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Estimating oxygen consumption of rainbow trout (*Oncorhynchus mykiss*) in a raceway: A Precision Fish Farming approach

E. Royer[a,*], F. Faccenda[b], R. Pastres[a]

[a] *Department of Environmental Sciences, Informatics and Statistics, Università Ca' Foscari Venezia, Via Torino 155, Venice Mestre, 30170, Italy*
[b] *Edmund Mach Foundation, Via Edmund Mach 1, 38010, San Michele all'Adige, Italy*

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ABSTRACT

The Precision Fish Farming (PFF) approach was applied to the estimation of fish oxygen consumption of rainbow trout in a raceway farm. A dynamic model, simulating the evolution of Dissolved Oxygen concentration, was identified: the daily oscillation of fish oxygen consumption rate was simulated by means of a sinusoidal function. The model was applied to the data set collected during a four-week field study, which was carried out in July 2019. Water temperature and Dissolved Oxygen concentration were measured with an hourly frequency in farm influent and effluent. Fish biomass was monitored on a daily basis by combining the data provided by a state-of-the art system for non-invasive estimation of fish weight distribution with mortality counting. The monitoring period was partitioned into two time-windows, as fish was not fed during the first two weeks. These windows were further partitioned into a calibration and validation set. Three model parameters, i.e. the average daily respiration rate, the amplitude of its daily oscillation, and its phase were estimated by fitting the model output to the time series of DO concentration in the effluent. The results of the calibration show that: 1) the daily average oxygen consumption rate is consistent with the literature; 2) the amplitude of the daily oscillation when fish is regularly fed is more than twice that estimated for fasting fish. The results of the validation suggest that the model could be used to implement a cost-effective automatic control of oxygen supply, based on the short-term prediction of oxygen demand.

1. Introduction

Dissolved oxygen (DO) is a fundamental parameter of intensive aquaculture: its concentration plays a key role in fish metabolism and can interact with other processes, such as bacterial activities or plankton metabolism, that may have a significant influence on its regulation (Lanari, 2007). Oxygenation is widely used in many land-based systems, including raceways (Lawson, 1995; Colt and Orwicz, 1991). In 2016, the European Union annual production of rainbow trout (*Oncorhynchus mykiss)* was about 185 thousand tons in volume and €615 million in value. Italy plays an important role in this context, accounting for 19 % of European production followed by Denmark and France (17 % and 14 %, respectively) (STECF - Scientific Technical and Economic Committee for Fisheries, 2018). After feeding and labour, DO is the one of the main cost items in trout farming: therefore, optimizing oxygen supply is of key importance in improving both profits and fish welfare.

Rainbow trout is a diurnal feeder (Boujard and Leatherland, 1992), whose metabolism shows a daily pattern, depending on both circadian rhythm and feeding regime (Bolliet et al., 2001, 2004; Heydarnejad and Purser, 2009). The daily pattern of oxygen consumption, in particular the postprandial uptake named Specific Dynamic Action (SDA) (McCue, 2006), has been well studied. However, most studies were carried out using respirometers, with specimen activity that is very different from that in a raceway farm, (Chabot et al., 2016; Eliason et al., 2008), or do not propose an analytical interpretation of oxygen consumption (Alsop and Wood, 1997; Gélineau et al., 1998). Furthermore, the application of the result of laboratory studies to largescale, fully operational farms is not straightforward and, in some instances, can be misleading (Colt and Maynard, 2019). In this regard, a set of methodologies for accurate DO consumption in a fish farm as well as of the rates of other by-product of fish metabolism was proposed and thoroughly discussed in (Colt and Maynard, 2019). This paper focused on the design of a raceway system, and, therefore, on setting safety boundaries for the oxygenation capacity (Colt and Orwicz, 1991).

On the other hand, the control and optimization of oxygen supply requires a fully dynamic approach, as DO concentration may rapidly

* Corresponding author.
E-mail address: edouard.royer@unive.it (E. Royer).

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change in relation to the concentration in the influent as well as to a set of processes occurring within a farm, such as fish consumption rate, photosynthesis, and bacterial activity, which are all affected by water temperature.

This paper aims at filling this gap by proposing a fully dynamic approach to the estimation of fish oxygen consumption, which represents the first attempt at applying the Precision Fish Farming (PFF) framework (Fore et al., 2018) to rainbow trout farming.

Precision Fish Farming (PFF) was introduced as an adaptation of the Precision Livestock Farming approach to aquaculture (Berckmans, 2004). PFF aims at supporting farmer decisions concerning the daily management of fish farms by improving accuracy, precision and repeatability in farming operations, thus leading to improve both animal welfare and productivity. These goals achieved by designing and implementing a control system, based on the integration of real time data and models. A PFF system is made up of a real-time observation component, a dynamic model and a "control" component, which provides support to decisions and may also implement decisions, based on the integration of model predictions and data from on-line sensors. The observation component provides real time data on a set of "animal variables" i.e. parameters related to the behavioural or physiological state of an animal, and environmental variables. The dynamic model predicts how animal variables dynamically vary in response to environmental variables. The control component finetunes a set of controllable inputs in order to ensure that the physiological state of a farmed organism is the desired one. In summary, in PFF, farm operations are supported by a four-step approach: Observe, Interpret, Decide, Act.

At present, the main challenges to the implementation of the PFF approach are: 1) the availability of real time data concerning animal variables; 2) the development of a new generation of reliable dynamic models predicting the evolution of animal variables with respect to those of the external forcings and control variables.

In such a perpective we implemented a dynamic mechanistic model of DO concentration which could be applied, in general, to raceways and RAS: the model is based on real time processing of water temperature and DO data, complemented by real time estimates of fish biomass, obtained from non-invasive monitoring of fish size distribution. The model was tested on a rainbow trout farm, as part of the activities of the GAIN (Green Aquaculture INtensification in Europe) H2020 EU funded project.

2. Material and methods

2.1. Case study

The PFF system was tested at a raceway trout farm located in Trentino-Alto Adige, Northern Italy. The company is associated the ASTRO Consortium (https://www.troteastro.it/). ASTRO has developed a standardized protocol which covers key aspects of farm management, such as: feeding, stocking density, water quality standards, quality of the final product (e.g. condition factor and flesh chemical-physical properties). The compliance with the protocol requirements is certified by a Protected Geographical Indication (IGP) label.

The farm comprises seven 200m long, 8m wide, and 0.8m deep raceways, which are covered by protection nets, in order to avoid bird predation, and equipped with oxygen supply systems. The influent quality varies in time, as the water is withdrawn from the Sarca river (Fig. 1).

2.1.1. Oxygenation system

Liquid oxygen is stored in a tank which supplies each raceway independently via a distribution network. Oxygen is gasified and then dissolved in water at atmospheric pressure using a Low Head Oxygenator (LHO) system designed and manufactured by the farmer himself. Oxygen supply to each raceway is made through a manual valve controlled by the farmer and set to a nominal value. The LHO is characterized by an Oxygen Transfer Efficiency (OTE) of 90 %

During the test of the PFF system, no emergency procedure was activated for raceway 6, and pure oxygen supply rate, LO2, remained at its nominal value (Table 2).

2.2. Oxygen consumption description

In general, DO dynamic in river water depends on advection and on three local processes, namely photosynthetic production, ecosystem respiration and oxygen exchange with the atmosphere (Cox, 2003). In order to simulate DO dynamic within a raceway, the following assumptions were made. 1) In this preliminary application of PFF, a "0D" DO dynamic model was applied, implicitly assuming that the raceway water s well mixed: in this case the mathematical representation of DO dynamic is an Ordinary Differential Equation (ODE); 2) Photosynthetic activity within the raceway was not taken into account; 3) The oxygen consumption term included only respiration due to farmed fish. 4) The exchange with the atmosphere was taken into account. The resulting model equation is given in Table 1, in which the first term represents the input and output of DO related to the volumetric flow rate (Q); the

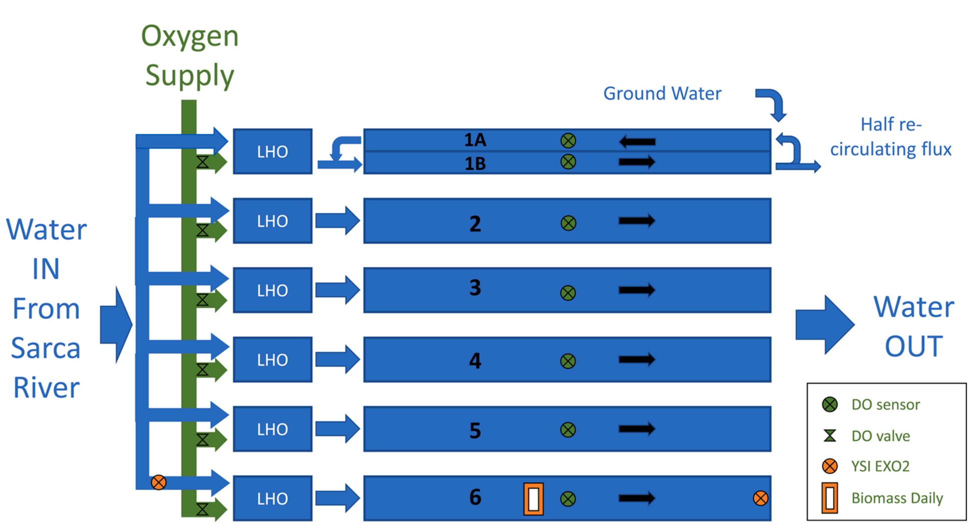

Fig. 1. Trout farm raceways and PFF system overview.

Table 1
Dissolved oxygen dynamic model.

Dissolved oxygen dynamic model		
DO mass balance equation		
$\frac{dx}{dt} = Q(x_{in} - x)/V + S + k_{rear}(DO_{sat} - x) - MR/V$		
Metabolic parameters		
R	oxygen consumption rate	[mg L^{-1} h^{-1} kg^{-1}]
State Variable		
x	DO concentration in the raceway	[mg L^{-1}]
External Forcings		
T_w	Water temperature	[°C]
x_{in}	influent concentration of DO	[mg L^{-1}]
M	Fish biomass	[kg]
Q	Water Flow rate	[L h^{-1}]
DO_{sat}	DO saturation concentration	[mg L^{-1}]
Control parameter		
S	oxygen supply rate	[mg L^{-1} h^{-1}]
Constant parameters		
V	Raceway Volume	[L]
k_{rear}	Reaeration rate	[h^{-1}]

Table 2
Functional expressions and parameters.

Functional expressions				
$R = (R_m + A(\cos 2\pi f(t + \varphi)))e^{p_k(Tw-15)}$				
$DO_{sat}(T_w) = 14.589 - 0.4\,T_w + 0.008\,{T_w}^2 - 0.0000661\,{T_w}^3$				
$S = \frac{0.9 * LO2\,.P.\,M_m}{N_a.k.[T_w(t) + 273.15]V}$				
Parameters				
p_k	0.07		Temperature coefficient	Myrick and Cech, 2000
k_{rear}	0.046	h^{-1}	Reaeration rate	Ciavatta et al., 2004
Q	158410^3	$L\,h^{-1}$	Volumetric flow rate	
$LO2$	2400	$L\,h^{-1}$	Oxygen supply rate	
P	101325	Pa	Atmospheric pressure	
M_m	31.998	$g\,mol^{-1}$	Oxygen molar mass	
N_a	6.022×10^{23}	mol^{-1}	Avogadro constant	
k	1.38×10^{-23}	$J\,K^{-1}$	Boltzmann constant	
V	1280	m^3	Raceway volume	
f	1/24	Hz	Frequency of sinusoidal respiration rate	
R	This study	$mg\,h^{-1}\,kg^{-1}$	Oxygen consumption rate	Tudor, 1999
DO_{sat}		$mg\,L^{-1}$	Oxygen Concentration at Saturation	Ginot and Hervé, 1994
S		$mg\,L^{-1}h^{-1}$	Oxygen concentration supply rate	

second one, the oxygen supply rate, S, which can be controlled by the farm; the third term the oxygen exchange with the atmosphere; and the fourth the oxygen consumption due to fish respiration (MR/V).

2.3. Estimation of oxygen consumption

In accordance with PFF terminology, a "feature variable" can be estimated based on measured animal variables and a "target variable", derived from feature variables, can be used in decision making. In this framework, R is a non-observable feature variable, which can be estimated by fitting the model output to a set of observations. DO concentration, x, was taken as a "target variable", as its level can be controlled by adjusting the oxygen supply, on the basis of predicted values of R, in order to keep DO at the desired level. R was estimated by minimizing the Root Mean Square Error, RMSE, Eq. (1):

$$RMSE = \sqrt{\frac{\sum_{i=1}^{i=n}(\hat{y}_i - y_i)^2}{n}} \tag{1}$$

in which n is the number of observations, y_i is the DO concentration in the effluent at time ti, $\hat{y}_i$ the DO concentration at time t_i estimated by the model.

The "metabolic parameter" R (Colt and Maynard, 2019) depends on several factors, i.e., water temperature, the circadian respiration pattern, and daily feeding pattern. Circadian rhythms of feeding and locomotor activity have been widely described (Sanchez-Vazquez and Tabata, 1997; Boujard and Leatherland, 1992). Several studies (Bolliet et al., 2001; Heydarnejad & Purser, 2008) demonstrated that trout metabolism and activity present a daily pattern as a consequence of a combination of two zeitgeber (a rhythmically occurring natural phenomenon which acts as a cue in the regulation of the body's circadian rhythms), Light Entrainment Oscillator (LEO) in a major part, and Feed Entrainment Oscillator (FEO) in a minor part. (Bolliet et al., 2004) highlighted a sinusoidal pattern of metabolism response of trouts to different feeding times. Therefore, in accordance with (Tudor, 1999) the daily fluctuation of oxygen consumption was simulated using a sinusoidal function and the effect of water temperature on R was modelled by means of an exponential function (Brigolin et al., 2010). The average daily rate, R_m, the amplitude of the oscillation A, and the phase, ϕ, were estimated in this study (see Table 2) by minimizing the goal function (Eq.1). The minimization was performed using the (Nelder and Mead, 1965) method implemented within the *opt* function from R stats package. This method is based on a Pascal algorithm from (Nash, 1990). In order to estimate both their expected values and their standard error, a procedure based on Monte Carlo method was adopted. The procedure consists in repeating the minimization of Eq. 1 by comparing the model output with a set of synthetic time series which are "consistent", from a statistical point of view with the observed one. The synthetic time series are obtained from the observe one by assuming that: 1) the pdf of each observation is independent; 2) DO data are normally distributed. The standard deviation of DO data was estimated from the sample distributions ofDO data obtained after de-trending the time series. 100 synthetic time series were then randomly extracted, taking DO observations as mean values. The minimization was repeated for each synthetic time series, thus obtaining empirical distributions of the three parameters which were consistent with the statistical hypothesis made on the pdf of the observed time series: mean values and standard error were then calculated.

Model performances were assessed using a well-established Goodness of Fit, GoF, methodology, i.e. the regression between observations and predictions (Pineiro et al., 2008). The model and the parameter estimation procedure were coded using R software core (version 3.4.0), within R Studio (version 1.0.143). The differential equation was solved using deSolve R package (version 1.21). DO concentrations measured in the effluent at the beginning of the period were taken as initial values for solving the model equation.

2.4. Monitoring strategy

The estimation of the parameters required as input time series of: concentration of DO in the effluent, x_{out}, water temperature, T_w, oxygen supply rate, S and fish biomass, M. The monitoring strategy for collecting these data is described in detail below. The monitoring system (Fig. 1) was installed in raceway 6: the influent flow rate was 0.44 m^3/s, which gives a residence time of 48 min, i.e. 0.8 h.

2.4.1. Animal variable

One of the goals of the H2020 project GAIN - Green Aquaculture Intensification in Europe (O'Donncha and Grant, 2019) is to test non-invasive methods for the monitoring of animal variables and to developnovel modelling approaches for the implementation of PFF on a range of aquaculture typologies, including rainbow trout raceways. Fish weight distribution was monitored in real time (Fig. 1) using Biomass Daily (BD), produced by Vaki and commercialized in Italy by Aquatrade L.t.d.

The system is being used to estimate weight distribution in Atlantic salmon cages (Lopez Riveros, 2017) but, to our knowledge, it has not been previously applied to rainbow trout in a raceway farm. Biomass Daily system consists of a 80 × 80 cm submerged frame connected to a sending box. A wireless connection allows data to be transferred to a remote computer.

The sensor, based on infrared technology, detects a signal whenever a specimen gets across the frame. Signals are postprocessed in real time and estimated individual weights are displayed on a dashboard. Daily statistics can be easily retrieved for further elaboration. All these data are remotely accessible through a web interface. Although the system requires some maintenance, e.g. weekly cleaning, it does not involve significant extraeffort for operators. The dashboard is userfriendly and data can be easily retrieved for post-processing. BD could also be used for non-invasive monitoring of a whole farm by moving the frame from one raceway to another: data gaps could be covered by interpolation or modelling. In this study, the frame was kept in raceway 6 for three months and the observations were compared with direct monthly sampling of 30 individuals.

2.4.2. External forcings and target variable

Water temperature, T_w and DO concentration were monitored every hour in the influent and effluent (Fig. 1) using two identical multiparametric automatic EXO2 sensors, commercialied by YSI. Probes were placed 70 cm below the water surface. DO measurements were made using an optical sensor whose precision was 0.1 mg L^{-1} within the range of observed values. Temperature measurements were obtained through a classical thermoresistance, with a 0.01 °C precision. The sensors include data loggers and can be connected to a cloud platform for visualizing and processing the data in real time. Time series were detrended using R software *detrend()* function and a Fourier analysis was then applied using R software *fft()* function. The spectral density associated with 24 h period was then quantified as a fraction of the whole spectral density.

3. Results

The test of the PFF system started on July 3rd, 2019 and ended on July 31st, 2019. The monitoring of weight distribution continued until November 8th, 2019, in order to test the performance of the monitoring device over a larger size range. The initial fish number, N = 20 472,was estimated using a fish counting device commercialized by Calitri Technology. The initial average fish weight was 1.08 kg, for a total estimated initial biomass of 22,109 kg, which gives an average stocking density of 17.27 kg/m^3. The daily mortality rate was estimated by direct counting of dead fish, in accordance with the daily husbandry practices.

Two feeding regimes were identified: from July 3rd to 16th fish were not fed or markedly underfed, as they were treated for the presence of a pathogen, *Lactococus Garviae*, while from July 17th to 31st they were given a full ration ranging between 0.4 % and 0.96 % of the average weight. These two time windows will be named FAST and FED hereafter.

Feed used was a commercial diet of 6 mm food pellets produced by Aller Aqua, containing 44 % of protein and 26 % of lipids. Fish were fed daily, around 9 AM. Feed was delivered manually, using a mobile gantry going from one side to another at a 10 m/s velocity, which means that feeding took about 20 min.

Fish were exposed to natural day/night cycle. Based on daily total solar irradiation data registered near the rearing site, this cycle was approximately 15 Light / 9 Dark (sunrise around 6 AM and sunset around 8 PM CEST).

3.1. Animal variables

The installation of the BD system was straightforward but, towards the middle of July, a technical problem concerning the connection of the frame to the receiver affected the quality of the data. Subsequently, data were regularly acquired until the beginning of November, when the device was transferred to another raceway. BD daily statistics, i.e. average weight and standard deviation, were based on 1325 detection per day, on average, i.e. about 6% of the population. The time series of average weight and standard deviation during the threemonthlong trial are presented in Fig. 2, which also shows the average weight estimated by collectively weighing 30 specimens, in accordance with farm husbandry practice.

Missing values, highlighted in grey in Fig. 2, were interpolated fitting a cubic spline in order to smooth the data and to better identify the trend on the whole period. As shown in Fig. 2, the interpolation compares the mean weight values estimated from direct sampling, in particular in late summer and autumn. The larger difference between BD estimates and direct sampling in early July, about 200 g, could be due to fish behaviour in relation to the introduction of the frame: at the beginning, bigger fish may get across it more frequently than smaller one.

3.2. External forcings and state variable

The time series of water temperature and DO measured in the influent and effluent are shown in Fig. 3a–b and their differences in Fig. 3c–d. Descriptive statistics, i.e. range, mean, median, standard deviation and Interquartile Range are presented in Table 3.

As one can see from Fig. 3a–b, both variables presented a clear daily pattern, driven by the primary production/respiration and heat exchange processes occurring in the Sarca river. Water temperature ranged approximately from 11.5 °C to 20.5 °C and the differences were slightly

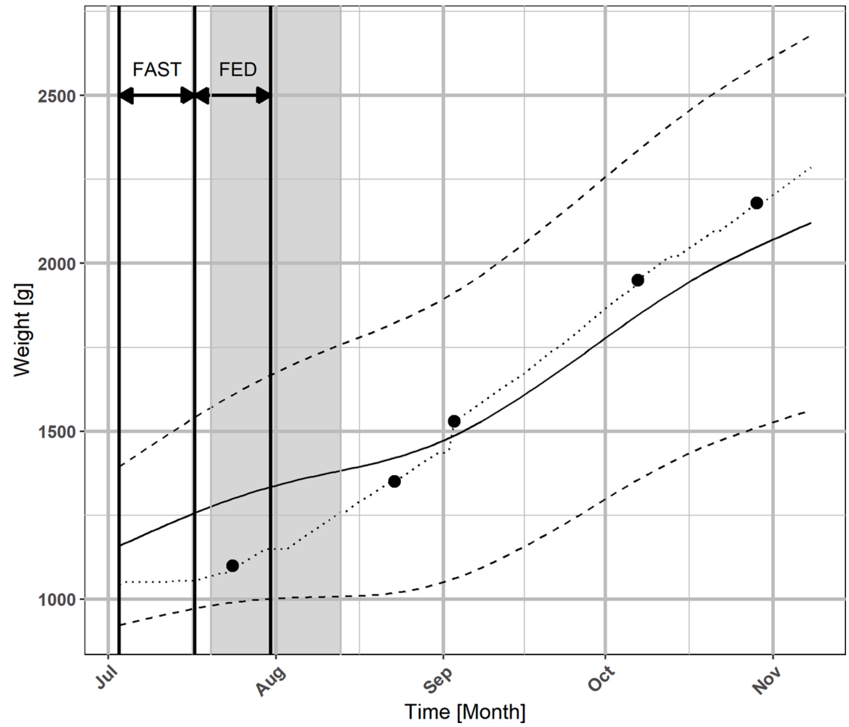

Fig. 2. Average fish weight (solid curve) and standard deviation range (dashed curves) estimated using Biomass Daily data, compared with mean values from direct sampling of 30 specimen (points) and farmer management software data (dotted curve). Interpolated data period is grey higlighted.

positive, probably due to dissipation of solar radiation energy into heat within the raceway. DO data, Fig. 3a, were characterized by a less regular pattern, which seems to be affected by the feeding regime. Overall, DO in the effluent was higher than that in the influent, see Fig. 3c, as oxygen was continuously supplied during the month of July: this suggests that the DO control system actually in place could be markedly improved. A daily pattern can also be noted in the T_w and DO differences, see Fig. 3c–d. In particular, DO daily oscillations were smaller and more regular during the FAST sub-window but became higher and characterized by higher noise during the last two weeks of July, when fish was fed in accordance with the feeding tables. The presence of a daily pattern is confirmed by the results of the Fourier analysis, summarized in the third column of Table 3, which shows the percentage of the spectral density associated with a daily period. The Fourier analysis confirms that DO differences were characterized by a significant daily component, which accounted for 42 % of the whole spectral density.

3.3. Oxygen consumption estimation

The oxygen consumption rate, R, was estimated for both the FAST and FED time windows, in order to detect changes due to feeding. The average daily value, $R_m$was estimated only on the FAST sub-window, in which the fish was not fed or underfed, as no differentiated trend can be identified in Fig. 3c. The other two parameters were calibrated on both windows, as, according to the literature, the daily pattern of oxygen consumption depends both on LEO and FEO. In order to test the model predictive capability, both the FAST and FED data set were partitioned into two subsets: the first one was used to estimate the parameters and the second one to validate the estimates.

The model output is compared with the time series of DO observations collected in each time window in Fig. 4a–d. A visual comparison shows that the model succeeds in simulating the daily pattern of DO dynamics observed in the FAST window, even though it underestimates the data in the validation sub-window. Furthermore, the model performance seems acceptable also in the first five days of the FED calibration sub-window. On the other hand, the model does not seem to capture the main features of DO dynamics observed in the FED validation window.

The results of the calibration are summarized in Table 4, which also presents the GoF indicators. As one can see, the phase ϕ, about two hours, is the same for both sub-windows but the amplitude A concerning the FED one is more than twice that obtained fitting the model to the FAST data set. A more detailed analysis of the daily pattern of DO dynamics highlights that the diel oscillation of water temperature can also play a non-negligible role in determining the daily pattern of fish oxygen consumption. The average hourly absolute values of DO supply, fish consumption and reaeration are represented in Fig. 5a and b for the FAST and FED windows, respectively. As one can see, the two main contributions to DO dynamics were fish respiration and oxygen supply. At constant water temperature, the oxygen consumption would be at its minimum around 10 PM and would peak around 10 AM, for both fasting and fed fish. However, due to the daily oscillation of water temperature (Fig. 3d), the oxygen demand kept increasing up to the early afternoon and peaked around 3 PM: this is clearly shown by Fig. 5a, concerning the FAST window. Such increase was more pronounced when fish was fed, due the post-prandial DO demand. It is also interesting to note that the average DO consumption was higher during the FED windows, when the average temperature was about 0.5 °C higher than that observed during the FED one. The reaeration contribution was much lower than fish respiration and oxygen supply. This finding could be related to the choice of the reaeration constant taken from (Ciavatta et al., 2004) which, however, is within the range given in (Cox, 2003) for small streams, i.e. 0.03 h^{-1} - 4.7 h^{-1}.

In terms of GoF indicators, see Table 4, the model calibration in the FAST time interval led to an RMSE of 0.19 $mgO_2.L^{-1}$ for the calibration and 0.32 $mgO_2.L^{-1}$ for the validation time series, and to higher values on FED sub-windows (0.49 $mgO2.L^{-1}$ for the calibration and 0.73 $mgO_2.L^{-1}$ for the validation). The results of the predicted vs observed regression indicates that the slope is close to 1 on the FAST period. However, taking into account the standard error, the null hypothesis, i.e. slope = 1, should be rejected at a 95 % confidence level. This means that the fraction of variance explained by the model could still be improved: the visual comparison of predicted and observed values suggests that

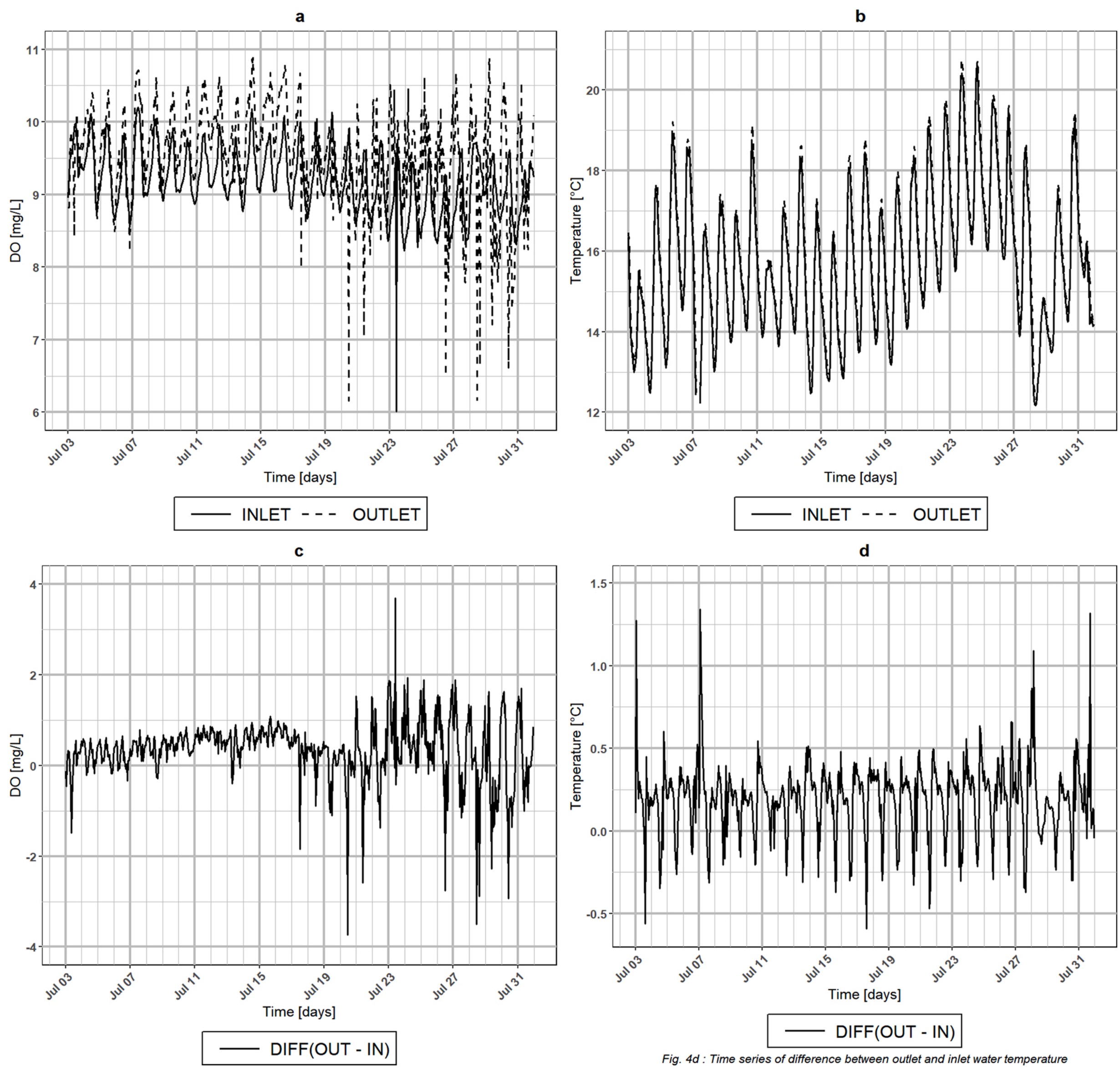

Fig. 3. Time series of DO concentration (a) and water temperature (b) in the influent and effluent and their differences (c and d).

Table 3
Descriptive statistics of the environmental variables.

Statistic	DO [mg L^{-1}]		Temperature [°C]	
	Influent	Effluent	Influent	Effluent
Range	6.0 - 8.9	6.1 - 10.8	11.26 – 20.68	11.54 – 20.75
Mean	9.2	9.5	15.74	15.93
Median	9.2	9.6	15.65	15.79
SD	0.4	0.7	1.84	1.84
Inter Quartile Range (IQR)	0.6	0.8	2.67	2.71
% Spectral density	67 %	46 %	80 %	81 %

differences are larger in the first two days. As one can expect, model performance is worse for the validation set: the bias, i.e. the intercept in Table 4, is higher compared with the calibration set. The results concerning the FED period gave a slope significantly different from 1 (0.13 for calibration and 0.11 for validation) and indicate a lack of fit and a higher bias with intercept value of 7.9 for calibration and 8.1 for validation.

4. Discussion

The one-month-long time series of water temperature, DO and fish biomass data collected during this study allowed the identification, calibration and validation of a dynamic DO model based on three main components (fish respiration, oxygen supply and reaeration) and the estimation of the daily pattern of trout oxygen consumption in a operational context. In this section, after comparing these results with the literature and discussing model limitations, an example of DO supply control, driven by the model results, will be presented: this would be representative of the fourth PFF step, i.e. "Act".

4.1. Estimation of DO consumption

The results of the estimation of the average daily oxygen consumption, R_m, are compared with literature values in Table 5.

Table 5 shows that the results obtained estimating R_m from a dynamic model are consistent with those presented in previous studies. Our findings are very close to those presented in (Briggs and Post, 1997), who measured field metabolic rates (FMR) of rainbow trout of about 1 kg using electromyogram telemetry. (Eliason and Farrell, 2014)

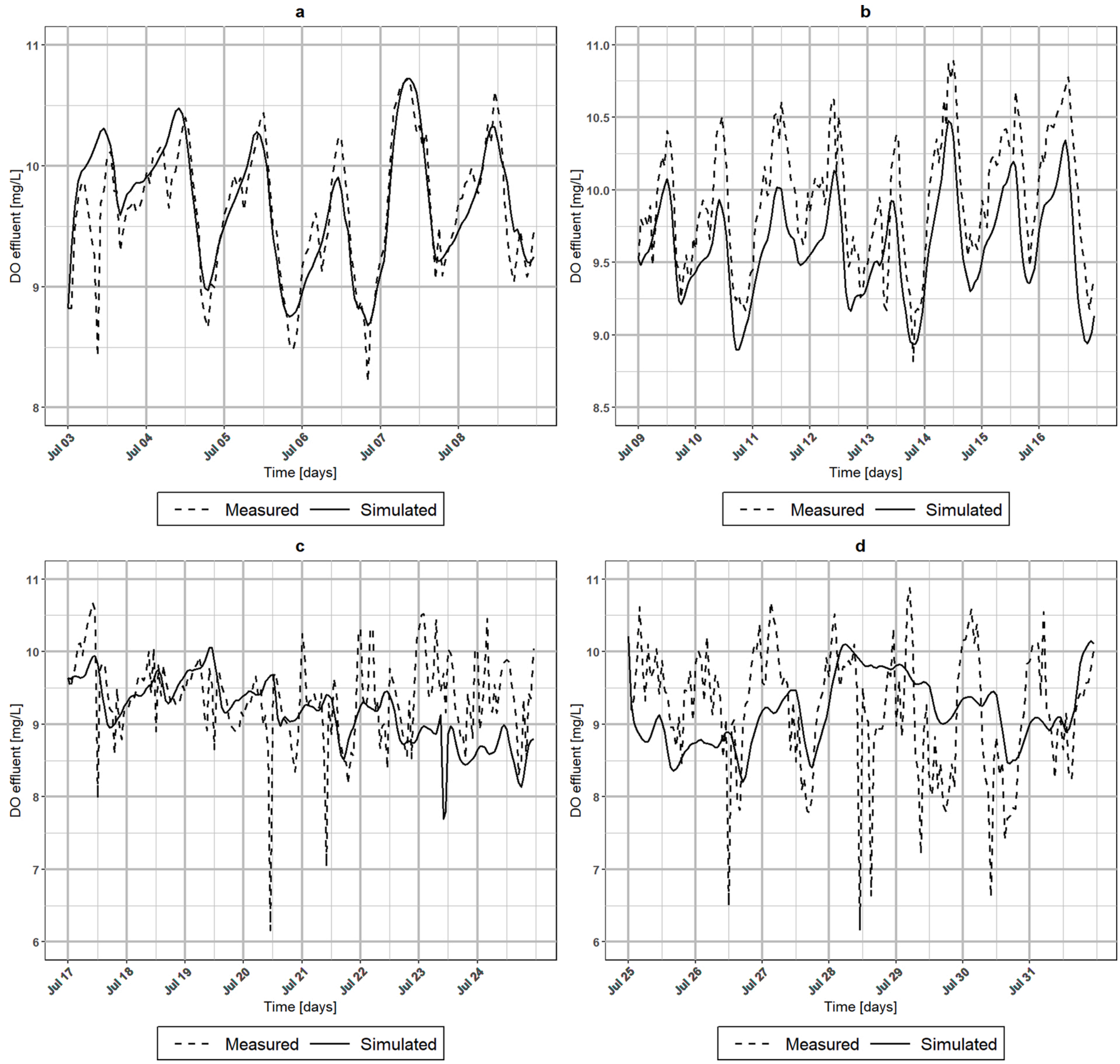

Fig. 4. Comparison between simulated and observed DO evolution concerning: a,b) model calibration and validation on the FAST time window; c,d) model calibration and validation on the FED time window.

Table 4
Results of the DO model calibration and validation.

	R_m [mg h^{-1} kg^{-1}]		101.5 ± 1.4
	A [mg h^{-1} kg^{-1}]		−12.1 ± 4.2
	ϕ [h]		1.9 ± 0.6
		RMSE	0.19
FAST	**Calibration**	Slope	0.81 ± 0.04
		Intercept	1.9 ± 0.4
		RMSE	0.32
	Validation	Slope	0.71 ± 0.03
		Intercept	2.5 ± 0.2
	A [mg h^{-1} kg^{-1}]		−29.3 ± 3.5
	ϕ [h]		1.9 ± 0.4
		RMSE	0.49
FED	**Calibration**	Slope	0.13 ± 0.05
		Intercept	7.9 ± 0.5
		RMSE	0.73
	Validation	Slope	0.11 ± 0.04
		Intercept	8.1 ± 0.3

investigated rainbow trout oxygen consumptions in normoxic and hypoxic conditions. Their estimate of the Standard Metabolic Rate, adjusted for temperature, is 91 mgO_2 h^{-1} kg^{-1}: as one can expect, this is lower than that of this study, as fish is free to move around in a raceway. Oxygen consumption of a population of juvenile rainbow trout in a tank reported by (Alsop and Wood, 1997) is also consistent with the values obtained in this study. The amplitude of the daily oscillation of R ranged from 13.0, for fasting fish, to 31.3 mgO_2 h^{-1} kg^{-1}, when feeding, thus leading to a peak consumption of 143 mgO_2 h^{-1} kg^{-1}. This value is, again, slightly higher than that found in (Eliason and Farrell, 2014) under normoxic condition, i.e. 1218 ± 5.9.

The phase of oxygen consumption did not change with respect to feeding indicating, at a constant temperature, a peak around 10 AM, i.e. about one hour after feeding and a minimum of R around 10 PM. This lag between feeding time and maximum DO consumption compares very well with the findings of (Gélineau et al., 1997), who reported a peak value one hour after feeding and could be explained by the fact that LEO is the dominant zeitgeber for rainbow trout metabolic rhythms with respect to FEO, as demonstrated by (Bolliet et al., 2004). It could also be related to a food-anticipatory activity, which takes place even when fish

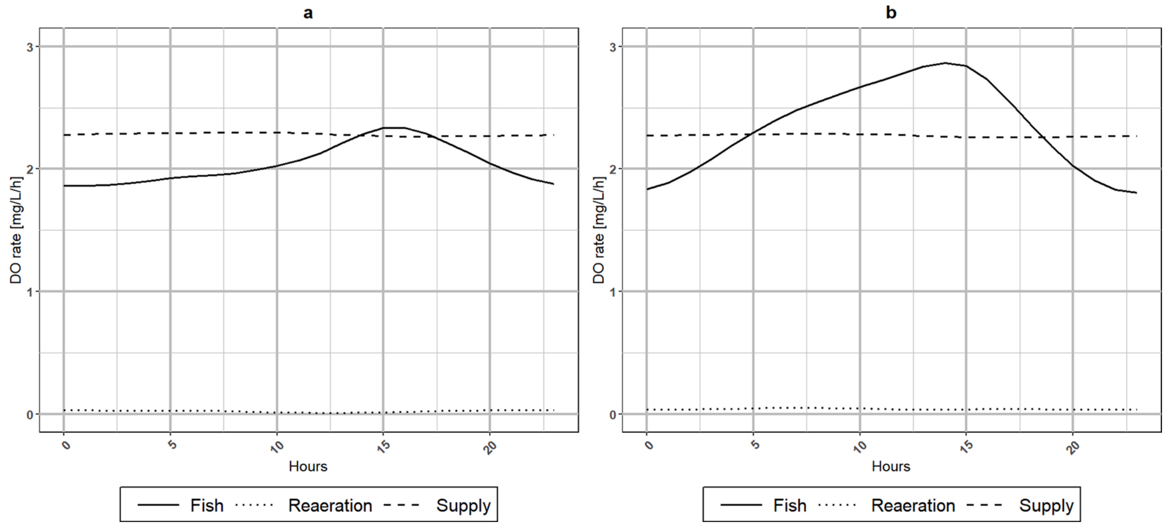

Fig. 5. Hourly mean DO rates on a) FAST sub-window b) FED sub-window.

Table 5
Mean metabolic rate Rm comparison.

Rm [mg h^{-1} kg^{-1}]	Water temperature [°C]	Method	Source
101.5 ± 1.4	15 °C	PFF - dynamic model	This study
130 ± 10	15 °C	electromyogram telemetry	Briggs and Post, 1997
96 - 192	15 °C	PO2 in tank measurement	Alsop and Wood, 1997
75 ± 3.4	10.75	Respirometer	Eliason & Farrel, 2014

are food-deprived, as suggested by (Bolliet et al., 2001), as a consequence of a regular feeding regime.

However, as shown in Fig. 5, taking into account the daily oscillation of water temperature, the oxygen consumption reaches its maximum value in the early afternoon both in the FAST and FED windows: this finding is consistent with the results presented in (Colt and Watten, 1988), who estimated a 4–6 hour lag. Overall, the above results indicated that the estimates of fish respiration rate succeeds in taking into account FEO, LEO, food-anticipatory activity as well as the effect of water temperature.

4.2. Model performance

The results suggest that the model gives a good description of the system during the FAST period. The estimate of the parameters, however, may have been affected by the uncertainty and potential bias in the time series of average weight, which is an important input. In fact, a discrepancy between DB estimates and those obtained from direct sampling was observed and it was necessary to interpolate the weight time series. As far as the first point is concerned, it should be pointed out that estimates from direct sampling are also affected by uncertainty, as the weight distribution of the sample may not be representative of that of the population. Therefore, such uncertainty cannot be removed but should be quantified by further testing the BD device. As far as the interpolation is concerned, growth curves are, in general, rather smooth: therefore, the lack of data in the second half of July definitely introduces another source of uncertainty, which, nevertheless, did not affect the estimation of the mean respiration rate. In practice, failures/interruption of any monitoring system may occur: in this case, the BD could be complemented by a growth model, which could supply reliable estimates of fish growth when the actual monitoring system fails.

The assumption of complete mixing can also be considered as a source of bias for the estimation of oxygen consumption in this specific case, as we were applying the model to a rather long raceway. A 1D model, which could simulate the dynamic of the spatial pattern of OD concentration along the raceway, is technically feasible. However, prior to the introduction of a more complex model structure, some basic ideas can be tested using simpler modelling approaches. The specific results obtained in this study could be improved by switching to a 1D transport-reaction model but the present model structure could already be applied to a large number of cases in which the hypothesis of complete mixing could safely be adopted. On the other hand, in this study, the model was fitted to the concentration of the effluent, which is likely to be the lower one: therefore, our findings may represent an overestimation of the average DO consumption. As a result, decisions based on such estimate could be considered robust as they ensure that DO supply is regulated on the basis of the "worst case" scenario.

However, the model limitation discussed thus far can hardly explain the lack of fit during the FED period, in which the daily pattern of DO concentration in the effluent was characterized by a higher average value and higher and less regular daily oscillations, compared with the previous fortnight.

These irregularities of daily oscillations in the effluent could be explained by the transition from a fasting condition to a feeding regime which is not fully steady. Indeed, on the FED period, feeding rations assume three different values in terms of ratio between feed quantity supplied to the raceway and fish biomass: 0% (for only two days), 0.43 % and 0.96 %. Such a variation could lead to a lack of regularity in DO consumption by fish.

As an alternative, rapid changes in the influent and in the dynamics of fish respiration could also be effectively dealt with using a different approach to the simulation of DO dynamic, based on data assimilation methods. In this framework, a non-observable feature variable, such as R, is included in the state vector. Both state variables are considered as stochastic ones: the trajectories of their expected values are estimated by solving a dynamic system, i.e. a system of ODE, until a DO observation becomes available. At this point, both state variables, i.e. DO and R are corrected in order to take into account the information provided by the observation. Different data assimilation algorithms are available: the Continuous-Discrete Extended Kalman Filter (CD-EKF) could be a good candidate, as it has already been tested in an application concerning aquaculture (Marafioti et al., 2012). All data assimilation algorithms, however, require the estimation of a set of hyperparameters concerning the probability density functions of the state variables: in practice, these parameters should be determined by prior calibration of a fixed parameter model. Therefore, the results presented can be considered as a preliminary step towards the full implementation of a DO model based on data assimilation.

4.3. Decide and Act: closing the loop

The time series of DO differences plotted in Fig. 3d clearly shows that the DO control system in place could be improved, as DO in the effluent was often higher than that in the influent. Furthermore, an emergency control system is activated, composed by one DO sensor positioned at the outlet of the raceway, two automated ON/OFF electro-valves (30 L/min) positioned at the oxygen entrance of the LHO, and a software. DO values are recorded every 15 min by the sensor. Three emergency thresholds are implemented: 1) if DO concentration is lower than 7.5 mg.L^{-1}, the first electro-valve is activated. 2) if DO concentration is lower than 6 mg.L^{-1}, the second electro-valve is activated. 3) if DO concentration is lower than 5 mg.L^{-1}, an alarm is set and a notification is sent to the farmer through GSM connection device. Once the DO value return above the threshold, corresponding electro-valve is closed. At present, automation only regards the alarm process whereas the nominal process has no automated control system.

The results presented in this study show, instead, that DO modulation during the day could be extremely relevant and could lead to a closed loop system for nominal oxygen supply management. Based on PFF approach and in a first approximation (because of model's limits highlighted in the previous sections), the model could be used as an estimator of unobserved state and included in a more ambitious system control oxygen supply using appropriate targets.

Two kinds of simulation were run using the recorded operational parameters and the oxygen model, assuming that nominal DO supply could be modulated.

1) The first regulation consists supplying the oxygen quantity that corresponds to fish demand of the next hour, taking into consideration a potential inertial effect of the raceway DO concentration and taking advantage of the forecast potential of the model. DO concentration in effluent that is more oxygenated than the influent does not make much sense: a control process could ensure that the DO level in the effluent is not higher than in the influent.
2) The second regulation consists in ensuring that the DO concentration within the raceway does not fall below a target value, in order to avoid the emergency increase in the oxygen supply rate, which is activated when DO concentration falls below 7.5 mg..

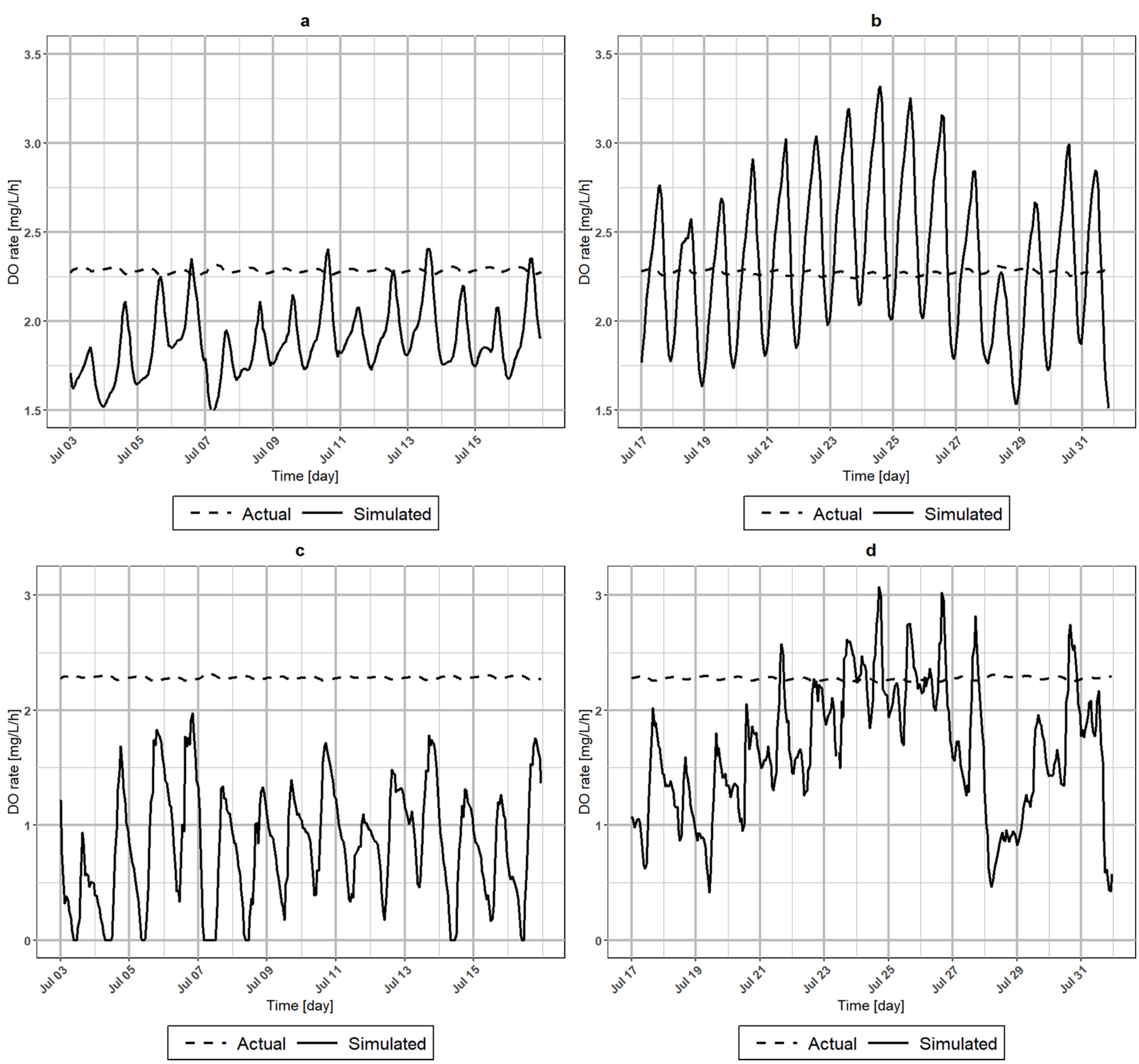

Fig. 6. Comparison of oxygen supplied by two automatized DO control systems with regard to the actual one: a) FAST - compensation of fish demand; b) FED - compensation of fish demand; c) FAST - target value of 8.5 mg L-1; d) FED - target value of 8.5 mg L-1.

Fig. 6 shows the comparison of the results of both simulations with the actual values in terms of oxygen supply for both FAST and FED sub-windows. The leads to a volume of oxygen that is cumulatively lower (721 m^3) than the actual one during the FAST sub-window of July (806 m^3). Within the same sub-window, opting instead for a target value of 8.5 $mg.L^{-1}$ leads to an oxygen supply that is even much lower (343 m^3) which could mean substantial cost savings. n the FED sub-window, the efficiency of both control a compensation of fish demand would cause (864 m^3). It means that during this period oxygen supply as often lower than the fish demand. To this regard, introducing a target value of 8.5 $mg.L^{-1}$ for DO concentration in the raceway would have been very pertinent (637 m^3) as it could have meant substantial cost savings even n this period.

These findings highlight the improvements margins of DO supply system, showing it may be suitable to build a control system that lower oxygen supply, ensuring at the same time fish welfare; In such a perspective, they also indicate that the DO model could be the core of an advanced and robust control system, aimed at optimizing the DO supply. Furthermore, the predictive capacity of the model could be used for early warning, i.e. for anticipating the activation of emergency procedures in case DO gets close to a tolerance threshold.

5. Conclusion

The results presented in this study indicate that the Precision Fish Farming approach can be effectively implemented in raceway trout farms and improve the management of DO supply, which represents one of the main cost items, after feeding and labour. Real time monitoring of water temperature and DO is in place in most rainbow trout farms: these data can be processed using the DO model proposed for estimating and predicting the daily pattern of oxygen consumption rates in relation to water temperature, fish biomass, and feed ration. The DO simulation model tested in this study includes an oxygen consumption term due to fish respiration which mimics the daily fluctuation of oxygen demand by means of a sinusoidal function. The phase of the sinusoidal term did not vary in response to changes in the feeding regime but, as expected, the amplitude significantly increased when fish was fed.

The modelling approach proposed also makes it possible to estimate specific DO respiration over a range of temperature and fish size, provided that time series of fish weight distribution are available. In this paper, time series were obtained by testing on rainbow trout Biomass Daily, a device for real time non-invasive monitoring of fish size distribution based on a technology previously applied to salmon farming. The device tested in this study may represent a suitable solution, but willneed further testing over a large range of fish size. Observations from BD could also be complemented by estimates provided by an individual growth model: such integrated monitoring system could be used to implement a cost-effective automatic control system for a whole farm, based on short-term predictions of oxygen demand in different basins stocked with trout of different size. Furthermore, it could also be used as a planning tool, for mid-term predictions of total DO consumption, based on feeding tables and/or individual growth models.

Furthermore, oxygen demand can also be used to estimate the energy necessary to fasting catabolism and SDA, thus to improve individual growth models based on the energy budget, including the explicit simulation of catabolic processes which lead to ammonia emissions. As DO demand can be affected by other factors which are difficult to quantify, e.g. water turbulence, turbidity, this parameter could be estimated using data assimilation methodologies in order to provide more reliable one-day-head predictions. Therefore, future perspective of this work includes: a) the development of a dynamic individual model of fish growth, in order to link oxygen consumption to catabolic processes b) the implementation of data assimilation methodologies in order to improve model performances and provide more reliable one-day-ahead forecasts of DO demand in relation to feed ration; c) the test of oxygenation system to allow modulation of oxygen supply according to reliable criteria.

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Declaration of Competing Interest

The authors reported no declarations of interest.

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Appendix A. Supplementary data

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