Intelligent Fish feeding through Integration of ENabling technologies and Circular principle

Grant Agreement (GA) No: 818036

D1.4 Digital twin of feeding process

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1. Introduction

This report is the final version for Deliverable 1.4, delayed from M30 to M40. The work is conducted as part of Task 1.4: “Experimental and in-silico assessments of feeding efficiency, biological responses and environmental impacts”; subtask 1.4.2 “In silico simulation of fish behaviour, physiology and metabolism (FishMet model)”.

2. FishMet- a digital twin for precision smart feeding

The FishMet digital twin is intended to help with the need to combine knowledge of the feeding biology of fish with finding optimal feeding strategies that enable us to optimise growth with minimal waste (smart feeding). FishMet is a simplified, digital representation of a fish built using computer-aided design and computer simulation.

FishMet aims at assisting users in decision-making regarding smart feeding strategies and can be integrated with other (e.g. AI-based) models.

The FishMet digital twin is a numerical model composed of virtual components and software modules operating on different levels. It is engineered to be able to communicate with iBOSS, the digital reality universe developed as part of the iFishIENCi project. Each component of the digital twin is therefore built to be integrated with iBOSS (see Deliverable 2.4) and the outside world: the fish farm automation system, the decision support system and the prediction/scenario modelling system. FishMet is therefore developed to use common application programming interface (APIs) for interoperability with other components.

3. FishMet- the biological basis

The prototype version of the model at the initiation of the project was based on conceptual in silico models and available published data that combined observations of fish behaviour and research-based knowledge about the feeding biology (appetite control, digestive physiology, and growth) in Atlantic salmon. The first version beyond the generic model was developed for rainbow trout, and additional data has been, and continue to be collected during experiments in the iFishIENCi project. An important feature of the model (Fig. 1; Fig 2) is that feed intake is a calculated output of the FishMet model together with behaviour related to feeding. One of the main aims of the model is to predict feed intake. This contrasts the common modelling approach in aquaculture that typically calculates growth based on a given feeding level (% of Body mass per day). The model include physiology, feeding behaviour and allocation of energy including growth, and key sensory, biological control mechanisms, but simplified to a level of precision that is useful and applicable in aquaculture. Modelling the impacts from the rearing environment on the key outputs, fish feeding behaviour, fish performance is another key element of the FishMet model.

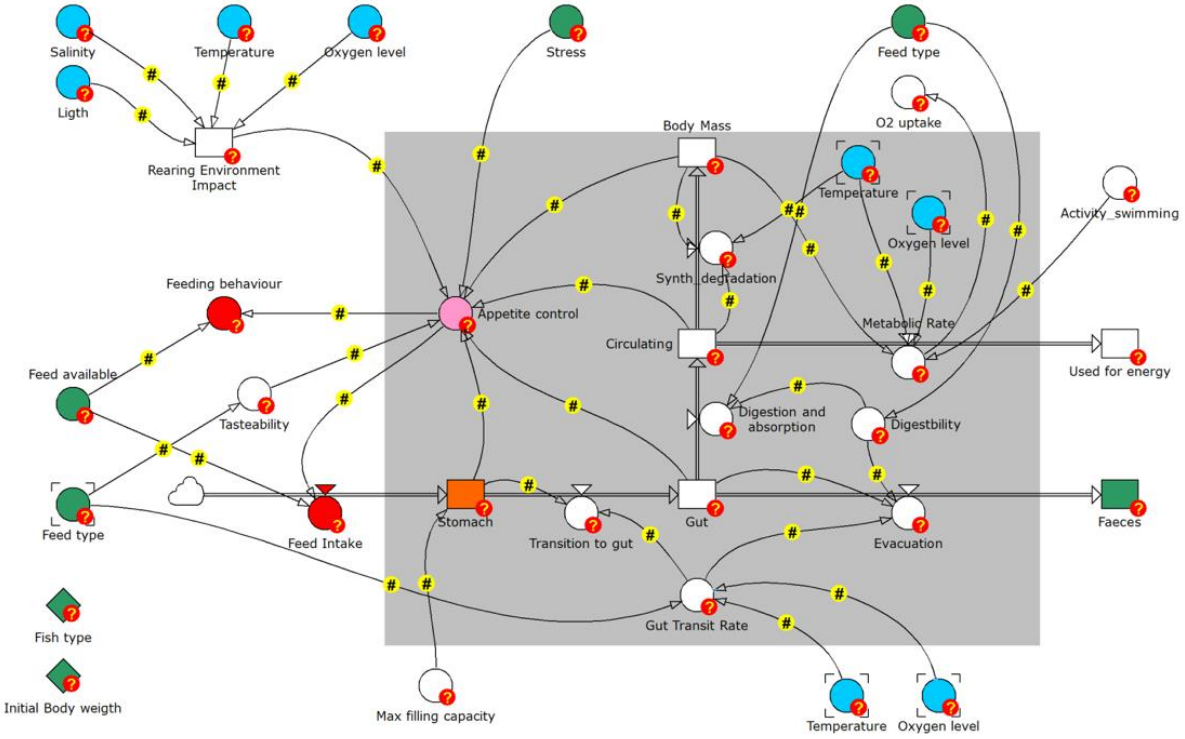


Fig 1. *Conceptual biological model for the prototype FishMet*

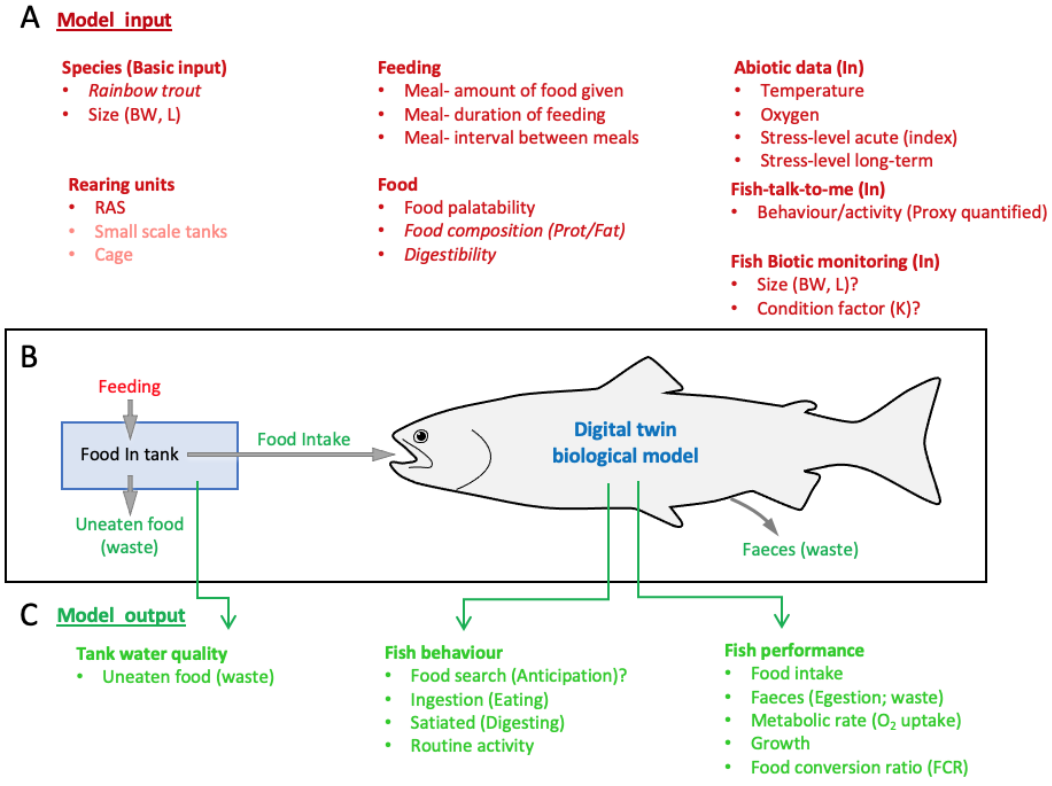


Fig 2. *Outline of main inputs and outputs for the FishMet model. The FishMet model is designed to work in an open environment and read monitoring data from iBOSS and compute output to the operator or provide data that can be pushed into smart feeding systems*

The factors described above are linked together in a simulation model that integrates knowledge of fish physiology and nutrition and describes food intake, stomach filling, and intestinal passage including digestion and absorption processes in fish followed by allocation into growth or energy metabolism. The conceptual model includes new knowledge about the orexigenic and anorexigenic factors that affect appetite and how peripheral factors such as stomach filling, digestion and plasma levels of nutrients affect feed intake. Gastric filling and intestinal passage are important elements, and a central part of the model focuses on the dynamic changes that occur due to variations in feeding amount and frequency as well as environmental factors such as temperature, and oxygen can affect appetite, feeding behaviour and ultimately food intake.

4. FishMet- the digital twin model

The FishMet model represents a mechanistic, nonparametric, process-based simulation model. It is different from a traditional analytic models based on equations. By incorporating mechanistic representation of various processes that control fish appetite, decision-making, behavior and feeding, FishMet can account for complexity, stochasticity, emergent effects etc. The computational part of the FishMet system follows the principles of **process simulations** and **agent-based modelling**[1]. It has been engineered from the start to provide extensibility, interoperability and a plugin-like application within a larger digital twin system. FishMet is a discrete time model running over numerous time steps with the resolution of 1 s. The model works at the fine-grained level of individual feed items and individual decisions of the fish. This potentially allow for complex simulations with variable feed, complex schedules, changing and stochastic environment. Given the power of modern computers, the computational efficiency of FishMet is more than adequate, e.g., a 24-h simulation requires a few seconds. This allows running multiple scenarios within a short time to aid decision support.

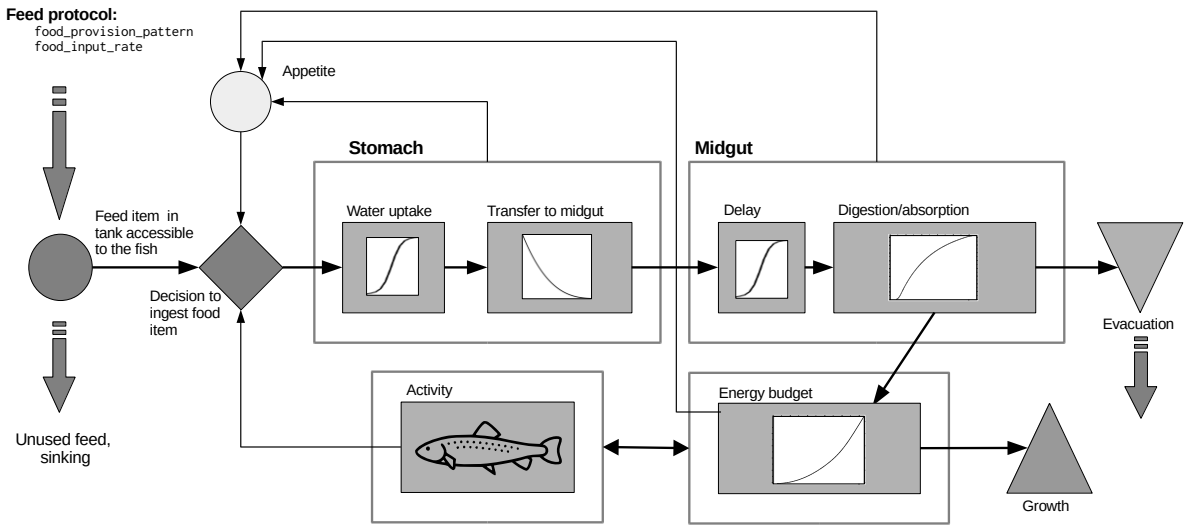


Fig. 3. *The FishMet model components implemented in fully functioning software code*

[1] Agent-based models (ABM) are computational models that simulate the actions and interactions of autonomous agents with the aim to assess their effects on the whole system. In ABM, a system is modelled as a collection of autonomous decision-making entities; *agents*

Continuous simulation: FishMet has been developed from the beginning as a process model. However, later development added the capability to perform open-ended continuous simulations. For example, one scenario is running FishMet to simulate repeated 24h periods continuously for a long time (with regular output of intermediate data), allowing to change the model parameters (e.g., feed protocol) at any moment. For example, such a scenario can provide a decision support for sudden unplanned perturbation in the modelled system. This, in turn, will allow its use for decision support, including simulation, monitoring, testing, predictive analytics, etc.

Feed protocol: The model flowchart (Fig. 3) starts from the food supply to the modelled environment. The food input can follow an arbitrary protocol, for example, two or three short meals, with any pattern of food provisioning or, for example, continuous ad libitum feeder operation. A simple regular feed schedule (e.g., Fig. 4) can be generated within the model, while complex, irregular, stochastic or disrupted patterns can be created by invoking helper scripts in Python, R or other programming languages.

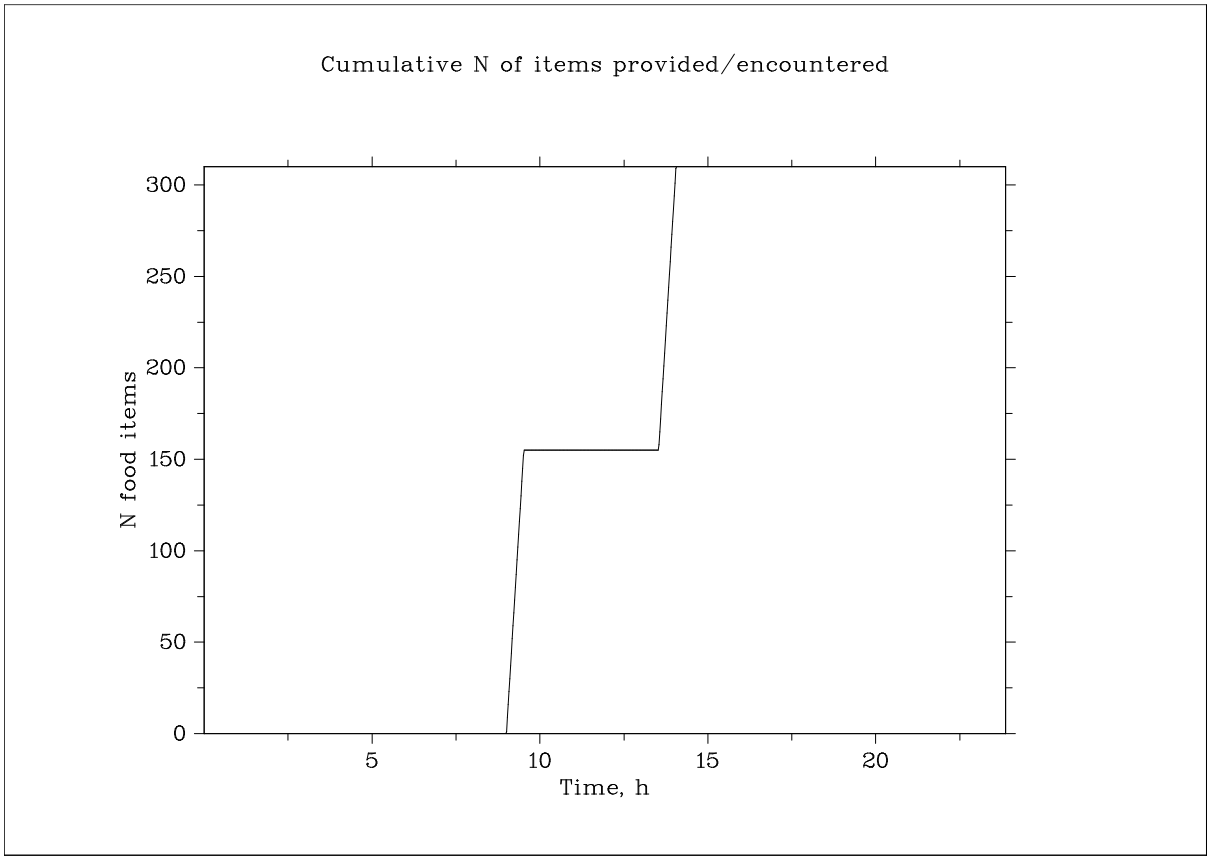


Fig 4. *An example of feed protocol with two daily meals (plot generated by the FishMet)*

Decision to eat: The modelled fish agent perceives every feed item and makes decision to eat or to ignore it based on its level of *appetite*. All ignored food items are lost (sinking).

Stomach: The consumed feed items transfer to and accumulate in the digestive tract of the model fish. For most fish the first receiving compartment is the stomach, and the current model is built for fish with gastric function. In the stomach, the feed items are subjected to water uptake following the logistic equation:

$$c_i = c_0 + \frac{c_{max} - c_0}{1 + a \times e^{-r \times t_i}}$$

where c_0 is the dry mass of the food item, c_{max} is the mass after water uptake, a and r are the parameters of the logistic equation.

Midgut: Following water uptake, the feed slowly transfers to the midgut. This pattern of the transfer is nonparametric and is defined by a table of experimentally determined reference points with smooth cubic spline interpolation function *I*.

$$c_i = c_{max} \times I(T_n, R_n, t_i)$$

where I defines the proportion of c_{max} remaining in the stomach at the time t_i that is defined by a cubic spline interpolation function (Phillips, 2000) with the grid values: abscissa T_n and ordinate R_n (Input parameters of the model). The interpolant *I* is normally a monotonously and nearly asymptotically decreasing function of time (Fig. 5). Because the pattern is non-parametric—defined by a data-driven interpolation function rather than a specific mathematical equation—any pattern can be easily implemented in the model even without theoretical understanding. This has a significant practical advantage because any pattern observed in fish at the actual farm environment can be easily modelled without regression analysis and need to select the best-fitting model. Yet, any theory-based equation can be easily translated to a set of reference points or (re)programmed directly.

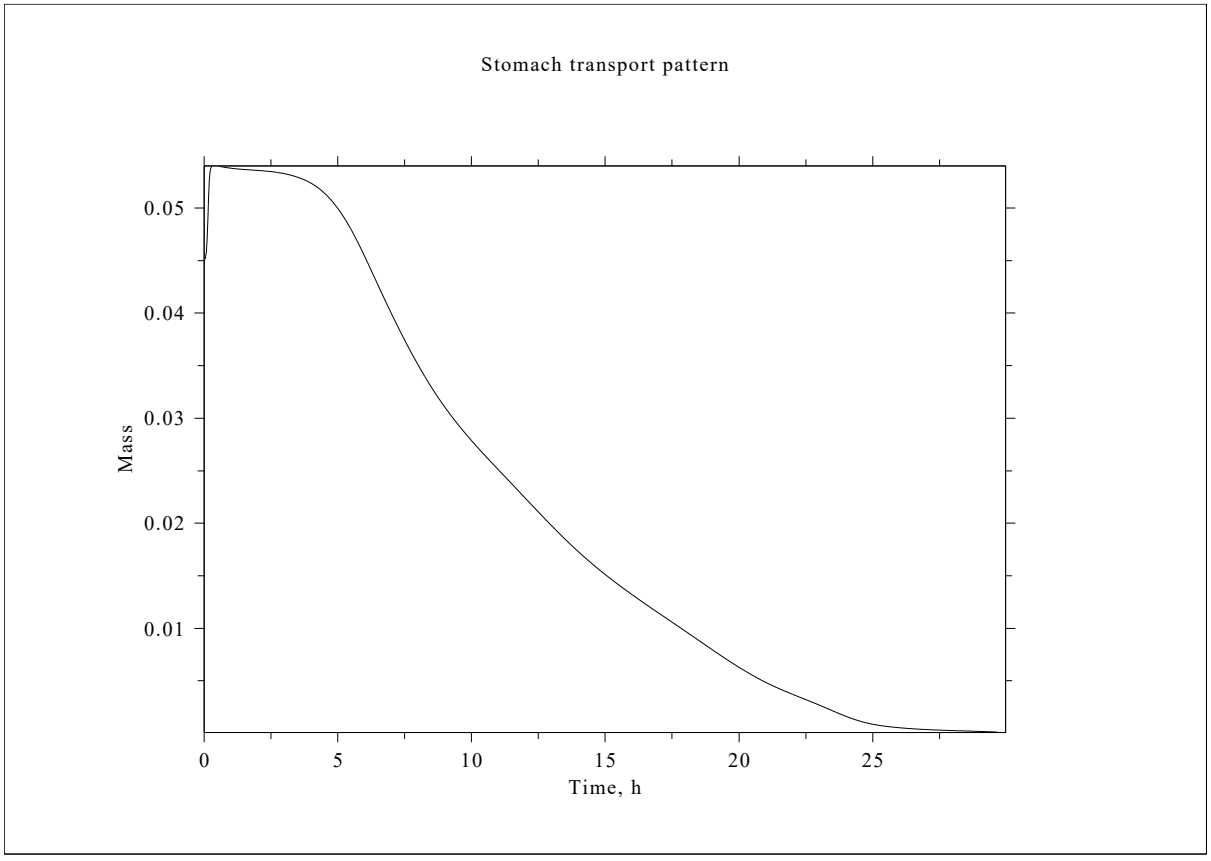


Fig 5. *Processing the feed in the fish stomach (illustrates of the shape of the nonparametric curve)*

Absorption: The food that transfer and is accumulated in the midgut is subjected to nutrient absorption. However, before absorption can occur the chyme (partly degraded feed items from the

stomach), needs to be digested to absorbable units. There is thus a digestion delay δ_d before the absorption process can start. Absorption is described by the following equation, based on the Michaelis-Menten dynamics (Fig. 6):

$$c_{i+1} = c_i - c_i \frac{r_{max} \sum c_i}{K + \sum c_i}$$

Here c_i and c_{i+1} are the mass of the feed item at the time step *i* and *i+1*, $\sum c_i$ accumulated feed mass in the midgut, r_{max} and *K* are the configurable Michaelis-Menten parameters.

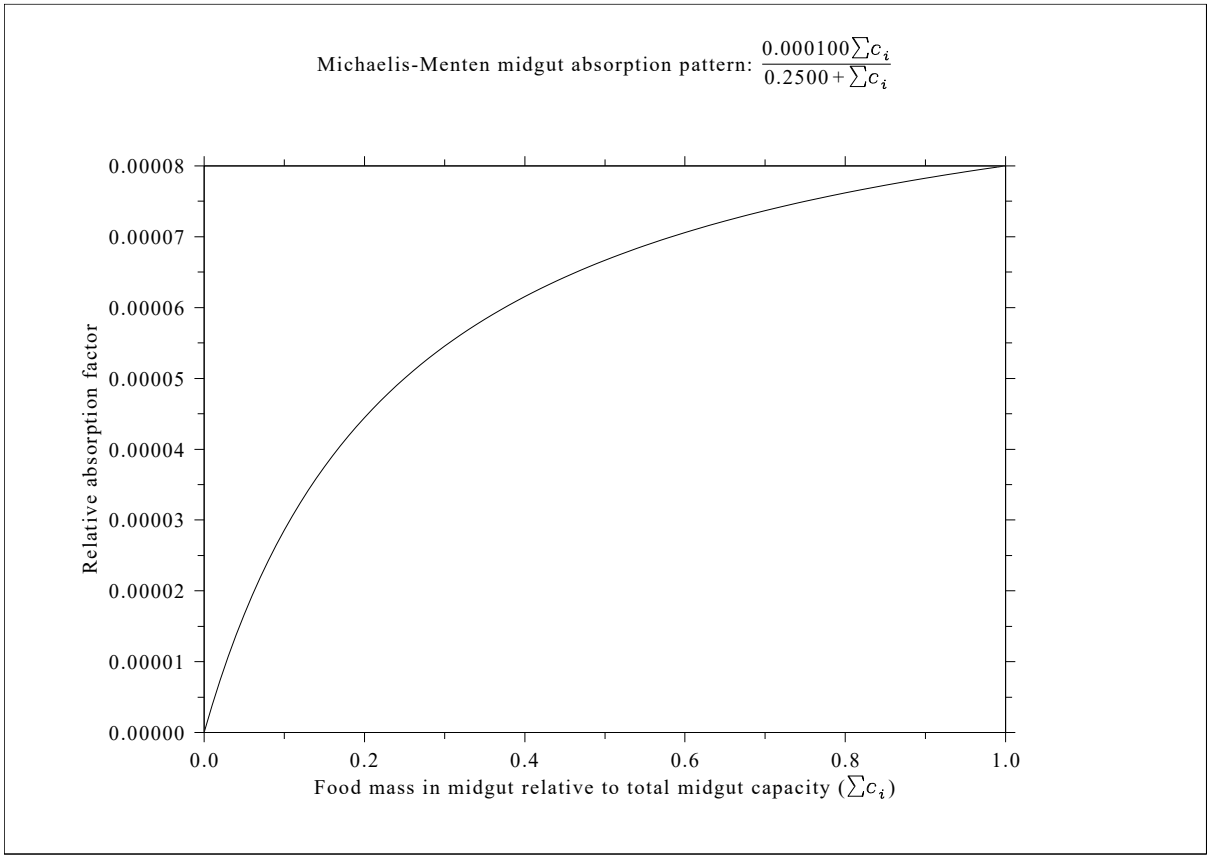


***Fig 6**. Parameters of the feed absorption in the fish midgut (illustrates the shape of the curve)*

The *maximum* extent of absorption is limited by the configurable *digestibility* of the feed (*A*).

Temperature effects: The pattern of food transport is nonparametrically defined by the interpolation over a grid vectors, but it also depends on the fish body mass and temperature. This dependency is again nonparametric and is determined by the stomach emptying matrix $T_{S=0}$ that describes the gastric emptying time for fish of different sizes at a range of temperatures based on empirical data on stomach emptying (e.g., as in Table 1). The adjustment is conducted in the following way. First, the stomach emptying time $\mathrm{T_{S=0}(t, m)}$ is calculated given the specific fish body mass *m* and ambient temperature *t* using cubic spline interpolation over the two dimensions (body mass and temperature). Second, an adjustment factor $\mathrm{a_T}$ is calculated as

$$a_T = \frac{T_{S=0}(t, m)}{Tn^i}$$

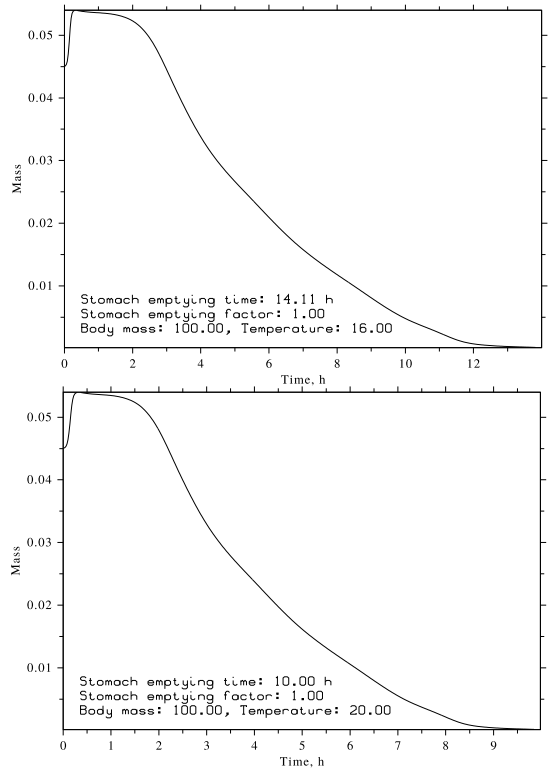


Fig 7. *An example of temperature adjustment for gastric emptying calculated by FishMet*

Table 1. *Example of stomach emptying time (h) data*

T°C	Fish mass			
	50 g	**100 g**	**300 g**	**500 g**
5	24	35	75	100
10	15	20	50	75
15	9	15	40	50
20	5	10	30	35
22	4	8	29	33

where $T_{S=0}(t,m)$ is the estimated stomach emptying time for the fish of mass *m* at the temperature t based on the stomach emptying matrix and Tn^i is the last value of the stomach transport pattern array for time that should correspond to zero amount of food in the stomach ($R_n^i = 0.0$). Finally, the time (abscissa) vector $\mathrm{T_n}$ of the stomach transport pattern is adjusted as $a_T \times T_n$ effectively "stretching" ($a_T > 1$) or "shrinking" ($\mathrm{a_T} < 1$) the time dimension to agree with the stomach emptying time given the fish mass and temperature. This algorithm allows representation of the temperature effects in absence of precise theoretical model solely on empirical (experimental or published) data. An example of such adjustment for stomach transport for 16 and 22 °C is given on Fig. 7 (example plots generated by the FishMet program).

Evacuation: All the food that is fully processed in the stomach and the midgut and have passed through the absorption process and remain in the system for more than a specific time M_{max} are then evacuated.

Activity and locomotion: FishMet assumes that fish locomotion has two major components: (a) the baseline U_b activity (swimming speed) that depends on the diurnal cycle (e.g., low at night, higher during the day) and (b) additional active locomotion/swimming $U_b\alpha_a$, such as increased swimming activity during feeding. Such appetite-linked activity involves the increased activity of the fish when its appetite level is non-zero. Here α_a is the multiplier factor applied to the baseline activity. The relationship between the fish appetite *A* and the factor α_a is defined by the linear equation:

$$\alpha_a = kA + b$$

Energetics: The dynamic energy budget of the fish is conditioned on the absorption calculation at each time step *i*. The overall energy budget of the fish is determined by this equation:

$$E_i = E_{i-1} + \mathrm{F}(\Delta a) - E_{SMR} - E_{AMR}$$

where E_i is the energy balance of the fish at time *i*, $\mathrm{F}(\Delta a)$ is the energy increment obtained from food absorption a, E_{SMR} is the energetic equivalent of the standard metabolic rate (SMR) and E_{AMR} is the energetic equivalent of the active metabolic rate (AMR) adjusted for a time step of the model. SMR in

the rainbow trout is based on the empirical data and cubic spline interpolation following the pattern depicted in Fig. 8.

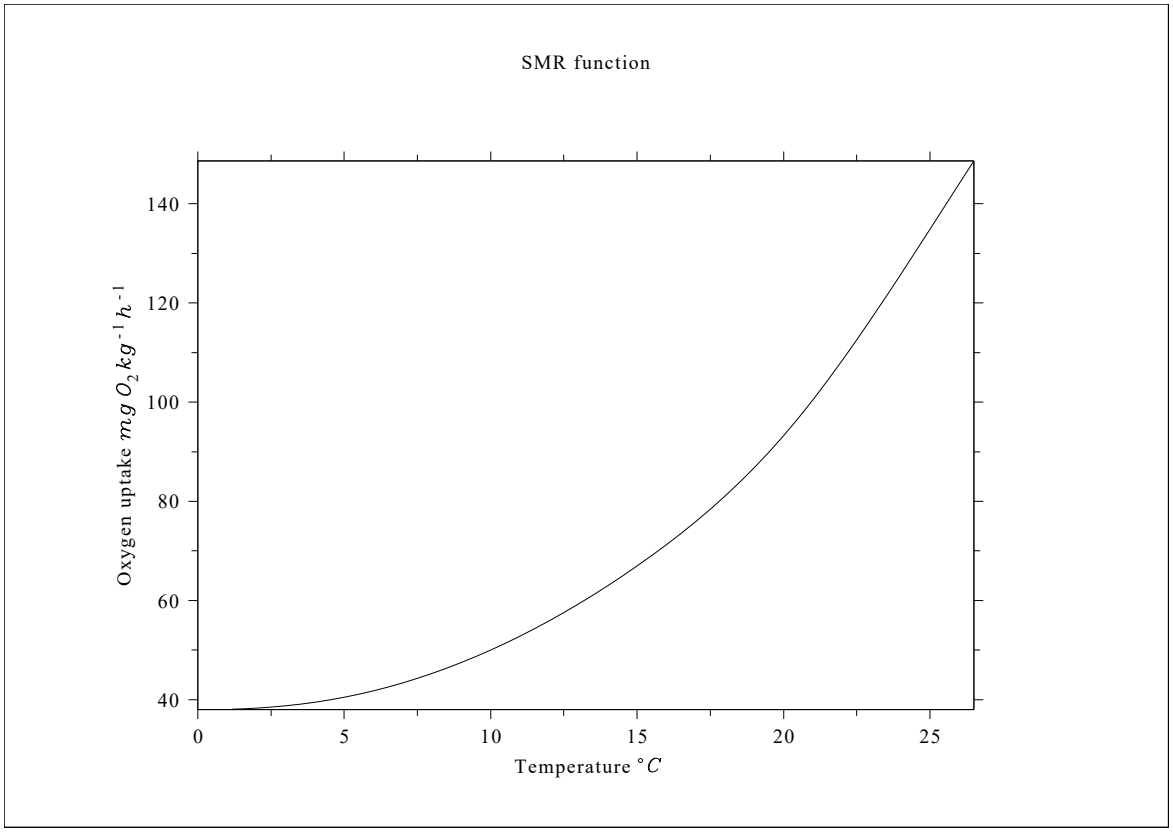


Fig 8. *Standard metabolic rate at different temperatures as modelled in FishMet*

Again, the nonparametric, data-driven nature of the SMR easily allows to adopt any experimental pattern without regression analysis and model selection. Therefore, the model can be easily adjusted to specific farm environment, fish line, etc. AMR is defined by the equation:

$$AMR = aM^bU^c$$

where *M* is the fish mass, *U* is the swimming speed, while *a*, *b*, *c* are model parameters.

Fish oxygen uptake $\mathrm{mg}\ O_2\ \mathrm{kg}^{-1}\ h^{-1}$ is then converted to absolute energy equivalent, kJ for the whole fish with the mass *M*. This conversion is based on computation of the volume of a specific mass of oxygen and energetic equivalent E_{SMR} of a specific volume of oxygen uptake. The AMR is determined by the baseline U_b and appetite-linked $U_b\alpha_a$ activity but can also be provided with an arbitrary pattern (e.g., generated by a helper script in Python or R) allowing to run complex and flexible simulation scenarios. Based on the dynamic energy budget obtained from a specific patterns of food supply, feed consumption and activity pattern, approximate *daily growth* of the fish will be calculated. An example energy budget of a fish is shown on Fig. 9.

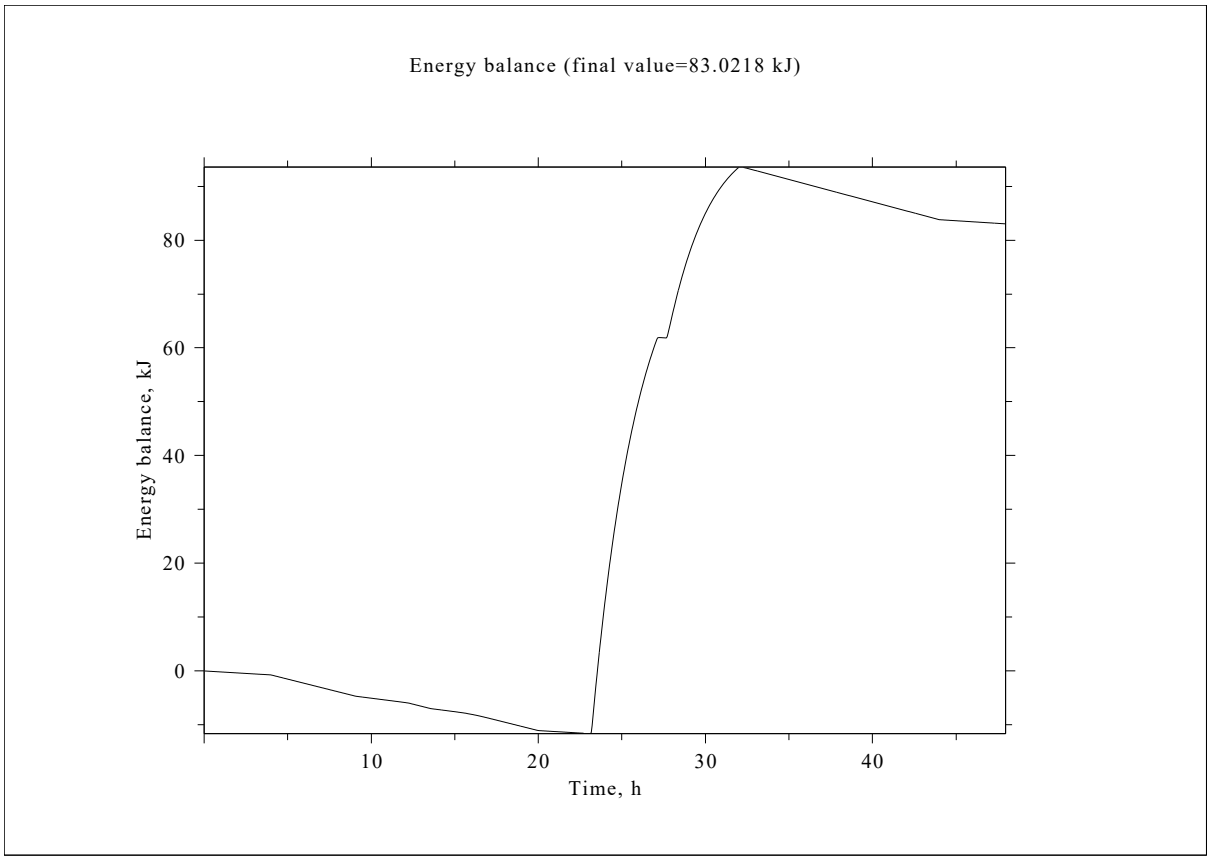


Fig 9. *An example of energy balance plot for a 48h simulation. Note the net positive influx of available energy after feed items are started to be absorbed at ca 23h.*

Appetite: the level of appetite (*A*) determines the probability that the modeled fish makes the decision to consume specific feed item. In the current model version, there are three factors determining the fish appetite: (a) stomach fullness, (b) midgut fullness and (c) overall energy balance (improving or worsening). Respectively, there are three appetite components: α_s. α_m, α_{SMR}.

The stomach and midgut appetite components are calculated based on the logistic equation:

$$1 - \frac{1}{1 + a \cdot e^{-r \cdot m}}$$

where *m* is the relative stomach (or midgut) filling, and *a* and *r* are adjustable parameters. A typical stomach appetite component α_s has the form shown at Fig. 10.

The energy appetite component is defined similarly, using this equation:

$$\frac{1}{1 + e^{-r \cdot (-\Delta_E - b)}}$$

where ΔE is the difference between the two consecutive average energy balance values in units of the SMR, *r* and *b* are adjustable parameters. The overall appetite is determined in such a way that the stomach component exclusively determines it at high stomach fullness (low stomach appetite component α_s) to avoid stomach overfilling:

$$\begin{cases} \alpha_s\ , \alpha_s < \alpha_{min} \\ max(\alpha_s, \alpha_m, \alpha_E) \end{cases}$$

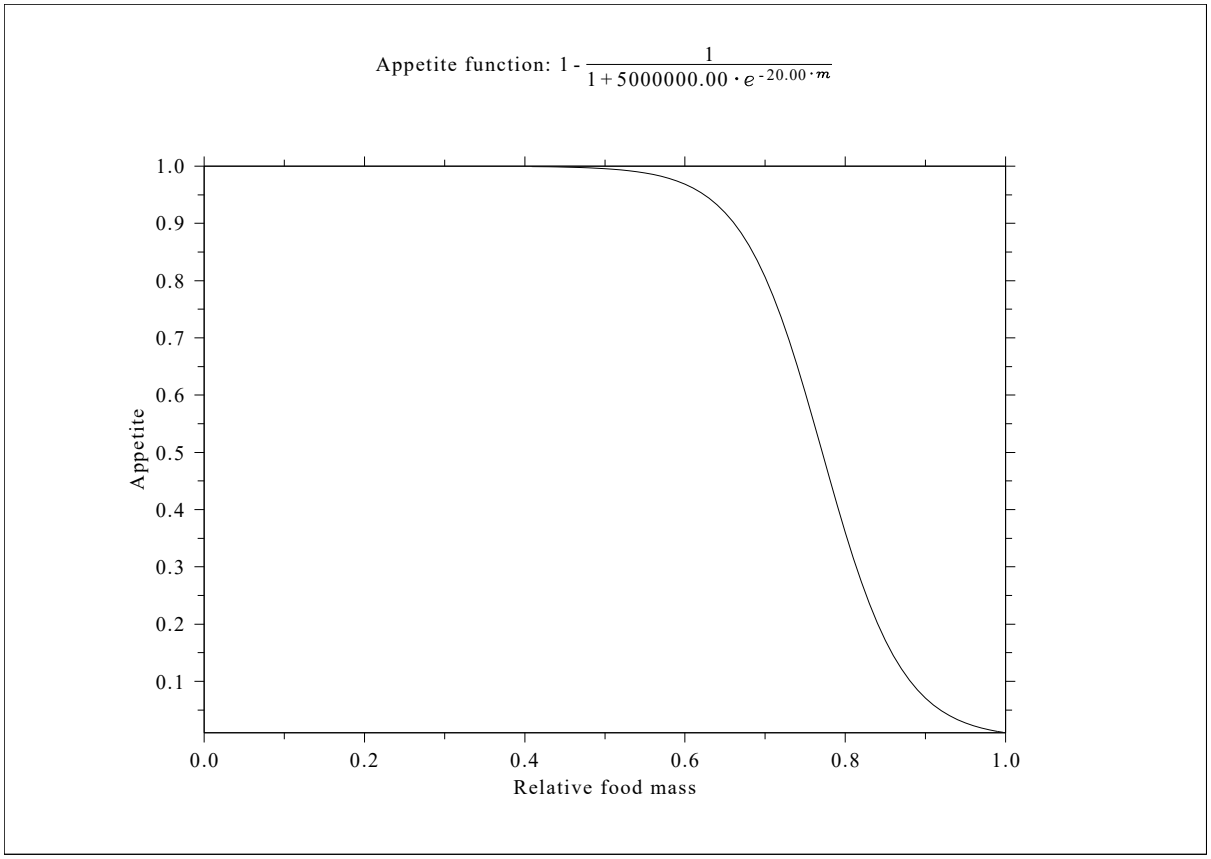


Fig 10. *The shape of the appetite function*

List of input parameters: The most principal parameters of FishMet are listed in Table 2.

Table 2. *Main input parameters of the FishMet model*

m_0	Fish body mass at the start of the simulation, g
S	Stomach filling capacity, g
G	Midgut filling capacity, g
c_0	Dry mass of the feed item, g
G	Gross energy content of the feed, MJ/kg
F	Food input rate during a meal, min^{-1}
Θ	Feeding schedule, a discrete time-based Boolean vector; identifies is food provided (meal) or not
u	Water uptake, relative to c_0, feed item mass after uptake is c_{max}
A	Digestibility, maximum absorption ratio
δ_i	Ingestion delay, time to complete the water uptake, min
δ_d	Digestion delay, time to the start of the absorption in the midgut, min
H	The duration of the simulation, h
t	Ambient water temperature, °C

5. FishMet implementation

○ Design principles

FishMet has been developed using these design principles:

1 It should have minimum software dependencies for the developer, application integration engineer and the end user. For example, it should not require installing other components, such as a language interpreter.
2 It should be portable across computing platforms, operating systems, and environments.
3 It should not depend on a single proprietary technology, thereby avoiding vendor lock-in. For example, open-source components with permissive license should be used. However, these should also be easy to substitute with commercial closed-source alternatives if necessary (e.g., to obtain a high-quality support subscription).
4 It should include an easy-to-use graphical interface, facilitating its use by non-IT-professionals. In particular, no programming experience should be required for simple application and no installation of other software components.
5 It should, simultaneously, make it possible to use FishMet system for complex applications requiring changing parameters and running diverse alternative models repeatedly. This is required for sensitivity analysis, model-free optimization using the Genetic Algorithm, Connectionist models or other machine learning techniques. Thus, the system should also be programmable. Such programmability assumes the software should be possible to run both on a typical desktop system and on a headless high-performance server system. This requires a pure terminal-based command-line interface.
6 Being a complex process simulation model, FishMet should be thoroughly documented, so it is easy to understand how this works and why. Therefore, any modification of the algorithm would be easy. In particular, any configuration files should preferably be in a human-readable plain-text format allowing arbitrary comments for explaining specific parameters, options etc.

○ General architecture

To meet the above design requirements, FishMet is programmed in Fortran 2008, which is a modern object-oriented compiled language. There are several benefits of Fortran over possible alternatives. Fortran is compiled, highly portable, and works on nearly any platform and operating systemt. There are several alternative high-quality compilers, free and commercial. Being a compiled language, Fortran implementations produce highly optimized machine instructions that ensures very fast computation, which is crucial for complex stochastic simulation models. Unlike other languages, modern Fortran natively supports parallel programming. Fortran standard is maintained by an ISO committee, thereby providing extremely long-tern software sustainability, and avoiding vendor lock-in. Finally, Fortran is easy to learn and use to a non-professional novice, much easier than alternative modern compiled languages such as C++. This makes it possible for non-professional programmers to contribute to further development of the code.

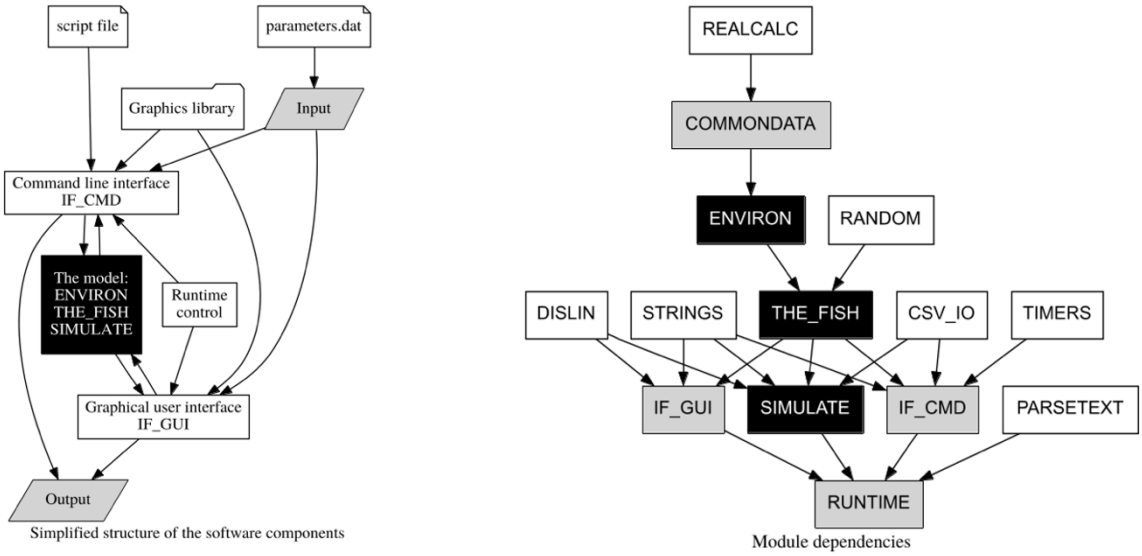


Fig 11. *FishMet- Simplified structure of software components (left) and main modules with their dependencies (right)*

FishMet has been developed to a modular architecture (Fig. 11). The main part of the system is its computational core (The model, black box in Fig. 11 left and black coloured modules in Fig. 11 right). This core is controlled through two different interface modules: the graphical user interface for using in the familiar point and click style (Fig. 12a) and the command line interface, implementing a command processor (Fig. 12b). This command processor can run various complex modelling scenarios using a user-provided batch script file. The default parameters of the model are obtained from the main plain-text configuration file that uses the same notation as the command processor. A portable graphic library, providing presentation-quality plotting capability links to both interface modules.

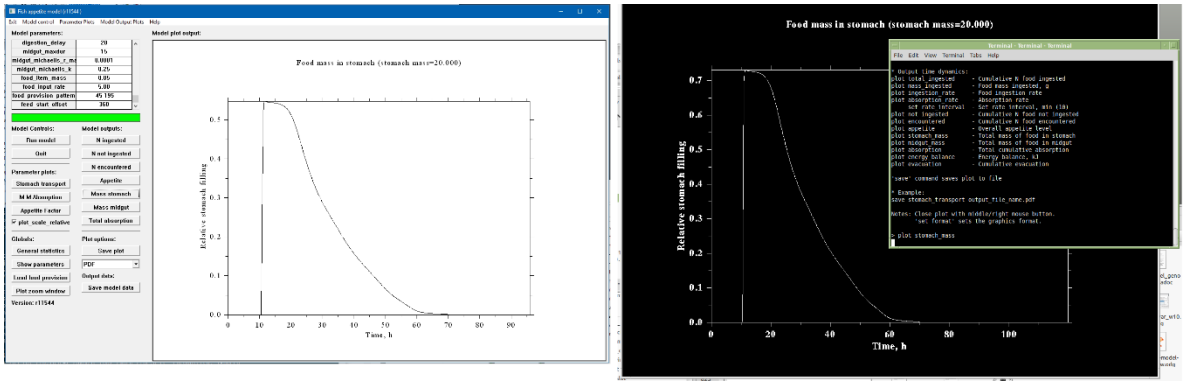


Fig 12. *The two interface models of the FishMet model: easy to use graphical and advanced command-line*

All components of FishMet are highly modular. This makes it easy to validate and test components (Oberkamp & Roy 2010), adapt or extend the software parts for different applications. For example, writing a new interface module using the internal API allows to build FishMet as a shared library to be called from a larger software system written in a different language. Simple applications for this are enabling FishMet simulations to be controlled from an R script or a Python program. The current version implements the executable program rather than callable library.

○ Model documentation.

The model code, especially the computational core, follows the literate programming paradigm (Knuth, 1992). This means that the code includes embedded documentation, explaining all details of the algorithm. The program build system includes several other documentation components, so that a developer documentation, comprehensive user manual and quick help can be automatically extracted and generated in several output formats, notably HTML and PDF. Detailed documentation for the FishMet model, including its description, implementation, and user manual, is developed parallel with the model itself and the computer code. A dedicated FishMet web site is built for this purpose. A working version of all the documentation is available at this link: http://fishmet.uib.no (Fig. 13). This link includes (1) brief man-style user manual on model parameters, configuration format and commands; (2) Detailed FishMet model description and user manual; (3) Detailed developer documentation on model implementation, program modules, file formats, subroutines, API etc.

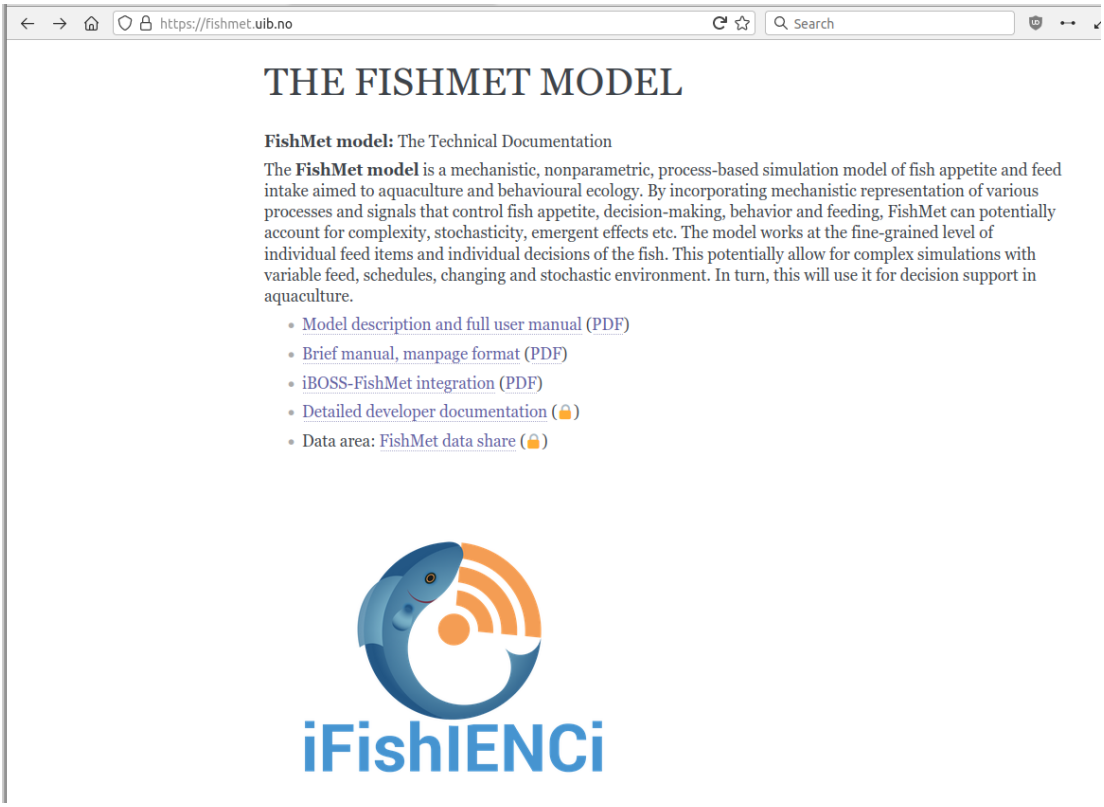


Fig 13. *Screenshot of preliminary FishMet technical documentation web site*

6. Experiments

To support the development of FishMet a set of ongoing experiments are being performed which are exploring relationships between the hunger state of fish and their behaviour.

○ Trial- Different feeding protocols, methods & feed intake analysis

▪ *System configuration*

Rainbow trout (*Oncorhynchus mykiss)* eggs (triploid) were acquired and reared in AquaBioTech (ABT) hatchery facilities according to ABT Standard Operating Procedure and industry standard hatchery conditions for *O. mykiss*. At an approximate mass of 15g/ind., the fish were transferred to a Recirculating Aquaculture System (RAS) which has been modified purposely for the iFishIENCi project. The system (Fig. 14) is fitted with circular 1500L, glass reinforced plastic tanks, heat pumps to maintain

optimal temperatures, overhead adjustable LED lights (9W) with sunrise/sunset simulation and set at a photoperiod of 12:12 light/dark, a Faivre drum filter (60µm mesh) for solids removal, ozonation through a venturi/cone diffuser at a rate of 30g/h for disinfection and disintegration of fine solids, UV irradiation units operating at 285nm and approximately 20mW/cm^2 for microbial inactivation, liquid oxygen injection to ensure adequate dissolved oxygen concentrations, and a protein skimmer to remove proteins, lipids, and other organic particulates.

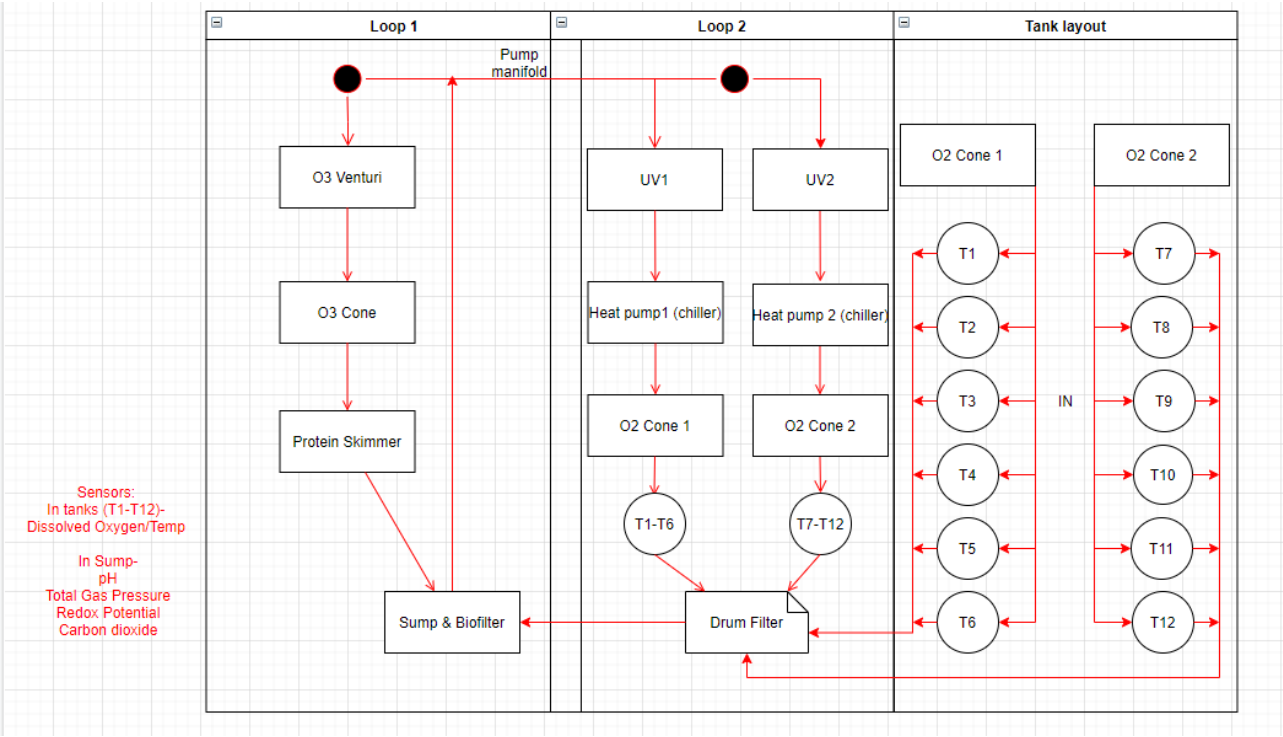


Fig 14. *General schematic of experimental recirculating aquaculture*

- *Methods for quantifying feed intake*

Feed intake was determined using infeed markers and X-radiography, according to the method presented by McCarthy et al. (1993). An extruded, slow-sinking diet (3.0mm) was marked with X-ray opaque, inert, glass Ballotini beads (0.4-0.6mm diameter). The diet was custom formulated and then produced by project partner TTZ Bremerhaven. Counts of beads visible in X-ray images of fish can be used to quantity feed consumption if they are in linear proportion to feed mass. Feed consumption values were estimated according to this method and linearly regressed against the actual mass of feed recovered from the digestive system (Fig. 15). The result R^2 value suggested this condition was met. Radiograph images were produced by exposing fish to X-rays for 30ms at a tube voltage of 50kV (Fig. 16). Counts from radiographs of known amounts of feed were used to produce a calibration line (Fig. 17), from which feed consumption during the ensuing trials was calculated.

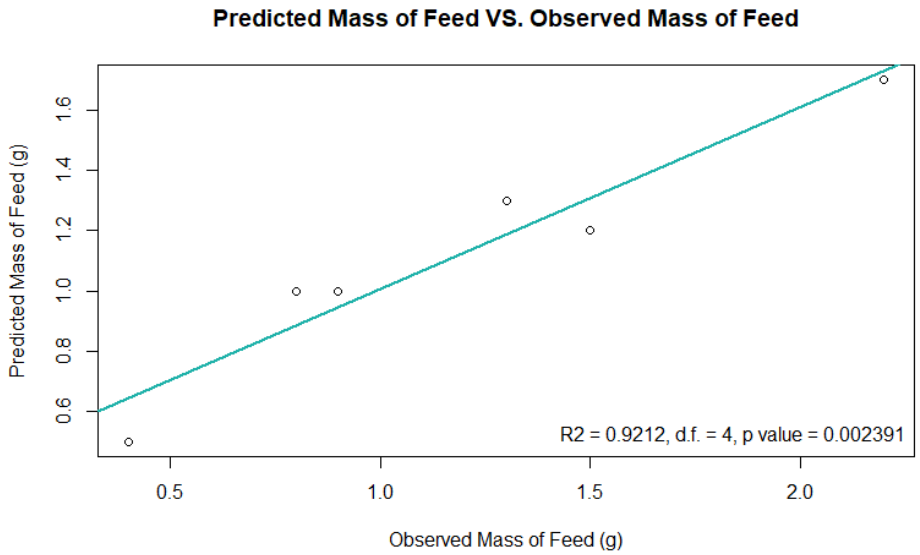


Fig 15. *Estimated feed mass linearly regressed against measured values of consumed feed*

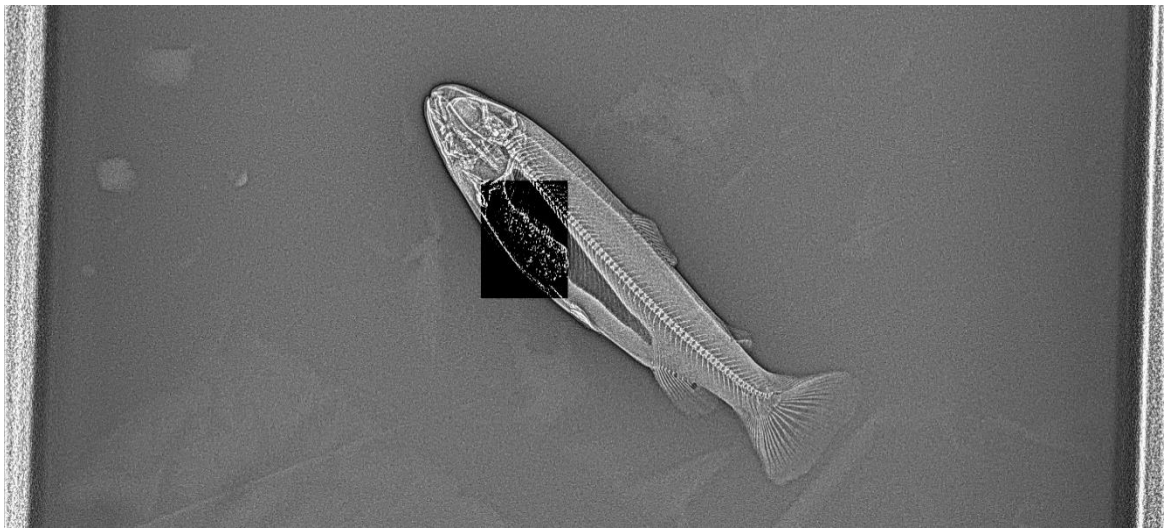


Fig. 16*. Example of a raw (cropped) image radiograph of rainbow trout post-feeding upon the Ballotini labelled diet*

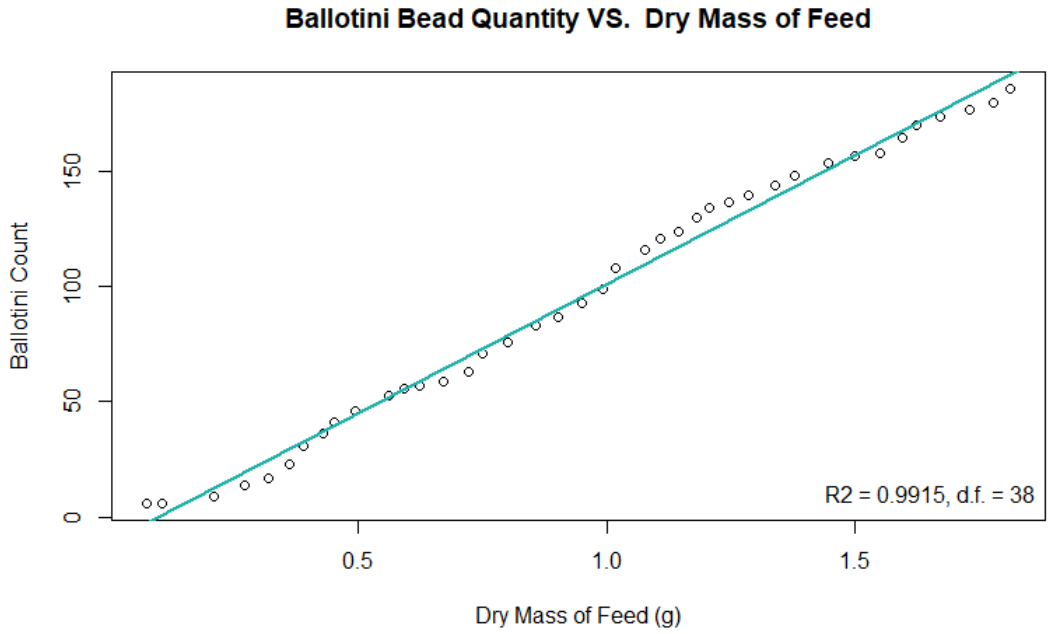


Fig. 17. *Linear regression plot of Ballotini bead count Vs dry mass of feed.*

- *Methods of behavioural analysis*

Pan-tilt-zoom IP cameras have been about the cultivation tanks (Fig. 18). The cameras are network interfaced by a network video recorder (HIKVision Embedded NVR DS-7600, Hangzhou Hikvision Digital Technology Co. Ltd, Hangzhou, China). During the experiments, feed was dispensed via two rotating drum, automatic feeders, placed on either side of the tank.

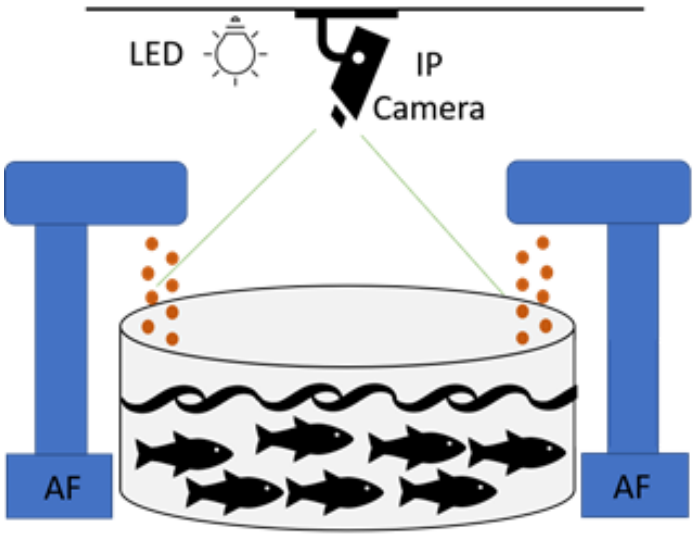


Fig. 18*. Diagram of experimental setup for behavioural analysis using IP camera*

Machine learning algorithms were trained (by iFishIENCi partner Bioceanor) to automatically target and track fish in video recording taking before, during, and after feeding events (Fig. 19). The training process was repeated until the average recognition rate was above 30% of the total number of fish.

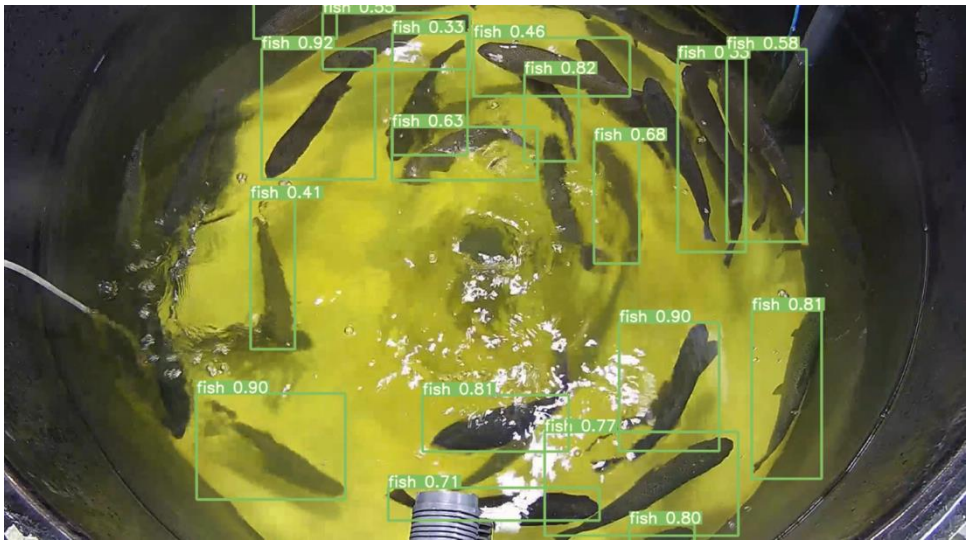
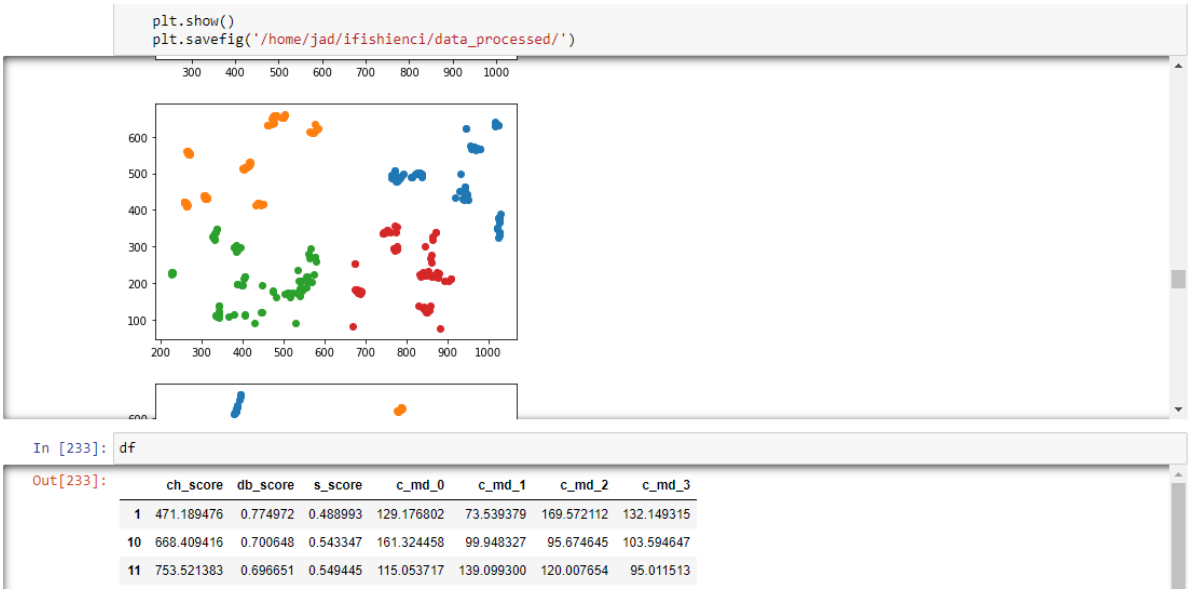


Fig. 19. *Example of a video frame extracted for training the machine learning algorithms*

The trials were designed in accordance with the aim of characterising clustering among fish relative to the presence or absence of satiation. Clustering is one of many potentially quantifiable behaviours and occurs at the group level. It was characterised for the first round of trials as the aggregation of individuals below feed dispensers is visually apparent prior to feeding events among fish conditioned to receiving meals at set times. This suggests it may be a useful component of a behaviour observation-based approach towards optimising feeding. It was also chosen because various methods for its determination are available, and algorithms were being developed by Bioceanor for applying these methods to video records of fish (Fig. 20).



	ch_score	db_score	s_score	c_md_0	c_md_1	c_md_2	c_md_3
1	471.189476	0.774972	0.488993	129.176802	73.539379	169.572112	132.149315
10	668.409416	0.700648	0.543347	161.324458	99.948327	95.674645	103.594647
11	753.521383	0.696651	0.549445	115.053717	139.099300	120.007654	95.011513

Fig. 20. *Example of a clustering plot generated as an output of the assessment using the k-means clustering algorithm.*

Behaviour was quantified using K-means clustering algorithms, which use vector quantisation to subset observed fish into clusters. Calinki-Harabasz (CH) scoring was then used to determine the relative distance between fish, both within and between clusters. High CH scores indicate high levels of clustering.

Radiograph images were stored a DICOM images, and the red-orange-yellow-white spectrum was edited using the open-source viewing and editing program, ezDICOM (Medical Viewer, Revision 16; Krug and Rorden, 2002). This improved clarity and emphasized contrast, enabling the expedient identification of beads. Images were then analyzed in the browser-supported version of the ImageJ software (ImageJ.JS v.0.3.13; ImJoy, 2020). The stomach region was delineated using ImageJ software and beads were manually counted using the multi-point counter tool. Four, fifteen second video segments were extracted taken from before, during and after feeding events using RStudio and the splitVideo function in the pathtrackr package (Harmer and Thomas, 2019).

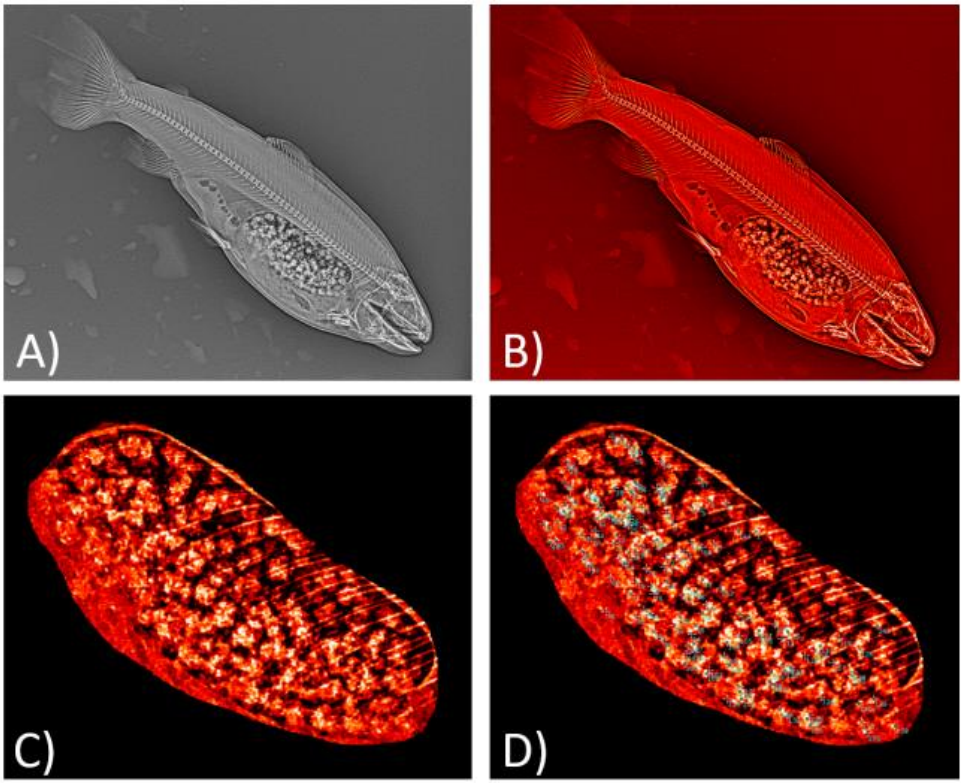


Fig. 21. *a) raw DICOM image, b) Colour scheme adjustment to enhance bead resolution, c) Stomach cut, d) Stomach cut with count markers*

- *Experimental protocol*

A total of three investigating feeding behaviour and stomach fullness was conducted according to the plan outlined in Table 3. Fish were conditioned to the feeding regime for one week and then starved for 24 hours prior to each experiment.

During the first experimental, behaviour was recorded for 45 minutes before feed was administered. Fish were fed to apparent satiation with the Ballotini labelled diet and behaviour continued to be recorded for 45 minutes following feeding cessation. Fish were radiographed immediately after the recording session ended. For the second experimental trial, fish were fed to satiation with an unlabelled diet. Radiography occurred after a second feeding event using the marked diet, and which

took place 6 hours after the first feeding ended. During the third experiment, fish were fed with unlabelled feed at the first feeding event and the second, which occurred 3 hours later. A further feeding event using the marked diet occurred 3 hours after the second, followed by X-radiography.

Table 3. *Trial plan designed to collect data for the FishMet model pertaining to very hungry fish (exp.1), moderately hungry fish (exp. 2) and fish in a low hunger state (exp.3)*

Experimental Plan		
Experiment 1	**Experiment 2**	**Experiment 3**
Record 45 min before feeding	Feed to apparent satiation (unlabelled diet)	Feed to apparent satiation (unlabelled diet)
Feed to apparent satiation (labelled diet)	2.) 6-hour digestion	2.) 3-hour digestion
Record 45 min after feeding	3.) Record 45 min before feeding	3.) Feed to apparent satiation (unlabelled diet)
Radiograph fish	Feed to apparent satiation (labelled diet)	4.) 3-hour digestion
	5.) Record 45 min after feeding	5.) Record 45 min before feeding
	6.) Radiograph fish	6.) Feed to apparent satiation (labelled diet)
		7.) Record 45 min after feeding
		8.) Radiograph fish

- *Results*
  - *Feed intake*

A trend of decreasing feed consumption as the frequency of feeding events increased was observed. Fish in experiment 1 consumed a mean (± s.d.) of 409 ballotini beads (± 138 beads; min = 131 beads, max = 739 beads). This corresponded to an estimated mean of 43 g of dry feed eaten per fish (± 14 g; min = 14 g, max = 77 g) contributing to 6.30% of fish body weight (± 2.36%; min = 2%, max = 12%). At Fish in Experiment 2 consumed a mean of 162 beads (± 34 beads; min = 106 beads, max = 252 beads) during the second meal. This represented an estimated mean of 17 g of eaten feed (± 4 g; min = 11 g, max = 26 g) responsible for 2.05 % of fish body weight (± 0.4%; min = 1%, max = 3%). Mean feed consumption during the final feeding event was 134 beads (± 68 beads; min = 39 beads, max = 257 beads). This corresponded to an estimated mean of 14 g of eaten feed (± 7 g; min = 4 g, max = 27 g) and 1.85% of fish body weight (± 1.14%; min = 0%, max = 4%).

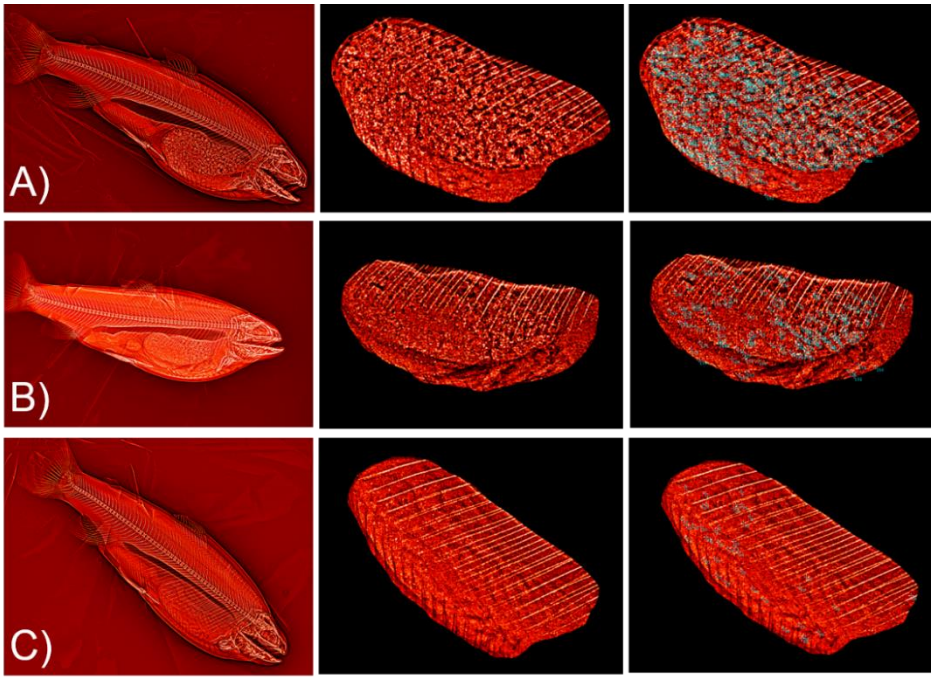


Fig. 22. *Edited radiographs, uncounted stomach images, and stomach images with completed bead counts (in blue) of fish sampled during experiment one (A), experiment two (B), and experiment 3 (C). The fish shown in A) consumed 468 beads or 49 g of feed worth 5% of BW; the fish in B) consumed 148 beads or 15 g of feed worth 2% of BW; and the fish in C) consumed 39 beads or 4 g of feed worth <1% of BW.*

Individual feed intake during experiment 1 and 2 fitted a normal distribution, and a binomial distribution in experiment 3. Differences between the consumption values of each experiment were found to be significant (trial 1 median = 44 g; trial 2 median = 16 g; trial 3 median = 12 g; $\chi 2 = 34.44$; d.f. = 2; p-value = 3.31 *10-8) when compared using a Kruskal-Wallis test.

Clustering appears to be a useful indicator of meal anticipation. However, anticipation is associated with conditioning to fixed feeding times and does not necessarily indicate hunger. Other behavioural indicators, such as aggression (occurrence frequency of distinct agnostic behaviours) and swimming speed, will now be assessed alongside continued analysis of clustering. Presentation of such behaviours will be analysed in terms of their relationship with feeding anticipation (anticipation being the consequence of conditioning) and relative hunger state, a proxy of which is assumed to be stomach fullness. Through a process of gradual experimentation and refinement, it is hoped that more succinct behavioural indicators of hunger state can be defined and subsequently identified in video images by machine learning algorithms. These indicators may be observable at the level of individual fish, the population, or both.

Table 4. *Descriptive statistics of feed intake dynamics acquired from radiography analysis of fish sampled during Experiment 1*

Descriptive Statistics of Experiment 1 Feed Intake Dynamics			
	Number of Beads Observed	**Estimated Mass of Eaten Feed (g)**	**Estimated Feed Percent of Fish Body Weight (%)**
Mean (**x̄**)	409	42	6.30
Standard Deviation (**σ**)	138	14	2.36
Standard Error of the Mean (**SEM**)	30.87	3.20	0.53
Coefficient of Variation (**CV**)	0.34	0.34	0.37

Table 5. *Descriptive statistics of feed intake dynamics acquired from radiography analysis of fish sampled during Experiment 2*

Descriptive Statistics of Experiment 2 Feed Intake Dynamics			
	Number of Beads Observed	**Estimated Mass of Eaten Feed (g)**	**Estimated Feed Percent of Fish Body Weight (%)**
Mean (**x̄**)	162	17	2.05
Standard Deviation (**σ**)	34	4	0.40
Standard Error of the Mean (**SEM**)	7.81	0.82	0.09
Coefficient of Variation (**CV**)	0.21	0.21	0.20

Table 6. *Descriptive statistics of feed intake dynamics acquired from radiography analysis of fish sampled during Experiment 3*

Descriptive Statistics of Experiment 3 Feed Intake Dynamics			
	Number of Beads Observed	**Estimated Mass of Eaten Feed (g)**	**Estimated Feed Percent of Fish Body Weight (%)**
Mean (**x̄**)	134	14	1.85
Standard Deviation (**σ**)	68	7	1.14
Standard Error of the Mean (**SEM**)	15.28	1.60	0.25
Coefficient of Variation (**CV**)	0.51	0.51	0.61

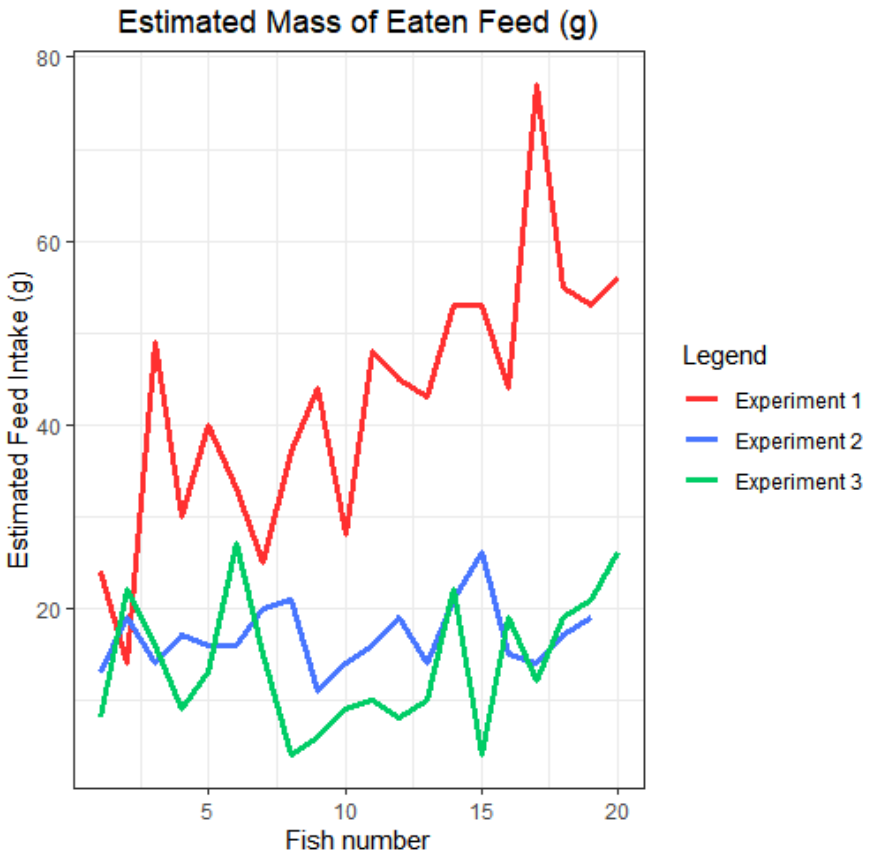


Fig. 23. *Line graph showing feed intake mass for each of the 20 fish in experiments 1, 2 & 3*

○ Trial- Feed intake vs temperature

The aim of the experiment was to determine the optimal feeding strategies in rainbow trout at three different temperatures (16±1°C; 18±1°C and 20±1 °C) through a gut transit study after starvation over varied time frames. The data set was used to calibrate the FishMet model for rainbow trout to different temperature

The trial was conducted at ABT facility. During phase 1 of the experiment, a total of 200 rainbow trout of about 100g were placed in a 650L cultivation tank and acclimatized for one week to the test temperature and hand-fed at apparent satiation with a control diet.

Two experimental diets (YTOX and CTRL) were formulated, to be isonitrogenous, isolipidic and isoenergetic. Yttrium oxide was added to the diet as an inert tracer (0.2% inclusion level), that is not absorbed, follows the transit of the digesta and can be analysed in the feed and faeces at low inclusion levels (Sorensen et al., 2002). This method is in use as routine for digestibility trials in feeding companies.

On the sampling day, fish were fed twice until apparent satiation with YTOX at 9:00 a.m. and with CTRL at 2:00 p.m (See *Table 7*).

The sampling was performed at three different times as specified in the table below. A total of 6 fish at each sampling point were netted into a bucket of fresh water and killed with an overdose of tricaine, followed by biometrics recording (Individual weight, total and fork length) and dissection.

The digestive tract was split in 3 parts (stomach; anterior + mid intestine, and posterior intestine) with a surgical ligature to avoid any migration of material. Each region was gently squeezed to remove the content, subsequently weighed, and stored in a separate container.

Phase 3 of the experiment was conducted 24h after Phase 2, and 6 fish were euthanized before feeding, following the same procedure previously described.

Samples were stored at -20 degree until further analysis.

At the end of each experiment fish were replaced in the cultivation tank, re-establishing the original number and the new temperature set to start the acclimation period of one week.

Each sample was dried for 24h in an oven at 60 °C and weighed. Data are shown in Tables 8-10. The wet and dry weight data were used for calibrating the FishMet model. Chemical analysis (Yttrium oxide) is still pending due to further and ongoing refinement of methodology to increase sensitivity at low volume samples in our lab.

Table 7. *Trial plan designed to collect temperature related data for the FishMet model*

Experimental Plan		
Experiment 1	**Experiment 2**	**Experiment 3**
Phase 1	**Phase 1**	**Phase 1**
Temperature set at 16±1°C	Temperature set at 18±1°C	Temperature set at 20±1°C
1 week acclimation	1 week acclimation	1 week acclimation
Phase 2	**Phase 2**	**Phase 2**
Sampling day: fish fed at apparent satiation at 9:00 a.m. with the test diet (YTOX)	Sampling day: fish fed at apparent satiation at 9:00 a.m. with the test diet (YTOX)	Sampling day: fish fed at apparent satiation at 9:00 a.m. with the test diet (YTOX)
Sampling after 20min; 1 hour; 2 hours	Sampling after 20min; 1 hour; 2 hours	Sampling after 20min; 1 hour; 2 hours
Sampling day: fish fed to apparent satiation at 2:00 p.m. with the control diet (CTRL)	Sampling day: fish fed to apparent satiation at 2:00 p.m. with the control diet (CTRL)	Sampling day: fish fed to apparent satiation at 2:00 p.m. with the control diet (CTRL)
Sampling after 20min; 1 hour; 2 hours	Sampling after 20min; 1 hour; 2 hours	Sampling after 20min; 1 hour; 2 hours
Starvation	Starvation	Starvation
Phase 3	**Phase 3**	**Phase 3**
Sampling at 9:00 a.m. prior feeding	Sampling at 9:00 a.m. prior feeding	Sampling at 9:00 a.m. prior feeding
Replacement of sampled fish	Replacement of sampled fish	NA

- *Results*

Table 8. *Biometrics and gut content acquired from fish sampled during Experiment 1 at* ***16°C***. Gut content weight after 24 h drying at 60°C

Time frames	N fish	Weight (g)	TL (cm)	FL (cm)	Stomach content (g)	AI+MI content (g)	PI content (g)	Total content (g)
AM sampling								
20 minutes post feeding	1	100	19.2	18.9	1.75	0.02	0.08	1.85
	2	134	20.5	20.2	4.33	0.02	0.07	4.42
	3	126	21.5	20.3	1.01	0.07	0.06	1.14
	4	108	19.3	19.0	4.20	0.12	0.08	4.40
	5	118	19.7	19.5	0.03	0.10	0.09	0.22
	6	98	18.3	17.6	0.12	NA	NA	0.12
1 hour post feeding	1	164	23.2	22.5	3.17	0.04	0.09	3.30
	2	130	21.7	21.5	1.95	0.06	0.05	2.06
	3	110	20.6	20.2	2.26	0.07	0.06	2.39
	4	104	19.5	19.2	2.19	0.05	0.02	2.26
	5	108	20.4	20.0	1.78	0.04	0.04	1.86
	6	102	19.8	19.5	0.04	0.05	0.05	0.13
2 hours post feeding	1	128	21.1	20.6	1.76	0.05	0.04	1.85
	2	102	19.7	19.5	0.09	0.02	0.02	0.13
	3	118	21.6	21.2	0.92	0.08	0.07	1.06
	4	116	20.5	19.8	1.18	0.05	0.06	1.30
	5	100	19.5	19.2	0.49	0.06	0.06	0.62
	6	146	22.0	21.5	NA	0.07	0.09	0.16
PM sampling								
20 minutes post feeding	1	128	20.9	20.5	2.73	0.07	0.06	2.86
	2	92	19.5	18.8	1.69	0.06	0.05	1.80
	3	76	18.2	17.7	1.62	0.10	0.07	1.79
	4	130	20.5	20.0	2.35	0.11	0.06	2.53
	5	142	20.0	19.5	3.92	0.10	0.21	4.23
	6	144	20.5	20.0	3.36	0.32	0.20	3.88
1 hour post feeding	1	110	20.3	20.1	2.12	0.08	NA	2.19
	2	186	23.5	23.0	3.70	0.17	0.18	4.04
	3	184	23.6	23.5	1.25	0.08	0.05	1.38
	4	128	20.5	20.3	1.82	0.08	0.08	1.98
	5	154	22.0	21.3	1.51	0.06	0.05	1.62
	6	118	19.6	19.3	NA	0.26	0.13	0.39
2 hours post feeding	1	120	20.6	20.2	0.50	0.07	0.03	0.60
	2	146	21.8	21.1	0.39	0.10	0.09	0.59
	3	134	20.8	20.0	1.07	0.11	0.08	1.25
	4	132	21.0	20.7	1.32	0.08	0.10	1.50
	5	154	21.3	21.8	3.35	0.19	0.16	3.70
	6	132	20.8	21.3	1.56	0.18	0.03	1.77
AM sampling (24 h after first sampling)								
before feeding	1	134	21.5	21.1	0.02	0.09	0.15	0.26
	2	114	20.7	20.5	NA	0.01	0.06	0.07
	3	194	18.9	18.4	NA	NA	NA	0.00
	4	132	21.5	21.2	NA	0.03	0.05	0.07
	5	114	20	19.8	0.24	0.09	0.09	0.41
	6	162	21.7	21.3	NA	0.08	0.12	0.20

Table 9. *Biometrics and gut content acquired from fish sampled during Experiment 2 at* ***18°C***. Gut content weight after 24 h drying at 60°C

Time frames	N fish	Weight (g)	TL (cm)	FL (cm)	Stomach content (g)	AI+MI content (g)	PI content (g)	Total content (g)
AM sampling								
20 minutes post feeding	1	166	22.5	21.8	2.27	0.06	0.09	2.42
	2	142	21.7	21.0	2.81	0.02	0.04	2.87
	3	158	22.2	21.6	2.30	0.09	0.04	2.43
	4	106	19.0	18.6	1.71	0.05	0.11	1.87
	5	186	23.0	22.5	2.90	0.03	0.14	3.07
	6	172	22.4	22.0	2.74	0.09	0.12	2.95
1 hour post feeding	1	176	23.1	22.5	4.19	0.02	0.06	4.27
	2	126	20.7	20.1	1.45	0.02	NA	1.47
	3	130	21.5	20.8	2.73	0.04	0.05	2.82
	4	176	22.6	21.8	3.22	0.03	0.11	3.36
	5	170	21.9	21.4	2.31	0.12	0.14	2.57
	6	176	23.9	22.6	1.39	0.08	0.08	1.55
2 hours post feeding	1	140	21.4	21.0	3.90	0.22	0.04	4.16
	2	204	23.4	22.7	3.04	0.12	0.10	3.26
	3	185	23.5	22.8	0.72	0.03	0.02	0.77
	4	154	21.8	21.5	4.97	0.16	0.09	5.22
	5	144	21.4	20.7	0.88	0.04	0.03	0.95
	6	204	24.1	23.7	3.16	0.08	0.12	3.36
PM sampling								
20 minutes post feeding	1	146	21.6	21.3	0.62	0.09	0.08	0.79
	2	178	22.5	22.0	4.04	0.04	0.16	4.24
	3	144	21.4	20.8	2.44	0.10	0.07	2.61
	4	184	23.3	22.6	2.40	0.13	0.16	2.69
	5	132	20.4	19.7	2.12	0.08	0.06	2.26
	6	154	22.2	21.8	1.15	0.11	0.09	1.35
1 hour post feeding	1	162	21.9	21.4	1.73	0.14	0.03	1.90
	2	196	23.7	23.1	1.92	0.13	0.11	2.16
	3	158	22.6	22.3	0.87	0.05	0.06	0.98
	4	146	20.4	20.1	2.38	0.09	0.09	2.56
	5	114	19.8	19.3	0.23	0.02	0.07	0.32
	6	130	20.5	20.0	0.39	0.29	0.17	0.85
2 hours post feeding	1	196	23.4	23.0	2.03	0.13	0.18	2.34
	2	193	22.8	22.4	0.44	0.16	0.12	0.72
	3	146	21.8	21.4	0.58	0.08	0.14	0.80
	4	196	22.6	22.3	0.46	0.08	0.15	0.69
	5	172	22.7	22.1	1.15	0.09	0.20	1.44
	6	155	22.3	21.6	2.43	0.08	0.12	2.63
AM sampling (24 h after first sampling)								
before feeding	1	124	19.5	19.2	NA	0.01	0.04	0.05
	2	130	19.6	19.3	NA	0.04	0.07	0.11
	3	136	19.4	19.0	NA	0.06	0.06	0.12
	4	142	20.5	20.0	0.05	0.05	0.08	0.18
	5	144	21.2	20.6	NA	0.02	0.04	0.06
	6	134	20.3	20.0	NA	0.05	0.12	0.17

Table 10. *Biometrics and gut content acquired from fish sampled during Experiment 3 at* ***20°C***. Gut content weight after 24 h drying at 60°C

Time frames	N fish	Weight (g)	TL (cm)	FL (cm)	Stomach content (g)	AI+MI content (g)	PI content (g)	Total content (g)
AM sampling								
20 minutes post feeding	1	172	22.9	22.5	2.62	0.02	0.10	2.74
	2	198	23.3	22.6	2.71	0.02	0.02	2.75
	3	220	23.8	23.1	1.75	0.11	0.07	1.93
	4	192	24.0	23.4	2.12	0.02	0.08	2.22
	5	94	18.5	17.9	3.79	0.02	0.03	3.84
	6	150	21.5	20.9	1.79	0.08	0.14	2.01
1 hour post feeding	1	182	33.4	32.8	1.58	0.02	0.04	1.64
	2	168	23.1	22.5	1.35	0.08	0.08	1.51
	3	136	20.9	20.3	2.80	NA	0.08	2.88
	4	278	25.1	25.1	4.44	0.09	0.19	4.72
	5	194	23.3	23.3	1.77	0.03	0.07	1.87
	6	142	21.6	21.3	3.65	0.06	0.04	3.75
2 hours post feeding	1	164	22.7	22.3	0.76	0.01	0.02	0.79
	2	182	22.8	22.4	3.51	NA	0.08	3.59
	3	172	22.7	22.0	3.05	NA	0.10	3.15
	4	144	20.9	20.9	1.04	0.03	0.12	1.19
	5	332	27.3	27.3	NA	0.04	0.13	0.17
	6	156	21.3	21.3	0.62	0.05	0.03	0.70
PM sampling								
20 minutes post feeding	1	180	22.5	22.0	0.83	0.02	0.06	0.91
	2	170	22.0	21.6	1.31	0.02	0.05	1.38
	3	178	22.4	22.0	1.50	0.06	0.17	1.73
	4	172	22.9	22.3	0.20	0.06	0.12	0.38
	5	134	21.3	20.8	1.20	NA	0.06	1.26
	6	186	23.0	22.4	1.68	0.09	0.07	1.84
1 hour post feeding	1	220	25.3	24.8	1.99	0.14	0.18	2.31
	2	186	23.4	22.8	0.79	0.08	0.11	0.98
	3	180	22.6	22.1	1.78	0.04	0.14	1.96
	4	164	21.6	21.2	1.85	0.11	0.11	2.07
	5	204	23.9	23.4	3.00	0.09	0.19	3.28
	6	160	22.3	21.8	2.56	0.07	0.08	2.71
2 hours post feeding	1	192	23.0	22.4	1.92	0.03	0.07	2.02
	2	152	21.3	20.7	0.25	0.06	0.10	0.41
	3	168	22.7	22.1	0.80	NA	0.03	0.83
	4	204	22.9	22.3	1.46	0.06	0.03	1.55
	5	92	18.0	17.4	0.08	0.01	0.05	0.14
	6	124	20.4	19.8	0.24	0.01	0.03	0.28
AM sampling (24 h after first sampling)								
before feeding	1	186	24.3	23.9	NA	0.01	0.11	0.12
	2	156	22.5	22.1	0.01	0.01	0.05	0.07
	3	132	20.5	19.8	NA	0.02	0.15	0.17
	4	190	24.0	23.4	NA	0.03	0.11	0.14
	5	184	23.2	22.5	NA	NA	0.01	0.01
	6	160	22.2	21.6	NA	0.02	0.06	0.08

7. Integration of FishMet with iBOSS and its use in commercial operations

Data produced and consumed by FishMet: FishMet accepts data on the environment conditions, the feeding protocol, various parameters of the fish morphology and physiology. The output of the model is then the pattern of the fish feeding behaviour (e.g., ingesting food items), its internal state (e.g., the level of appetite) and parameters of the gastrointestinal system functioning (e.g., stomach and gut fullness, absorption, evacuation of faeces). There are also approximations for growth, FCR, and oxygen uptake. Due to the stochastic simulation nature of the model can, any kind of realistic stochastic input can be used, notably individual and group differences between the fish

Data exchanged with the iBOSS cloud during development phase: FishMet can input data on characteristics of the food (e.g., size, gross energy), the feeding protocol, basic biological characteristics of the fish (including group and individual variability) as well as key environmental data (temperature and oxygen). Then it will output prediction data on feed intake, activity, individual internal state (e.g., appetite) energetic status and growth.

Data exchanged with the iBOSS cloud during operational phase (to provide recommendations and decision support): iBOSS system can send the data on the environment (e.g., physical conditions like temperature and dissolved oxygen, feeding protocol etc.) and in return obtain model predictions on appetite, feeding behaviour of the fish, feed conversion ratio, waste (uneaten food) and physiological state. Any stochastic data can be used, making it possible to test diverse scenarios, even those that are difficult or impossible to implement through experimentations.

FishMet computational server. A Linux-based computational server has been set up using the NREC IaaS cloud infrastructure. It communicates with the iBOSS via an authenticated https and secured connection (ssh/sftp).

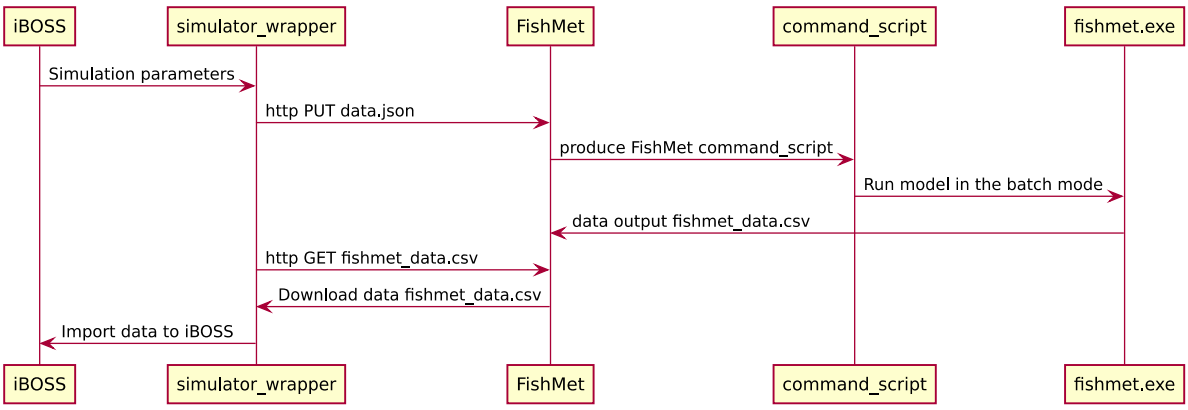


Fig 24. Sequence diagram showing the integration of iBOSS and FishMet

The flow of the interaction: A common http and sftp-based API (application programming interface) was devised for interacting the iBOSS system with the FishMet model implemented on its computational server. This interface includes an input JSON data structure that is sent to the FishMet computational server as well as the output data in CSV format. The flow of the interaction between the iBOSS and FishMet is depicted on Fig. 24. Overall, the interactions are initiated by iBOSS. First, input data (JSON) is transferred from iBOSS simulator wrapper to the FishMet server (https PUT). This triggers generation of the model command script, running of the FishMet model executable and storing the output data on the server. Then, the data are pulled to iBOSS using https GET request. Manual access to the data can also be done using standard authenticated WebDAV protocol. The model and the data can also be controlled (e.g., new versions of the source code deployed) via standard ssh/sftp protocols.

Who and when to trigger the execution of FishMet simulation: The FishMet simulations can be conducted directly by the end users, both manually using the graphical interface and through programming complex scenarios in model scripts. Additionally, simulations can be automatically administered by the iBOSS system. The latter case includes provision of iBOSS machine-generated modelling scenarios for fully automatic functioning.

FishMet can be triggered and running on-demand. The multiple types of user interface implemented in the FishMet model allow diverse scenarios for running it on demand. These range from the program own graphical interface, allowing simple application by a general non-IT user and running complex simulation batches by programming the model through its scriptable command line interface. Other kinds of interfaces can be added in future by writing new modules, e.g., a callable shared library. The algorithm of the model can also be altered through editing the source code. The later possibility is facilitated by the modular implementation design of FishMet and its embedded documentation.

The initial model is developed for the rainbow trout. However, most parts of the model are sufficiently generic, so that the algorithms can be adapted to other species (by different parametrization with minimal adaptation of the source code), such as seabass and Atlantic salmon. He rainbow trout parametrization was based on published literature data and experiments conducted at ABT.

Description for interface to iBOSS is detailed and discussed in deliverable D2.4

8. Future directions

This deliverable describes the actual status of the FishMet model at the time of reporting (end of September 2022). This includes the status for the conceptual model, software engineering, programming, and the outcome of the feeding behaviour experiments, and the feed intake measurement methodology. The model also includes fish size, growth, and energy balance, and is adopted to different environmental conditions (temperature). The implementation has been done by combining literature data with trials at ABT. Basic sensitivity analysis was also conducted to aid in selection of the internal parameters of the model.

The directions for improving the FishMet model include further development and implementation of interaction with environmental parameters. New modules will be added to FishMet to represent such crucial components of the fish as

(1) fish stress and its signalling pathway to the appetite;

(2) characteristics of the visual system (visual acuity, water clarity, illumination level) to allow dynamic recalculation of the visual range for detecting feed particles, perception error due to fish stress, attention and environmental factors. This will provide a more realistic assumption under a wide range of environments than perfect detectability of the feed;

(3) palatability of the feed that will allow a more realistic representation of experimentally-determined subjective taste preferences by the fish.

The main components of the model algorithm and parameter will continue to undergo testing and validation with the new literature and experimental data sets. Next, we will adapt the model to a new species, the Asian seabass. This work will be based on acquiring data in trials at ABT combined with published literature data. The latest activity is part of the exploitation plan of FishMet after the end of the current project.

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