in Table A2 of Appendix A1. All model parameters are described in Table A3 of Appendix A1.

2.1 Parameter estimation

The DEB parameters can be either estimated as described in Marques *et al.* (2019) and Stavrakidis-Zachou *et al*. (2019), using the freely downloadable DEBtool software (http://www.bio.vu.nl/thb/deb/deblab/) and a number of zero- and uni-variate data sets, or be retrieved from the AmP collection (AmP2019).

For gilthead sea bream and Atlantic salmon, parameters were estimated using data obtained from the literature and from partners of the AQUAEXCEL^{2020} project. For rainbow trout, the parameter values were retrieved from AmP *Oncorhynchus mykiss*, version 2017/10/30 (bio.vu.nl/thb/deb/deblab/add_my_pet/entries_web/Oncorhynchus_mykiss/Oncorhynchus_mykiss_res.html). The add-on module for digestion was calibrated using additional nutritional data.

2.2 Input–output for the AquaFishDEB model

The inputs and outputs for the model are summarized in Tables 1 and 2. The inputs of the model include the name of the farmed species (Species name) and the physicochemical parameters of the tank water (Water chemistry); namely, temperature, oxygen, salinity, and pH. Temperature affects the rates of the model (see Appendix A1, eq. A1), while oxygen concentration, salinity and pH act as red flags when their values fall outside a pre-specified range.

Fish size is given as the average initial wet weight (g) at the start of the experiment for a desired initial number of fish (Fish group size). Mortality refers to the percentage of the initial fish group size that was lost by the end of the experimental period (d). Feeding level can be *ad libitum*, referring to the maximum feed intake, or restricted, given as the amount of food equal to the percentage of initial body weight (BW). Restricted feeding allows for the adaptation of the % BW d^{-1} through intermediate weighing at an interval defined by the user. Food composition is given in grams of crude protein (P), crude fat (F), crude ash (A), and Nitrogen Free Extract (NFE) per kg of feed dry weight, and dry matter (DM) as g kg^{-1} of feed fresh weight, while the apparent digestibility is given as % of the DM (or nutrient) retained by the fish after faecal loss has been accounted for. Alternatively, default feeds, which are the recommended Food and Agriculture Organization standard feeds based on the production stage, can be used.

Table 1: Summary of inputs for the AquaFishDEB model

Description	Parameter	Units
Species name	Atlantic salmon	-
	Gilthead seabream	-
	Rainbow trout	-
Water Chemistry	Water temperature	^oC
	Salinity	psu
	Dissolved oxygen	mg l^{-1}
	pH	-

Fish size	Initial wet weight	g
Fish group size	Initial number of fish	#
	Mortality	%
Experimental period	Time	d
Feeding level	Ad libitum	-
	Restricted	% BW d^{-1}
	Restricted (adapted feeding)	% BW d^{-1}
Feeding frequency	Number of meals	# d^{-1}
	Time interval between meals	h
Feed composition	Dry matter (DM)	g (kg feed DM)$^{-1}$
	Crude protein (P)	g (kg feed DM)$^{-1}$
	Crude fat (F)	g (kg feed DM)$^{-1}$
	Crude ash (A)	g (kg feed DM)$^{-1}$
	Nitrogen free extract (NFE)	g (kg feed DM)$^{-1}$
Apparent nutrient digestibility	Dry matter (DM)	%
	Crude protein (P)	%
	Crude fat (F)	%
	Crude ash (A)	%
	Nitrogen free extract (NFE)	%

The outputs of the model include information on growth performance, waste production and gaseous exchange. The number of fish and the total fish biomass are predicted as functions of time, taking into account the input mortality rate. The feed conversion efficiency (FCR), total feed intake as well as waste production and gaseous exchange are also given as functions of time. Faecal dry matter and faecal loss-N are given in g per kg of feed DM and the total waste production as feacal and non faecal loss-N in g N h^{-1}. O_2 consumption and CO_2 production are predicted for the total fish biomass as well as per kg of fish hourly.

Table 2: Summary of outputs for the AquaFishDEB model

Description	Parameter	Units
Growth	Number of fish	#
	Body size	g $fish^{-1}$
	Biomass	g
	Feed intake	g d^{-1}
	Feed conversion ratio	-
Waste production	Faecal dry matter	g h^{-1}
	Faecal loss-N	g N h^{-1}
	Non faecal loss-N (TAN)	g N h^{-1}
Gaseous exchange	Oxygen consumption	g h^{-1}
	Oxygen consumption	mg kg^{-1} h^{-1}
	Carbon dioxide production	g h^{-1}
	Carbon dioxide production	mg kg^{-1} h^{-1}

2.3 Integration

The AquaFishDEB model can be used either as a stand-alone model or integrated with the water treatment model as a component of the virtual lab. The AquaFishDEB and the water treatment models are linked bidirectionally in such a way that the waste and gaseous outputs (described in Table 2 under the 'waste production' and 'gaseous exchange' categories) predicted by AquaFishDEB are fed as input into the water treatment model for further calculations. Feedback from the water treatment model regarding the dissolved oxygen concentration in the tank can also be used as input to AquaFishDEB. Values of oxygen concentration that fall outside the predefined for the species range will act as warnings for unsuitable rearing conditions for fish growth. The AquaFishDEB model could also be linked with the flow model in different ways. Increase of the current velocity may affect feeding as well as metabolic costs. These effects could indirectly be modeled by decreasing food availability (e.g., decrease the parameter k_X, see Tables A2 and A3) and increasing maintenance cost (e.g., increase the parameter $[\dot{p}_M]$, see Tables A1 and A3).

3 Results

3.1 DEB parameters

Comparison of the DEB model predictions with observed data are presented in Tables A5-A6 and Figures A2-A3 in the Appendix A4 for Atlantic salmon and gilthead seabream and in AmP *Oncorhynchus mykiss* (v. 2017/10/30) for rainbow trout. The parameter estimation resulted in an acceptable goodness of fit, quantified by the mean relative error (MRE) (0.157 for Atlantic salmon, 0.197 for gilthead seabream and 0.101 for rainbow trout), giving an overall good match between predictions and observations for all species. Notice that MRE takes values in the interval [0, ∞); values close to 0 mean that the model predictions are close to the data.

In order to estimate the specific parameters of the digestion-assimilation module, additional data were use such as those relating to the gastric evacuation time for each species. The data and the associated predictions are given in Figure A4 in the Appendix A4.

All AquaFishDEB parameter values for the Atlantic salmon, gilthead seabream and rainbow trout are given in Table 3. Detailed description of these parameters can be found in are given in Table A3 (Appendix).

Table 3: AquaFishDEB parameter values

Symbol	Units	Atlantic salmon	Gilthead seabream	Rainbow trout
$\{\dot{p}_{A_m}\}$ (*)	J/cm^2.d	212.3	30.98	2511.6
$\dot{v}$ (*)	cm/d	0.0307	0.0846	0.0325
κ (*)	-	0.9160	0.9617	0.6192
$\kappa_{X_P}, \kappa_{X_{nP}}, \kappa_{X_{NFE}}$ (**)	-			
κ_X (**)	-			
κ_P (**)	-			
κ_R (***)	-	0.95	0.95	0.95
$[\dot{p}_M]$ (*)	J/cm^3.d	70.23	21.78	343.9
$[E_G]$ (*)	J/cm^3	5230	50.97.3	52.67
E_H^b, E_H^j, E_H^p (*)	J	18.2, 2.3 10^4,	5 10^{-2}, 3.1 10^2,	43.3, 854.1,

		$3.8\ 10^5$	$1.1\ 10^5$	$3.9\ 10^6$
$\dot{k}_J$ (***)	1/d	0.002	0.002	0.002
T_A (*)	K	6617	8000	8000
δ_g (****)	-	0.1		1.9
$\{\dot{J}_{Xg_m}\}$. (****)	1/cm^2.d	$1.6\ 10^{-3}$		$1.5\ 10^{-2}$
$\mu_X^{\max}$ (****)	kJ/mol	684	650	650

(*) Parameter values are estimated using the AmP procedure (Marques et al., 2019)

(**) Parameter values are calculated based on food composition

(***) Parameter values are fixed

(****) Parameter values are tuned to match experimental data

3.2 Model outputs

Using the Atlantic salmon as an example, we present the model performance. Figures 3 and 4 illustrate some of the main model outputs for a given set of inputs. The growth and feeding characteristics are daily outputs, while the waste production and gas exchange hourly. The hourly output shows also the fluctuations due to feeding schedule.

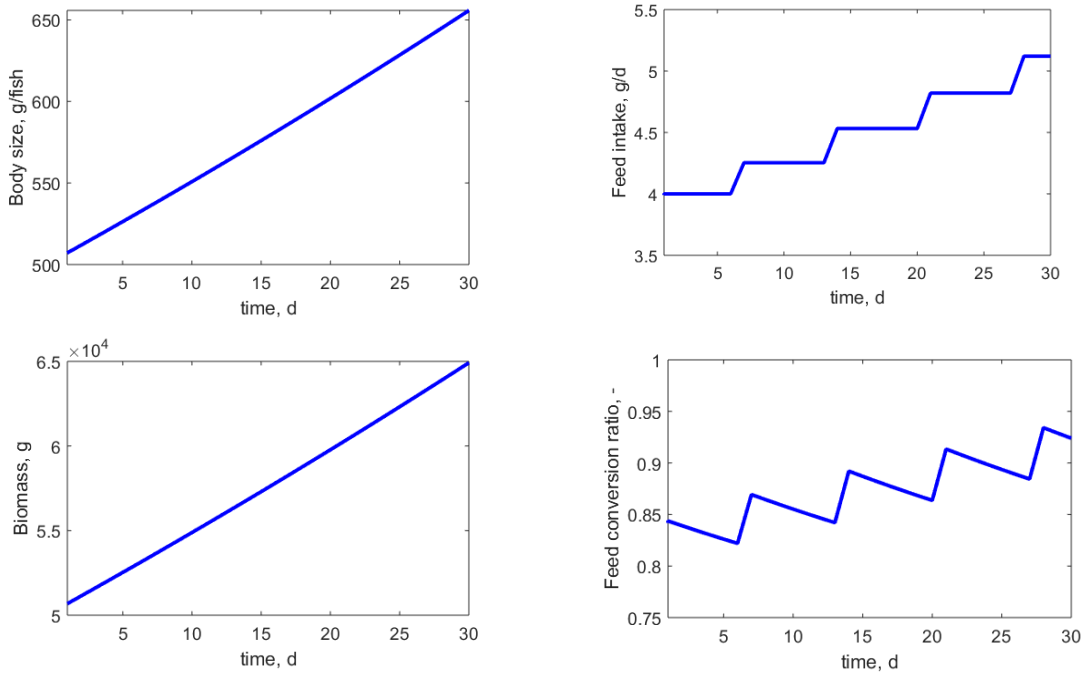

Figure 3: Growth performance of Atlantic salmon for a given set of inputs (temperature: 9oC; feeding type: adapted (every 7 days); feeding level: 1% BW, two meals per day; initial population: 100 fish; feed composition: 45% protein, 20% fats, 10% ash, 15% NFE, 10% moisture). Left: body size and total biomass; Right: individual feed intake and FCR.

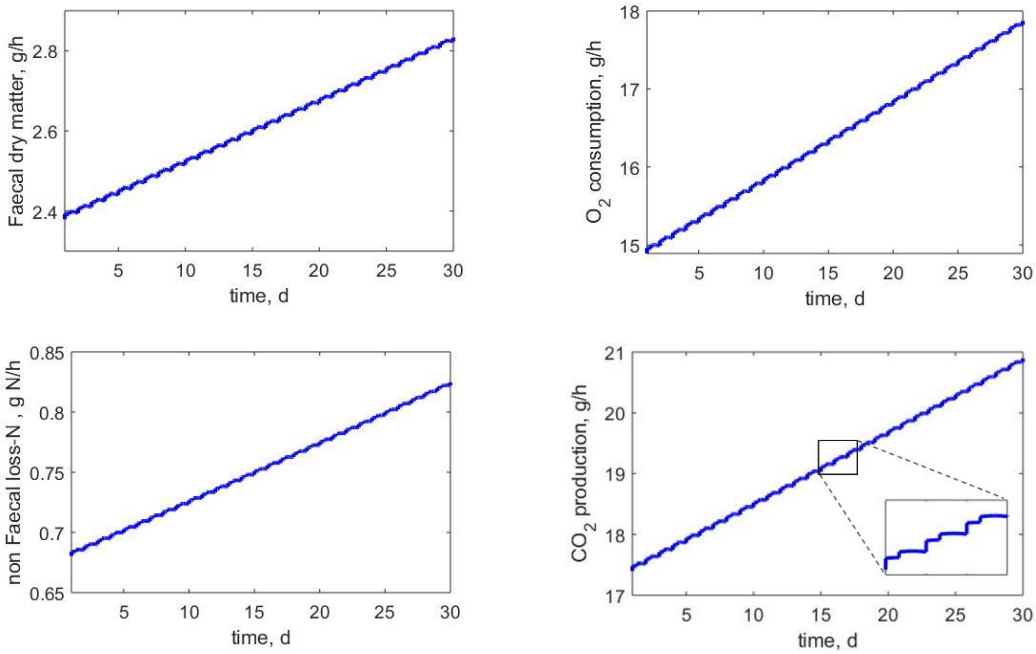

Figure 4: Waste production and gaseous exchange for Atlantic salmon for a given set of inputs (temperature: 9oC; feeding type: adapted (every 7 days); feeding level: 1% BW, 2 meals per day; initial population: 100 fish; feed composition: 45% protein, 20% fats, 10% ash, 15% NFE, 10% moisture). Left: total solids production and nitrogenous excretion rates; Right: total oxygen consumption and carbon dioxide production rates.

Additionally, Figure 5 shows some of the model capabilities by capturing the effects of different rearing conditions on model outputs. Growth is strongly linked with the amount of feed consumed (Figure 5, left column) where a higher ration results in higher weight gain. Moreover, the effect of different feeding frequency on oxygen consumption and that of diet composition on the production of nitrogenous waste are shown in Figure 5 (right column). The examples show how a diet rich in protein also translates in high production of Total Ammonia Nitrogen while an increase in feeding frequency (two meals per day instead of one) can result in lower daily fluctuations of gas exchanges.

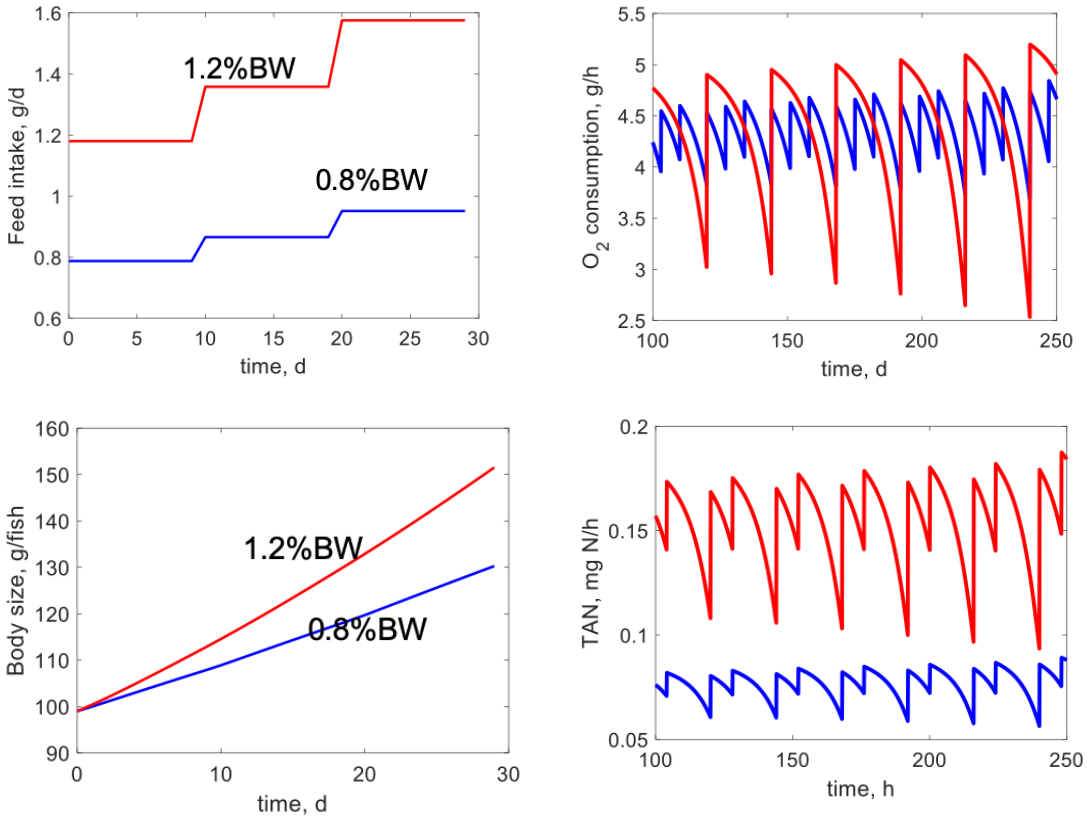

Figure 5: Feed intake and body size (left column) for restricted and adapted feeding at different feed levels. Oxygen consumption (upper right) at different feeding frequencies and total ammonia nitrogen excretion (bottom right) for different feed composition (red line more protein).

Furthermore, on the capabilities of the model, an emerging property of the model is that it captures the effects of food composition on assimilation, which in essence translates to the effects of protein-energy (PE) ratio in the diet. As seen in Figure 6, plotting assimilation rate as a function of the protein fraction in the food, results in a graph resembling an inverted parabola. Food either low or high in protein, results in low assimilation rate. Moreover, the shape of the curve is influenced by the non-protein component of the food, which means that for a fixed fraction of protein assimilation depends on the fat and carbohydrate contents (which determine energy content). It therefore follows, that for given ratio of fats and carbohydrates, there exists a specific protein fraction where assimilation is maximized.

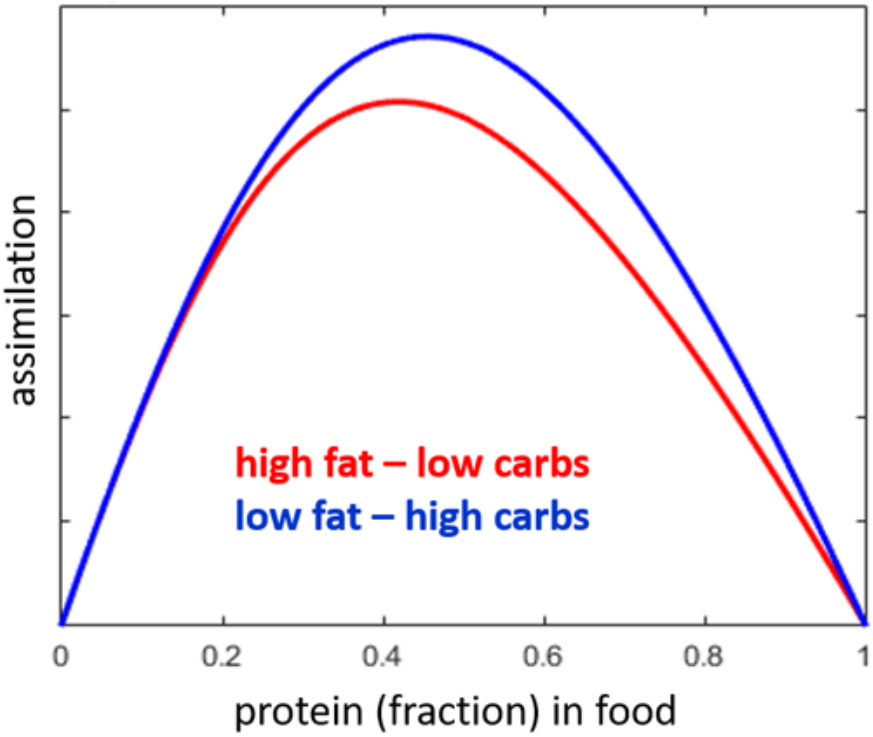

Figure 6: Assimilation as a function of the fraction of protein in the food. Colours indicate different fat and carbohydrate contents for the non-protein component of the food

3.3 Model validation

Model validation was performed via comparison of the model predictions to data obtained from the literature. For each species, data on weight, oxygen consumption, carbon dioxide production and total ammonia nitrogen (TAN) excretion were used. The datasets included different fish sizes, temperatures, and diets that differed in quantity as well as quality. For each dataset, a simulation was run using as input the rearing conditions (temperature, trial duration, initial size, feed composition, ration size, and feeding schedule) of the respective study. Then, for each species model predictions were plotted against the actual measurements (Figures 7-9), while they were also grouped according to the type of data (growth, gaseous exchange, TAN excretion). In each graph, measures of error were computed, namely the Mean Relative Error (MRE) and the Symmetric Mean Relative Error (SMRE) as described in Shcherbakov *et al.* (2013). Moreover, the line of equality (y=x) was inserted for visualizing complete agreement between model predictions and observations. The distance of points from the line signifies deviations of predictions to observations.

Generally, such deviations were low for all species (Figures 7,8,9) as supported by low values of MRE and SMRE, which did not exceed 0.69 and 0.36, respectively. The model performed reasonably well and was able to capture the diverse nature of the inputs of the validation datasets. Particularly for growth (Figures 7,8,9, top left), the level of agreement between predictions and observations was very high across species with Atlantic salmon exhibiting the lowest MRE (0.09) and SMRE (0.05) followed by rainbow trout (MRE=0.17, SMRE=0.09) and seabream (MRE=0.45, SMRE=0.36). Deviations were higher for gaseous exchange and nitrogenous waste with MRE ranging between 0.48-0.69 and SMRE between 0.18-0.36, but points were generally scattered across both sides of the agreement line showing no particular bias for over- or under-estimation. However, for Atlantic salmon, the model tended to overestimate oxygen consumption when in cases where fish were of small size and/or feeding

was provided *ad libitum* (Figures 7). This bias was not exhibited at low feeding levels or at intermediate and large fish sizes.

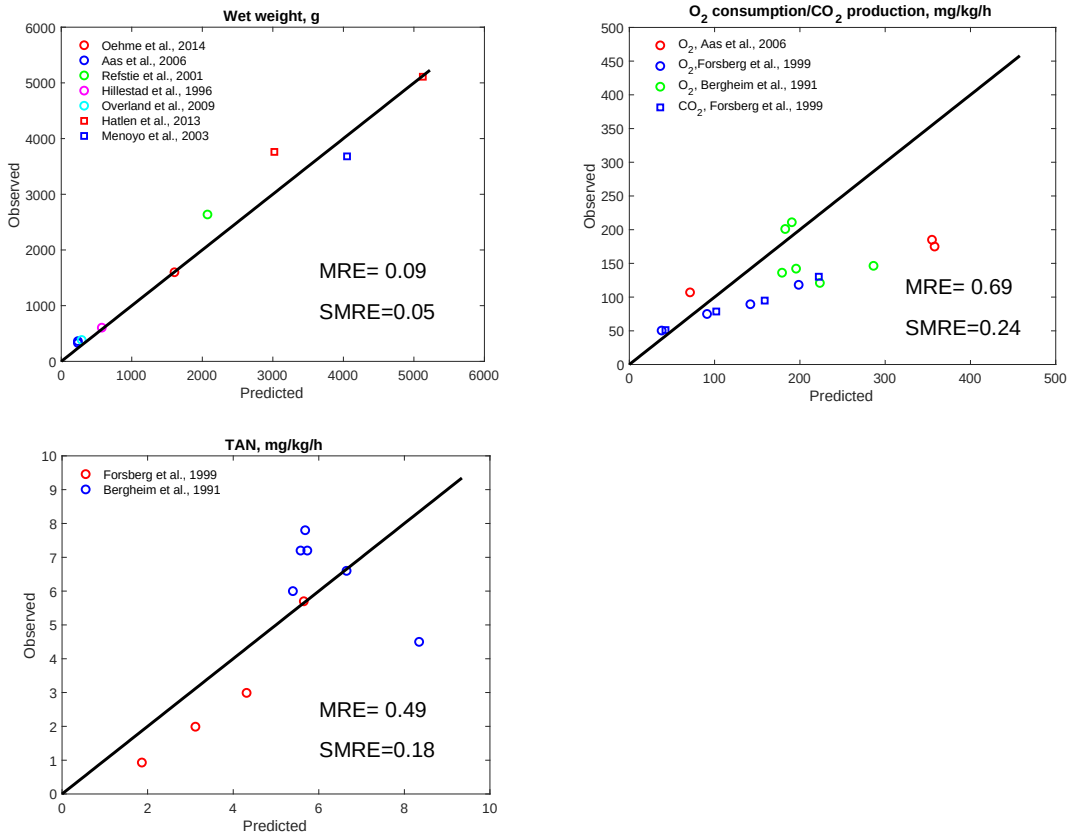

Figure 7: Model validation for Atlantic salmon. Observations vs. predictions, with line of equality.

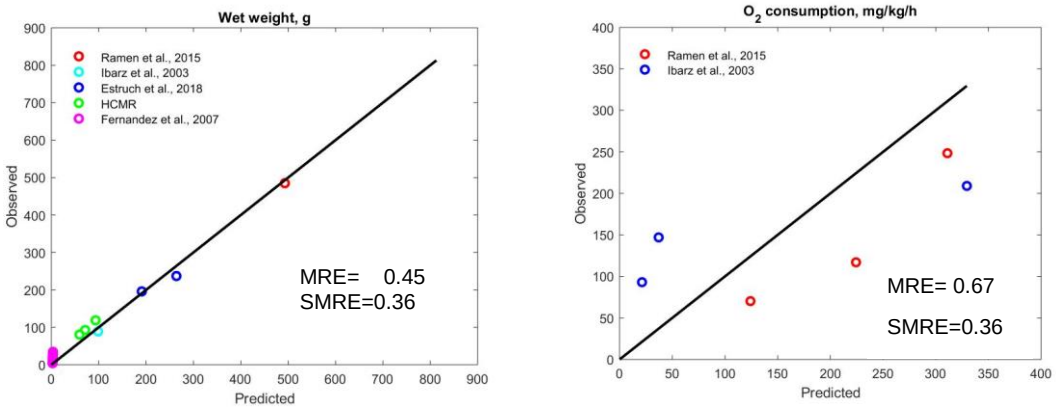

Figure 8: Model validation for gilthead seabream. Observations vs. predictions, with line of equality.

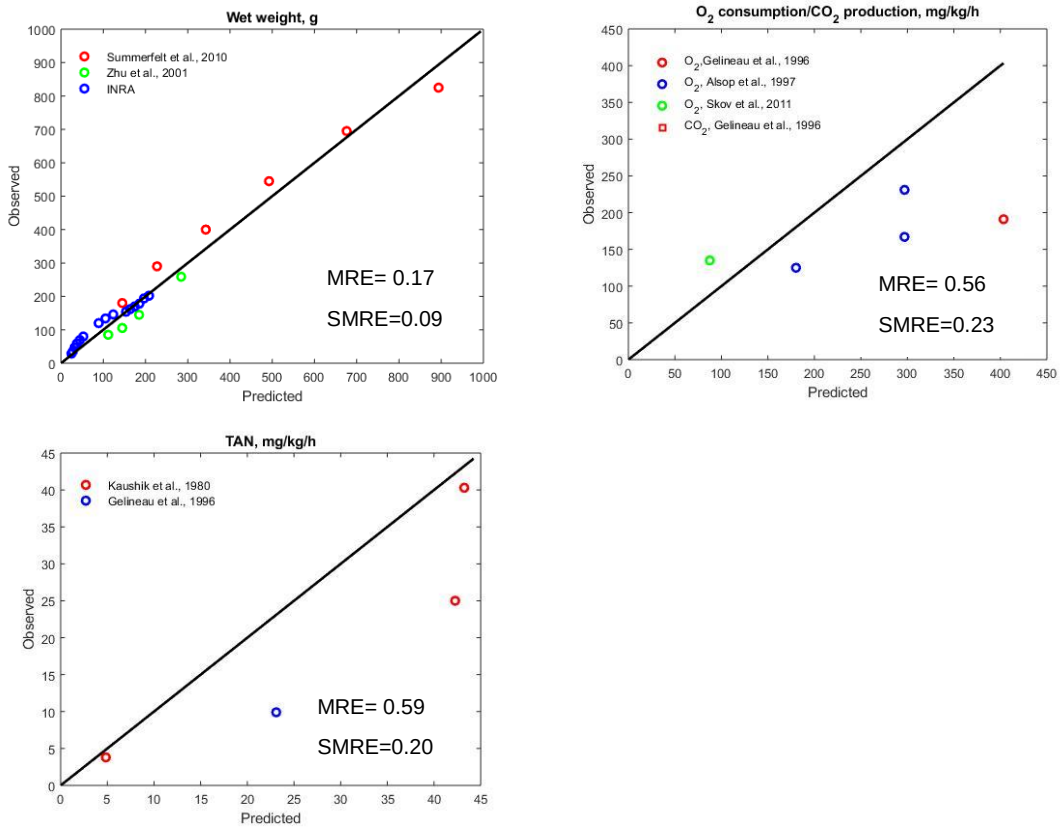

Figure 9: Model validation for rainbow trout. Observations vs. predictions, with line of equality.

3.4 Sensitivity analysis

Sensitivity analysis was performed to investigate the dependency of the various model outputs to model inputs. Key input parameters (e.g., temperature, feed ration, feed composition and feeding schedule) were modified by +10% and the effects on each output was quantified by the sensitivity index (SI), computed as the mean relative deviation of each simulation (X^1) from the reference (X^0):

$$SI = \frac{1}{n}\sum_{i=1}^{n}\frac{X_i^1 - X_i^0}{X_i^0}\ 100 \quad (1)$$

where n represents the number of simulation days. Negative values indicate that an increase in the input parameter causes a decrease in the output. Because the same tendencies were observed for all species, we here use Atlantic salmon as example. Figure 10 shows the mean relative change of the output over the course of a short experiment (10 days) for a 10% increase in key inputs. We observe that temperature changes affect the total ammonia nitrogen, oxygen consumption, faecal dry matter, and FCR, but to a lesser extent feed intake and growth. Moreover, FCR with waste production and oxygen consumption show an opposite dependency (Figure 10, a). In addition, waste production and oxygen consumption have a similar dependence on feed ration change (Figure 10, b). Regarding the composition of the diet, as expected TAN was the most sensitive output to changes in the protein content, while solids and oxygen consumption showed intermediate dependency (Figure 10, c). Finally, we observed that the outputs are not sensitive to the increase of the daily meal number (from 1 to 2); the sensitivity will slightly increase for high feed rations (Figure 10, d).

This is by no means an exhaustive sensitivity analysis, but it gives an indication of the sensitivity of the model outputs to changes in model inputs. Different combinations of fish size and rearing conditions may affect the value of the sensitivity index, but we expect not to alter considerably the general trends.

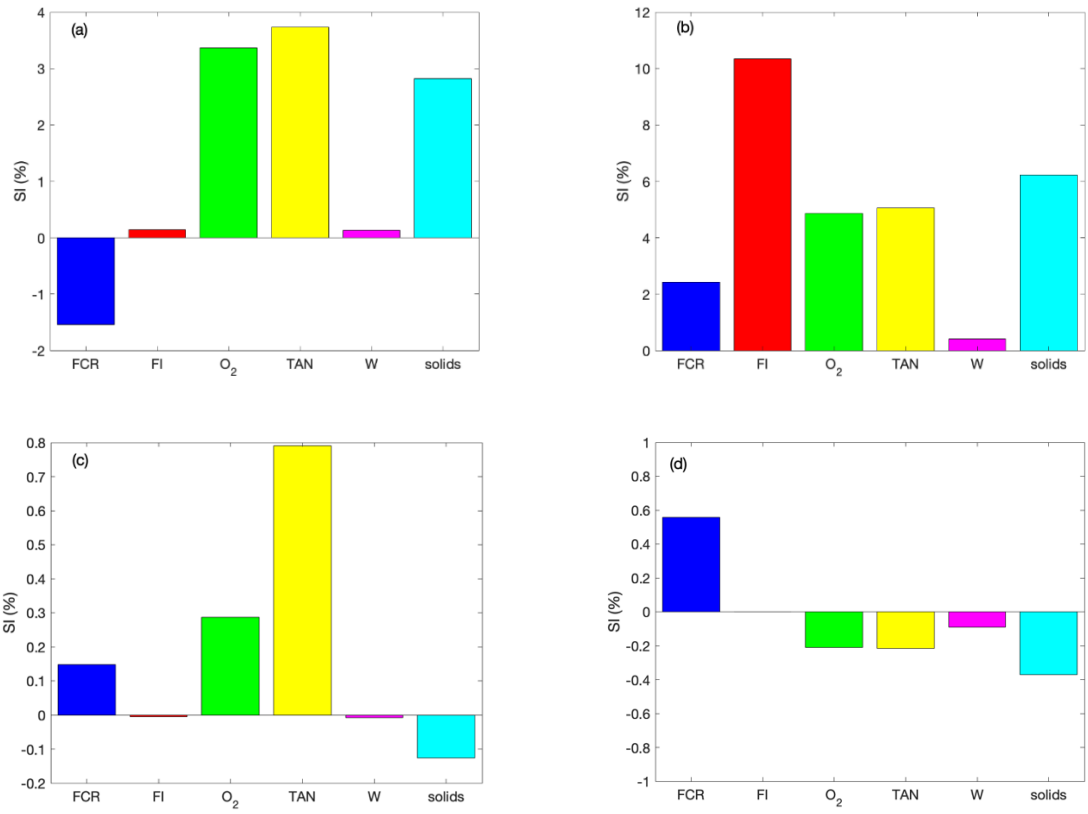

Figure 10: The sensitivity index (SI), computed as the mean, over the course of a 10-day experiment, relative deviation of each simulation from a reference value (eq. 1), for a 10% increase in (a) temperature, (b) feeding, (c) feed protein and (d) daily meal number (from 1 to 2). Bars denote feed conversion ratio (FCR), feed intake (FI), oxygen consumption (O2), total ammonia nitrogen (TAN), weight (W), and faecal dry matter (solids).

4 Discussion and Conclusions

We here describe the development and functionality of the AquaFishDEB model, which is one of the main components of the AQUAEXCEL2020 virtual laboratory. The model has been parameterised and then validated against data obtained from literature. It captures the effects of the considered variables with acceptable accuracy and generates the model outputs described in the Grant Agreement 652831 AQUAEXCEL2020 relating to growth, feed consumption, waste production and gaseous exchange.

That being said, it is important to document the limitations of the model to ensure its correct use and avoid misinterpretation of the results. As stated in the model description, temperature affects the rates of the model, while oxygen concentration, salinity and pH act as red flags when their values fall outside a pre-specified range. This approach was followed due to the considerable knowledge gaps that relate these parameters with fish performance and the low availability of relevant data. Therefore, the applicability of the model is limited to a set of rearing conditions that the species normally encounter which renders it unsuitable for simulating hypoxic or ocean acidification scenarios. Even with respect to temperature, while literature is ample for the typical species-specific temperature range, information on the biological responses on the edges of this range is scarce. Thus, the model functions well for the species-specific typical temperature range but may exhibit deviations from experimental observations close to its edges. Another consideration to take into account is that although the model is developed to capture all life stages, data for parameterization cover larval and juvenile life stages, validations, however, were mainly performed for the production stage where data was mostly available. Consequently, the model can capture all life stages but may be less accurate for larval stages or for large reproducing individuals. Moreover, spawning events are not included in AquaFishDEB; a feature that would require the development of an extra

reproduction module to account for species-specific reproductive traits. Finally, it is important to note that feed spill is also not included in the model. The model assumes that all the provided feed is consumed by the fish as long as the maximum capacity M_{gm} has not been reached. Beyond that point any excess feed is assumed to be removed from the system and not anymore available to the fish. The fact that the model does not include feed spill may be accountable for the overestimation of O_2 consumption when fish fed *ad libitum*.

The final version of flow files model has been developed, we have successfully been able to integrate it into the Virtual Laboratory in Bjørnson *et al*. (2019) and a demonstration of this is provided in the Appendix A5. The role of AquaFishDEB is pivotal for the function of the virtual laboratory because of its interlinkage with the other components and predominantly the water treatment model. Currently, the AquaFishDEB model is directly integrated only with the water treatment model, where the waste production and gaseous exchange predicted by the former are used as inputs for the latter. Although the link between the two models is uni-directional, potential two-way integrations could be developed. Future work could explore the effects of water quality on the biological performance of the species explicitly instead of the adopted red flag approach, provided that there are relevant data available for the development and parametrization of extra modules. This could include effects of the toxicity of the nitrogenous compounds (TAN, nitrite, nitrate) on processes such as assimilation and maintenance, which would therefore affect biological performance. Moreover, the flow model is not explicitly linked to the AquaFishDEB and is used as a stand-alone model. However, potential integrations could also be established, such as those relating current velocity to feeding and metabolic costs (2.3). The prototype version of the virtual lab showed the integration of the first two models (AquaFishDEB and water treatment model) to be functional and this will further be expanded in the final version.

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Appendix

A1 Model equations and parameters

Tables A1 and A2 give the equations of the AquaFishDEB model. Table A3 defines all model parameters. For a more comprehensive description of the DEB theory and a full list of the equations and the nomenclature used we refer to Kooijman (2010), Stavrakidis-Zachou *et al*. (2019).

Table A1: State variables, energy fluxes and dynamics of the DEB model. Brackets [.] indicate quantities expressed per unit of structural volume and braces {.} per unit of structural surface area.

State variables	
V, $L = V^{1/3}$	Structural body volume (cm^3), Volumetric structural length (cm)
E, $[E] = E / V$	Energy in reserve (J), Reserve density (J/cm^3)
E_H, E_R	Energy investment (J) into maturation, - to reproduction
M_X	Mass content of the food in the gut (mol)
Fluxes	
$\dot{p}_A$	Assimilation rate: $\dot{p}_A = \{\dot{p}_{A_m}\} f L^2$, with $f = \frac{M_X}{M_X + M_K^X}$ and $M_K^X = \frac{\{\dot{J}_{EA_m}\}}{\{\dot{J}_{Xg_m}\}} \left(\left(y_{EX_P} a_P\right)^{-1} + \left(y_{EX_{nP}}(1-a_P)\right)^{-1} - \left(y_{EX_P} a_P + y_{EX_{nP}}(1-a_P)\right)^{-1} \right)$
$\dot{p}_C$	Reserve mobilization rate: $L^3[E](\dot{v}/L - r)$ with $r = \frac{\frac{\kappa[E]\dot{v}}{L} - \dot{p}_S}{[E_G] + [E]\kappa}$
$\dot{p}_S$	Somatic maintenance rate: $[\dot{p}_M] L^3$
$\dot{p}_J$	Maturity maintenance rate: $\dot{k}_J \min\{E_H, E_H^p\}$
$\dot{p}_G$	Growth rate: $\kappa \dot{p}_C - \dot{p}_S$
$\dot{p}_R$	Energy flux to maturation/reproduction: $(1-\kappa)\dot{p}_C - \dot{p}_J$
$\dot{p}_D$	Dissipating power: $\dot{p}_S + \dot{p}_J + (1-\kappa_R)\dot{p}_R$
Dynamics	
$\frac{d}{dt} V = rV$	
$\frac{d}{dt}[\mathrm{E}] = [\dot{p}_A] - [E]\dot{v}/L$	
$\frac{d}{dt} E_H = \dot{p}_R (E_H < E_H^p)$	
$\frac{d}{dt} E_R = \dot{p}_R (E_H \geq E_H^p)$	
$\frac{d}{dt} M_X = -(y_{X_P E} + y_{X_{nP} E} + y_{PE}) \dot{p}_A / \mu_E$	

Table A2: Model equations that produce the output quantities. The equations use quantities defined in Table A1.

Wet weight (g)	$W = d_{Vw}\left(V + (E + E_R)\frac{w_{Ed}}{d_{Ed}\mu_E}\right)$
Group size	$\frac{dN}{dt} = mN$, with m the mortality rate
Feeding rate (g/d)	$\dot{J}_X = \min\left(w_X \frac{d_{Xw}}{d_{Xd}}(M_{gm} - M_X),\ \dot{k}_X W\right)$ with $M_{gm} = s[M_{gm}]V$ and $s = (1 - \mu_X/\mu_X^{\max})$
Feed conversion ratio	$FCR = \frac{\dot{J}_X}{dW/dt}$
Faeces production (g/d)	$\dot{J}_P = \frac{y_{PE}}{\mu_E}\dot{p}_A$
Faecal loss-N (g/d)	$\dot{J}_{PN} = 14 n_{NP}\frac{y_{PE}}{\mu_E}\dot{p}_A$
Non faecal loss-N	$\dot{J}_N = \eta_{ND}\dot{p}_D + \eta_{NG}\dot{p}_G$
Oxygen consumption	$\dot{J}_O = \eta_{OA}\dot{p}_A + \eta_{OD}\dot{p}_D + \eta_{OG}\dot{p}_G$
Carbon dioxide production	$\dot{J}_C = \eta_{CA}\dot{p}_A + \eta_{CD}\dot{p}_D + \eta_{CG}\dot{p}_G$

Table A3: Description of all parameters in the AquaFishDEB model.

Symbol	Units	Interpretation
$\{\dot{p}_{A_m}\}$	J/cm^2.d	Surface-specific max assimilation rate
$\dot{v}$	cm/d	Energy conductance
κ	-	Allocation fraction to soma
$\kappa_{X_P}, \kappa_{X_{nP}}, \kappa_{X_{NFE}}$	-	Digestion efficiency of protein, lipid and NFE to reserves
κ_X	-	Digestion efficiency of food to reserves
κ_P	-	Faecation efficiency of food to faeces
κ_R	-	Reproduction efficiency
$[\dot{p}_M]$	J/cm^3.d	Volume-specific somatic maintenance rate
$[E_G]$	J/cm^3	Specific costs for structure
E_H^b, E_H^j, E_H^p	J	Maturity threshold at birth, metamorphosis, puberty
$\dot{k}_J$	1/d	Maturity maintenance rate coefficient
μ_*	J/mol	chemical potentials of $*$= X(food), P(product), V(structure), E(reserves)
w_*	g/mol	molecular weights of $*$
d_*	g/cm^3	specific density of $*$
$n_{C*}, n_{H*}, n_{O*}, n_{N*}$	-	chemical index of elements (C,H,O,N) in organic compounds *
auxiliary parameters		
T_A	K	Arrhenius temperature
δ_g	-	Gut-volume shape coefficient
$\{\dot{J}_{Xg_m}\}$	1/cm^2.d	Surface-specific max digestion rate
$\mu_X^{\max}$	J/mol	Maximum chemical potential of food

a_P	-	Fraction of protein in food
$[M_{gm}] = \delta_g d_X / w_X$	mol/cm^3	Volume-specific max capacity of the gut (dry weight)
$\{\dot{J}_{EA_m}\} = \{\dot{p}_{A_m}\}/\mu_E$	mol/cm^2 d	Surface-specific max assimilation rate
$\kappa_{X_{nP}} = \kappa_{X_L} a_L + \kappa_{X_{NFE}}(1 - a_L)$	-	Digestion efficiency of non-protein to reserves, with a_L the fraction of lipids in the non-protein part of food
$y_{EX_P} = \kappa_{X_P} w_{X_P} / w_E$	mol P/mol E	yield of reserve on protein
$y_{EX_{nP}} = \kappa_{X_{nP}} w_{X_{nP}} / w_E$	mol nP/mol E	yield of reserve on the non-protein
$\kappa_X = \theta_P \kappa_{X_P} + \theta_L \kappa_{X_L} + \theta_A \kappa_{X_A} + \theta_{NFE} \kappa_{X_{NFE}}$	-	Digestion efficiency of food to reserves, where θ are the fractions of protein, lipid, ash, and NFE in food and κ their respective digestibilities.
$\kappa_P = \theta_P(1 - \kappa_{X_P}) + \theta_L(1 - \kappa_{X_L}) + \theta_A(1 - \kappa_{X_A}) + \theta_{NFE}(1 - \kappa_{X_{NFE}})$	-	Digestion efficiency of food to faeces, where θ are the fractions of protein, lipid, ash, and NFE in food and κ their respective digestibilities.
$y_{PE} = \frac{\kappa_P \mu_E}{\kappa_X \mu_P}$	-	yield of faeces on reserve
$\dot{k}_X$	-	Food as fraction of body (wet) weight

All physiological rates depend on temperature. For a species-specific range of temperatures, the temperature effect is quantified by the Arrhenius relationship (Kooijman, 2010). For T_A the species-specific Arrhenius temperature, the rate of a physiological process $\dot{k}$ at temperature T is given by

$$\dot{k}(T) = \dot{k}_1 \exp\left(\frac{T_A}{T_1} - \frac{T_A}{T}\right) \quad \text{(A1)}$$

where $\dot{k}_1$ the rate at a chosen reference temperature, here T_1=293K.

A2 Digestion-Assimilation module

The stomach/gut, which is still an "environment" for the fish, is used to smooth out fluctuations in food availability. For simplicity, we will not distinguish between stomach and gut and from now on we will refer to it as gut. The shape of the digestive system resembles that of a (tube) cylinder. Thus, the volume of the gut of length L_λ and diameter L_φ is $V_g = \pi L_\lambda L_\varphi^2/4$ and the surface area of contact between gut and gut content is $A_g = \pi L_\lambda L_\varphi$. Following Kooijman's (2010) approach, if the secretion rate of enzymes is constant and the deactivation is a first-order process, the dynamics of the amount of active enzymes, M_g, in the gut, is given by $\frac{d}{dt}M_g = \{\dot{J}_g\}\pi L_\lambda L_\varphi - \dot{k}_g M_g$, where $\{\dot{J}_g\}$ is the constant secretion rate of enzyme per unit of gut wall surface area and $\dot{k}_g$ is the decay rate of enzyme activity. We assume that the concentration of active enzymes reaches an equilibrium fast. The equilibrium amount of active enzymes is $M_g = \{\dot{J}_g\}\pi L_\lambda L_\varphi / \dot{k}_g$. For isomorphs, we can assume that the gut length L_λ and diameter L_φ are proportional to structural length L. Consequently, the equilibrium amount of active enzymes is proportional to surface area, L^2. Enzymes attack food break them down and produce products that will then be absorbed through the gut wall and form the generalized reserve molecules. Let M_X be the mass of food (in mol) in the gut. The products are produced by digestion at a rate proportional to $M_g M_X$, i.e., $\dot{J}_d = \{\dot{J}_{Xgm}\}L^2 M_X$, where $\{\dot{J}_{Xgm}\}$ is the maximum surface-specific rate of digestion.

Food is a mixture of proteins, lipids and carbohydrates. Suppose that a fraction a_P of food is protein and the remaining $1 - a_P$ are the lipids and carbohydrates. From now on we will call them protein, X_P, and non-protein, X_{nP}, component. The products of the digestion process will also be a mixture of proteins, lipids and carbohydrates. Consequently, a fraction a_P of the food in the gut is protein and the remaining $1 - a_P$ non-protein. The composition of each compound

is represented as a "generalized" compound with fixed stoichiometry. The protein compound of the food is denoted by $CH_{n_{HX_P}}O_{n_{OX_P}}N_{n_{NX_P}}$, the non-protein by $CH_{n_{HX_{nP}}}O_{n_{OX_{nP}}}$, the reserves by $CH_{n_{HE}}O_{n_{OE}}N_{n_{NE}}$ and the faeces by $CH_{n_{HP}}O_{n_{OP}}N_{n_{NP}}$. The assimilation process can be described by the macro-chemical equation:

$$y_{EX_P}CH_{n_{HX_P}}O_{n_{OX_P}}N_{n_{NX_P}} + y_{EX_{nP}}CH_{n_{HX_{nP}}}O_{n_{OX_{nP}}} \rightarrow$$

$$CH_{n_{HE}}O_{n_{OE}}N_{n_{NE}} + y_{PE}CH_{n_{HP}}O_{n_{OP}}N_{n_{NP}} + \text{mineral products } (H_2O,\ CO_2, N-\text{wastes}),$$

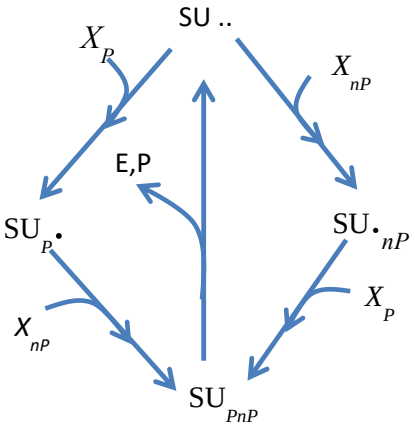
where y_{EX_P} and $y_{EX_{nP}}$ are the molecules of protein and non-protein required to form a molecule of reserve and y_{PE} the molecules of feaces produced.

The absorption of the products through the gut wall and transformation into reserves (assimilation process) is modeled using the synthesizing unit (SU) concept of DEB theory (Kooijman, 2010). The SUs are generalized enzymes that bind and process one or more substrates to form one or more products. An SU can be in the unbound state, waiting for the arrival of one or more substrates, or in the bound state, processing those substrates. We assume that the two complementary substrates of protein and non-protein are processed in parallel to produce the generalized reserves, E. An SU processing two substrates can be either in binding or processing state. Let $\theta_{..}$ be the fraction of SUs in the binding state, waiting for the required molecules of protein or non-protein to be bound, $\theta_{P.}$ and $\theta_{.nP}$ the fraction of SUs waiting for molecules of the missing substrate to be bound, and θ_{PnP} the fraction of SUs in the processing state.

Digestive enzymes break down food particles, M_X, which are then bound to free SUs to form the reserves. Let j_{X_P} and $j_{X_{nP}}$ be the arrival fluxes of the protein and non-protein substrates. The interactions of the two substrates into products (reserves and faeces) and the dynamics of the SUs are given in Figure A1. Assuming rapid convergence to steady state, the production flux then amounts to $j_{EA} = \dot{k}\theta^*_{PnP}$, where θ^*_{PnP} is the fraction of SUs in the processing state.

Thus,

$$j_{EA} = \frac{1}{\dot{k}^{-1} + \left(y_{EX_P}j_{X_P}\right)^{-1} + \left(y_{EX_{nP}}j_{X_{nP}}\right)^{-1} - \left(y_{EX_P}j_{X_P} + y_{EX_{nP}}j_{X_{nP}}\right)^{-1}} \quad \text{(A2)}$$



Dynamics of the fractions of the SUs at different states.

$$\frac{d\theta_{..}}{dt} = -(y_{EX_P}j_{X_P} + y_{EX_{nP}}j_{X_{nP}})\theta_{..} + \dot{k}\theta_{PnP}$$

$$\frac{d\theta_{P.}}{dt} = y_{EX_P}j_{X_P}\theta_{..} - y_{EX_{nP}}j_{X_{nP}}\theta_{P.}$$

$$\frac{d\theta_{.nP}}{dt} = y_{EX_{nP}}j_{X_{nP}}\theta_{..} - y_{EX_P}j_{X_P}\theta_{.nP}$$

$$\frac{d\theta_{PnP}}{dt} = y_{EX_{nP}}j_{X_{nP}}\theta_{P.} + y_{EX_P}j_{X_P}\theta_{.nP} - \dot{k}\theta_{PnP}$$

$$\theta_{..} + \theta_{P.} + \theta_{.nP} + \theta_{PnP} = 1$$

Equilibrium fraction of SUs in the processing state:

$$\theta^*_{PnP} = \left(\dot{k}^{-1} + \left(y_{EX_P}j_{X_P}\right)^{-1} + \left(y_{EX_{nP}}j_{X_{nP}}\right)^{-1} - \left(y_{EX_P}j_{X_P} + y_{EX_{nP}}j_{X_{nP}}\right)^{-1}\right)^{-1}$$

Figure A1: The interaction of the protein and non-protein substrates are processed in parallel to produce the generalized reserves, E, and faeces, P.

We associate the arrival fluxes j_{X_P} and $j_{X_{nP}}$ with the digestion rate, J_d. Thus, $j_{X_P} = a_P \dot{b} M_X$ and $j_{X_{nP}} = (1 - a_P)\dot{b} M_X$, where the association rate $\dot{b} = \{\dot{J}_{Xgm}\}L^2$. We link the association rate $\dot{b}$ with the maximum digestion rate and the dissociation rate $\dot{k}$ with the maximum specific assimilation rate, $\dot{J}_{EA_m}$, both relate to the surface area of the contact between gut and gut content A_g, which is taken proportional to L^2. Thus, $\dot{b} = \{\dot{J}_{Xgm}\}L^2$ and $\dot{k} = \{\dot{J}_{EA_m}\}L^2$, where $\{\dot{J}_{Xgm}\}$ and $\{\dot{J}_{EA_m}\}$ are the surface-specific maximum digestion and assimilation rates, respectively.

The rate of reserve formation or assimilation rate, $\dot{J}_{EA}$ (mol/d), equals

$$\dot{J}_{EA} = \dot{J}_{EA_m} \frac{M_X}{M_X + \frac{\{\dot{J}_{EA_m}\}}{\{\dot{J}_{Xgm}\}}\left(\left(y_{EX_P} a_P\right)^{-1} + \left(y_{EX_{nP}}(1-a_P)\right)^{-1} - \left(y_{EX_P} a_P + y_{EX_{nP}}(1-a_P)\right)^{-1}\right)} \quad \text{(A3)}$$

which can be written as

$$\dot{J}_{EA} = \{\dot{J}_{EA_m}\} f L^2 \quad \text{(A4)}$$

where f represents the scaled functional response given by

$$f = \frac{M_X}{M_X + M_K^X} \quad \text{(A5)}$$

and M_K^X the half saturation constant given by

$$M_K^X = \frac{\{\dot{J}_{EA_m}\}}{\{\dot{J}_{Xgm}\}}\left(\left(y_{EX_P} a_P\right)^{-1} + \left(y_{EX_{nP}}(1-a_P)\right)^{-1} - \left(y_{EX_P} a_P + y_{EX_{nP}}(1-a_P)\right)^{-1}\right) \quad \text{(A6)}$$

The mol-based assimilation rate (eq. A4) can be converted in energy-based as

$$\dot{p}_A = \{\dot{p}_{A_m}\} f L^2 \quad \text{(A7)}$$

where $\{\dot{p}_{A_m}\} = \mu_E \{\dot{J}_{EA_m}\}$, with μ_E (J/mol) the chemical potential of reserves.

The rate of faeces production is proportional to reserve formation rate (eq. A4 or A7) and is equal to

$$\dot{J}_P = y_{PE}\{\dot{J}_{EA_m}\} f L^2 = \frac{y_{PE}}{\mu_E}\dot{p}_A \quad \text{(A8)}$$

where y_{PE} is the yield of faeces on reserve (mol P/mol E) and the rates at which the protein and non-protein part of food are used are

$$\dot{J}^+_{X_P} = \{\dot{J}_{EA_m}\} f L^2 / y_{EX_P} \quad \text{and} \quad \dot{J}^+_{X_{nP}} = \{\dot{J}_{EA_m}\} f L^2 / y_{EX_{nP}} \quad \text{(A9)}$$

Next we derive the food dynamics in the gut. If M_X is the mass of food in the gut in mol ($w_X M_X$ stomach content in grams), between meals, the rate of change in gut content is given by

$$\frac{d}{dt} M_X = -\left(\dot{J}^+_{X_P} + \dot{J}^+_{X_{nP}} + \dot{J}_P\right) = -(y_{X_P E} + y_{X_{nP} E} + y_{PE})\{\dot{J}_{EA_m}\} f L^2 \quad \text{(A10)}$$

where the first two terms represent the food absorbed by the gut to form reserves (eq. A9) and the third that lost as faeces (eq A8).

The maximum storage capacity of the gut, M_{gm}, (in mol) is assumed to be proportional to its structural volume and is written as $[M_{gm}]V$, where $[M_{gm}]$ (mol/cm^3) is the volume-specific maximum capacity of the gut for food, which depends on the geometry of the gut as well as the type of food (energy content, density etc.). In addition, voluntary food intake depends on food composition and is inversely related to energy content, with energy-rich foods reducing hunger fast and resulting in low food intake (Fountoulaki *et al.*, 2005). To account for the effect

of food rich in energy on food intake we define the stress factor $s = (1 - \mu_X/\mu_X^{\max})$, with μ_X the chemical potential of food and $\mu_X^{\max}$ the maximum chemical potential of food. The stress factor s then reduces the maximum capacity by the factor s, thus $M_{gm} = s[M_{gm}]V$.

If fish is fed in meals as fraction, $\dot{k}_X$, of their body (wet) weight, W, the amount of food given per meal per day is $\dot{k}_X \frac{W}{w_X}$ (mol/d), where w_X is the molecular weight of food (units is mol/g). To convert it into dry mass it must be multiplied by the ratio of dry-to-wet mass of food $\frac{d_{Xd}}{d_{Xw}}$, where d_{Xd} and d_{Xw} are, respectively, the specific density of dry and wet food (g/cm^3). The amount of feed consumed is based on the minimum between the deficit of the gut (i.e., the 'gut-space' that can still be filled by the individual) and the food provided. The amount of food consumed per meal (in mol/d) is given by

$$\dot{J}_X = \min\left(\mathrm{s}[M_{gm}]L^3 - M_X,\ \dot{k}_X \frac{d_{Xd}}{d_{Xw}} \frac{W}{w_X}\right) \quad \text{(A11)}$$

The wet mass of food consumed in g/d is given by

$$\dot{J}_X = \min\left(w_X \frac{d_{Xw}}{d_{Xd}} \mathrm{s}[M_{gm}]L^3 - M_X,\ \dot{k}_X W\right) \quad \text{(A12)}$$

A3 Derivation of chemical indices

The chemical indices express the relative abundance of hydrogen (H), oxygen (O) and nitrogen (N) relative to carbon (C). Therefore, if the proximate composition of feed, structure, and reserve in terms of macronutrients such as protein (P), lipids (L) and carbohydrates (NFE) as well as the elemental composition of these macronutrients is known, then the chemical indices can be calculated (see Table A4). We here assumed a fixed elemental composition in terms of carbon, hydrogen, oxygen and nitrogen (n_{C*}, n_{H*}, n_{O*}, n_{N*}) for the various macronutrients (where * could be P, L or NFE). The values (table) were calculated by obtaining the amino and fatty acid profiles of feeds as well as fish of various sizes, life stages, nutritional conditions and geographical origins and then using the molecular formulas of their constituent monomers to construct typical animal proteins, lipids and carbohydrates (Nurhan *et al.*, 2007; Knox *et al.,* 1988; Wilson and Cowey, 1985; Timberg *et al.,* 2011; Haliloglu, 2002; Diana *et al.*, 1990).

Given the proximate composition (P, L, NFE) of an organic compound, the chemical indices can be calculated using the equations in Table A4. In addition, the gross energy (GE) of those compounds is given by:

$$GE = (23.6P + 39.5L + 17.7NFE) \quad (13)$$

where 23.6, 39.5 and 17.2 kJ g^{-1} are the combustible energy contents of P, L and NFE, respectively, as typically used in fish research (Schrama *et al.*, 2018). Finally, if the digestibilities (ADC=Apparent Digestibility Coefficients) of macronutrients in the food ($\kappa_{X_P}, \kappa_{X_L}, \kappa_{X_{NFE}}$) are also known, then it follows that the digestibility of GE will be:

$$\kappa_{X_{GE}} = \frac{23.6P\kappa_{X_P} + 39.5L\kappa_{X_L} + 17.7NFE\kappa_{X_{NFE}}}{GE} \quad \text{(A14)}$$

The chemical potential of food (μ_X) and fecal (μ_P) are, respectively,

$$\mu_X = 1000\, GE\, w_X \quad \text{(A13)}$$

and

$$\mu_P = 1000\left(1 - \kappa_{X_{GE}}\right) GE \frac{w_P}{1 - \kappa_{X_{DM}}} \quad \text{(A14)}$$

where w_X and w_P are the molecular weights of food and faeces, respectively, and calculated as $w_X = 12n_{CX} + 1n_{HX} + 16n_{OX} + 14n_{NX}$ and $w_{XP} = 12n_{CP} + 1n_{HP} + 16n_{OP} + 14n_{NP}$. The chemical indices are food type dependent and their calculation is given by the equations in Table A4. The gross energy (GE) and its digestibility $\kappa_{X_{GE}}$ are calculated in eq. A13 and A14, while the digestibility of dry matter $\kappa_{X_{DM}}$ is food specific.

Table A4: The chemical indices of the organic compound * (food, structural biomass, or reserve) calculated from the three macronutrients (protein (P), lipids (L) and carbohydrates (NFE)).

$$n_{O*} = \frac{12(n_{OP}\theta_P + n_{OL}\theta_L + n_{ONFE}\theta_{NFE})}{16(n_{CP}\theta_P + n_{CL}\theta_L + n_{CNFE}\theta_{NFE})}$$

$$n_{H*} = \frac{12(n_{HP}\theta_P + n_{HL}\theta_L + n_{HNFE}\theta_{NFE})}{(n_{CP}\theta_P + n_{CL}\theta_L + n_{CNFE}\theta_{NFE})}$$

$$n_{N*} = \frac{12n_{NP}\theta_P}{14(n_{CP}\theta_P + n_{CL}\theta_L + n_{CNFE}\theta_{NFE})}$$

The chemical indices of the macronutrients # (P,L,NFE)

	$n_{C\#}$	$n_{O\#}$	$n_{H\#}$	$n_{N\#}$
$Protein$	44.9	30.4	7.6	16.2
$Lipid$	76.7	11.4	11.5	-
NFE	44.4	49.4	6.2	-

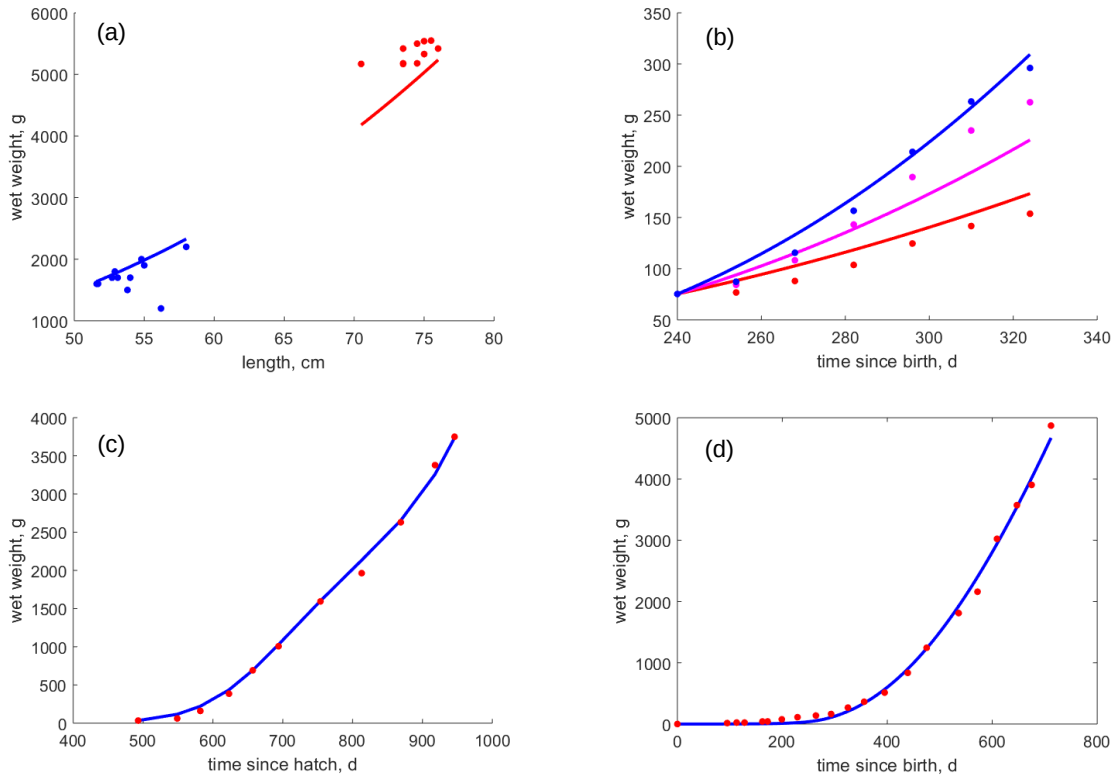
A4 Parameterisation data and model predictions

Table A5: Comparison of model predictions with observed data for Atlantic salmon.

Symbol (unit)	Interpretation	T (ºC)	Observations	Predictions
$a_h\ (d)$	Age at hatch	8	65	57.8
$a_h\ (d)$	Age at hatch	11	40	45.08
$a_b\ (d)$	Age at birth	8	40.6	101.7
$a_b\ (d)$	Age at birth	11	80	79.34
$a_m\ (d)$	Life span	10	1825	1824
$L_0\ (cm)$	Egg diameter		0.62	0.22
$L_h\ (cm)$	Length at hatch		1.9	1.89
$L_b\ (cm)$	Length at birth		2.5	1.91
$L_j\ (cm)$	Length at metamorphosis		20	19.83
$L_i\ (cm)$	Ultimate total length		120	119.4
$W_w^0\ (cm)$	Wet weight of egg		0.105	0.224
$W_d^0\ (cm)$	Dry weight at birth		0.0396	0.0153
$W_w^b\ (cm)$	Wet weight at birth		0.766	0.0836
$W_w^j\ (cm)$	Wet weight at metamorphosis		90	92.95
$W_w^p\ (cm)$	Wet weight at puberty		2000	1221
$W_w^i\ (cm)$	Ultimate wet weight		$2\ 10^4$	$2.03\ 10^4$

Table A6: Comparison of model predictions with observed data for gilthead seabream.

Symbol (unit)	Interpretation	T (ºC)	Observations	Predictions
$a_h\ (d)$	Age at hatch	17.5	3	2.57
$a_b\ (d)$	Age at birth	17.5	8	5.08
$a_j\ (d)$	Age at metamorphosis	19	100	119.8
$a_p\ (d)$	Age at puberty	19	1095	389.2
$a_m\ (d)$	Life span	20	4015	4018
$L_h\ (cm)$	Length at hatch		0.226	0.155
$L_b\ (cm)$	Length at birth		0.363	0.198
$L_j\ (cm)$	Length at metamorphosis		2.8	1.146
$L_p\ (cm)$	Length at puberty		36.5	24.35
$L_i\ (cm)$	Ultimate total length		70	78.1
$W_w^b\ (cm)$	Wet weight at birth		$1.6\ 10^{-4}$	$2.7\ 10^{-4}$
$W_w^j\ (cm)$	Wet weight at metamorphosis		1.5	1.62
$W_w^p\ (cm)$	Wet weight at puberty		500	504.8
$W_w^i\ (cm)$	Ultimate wet weight		$1.72\ 10^4$	$1.66\ 10^4$



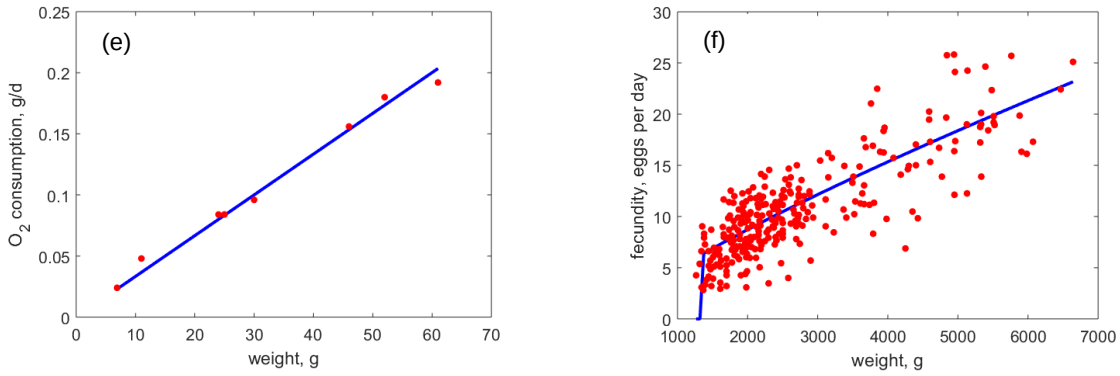
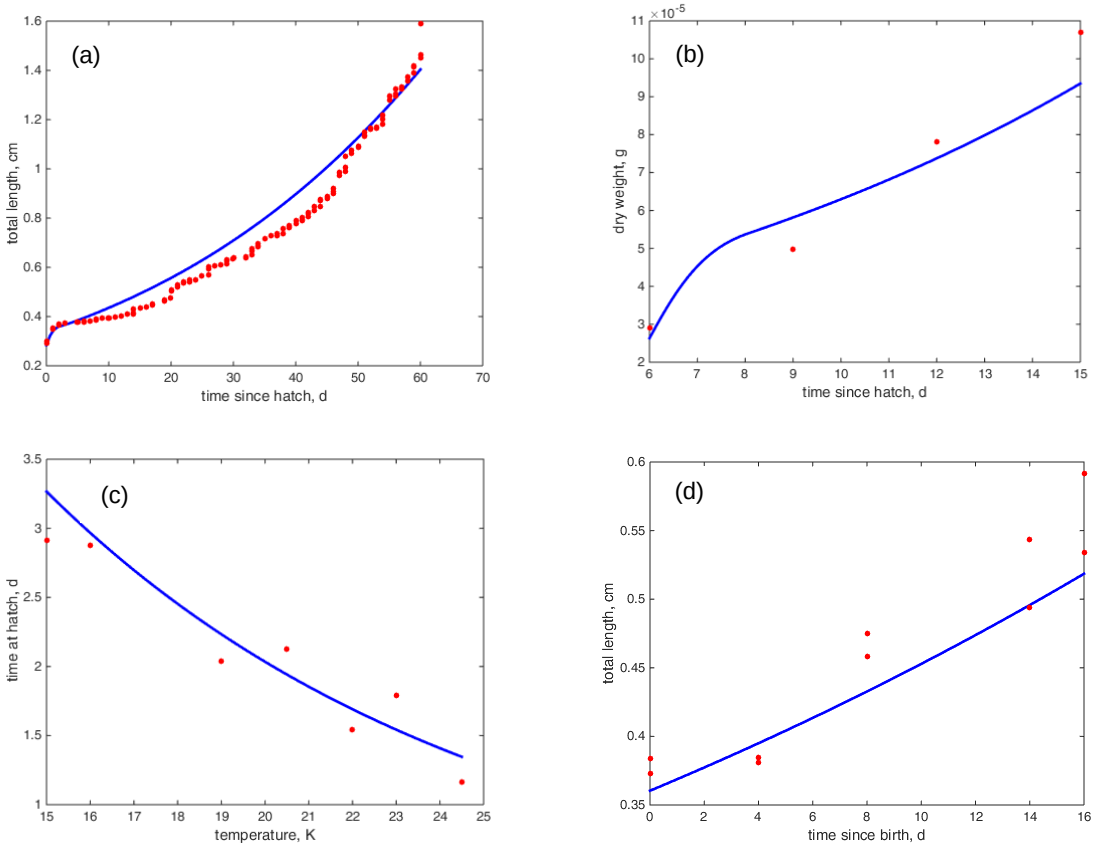


Figure A2: Comparison of model predictions (lines) to observations (points) for Atlanitic salmon: (a) weight as function of length (Palstra et al., 2007, INRA (unpublished)), (b) weight-at-age at different constant temperatures (colours) (Handeland et al., 2008), (c) weight-at-age at ambient temperature (Palstra et al., 2007), (d) weight-at-age at constant temperature (Davidson et al., 2016), (e) oxygen consumption for different fish sizes (Cook et al., 2000), (f) fecundity as a function of weight (Eyto et al., 2015).



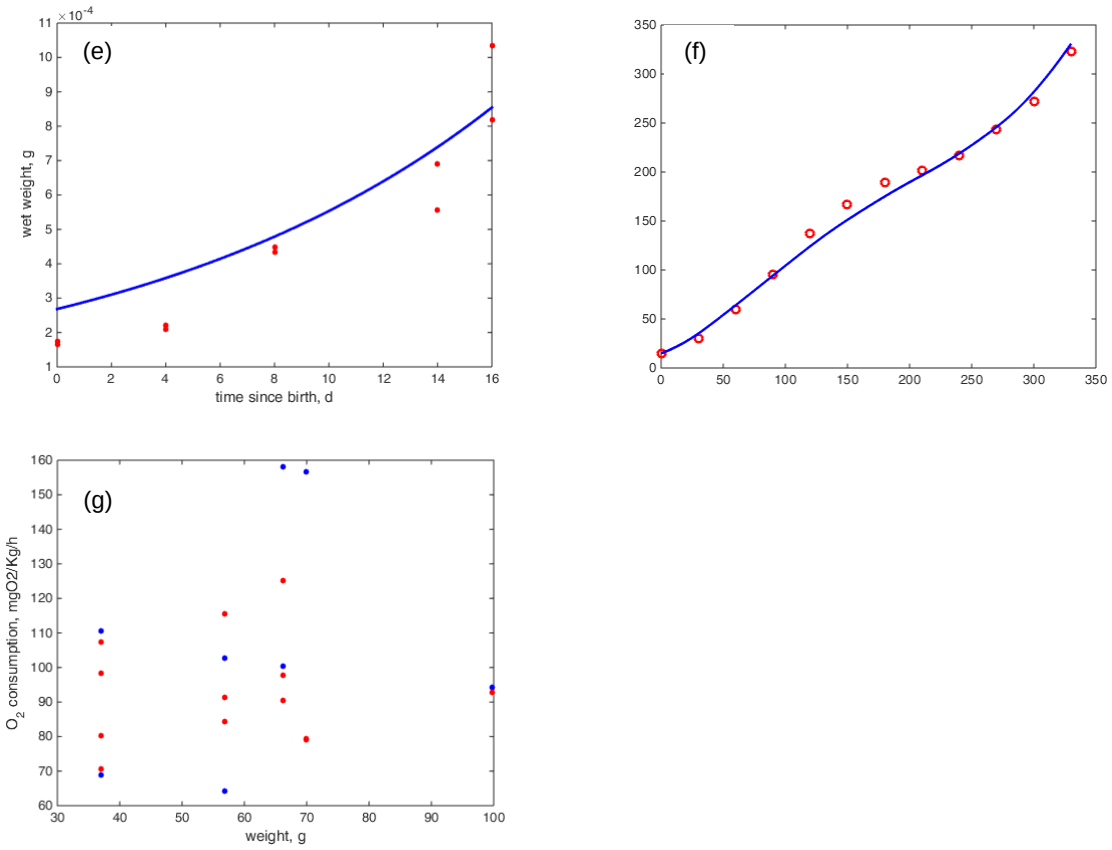
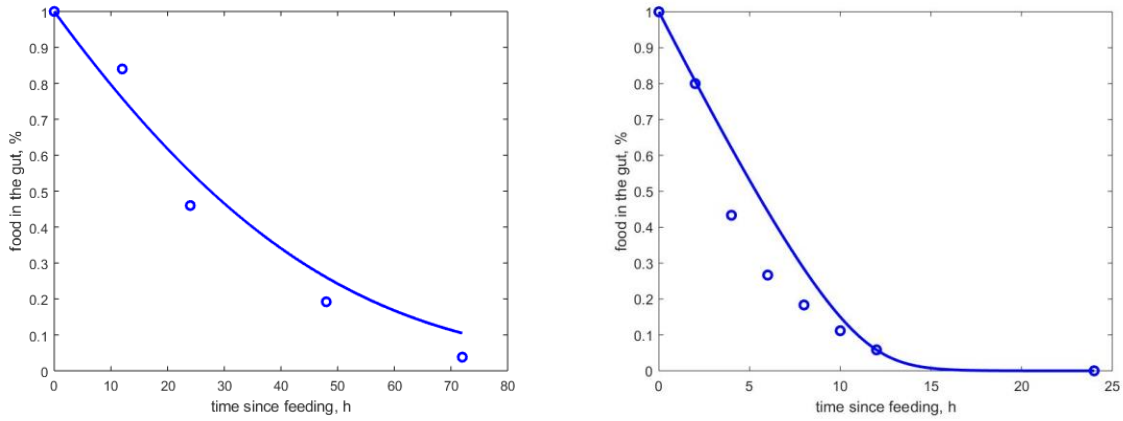


Figure A3: Comparison of model predictions (lines) to observations (points) for gilthead sea bream: (a) length-at-age (Lika et al., 2014), (b) dry weight-at-age (Parra and Yufera, 2000), (c) time at hatch at different temperature (Yufera et al., 2011), (d) length-at-age (HCMR, unpublished), (e) length-at-age (HCMR, unpublished), (f) weight-at-age at ambient temperature (Brigolin et al., 2010), (g) oxygen consumption for different fish sizes at different constant temperatures (red observations, blue prediction) (Guinea and Fernandez, 1997).



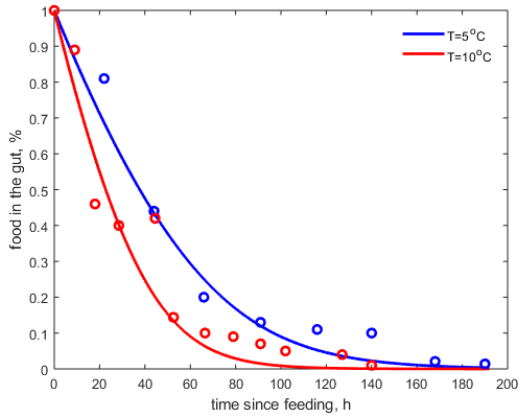


Figure A4: Data on gastric evacuation used for the parametrization of the digestion-assimilation module. Points indicate observations and lines model predictions. Data obtained from Handeland et al., 2008 for Atlantic salmon (upper left), Nikolopoulou et al., (2011) for gilthead seabream (right), and Dana (1984) for rainbow trout (lower left).

A5 Demonstrations in the Virtual Lab

In this section, we demonstrate the configuration and use of growth model in the Virtual Laboratory, through the configuration of a simple experiment. **Erreur ! Source du renvoi introuvable.** shows the main page of the simulation area (https://ae2020virtuallab.sintef.no/). The user may choose to review old experiments or launch a new one using the Experiment Wizard button. For our demonstration, we will continue on to the Wizard.

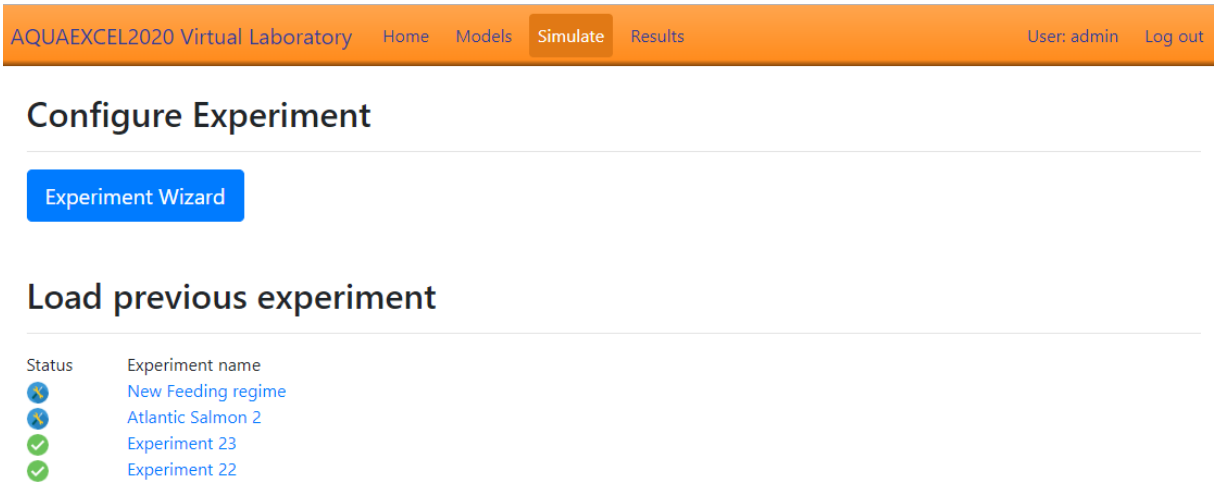


Figure A5: Simulation main page

Erreur ! Source du renvoi introuvable. shows the page that loads when the user starts the simulation wizard. The wizard is split into five subpages: *Experiment*, *Infrastructure*, *Biomass*, *Parameters* and *Simulate*. The user is first sent to the Experiment page where they may choose a name for the new experiment. This is the name that will be linked in the main simulation page under Previous Experiments, see **Erreur ! Source du renvoi introuvable.**.

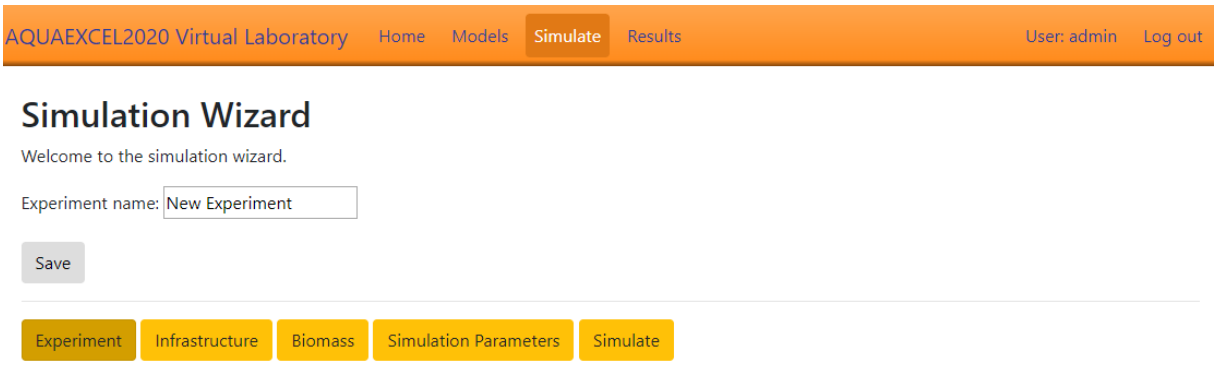


Figure A6: Simulation Wizard

Erreur ! Source du renvoi introuvable. demonstrates this where the user has validated a new experiment name and is currently configuring the water treatment model. Water chemistry parameters which are used as input by the AquFishDEB model such as water temperature, salinity, pH, and oxygen concentration are also included here.

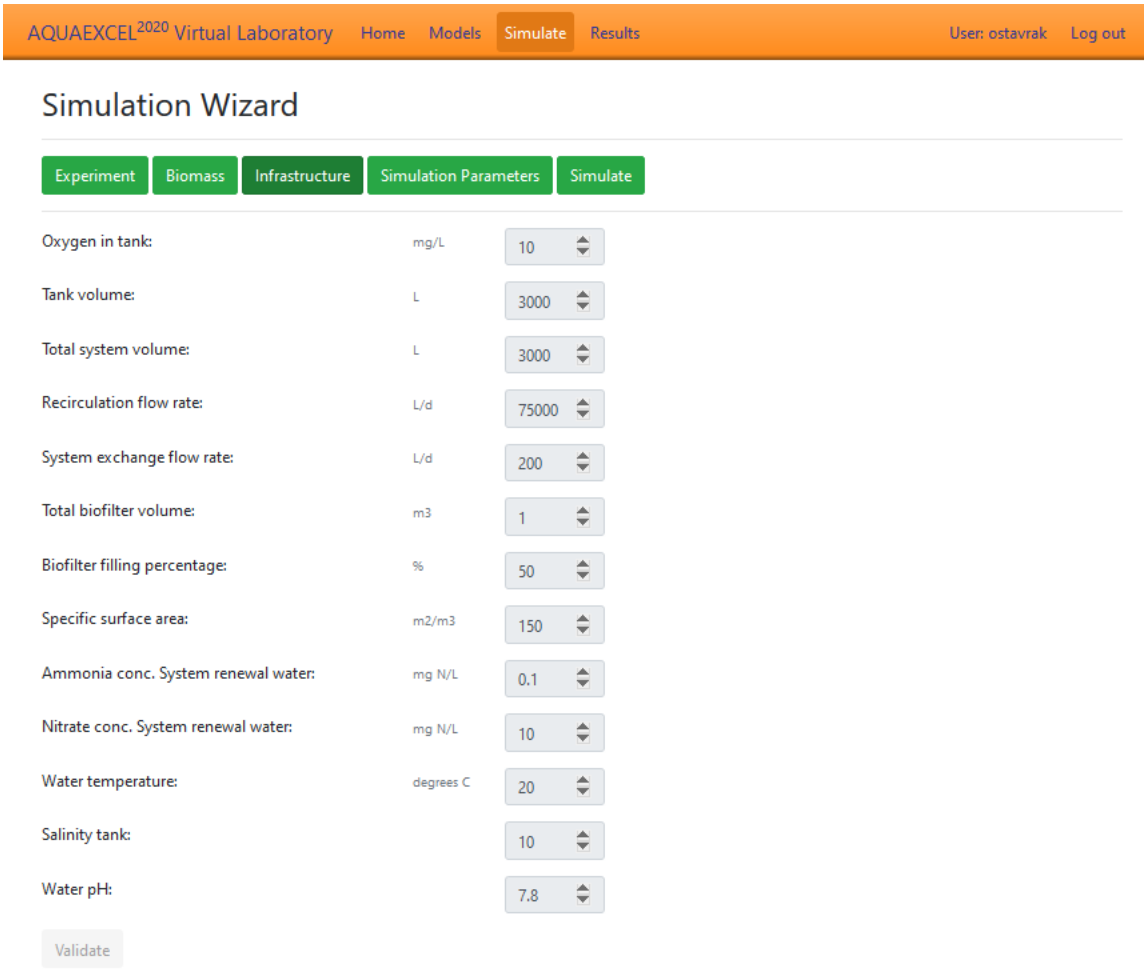


Figure A7: Simulation Wizard Infrastructure Configuration

It is possible to go through the experiment wizard in any order by pushing the five different buttons. **Erreur ! Source du renvoi introuvable.** demonstrates this when we have validated the parameters for the water treatment model and skipped ahead to the Simulate button. Here we get a status of what has been configured. The Virtual Laboratory will not let us simulate

until all parameters are set and validated, at which point the Simulate button will activate and the user may start the simulation.

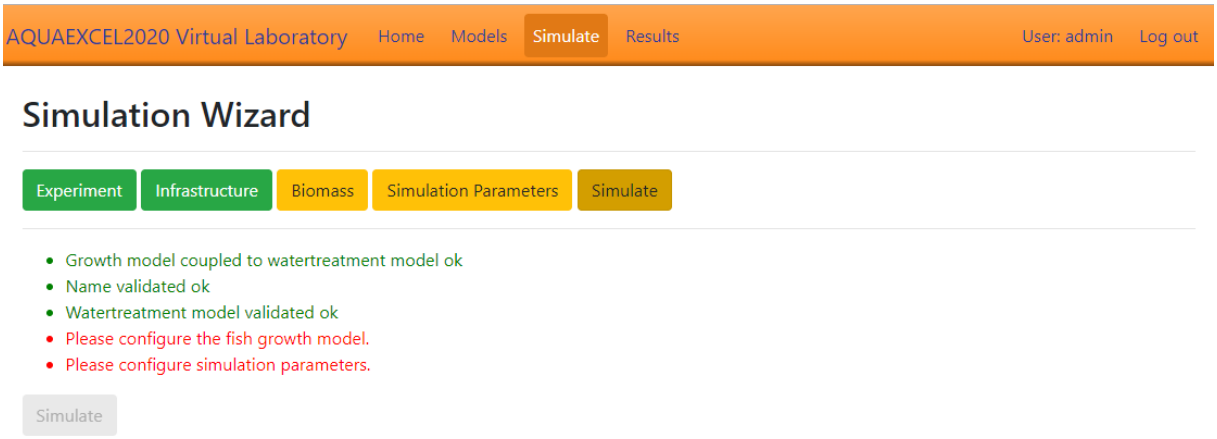


Figure 11: Simulation Wizard Simulation Start

While parameters such as water chemistry and the experimental duration are given in the *Infrastructure* and *Simulations Parameters* subpages respectively, most of the biological inputs for the AquaFishDEB model are given in the *Biomass* subpage (Figure A9). At this stage, the inputs that are available for each species are the *Initial number of fish*, the *Initial mean weight*, *the number of meals per day*, and the *Interval between meals*. Inputs regarding the feed composition and ration size will be incorporated in the final version of the Virtual Laboratory.

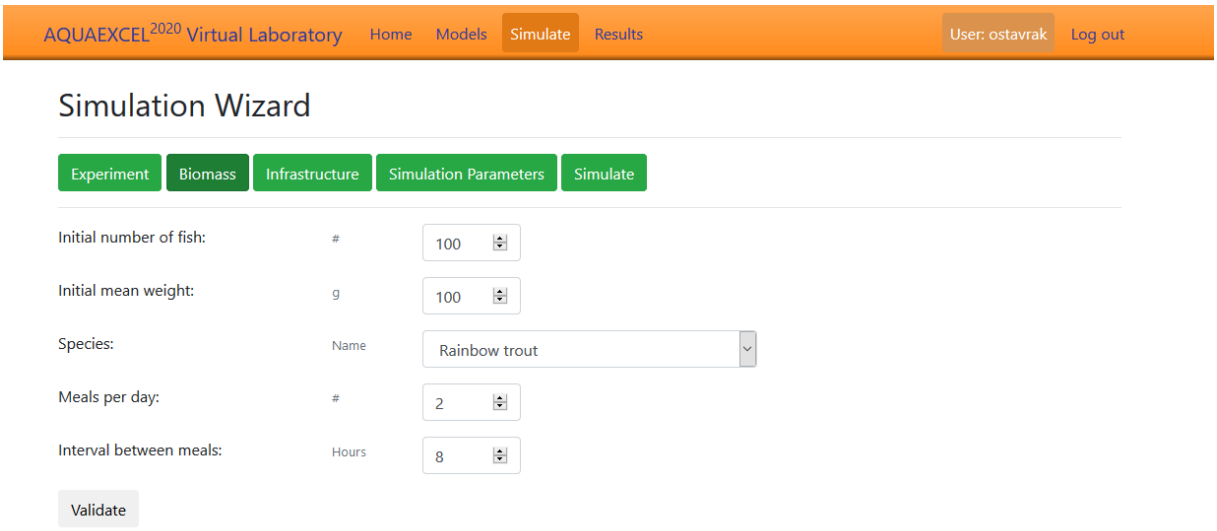


Figure 12: Simulation Wizard Biomass configuration

Once the simulation finishes the experiment results will be available from the Results page. **Erreur ! Source du renvoi introuvable.** shows the listing of different experiments that have been successfully simulated.

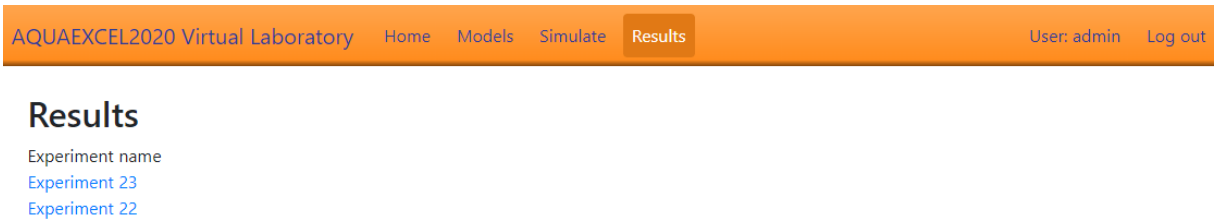


Figure A10 Results Main Page

The user may browse individual data series from the subpages of the individual experiments. **Erreur ! Source du renvoi introuvable.** illustrates two examples of the AquaFishDEB results in the Virtual Laboratory, the growth rate of individual fish for the first 20 days of our experiment (left), and the resulting oxygen consumption for a group of 100 individuals (right).

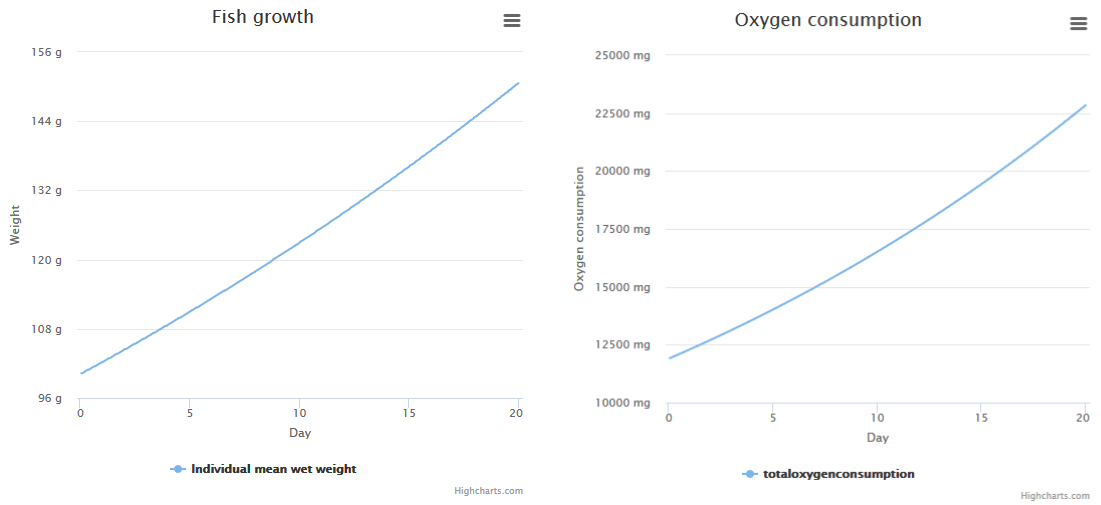


Figure A13: Results Page. Individual weight progression for a 20 day experiment (left) and the respective oxygen consumption for a group of 100 fish (right).

Glossary

AQUAEXCEL^{2020}: AQUAculture Infrastructures for EXCELlence in European Fish Research towards 2020

DEB: Dynamic Energy Budget

Document information

EU Project N°	652831	**Acronym**	AQUAEXCEL[2020]
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Project website	www.aquaexcel.eu		

Deliverable	N°	**D5.6**	Title	Final models for growth, feed intake and waste production simulation
Work Package	N°	**5**	Title	Virtual laboratories and modelling tools for designing experiments in aquaculture research facilities

Date of delivery	**Contractual**	23/12/2019 (Month 50)	**Actual**	15/1/2020 (Month 51)
Dissemination level	X	**PU Public, fully open, e.g. web**		
		CO Confidential, restricted under conditions set out in Model Grant Agreement		
		CI Classified, information as referred to in Commission Decision 2001/844/EC.		

Authors (Partner)	Konstadia Lika, Orestis Stavrakidis-Zachou, Nikos Papandroulakis			
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Issue Date	**Revision N°**	**Author**	**Change**
23/12/2019	1		First version
27/12/2019	2		Feedback from WP leader

Annex: Check list

Deliverable Check list (to be checked by the "Deliverable leader")

	Check list		Comments
BEFORE	I have checked the due date and have planned completion in due time	X	*Please inform Management Team of any foreseen delays*
	The title corresponds to the title in the DOW	X	*If not please inform the Management Team with justification*
	The dissemination level corresponds to that indicated in the DOW	X	
	The contributors (authors) correspond to those indicated in the DOW	X	
	The Table of Contents has been validated with the Activity Leader	X	*Please validate the Table of Content with your Activity Leader before drafting the deliverable*
	I am using the AQUAEXCEL[2020] deliverable template (title page, styles etc)	X	*Available in "Useful Documents" on the collaborative workspace*
The draft is ready			
AFTER	I have written a good summary at the beginning of the Deliverable	X	*A 1-2 pages maximum summary is mandatory (not formal but really informative on the content of the Deliverable)*
	The deliverable has been reviewed by all contributors (authors)	X	*Make sure all contributors have reviewed and approved the final version of the deliverable. You should leave sufficient time for this validation.*
	I have done a spell check and had the English verified	X	
	I have sent the final version to the WP Leader, to the 2nd Reviewer and to the Project coordinator (cc to the project manager) for approval	X	*Send the final draft to your WPLeader, the 2nd Reviewer and the coordinator with cc to the project manager on the 1st day of the due month and leave 2 weeks for feedback. Inform the reviewers of the changes (if any) you have made to address their comments. Once validated by the 2 reviewers and the coordinator, send the final version to the Project Manager who will then submit it to the EC.*