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Modelling fish growth and composition: a pathway to optimize feeding and rearing practices

Andreia I. G. Raposo

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INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR

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Tese de candidatura ao grau de Doutor em Ciências Animais, Especialidade em Nutrição, apresentada ao Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto.

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"*The sea, once it casts its spell, holds one in its net of wonder forever.*"

Jacques Cousteau

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Abstract

Aquaculture fish production has increased in recent decades as an answer to the challenges of food security for an increasing world population, and as a way of reducing pressure on natural stocks. However, this increase resulted in the need to develop new techniques and technologies to improve production efficiency, contributing to the improvement of the quality of fish products, as well as to the reduction of production costs and the ecological footprint. Over the last decade, interest in precision fish farming and feed formulation has been growing, and a key aspect is the development of accurate mathematical models that support it. Thus, the main focus of this work was to develop different mathematical models to predict the growth and body composition of Nile tilapia (*Oreochromis niloticus*), in order to optimize feed formulations and rearing practices for this species, and validate them against independent data. Another objective was to extend this framework to other economically relevant species (e.g., gilthead seabream, European seabass, rainbow trout, Atlantic salmon, Senegalese sole and turbot) in order to better understand the similarity relationships between species. To achieve the stated goals, data on fish growth and body composition was collected from growth trials published in the scientific literature, and a structured approach to model development was undertaken, by comparing different model designs and calibration methods under objective measures of generalization capacity (e.g., cross-validation error), before calibrating and validating the final model. Comparison between species was achieved by re-calibrating the models developed for tilapia with data for other species, and evaluating changes in model parameters and predictions. The results of this work contribute to the development of Nile tilapia precision farming, through the development and validation of growth and body composition models. They also contribute to a better understanding of certain questions of scientific (e.g., plausibility of universal metabolic scaling, and of isometry in Nile tilapia) and technical nature (e.g., optimal error models and calibration methods) regarding the modelling of growth and body composition in Nile tilapia and other economically relevant species.

In conclusion, this research contributes for advances in the field of aquaculture and fish nutrition by providing valuable tools for precision feed formulation and fish farming and by enhancing the understanding of growth and body composition modelling for various economically important species, paving the way for more efficient and environmentally responsible aquaculture practices in the future.

Resumo

A produção de peixe em aquacultura tem aumentado nas últimas décadas como resposta aos desafios da segurança alimentar de uma população mundial crescente e como forma de reduzir a pressão sobre os stocks naturais. No entanto, este aumento resultou na necessidade de desenvolver novas técnicas e tecnologias para melhorar a eficiência da produção aquícola, melhorando a qualidade dos produtos e reduzindo os custos de produção e a pegada ecológica. Na última década, o interesse pela aquacultura de precisão tem vindo a crescer, sendo um aspeto fundamental o desenvolvimento de modelos matemáticos precisos. Assim, o foco principal deste trabalho foi desenvolver diferentes modelos matemáticos para prever o crescimento e a composição corporal da tilápia do Nilo (*Oreochromis niloticus*), para otimizar as formulações de rações e as práticas de cultivo para esta espécie, e validá-los contra dados independentes. Outro objetivo foi alargar esta metodologia a outras espécies economicamente relevantes (p. ex., dourada, robalo, truta arco-íris, salmão, linguado e pregado), para compreender melhor as relações de semelhança entre espécies. Para atingir os objetivos propostos, foram recolhidos dados sobre o crescimento e a composição corporal dos peixes publicados na literatura científica. Foi também seguida uma abordagem estruturada para o desenvolvimento de modelos, comparando diferentes conceções de modelos e métodos de calibração, através medidas objetivas de capacidade de generalização (p. ex., erro de validação cruzada), antes de calibrar e validar o modelo final. A comparação entre espécies foi feita através da recalibração dos modelos desenvolvidos para a tilápia, com dados de outras espécies, e da avaliação das alterações nos parâmetros e previsões do modelo. Os resultados deste trabalho contribuem para o desenvolvimento da aquacultura de precisão da tilápia do Nilo, através do desenvolvimento e validação de modelos de crescimento e composição corporal. Contribuem também para uma melhor compreensão de certas questões de natureza científica (p. ex., plausibilidade do expoente metabólico universal e da isometria na tilápia do Nilo) e técnica (p. ex., modelos de erro ótimos e métodos de calibração) relativas à modelação do crescimento e da composição corporal da tilápia do Nilo e de outras espécies economicamente relevantes.

Em conclusão, esta investigação contribui para os avanços no domínio da aquacultura e nutrição de peixes, fornecendo ferramentas valiosas para a aquacultura de precisão e melhorando a compreensão da modelação do crescimento e da composição corporal de várias espécies economicamente importantes, abrindo caminho para práticas de aquacultura mais eficientes e ambientalmente mais responsáveis no futuro.

List of published scientific papers

Articles published in journals indexed in ISI Web of Knowledge:

Raposo, A. I. G., Soares, F., Conceição, L. E. C., Valente, L. M. P., & Silva, T. S. (2023). Development and evaluation of Nile tilapia (Oreochromis niloticus) body composition models. *Aquaculture*, 564, 739039. https://doi.org/10.1016/j.aquaculture.2022.739039

Soares, F. M., Raposo, A., Mendes, R., Azevedo, M., Dias, J., Nobre, A., Conceição, L. E. C. & Silva, T. S. (2023). ficoEst, a tool to estimate the body composition of farmed fish. *Aquacultural Engineering*, *103*, 102364. https://doi.org/10.1016/J.AQUAENG.2023.102364

Raposo, A. I. G., Soares, F., Conceição, L. E. C., Valente, L. M. P., & Silva, T. S. (2023). Development and evaluation of Nile tilapia dynamic growth and body composition models. *Aquaculture,* 578 (2024), 740032. https://doi.org/10.1016/j.aquaculture.2023.740032

Raposo, A. I. G., Soares, F., Conceição, L. E. C., Valente, L. M. P., & Silva, T. S. (2023). Modelling growth and body composition: a comparative analysis of seven farmed commercial fish species. (*submitted to Aquaculture*)

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Chapter 1 - Introduction

1.1. Current sustainability issues in aquaculture

Sustainability is a term that refers to “the ability of human societies and natural ecosystems to meet the needs of the present without compromising the ability of future generations to meet their own needs”, according to the Brundtland definition (Brundtland, 1987). It involves balancing economic, social, and environmental considerations to ensure that development is pursued in a way that is sustainable in the long term. In today's world, sustainability is more important than ever. The increase of world population and consequently human activities have put tremendous pressure on the natural world. Therefore, it is imperative to adopt adequate strategies in order to avoid environmental degradation, social and economic inequalities, and food and water scarcity.

Fish consumption is growing with the increase of the world population. In fact, between 1961 and 2017, the global fish consumption increased at an average annual rate of 3.1%, outpacing the annual world population growth (1.6%) for the same period, as well as the growth in other animal protein food sectors (meat, dairy, milk, etc.) (FAO, 2020b). Within this period, the aquaculture sector has clearly emerged as an essential factor in reducing the pressure in fish wild stocks, while improving food security. Additionally, global fish production in 2020 reached 178 million tons, with aquaculture representing 49% of the total production (FAO, 2022). The growth and intensification of the aquaculture sector have created new sustainability challenges that call for efficient fish production with high nutritional value, while ensuring sector profitability (Boyd et al., 2020). In this sense, different strategies are being used in the aquaculture sector to tackle sustainability challenges (Figure 1).

In the last 30 years, the aquafeed industry has been dealing with a significant pressure to replace the traditional feed ingredients like fish meal and fish oil with alternative ingredients (e.g., plants, algae, insects, yeasts, bacteria, processed animal proteins) which offer a wide range of nutritional profiles (Boyd et al., 2020). Nowadays, significant efforts have been made to adopt sustainable fishing practices, so the main concern lies in the availability and high costs of fish-based ingredients. Consequently, these fish-based ingredients have found more extensive use in feeding small fish juveniles, where the evidence of their benefits is more pronounced (Jackson, 2006; Tacon & Metian, 2008; Turchini et al., 2009), while alternative ingredients have progressively displaced them from grow-out formulas. Nevertheless, there are many factors that define the formulation of a good feed: nutritional requirements of the species, feed production technology, feed ingredient source, their impact on physical properties of the pellets, and chemical composition – all these factors must be considered. Thus, for fish feeds to meet both fish nutritional demands and cost-minimization criteria, commercial feed mills are increasingly adopting a “precision feed formulation”. This enables feed mills to cope with a variable and diverse range of commodity and specialized ingredients, that often display strong fluctuations in terms of availability and cost. Therefore, instead of static formulas with fixed ingredient proportions, the feed formulation adapts dynamically. It responds to specific purposes and market conditions, allowing for variable ingredient proportions within the nutritional specifications. The challenge is that this requires prior knowledge on the nutritional requirements of a species, to ensure a precise balance of nutrients in fish diets to meet the nutritional needs of the target species at different life stages. Thus, factors such as growth rate, health and environmental conditions must be considered. Furthermore, to ensure that the flexibility in formulation does not compromise the (nutritional) formulation targets, precise characterization of ingredients is essential. This involves not only considerations in terms of ingredients cost and carbon footprint, but also in terms of composition and digestibility, Overall, this is a big challenge for feed formulators, along with the difficulty of finding alternative ingredients as highly nutritious as fish meal or fish oil (Daniel, 2018; Hodar et al., 2020). Promising results have emerged from academic and industrial research in the last years, but much work remains to be done to increase the sustainability, reduce carbon footprint, improve circularity, reduce costs and increase scalability of alternative ingredients in the formulation of commercial diets (FAO, 2020a).

Besides the need to find and use alternative ingredients, there are also pressures to increase production in cultivation systems while maintaining product quality, profitability and reducing environmental impacts. An efficient production system depends strongly on monitoring and prediction of several aspects that determine the success of fish production. In this context, within the last decade there has been a significant effort towards “precision fish farming” (PFF). PFF is a concept that aims to revolutionize aquaculture by applying control-engineering principles to fish production (Føre et al., 2018). To achieve this, PFF focuses on improving accuracy, precision and repeatability in farming operations while facilitating autonomous and continuous monitoring of fish biomass and behaviour (Føre et al., 2018). This means that data-driven management is done to improve the farmer's ability to monitor, control and document biological processes on fish farms, including monitoring environmental conditions and extracting insights from fish behaviour to inform operational decisions (O’Donncha et al., 2021). The integration of modern information technology, such as artificial intelligence, also plays a crucial role in the advancement of PFF. Yang et al. (2020) highlights that artificial intelligence is a key technology for developing intelligent decision-making systems in aquaculture. It enables data-driven production and decision-making through the utilization of sensors, big data, the internet, and camera devices (Bownik & Wlodkowic, 2021; Hu et al., 2020; Wang et al., 2021; Zhou et al., 2018). For instance, sensors collect real-time data on environmental parameters, while models analyse this data to predict fish growth, feeding requirements and health. In turn, automation systems enable precise control of feeding and water quality based on the sensor and model outputs. This close integration allows for data-driven decision-making, optimized resource utilization and reduced environmental impact, enhancing the profitability and long-term sustainability of aquaculture operations.

In the 1990s, it was argued that promoting the production of omnivorous species instead of carnivorous species would enhance sustainability in aquaculture. This was because species like salmon required substantial amounts of protein, which, at that time, were largely supplemented through fish meal and fish oil, leading to overfishing and environmental degradation (Naylor et al., 2000). However, a significant progress has been made in fishery management and fish feed industry to address these concerns. Currently, fishmeal and fish oil are derived mostly from small pelagic fishes, which are among the most sustainable of all fisheries (Hilborn et al., 2022). Most fisheries in developed nations of the world are now managed by independently set quotas based on targeting a maximum sustainable yield (MSY) and operate within those quota systems (Glencross, 2023). Thus, a reduction in fishing pressure has been central to that success. Moreover, Glencross (2023) also stated that as of 2021 almost a third of all fishmeal and more than 50% of all fish oil are coming from food production by-products of fishery and aquaculture produce comprising a circular nutrient recovery and thus contributing to aquaculture sustainability. Over the past 20 years, research efforts have led to a significant decrease in the Feed Conversion Ratio (FCR), particularly for salmon, which has decreased from 1.54 to 1.27 (Glencross, 2023; Henriksson et al., 2021). Apart from improvements in FCRs, there has been a reduction in the utilization of fishmeal and fish oil in diet formulation, resulting in a decline in sustainability metrics like Life Cycle Assessment (LCA) (Glencross, 2023; Henriksson et al., 2021). Therefore, the composition of aquaculture diets has undergone significant changes (Kaushik & Troell, 2010; Tacon et al., 2009; Tacon & Metian, 2015). For instance, feeds used for farmed Atlantic Salmon (*Salmo salar*) in Norway have seen a reduction in total fish protein inclusion from 65% in 1990 to under 15% in 2016, largely replaced by plant-based proteins, oils, and carbohydrates (Aas et al., 2019). Similarly, tilapia feeds decreased from approximately 30% fish protein in 1995 to nearly 6% in 2019 (Henriksson et al., 2021). This shift in diet composition has led to some trophic levels being interpreted differently in the aquaculture sector, prompting authors to suggest that the designation of trophic levels in aquaculture requires further examination to develop policy positions and best practice guidelines for improving aquaculture sustainability (Cottrell et al., 2021). As a result of increased research and development efforts, production systems for carnivorous species have become more efficient, while omnivorous species, such as tilapia, have seen more modest performance gains due to limited research and development in breeding and feeding practices, as well as limited access to quality feed (FAO, 2019). However, tilapia is a fast-growing species that can be raised in small-scale operations, making it accessible to small-scale farmers, providing a source of income and contributing to food security. As a result, Nile tilapia is a valuable source of protein, particularly in developing countries where aquaculture is an important industry, providing socio-economic benefits to those communities (Tacon et al., 2009).

In summary, the development of tools to improve precision of feed formulation and fish farming practices can be seen as essential steps for a sustainable aquaculture sector. This is particularly important for species such as Nile tilapia that, despite having high socio-economic relevance and low trophic level, are still often reared under suboptimal practices.

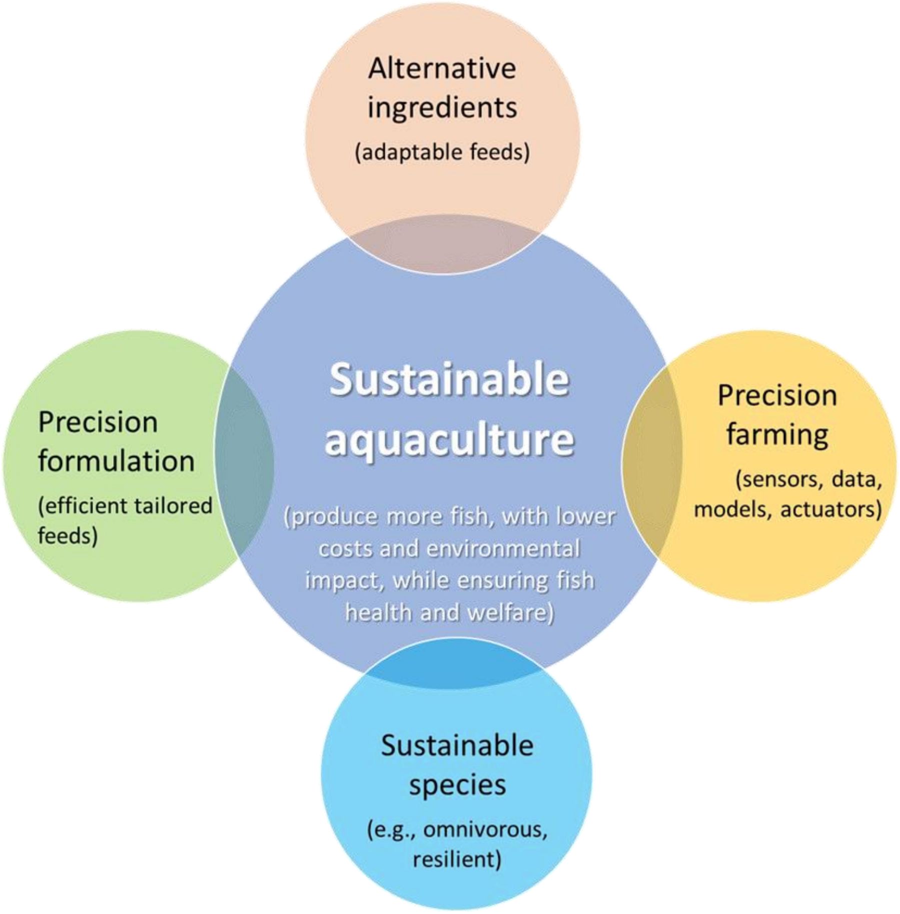

Figure 1 – Diagram illustrating the different strategies that are used to tackle sustainability challenges in the aquaculture sector.

1.2. Tilapia aquaculture production

Tilapia is a popular fish in many parts of the world, including Asia, Africa and Latin America, and is becoming increasingly popular also in North America and Europe. The expansion of aquaculture production of tilapia is evident in the relative growth rates of global *per capita* consumption in recent years (FAO, 2018). The aquaculture production of Nile tilapia *Oreochromis niloticus* between 2000 and 2020 has increased 350% (FAO, 2022) and its global production is expected to reach 7.3 million tons in 2030 (FAO, 2020a). There are several reasons that justify the economic relevance and interest in Nile tilapia production. Firstly, tilapia is known for its rapid growth rate which allows it to reach market size in a relatively short period of time. Additionally, it is able to tolerate a wide range of environmental conditions - low oxygen levels, high temperatures and salinity - without suffering adverse effects (Burggren et al., 2019; Lawson & Anetekhai, 2011; Pandit & Nakamura, 2010; Watanabe et al., 1985). In addition, tilapias are resistant to disease and parasite outbreaks. Therefore, tilapia can be successfully farmed in a variety of environments and production systems (El-Sayed, 2006), which makes them an attractive choice for fish farmers.

1.2.1. Current challenges in tilapia production

Nile tilapia is one of the most important freshwater fish species globally, with high demand in both domestic and international markets. However, the production of Nile tilapia faces several challenges and constraints. One of the main challenges facing Nile tilapia production is the social-economic difficulties in Asiatic countries where most production is concentrated. Many farmers in developing countries face numerous challenges, including inadequate funding, poor infrastructure, and lack of access to modern technology (Antwi et al., 2017; Arifianto, 2022; Arumugam et al., 2023; Minapoli, 2022; Moyo & Rapatsa, 2021; Munguti et al., 2022; Singh, 2019; Toledo et al., 2008). These challenges lead to low productivity, high production costs, and low profitability, which limits the potential for expansion of Nile tilapia production. In 2022, the rise in feed prices has become a major concern for farmers, as it is increasingly challenging to achieve cost-efficiency in production. This creates a significant challenge for farmers who must manage their costs while ensuring their product remains competitive in the market. Adding to this challenge is the issue of fluctuating prices in local markets, which can vary widely between regions (Arifianto, 2022). This can create significant disparities in profitability for farmers and limit their ability to expand their businesses.

Another major constraint of Nile tilapia production is the lack of education and technical knowledge among farmers. Most farmers in developing countries lack the necessary knowledge and skills to efficiently produce Nile tilapia. This lack of knowledge leads to poor management practices, which results in low-quality fish and high mortality rates. Furthermore, farmers lack access to information on market trends and the latest technologies, which limits their ability to make informed decisions regarding their production. In turn, those with access to knowledge and that use new technologies, still face challenges due to their dependence on traditional markets (Minapoli, 2022). The adoption of new technologies often requires significant additional costs, making it difficult to compete in the short term with farmers who do not use such innovations. Some farmers have started selling frozen fish (Eltholth et al., 2015; Minapoli, 2022; Ogello et al., 2022) as a strategy to overcome some of the challenges associated with the traditional market, such as the need to transport live fish long distances or maintain stock for extended periods.

The availability and quality of fry are critical for the success of Nile tilapia farming and are strongly affected by diseases outbreaks and environmental issues (e.g., floods and upwellings in lakes can affect temperature, dissolved oxygen, salinity and water pH). Bacterial diseases such as infectious *Aeromonas* sp. and *Streptococcus* sp. can cause mass mortality and reduce survival rates to values as low as 30% (Amal & Zamri-Saad, 2011; Mai-xin, 2010; Puneeth et al., 2022). Parasites and fungi may also affect fry during the hatchery and nursery stages (Akoll et al., 2012; Awosolu et al., 2018; Chauhan, 2014). Furthermore, Tilapia Lake Virus (TilV) has been emerging in tilapia production worldwide, affecting all live stages of tilapia, causing up to 90% mortality (Aich et al., 2022; Jansen et al., 2019; Nicholson et al., 2020). The supply of high-quality fry is still limited, and too costly for many small-scale farmers, which limits their ability to expand their production. To overcome the diseases and environmental challenges, several tilapia strains have been selected in the last two decades. Overall, the efforts have been concentrated on the selection of more disease resistant (Adamek et al., 2022; Chen et al., 2022; Kayansamruaj et al., 2023; Zhu et al., 2021), with faster growth rates (Dos Santos et al., 2022; Herkenhoff et al., 2020; Trinh et al., 2021) and higher tolerance to environmental conditions (Qin et al., 2022; Setyawan et al., 2022; Washim et al., 2022).

In order to overcome some of these challenges, it is important that tilapia farmers have wider access to technological tools for precision farming. In particular, mathematical models of Nile tilapia biology can be a valuable asset in this context, contributing to a better understanding, monitoring and prediction of relevant processes in tilapia farming and, thus, their optimization.

1.3. Mathematical models and their use in aquaculture

A mathematical model is a simplified and formal representation of a complex process or system using mathematical equations or formulas. It aims to capture the essential characteristics and behaviour of a real system, enabling the analysis and understanding of its dynamics, prediction-making and problem-solving. By abstracting the system using mathematical terms, it is possible to study its properties, draw logical deductions, and even predict future behaviour.

This capacity of mathematical models to encapsulate prior information about a process and predict future behaviour makes them key elements in the development and application of precision fish farming and feed formulation strategies. Thus, mathematical modelling should be seen as a powerful tool that can help farmers optimize production processes, improve productivity and ensure the sustainability of the industry and, in this sense, different types of models can be (and have been) developed and applied for different purposes in aquaculture.

1.3.1. Types of models

The pursuit of accurate prediction and understanding of complex systems has led to the development of many different types of models and modelling approaches. An important distinction that has to be made between model types relates to their time dependency, where we can consider *static models* and *dynamic models*. *Static models* do not consider time as a variable or factor and are thus appropriate to use when the process to be predicted is essentially static. Conversely, in *dynamic models*, time is an explicit or implicit variable and can be used to simulate the behaviour of the system along time under different scenarios. However, under specific circumstances, such as when equilibrium or steady-state conditions can be assumed, it becomes feasible to treat a dynamic process as if it was static. In such cases, static models can be employed to represent the underlying dynamics of the process. Furthermore, it is often also possible to model a dynamic variable using a static model by using a prediction variable that works as a proxy of time (e.g., fish weight or fish length). Furthermore, *dynamic models* can be distinguished according to the time category: continuous or discrete. In continuous dynamic models, the models use differential equations to describe the changes in the system being studied over time (Cuenco, 1989). These models assume that the changes in the system occur continuously and smoothly over time, without any sudden jumps or changes. Discrete dynamic models, on the other hand, use difference equations to describe the changes in the system and assume those changes can occur in discrete steps or intervals, with sudden jumps or changes between these intervals (Cuenco, 1989).

There are several mathematical modelling approaches, with some of them being extremely simple and others more complex, depending on the level of understanding on which the models are based on (Dumas et al., 2010; Marion & Lawson, 1995). *Empirical or phenomenological models* usually are often simpler and merely seek to describe a relation between more than two variables, and not necessarily explain their underlying mechanisms (Dumas et al., 2010; Sun et al., 2016a). In aquaculture, it is common to use empirical models (e.g., relative/specific growth rate, feed conversion rate or thermal growth coefficient) as the underlying parameters are easily determined. The main advantage of using simple models is that they are easy to calibrate and require little information. Conversely, they often lack a clear biological interpretation and/or neglect fundamental properties of aquatic animals (e.g., variations in growth trajectory across life stages) (Cuenco, 1989; Ellner & Guckenheimer, 2006). Another problem is that they only work well under specific conditions (e.g., they assume that fish are being well fed; they only work in the context for which they have been calibrated) and are insensitive to important factors that affect the growth and composition of fish (e.g., protein content in the diet). Alternatively, *mechanistic or explanatory* models rely on a theory or hypothesis that explains the nature of the systems, hence being capable of both describing a particular set of data and explain why the observed relationships exist, at least in theory (Fishwick & Modjeski, 1991), but tend to be more complex and difficult to calibrate compared to empirical models. Mechanistic models provide insight into relevant factors and their relationships. They help to identify key factors to incorporate into a factorial design, thereby suggesting appropriate factor levels based on underlying mechanisms. Since they try to follow (as closely as possible) the mechanism that generates the data, it is expected that, when well calibrated, they generalize and extrapolate better (for unknown situations) compared to empirical models.

Models can also be classified or distinguished based on the type of outcome they predict. In *deterministic models*, a set of equations is used to describe the expected behaviour of a system. These models are calibrated assuming that there is randomness in the outputs of the process being predicted, but this randomness (or noise) is simply considered a nuisance and not replicated in the outputs of the model. Furthermore, these models are relatively easy to interpret and can be used to test various hypotheses and scenarios. On the other hand, *stochastic models* not only assume that there is inherent variability in the system being studied, like *deterministic models* do, but also seek to incorporate this variability or uncertainty into the model outputs. However, *stochastic* *models* are generally more complex and computationally intensive than *deterministic models,* and can also be more difficult to interpret.

1.3.2. Modelling process

The modelling process involves creating simplified representations of complex systems in order to better understand and predict their behaviour. However, modelling is not a simple task and involves a series of actions that require careful consideration and attention to detail (see Figure 2). Firstly, it is necessary to identify the problem or system to be modelled and the model's goal. Then, an appropriate modelling approach and methodology should be selected, considering the specific context and requirements of the model. However, accuracy is not guaranteed without careful consideration of the following steps in the modelling process:

I. Data collection

The step of data collection is one of the most important steps in modelling. Models rely on data to accurately represent the behaviour of the system being studied, and the quality of the data used to develop the model directly affects the accuracy and reliability of its predictions (Budach et al., 2022; Klein & Rossin, 1999). Thus, the development of an optimal model depends on the quantity and quality of the collected data. However, collecting high-quality data can be a challenging task, and may involve the use of specialized equipment, such as water quality sensors or fish tracking devices, as well as skilled personnel to collect and analyse the data. As an alternative, or as a complement, collecting data from the scientific literature allows researchers to access a large amount of existing data from a variety of sources. Nevertheless, literature sources may use different units of measurement, sampling methods, or definitions of key variables, which can make it difficult to compare and integrate data from multiple sources. In addition, data may be incomplete or missing, or may have been collected using methods that are not well-documented, which can make it difficult to assess their reliability and validity. Moreover, it can be a time-consuming and resource-intensive process.

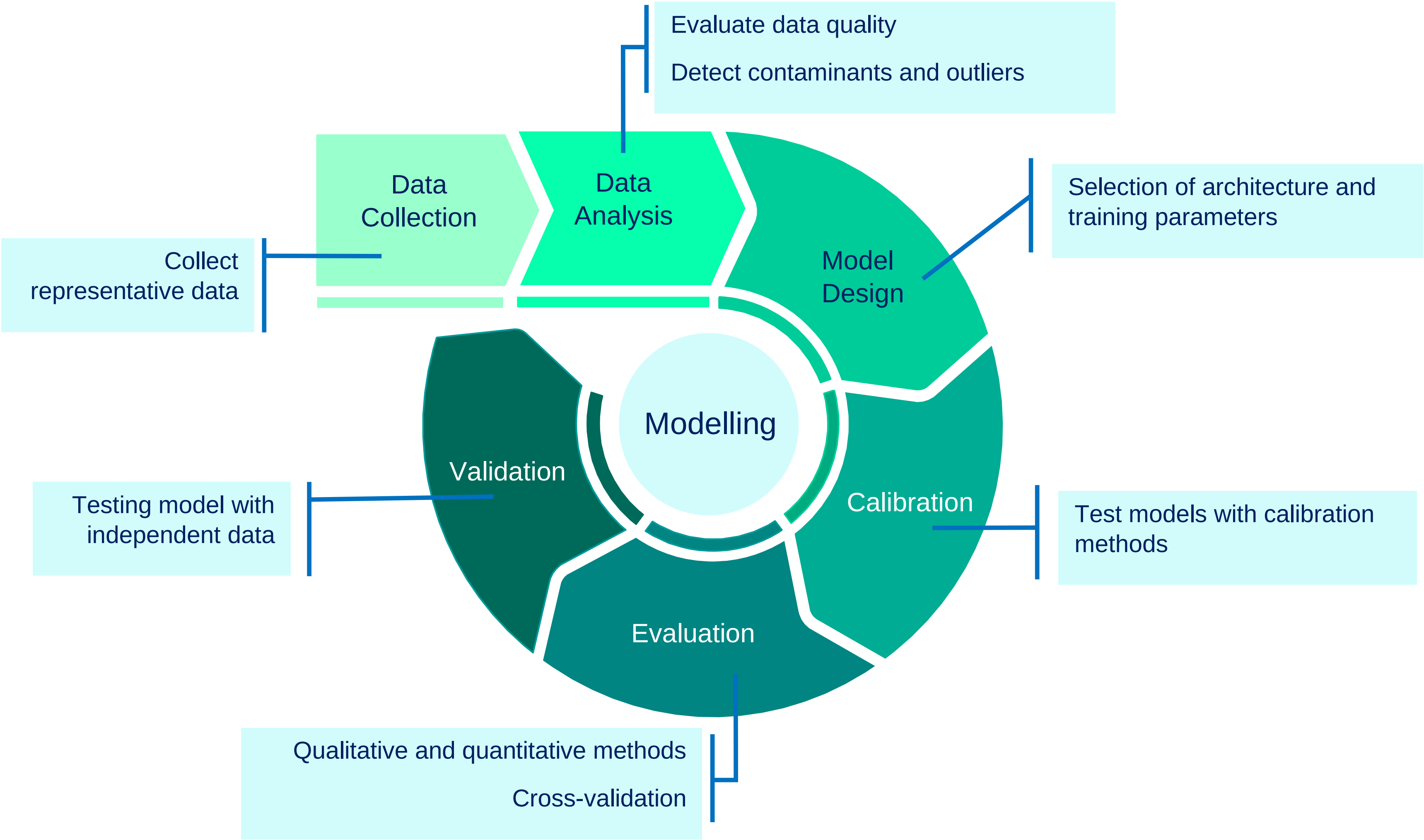

Figure 2 – Diagram displaying the different steps in the modelling process after identifying the problem or system to be modelled, the models' goal and the appropriate modelling approach and methodology.

II. Data analysis

After data collection, a careful analysis is imperative to evaluate data quality and detect any possible contaminants and outliers. In order to do that, data should be first analysed using a series of practices to summarize and describe the set of data in a simple way, such as:

- Analysis of univariate descriptive statistics such as the mean (average), median, minimum, maximum and standard deviation. These statistics give a general overview of the central tendency, variability, and range of a dataset;
- Univariate analysis of distributions, to identify any underlying patterns or trends in the data, as well as any variations from expected patterns by using histograms, boxplots and Q-Q plots.

Furthermore, conducting an exploratory multivariate analysis of the data is crucial to detect outliers, gain insight into the behaviour of the data, understand the assumptions that can be made, and anticipate the expected outcomes of the predictors. One common technique for exploratory analysis is to analyse correlations between variables, which can be visualized using scatterplots and correlograms, or use tools like Principal Component Analysis (PCA), when handling higher-dimensional datasets. This allows for the assessment of whether pairs of variables are related and, if so, whether they tend to move in the same or opposite directions.

III. Model design

It is important to understand how the system to be modelled works in order to design reasonable models. However, sometimes the information available about a process is insufficient. In this case, the use of statistical tools to explore relationships between data (e.g., linear and non-linear regression models, classification and clustering tools) can be beneficial to explore relationships between data and derive insights from the available information.

In constructing models, the relationship between input variables and the output variable is a critical consideration (Cuenco, 1989). Many equations are available to define these relationships (see Table 1 for a non-exhaustive list of examples). Models can be constructed with different number of parameters (e.g., polynomial equations with different degrees), being the models more complex as the number of parameters increase. However, more complex models do not necessarily mean that they are more accurate. In fact, in some cases, it is preferable to use a simpler model (Katsikopoulos et al., 2018; Wenger & Olden, 2012). Following *Occam's razor*, which states that "*the simplest explanation is most likely the right one*", a model with as few variables and hypotheses as possible should be selected from among multiple appropriate and possible explanations for the same set of data (Sober, 1990). Incorporating additional parameters to capture more nuanced relationships between variables can improve model accuracy and predictive power. However, it may also increase the risk of the model to perform exceedingly well on the training data, but then fail to generalize well to unseen or new data (i.e., model overfitting). This may occur when the model becomes too complex and captures not only the underlying patterns but also the noise or random fluctuations present in the training data. Thus, it is important to carefully consider model complexity and balance it against the amount and quality of available data. This can help avoid overfitting and ensure that the model is both accurate and generalizable to new data. By selecting an appropriate level of complexity, models can be constructed to effectively represent the relationship between input and output variables while maintaining interpretability and generalizability.

Table 1 - Several univariate functions commonly used to model biological processes. y = dependent variable; x = independent variable; e = base of the natural logarithm; A, B, n > 0 = parameters of the equation (adapted from Cuenco, 1989).

	Function	Nº of parameters
Linear	$y = Ax$	1
Affine	$y = Ax + B$	2
Exponential	$y = Ae^{nx}$	2
Power function	$y = Ax^n$	2
Exponential saturation	$y = A(1 - e^{-nx})$	2
Modified power function	$y = Ax^n + B$	3

IV. Model calibration

Calibrating a model is a process of finding the optimal set of parameters, based on given datasets (i.e., observations and corresponding input sets), in order to minimize a measure of prediction error. The objective is to maximize the model's ability to accurately predict outputs for any valid input by minimizing prediction errors on the calibration data. It is important to note that the determination of the "optimal" parameter set, as well as the method employed to identify it, typically rely on the specific measure of "prediction error" being utilized.

The assumptions that are made to adjust the model to the calibration data are other important factors to consider in model calibration. Assumptions are the simplifying conditions or constraints imposed on the model to make it tractable or representative of the real-world system, and thus can vary depending on the specific modelling context and goals. For example, to calibrate models, the equation normally used is one of the following, depending on the different assumptions:

$$y = f(x,\beta) + \varepsilon_{additive} \qquad (1)$$

$$y = f(x,\beta) \times \varepsilon_{multiplicative} \qquad (2)$$

where y are measurements, $f(x,\beta)$ is the linear or nonlinear functional, x is the vector of known inputs, β is the vector of unknown parameters to be estimated, $\varepsilon_{additive}$ the additive error and $\varepsilon_{multiplicative}$ is the multiplicative error. In equation (1), it is assumed that the error is additive, meaning that the size of the error will not change with the size of the measurements (e.g., the measurements of fish with 10 g or 100 g of body weight are going to display errors of 1 g). On the other hand, in equation (2) there is the assumption that the error is multiplicative, meaning that the error size is going to change with the sizes of measurements (e.g., the measurements of fish with 10 g of body weight are going to have errors of 1 g, while fish with 100 g of body weight will have 10 g) (Figure 3). Essentially, the first equation assumes that the size of errors is constant on an absolute scale, while the second assumes that the size of errors is constant on a relative scale. Therefore, determining whether the error is additive or multiplicative is critical to accurately parameterize the model and evaluate its performance (Gaganis, 2009). Failing to account for the correct type of error can lead to biased estimates of the model parameters and inaccurate predictions. Moreover, different assumptions can lead to different calibration processes because they determine the nature of the relationships to be captured and the parameters to be estimated. The calibration process will involve techniques specific to the model assumptions, such as least squares estimation, maximum likelihood estimation, or Bayesian inference.

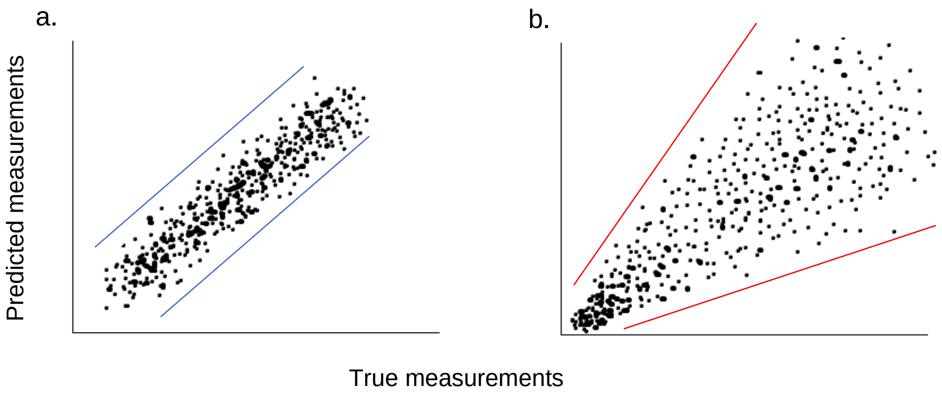

Figure 3 - True measurements vs. predicted measurements in the presence of additive error (a.) and multiplicative error (b.), when plotted on an absolute scale (adapted from Wu et al. (2019)).

The most important and straightforward way of evaluating model calibrations is to use quantitative metrics that are either positively (in the case of “goodness-of-fit” metrics) or negatively correlated (in the case of “error” metrics) with reasonable measures of prediction quality. The ideal quantitative metric depends on the specific modelling objectives, the nature of the data being modelled and the noise model assumed, so it is common for people to simultaneously consider more than one metric when evaluating models and calibrations. Some examples of the most common metrics are:

a. The R-squared measure of goodness-of-fit

The R-squared is the proportion of the variance in the dependent variable that is predicted from the independent variable(s). It provides information on how well the observed results are replicated by the model, though it is highly sensitive to outliers (Gaganis, 2009; Smith et al., 1996). Values of R^2 lie between 0 and 1, with higher values indicating better goodness-of-fit. The formula can be expressed as:

$$R^2 = 1 - \frac{Residual\ sum\ of\ squares}{Total\ sum\ of\ squares},$$

$$R^2 = 1 - \left[\frac{\sum_i (\hat{y}_i - y_i)^2}{\sum_i (y_i - \bar{y})^2}\right]$$

where $\hat{y}_i$ is the predicted value, y_i is the observed value and $\bar{y}$ is the arithmetic mean of y (Ellis, 1972).

b. Root Mean Square Error (RMSE)

This metric expresses the standard deviation of the residuals (prediction errors) and it reveals how closely the data is clustered around the line of best fit. While this metric is also sensitive to outliers, it is particularly useful for measuring additive errors. It is also an effective criterion for assessing the accuracy of a model, with a lower value indicating higher accuracy, being 0 the lower limit (Kim & Kim, 2016). The formula is expressed as:

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

where, $\hat{y}_i$ is the predicted value, y_i is the observed value and n the number of data points. In normal (least squares) linear regression, this is the error that is explicitly minimized.

c. Mean absolute error (MAE)

The MAE provides an average assessment of the absolute errors between predictions and observations, and it offers insight into the bias and variance of the model predictions. Like the RMSE, it is appropriate for gauging additive errors, while being less susceptible to outliers. The MAE is calculated as:

$$MAE = \frac{\sum_{i=1}^{n} |\hat{y}_i - y_i|}{n}$$

where, $\hat{y}_i$ is the predicted value, y_i is the observed value and n the number of data points (Willmott & Matsuura, 2005). This is the error measure that is explicitly minimized when applying least absolute deviation regression methods.

d. Mean absolute percentage error (MAPE)

The MAPE is the mean or average of the absolute percentage errors of predictions and is commonly employed as an indicator of a model's prediction accuracy. This measure is advantageous because it is scale-independent and provides errors in terms of percentages, which are simple to comprehend (Swamidass, 2000). Additionally, since absolute percentage errors are used, the issue of positive and negative errors cancelling each other out is avoided (Swamidass, 2000). This metric is less sensitive to outliers and is well-suited for evaluating multiplicative errors. A lower MAPE score indicates greater accuracy. This metric is calculated as:

$$MAPE = \frac{1}{n} \Sigma_{i=1}^{n} \left| \frac{y_i - \hat{y}_i}{y_i} \right| \times 100$$

where, $\hat{y}_i$ is the predicted value, y_i is the observed value and n the number of data points.

e. The coefficient of residual mass (CRM)

The CRM provides information about bias: the tendency of the model to underestimate (CRM>0) or to overestimate (CRM<0), with the optimum = 0 (Bonfante et al., 2010; Smith et al., 1996). This is a scale-independent metric and sensitive to outliers. The equation is expressed as:

$$CRM = \frac{(\Sigma_{i=1}^{n} y_i - \Sigma_{i=1}^{n} \hat{y}_i)}{\Sigma_{i=1}^{n} y_i}$$

where, $\hat{y}_i$ is the predicted value, y_i is the observed value and n the number of data points (Bonfante et al., 2010; Smith et al., 1996).

Different error metrics and assumed error models generally lead to different calibration processes, as they define the optimization problem during calibration. Since the aim of the calibration is to minimise the chosen error metric, this optimisation process may require different algorithms or techniques, depending on the properties of the metric.

In many cases, outliers in the data can have a significant impact on the model performance, leading to inaccurate predictions. Therefore, it is important to consider the presence of outliers and their effect on the model during the calibration process. One approach to dealing with outliers is to perform robust calibration, which aims to minimize the influence of outliers on the model parameters (Hodge & Austin, 2004; Pell, 2000; Rousseeuw & Hubert, 2011). One example of a robust calibration method is robust linear regression, which is a variation of linear regression that uses a different loss function to down weight the contribution of outliers (Sadouk et al., 2020). This approach is particularly useful when the data contains a few extreme values that can significantly affect the estimated regression coefficients. Another approach is to use mixed-effects models, which allow for the incorporation of both fixed and random effects (Fang, 2011; Pinheiro & Bates, 2000; Schielzeth et al., 2020). This can be useful when there is heterogeneity in the data, such as different experimental conditions or sampling locations, which can lead to different sources of variation. By accounting for this heterogeneity, mixed-effects models can produce more accurate predictions.

V. Evaluation of models and calibration methods

This step involves testing *the process of obtaining a calibrated model* from a calibration dataset, which involves the combination of the model and a specific calibration method. The main objective is to verify that the process used to derive the final model, calibrated with all the calibration data and prepared for validation against independent data, results in models that demonstrate strong performance when evaluated with data that was not included in the calibration set. In other words, the objective is to ensure that the calibrated models exhibit low generalization errors (e.g., a measure of model accuracy when predicting future samples), indicating their ability to accurately predict outcomes beyond the data used for calibration. Simply using “calibration error” (e.g., a measure of model accuracy on previously seen samples) as a proxy is overly optimistic and can lead to the selection of excessively complicated models that generalize poorly. A good option to estimate the generalization error, when no independent validation data is available, is to use cross-validation errors.

The principle of the cross-validation (CV) is to split the data in equal *k* folds to estimate the error of each algorithm. Consequently, a portion of the data is utilized to train each “combination of model + calibration method” (the training sample), while the remaining data is used to gauge the algorithm's error (the validation sample). The algorithm with the lowest estimated error is then selected (Arlot & Celisse, 2010; Hastie et al., 2001). The optimal number of folds (*k*) utilized to split the data is dependent on the data's structure. However, when the goal is model selection for estimation purposes, a recommended range for *k* is between 5 and 10. This is due to the fact that statistical performance does not improve beyond a certain point with higher values of *k* (Arlot & Celisse, 2010). Moreover, as the *k* value decreases, less data is available for the algorithm to be calibrated (training) – e.g. in a 10-fold CV, 90 % of the data is used as a training data set and 10 % is used for testing (validate) the algorithm; in a 5-fold CV, 80 % of data is used for training and 20 % is used to test; and in a 2-fold CV, 50 % of the data is used for calibration and 50 % to test the algorithm. There is also the Leave-one-out cross-validation (LOOCV) method. In this case, the *k* is equal to the number of the data set ($k = n$), so this method uses every data point to train the algorithm except one, using the left-out point for testing the algorithm (with this process being then applied for every other point). The LOOCV is a deterministic method, so there is no need to repeat the process, whereas in *k*-fold CV it is advisable to repeat it ensuring a large number of data split combinations are covered. In addition, the LOOCV performs a low-error bias estimation and it is asymptotically optimal. Nevertheless, it suffers from a large variability in the prediction error, especially when there are outliers, and is computationally expensive (Arlot & Celisse, 2010). Therefore, a cross-validation with k < n usually is the best approach to obtain an algorithm with a small error. However, it must be considered that cross-validation with lower *k*-values is more restrictive and therefore imposes great penalty on more complex models, compared to cross-validation with higher *k*-values, which are more flexible (Hastie et al., 2001).

When cross-validation is used, a relevant error metric (e.g., MAPE) can be calculated for each fold. Then, the average of the respective metric values across all folds is calculated. Thus, by using cross-validation, it is possible to obtain a more accurate estimate of the performance of the model calibration method as it is evaluated on multiple independent test sets, reducing the risk of overfitting.

Besides quantitative criteria, it is also important to consider qualitative criteria during model evaluation. The qualitative analysis provides relevant information about the data (e.g., differences, trends, types of error and distribution patterns of the simulated and observed values), highlighting the qualities of model structure (Smith et al., 1996). These methods usually comprehend the residual analysis and refer to visual evaluation of plots, like normal Q-Q plots, residuals vs. fitted, scale-location or Cook’s distance plots. Basically, it involves examining the differences between the predicted values from a model and the actual observed values, which are known as residuals. These residuals represent the errors in the model and provide insights into how well the model is performing. Furthermore, it allows to check the assumptions of the model (Andel, 1997; Verran & Ferketich, 1987). For example, Figure 4 illustrates an example of models with different assumptions calibrated with the same data predicting the water component of fish. Despite differences in their behaviour, the models perform as expected (e.g., water composition approaching zero as body weight also approaches zero) and they fit the provided data adequately. Residual plots can reveal patterns in the data that the model did not capture, such as heteroscedasticity or nonlinearity, which can suggest that the model may need to be refined or a different approach may be required to improve the accuracy of the model and the reliability of its predictions. Moreover, it assists in determining whether the model is overfitting (i.e., model is too complex and captures noise in the data, which leads to poor performance on new data) or underfitting (i.e., model is too simple and fails to capture important patterns in the data). Therefore, it is a powerful tool for understanding the strengths and weaknesses of a model.

In summary, when selecting a model type and calibration method, it is important to consider objective criteria (e.g., cross-validation), particularly given that multiple models may perform well in adapting to the available data and exhibit low calibration errors, yet their predictions may still be inaccurate when presented with independent data. Therefore, it is essential to assess both qualitative and quantitative indicators to measure the models' accuracy and reliability. By doing so, one can identify the most appropriate model that not only fits the available data, but also has good generalization capabilities, which is crucial for making reliable predictions.

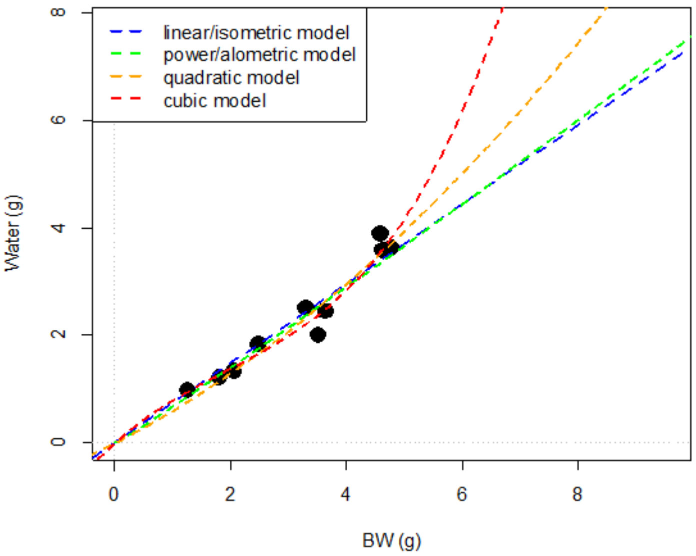

Figure 4 - Scatter plot illustrating the behaviour of different types of models when trying to fit body composition data. Herein models were developed with different assumptions in order to predict the absolute amount of water (in grams) in function of the fish body weight. All models were calibrated with the same dataset and seem to fit well the data, but present different behaviours, particularly when extrapolated.

Thus, overall, the process of obtaining a calibrated model needs to be evaluated against various criteria to select the best combination of "model + calibration," including:

- **Quantitative**
    - Low empirical (i.e., training/calibration set) errors: these errors refer to the model's ability to predict well with the data it has seen during the training phase. A good model should be able to capture the important patterns in the training data and accurately predict the response variable.
    - Low generalization (i.e., independent/validation set) errors: these errors refer to the model's ability to predict well with data it has *not* seen during the training phase (e.g., estimated using *k*-fold CV). A good model should be able to capture not only the important patterns in the training data, but also provide accurate predictions of the response variable for *unseen* data.

- **Qualitative**
    - Residual dependence: the residuals should reflect a non-pathological fit, meaning that they should not display any systematic patterns or trends. If residuals exhibit systematic patterns or trends, this suggests that the model has not captured all the relevant information in the data and may need further refinement.
    - Residual distribution: the residual distribution should be consistent with assumed error distribution (e.g., normality, constant variance). If the residuals' distribution is not consistent with the assumed error distribution, this may suggest that the model's assumptions are incorrect or that there are outliers in the data that need to be addressed.

VI. Validation/testing of calibrated models

Once the evaluation of various combinations of "model + calibration method" has been conducted, and the appropriate method(s) have been selected based on cross-validation results, these chosen combinations are applied to the entire calibration dataset to generate the final models. However, for a thorough validation of these models, it is crucial to assess their performance against truly independent data (i.e., data that was not involved at *any* previous stage of the modelling process), both quantitatively and qualitatively. This evaluation is essential to obtain an accurate estimate of the model's ability to predict unseen data. By assessing the models against this independent dataset, we can gain valuable insights into their predictive capabilities and understand how well they generalize to new and unobserved data. Until here, several changes can be done in the modelling process (e.g., collect more data to calibrate the models, try different models or calibration methods). However, after performing the validation step, no changes in the model calibration processes should be done based on those results, or else the choice of the optimal models is no longer guaranteed to be unbiased, since the used data is no longer independent from the model construction process.

The evaluation of the models is done based on the methodology described in the previous step (e.g., calculation of metrics and residual analysis), and at the end of this step, if all prior steps have been executed accurately, a final list of calibrated models with unbiased estimates of their generalization errors is obtained. This provides an objective foundation for selecting the prediction model to use and informs the user on the expected magnitude of future prediction errors when using the model.

1.3.3. Models used in aquaculture

In a general sense, mathematical models can be (and have been) used with different purposes within the aquaculture sector. These include models focused on the biology of marine organisms, which can be used (e.g.) to estimate and predict fish growth (Bar et al., 2008; Conceição et al., 1998; Hua et al., 2010; Kuebutornye et al., 2020; Machiels & Henken, 1986; Oviedo-Rondón et al., 2014; Santos et al., 2019; Soares et al., 2023; Strand, 2005), energetic and nutritional requirements (Bavčević et al., 2020; Cho & Bureau, 1995; Hartman & Brandt, 1995; Lupatsch, 2003; Lupatsch et al., 1998, 2003, 2010; Nobre et al., 2019), body composition (Breck, 2014; Chowdhury et al., 2013; Chowdhury & Bureau, 2009; Dumas et al., 2010; Shizari, 2020), feed intake (Sun et al., 2016b), metabolic kinetics (Rønnestad & Conceição, 2012), waste outputs (Cho & Bureau, 1998; Chowdhury et al., 2013; Lupatsch & Kissil, 1998; Nobre et al., 2019; Wik et al., 2009; Yulisa et al., 2023) or susceptibility to diseases (Alaliyat & Yndestad, 2015; Ferreira et al., 2021; Mikkelsen et al., 2009; Salama & Rabe, 2013; Thrush et al., 2011), as well as many other types of models focused on other important aspects of aquaculture, such as (e.g.) the use of spatial models to estimate environmental variables within cages (Alver et al., 2022), or the use of geospatial models for optimal cage placement (Koniyo & Kasim, 2017; Zeichen et al., 2022). Many of these processes are complex, interdependent, and influenced by a variety of factors, including genetics, environmental conditions, feed intake, and water quality, making them challenging to represent using simple mathematical models.

In the context of precision feed formulation and fish farming, understanding how fish assimilate energy and nutrients from the feed to grow is essential, and mathematical models provide a useful formalization of this knowledge. In specific, mathematical models of fish growth and body composition are straightforward tools that enable aquaculture managers to manage production parameters, such as feeding rates, feed type and stocking density, such that growth and nutrient utilization is maximized, while mortality rates and feed conversion rates are minimized. Additionally, they offer an objective and practical way to describe growth patterns, being a useful tool to estimate the time needed to reach a certain target weight (e.g., market weight) (Bureau et al., 2000; Mansano et al., 2017).

1.3.4. Current challenges in modelling

Mathematical models have become an indispensable tool in the aquaculture sector, as they enable predictions on how various factors impact the efficiency of fish production. However, in many published studies, authors do not clearly explain the criteria that drove their choice of models. Often, no details are provided on the calibration methods used, so there is no way to understand how the process of adjustment of the model parameters was done. Moreover, it is common to find scientific articles in which authors only rely on goodness-of-fit or error metrics that are evaluated on the data used for calibration when selecting a model. However, this approach may not provide a clear understanding of the model's generalization capabilities. Therefore, there is a need for standardization and clear reporting of methods in fish modelling research to facilitate comparability and reproducibility. Overall, it is important to test different combinations of models and calibration methods in order to explore different assumptions. Moreover, the use of both qualitative and quantitative model evaluation methods – rather than just relying on one or two goodness-of-fit measures – and their combination with cross-validation methods (or other validation method), is essential to obtain robust and predictive models which can generalize to unseen samples. In addition, the documentation of the whole process is equally important so that information can be easily shared and understood by the interested parties.

Another important problem related to most mathematical models used in the aquaculture sector is that they are often very simple and lack the ability to predict the effect of nutritional factors on fish growth and composition. Thus, there is a need to assist the industry in transitioning from simpler models to more advanced models. This is because advanced models have the ability to predict not only fish growth, but also fish body composition, feed requirements and production waste, under different (and varying) contexts. Thus, advanced models can help reduce production costs and minimize waste. Nevertheless, it is necessary to develop these mathematical models based on biological, biochemical, and statistical principles in order to ensure high-quality and robust predictions.

Ultimately, the interconnection between the body composition and growth of fish poses a challenge in modelling them accurately. Therefore, to develop high-quality growth models for tilapia it is important to have a prior comprehensive understanding and predictive capability of body composition. This knowledge not only facilitates the development of mathematical models to estimate feed requirements and waste production, but also ensures the accuracy and reliability of the overall growth modelling process.

1.4. Prediction of fish body composition

1.4.1. Fish body composition

The whole-body composition of fish is usually referred to as proximate composition and it comprehends the components of moisture, crude protein, crude lipid (ether extract), crude fibre, crude ash (minerals) and nitrogen-free extracts (NFE) (Greenfield & Southgate, 2003). The word “crude” is often used before some terms (e.g., protein) to emphasize that it is a determination of a group of closely related substances together (Hart & Fisher, 1971) (Table 2). The proximal composition is usually expressed as a percentage of the original weight of the sample, though in some cases it is given as a percentage of the dry weight. Generally, in routine analysis, moisture, crude protein, crude lipid and crude ash, are determined through chemical procedures and then the NFE is calculated by subtracting the sum of these percentages from 100%. It can also be referred as the “carbohydrates by difference", or "total carbohydrates" (Hart & Fisher, 1971). Although present in the body composition of fish, carbohydrates are often neglected due to their small quantity and, therefore, usually ignored in chemical analysis (Aitken et al., 2001; Breck, 2014; Brett & Groves, 1979). The body composition in fish results from the uptake and utilization of nutrients from the diet. Proteins, lipids and other nutrients are broken down into their basic components during digestion and absorbed into the intestine and further transported to the bloodstream (Bakke et al., 2010; Webster & Lim, 2002). These components are then transported to the different tissues and organs of the fish, where they are utilized for energy production, growth, and maintenance.

Table 2 - Target substances and other substances that can be present when the different whole body proximate composition groups are analysed.

Proximate composition	Target substances	Other substances
Moisture	Water	Volatile substances
Crude ash	Minerals	Organic residue
Crude lipid (ether extract)	Triacylglycerides, phospholipids, fatty acids, steroids	Liposoluble vitamins, waxes, lipoproteins, carotenoids
Crude protein	Proteins, peptides, amino acids	Non-protein compounds (e.g., nucleotides, vitamins, polyamines or other kind of amines)

Nutrients in excess are often stored as fat, which can be further mobilized as an energy source when needed. The body composition of fish can be affected by multiple factors – such as, species, genotype, environment, diet – during their life cycle, with consequences for many biological functions of fish (e.g., appetite, reproduction) (Breck, 2014).

Direct methods for body composition assessment

There are distinct methods for measuring the chemical composition of food products, including fish, which involve analytical methodologies standardized by the Association of Analytical Chemists (AOAC, 2005). The most commonly used analytical methods for measuring the components of fish body composition are described below.

Moisture/water content

Water content is usually measured by oven-drying samples at 105 ºC and then calculating the loss of mass after total evaporation of water, which is until constant weight is achieved (Aitken et al., 2001; Aurand et al., 1987):

$$Water\ content\ (\%WW) = \frac{sample\ wet\ weight - sample\ dried\ weight}{sample\ wet\ weight} \times 100$$

Minerals/ash

Minerals are the inorganic components of fish, often called ash because of the method of measuring them. To estimate this fraction, dry samples are weighted and then, organic compounds are either decomposed or released under high temperatures (500 – 600 °C); the remaining residue is then weighted (Aurand et al., 1987). The amount of ash (crude ash) is then estimated as the remaining mass after incineration of the organic matter (Aitken et al., 2001):

$$Crude\ ash\ (\%WW) = \frac{weight\ of\ ash}{weight\ \ sample} \times 100$$

Crude lipid

The most common methods to assess crude lipid from a sample is through an organic solvent extraction. The solvent is added to a dry, ground sample to dissolve the fats and all liposoluble substances, and then evaporated, leaving a mass that is weighted to calculate the percentage of crude lipid (Aitken et al., 2001; Aurand et al., 1987; Hart & Fisher, 1971):

$$Crude\ fat\ (\%WW) = \frac{weight\ of\ fat\ extract}{weight\ \ sample} \times 100$$

Crude lipid in fish is usually determined by gravimetric methods, such as (Bligh & Dyer, 1959) and Soxhlet (Aitken et al., 2001; Aurand et al., 1987). In these methods lipophilic substances are extracted with nonpolar and polar organic solvents to eliminate the bonds between sample matrix and lipids (Srigley & Mossoba, 2017). Bligh & Dyer (1959) recommended to use chloroform and methanol to obtain an organic phase and then the solvent is evaporated to obtain the fat content. In Soxhlet, ether and hexanes are used in multiple extraction cycles in a specialized glassware setup (Srigley & Mossoba, 2017).

Crude protein

Crude protein content is generally assessed by determining the percentage of nitrogen (as NH3) and assuming that this nitrogen was released from protein during digestion (Aurand et al., 1987; Mariotti et al., 2008). The nitrogen content of many proteins is about 16 %, so the crude protein in a certain fish sample is conventionally estimated by multiplying the determined nitrogen content by a nitrogen-to-protein conversion factor of 6.25 (Aitken et al., 2001; Aurand et al., 1987; Hart & Fisher, 1971). One standard method for determining nitrogen is the Kjeldahl procedure. The nitrogen content in fish can be calculated by:

$$N(\%) = \frac{0.7(V_1 - V_0)}{M}$$

where V_1 is the mean volume in millilitres (mL) of 0.1 M hydrochloric acid required to neutralize or react with the nitrogenous compounds present in the fish sample, V_0 is the mean volume in millilitres (mL) 0.1 M hydrochloric acid required for blank (control sample that contains no nitrogenous compounds) and M is the actual weight of the sample being analysed in grams (Aitken et al., 2001). Nowadays, the Dumas method (nitrogen combustion) i is also widely used to assess the crude protein content. During the sample combustion, nitrogen is released and quantified by gas chromatography (Nielsen, 2014).

Estimation of body composition by indirect non-invasive methods

Mathematical models that relate body composition to measurable variables such as body weight, length, condition factor, conductivity or impedance (Copeland et al., 1999; Cox & Hartman, 2005; Eyduran et al., 2010; Hanson et al., 2011; Muchlisin et al., 2017; Naeem & Salam, 2010) are increasingly used due to their non-destructive nature, allowing repeated measurements over time, and avoiding analysis costs. These methods can comprehend two main approaches: methods that rely on specific instrumentation, and models that rely on easily and trivially measured variables. Both approaches have their unique characteristics and serve different purposes. Methods that involve specific instruments, such as TOBEC (total body electrical conductivity) (Barziza & Gatlin, 2001; Brown et al., 1993), BIA (bioelectrical impedance analysis) (Duncan, 2008; Fitzhugh et al., 2010; Hartman et al., 2015; Pothoven et al., 2008), CT scans (computed tomography) (Ceballos-Francisco et al., 2020; Ding et al., 2019) and DEXA (dual-energy x-ray absorptiometry) (Hussain et al., 2013; Johnson et al., 2017; Ndiaye et al., 2020; Wood, 2004), offer advanced and accurate determination of fish body composition. Some of these techniques provide detailed information about tissue types, organ sizes and body shape, allowing for precise estimation of different components of body composition. However, these methods can be costly and require expertise.

On the other hand, models that rely on easily and trivially measured variables (e.g., weight, length) are the most economical indirect non-invasive methods for estimating fish body composition. In fact, it is crucial to have models based on trivial measurements as baseline references. These baseline models require minimal data inputs and provide a benchmark for evaluating the performance of more complex techniques. Thus, any indirect methodology that utilizes instrumentation should be rigorously evaluated against these baseline models to ensure its reliability and effectiveness in fish body composition estimation.

Usually, empirical models, which are based on the analysis of data from large numbers of fish of different species, sizes and conditions, are used for this purpose. These models use regression analysis to relate body composition to variables such as body weight and length (Breck, 2014; Chowdhury et al., 2013; Chowdhury & Bureau, 2009; de Castro Silva et al., 2015; Dumas et al., 2010; Furuya et al., 2014; Shizari, 2020). Furthermore, these models enable the extraction of more information from the data (e.g., farmers can use their production data to estimate environmental impacts) which may contribute for important decisions in aquaculture management.

1.4.2. Fish body composition models

The relationship between body weight and body components is usually described either with isometric and/or allometric models (Dumas et al., 2010):

$$Isometric \qquad y = a \times body\,weight$$

$$Allometric \qquad y = a \times body\,weight^{b}$$

where *a* is the scaling factor and *b* is the BW exponent of the model. The values of *a* and *b* are estimated using statistical techniques such as regression analysis, and determine the scaling relationship between body weight and the specific body component (*y*). The isometric model assumes that the proportion between body weight and a specific body component (*y*) remains constant regardless of the size or weight of the fish. On the other hand, the allometric model assumes that the proportion between body weight and body components changes as the fish grows in size. Furthermore, it allows examination of how the proportion of a body component changes with increasing fish size, as it recognises that different body components can grow at different rates relative to body weight.

Implicitly, in body composition models, the absolute amount of each component is assumed to follow either a linear relationship (isometric models) or a power law relationship (allometric models) with body weight. However, the different body components of fish can also be predicted using different models. Breck (2014), for instance, used a linear regression to obtain equations to predict protein and ash from water, in several freshwater species. Then, the fish' water mass was used to predict protein, ash and lipids by subtracting the previous components from wet weight. This is a good concept to develop a model that only requires water composition and body weight of fish.

The modelling of fish body composition is typically approached from a static perspective, providing a simplified representation of the relationship between body components and body weight. This provides an understanding of the general patterns and proportions of the different body components at a given time, which can serve as a starting point for further research. It can also be refined or complemented with dynamic models that consider changes in body composition over time.

1.4.3. Challenges in predicting the body composition of fish

Predicting the body composition of fish poses several challenges due to the complexity and dynamic nature of fish physiology. One of the main challenges is the inter- and intra-species variability, where different fish species and individual fish within a species exhibit variation in their body composition.

There are also several challenges associated with data collection that can contribute to difficulties in predicting fish body composition. One major challenge is the lack of uniform language and reporting among authors. For example, some authors may refer to "whole-body" composition but actually use the carcass (i.e., fish without internal organs) in their chemical analysis. Additionally, some authors report values on a dry weight basis, others on a wet weight basis. Also, some body components are measured indirectly by difference, which in some cases contributes to dubious data quality. Some of these issues are easy to identify than others, but they all can contribute to prediction errors if not detected early in the analysis.

Another challenge in predicting the body composition of fish lies in the lack of standardized methods to predict fish body composition. In the scientific literature, different methodologies have been used to predict the body composition of fish. Comparing and reconciling data obtained from different methods becomes difficult, hindering the establishment of consistent models. Additionally, many methods have some inherent problems being either poorly described or lacking explanation about the model’s choice. For instance, (Breck, 2014), who developed a model to estimate fish body composition that only requires water composition and body weight of fish, compared the predicted to the observed values of lipids, by linear regression relying only on a F-test, RMSD and R^2 to evaluate the obtained models. However, information about the calibration methods was not provided, nor a cross-validation or an independent validation was performed. In turn, both TOBEC and BIA are methods that require calibration using data specific to the fish species being studied. These methods present some limitations when used in fish. For instance, with BIA, the accuracy of the method can be affected by factors such as fish size, shape and fish body orientation, as well as water conductivity and temperature (Ducan, 2008; Hartman et al., 2011;

Pothoven et al., 2008; Vue et al., 2015). TOBEC in turn, can be less accurate for species with a more irregular body shape and less accurate on measuring protein content (Robin et al., 2002). Image-based methods also present some challenges for predicting fish body composition. The CT scan is not only expensive but also involves exposure to X-radiation. Moreover, such equipment is not suitable for field use. Regarding the use of DEXA in fish, there are still few studies conducted, and there appears to be an underestimation of fat due to bias in the quantification of the ratio of water/fat-free tissues (hydration) (Johnson et al., 2017; Lovett et al., 2019; Ndiaye et al., 2020; Wood, 2004).

Model assumptions can also present some challenges when predicting fish body composition. Models often rely on assumptions, such as linear relationships between body weight and body components or constant growth patterns. However, these assumptions may not hold true for all species or under different environmental conditions, potentially introducing inaccuracies in predictions. Moreover, authors often fail to provide explicit information regarding the calibration methods and other inherent processes involved in modelling. This lack of transparency makes it challenging to understand the specific assumptions that were considered during the development and calibration of the model.

Regarding Nile tilapia (*Oreochromis niloticus*), there are contradictory opinions concerning the type of model that should be used when predicting body composition. Some authors defend the use of isometric models (de Castro Silva et al., 2015; Hanley, 1991), while others use different models for different components (Chowdhury et al., 2013; Chowdhury & Bureau, 2009; Shizari, 2020; Van Trung et al., 2011). Chowdhury et al. (2013) tested isometric and allometric models on Nile tilapia body composition and reported that the allometric equations may be better suited to predict the lipid content of fish with body weight below 5 g, though the isometric models had better predictions overall. But no explanation was given to support this statement. In addition, the authors mentioned that an independent data set was used to validate the model, but no information was provided on the method used, whilst only residuals sum squares (RSS) and the R^2 were used to choose the models.

Overall, it is common to come across various body composition models for a given species, and some of these models may even use different types of models applied to different body components. However, the reasoning behind these choices is often unclear or not well explained. Moreover, it is common to encounter situations where different authors use varying methods to calculate the same parameters, making it challenging to compare and integrate results. For instance, some authors may use length-based models, while others use weight-based models to estimate fish body composition. Such discrepancies can lead to confusion, inconsistencies, and difficulties in interpreting the data. Furthermore, all studies mentioned above that used the isometric model shared something in common: they all (presumably) tested the isometric model calibrated under the assumption of additive constant-variance errors. In fact, it is the most straightforward choice. However, it is imperative to understand if one's data displays additive or multiplicative error through the residual analyses.

1.5. Prediction of fish growth

1.5.1. Fish growth

On a basic level, fish growth can be defined as an increase in body weight and length that occurs due to the accumulation of tissues such as muscle, bone and fat (Bureau et al., 2000). In simple terms, the growth of fish relies on the food they eat, and whether their nutritional needs are met, which in turn will also determine their body composition. In an aquaculture production context, fish seldom have access to natural food, so nutrients (e.g., protein, fat, minerals) for growth are usually supplied through formulated feeds. An important aspect to consider is that macronutrients in fish feeds do not get directly assimilated into fish tissues: they must first be digested into simple molecules, absorbed, transported and often transformed, before being accumulated in the fish body. All these processes, and many other processes involved in the maintenance of life, require additional costs in terms of nutrients and energy. Thus, since energy is obtained through the oxidation of nutrients, this implies that the supply of nutrients in feeds must cover not only their use as building blocks for tissue growth, but also their use as energetic fuel for both growth-related and maintenance-related metabolism. Furthermore, there are nutrients and energy losses during the growth and physiological processes, which will determine the nutrient and energy retention. Thus, the determining factor in growth lies in the balance of retained nutrients, specifically protein, and energy after the metabolic needs of maintenance and other physiological processes have been met (Lupatsch et al., 1998). Maintenance costs cover a range of energy expenditures necessary for basic survival, including activities such as swimming and maintaining body temperature, and are influenced by body size and temperature (Figure 5). In summary, the nutritional and energetic requirements of a certain fish species are generally estimated based on the assumption that the total requirement can be obtained by summing the requirements for maintenance and growth (Booth et al., 2010).

Thus, to formulate appropriate feeds, it is important to understand the nutritional and energetic requirements of fish: what nutrients and in which quantities should be present in a fish feed such that they are supplied with enough building blocks and fuel to support both their maintenance as well as their growth? This is a challenging question to address since the amount of energy and nutrients required for fish to growth and maintain their biomass change throughout their lifespan, as do cell structure responses and functions (Mansano et al., 2017). Thus, and given that fish growth is directly associated with changes in the amount of water, protein, lipids and minerals retained in fish body, achieving a better understanding of nutritional requirements, feed formulation and optimal feed practices depends on a solid knowledge on both the dynamics of fish growth and the composition of the accumulated biomass. In practice, the processes of growth and nutrient retention in fish are highly complex, being affected by a wide range of factors (Figure 6). These factors can be intrinsic (e.g., fish species, fish strain/genetic factors, fish size, swimming activity, maturity, metabolic/energetic status) or extrinsic, which can be subdivided in abiotic (e.g., temperature, dissolved oxygen, photoperiod, pH, ammonia, nitrite, salinity) and biotic (e.g., diets, feeding rate and frequency) (Bureau et al., 2000; Cuenco, 1989; Dumas et al., 2010; From & Rasmussen, 2017; Mansano et al., 2017; Sun et al., 2016a). Furthermore, intrinsic and extrinsic factors can interact in various ways, which further constitutes a challenge when studying fish growth and nutrient retention.

In general, it has been reported that fish can drive their energy needs mostly from lipids when fed high-fat diets and thus spare protein to be used primarily to growth (i.e., protein-sparing effect) (Orire & Sadiku, 2011; Vergara et al., 1996). However, to achieve the protein-sparing effect, fish need to be fed a well-formulated diet that meets the nutritional requirements of the target species. In the specific case of Nile tilapia, its primary energy sources are, like for many other species, lipids, carbohydrates, and proteins. However, the omnivorous nature of tilapia allows for more efficient carbohydrate digestion compared to highly carnivorous fish species. This makes the replacement of lipid by non-protein energy more straightforward, without compromising growth performance and reducing the feed costs (Kabir et al., 2020; Maas et al., 2019, 2020; Peres et al., 2022). However, the amount of energy that this species is able to extract from different carbohydrates can vary, and their ability to digest certain types of carbohydrates may be limited (Anderson et al., 1984; Maas et al., 2020; NRC, 2011; Shiau & Chen, 1993; Shiau & Lin, 1993).

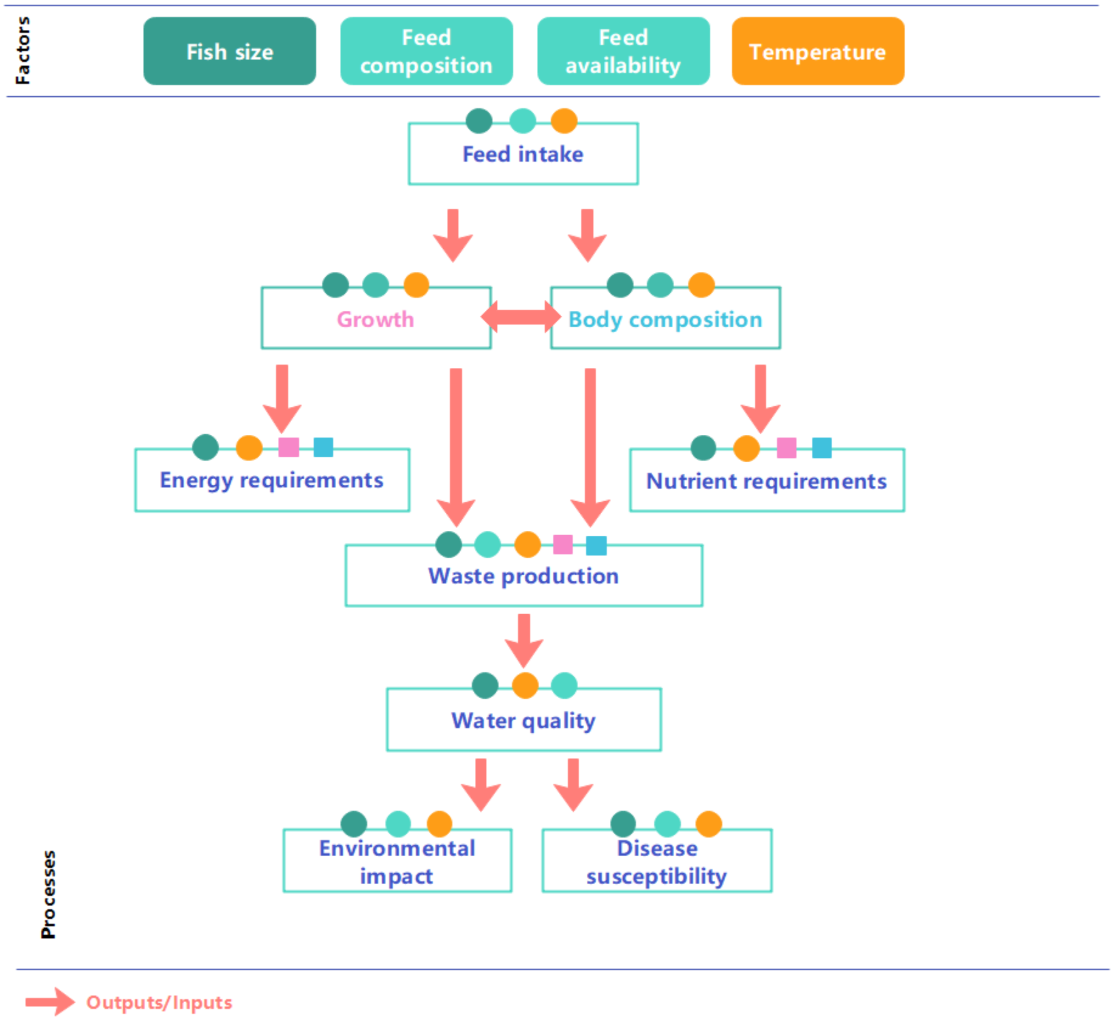

Figure 5 – Diagram illustrating the relationships between growth, body composition, nutritional and energy requirements, as well as the influence of various nutritional and environmental factors. The full circles represent the effects that different factors (green for fish size, light green for feed aspects and orange for temperature) can have on the respective processes, while the full squares denote the influence of certain processes (pink for growth and light blue for body composition) on others. Arrows indicate the flow of outputs from one process to serve as inputs for others.

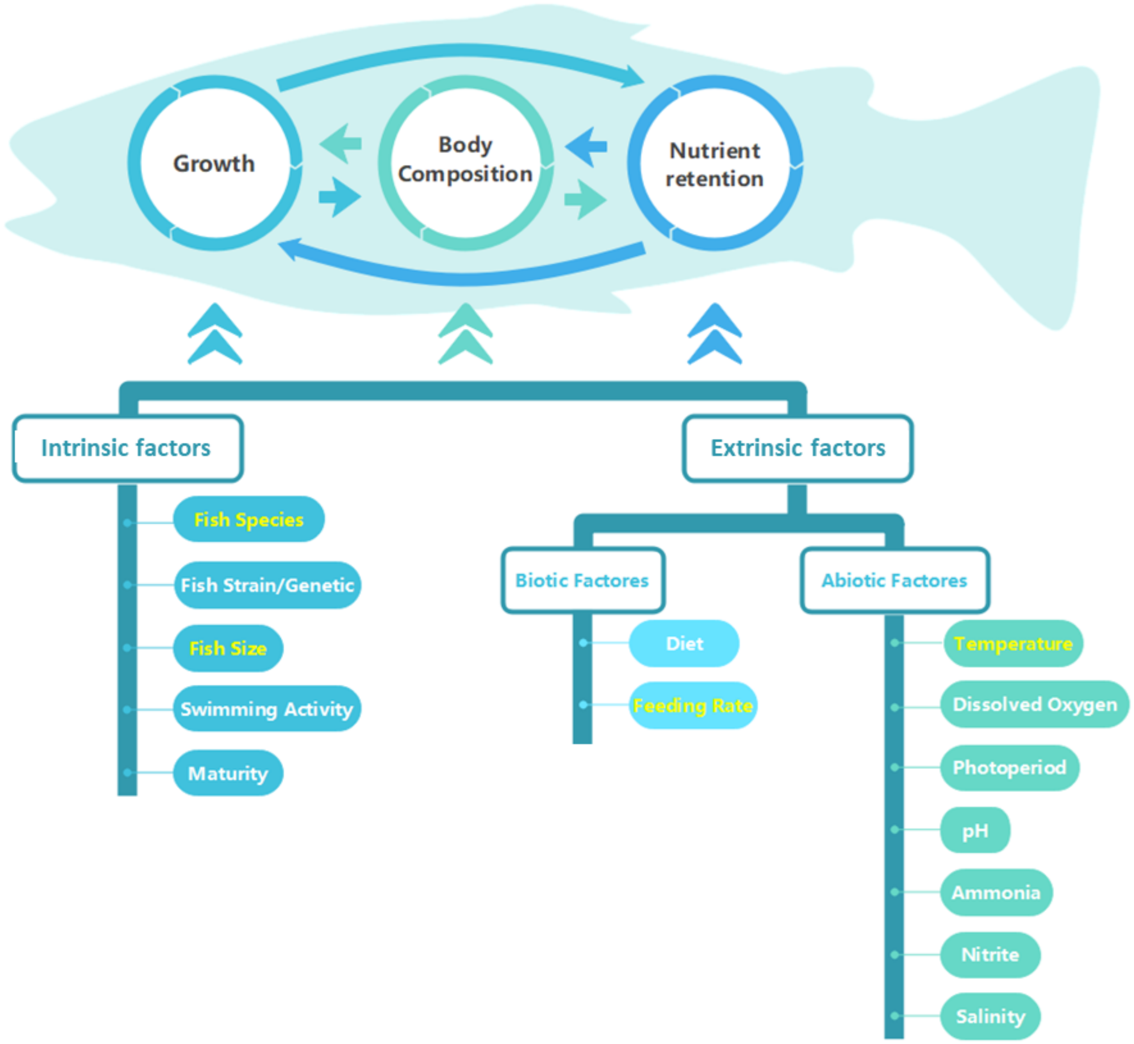

Figure 6 – Diagram illustrating the relationships among the processes of growth, body composition, and nutrient retention in fish, highlighting some of the various factors that may influence these processes. The factors marked in yellow indicate some of the most important ones, that are the main focus of this work.

1.5.2. Fish growth models

Since growth is a dynamic process, the use of dynamic mathematical models is essential for understanding the variation in the growth of animals as a function of time (age) (Mansano et al., 2017). These models can consider various environmental and physiological factors that can influence growth, such as temperature, feeding rates, genetic factors and disease. Depending on the model, these equations may be based on empirical observations, biological principles or a combination of both. They are often used to optimize feeding regimes and predict fish growth in different environments with the aim of accurately predicting growth patterns. By obtaining this information, it is then possible to adjust feeding schedules and other management practices to ensure that fish reach their target size in a time-efficient manner.

In terms of mathematical methods, simple fish growth models can often be solved by analytical integration of ordinary differential equations (Jones et al., 2009). However, complex models often require the use of numerical methods to solve the system of equations that describe the growth and energy balance of the fish (Dumas et al., 2010; Teleken et al., 2017). In both cases (simple and complex growth models) equations must refer to measurable traits of the fish or its environment (Cuenco, 1989).

Simple growth models

In the aquaculture sector, the most commonly used simple fish growth models are the Specific Growth Rate (SGR), the Thermal-unit Growth Coefficient (TGC), and the feed conversion rate (FCR) models. These models are considered simple models, since they are more empirical and based on simple relationships between growth and environmental or physiological variables and tend to assume that these relationships are constant over time.

Specific Growth Rate (SGR)

The SGR model is the simple model that is most often used to describe growth as a function of time. This model assumes that growth rate is proportional to the current weight. Therefore, growth can be expressed as a relative increase in body weight per unit of time and the following differential form is used:

$$\frac{dW}{dt} = SGR \times W$$

dW/dt is the rate of change in weight (i.e., growth rate) with respect to time (i.e., the growth rate) and *SGR* is a constant that represents the relative rate at which fish are growing. When integrated assuming constant SGR, the following expression is obtained:

$$W_t = W_0 \times e^{(SGR \times t)}$$

where W_0 is the initial weight, W_t is the final weight and t is time in days. The SGR parameter itself can be readily estimated from data, by using a rearranged version of the integrated form (Bureau et al., 2003):

$$SGR = \frac{log(W_t) - log(W_0)}{t}$$

From the integrated form, we can see that the SGR model assumes indefinite exponential growth. However, it has been shown that this assumption is not entirely realistic, even at relatively short timescales. As a result, growth predictions based on the SGR model may need to be recalculated when the predicted growth curve deviates significantly from the observed growth trajectory (Brett, 1979; Dumas et al., 2010).

Thermal-unit Growth Coefficient (TGC)

Theoretically, the *TGC* model allows the use of growth data collected from fish of given size at one temperature to predict the growth of fish of a different size when held at other temperatures (Jobling, 2003). This makes the model more flexible than the SGR model. The application of this model in fish, specifically for salmonids, was initially conducted by Iwama & Tautz, 1981 and it assumes the following differential form:

$$\frac{dW}{dt} = \mu \, T \, W^{b}$$

where μ (> 0) has units of g^{1-b} $(°C\ Day)^{-1}$, *T* is water temperature (°C) and the allometric exponent *b* (> 0) is dimensionless (Iwama & Tautz, 1981). When this expression is integrated assuming constant μ and T, the following expression is obtained:

$$W_t = \left\{ {W_0}^{1-b} + [(1-b) \times \mu \times (T \times t)] \right\}^{\frac{1}{1-b}}$$

If, furthermore, a standard value of 2/3 is assumed for b and a "$\frac{TGC}{1000} = (1-b) \times \mu$" substitution is performed, the following expression is obtained:

$$W_t = \left\{ \sqrt[3]{W_0} + \left[\left(\frac{TGC}{1000} \right) \times (T \times t) \right] \right\}^{3}$$

where W_0 is the initial weight, W_t is the final weight, *T* is the average temperature in °C and *t* is time in days (Jobling, 2003). Furthermore, the TGC parameter can be easily estimated from available data by utilizing a rearranged version of the integrated form (Jobling, 2003):

$$TGC = \left[\frac{\left(\sqrt[3]{W_t} - \sqrt[3]{W_0} \right)}{(T \times t)} \right] \times 1000$$

Feed conversion ratio (FCR)

The *FCR* is a commonly used metric for measuring the efficiency of fish in converting consumed feed into body mass, and is obtained by dividing the total amount of feed given by the total weight gain during a specific period of time. A low *FCR* indicates that the fish are converting feed efficiently and growing quickly, while a high *FCR* indicates that the fish are not converting feed efficiently and may be experiencing slower growth rates. Whenever FCR is used as a metric, the growth rate is implicitly assumed to be independent of fish size or temperature, and simply proportional to feed rate:

$$\frac{dW}{dt} = \frac{1}{FCR}\,FI$$

where FI represents feed intake in (e.g.) g/day. When integrated assuming a constant FCR, we obtain the following expression:

$$W_t = W_0 + \frac{1}{FCR}\,cumFI$$

where cumFI represents the cumulative feed intake (e.g., in g) between “time = 0” and “time = t”.

The FCR growth model is often used in practice as a prediction tool in fish farming. Nevertheless, factors such as water quality, stocking density, and temperature can also affect fish growth (Abdel-Tawwab et al., 2019; Azim et al., 2003; Gislason et al., 2010; Pauly, 1979; Viadero, 2004), which should be taken into consideration when using the *FCR* model as a prediction tool. The advantages of the *FCR* growth model is its simplicity, not requiring complex data and thus being very practical. However, this simplicity can also be seen as a disadvantage, since it does not consider feed properties, temperature or energy metabolism, which makes the *FCR* a model that provides limited predictive power.

Complex growth models

Complex growth models consider changes in the environment and other factors over time, and may be better suited for predicting growth under more complex and variable conditions. Bioenergetic and nutrient-based models are examples of complex growth models that are commonly used in aquaculture to estimate the growth and feeding requirements of fish species. These models are based on the principles of energy and nutrient balance:

Net balance = gain – losses

Thus, mathematical equations are used to simulate the flow of energy and nutrients through the fish's body over time (Dumas et al., 2010).

Bioenergetic growth models

Bioenergetic growth models generally relate growth to the accumulation of energy in fish body and are structured to ensure that the predictions comply with energy conservation: fish cannot retain more energy than their intake of energy (Kooijman & Kooijman, 2010). This implies that they are sensitive to important properties of the feed (e.g., digestible energy content), which makes them suitable for situations where simple growth models can be inadequate (e.g., simulating growth under time-varying energy intake levels). In a general sense, bioenergetic models start with an energy budget:

$$E_{intake} = E_{retention} + E_{losses}$$

and then use it to express energy retention as the difference between a "intake/gain" (or "anabolic") term and a "loss" (or "catabolic") term:

$$E_{retention} = E_{intake} - E_{losses}$$

$$\frac{dE}{dt} = E_{intake} - E_{losses}$$

A growth model can then be obtained from this relationship by assuming a relationship between fish body energy (E) and fish body weight (W), and applying the chain rule:

$$\frac{dW}{dt} = \frac{dW}{dE}\frac{dE}{dt}$$

$$\frac{dW}{dt} = \frac{dW}{dE}(E_{intake} - E_{losses})$$

If we assume an allometric model, then the relationship between energy and weight is $E = a \times W^b$, which is the same as saying that $W = \left(\frac{1}{a}\right)^{1/b} \times E^{1/b}$. Thus:

$$\frac{dW}{dE} = \left(\frac{1}{a}\right)^{1/b} \times (1/b) \times E^{\frac{1-b}{b}}$$

Under a simple isometric model, fish have a constant energy density (δ_E). In this case, the b parameter is 1, so the expression further simplifies to:

$$\frac{dW}{dE} = \frac{1}{a} = \frac{1}{\delta_E}$$

Thus, in this case, the resulting bioenergetic growth model would be:

$$\frac{dW}{dt} = \frac{1}{\delta_E}(E_{intake} - E_{losses})$$

As we explore the mechanisms underlying fish growth, a critical component is understanding how energy flows through these organisms. Bioenergetic models can be used to predict not only fish growth, but also feed requirements and waste outputs (Brigolin et al., 2010). Thus, they offer a sound basis for aquaculture models, since all major components are comprised: food consumption (frequently the most important cost item), excretion (excretion products pollute the environment and affect growth), respiration (dissolved oxygen is a major limiting factor) and growth itself (Cuenco, 1989). In this regard, bioenergetic models enable the description of energy partitioning from dietary sources for catabolism and anabolism processes, with a factorial approach (Bureau et al., 2003). Figure 7 shows the partitioning of dietary energy yielding components as a relatively simple way of looking at dietary component utilization by fish. Basically, it describes the partitioning of food energy into excretory energy (FE, UE and ZE), heat production/increment (HiE) and recovered/retained energy (RE) (Brett & Groves, 1979; Xie et al., 2011). The RE can therefore be calculated as:

$$\mathrm{RE} = \mathrm{IE} - \mathrm{FE} - (\mathrm{UE} + \mathrm{ZE}) - \mathrm{HiE}$$

where IE represents the E_{intake} component, while FE+(UE+ZE) +HiE represents the E_{losses} component (Xie et al., 1997, 2011)

.

However, this type of model can be converted to a simpler equation, where the losses are subdivided into two components: intake-dependent losses (*COG*DE*) and intake-independent losses (*FM*) – the advantage of this approach is that it is simpler (i.e., has less parameters) and thus easier to calibrate:

$$\mathrm{RE} = (1 - \mathrm{COG}) \times \mathrm{DE} - \mathrm{FM}$$

or

$$\mathrm{RE} = k_e \times \mathrm{DE} - \mathrm{FM}$$

where *COG* represents the costs of growth – in this case the costs of energy deposition (Bureau et al., 2000; Conceição et al., 1998) – usually equated with the specific dynamic action (SDA), DE is the digestible energy, used for growing and maintenance, and *FM* the fasting maintenance losses, which is usually assumed to depend on body weight and water temperature. A common expression for the fasting maintenance costs is:

$$FM(BW,T) = a \times BW^{b} \times e^{c \times T}$$

where *BW* is the body weight, *T* is temperature and *a*, *b* (exponent that determines the variation in metabolic rate as a function of body weight) and *c* are estimated parameters.

Furthermore, although the cost of growth (*COG*) in mammals has been shown to be constant (Kirkwood & Webster, 1984), with fish being aquatic animals in constant motion, it is difficult to perform experiments that are representative of reality. However, empirical models demonstrated that the *COG* (i.e., one minus the slope of the *RE* in function of *ME* or *DE*) was fairly constant and easy to predict (Bureau et al., 2000). Thus, the term *COG* represents the metabolic losses dependent on the level of digestible energy intake (*DE*) and can also be expressed as $k_e = 1 - COG$ (energy retention efficiency coefficient).

In order to better understand the energetic costs and benefits of fish growth, reproduction and other physiological processes, models that describe the energy balance of these organisms have been developed. For example, static energy budget models (SEB), which uses a set of allometric functions to describe the relationship between rates of energy budget parameters in fish (e.g., food consumption, growth, and respiration) and fish size as modified by abiotic factors like temperature (van der Veer et al., 2009). In turn, the dynamic energy budget (DEB), aims to determine the quantitative aspects of metabolism explicitly based on the conservation of mass, isotopes, energy and time, including the inherent loss of energy associated with all processes (Kooijman & Kooijman, 2010; Nisbet et al., 2000, 2012; Sousa et al., 2010). In other terms, they focus on describing the rates at which the organism assimilates and utilizes energy and elemental matter from food for its maintenance, growth, reproduction and development in a dynamic environment (Nisbet et al., 2000, 2012).

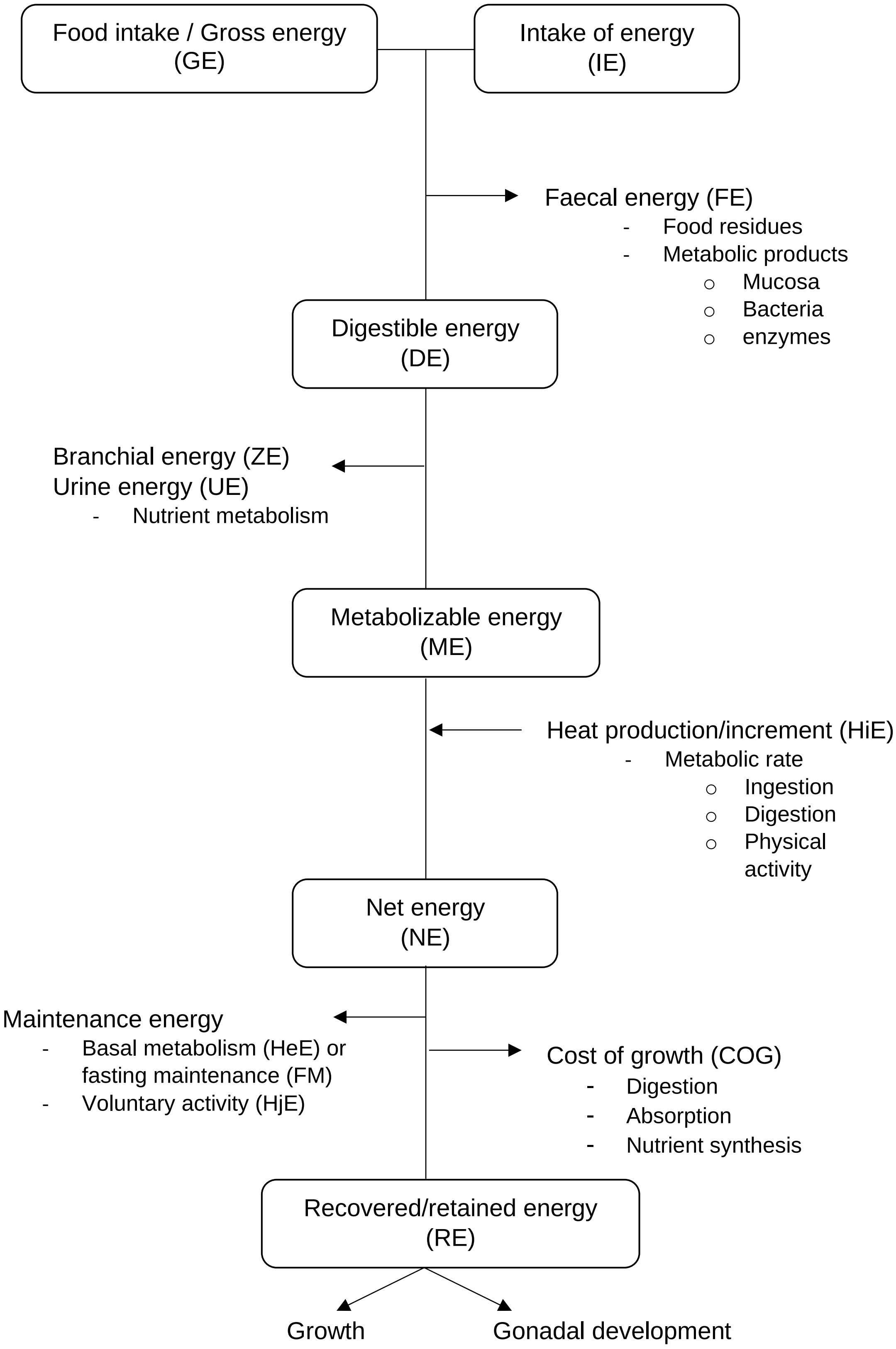

Figure 7 – Diagram illustrating the partitioning of dietary energy yielding components. Adapted from Smith, (1980) and Bureau et al. (2003).

Nutrient-based models

Nutrient-based models consist of mathematical descriptions of the relationships between nutrient deposition and weight gain that ensure the principle of conservation of mass (Dumas et al., 2010). In contrast to bioenergetic models, which assume that growth is driven by energy intake and utilization (Phan et al., 2019), nutrient-based models focus on the role of specific nutrients in organism growth and development (Konnert et al., 2022ab). These models are more complex because they make distinction between different types of nutrients, beyond relying on a "total energy" budget. Furthermore, some of them consider the availability and utilization of nutrients, often relying on metabolic pathways of nutrients and their precursors, and representing energy in terms of ATP (Dam & Penning De Vries, 1995; Dumas et al., 2010; Hua et al., 2010). Thus, they generally consider the composition of diets in terms of macronutrients or even at the level of micronutrients (e.g., amino acids, fatty acids). These models, besides being used to predict growth, can be used to optimize fish feed formulations and feeding strategies, as well as to evaluate the environmental impacts of fish farming.

Most nutrient-based models can be considered *nutrient budget models*, since they consider nutrient inputs, outputs and internal nutrient storage, following a mass balance (similar to the energy balance implicit in bioenergetic models). For example, (Dam & Penning De Vries, 1995) developed and calibrated a model to predict the growth and fat percentage of *Oreochromis niloticus* and *Oncorhynchus mykiss* under different feeding levels and feed compositions. The developed model considered as main state variables the amount of body protein and fat, which were driven by synthesis and oxidation processes. Therefore, the model accounted for factors such as, feeding rate, feed composition, digestibility of feed components, temperature and on the stoichiometry of the reaction equations of biosynthesis. In addition, it considered the amount of energy consumed through feeding, the energy expended in various physiological processes and the energy used for growth (i.e., description of the energy budget of the fish). The results showed that the model was able to accurately predict growth and fat content on both species. The work of Lupatsch (2003) was also of great importance and contributed significantly with critical findings regarding the protein and energy metabolism. In the work of Lupatsch (2003), a set of growth and metabolism experiments were performed which allowed the quantification of energy and protein fluxes as a function of body weight and feed intake, digestible feed intake (discounting faecal losses), fasting metabolism and retention. The study focused on determining the optimal energy and protein requirements of gilthead seabream (*Sparus aurata*) for efficient production. For this purpose, a factorial approach was used to determine the energy and protein requirements, by systematically varying the levels of dietary protein and lipids, while keeping the other nutrients constant. The growth performance, feed intake and nutrient utilisation of the fish were measured to determine the optimum dietary energy and protein requirements. For this, once more, one of the approaches was to take into consideration the contribution of protein and energy from diets. In this case, the author quantified the energy and protein requirements according to the factorial model with the addition of a parameter for the efficiency of dietary energy and protein to deposit new body tissues as growth. The author has thus shown that the parameters derived from energy and protein demand can be used to develop models that dictate the required dietary composition, at least in terms of protein and energy, for fish at any stage of their life cycle. Additionally, by defining the maintenance and growth requirements of fish, an energy budget can be derived to quantify the energy fish need to consume to reach their growth potential, at any specific temperature, and part of their growth cycle (Lupatsch, 2003). Following this framework, Nobre et al. (2019) developed an energy and protein flux model (EP) to simulate fish production in commercial farms. This type of model considers not only the energy requirements of a species (as bioenergetic models do), but also its protein requirements and translate those into individual weight gain and proximate composition over time (Nobre et al., 2019). In these types of models, besides feed composition, the initial fish body weight, temperature, feed intake and feed digestibility are also considered. Moreover, the model avoids including the fish energy and nutrient function decoupled from body weight as suggested by (Canale & Breck, 2013). Thus, these types of models constitute a useful tool to simulate fish growth in a commercial farm, where field data is limited (Nobre et al., 2019).

Another type of nutrient-based models are the *metabolic flux models.* These models try to replicate all the major metabolic pathways of the fish. Thus, these models consider the influence of the amino acid composition of the feed and may simulate the fundamental physiological and metabolic mechanisms of fish, such as amino acid oxidation and conversion, protein synthesis and degradation, glycolysis, gluconeogenesis, glycogenesis and glycogenolysis. For example, (Bar et al., 2007) developed a model that could predict growth, body composition, and first limiting amino acid for a variety of diets over time in Atlantic salmon. Such model provides a detailed description of the flow of nutrients and metabolites through major metabolic pathways in growing tissues as a function of time. Despite being an incomplete representation of nutrient metabolism in fish, the model demonstrated good predictive capability. Further on, Hua et al. (2010) adapted a non-ruminant model to describe the utilization of energy-yielding nutrients and metabolites for body protein deposition (Pd) and body lipid deposition (Ld) in fish whole-body. The model is based on rules that define how retained nutrients are partitioned between Pd and Ld, which are constrained by three key parameters: PdMax (the maximum daily rate of Pd), minLP (the minimum ratio of whole-body lipid to protein mass), and the maximum efficiency of using intake of the first limiting dietary amino acids (AAs) for Pd. The authors, however, noted significant discrepancies between the model predictions and experimental observations. While the model accurately predicted energy retention, it did not always accurately predict growth rate, Pd, and Ld. The authors suggest that these discrepancies are likely due to differences in nutrient utilization and partitioning mechanisms between fish and non-ruminant animals. Although *metabolic flux models* may not always provide optimal predictions, they offer valuable insights into the process of fish growth and the underlying factors that influence it.

1.5.3. Challenges in predicting the growth of fish

The models most commonly used in the aquaculture sector are very simple and lack the ability to predict the effect of some environmental, nutritional and genetic factors on fish growth and composition. They often ignore feed properties, lack a clear biological interpretation and/or neglect fundamental properties of aquatic animals (e.g., variations in growth trajectory across life stages) (Dumas et al., 2010; Sun et al., 2016a). Additionally, they only work well when it is assumed that the fish are in optimal rearing conditions, and factors such as diet composition are not considered. For instance, the SGR model presents serious problems: it assumes that fish growth is exponential and unbounded, which can lead to gross overestimation of weight when data is extrapolated (Bureau et al., 2000; Dumas et al., 2010). Moreover, it does not consider the influence of environmental factors on fish growth, such as temperature, water quality and food availability, which can significantly impact fish growth rates. Therefore, it may not accurately represent long-term growth trajectories or capture growth patterns beyond the experimental or observational period. The TGC model on the other hand, incorporates temperature as a key factor in the growth equation, allowing for a more accurate representation of the temperature-growth relationship. However, the model works over assumptions, such as that growth increases in a steady and predictable way when temperature increase and that growth is proportional to temperature expressed in degrees Celsius (Jobling, 2003). As Jobling (2003) and Jobling et al. (1994) demonstrate, growth does not necessarily follow a steady increase with increasing temperature. Thus, a TGC calculated under certain temperature conditions does not necessarily lead to good growth predictions at other temperature ranges, even if covered by the growth curve’s temperature profile (see Jobling (2003) for more details). The FCR model, in turn, although it considers the feed intake of the fish, does not consider temperature, fish size and feed properties, which can often result in misleading predictions (particularly at very high or very low feed intake rates).

While complex models seek to tackle some of the limitations previously described (by, e.g., explicitly considering feed properties as relevant factors), they may also present problems and limitations. One important aspect is that, given the relationships between growth, requirements and maintenance costs, the development of complex growth models requires some levels of understanding about these processes and how they inter-relate. For instance, regarding bioenergetic models, (Canale & Breck, 2013) reported that, when conventional bioenergetic models are incorrectly applied to simulate fish growth or feed consumption, and it is assumed that the energy density of a fish is a function of its wet weight or a time-independent function, the fundamental principles of energy conservation are disregarded. Thus, the errors associated with bioenergetics models depend on how quickly the energy density of fish changes with size (Canale & Breck, 2013). Assuming a constant energy density for fish can avoid mathematical errors, but may not be suitable for most applications and can lead to significant biological inaccuracies. Additionally, bioenergetic models may oversimplify the complex physiological processes involved, potentially leading to inaccurate results. For example, they often rely on simplified assumptions about energy allocation, nutrient utilization, and metabolic processes, and thus do not fully capture the dynamic nature of fish physiological systems. DEB models, while not purely bioenergetic due to the fact that they additionally consider mass balances, do not distinguish different types of nutrients (e.g., lack of information on the chemical composition, such as, moisture, protein, lipid and ash of biomass gain) (Dumas et al., 2010; Kooijman & Kooijman, 2010), which limits their capacity to accurately predict the effect of essential nutrients on fish growth. Finally, nutrient-based models also have limitations that need to be considered. For instance, the parameterization of these models can be challenging due to the lack of accurate species-specific data on nutrient requirements and utilization efficiencies. Moreover, as simplifications of reality, they may overlook important factors, such as minor essential nutrients or metabolic pathways, which can restrict the predictive capacity of these fish growth models. Moreover, the individual variability within fish populations, in addition to the complexity of nutrient-growth interactions, can also pose challenges in accurately representing these processes in models.

Overall, simple fish growth models are still used as standard in the industry, while nutritional models are mainly used by the scientific community. There is a need to explore and develop models that are easy to use by fish farmers and still have the ability to predict fish growth and composition based on fundamental properties of fish metabolism. Therefore, it is imperative for the scientific community to dedicate more effort to overcome some scientific (e.g., biological and individual fish variability) and technical (e.g., tools to easily apply and interpret the models) issues regarding the implementation of these models by the industry and thus bridge the gap between the two.

Objectives

The work presented in this thesis aimed to advance the current state-of-the art in mathematical modelling and prediction of body composition and growth for economically relevant fish species, contributing to the improvement of efficiency and sustainability in aquaculture. To do so, the following main objectives were set:

1) Develop a reference static body composition model for Nile tilapia using cross-validation to choose the best combination of model and calibration method;

2) Develop a reference dynamic growth and body composition model for Nile tilapia using cross-validation to choose the best combination of model and calibration method;

3) Provide a robust validation of the reference models developed using independent datasets;

4) Extend the developed framework to other commercially relevant fish species and use it to compare them in terms of model parameters, to evaluate possible differences in, e.g., growth potential, body composition, physiology and metabolism.

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Chapter 2

Development and evaluation of Nile tilapia body composition models

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Development and evaluation of Nile tilapia (*Oreochromis niloticus*) body composition models

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Abstract

Mathematical models can be used as an alternative to conventional analytical methods (AOAC), allowing to indirectly estimate the body composition of fish without, in some cases, being necessary to sacrifice animals. To develop models with high predictive capacity, in addition of having access to representative data, it is important to use calibration methods that minimize the estimation of the generalization error.

In this work, Nile tilapia whole-body composition data were collected from 138 scientific publications, covering fish from 0.01 g to 1470 g. Predictive models were obtained for each body component using different combinations of models and calibration methods. The different combinations were evaluated through cross-validation approaches in order to select the models with the best predictive capacity. Such models were compared against other published Nile tilapia body composition models, using an independent validation dataset.

The results show that model predictions are greatly affected by the type of model, calibration method and amount of calibration data available. Models calibrated under the assumption of multiplicative error had better predictive capacity than those calibrated assuming additive error, which indicates that, in this particular case, performing regression on log-transformed data, even for isometric models, is advantageous. From the models tested, the ones with the best predictive capacity are the allometric model (*al_mu*; calibrated assuming multiplicative error) and a robust hybrid model (*hyb_rob*; ensemble of isometric and allometric models, calibrated assuming multiplicative error using a robust regression method), with both having good prediction capacity when compared with models published by other authors.

Although the results obtained support the hypothesis that Nile tilapia body composition is essentially allometric, isometric models can also potentially be used without much performance loss, if they are calibrated assuming multiplicative error (i.e., using log-transformed data).

Keywords: Mathematical models, Body composition, Nile tilapia, Regression analysis, Prediction tools

1. Introduction

Currently, there is pressure to sustainably increase fish farming production while keeping production profitability, good product quality, and fish welfare and environmental impacts within acceptable limits. An efficient and sustainable fish farming system depends strongly on informed management decisions, based on comprehensive monitoring of several indicators (e.g., environmental, economic, quality, welfare, health) that determine the state and success of production. In an aquaculture context, the measurement of body composition is important for the determination of mass balances which can be applied in the estimation of nutrient retention (or nutrient retention efficiencies), estimation of nutritional requirements and/or estimation of environmental impacts. Usually, the body composition of fish is determined using standard analytical methods (e.g., AOAC methods). On the other hand, mathematical models can also be used to estimate the body composition of fish and thus reduce the associated cost. Additionally, there is no need to use toxic chemicals, it is less time consuming and, in some cases, does not require euthanizing fish. Thus, body composition models can be useful tools to estimate body composition of fish in a low-cost way when it is otherwise logistically and/or economically unviable.

The most common body composition models available in the scientific literature use linear regression analysis to predict the body composition of fish through the relationships between body weight (BW) and the main body composition components: water, crude protein, crude lipids and ash (Lupatsch et al., 2003; Dumas et al., 2007). The use of this specific method usually implies either an isometric relationship measured under additive error (if data is not transformed) or an allometric relationship measured under multiplicative error (if data is log-transformed). In the specific case of Nile tilapia (*Oreochromis niloticus*), there are several published models and it is interesting to note that some authors only use isometric models (Hanley, 1991; Chowdhury et al., 2013; de Castro Silva et al., 2015), while others use different models for different components (Chowdhury et al., 2013; Chowdhury & Bureau, 2009; Shizari, 2020; van Trung et al., 2011), with the justification associated with these choices being unclear, as well as the consequences in terms of predictive capacity. For instance, Chowdhury & Bureau (2009) report in their work an isometric relationship for water and crude protein, and an allometric relationship for crude lipids and ash, with respect to BW. There are other authors that also support an isometric relationship between BW and crude protein in Nile tilapia (Chowdhury et al., 2013; Chowdhury & Bureau, 2009; de Castro Silva et al., 2015; Hanley, 1991; van Trung et al., 2011). However, Chowdhury et al. (2013) findings suggest that the relationship between BW and crude lipids can be explained by isometry, and that the same is applicable for the relationship between BW and crude protein. The authors found, however, that lipid growth in tilapia below 5 g can also be described by allometry. Thus, overall, there is no clear scientific consensus regarding the relationship between BW and body composition in Nile tilapia (and the best way to model it). This is compounded by the general lack of information regarding the methods of development, calibration and validation of the reported models: the criteria for choosing the type of model assumed and the method used to calibrate it are often subjective (e.g., based on visual evaluations), suboptimal (e.g., based on calibration error metrics/goodness-of-fit measures) or simply unreported, which hampers an objective evaluation of the relative merit of the competing underlying hypotheses (e.g., isometry *vs.* allometry).

According to statistical learning theory, a model calibrated under a specific method is optimal if it minimizes the estimation of the generalization error (i.e., the expected error when predicting unseen data) or also called validation error. Thus, to objectively evaluate the adequateness of a particular model (or calibration method), it is essential not only to evaluate how well it fits the data used to calibrate it (i.e., calibration error), but also to estimate how well it performs against independent data (i.e., cross-validation error). In order to do that, the developing models are subjected to the standard method of cross-validation. In such approaches, data is split in *k* folds and part of the data is used to train each algorithm (the training sample), while the remaining part is used to estimate the error of the algorithm (the validation sample), with the process being repeated for different partitions (Arlot & Celisse, 2010; Hastie et al., 2001). Although there other alternative (and potentially less computationally demanding) methods to compare models in regards to their generalization errors , such as the Akaike information criterion (AIC) (Akaike, 1973; Stone, 1977; Zhang & Yang, 2015), the Bayesian information criterion (BIC) (Schwarz, 1978; Findley, 1991), or the generalized cross-validation (GCV) (Craven and Wahba, 1978; Chaves et al., 2019), these methods generally rely on asymptotic approximations and do not display the same quasi-universality properties as CV methods do (see section 10.4 of Artlot & Celisse, 2010).

In this work, we explore in detail different body composition models and calibration methods, using objective criteria based on cross-validation approaches, to determine the best combination of model and calibration method for Nile tilapia.

2. Materials and methods

2.1 Data collection

Extensive research was carried out to collect Nile tilapia whole-body composition (i.e., water, crude protein, crude lipids and ash) and respective whole-body weight data from scientific literature sources. Since carbohydrates are present in the body composition of fish in small quantities (Brett & Groves, 1979; Breck, 2014), and usually unreported, they were left out of this analysis. Overall, only studies in which the whole fish content (usually referred by authors as “whole-body” or “carcass” composition) was determined according to the standard analytical methods of the Association of Official Analytical Chemists (AOAC) were used. In addition, data in which the sum of components exceeded 102 % (sum of water, crude lipids, crude protein and ash) were assumed to be contaminated by measurement or reporting errors and therefore excluded from the analysis. Data expressed on a dry matter basis were converted into a wet weight basis, according to Shearer’s (1994) recommendation.

2.2 Evaluation of models and calibration methods

Several models were developed from simple (e.g., isometric models) to more complex (e.g., polynomial and ensemble averaging models), and calibrated based on different calibration methods (e.g., least squares vs. robust calibration; assumption of additive errors vs. multiplicative errors). To further make a fair comparison with Nile tilapia body composition models from other authors, we implemented similar models, such as mixed models that use isometric submodels to predict water and crude protein, and allometric submodels to predict crude lipids and ash (following the *Chow_bureau* model in Chowdhury & Bureau (2009)) (Table 1). In addition, we used an allometric model following the 95 % confidence interval (CI95%) to determine the intervals for body weight exponents. Therefore, 23 combinations of "model + calibration method" were developed models were developed and pre-analysed. However, only 6 of them were ultimately selected as being reliable enough for validation purposes (Table 1).

Regression analysis was performed for each body component and models were tested with different calibration methods. The “model + calibration method” combinations were evaluated with qualitative (prediction and residual diagnostic plots) and quantitative methods, with several specific error metrics being considered, such as root mean square error (RMSE), as root mean square log error (RMLSE), mean absolute percentage error (MAPE) and coefficient of residual mass (CRM), described as follows:

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

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

$$MAPE = \frac{1}{n} \sum_{i=1}^{n} \left| \frac{y_i - \hat{y} i}{y_i} \right| \times 100 \tag{3}$$

$$CRM = \frac{(\sum_{i=1}^{n} y_i - \sum_{i=1}^{n} \hat{y}_i)}{\sum_{i=1}^{n} y_i} \tag{4}$$

where, $\hat{y}_i$ is the predicted value, y_i is the observed value and n the number of data points.

For an objective quantitative evaluation of the different combinations, in addition to calibration error metrics, focus was given on cross-validation errors obtained through different cross-validation methods (leave-one-out cross-validation (LOOCV), 100 repetitions k-fold cross-validation with k=10, k=5, k=2), to ensure that the chosen models display the best performance when predicting outside their calibration set. To determine the risk of overfitting, cross-validation was performed with different values of k in order to subject the combinations to different levels of "difficulty", since, as the value of k decreases, less data is available for the model to be calibrated.

All analyses were performed using R version 4.1.2 (R Core Team, 2021), where the *rlm* function from the MASS package (Venables & Ripley, 2002) was used to calibrate the robust models.

2.3 Validation of calibrated models

The body composition models were validated using unpublished independent body composition data from a Nile tilapia (*Oreochromis niloticus*) growth trial. In this trial, fish of different initial body weights were fed *ad libitum* with a range of experimental diets (with crude protein content between 28 and 36 %, and gross energy content between 17.8 and 19.8 MJ/kg), 2 times per day, for 141 days. The dataset consists of the measured whole-body composition of 29 fish with body weights ranging from 54 g to 454 g. Frozen whole-body samples were minced without thawing, using a meat grinder and moisture content was determined (105 °C for 24 h). Freeze-dried whole fish were finely milled and homogenised prior to further analysis. Samples were then analysed for ash content (550 °C for 6 h), crude protein (N x 6.25, Leco N analyser, Model FP-528, Leco Corporation, USA) and crude lipid (petroleum ether extraction, Soxtherm, Germany).

The performance of models published by other authors (Chowdhury & Bureau, 2009; Chowdhury et al., 2013; Shizari, 2020) were also validated with the same independent dataset, in order to make a fair comparison against the models developed in this study (Table 2).

3. Results

3.1 Data analysis

The data was collected from 138 sources (with 946 sets of measurements), covering fish with body weights ranging from 0.01 g to 1470 g. However, data referred to fish weighing mostly < 500 g, with half of the data corresponding to fish between 14.5 g and 116.3 g (Figure A.1), with a median weight of 38.1 g.

Nile tilapia's body components seemed to be relatively constant when expressed in a relative basis (as a percentage of total body weight), displaying a median of 73.5 % for water, 15.3 % for crude protein, 5.9 % for crude lipids, and 4.0 % for ash. However, it should be pointed out that components seemed to vary with body weight (Figure 1). Regarding the sum of components, most data summed up to below 100 % with a median of 99.4 % (IQR = 100.0 – 98.5 %).

3.2 Evaluation of models and calibration methods

In general, all the models seem to have fitted the data reasonably well and presented similar calibration errors (Figure 2). In a first analysis, based on the calculated metrics, 8 out of 23 models showed to have acceptable cross-validation errors (Table 2), with a mean absolute percentage error (MAPE) of the order of ≈ 4 % for water, ≈ 10 % for crude protein, ≈ 45 % for crude lipids and ≈ 26 % for ash. The calibration errors were slightly higher than the cross-validation errors across all CV methods, except for the 2-fold CV, in which the calibration errors were slightly lower than the cross-validation errors. Also, the relative order of models listed in Table 2, in terms of calibration and cross-validation errors, depended on the CV method used: CV with lower *k* (e.g., 2-fold CV) tend to privilege the simpler models (e.g., *al_mu*: allometric model calibrated under the assumption of multiplicative error), being the al_mu model the one with lower errors, whereas CV with higher *k* (e.g., LOOCV) seem to benefit the more complex models (e.g., *lcub*: cubic model in log-space calibrated under the assumption of multiplicative error; *lcub_rob*: the robust form of *lcub*) (Table 2). In a second analysis, the performance of the 8 models was evaluated. Therefore, although the lcub and the lcub_rob models displayed the lowest CV errors when using the LOOCV method, they seemingly had problems predicting the body composition of the fish with the lowest and highest body weights, with the sum of components for such extrapolations exceeding the total body weight by 2% (Figure A.2). This suggested that these models where overly complicated and at risk of overfitting the calibration data and/or providing unreasonable predictions. Therefore, the lcub and lcub_rob models were subsequently excluded from downstream validation.

Overall, isometric models seem to have had higher calibration and cross-validation errors compared to allometric models. The errors were also higher when models were calibrated under the assumption of additive error rather than multiplicative error.

Considering all quantitative (calibration and cross-validation metrics) and qualitative criteria (analysis of predictions, residuals and violation of constraints), 6 models from a list of

23 were chosen for downstream validation and comparison with other published models. Across all cross-validation methods (Table 3), 6 models (Table 4), the *al_mu* model (allometric model calibrated under the assumption of multiplicative error) displayed the best performance. Comparing the values predicted by these 6 models with the observed values (Figure 3), for the fish weight range where most data has been observed, most models did not diverge much from each other. The biggest differences were found for smaller and larger fish weights, where data were sparser, and as well for the water component, where there was greater disagreement between models. Furthermore, a clear qualitative difference between the *woutwater_iso* (isometric model) and the allometric models was seen. Visual inspection of the observed data suggested trends with nonzero slopes, being particularly visible in terms of the amount of water and crude lipids in Figure 3, which give additional support to the hypothesis that Nile tilapia body composition does not follow an exactly isometric pattern. Regression analysis further supported this: the body weight exponents recovered by linear regression of log-transformed data were close to 1, but the exponent of value 1 were not within any of the calculated 95% confidence intervals (as shown in Table 5).

The residuals in log-space of all models generally followed an approximately normal distribution with approximately constant variance. Further analysis demonstrated a negative correlation between the residuals of water and the residuals of crude protein and crude lipids. This is possibly related to laboratory errors during the determination of water content, which can affect the accurate determination of the other components (Breck, 2014).

3.3 Validation of calibrated and published models

Out of all tested models (6 best developed models, plus the other authors models), the *hyb_rob* and the *al_mu models* (developed in the current study) display the best performance: both models present validation errors that are either similar or lower than the calibration errors, with a validation MAPE of the order of ≈ 3 - 5 % for water, ≈ 5 - 9 % for crude protein, ≈ 40 % for crude lipids, and ≈ 20 % for ash (Table 6). Model hyb_rob, which was the one with the best prediction capability, was able to predict well the validation data regarding all components, including lipid content. Model al_mu on the other hand, presented a bigger variation between the predicted and the observed lipid content, but good predictions for the rest of the components. Thus, overall, both models predict the validation data well (compared to other competing models), demonstrating their good capacity to predict the body composition of Nile tilapia (Figure 4 and 5).

Though three of the models previously reported in the literature (namely, *Chowdhury2013_mixed*, *Shizari2020_alo* and *Shizari2020_iso*) display validation errors comparable to the best models developed in this study (*hyb_rob* and *al_mu*), some shortcomings of these and other published models can be noted. They show relatively higher validation errors for crude lipids and ash (see Table 6), higher errors for crude lipids when tested against our calibration dataset (see Figure 6), and/or displayed predictions of the individual components which, when added together, are inconsistent with the fish body weight (see Figure B.1 for an example with the *Chow_bureau* model).

Nevertheless, in general, all tested models show fair predictions for Nile tilapia body composition and display lower validation errors than calibration errors, suggesting that there is no model overfitting (Figure 6).

Analysing the residuals of all tested prediction models, when predicting the validation dataset, a strong negative correlation is visible between the errors of water and crude lipids (see Figure 7 for an example with the *hyb_rob* and *al_mu* models). Thus, when the models underestimate one component, the other tends to be overestimated, and vice-versa, which suggests that these two components were affected by a common factor. More detailed analysis confirms that this effect seems to be related to the nutritional state of fish (i.e., related to the different nutritional factors fish were subjected to): data related to fish subjected to high-energy diets display higher-than-predicted crude lipid contents, while data related to fish subjected to restricted feeding conditions display lower-than-predicted crude lipid contents (Figure 8 – a. and b.). The opposite pattern can be seen for water content (Figure 8 – c. and d.).

4. Discussion

Cross-validation methods, which provide an estimate of the expected generalization error of several models and calibration methods, complemented with the application of other quantitative and qualitative analyses, allow to more objectively identify the best combination of “model + calibration method" to predict the body composition of Nile tilapia. This reduces reliance on subjective and arbitrary decisions about the structure of models and how best to calibrate them. Even so, it could be said that there is still some degree of subjectivity, since one can choose to apply different cross-validation methods to estimate the generalization error (e.g., different cross-validation approaches, along with alternatives such as AIC, BIC, GCV). Thus, this work sought to reduce this problem by considering different CV methods and evaluating whether there is an agreement among methods or not. In fact, through the use of CV methods with different *k* values, it was possible to see a certain general agreement: models that perform poorly according to one CV method also tend to perform poorly according to the others. Similarly, models that perform well with one CV method tend to perform at least reasonably when evaluated under other CV methods. However, this consensus was not exact since the different CV methods do not exactly agree on the order/ranking of the models according to the calibration and validation errors. More restrictive methods (k-fold CV with low *k*) tend to give preference to simpler models (e.g., penalise complex models more) than the less restrictive methods (k-fold CV with high *k* or leave-one-out CV). This is mainly due to the fact that as the value of *k* decreases, less data is available for the algorithm to be calibrated (training), which increases the risk of miscalibration for more complex models. Therefore, the amount of data available to calibrate the model seems to affect the prediction capacity, with more complex models generally requiring larger amounts of data for effective calibration.

Another important observation is that models calibrated under the assumption of multiplicative error seem to have a better predictive capacity than those in which error was assumed to be additive. This suggests that regression in "log-space" is not only convenient, but advisable, even for isometric models, as it helps to ensure that the assumptions of normal distribution of errors and homogeneity of variance are met. Though log-transformation of data does not automatically mean that the assumptions of homoscedasticity and normality of errors are met, the log-transformation of data does reduce heteroscedasticity and lead to better model calibrations in this particular case (regardless of whether an isometric or allometric model is assumed). Most of the published studies that have used the isometric model have something in common: they all (presumably) tested the isometric model calibrated under the assumption of additive constant-variance errors. In fact, it is the most obvious choice, as the majority of statistical methods assume additive error. However, it is imperative to evaluate (e.g., through residual analyses and cross-validation methods) whether such assumption is valid, as this affects the performance of models. Thus, it can be concluded that the use of complementary evaluation methods is useful, as it ensures that the selected combinations of “model + calibration method” perform well under different conditions, and also that the hypothesis of the presence of multiplicative error is more likely to be correct than that of additive error.

Despite the usefulness of CV methods to compare the expected validation error of different combinations of "model + calibration method”, the validation error for the final selected combination, as well as the comparison with other published models, should always be made using a completely independent dataset (i.e., one that has not been used during the cross-validation process neither for the calibration of any of the tested models). In this work, a challenging validation dataset was used, corresponding to Nile tilapia of different sizes and subjected to different nutritional conditions, which allowed us to demonstrate the effectiveness of the model development and evaluation process. The results demonstrate the good capacity of the resulting models to generalize in novel contexts, since no overfitting of the models was apparent. In general, the models developed to predict Nile tilapia body composition presented lower validation errors than those previously reported in the scientific literature (i.e., Chowdhury & Bureau, 2009; Chowdhury et al., 2013; Shizari, 2020). This can be explained by the use of a large calibration dataset, but also by the tools and methodologies that were used to iteratively develop, calibrate and evaluate the predictive capacity of the developed models. Since the *hyb_rob* and *al_mu* models display lower calibration and validation errors than other alternatives, we recommend their use to predict Nile tilapia body composition as a function of body weight.

Allometry has been used to describe the effect of body weight (BW) on the body composition of several animals, including fish. Though there seems to be a lack of consensus regarding the type of model that is optimal to describe the body composition of Nile tilapia, with some authors suggesting that an isometric model may be more appropriate than an allometric model, in the present work, most results are indicative of a generally allometric pattern when describing the body composition of Nile tilapia. In particular, visual inspection of the observed data, which suggests trends with nonzero slopes (particularly visible in terms of the relative amount of water and crude lipids), along with the good quantitative performance of the allometric models, gives support to the notion that the body composition of Nile tilapia does not follow an exactly isometric pattern. Furthermore, the estimation of 95% confidence intervals for the BW exponents presents intervals that do not include the value 1 for all components, causing the null hypothesis of isometry to be statistically rejected. Considering the results obtained through the validation process, allometric models, like the *hyb_rob* and the *al_mu*, seem to have quantitatively better prediction capacity than isometric models. Moreover, models with greater flexibility than allometric models, such as the *lcub* and *lcub_rob*, while appearing to be quantitatively good in some cases (e.g., LOOCV), showed signs of overfitting in other cases (e.g., 2-fold CV).

According to the results obtained, the relative amounts of water and crude lipids in Nile tilapia clearly depend on BW, which is consistent with reports from other studies (Salam & Davies, 1994; Lupatsch et al., 2001; Bureau et al., 2003; Dumas et al., 2010; van Trung et al., 2011; de Castro Silva et al., 2015). On the other, the relative amounts of crude protein and ash do not seem to depend as clearly on BW, unlike what Xie et al., (1997) reported for Nile tilapia. Moreover, in contrast to what Chowdhury & Bureau (2009) reported, in this study no isometric relationship was found for water with respect to BW. In fact, the model that was developed based on the logic described in Chowdhury & Bureau (2009) showed higher calibration and validation errors. Overall, it seems that some components are more correlated to BW than others. This is probably why the hybrid model in its robust form (*hyb_rob*), which provides estimates that are a compromise between an allometric and an isometric model using an outlier-resistant calibration method, presents better predictive capacity than the pure allometric model.

Though the results of this study support the hypothesis that Nile tilapia body composition is essentially allometric, given that the BW exponents are effectively close to 1 (e.g., quasi-isometry), it may be justifiable to use isometric models, as well as intermediate models between a purely isometric and allometric model (e.g., *hyb_rob* model). It is particularly relevant to consider these types of approaches when few data are available to calibrate the models.

Besides genetic and other intrinsic factors (like BW), the body composition of fish can also be affected by different environmental and dietary factors during their life cycle (Dumas et al., 2007, 2010; Shizari, 2020). This study provides evidence of such effects in the context of Nile tilapia body composition, since there was a direct relationship between prediction errors and the nutritional factors fish were subjected to. For example, crude lipids are consistently overestimated and water consistently underestimated for fish fed low-energy diets or subjected to restricted feeding conditions, while the opposite effect was observed for fish fed high-energy diets. The analysis of validation residuals demonstrates these patterns in all models developed in this study, as well as in models from other authors, which supports the hypothesis that nutrition has an important effect on Nile tilapia body composition. This may, at least in part, explain the generally higher prediction errors obtained across all models when trying to predict Nile tilapia body composition.

This sensitivity of Nile tilapia body composition to nutritional factors and, in general, to their nutritional state, suggests that the developed Nile tilapia body composition models can be used as a reference to evaluate their nutritional state. By comparing measurements against a reference model, it may be possible to detect situations of nutritional deficiency (e.g., relatively low crude lipid and high water content) or excess (e.g., relatively high crude lipid and low water content).

5. Conclusion

The predictions of the Nile tilapia body composition models developed in this study were consistent with that of other published models. However, some models developed in this study, such as the hyb_rob (ensemble of isometric and allometric models, calibrated assuming multiplicative error using a robust regression method) and al_mu (allometric model calibrated under the assumption of multiplicative error), demonstrated better performance when tested against an independent dataset, compared to other published models. This highlights the importance of having a good and vast calibration dataset and of using cross-validation to evaluate model performance, prior to the validation step. This methodology also allowed us to conclude that Nile tilapia body composition follows a quasi-isometric allometric pattern, which means that both isometric and allometric models seem to provide reasonable predictions, as long as they are calibrated under the assumption of multiplicative error. This also suggests that when few data are available to calibrate models it may be preferable to use isometric models, as well as ensemble of isometric and allometric models. Furthermore, a relation between prediction errors and the nutritional factors was observed, which implies that the composition models can also be used as a reference to evaluate fish nutritional state and detect situations of nutritional deficiency. Therefore, mathematical models can be used both as a practical and low-cost tool to estimate the body composition and as reference models to assist in the interpretation of body composition data.

Finally, this study shows the importance of testing body composition models and their assumptions, in order to ensure high predictive capacity. It also underlines the importance of making an evaluation of models based on objective criteria (e.g., combination of qualitative and quantitative criteria with cross-validation methods) which is a necessary strategy to help us answer both scientific questions (e.g., “which is more plausible: isometry or allometry?”) and technical questions (e.g., “what is the nature of measurement errors?”, “what is the best method to calibrate each model?”).

In the future, it will be interesting to explore the relationships between the different components of fish body composition. Breck, 2014 reported that there is a strong relationship between fish weight and the amount of water and ash. Therefore, it would be interesting to use the water and ash measurements and relate them to fish weight to predict the other components of body composition (i.e., protein and lipids). Thus, the model predictions would not only be based on fish weight. The study and definition of this type of relationship could lead to the possibility of replacing the direct methods for determining some components of body composition by indirect ones, at least for some purposes.

Acknowledgements

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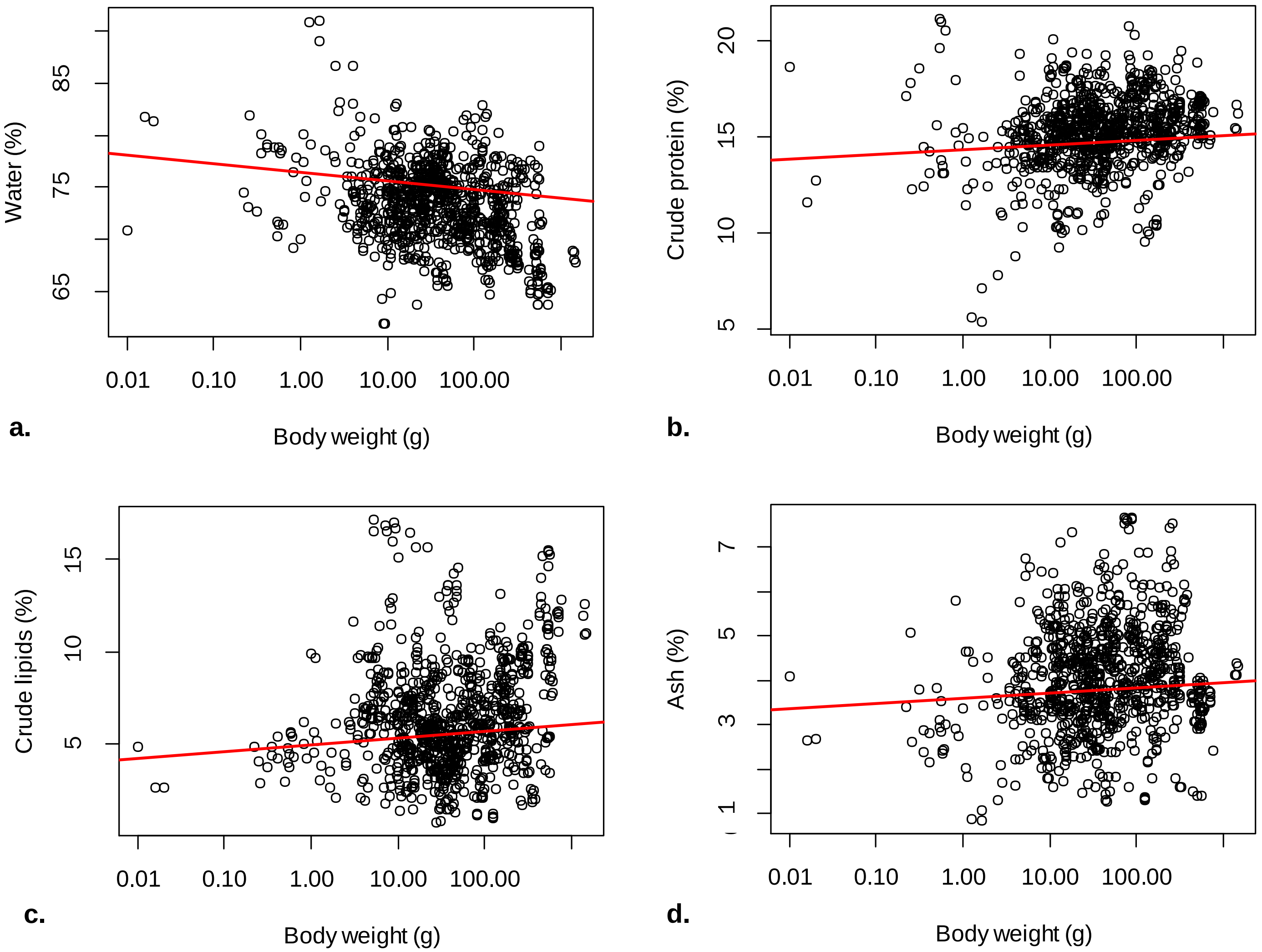


Figure 1 - Overview of the calibration data: relative abundance of water (a.), crude protein (b.), crude lipid (c.) and ash (d.) in relation to whole body weight (BW(g)) of Nile tilapia (O. niloticus); red line represents the linear regression of each component in function of Nile tilapia body weight in log scale.

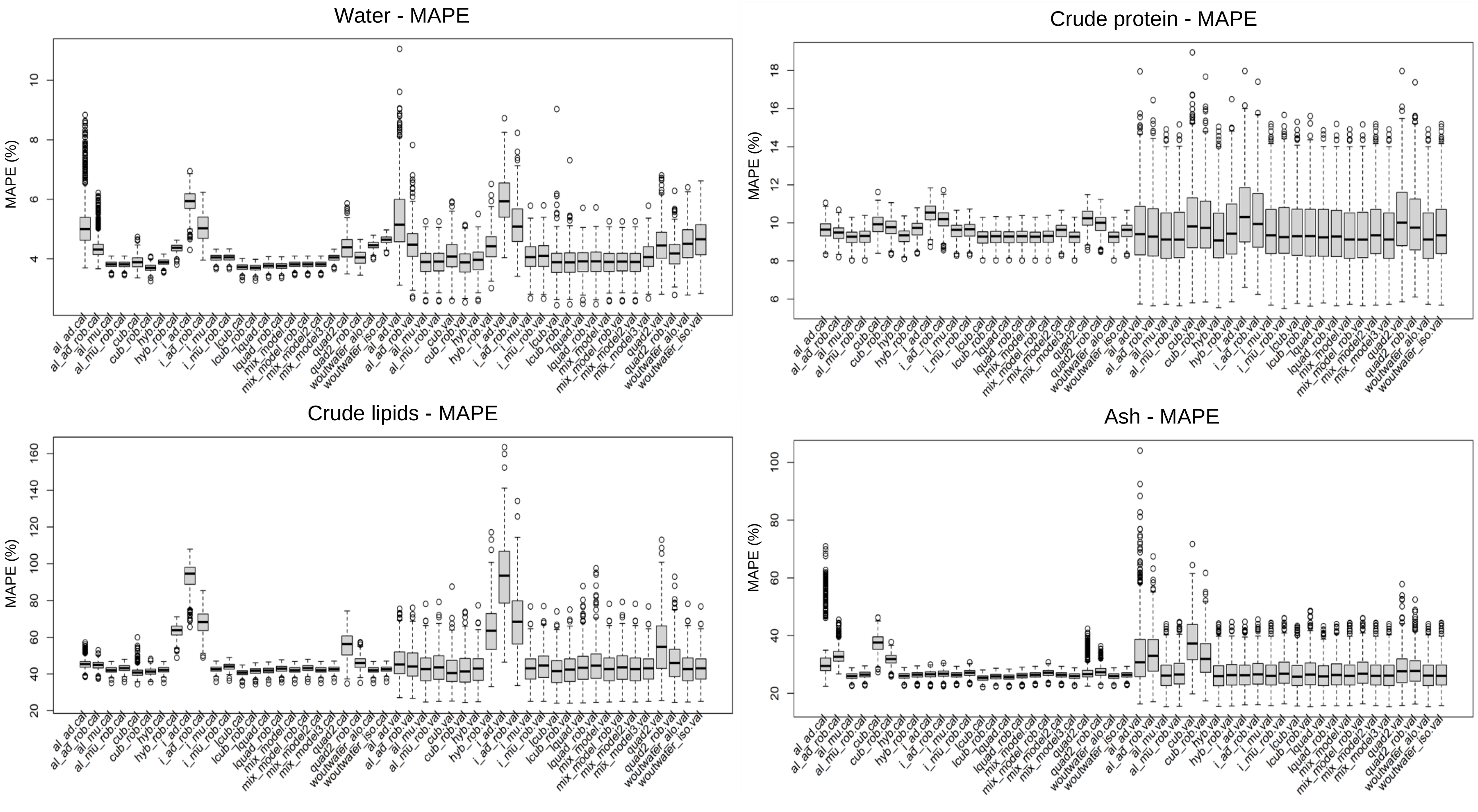


Figure 2 - Mean absolute percentage error (MAPE) for calibration and validation errors of the 23 models, when applying a 5-fold cross-validation.

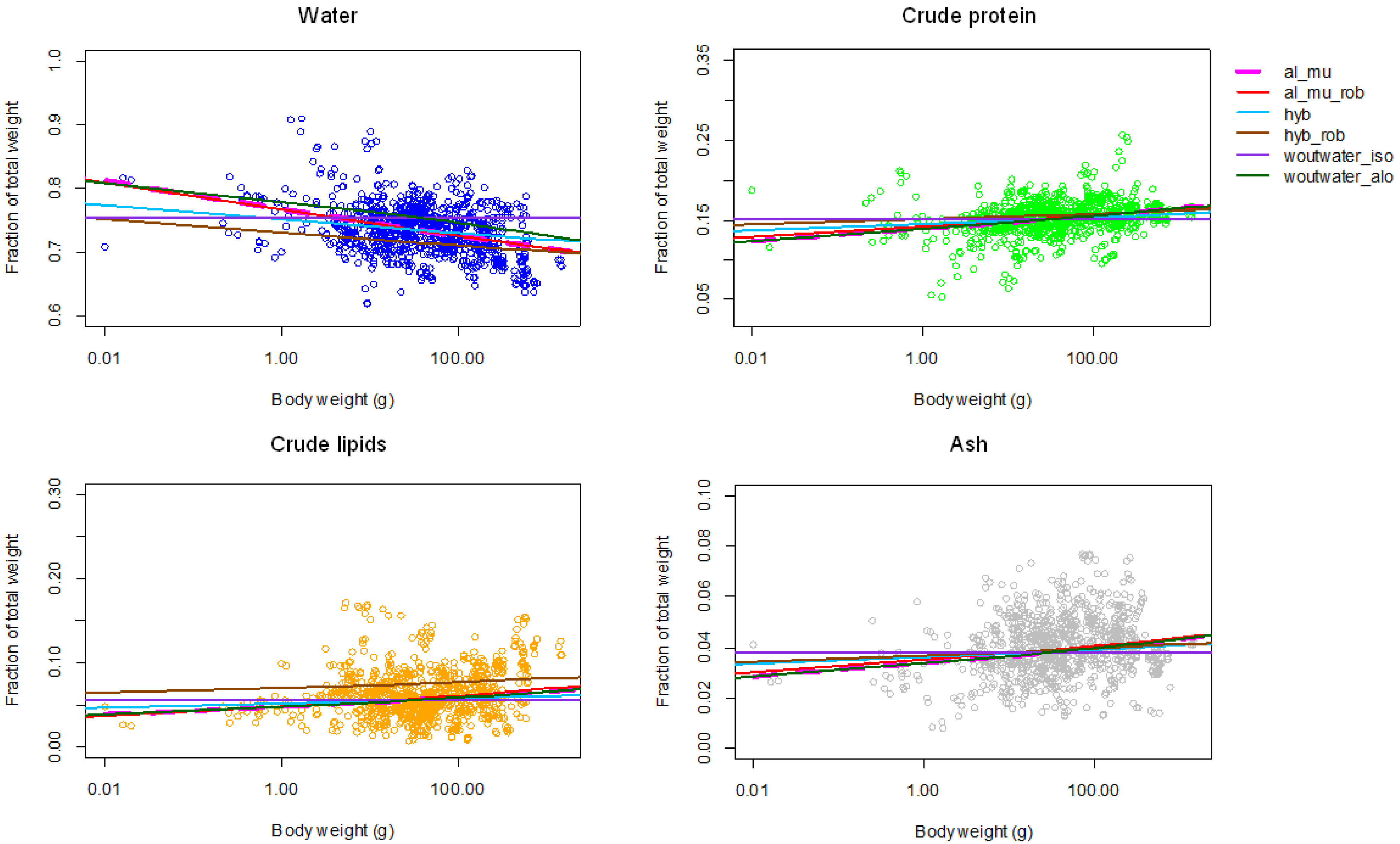


Figure 3 – Scatter plots showing each component (in relative terms) as a function of body weight (log scale). Points represent measured data, while lines represent the predictions of the 6 chosen models.

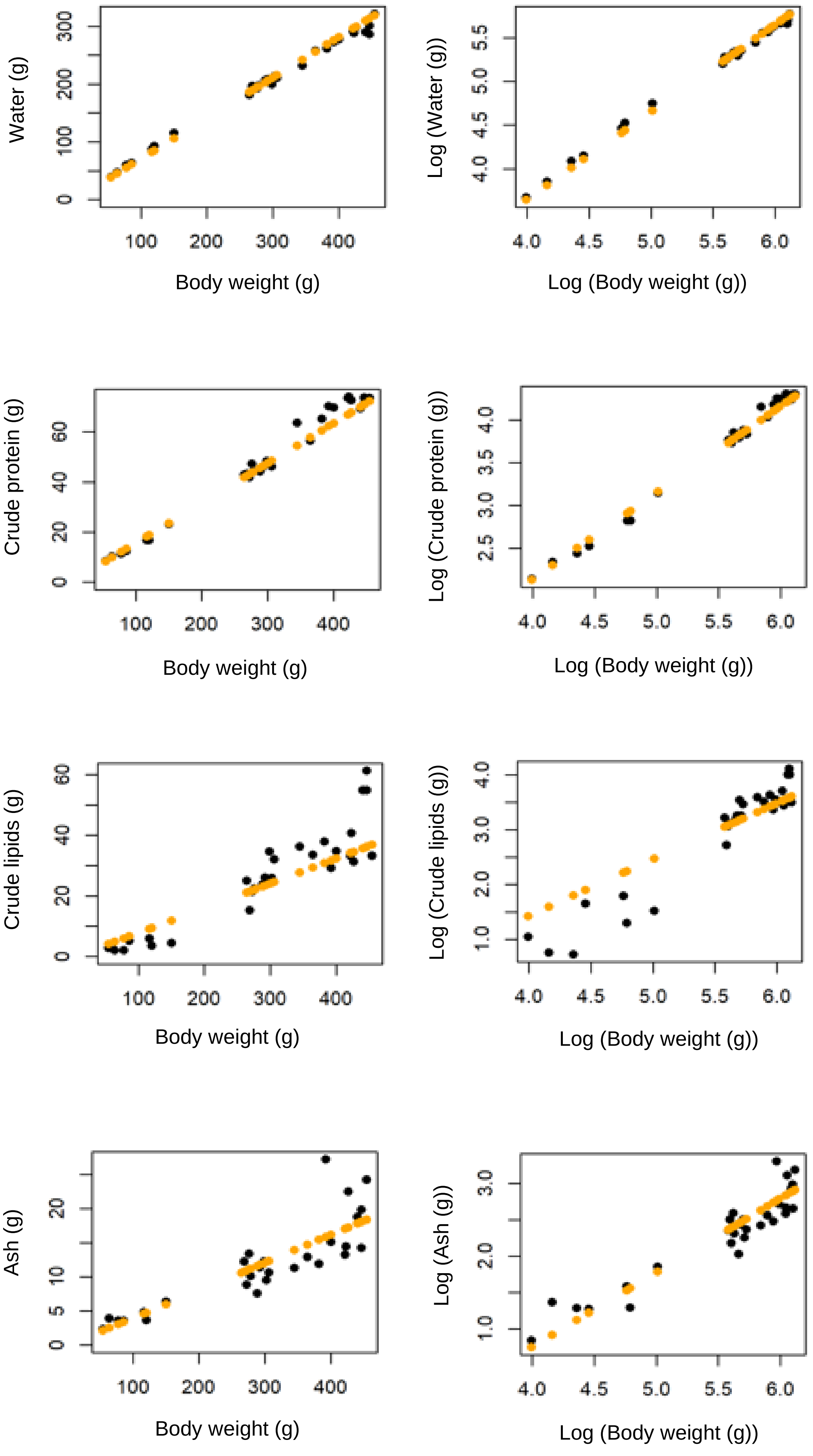


Figure 4 – Scatter plot of the independent data (black) against the predictions (orange) of the hyb_rob model.

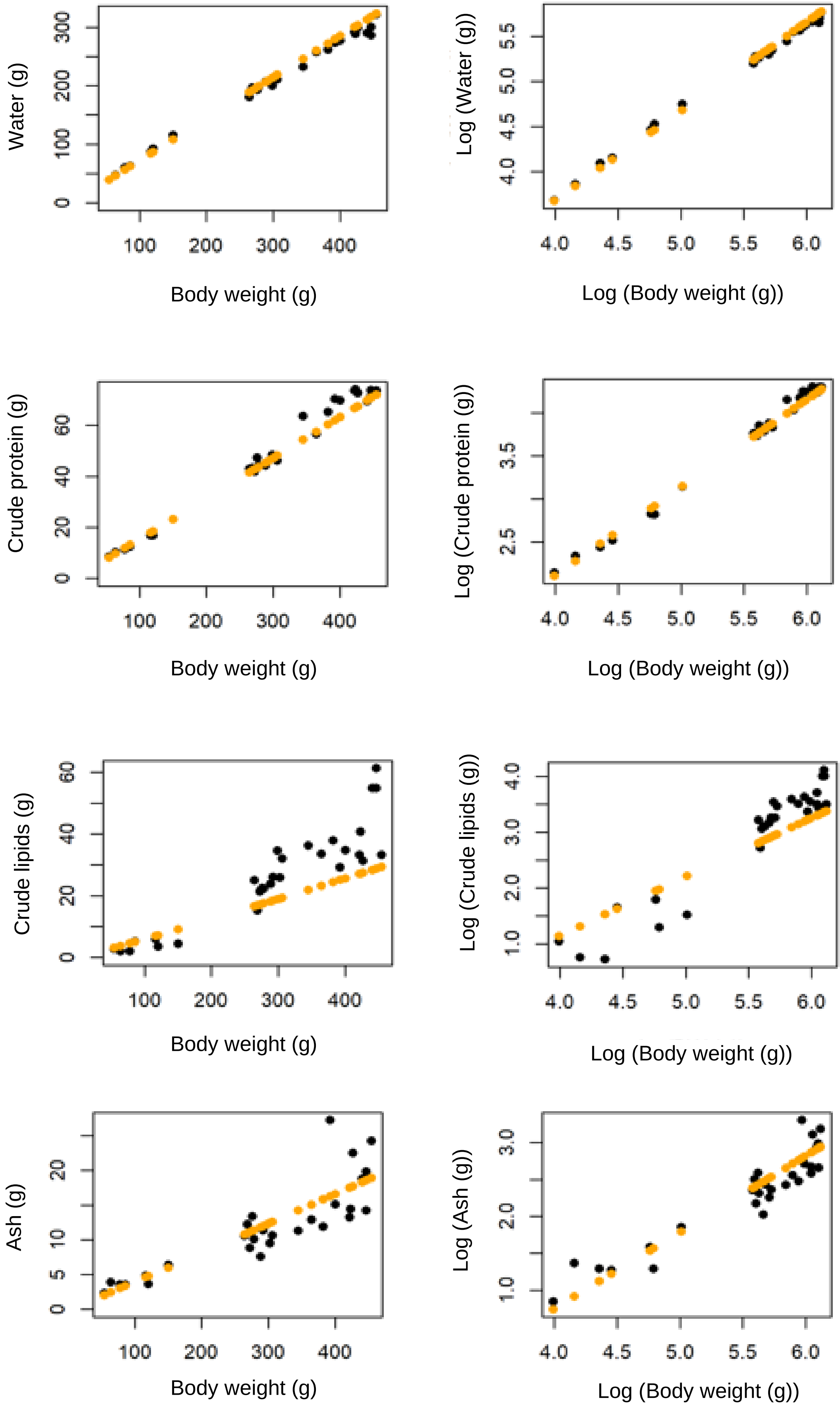


Figure 5 - Scatter plot of the independent data (black) against the predictions (orange) of the al_mu model.

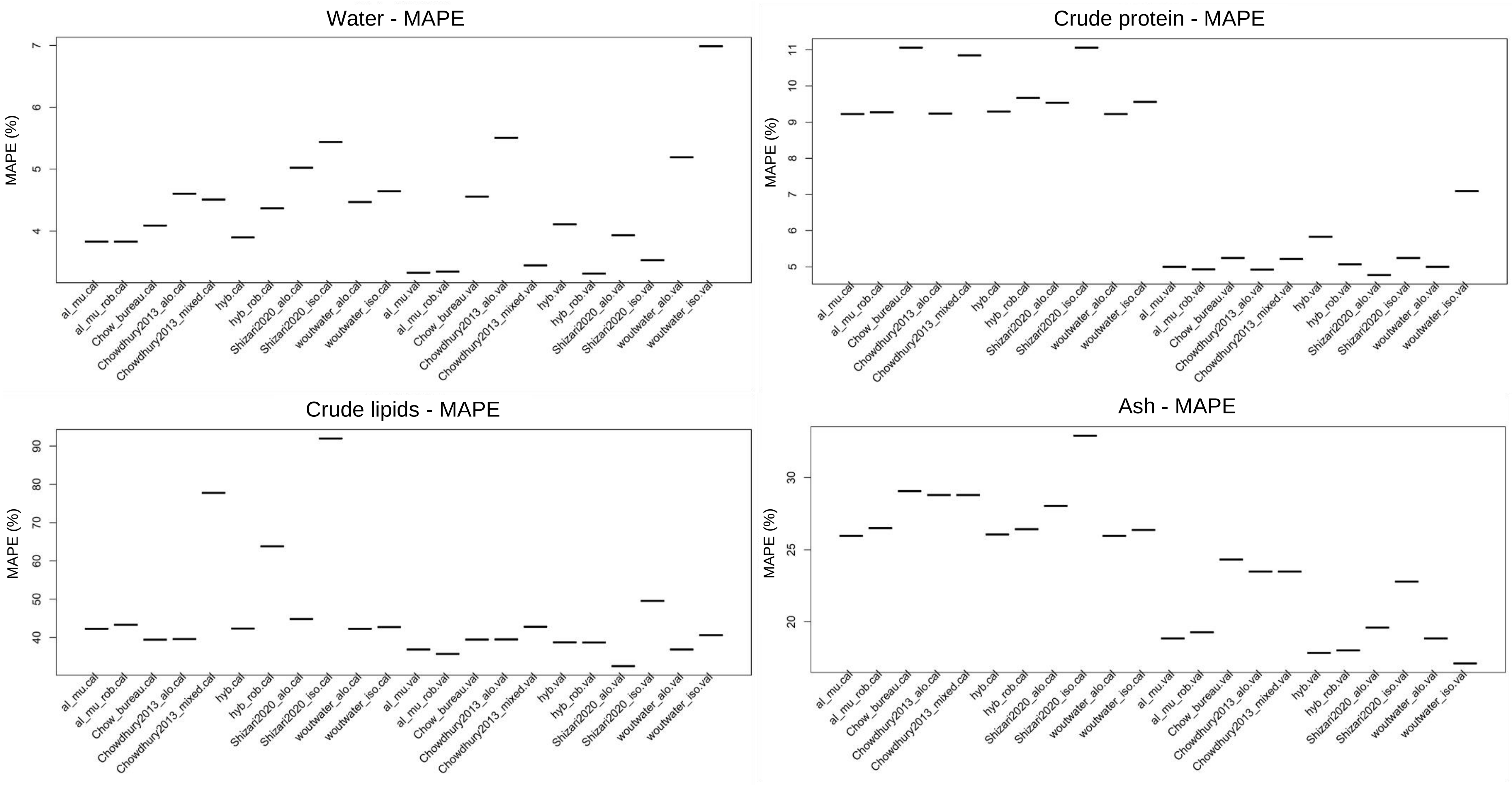
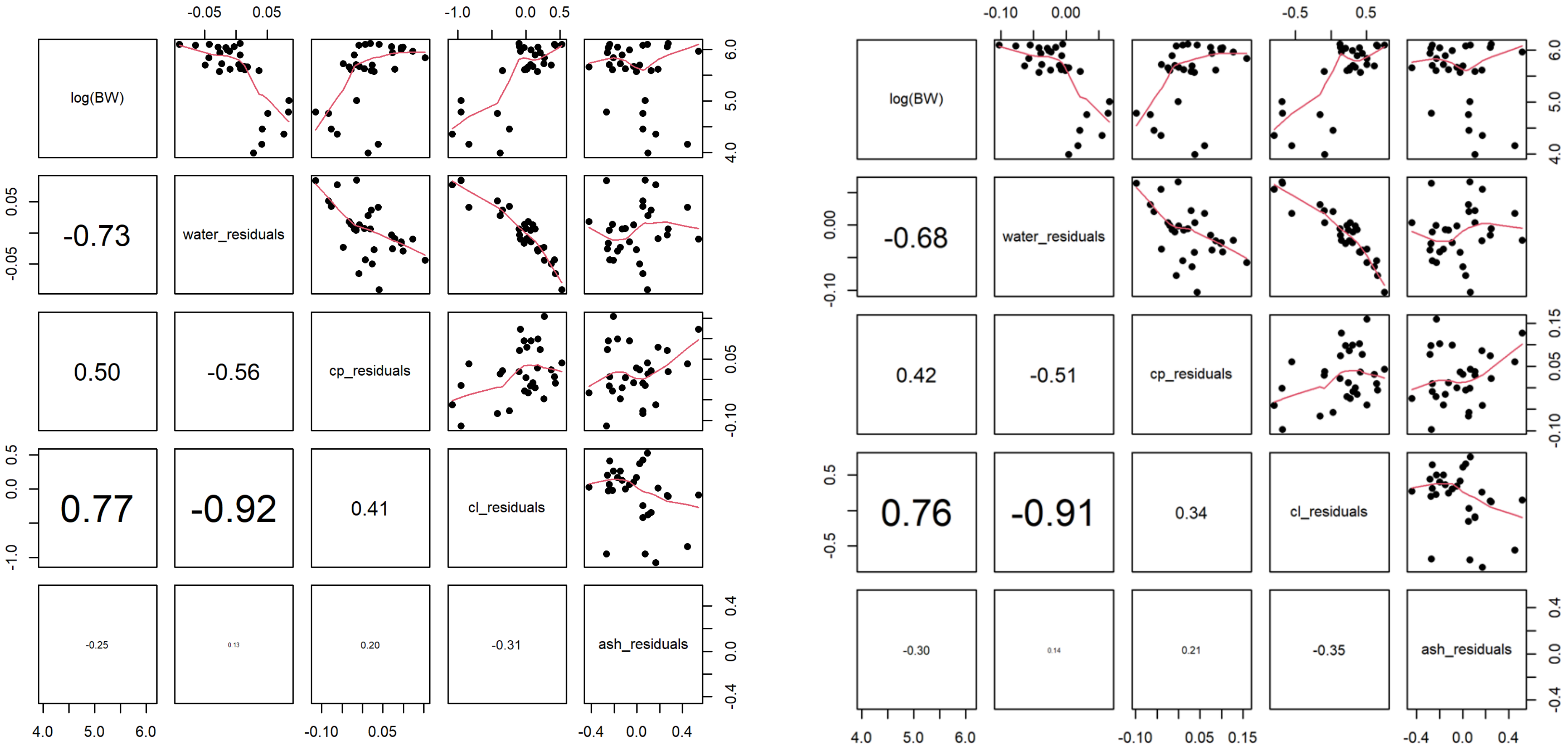


Figure 6 - Mean absolute percentage errors (MAPE) for the different models, measured against the whole calibration dataset and against an independent validation dataset.



a.

b.

Figure 7 - Scatter plots showing the relationships between log(BW) and the prediction residuals obtained when predicting the independent dataset using the developed models: a. model hyb_rob; b. model al_mu.

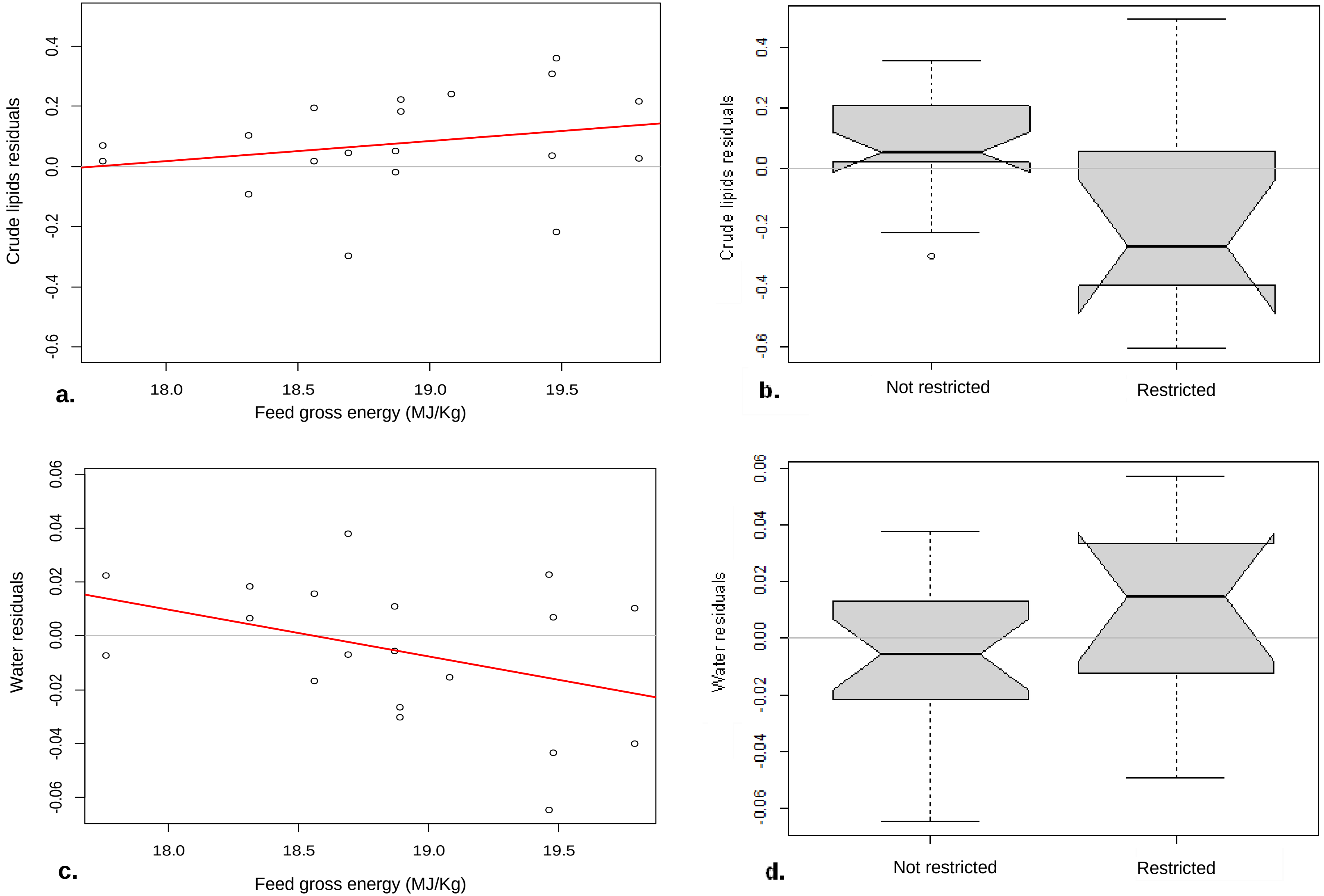


Figure 8 - Scatter plot of crude lipids (a.) and water (c.) residuals in function of feed energy. Box plots of crude lipids (b.) and water (d.) residuals in function of fish feeding regime. The residuals of both components were calculated with an allometric model.

Table 1 – Overview and description of the models developed and the models used from other authors.

Model	Equation	Description
al_mu	$y = a \times BW^b$	Linear regression after log-transformation
al_mu_rob	$y = a \times BW^b$	Robust linear regression after log-transformation
hyb	$y = a_1 \times BW + a_2 \times BW^b$	Predicts the average of the *i_mu* and *al_mu* predictions
woutwater_alo	$y = a \times BW^b$	Predicts components using allometric models, except for water, which is predicted by difference (to ensure sum of components is 100%)
hyb_rob	$y = a_1 \times BW + a_2 \times BW^b$	Predicts the average of the *i_mu* and *al_mu* predictions
woutwater_iso	$y = a \times BW$	Predicts components using isometric models, except for water, which is predicted by difference (to ensure sum of components is 100%)

Models already published by other authors		
[1]*Chowdhury2013_mix*	$y = a \times BW$ $y = a \times BW^b$	Predicts crude protein and lipids using isometric models and allometric model to predict ash. Water is predicted by difference (to ensure sum of components is 100%)
[1]*Chowdhury2013_alo*	$y = a \times BW^b$	Predicts components using allometric models, except for water, which is predicted by difference (to ensure sum of components is 100%)
[2]*Shizari2020_iso*	$y = a \times BW$	Predicts components using isometric models, except for water, which is predicted by difference (to ensure sum of components is 100%)
[2]*Shizari2020_alo*	$y = a \times BW^b$	Predicts components using allometric models, except for water, which is predicted by difference (to ensure sum of components is 100%)
[3]*Chow_bureau*	$y = a \times BW$ $y = a \times BW^b$	Predicts water and crude protein using isometric models. Crude lipids and ash are predicted with allometric models.

[1] Chowdhury et al. (2013)
[2] Shizari (2020)
[3] Chowdhury & Bureau (2009)

Table 2 - Equations and coefficients of the models published by other authors to estimate Nile tilapia body composition.

		Water (g)		Crude protein (g)		Crude lipids (g)		Ash (g)	
Model	Equation	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
[1]*Chowdhury2013_mix*	$y = a \times BW$	-	-	0.160	-	0.085	-	-	-
	$y = a \times BW^b$	-	-	-	-	-	-	0.036	1.039
[1]*Chowdhury2013_alo*	$y = a \times BW^b$	-	-	0.141	1.020	0.044	1.042	0.036	1.039
[2]*Shizari2020_iso*	$y = a \times BW$	-	-	0.161	-	0.093	-	0.045	-
[2]*Shizari2020_alo*	$y = a \times BW^b$	-	-	0.131	1.033	0.039	1.115	0.023	1.100
[3]*Chow_bureau*	$y = a \times BW$	0.728	-	0.161	-	-	-	-	-
	$y = a \times BW^b$	-	-	-	-	0.042	1.047	0.036	1.047

[1] Chowdhury et al. (2013)

[2] Shizari (2020)

[3] Chowdhury & Bureau (2009)

Table 3 – Cross-validation errors of 8 out of 23 models chosen in the first analysis for each method of cross-validation.

	RMSE (g)				**RMLSE (log(g))**				**MAPE (%)**				**CRM (g)**			
LOOCV	Water	Crude protein	Crude lipids	Ash	Water	Crude protein	Crude lipids	Ash	Water	Crude protein	Crude lipids	Ash	Water	Crude protein	Crude lipids	Ash
lcub	3.43	1.38	2.56	1.04	0.04	0.10	0.39	0.26	3.69	9.96	42.65	27.10	0.00	0.00	0.08	0.04
lcub_rob	3.52	1.37	2.63	1.04	0.04	0.10	0.38	0.33	3.69	9.93	43.85	27.46	0.00	0.01	0.11	0.07
al_mu	3.72	1.37	3.31	1.09	0.04	0.10	0.40	0.26	3.74	9.84	44.53	27.48	0.00	0.00	0.09	0.05
al_mu_rob	3.74	1.37	3.19	1.10	0.04	0.10	0.40	0.26	3.75	9.84	45.73	27.87	0.00	0.01	0.12	0.07
hyb	4.09	1.39	3.47	1.03	0.04	0.10	0.40	0.26	3.80	9.85	44.44	27.29	0.00	0.01	0.09	0.04
woutwater_alo	4.68	1.37	3.31	1.09	0.05	0.10	0.40	0.26	4.39	9.84	44.53	27.48	0.03	0.00	0.09	0.05
hyb_rob	3.83	1.36	3.18	1.04	0.05	0.10	0.47	0.26	4.32	10.11	67.55	27.55	0.02	0.02	0.46	0.06
woutwater_iso	5.75	1.50	3.64	1.01	0.05	0.11	0.40	0.26	4.58	10.03	44.66	27.36	0.03	0.01	0.08	0.04
10-Fold CV																
lcub_rob	6.47	2.00	5.22	1.71	0.05	0.13	0.46	0.31	3.87	9.59	42.80	26.80	0.00	0.00	0.02	0.00
lcub	6.31	2.02	5.16	1.73	0.05	0.13	0.47		3.87	9.59	41.88	26.21	0.00	0.00	0.06	0.03
al_mu	6.88	2.00	6.88	1.75	0.05	0.13	0.47	0.31	3.89	9.42	43.05	26.60	0.01	0.00	0.14	0.02
al_mu_rob	6.92	2.01	6.53	1.78	0.05	0.13	0.47	0.31	3.89	9.44	44.12	21.99	0.01	0.00	0.10	0.03
woutwater_alo	8.94	2.00	6.88	1.75	0.05	0.13	0.47	0.31	4.51	9.42	43.05	26.60	0.04	0.00	0.14	0.02
hyb	7.82	2.06	7.32	1.64	0.05	0.13	0.47	0.31	3.94	9.44	42.99	26.55	0.02	0.02	0.18	0.01
hyb_rob	6.78	1.99	5.89	1.67	0.06	0.13	0.56	0.31	4.41	9.78	64.66	26.85	0.01	0.01	0.10	0.00
woutwater_iso	11.42	2.33	7.79	1.60	0.06	0.13	0.48	0.32	4.66	9.67	43.26	26.73	0.05	0.03	0.21	0.04
5-Fold CV																
lcub_rob	6.87	2.07	5.66	1.81	0.04	0.13	0.47	0.32	3.88	9.61	42.76	27.06	0.00	0.00	0.03	0.01
al_mu	7.30	2.07	7.50	1.82	0.05	0.13	0.48	0.32	3.88	9.42	42.88	26.50	0.01	0.00	0.17	0.02
lcub	6.70	2.09	5.62	1.84	0.05	0.13	0.47	0.32	3.91	9.63	41.85	26.32	0.00	0.01	0.08	0.03
al_mu_rob	7.35	2.07	7.14	1.86	0.05	0.13	0.48	0.32	3.89	9.41	43.92	27.03	0.01	0.00	0.14	0.03
hyb	8.34	2.12	8.06	1.71	0.05	0.13	0.48	0.32	3.94	9.42	42.84	26.46	0.02	0.02	0.21	0.01
woutwater_alo	9.55	2.07	7.54	1.82	0.06	0.13	0.48	0.32	4.51	9.39	42.88	26.50	0.04	0.00	0.17	0.02
hyb_rob	7.13	2.04	6.34	1.74	0.06	0.13	0.56	0.32	4.41	9.75	64.18	26.76	0.01	0.01	0.06	0.00
woutwater_iso	12.42	2.42	8.62	1.66	0.06	0.13	0.48	0.32	4.66	9.65	43.09	26.65	0.06	0.04	0.24	0.04

(continuation)	**RMSE (g)**				**RMLSE (log(g))**				**MAPE (%)**				**CRM (g)**			
2-Fold CV	Water	Crude protein	Crude lipids	Ash	Water	Crude protein	Crude lipids	Ash	Water	Crude protein	Crude lipids	Ash	Water	Crude protein	Crude lipids	Ash
al_mu	7.59	2.17	8.02	1.90	0.05	0.13	0.48	0.32	3.91	9.49	43.20	26.72	0.01	0.00	0.19	0.02
al_mu_rob	7.67	2.12	7.58	1.93	0.05	0.13	0.48	0.32	3.92	9.45	44.21	27.20	0.01	0.00	0.15	0.03
hyb	8.66	2.15	8.58	1.78	0.05	0.13	0.48	0.32	3.95	9.48	43.08	26.64	0.02	0.02	0.22	0.01
woutwater_alo	9.88	2.17	8.02	1.90	0.06	0.13	0.48	0.32	4.54	9.49	43.20	26.72	0.04	0.00	0.19	0.02
lcub_rob	7.70	2.19	7.05	1.99	0.05	0.14	0.48	0.33	4.00	9.68	43.17	29.54	0.00	0.00	0.03	0.11
lcub	7.49	2.26	7.11	2.06	0.05	0.14	0.49	0.33	4.03	9.73	42.23	28.12	0.00	0.00	0.07	0.03
hyb_rob	7.56	2.08	6.71	1.81	0.06	0.13	0.56	0.32	4.44	9.78	63.80	26.86	0.01	0.01	0.03	0.00
woutwater_iso	12.98	2.47	9.20	1.73	0.06	0.13	0.49	0.32	4.68	9.70	43.28	26.81	0.06	0.04	0.26	0.04

Table 4 - Parameters of the calibrated equations of the 6 best models to predict body components in grams.

		Water (g)		Crude protein (g)		Crude lipids (g)		Ash (g)	
Model	Equation	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
al_mu	$y = a \times BW^b$	0.763	0.989	0.141	1.019	0.047	1.053	0.034	1.034
al_mu_rob	$y = a \times BW^b$	0.764	0.989	0.141	1.019	0.047	1.053	0.034	1.034
hyb	$y = a_1 \times BW + a_2 \times BW^b$	^{a1}0.366 ^{a2}0.382	0.989	^{a1}0.076 ^{a2}0.071	1.019	^{a1}0.028 ^{a2}0.023	1.053	^{a1}0.019 ^{a2}0.017	1.034
woutwater_alo	$y = a \times BW^b$	-	-	0.141	1.019	0.047	1.053	0.034	1.034
hyb_rob	$y = a_1 \times BW + a_2 \times BW^b$	^{a1}0.367 ^{a2}0.382	0.989	^{a1}0.077 ^{a2}0.071	1.019	^{a1}0.030 ^{a2}0.023	1.053	^{a1}0.020 ^{a2}0.017	1.034
woutwater_iso	$y = a \times BW$	-	-	0.151	-	0.057	-	0.038	-

Table 5 – Lower and upper bound of the 95 % confidence intervals for body weight exponents, estimated assuming the al_mu model, following the approximation CI95% = mean ± (2 x standard error).

	95 % confidence intervals	
	Lower bound	Upper bound
Water	0.987	0.991
Crude protein	1.003	1.014
Crude lipids	1.033	1.073
Ash	1.020	1.047

Table 6 – Estimates of the generalization errors of the compared models predicting unseen data (validation errors). The greener cells indicate lower estimates of the generalization errors, and the reddish ones indicate higher ones.

	Water				**Crude protein**				**Crude lipids**				**Ash**			
Models	RMSE (g)	RMLSE (log(g))	MAPE	CRM (g)	RMSE (g)	RMLSE (log(g))	MAPE	CRM (g)	RMSE (g)	RMLSE (log(g))	MAPE	CRM (g)	RMSE (g)	RMLSE (log(g))	MAPE	CRM (g)
hyb_rob	8.34	0.04	3.31	0.01	3.59	0.07	5.07	0.03	8.15	0.43	38.64	0.10	3.20	0.22	18.02	0.00
al_mu	10.02	0.04	3.33	0.03	3.71	0.07	5.00	0.04	11.77	0.44	36.83	0.29	3.20	0.23	18.85	0.02
Chowdhury2013_mixed	9.19	0.04	3.45	0.02	3.43	0.07	5.22	0.03	7.63	0.45	42.80	0.05	3.57	0.26	23.48	0.13
Shizari2020_alo	12.04	0.05	3.93	0.04	3.47	0.06	4.77	0.03	8.76	0.38	32.48	0.16	3.18	0.24	19.60	0.00
al_mu_rob	10.07	0.04	3.34	0.03	3.51	0.06	4.93	0.03	10.95	0.42	35.68	0.26	3.23	0.23	19.27	0.04
Shizari2020_iso	7.98	0.05	3.53	0.01	3.33	0.07	5.24	0.02	7.11	0.49	49.55	0.04	3.48	0.25	22.79	0.11
woutwater_alo	15.81	0.06	5.19	0.06	3.71	0.07	5.00	0.04	11.77	0.44	36.83	0.29	3.20	0.23	18.85	0.02
hyb	12.40	0.05	4.11	0.04	4.50	0.08	5.83	0.06	12.74	0.47	38.69	0.33	3.21	0.22	17.85	0.01
Chow_bureau	13.56	0.05	4.56	0.04	3.33	0.07	5.24	0.02	13.97	0.51	39.44	0.38	3.65	0.26	24.32	0.14
Chowdhury2013_alo	16.66	0.06	5.51	0.06	3.55	0.06	4.92	0.03	13.92	0.51	39.47	0.38	3.57	0.26	23.48	0.13
woutwater_iso	20.89	0.08	6.99	0.08	5.39	0.09	7.09	0.08	13.74	0.51	40.58	0.37	3.28	0.22	17.12	0.05

Appendixes

Table A.1 - List of references from which the data used was retrieved.

Source	DOI/URL
Abdelghany & Ahmad, 2002	DOI: 10.1046/j.1365-2109.2002.00689.x
Abdelghany et al., 1998	DOI: 10.1046/j.1365-2109.1998.00219.x
Abdelghany et al., 2002	DOI: 10.1111/j.1749-7345.2002.tb00507.x
Abdel-Hakim et al., 2010	DOI: 10.21608/EJABF.2010.2058
Abdel-Tawwab & Ahmad, 2009	DOI: 10.1111/j.1365-2109.2009.02254.x
Abdel-Tawwab et al., 2006	DOI: 10.1300/J028v18n03_02
Abdel-Tawwab et al., 2008	DOI: 10.1016/j.aquaculture.2008.03.055
Abdel-Tawwab et al., 2010	DOI: 10.1016/j.aquaculture.2009.10.027
Abdel-Tawwab et al., 2015	DOI: 10.1007/s10499-015-9882-y
Abonorag et al., 2018	DOI: 10.1016/j.fsi.2018.05.051
Abozaid et al., 2016	http://www.aensiweb.net/AENSIWEB/rjfh/rjfh/2016/Oct-Dec/10-15.pdf
Afuang et al., 2003	DOI: 10.1046/j.1365-2109.2003.00920.x
Ahmad et al., 2005	DOI: 10.1111/j.1365-2109.2004.01201.x
Ahmad et al., 2008	https://www.cabdirect.org/cabdirect/abstract/20133318727
Ahmad et al., 2011	DOI: 10.1080/10454438.2011.626350
Ahmad et al., 2011	DOI: 10.1080/10454438.2011.626350
Al-Asgah & Ali, 1997	DOI: 10.1051/animres:19970404
Al-Hakim et al., 2013	DOI: 10.21608/EJABF.2013.2169
Ali & Al-Asgah, 2001	DOI: 10.1051/animres:2001119
Ali & El-Feky, 2013	DOI: 10.21608/ejap.2013.93679
Ali et al., 2003	DOI: 10.33997/j.afs.2003.16.1.007
Ali et al., 2007	DOI: 10.1111/j.1439-0426.2007.00897.xU
Ali et al., 2008	https://ageconsearch.umn.edu/record/276669
Ali et al., 2016	DOI: 10.1016/j.ejar.2016.06.004
Ayisi et al., 2017	DOI: 10.1016/j.aaf.2017.02.001
Azaza et al., 2008	DOI: 10.1007/s10499-008-9220-8
Badwy et al., 2008	https://www.researchgate.net/publication/267768354
Bahnasawy, 2009	DOI: 10.3923/pjn.2009.674.678
Bake et al., 2014	DOI: 10.11648/j.aff.20140304.14
Belal et al., 2004	DOI: 10.1111/j.1749-7345.2004.tb00110.x
Belal et al., 2015	DOI: 10.12720/jomb.4.6.457-460
Belal, 1999	DOI: 10.1111/j.1749-7345.1999.tb00877.x
Belal, 1999a	DOI: 10.1046/j.1365-2109.1999.00323.x
Biswas & Takeuchi, 2003	DOI: 10.1046/j.1444-2906.2003.00720.x
Boonanuntanasarn et al., 2018	DOI: 10.1016/j.aquaculture.2018.07.011
Boonanuntanasarn et al., 2018a	DOI: 10.1016/j.aquaculture.2017.09.032
Cao et al., 2008	DOI: 10.1111/j.1365-2095.2007.00508.x
Chakraborty et al., 2011	DOI:10.4194/trjfas.2011.0117
Cheng et al., 2019	DOI: 10.46989/001c.20972
Cho & Jo, 2002	DOI: 10.1111/j.1749-7345.2002.tb00477.x

Chowdhuru, 2011	http://hdl.handle.net/11250/186022
Daudpota et al, 2016	https://www.researchgate.net/publication/288990411
Daudpota et al., 2014	https://www.researchgate.net/publication/276273338
Devic et al., 2017	DOI: 10.1111/anu.12573
Ebrahim et al., 2007	http://arabaqs.org/journal/vol_2/1/Text%2007%20-%2003.pdf
Eid et al., 2008	https://www.cabdirect.org/cabdirect/abstract/20133318679
El Sayed, 2003	DOI: 10.1016/S0044-8486(02)00252-1
El-Hakim et al., 2007	DOI: 10.21608/ejabf.2007.1937
El-Haroun et al., 2006	DOI: 10.1111/j.1365-2109.2006.01584.x
El-Kasheif et al., 2011	DOI: 10.21608/EJABF.2011.2082
El-Saidy & Gaber, 2003	DOI: doi.org/10.1046/j.1365-2109.2003.00914.x
El-Saidy & Gaber, 2005	Doi: 10.1111/j.1365-2109.2004.01201.xr2004
El-Saidy & Hussein, 2015	DOI: 10.5376/ija.2015.05.0003
El-Saidy et al., 2012	https://www.researchgate.net/profile/Deyab-El-Saidy/publication/268503324
El-Sayed et al., 1992	DOI: 10.1016/0044-8486(92)90278-S
El-Sayed et al., 1996	Doi: 10.1046/j.1365-2109.1996.00776.x
El-Sayed et al., 2006	https://cals.arizona.edu/azaqua/ista/ISTA7/Abstracts/Date_pits_ISTA7.doc
El-Sayed et al., 2013	DOI: 10.1111/anu.12031
El-Sayed, 1992	DOI: 10.1111/j.1365-2109.1992.tb00607.x
El-Sayed, 1998	DOI: 10.1046/j.1365-2109.1998.00199.x
El-Sayed, 2002	DOI: 10.1046/j.1365-2109.2002.00700.x
El-Shafai et al., 2004	DOI: 10.1111/j.1365-2109.2004.01055.x
Ergün et al., 2009	DOI: 10.1007/s10499-008-9207-5
Fasakin et al., 2001	DOI: 10.1300/J028v11n04_09
Fasakin et al., 2005	DOI: 10.1016/j.aquaculture.2005.02.059
Focken et al., 2000	https://www.researchgate.net/profile/Ulfert-Focken/publication/267790564
Furuya et al., 2005	https://www.researchgate.net/publication/262473959
Gaber & Hanafy, 2008	DOI: 10.1080/10454430802329721
Gaber et al., 2014	DOI: 10.4236/gep.2014.22010
Gaber, 2005	DOI: 10.1111/j.1749-7345.2005.tb00338.x
Gaber, 2006	DOI: 10.1111/j.1365-2109.2006.01517.x
Gaber, 2006a	DOI: 10.1111/j.1749-7345.2006.00008.x
Gan et al., 2016	DOI: 10.1111/jai.12997
Gao et al., 2015	DOI: 10.1007/s00343-015-4246-z
Gaye-siessegger et al., 2006	DOI: 10.1007/s10695-006-9000-1
Gaye-Siessegger et al., 2007	DOI: 10.1111/j.1095-8649.2007.01469.x
Goda et al., 2012	https://www.researchgate.net/publication/285160866
Güroy et al., 2007	https://journals.tubitak.gov.tr/veterinary/vol31/iss2/2
Hafedh, 1999	DOI: 10.1046/j.1365-2109.1999.00343.x
Hanley, 1991	DOI: 10.1016/0044-8486(91)90224-U
Hassaan et al., 2014	DOI: 10.1016/j.ejar.2014.04.001
Hussein et al., 2016	https://jades.journals.ekb.eg/article_67386_a8973faf8660e3cc11ded3448f9efd93.pdf

Jatta, 2013	https://www.grocentre.is/static/gro/publication/260/document/jatta13prf.pdf
Jiang et al., 2019	DOI: 10.1111/anu.12983
Kaya & Bilgüven, 2015	https://dergipark.org.tr/tr/download/article-file/154224
Khalil et al., 2012	https://www.researchgate.net/publication/258927420
Khalil et al., 2015	https://www.researchgate.net/publication/279513431
Leal et al., 2009	DOI: 10.1007/s10499-009-9284-0
Li et al., 2011	DOI: 10.1016/j.anifeedsci.2011.09.002
Madalla, 2008	http://hdl.handle.net/1893/795
Mambrini & Kaushik, 1994	DOI: 10.1016/0300-9629(94)90152-X
Michelato et al. 2013	DOI: 10.1590/S1516-35982013000100002
Michelato et al., 2016a	DOI: 10.1016/j.aquaculture.2016.06.038
Michelato et al., 2016b	DOI: 10.1016/j.aquaculture.2016.02.022
Mohammad et al., 2012	https://www.researchgate.net/publication/294697795
Mostafa et al., 2009	http://ajbasweb.com/old/ajbas/2009/1234-1245.pdf
Mugo-bundi et al., 2013	DOI: 10.1111/are.12181
Nobrega et al., 2016	DOI: 10.1016/j.aquaculture.2016.12.026
Ogunji & Wirth, 2002	DOI: 10.46989/001c.20316
Opiyo et al., 2019	DOI: 10.1016/j.sciaf.2019.e00103
Pan et al., 2003	DOI: 10.1016/S0044-8486(03)00306-5
Pechsiri & Yakupitiyage, 2005	DOI: 10.1111/j.1365-2109.2004.01182.x
Pereira et al., 2017	DOI: 10.1016/j.aquaculture.2017.01.033
Pouomogne et al., 1997	DOI: 10.1016/S0044-8486(97)00091-4
Reda et a., 2016	DOI: 10.1016/j.fsi.2016.01.040
Richter et al, 2003	DOI: 10.1016/S0044-8486(02)00497-0
Ritcher, 2019	https://tede2.uepg.br/jspui/handle/prefix/2877
Schamber et al., 2013	DOI: 10.1007/s10499-014-9776-4
Schneider et al., 2004	DOI: 10.1111/j.1365-2109.2004.01179.x
Schulz et al., 2007	DOI: 10.1111/j.1365-2109.2007.01699.x
Sena et al., 2012	DOI: 10.4025/actascianimsci.v34i3.1317
Shahkar et al., 2014	DOI: 10.1080/09712119.2014.928626
Shalaby et al., 2006	DOI: 10.1590/S1678-91992006000200003
Siddhuraju & Becker, 2003	DOI: 10.1046/j.1365-2109.2003.00836.x
Siddiqui et al., 1988	DOI: 10.1016/0044-8486(88)90007-5
Silva et al., 2014	DOI: 10.1007/s10811-014-0453-9
Silva et al., 2015	DOI: 10.1590/S1806-92902015000400001
Soltan et al., 2002	https://www.researchgate.net/publication/273023780
Soltan et al., 2009	https://dx.doi.org/10.3923/pjn.2009.395.407
Subandiyono & Hasturi, 2020	http://www.bioflux.com.ro/docs/2020.2468-2476.pdf
Telli et al., 2014	DOI: 10.1016/j.fsi.2014.05.025
Tian et al., 2013	DOI: 10.1016/j.aquaculture.2013.01.032
Tian et al., 2014	DOI: 10.1007/s10695-014-0001-1
Tian et al.,2017	DOI: 10.1017/S0007114517003063
Tidwell et al., 2000	DOI: 10.1111/j.1749-7345.2000.tb00912.x
Tidwell et al., 2007	DOI: 10.1111/j.1749-7345.2000.tb00912.x
Trosvik et al., 2012	DOI: 10.1111/j.1749-7345.2012.00595.x

Trosvik et al., 2013	DOI: 10.1080/01448765.2013.810123
Urbich, 2020	https://tede2.uepg.br/jspui/handle/prefix/3040
Valente et al., 2015	DOI: 10.1007/s10811-015-0590-9
Vechklang et al., 2012	DOI: 10.1080/10454438.2012.678786
Wee & Ng, 1986	DOI: 10.1111/j.1365-2109.1986.tb00094.x
Wu et al., 2015	DOI: 10.1007/s10499-014-9877-0
Xie et al., 1997	DOI: 10.1016/S0044-8486(97)00142-7
Ye et al., 2016	DOI: 10.1111/jai.13004
Yigit & Olmez, 2011	DOI: 10.1111/j.1365-2095.2010.00789.x
Younis et al., 2017	DOI: 10.1016/j.sjbs.2017.06.012
Yousef et al., 2014	DOI: 10.21608/jalexu.2014.160513

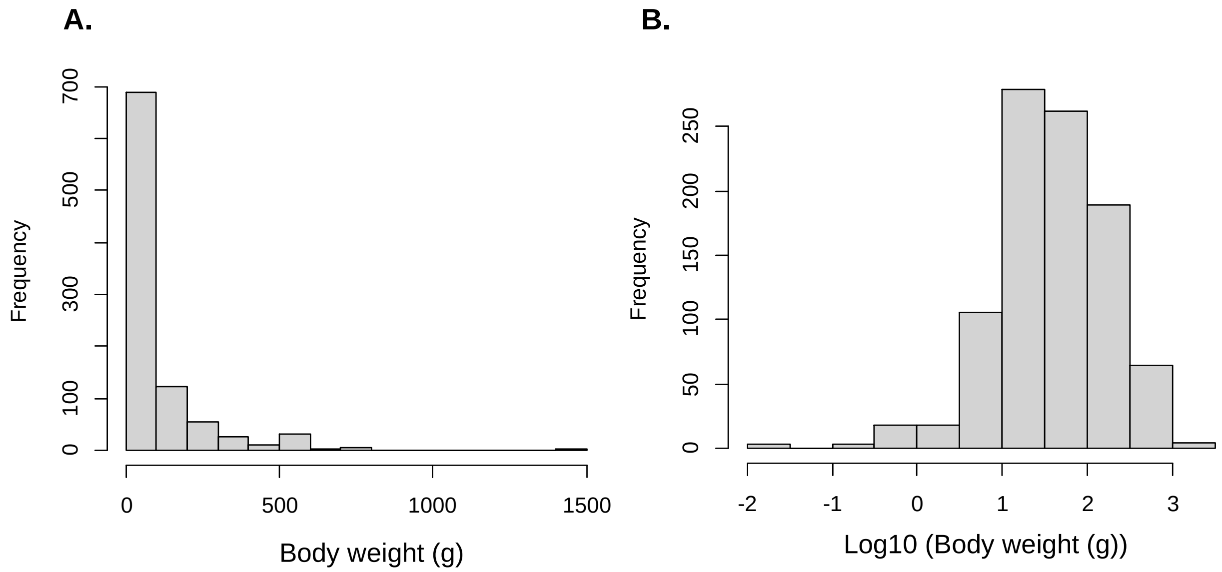


Figure A.1 - A) Nile tilapia (O. niloticus) body weight histogram in absolute scale (g); B) Nile tilapia body weight histogram in log-scale.

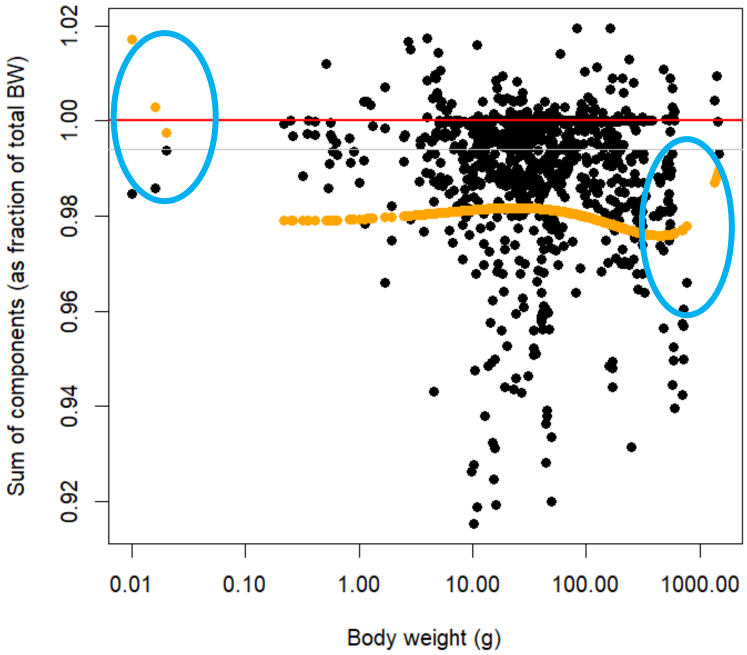


Figure A.2 - Behavior of the lcub model. Measured (black) and predicted (orange) sum of body components (as fraction of total body weight) of Nile tilapia (O. niloticus) in relation to the body weight in log-scale; median measured value for the sum in grey and the limit of 100% in red. Blue circles indicate the problematic areas.

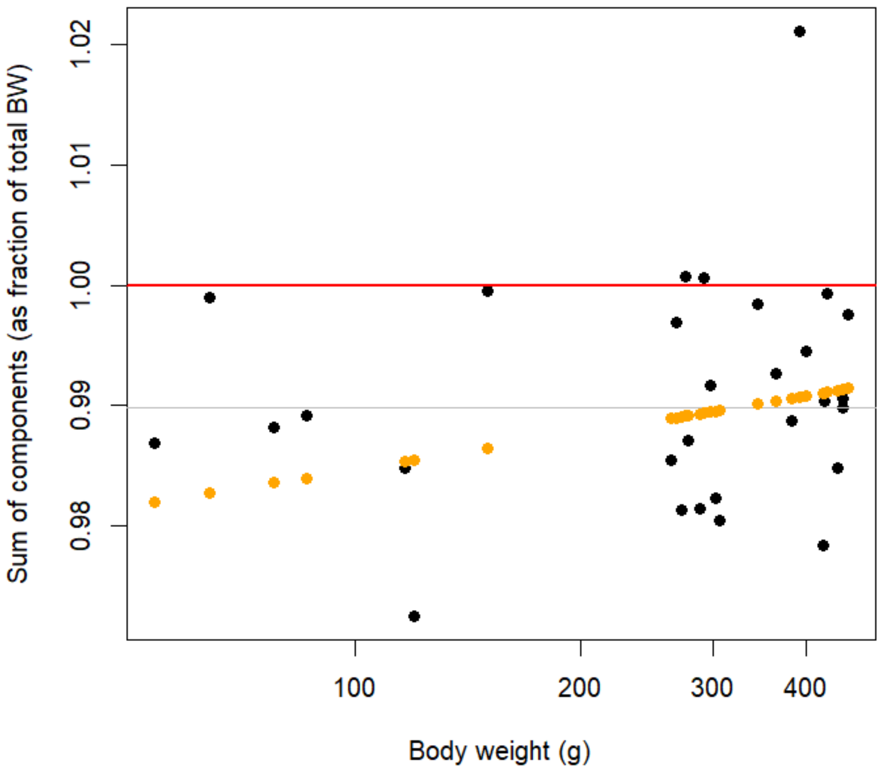


Figure. B.1 - Sum of individual component predictions of the Chow_bureau model (which uses isometric models to predict water and protein, and allometric models to predict lipids and ash). Independent data (black) and predicted (orange) sum of body components (as fraction of total body weight) of Nile tilapia (O. niloticus) in relation to the body weight in log-scale; median measured value for the sum in grey.

Chapter 3

ficoEst, a tool to estimate the body composition of farmed fish

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ficoEst – a tool to estimate the body composition of farmed fish

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Abstract

ficoEst – Fish Composition Estimator is a public web tool to estimate the whole-body proximate composition of farmed fish (https://webtools.sparos.pt/ficoest/). The tool was designed for researchers in fish nutrition and fish farmers, and is available for six commercially relevant species: gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*), meagre (*Argyrosomus regius*), rainbow trout (*Onchorhynchus mykiss*), Atlantic salmon (*Salmo salar*), and Nile tilapia (*Oreochromis niloticus*). ficoEst uses three different types of mathematical models (BC1, BC2, and BC3) to estimate the body composition of fish in terms of crude protein, crude lipids, water, ash, phosphorus, and energy. The models differ in the input data used to perform the estimates. BC1 models consider only body weight, BC2 models consider both body weight and water, and BC3 models consider body weight, water, and ash as inputs. The model evaluation results demonstrate that considering water and ash as additional inputs to body weight (BC3 models) significantly improves the accuracy in predicting some body composition components, such as crude lipids (e.g., up to 67.9% and 28.1% more accurate, compared to BC1 and BC2 models, respectively, depending on the species considered). ficoEst can be used as a complementary tool to analytical methods to obtain additional information about fish body composition. As a public web tool, ficoEst has the potential to be a valuable resource for researchers and fish farmers interested in estimating the body composition of farmed fish.

Key-words: Fish farming; Aquaculture; Body composition; Mathematical modelling.

1. Introduction

Fish whole-body proximate composition is an important aspect commonly measured in fish nutrition studies and in commercial fish farming activities. Research in fish nutrition often relies on body composition measurements as a way to calculate nutrient retention, namely to estimate nutrient requirements (Caceres-Martinez et al., 1984; Lupatsch et al., 1998; Tulli et al., 2010; Van Trung et al., 2011), evaluate the nutritional value of ingredients and aquafeeds (Fournier et al., 2003; Encarnação et al., 2006;

Glencross et al., 2008; Dias et al., 2009), and quantify nutrient waste outputs (Cho et al., 1994; Azevedo et al., 1998; Dias et al., 2005). In addition, fish farming companies usually measure the body composition of fish during product quality assessment activities, since it is related to several nutritional and organoleptic characteristics (Freitas et al., 2020).

Typically, fish body composition is estimated through analytical methods, such as the ones described by the Association of Official Analytical Chemists (AOAC, 2005). Despite being reliable and robust, analytical methods are not always a viable option to estimate the body composition of fish, since they are time-consuming and expensive. This can translate into a limitation of the number of samples collected for body composition analysis, which can hinder a detailed analysis of nutrient flux in fish and more regular assessments of product quality and environmental impact.

The relationships between the different body composition components, and between them and body weight, are well described in the literature (Shearer et al., 1994; Lupatsch et al., 1998, 2001, 2003b; Dumas et al., 2007; Chowdhury et al., 2013; Breck, 2014; Raposo et al., 2023). As a result, several researchers have proposed mathematical models as practical tools to estimate body composition in fish (Yeannes and Almandos, 2003; Dumas et al., 2010; Chowdhury et al., 2013; Breck, 2014; Raposo et al., 2023). However, most of the models developed so far are only made available as mathematical notation in published material. To the best of our knowledge, there is no publicly available tool at this time, based on mathematical models, that can be used to estimate the proximate composition of farmed fish. In our view, implementing such a tool and making it freely available to the public could be of great interest and utility for various agents that work in research and industrial environments within the fish farming sector.

Here we present ficoEst – Fish Composition Estimator, a public web tool directed to researchers and fish farmers, developed and implemented to be used as a simple method of estimating the body composition of farmed fish (https://webtools.sparos.pt/ficoest/). This tool uses mathematical models to provide estimates on the body composition of different fish species, i.e., gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*), meagre (*Argyrosomus regius*), rainbow trout (*Onchorhynchus mykiss*), Atlantic salmon (*Salmo salar*) and Nile tilapia (*Oreochromis niloticus*). All models behind ficoEst were calibrated and validated at the species level using datasets mostly collected from the scientific literature. ficoEst was developed to serve as a complementary tool, supporting studies on fish nutrition and increasing the information gathered at the farm level when analytical methods may not be feasible.

2. Materials and methods

The entire process of making ficoEst available consisted of four main steps, briefly described in the diagram in Figure 1 and in detail in the following subsections.

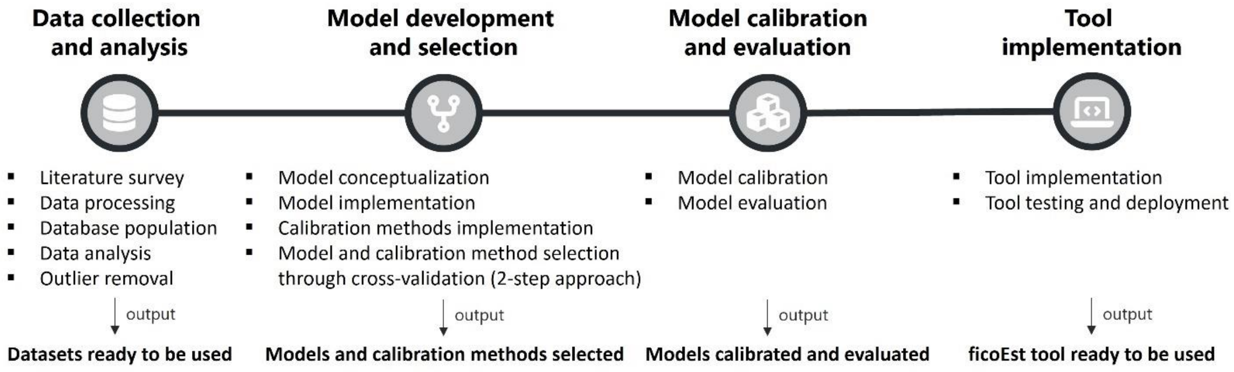


Figure 1. Diagram illustrating the four main steps behind ficoEst development.

2.1. Data collection and analysis

Datasets on the whole-body proximate composition of gilthead seabream, European seabass, meagre, rainbow trout, Atlantic salmon and Nile tilapia were collected from the scientific literature and from trials carried out by Sparos Lda., and its partners, in R&D projects. All datasets were stored in a database, following ‘tidy data’ principles (Wickham, 2014), and processed whenever necessary to ensure standardization and uniformity of data.

Figure 2 shows a diagram illustrating the general structure of the database, which includes, in addition to whole-body proximate composition data, other relevant information related to the context in which the fish were reared and the data generated. Table 1 includes a summary of the datasets included in the database, per species.

After the data collection and processing phase, data were visualized and analysed at the species level, in the R software (R Core Team, 2023). During this process, outliers were removed after sum of components analysis and the main relationships between body weight, body composition components and other variables (e.g., feed composition) were characterized. The outcomes of this process were crucial in providing a solid foundation and guidance for the model development phase.

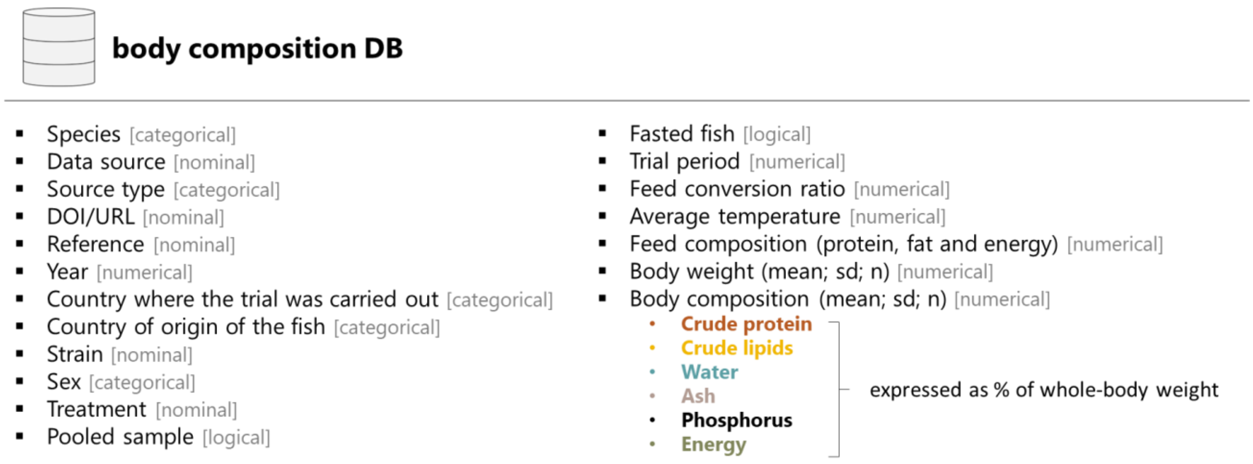


Figure 2. Diagram describing the main variables included in the body composition database (DB).

Table 1. Summary of the datasets included in the body composition database, per species.

Attributes	Units	Gilthead seabream	European Seabass	Meagre	Rainbow trout	Atlantic salmon	Nile tilapia
Data points	-	442	161	152	331	368	392
Data sources	-	69	30	37	49	51	97
Year range	-	1977 – 2019	1983 – 2020	2010 – 2022	1972 – 2019	1974 – 2020	1986 – 2020
Countries	-	11	8	6	11	8	23
Body weight range	g	0.1 – 582.1	4.7 – 482.0	0.2 – 1280.8	1.6 – 3251.3	0.2 – 4950.0	0.1 – 1302.2
Temperature range	°C	17.5 – 27.0	13.4 – 28.8	17.0 – 34.0	6.0 – 19.4	2.0 – 23.0	17.0 – 32.0

2.2. Model development and selection

Different types of models and calibration methods were developed and tested, aiming to select the best methods in estimating the body composition of fish. All developed models fall into one of the following three families, differing from each other in terms of the type of input data considered (all expressed in a wet basis):

(i) BC1: models that consider only the body weight of fish as input;

(ii) BC2: models that consider the body weight and water percentage of fish as inputs;

(iii) BC3: models that consider the body weight, water and ash percentage of fish as inputs.

Differences between models belonging to the same family are essentially related to approaches used to estimate the body composition components. Some models estimate a specific component using isometric or allometric functions, or a mix of both by using ensemble averaging. Other models estimate a specific component simply by difference, assuming that the sum of crude protein, crude lipids, water and ash comprise the whole-body composition of fish (i.e., should add up to about 100% when expressed as a percentage). To estimate the energy content a single method was used for all models, where energy is calculated based on protein and lipids content using the energetic coefficients estimated by Lupatsch et al., 2003a ($protein_{energy} = 23.1$ kJ/g and $lipids_{energy} = 35.8$ kJ/g). Carbohydrate contents in fish body normally represent less than 0.14% (Breck, 2014), and were thereby considered negligible. An overview of all developed models is available as Supplementary Material.

For each model, different calibration methods were implemented in the R software based on various regression techniques, such as least squares (*lm* function from the 'stats' package; R Core Team, 2023), Huber loss minimization (*rlm* function from the 'MASS' package; Venables and Ripley, 2002), mixed-effects (*lme* function from the 'nlme' package; Pinheiro and Bates, 2000), and seemingly unrelated regressions (*systemfit* function from the 'systemfit' package; Henningsen and Hamann, 2007).

The model and the respective calibration method to use per family (i.e., BC1, BC2 and BC3) were selected through cross-validation (5-fold with 2 repetitions) following a two-step approach (Figure 3). In the first step, all models within a family were compared to each other, when calibrated using the two simplest calibration methods (i.e., least squares and Huber loss minimization). In the second step, all calibration methods related to the models selected in the previous step were compared to each other, aiming to select the most appropriate one. In both steps, several error metrics were used in cross-validation to assess model performance (e.g., mean absolute percentage error, absolute error, root mean squared error, mean squared error, mean percentage error), but decisions were taken primarily based on the mean absolute percentage error (MAPE; equation 1).

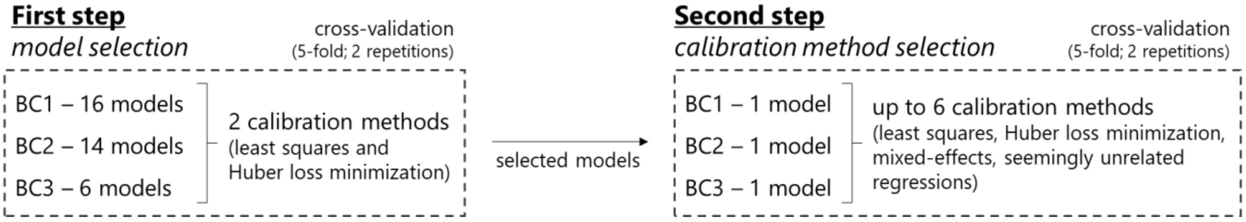


Figure 3. Diagram illustrating the two-step approach followed to select the model and calibration method per family.

2.3. Model calibration and evaluation

Model calibration and evaluation were performed using the dataset available for each species (without outliers). For each model family, calibration was done using the previously selected model and calibration method combination. Model evaluation was focused on providing an estimate of the accuracy in predicting each body composition component, calculated here as the mean absolute percentage error (MAPE; equation 1):

$$MAPE\ (\%) = \frac{100}{n}\sum_{i=1}^{n}\left|\frac{P_i - O_i}{O_i}\right| \tag{1}$$

where n is the number of predicted-observed value pairs, P_i is the predicted value, and O_i is the observed value. This metric provides an indication of the average deviation models are expected to have for each body composition component. Nonetheless, it should be noted that part of the error associated to this metric (and others) is not only directly related to the model itself, but inevitably contains part of the error associated with the data. Most of the empirical datasets used to calibrate and evaluate the models come from published studies, where only the group-level mean and standard deviation (sd) values are presented. This means that the data contains inherent variation from multiple sources (e.g., technical methods, biological replicates), often aggregated and presented in scientific publications as a single measure (e.g., standard deviation or standard error). Here, the variation associated with observed data was estimated as the average coefficient of variation, using the following formula ($PE_{observations}$, percentage error of observations):

$$PE_{observations}\ (\%) = \frac{100}{n}\sum_{i=1}^{n}\left(\frac{sd\_value_i}{mean\_value_i}\right) \tag{2}$$

where n is the number of mean-sd value pairs, $mean\_value_i$ is the observed mean value of a body composition component, and sd_value_i is the observed standard deviation value of a body composition component. This metric can be interpreted as the uncertainty of the observed values and used to relativize the model evaluation errors, since it is expressed in the same basis (i.e., in percentage).

2.4. Tool implementation

All modules that comprise the ficoEst tool were implemented in R. The back-end engine consists of different functions used to: compile, process, and plot data; calibrate, evaluate and run models; and generate reports. The front-end (i.e., user interface) consists of functions used to create input controls, informational, and navigational elements.

The following R packages were used in the back-end: ‘readxl’ (Wickham and Bryan, 2023), ‘openxlsx’ (Schauberger and Walker, 2023), ‘dplyr’ (Wickham et al., 2023), ‘ggplot2’ (Wickham, 2016), ‘patchwork’ (Pedersen, 2022), ‘GGally’ (Schloerke et al., 2021), ‘officer’ (Gohel, 2023), ‘stringr’ (Wickham, 2022), ‘yardstick’ (Kuhn et al., 2022), ‘MASS’ (Venables and Ripley, 2002), ‘nlme’ (Pinheiro and Bates, 2000), and ‘systemfit’ (Henningsen and Hamann, 2007); and the following ones in the front-end: ‘shiny’ (Chang et al., 2022), ‘shinyjs’ (Attali, 2021), ‘shinyBS’ (Bailey, 2022), ‘shinydashboard’ (Chang and Borges, 2021), ‘shinydashboardPlus’ (Granjon, 2021), ‘shinybusy’ (Meyer and Perrier, 2022), ‘shinymanager’ (Thieurmel and Perrier, 2022), and ‘DT’ (Xie et al., 2023).

3. Results and discussion

3.1. Data analysis

The subsections below present just a small snapshot of the data analysis performed, with particular focus on the sum of components and the relationships between body weight and body composition components. For more details on the analysis carried out per species, see the data analysis reports available in the Supplementary Material.

3.2. Sum of body composition components

Body composition data were initially evaluated in terms of the sum of the main components that make up the fish (i.e., crude protein, crude lipids, water and ash). These four components, when expressed as a percentage, should add up to about 100%. Small deviations may occur as the data inevitably have variation associated with them and, in addition, fish is also made up of carbohydrates (not considered in this work due to the scarcity of data collected for this component and the fact that it typically constitutes less than 0.14% of the whole-body composition of fish; Breck, 2014). However, data points that show large deviations in the sum of components from 100% should be considered unreliable, as it is a sign of having one or more components misestimated. To objectively define a range based on which to consider the data points as unreliable, the average variation of each component (i.e., mean standard deviation) was chosen. In the data we collected per species, the sum of the mean standard deviations of body composition components is around 2.50 percentage units, with the exception of salmon which presents lower values (seabream: 2.39%; seabass: 2.43%; meagre: 2.78%;

trout: 2.44%; salmon: 1.43%; tilapia: 2.91%; see the data analysis reports available in the Supplementary Material). In this sense, all data points where the sum of crude protein, crude lipids, water and ash was outside the 100.00 ± 2.50% range were considered as outliers and removed. At the species level, this accounted for the following percentages of data points removed: 7% for gilthead seabream, 18% for European seabass, 15% for meagre, 3% for rainbow trout, 5% for Atlantic salmon, and 14% for Nile tilapia.

The data were not normalized to add up to 100%, although some of the raw data collected were already in a normalized form and, therefore, were kept as they were. The fact that the components do not add up to exactly 100% can be caused by poor estimation of a single component. In such cases, normalizing the data will inevitably propagate the error to other components, which is undesirable for model calibration and evaluation purposes.

3.3. Relationships between body weight and body composition components

The relationships between body weight and body composition components are something that has already been described in the literature for almost all fish species considered in this work (with the exception of meagre), e.g., gilthead seabream (Lupatsch et al., 1998, 2003b), European seabass (Lupatsch et al., 2001), rainbow trout (Dumas et al., 2007; Breck, 2014), Atlantic salmon (Shearer et al., 1994), and Nile tilapia (Chowdhury et al., 2013; Raposo et al., 2023). Hence, this section does not bring any new findings in relation to what has already been presented in other works. The main objective here is to provide a consolidated view of how the whole-body proximate composition of the target fish species varies across the body weight range, what are the strongest correlations that can be found in data, and the rationale behind the developed models.

Figure 4 shows the variation in body composition (expressed as % of body weight) across the body weight range, where common patterns can be found among species. In general, body composition varies more markedly in smaller fish, tending to stabilize or changing at lower rates as body weight increases. When expressed as a percentage, water decreases, crude protein and lipids increase (at different rates though), and ash does not significantly change with increasing body weight. All species show similar magnitude of change in crude protein content across the body weight range, with greater rates of change in the lower body weight range. However, the same does not occur with crude lipids and water. Despite similar trends shared across species for these two components (i.e., crude lipids increase and water decreases as body weight increases), the magnitude of such changes is not the same among species. The magnitude of change in crude lipids and water as body weight increases is much greater in gilthead seabream, European seabass, rainbow trout and Atlantic salmon than in meagre and Nile tilapia.

All of the above suggests that, for each model family, a model with a common structure can be used to describe the body composition of the target fish species (since the pattern of change of each component, across the body weight range, is similar between species), but it needs to be calibrated specifically for each species (since the magnitude of change of some components, across the body weight range, is different between species).

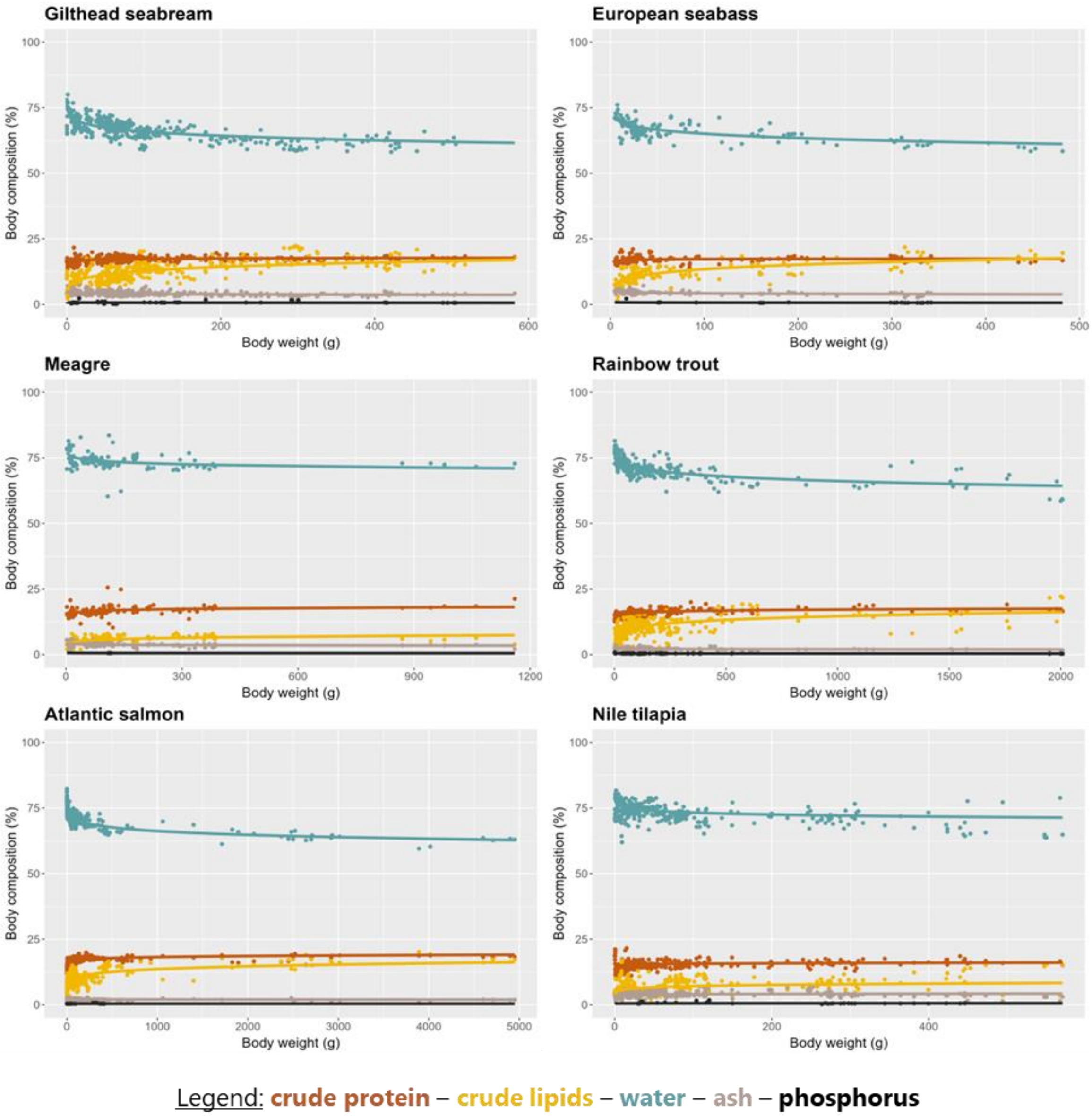


Figure 4. Scatter and line plots showing the relationships between body composition components (i.e., crude protein, crude lipids, water, ash and phosphorus), expressed as percentage, across the body weight range, for each species.

In addition, Figure 5 shows the variation in body composition (expressed as % of lipid-free mass) across the lipid-free mass range (displayed in $\log_{10}$ scale). For most species, the variation of crude lipids as a fraction of lipid-free mass is higher compared to the other components (with the exception of meagre). The higher variation in crude lipids is essentially related to the fact that most lipids are an energy-storage component and, therefore, prone to the fluctuations that arise from the balance of energy inputs (that come from feeding) and outputs (essentially from metabolism and activity), which are generally dependent on the context in which the fish are reared. As protein, water and ash are mostly related to structural functions, this means that these components are constrained to each other and, therefore, show less variation when expressed on a lipid-free mass basis. For a more complete description and discussion about these relationships, see for example the work of Breck (2014).

Correlation matrices were generated for each species, to assess the correlation coefficients between body composition components and between these and body weight. Table 2 shows the strongest correlations found in data (log-transformed), when expressed as absolute values (i.e., in grams or kJ). For other correlations see the data analysis reports available in the Supplementary Material.

Table 2. Strongest correlation coefficients found in data (log-transformed), when expressed as absolute values (i.e., in grams or kJ), for each species.

Pairs	Gilthead seabream	European seabass	Meagre	Rainbow trout	Atlantic salmon	Nile tilapia
log(body weight) – log(crude protein)	0.999	0.998	0.997	0.998	0.999	0.999
log(body weight) – log(water)	0.999	0.999	0.999	1.000	1.000	0.999
log(crude protein) – log(energy)	0.994	0.996	0.994	0.991	0.990	0.992
log(crude lipids) – log(energy)	0.993	0.997	0.989	0.994	0.988	0.983

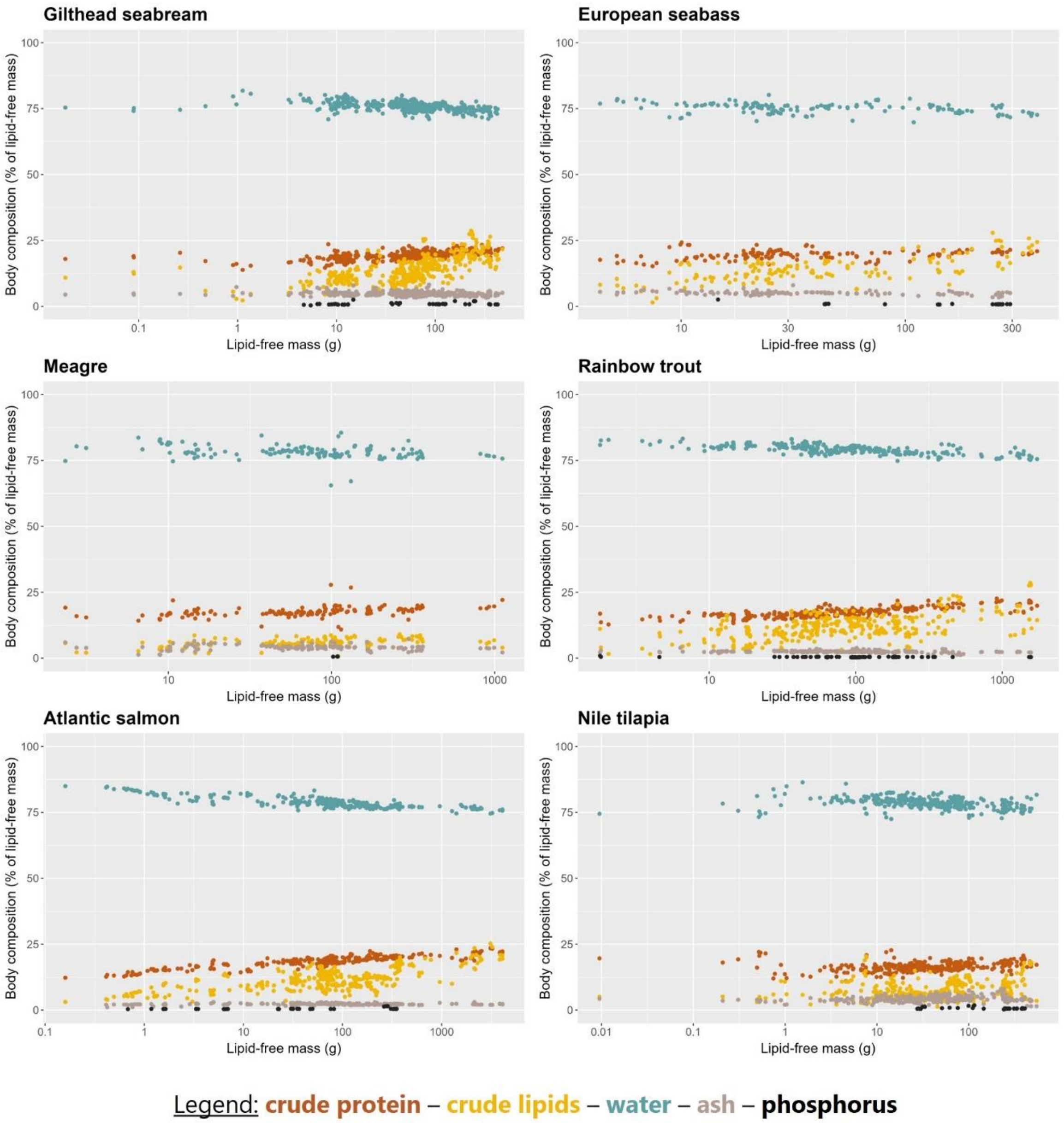


Figure 5. Scatter plots showing the relationships between body composition components (i.e., crude protein, crude lipids, water, ash and phosphorus), expressed as percentage of lipid-free mass, across the lipid-free mass range (displayed in log10 scale), for each species.

Strong correlations have been found between body weight and crude protein, as well as between body weight and water. This suggests that these two components of body composition can be better predicted based on body weight. In contrast, the remaining components, namely crude lipids and ash, have lower correlation coefficients and a wider variation across the body weight range. Therefore, estimating these components solely as a function of body weight may not guarantee accurate estimates in all cases. To improve estimates, it is recommended to consider water and ash content as model inputs, in addition to body weight. By doing so, the variation inherent to ash that cannot be explained by body weight can be eliminated, and the estimates for other components can be improved. Additionally, using water and ash fractions as inputs allows for the estimation of crude protein content based on body weight, while crude lipids can be estimated by difference (i.e., by subtracting the other components from 100%), which reduces the inherent high variation associated with this component (see Figure 5 which illustrates the high variation of crude lipids, compared to the other components, when expressed as % of lipid-free mass). These recommendations are the basis for the BC2 and BC3 models, which use water and ash content as inputs. Since these two components are relatively easy and cheap to estimate using analytical methods, this approach stands out as a practical solution for estimating the body composition of fish.

The expected strong correlations found between crude protein and energy, as well as between crude lipids and energy, reinforce the use of energetic coefficients applied to crude protein and crude lipids as a viable option to estimate the energy content.

3.4. Selected models and calibration methods

The overall results of the two-step cross-validation process indicate that, from the list of developed models and calibration methods, the best combinations per model family are the following: (i) for the BC1 family, the best model is the BC1.10 when calibrated with the Huber loss minimization method; (ii) for the BC2 family, the best model is the BC2.14 when calibrated with the Huber loss minimization method; and (iii) for the BC3 family, the best model is the BC3.6 when calibrated also with the Huber loss minimization method. Although there was not a full consensus from cross-validation results (i.e., the best combination in terms of model and calibration method differed between some species), we were able to select a combination of model and calibration method, per model family, that ensures performance that practically matches the best solution across all species. These results can be explored in more detail in the cross-validation reports available in the Supplementary Material. Table 3 presents a simplified description of the selected models.

Table 3. Simplified description of the selected models per family, i.e., BC1.10, BC2.14, and BC3.6.

Model and inputs	Description
BC1.10 Inputs: body weight (g)	Absolute values $crude\ protein\ (g) = protein_{scale} \times body\ weight\ (g)^{protein\_exp}$ $crude\ lipids\ (g) = lipids_{scale} \times body\ weight\ (g)^{lipids\_exp}$ $water\ (g) = water_{scale} \times body\ weight\ (g)^{water\_exp}$ $ash\ (g) = ash_{scale} \times body\ weight\ (g)^{ash\_exp}$ $phosphorus\ (g) = phosphorus_{scale} \times ash\ (g)$ $energy\ (kJ) = protein_{energy} \times crude\ protein\ (g) +\ lipids_{energy} \times crude\ lipids\ (g)$ Relative values $crude\ protein\ (\%) = (crude\ protein\ (g))/(body\ weight\ (g)) \times 100$ $crude\ lipids\ (\%) = (crude\ lipids\ (g))/(body\ weight\ (g)) \times 100$ $water\ (\%) = (water\ (g))/(body\ weight\ (g)) \times 100$ $ash\ (\%) = (ash\ (g))/(body\ weight\ (g)) \times 100$ $phosphorus\ (\%) = (phosphorus\ (g))/(body\ weight\ (g)) \times 100$ $energy\ (kJ/g) = (energy\ (kJ))/(body\ weight\ (g))$
BC2.14 Inputs: body weight (g) water (%)	Absolute values $water\ (g) = water\ input\ (\%) \times body\ weight\ (g)$ $ash\ (g) = ash_{scale} \times body\ weight\ (g)^{ash\_exp}$ $rest\ (g) = body\ weight\ (g) - (water\ (g)\ + ash\ (g))$ $lpratio = e^{(lpratio_{int} + (lpratio_{water_{lin}} \times \log(water_{frac})) + \left(lpratio_{water_{qua}} \times \log(water_{frac})^2\right))}$ $crude\ protein\ 1\ (g) = rest\ (g) - (1/(1 + lpratio))$ $crude\ protein\ 2\ (g) = protein_{scale} \times body\ weight\ (g)^{protein\_exp}$ $crude\ protein\ (g) = mean(crude\ protein\ 1\ (g), crude\ protein\ 2\ (g))$ $crude\ lipids\ (g) = body\ weight\ (g) - (water\ (g) + ash\ (g) + crude\ protein\ (g))$ $phosphorus\ (g) = phosphorus_{scale} \times ash\ (g)$ $energy\ (kJ) = protein_{energy} \times crude\ protein\ (g) +\ lipids_{energy} \times crude\ lipids\ (g)$ Relative values $crude\ protein\ (\%) = (crude\ protein\ (g))/(body\ weight\ (g)) \times 100$ $crude\ lipids\ (\%) = (crude\ lipids\ (g))/(body\ weight\ (g)) \times 100$ $water\ (\%) = water\ input\ (\%)$ $ash\ (\%) = (ash\ (g))/(body\ weight\ (g)) \times 100$ $phosphorus\ (\%) = (phosphorus\ (g))/(body\ weight\ (g)) \times 100$ $energy\ (kJ/g) = (energy\ (kJ))/(body\ weight\ (g))$
BC3.6 Inputs: body weight (g) water (%) ash (%)	Absolute values $water\ (g) = water\ input\ (\%) \times body\ weight\ (g)$ $ash\ (g) = ash\ input\ (\%) \times body\ weight\ (g)$ $rest\ (g) = body\ weight\ (g) - (water\ (g)\ + ash\ (g))$ $lpratio = e^{(lpratio_{int} + (lpratio_{water_{lin}} \times \log(water_{frac})) + \left(lpratio_{water_{qua}} \times \log(water_{frac})^2\right))}$ $crude\ protein\ 1\ (g) = rest\ (g) - (1/(1 + lpratio))$ $crude\ protein\ 2\ (g) = protein_{scale} \times body\ weight\ (g)^{protein\_exp}$ $crude\ protein\ (g) = mean(crude\ protein\ 1\ (g), crude\ protein\ 2\ (g))$ $crude\ lipids\ (g) = body\ weight\ (g) - (water\ (g) + ash\ (g) + crude\ protein\ (g))$ $phosphorus\ (g) = phosphorus_{scale} \times ash\ (g)$ $energy\ (kJ) = protein_{energy} \times crude\ protein\ (g) +\ lipids_{energy} \times crude\ lipids\ (g)$ Relative values $crude\ protein\ (\%) = (crude\ protein\ (g))/(body\ weight\ (g)) \times 100$ $crude\ lipids\ (\%) = (crude\ lipids\ (g))/(body\ weight\ (g)) \times 100$ $water\ (\%) = water\ input\ (\%)$ $ash\ (\%) = ash\ input\ (\%)$ $phosphorus\ (\%) = (phosphorus\ (g))/(body\ weight\ (g)) \times 100$ $energy\ (kJ/g) = (energy\ (kJ))/(body\ weight\ (g))$

3.5. Model calibration and evaluation

All models selected in the previous step were calibrated for each species with the selected calibration method (i.e., Huber loss minimization). Subsequently, the models were validated using the same datasets used for calibration purposes. The model evaluation results for BC1.10, BC2.14 and BC3.6 are presented in Figures 6 to 8, respectively.

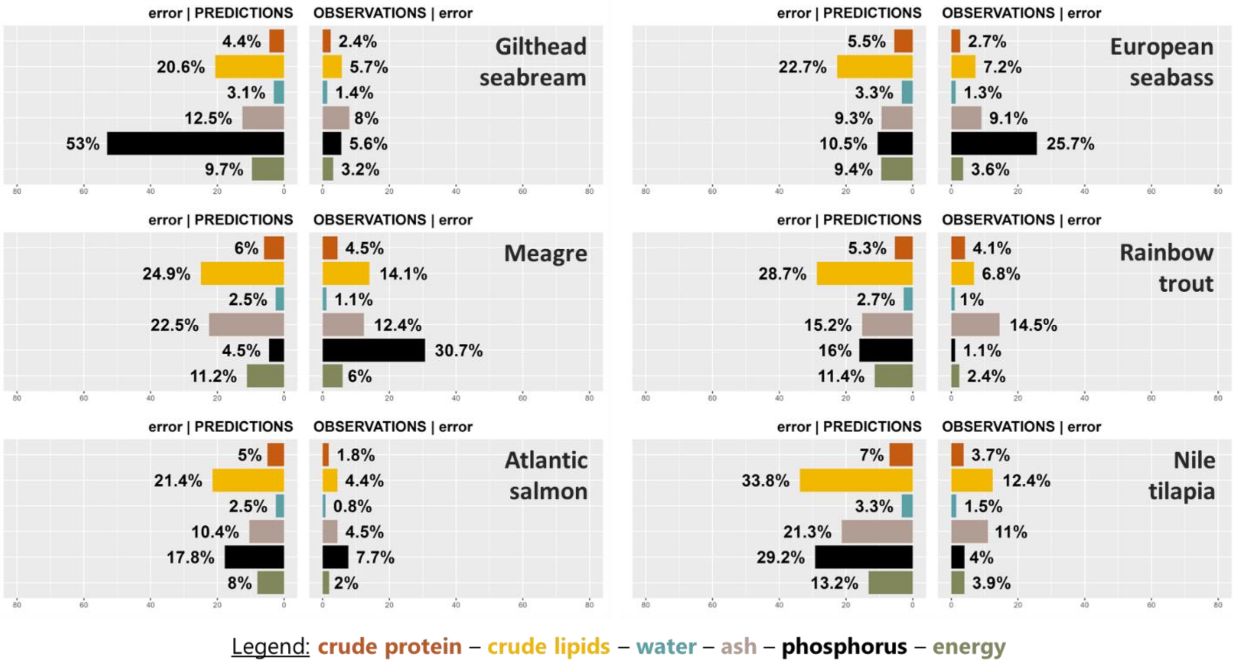


Figure 6. BC1.10 model evaluation plots, for each species. Inside each plot, the bars on the left represent the error associated with the model predictions, given by the mean absolute percentage error (MAPE), and the bars on the right the error associated with the data used to validate the model, given by the percentage error of observations (PE$_{observations}$).

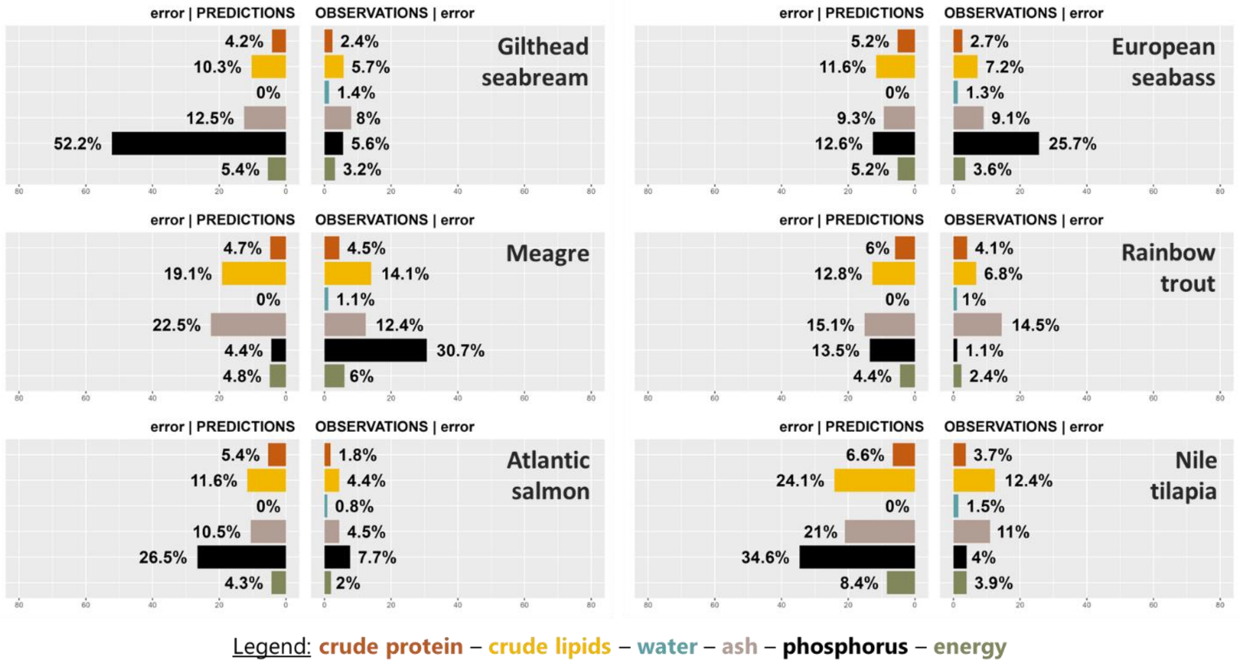


Figure 7. BC2.14 model evaluation plots, for each species. Inside each plot, the bars on the left represent the error associated with the model predictions, given by the mean absolute percentage error (MAPE), and the bars on the right the error associated with the data used to validate the model, given by the percentage error of observations ($PE_{observations}$). For this model, the error associated with water is defined as 0%, since this component is provided as input.

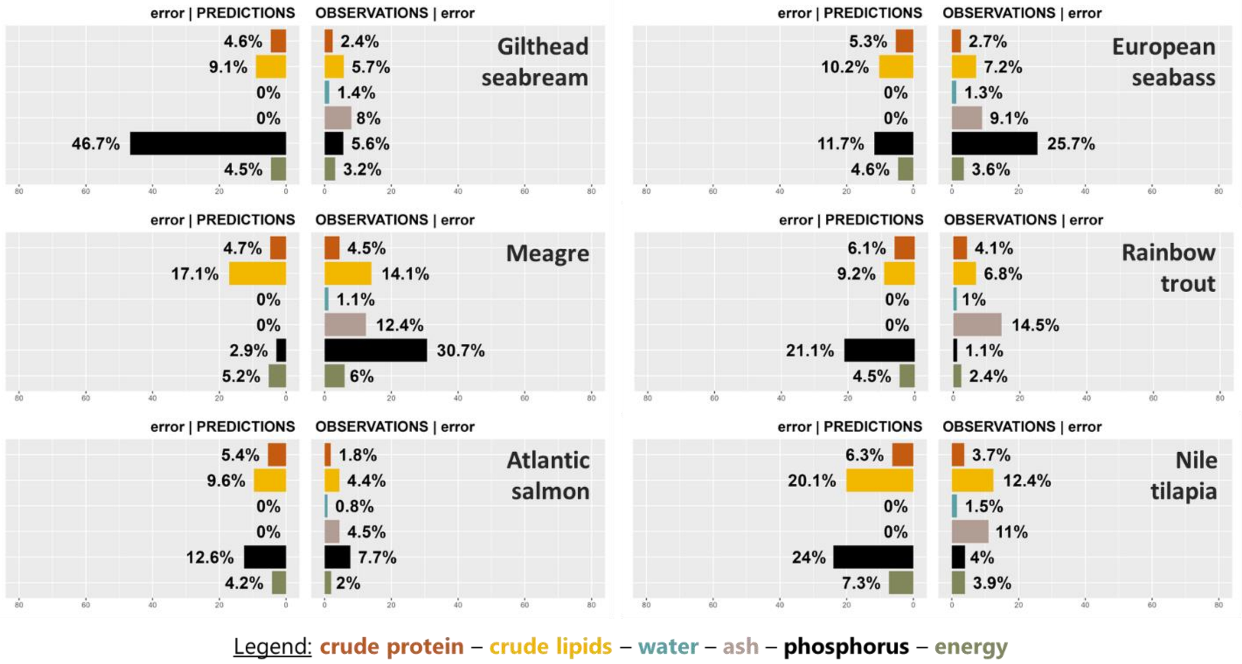


Figure 8. BC3.6 model evaluation plots, for each species. Inside each plot, the bars on the left represent the error associated with the model predictions, given by the mean absolute percentage error (MAPE), and the bars on the right the error associated with the data used to validate the model, given by the percentage error of observations ($PE_{observation}$s). For this model, the errors associated with water and ash are defined as 0%, since these components are provided as inputs.

In general, for all models and species, the largest prediction errors found are related to crude lipids (20.6 – 33.8% for BC1.10; 10.3 – 34.1% for BC2.14; 9.1 – 20.1% for BC3.6). The BC1.10 and BC2.14 models also show large prediction errors for ash when compared to other components (e.g., crude protein and energy), although generally lower than the error found for crude lipids (at least for the BC1.10 model). The large prediction errors found for phosphorus in some species (i.e., gilthead seabream, rainbow trout and Nile tilapia) seems to be related to the high variability of values reported in the different data sources considered and also to the relatively small number of data points collected for this component. For all species, considering water and ash as additional inputs to body weight (e.g., BC2.14 and BC3.6) improves the crude lipids estimates, but does not guarantee improvements for other body composition components, such as crude protein and energy.

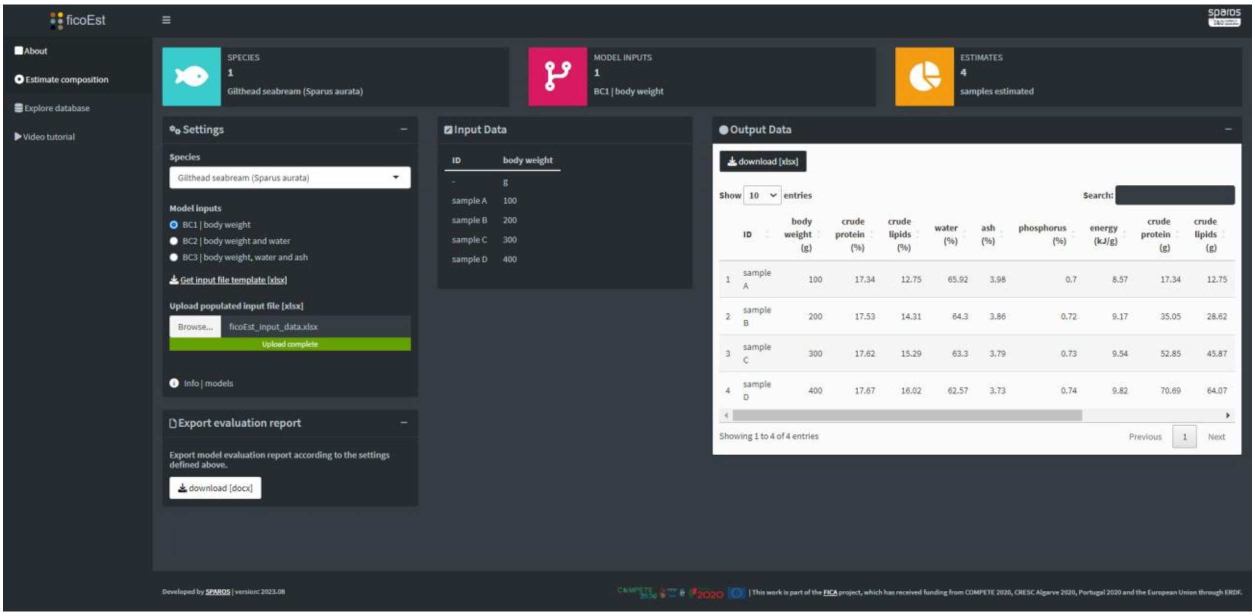
The error in the data used to calibrate and evaluate the models, specifically the observation error ($PE_{observations}$), is generally lower for water, crude protein, and energy, but higher for crude lipids, ash, and phosphorus, in all species. It is important to note that model evaluation errors, in addition to including the error due to an incomplete fit of the model to the data, are also compounded in part by inherent variation in observational data. Therefore, the observation error can be used as a rough estimate of the variation associated with observational data and considered to relativize the model errors. Model evaluation errors close to observation errors indicate that, on average, the values predicted by the model are within the variation in the observational data. For example, in the case of meagre, the BC3.6 model evaluation error for crude protein (4.7%) is close to observation error (4.5%), which means that, on average, the model error is similar to the variation in the observational data used to evaluate the model.

3.6. Tool overview

ficoEst is freely available as a public web tool (https://webtools.sparos.pt/ficoest/). Its user interface is reactive and currently consists of four different pages (version 2023.08):

1. *About*, where users can access general information about the tool;
2. *Estimate composition*, where users can select the species and model, enter input data, and get body composition estimates. In addition, it is also possible to download a model evaluation report for the selected model and species;
3. *Explore database*, where users can explore the body composition data available in the database and export a data analysis report for each species;
4. *Video tutorial*, where users can watch a quick video tutorial illustrating how the tool can be used to estimate the body composition of fish.

Figures 9 and 10 show snapshots of the ficoEst user interface (Estimate composition and Explore database pages, respectively). To enter input data, users must have access to Microsoft ExcelTM, LibreOffice Calc, or other similar software that allows editing spreadsheets in XLSX (Office Open XML) format.



SPECIES 1 Gilthead seabream (Sparus aurata)

MODEL INPUTS 1 BC1 | body weight

ESTIMATES 4 samples estimated

Species: Gilthead seabream (Sparus aurata)

Model inputs
- ● BC1 | body weight
- ○ BC2 | body weight and water
- ○ BC3 | body weight, water and ash

Upload populated input file [xlsx]: ficoEst_input_data.xlsx

ID	body weight
-	g
sample A	100
sample B	200
sample C	300
sample D	400

Show 10 entries   Search:

	ID	body weight (g)	crude protein (%)	crude lipids (%)	water (%)	ash (%)	phosphorus (%)	energy (kJ/g)	crude protein (g)	crude lipids (g)
1	sample A	100	17.34	12.75	65.92	3.98	0.7	8.57	17.34	12.75
2	sample B	200	17.53	14.31	64.3	3.86	0.72	9.17	35.05	28.62
3	sample C	300	17.62	15.29	63.3	3.79	0.73	9.54	52.85	45.87
4	sample D	400	17.67	16.02	62.57	3.73	0.74	9.82	70.69	64.07

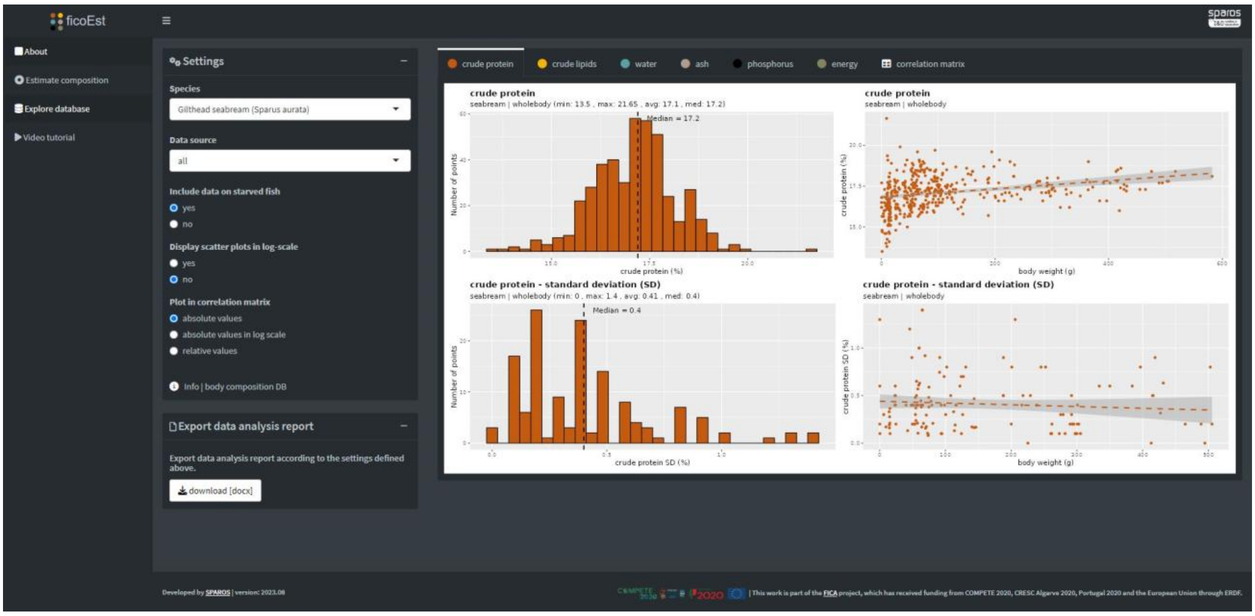
Showing 1 to 4 of 4 entries

Export model evaluation report according to the settings defined above.

Developed by SPAROS | version: 2023.08

| This work is part of the FICA project, which has received funding from COMPETE 2020, CRESC Algarve 2020, Portugal 2020 and the European Union through ERDF.

Figure 9. Snapshot of ficoEst (version 2023.08) Estimate composition page.



Species: Gilthead seabream (Sparus aurata)

Data source: all

Include data on starved fish
- ● yes
- ○ no

Display scatter plots in log-scale
- ○ yes
- ● no

Plot in correlation matrix
- ● absolute values
- ○ absolute values in log scale
- ○ relative values

crude protein
seabream | wholebody (min: 13.5 , max: 21.65 , avg: 17.1 , med: 17.2)

crude protein
seabream | wholebody

crude protein - standard deviation (SD)
seabream | wholebody (min: 0 , max: 1.4 , avg: 0.41 , med: 0.4)

crude protein - standard deviation (SD)
seabream | wholebody

Export data analysis report according to the settings defined above.

Developed by SPAROS | version: 2023.08

| This work is part of the FICA project, which has received funding from COMPETE 2020, CRESC Algarve 2020, Portugal 2020 and the European Union through ERDF.

Figure 10. Snapshot of ficoEst (version 2023.08) Explore database page.

4. Conclusions

Measuring the whole-body proximate composition of fish is an essential step in several research activities in fish nutrition, as well as in product quality control and environmental impact assessment activities carried out in fish farming enterprises. So far, analytical methods have been the most widespread, and sometimes the only, approach to estimate the proximate composition of fish. Most of the times analytical methods are sufficiently robust and reliable. However, they are time-consuming and relatively expensive, thus bringing some limits to the regular determination of fish composition.

The tool presented here was developed with the purpose of serving as a complementary approach to determine the whole-body proximate composition of fish. When analytical methods are not a viable option, ficoEst can be used to generate additional information. The back-end engine of this tool uses different mathematical models to perform estimates for the main body composition components (i.e., crude protein, crude lipids, water, ash, phosphorus and energy). All models were calibrated and evaluated with empirical datasets, most of them collected from the scientific literature and, therefore, previously validated by the scientific community.

The use of mathematical models to estimate the body composition of fish is a solution widely proposed and explored by several authors in the past. However, most models developed and published in the scientific literature are not implemented in a publicly available ready-to-use format. As a public web tool, ficoEst has the potential to be a valuable resource for researchers and fish farmers interested in estimating the body composition of farmed fish.

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CRediT authorship contribution statement

Filipe Soares: Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Data curation, Writing—Original draft preparation, Writing—Review and editing, Visualization, Funding acquisition. **Andreia Raposo:** Investigation, Data curation, Writing—Review and editing. **Rodrigo Mendes:** Investigation, Data curation, Writing—Review and editing. **Marina Azevedo:** Investigation, Data curation, Writing—Review and editing. **Jorge Dias:** Resources, Writing—Review and editing, Supervision, Project administration, Funding acquisition. **Ana Nobre:** Writing—Review and editing. **Luís E. C. Conceição:** Resources, Writing—Review and editing, Supervision, Project administration, Funding acquisition. **Tomé**

Silva: Conceptualization, Methodology, Software; Validation, Formal Analysis, Investigation, Data curation, Writing—Review and editing. **Declaration of Competing Interest** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. **Data Availability** The data that has been used is confidential.

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Appendix A. Supporting information Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aquaeng.2023.102364.

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Chapter 4

Predicting fish body composition: can water and ash inputs improve estimations?

1. Introduction

In aquaculture, body composition is important to be measured or estimated, as it can affect the quality of the fish and its market value (Azam et al., 2004; Breck, 2014; Jobling, 2001). Fish body composition can be directly assessed by analysing its chemical composition which comprehends crude protein, crude lipids, ash, and water (e.g., AOAC methods). These analyses can be time-consuming and expensive, so there is a need for more practical and cost-effective methods. Mathematical models can be an alternative and present several advantages over chemical analysis as they are less time-consuming and less costly. In fact, mathematical models can predict body composition in a matter of minutes to hours, depending on the complexity of the model and the computational resources available (Chowdhury & Bureau, 2009; Breck et al., 2014; Soares et al., 2023a). Another advantage of using models is that, as a complementary practical method, they can contribute to a more comprehensive assessment of body composition, allowing to extract more information from data (e.g., farmers can use their production data to estimate environmental impacts) and provide additional information that may be important for optimizing production (Soares et al., 2023b). Moreover, mathematical models can also be used to predict body composition in near real-time (e.g., by using dynamic models coupled to data management software), allowing for rapid adjustments to feeding practices and diet formulations. This can ultimately lead to improved growth, health and production profitability. Thus, mathematical models can provide a cost-effective solution for estimating the body composition of fish in situations where it is impractical or financially unfeasible to do so through conventional means, as suggested by Raposo et al., 2023.

Different models to predict fish body composition have already been developed for several fish species (Bar et al., 2007; Breck, 2011, 2014; Chowdhury et al., 2013; Chowdhury & Bureau, 2009; de Castro Silva et al., 2015; Hanley, 1991; Raposo et al., 2023; van Trung et al., 2011). Usually, they are based on linear regressions between body weight (BW) and the main body composition components (e.g., water, crude protein, crude lipids and ash) (Dumas et al., 2010), implying either an isometric or allometric relationship. In Raposo et al. (2023) work, the authors developed and thoroughly evaluated different types of body composition models for Nile tilapia and obtained a robust hybrid model (ensemble of isometric and allometric models using robust regression), with a good prediction capacity when compared with models published by other authors. SPAROS developed a tool – ficoEst (https://webtools.sparos.pt/ficoest/) - which comprehends different types of models that uses body weight, water and/or ash content as inputs to estimate the remaining components (i.e., crude protein, crude lipids, phosphorus and energy).

Herein, we compared the performance of the *BCRA* model developed by Raposo et al. (2023) against the ficoEst models, for 5 commercial fish species (Nile tilapia *Oreochromis niloticus,* Atlantic salmon *Salmo salar,* gilt head seabream *Sparus aurata*, European seabass *Dicentrarchus labrax* and rainbow trout *Oncorhynchus mykiss*). The main objective was to assess whether considering information about water and ash content improves the accuracy of models to estimate other body composition components.

2. Materials and methods

2.1. Data collection and processing

The dataset used in this study is composed of data collected from the scientific literature and from trials developed in the framework of projects coordinated by SPAROS, covering a varied range of fish body weights, rearing and feed conditions (see Table 1 and Appendice 1.A). Data concerning carbohydrates were not considered, due to their minimal contribution to fish whole body composition and because they are usually not reported in literature (Breck, 2011). Moreover, only cases where the whole fish content (described as “whole-body” composition) was referred and determined following the methods described by the Association of Official Analytical Chemists (AOAC) were used. Additionally, in some cases, data was processed with the use of conversion methods to convert diet and whole-body composition from dry weight to wet weight, to calculate the energy content of diets, and to convert coefficient of variation (CV) and standard error (SE) to standard deviation (SD). Furthermore, based on the analysis of the sum of the mean standard deviation of each body composition component, only datasets in which the sum of components ranged from 97.5% to 102.5% were considered, in order to exclude contamination by measurement or reporting errors. Correlations between body composition components, and these and body weight, were also evaluated in relative and absolute terms (Appendice 2).

2.2. Model evaluation and calibration methods

In this work different models from a tool developed by SPAROS to predict the body composition of different species (ficoEst, available at https://webtools.sparos.pt/ficoest/), were tested against a model previously published by Raposo et al. (2023). The ficoEst tool includes three different families of body composition models: *BC1*, relies on body weight as input; *BC2*, uses body weight and water percentage as inputs; *BC3,* considers body weight, water and ash percentage as inputs. In turn, the model published by

Raposo et al. (2022), herein designated as *BCRA*, is an ensemble averaging of isometric and allometric models in which considers only body weight as input (see Table 2 for details about the different models).

Regression analysis was performed for each body composition component and the models were evaluated both qualitatively, by visually observing model behaviour, and quantitatively, by calculating the mean absolute percentage error (MAPE) between observed and predicted values for each component, as follows:

$$MAPE\ (\%) = \frac{100}{n}\sum_{i=1}^{n}\left|\frac{P_i - O_i}{O_i}\right|$$

where:

n	is the number of predicted - observed value pairs
P_i	is the predicted value
O_i	is the observed value

In addition, a method of cross-validation with 5-folds and 10 repetitions was used to assess the model performance. All analyses were performed using R version 4.1.2 (R Core Team, 2021).

2.3. Model validation

Models were validated using independent datasets (i.e., not used during the calibration process) and evaluated qualitatively and quantitatively (i.e., MAPE), as described in section 2.2.

Furthermore, to assess the uncertainty of the observed values, the mean percentage error (PE) was calculated based on the *mean±sd* values of the pairs that were used to calibrate and validate the models, as follows:

$$Observations\ PE\ (\%) = \frac{100}{n}\sum_{i=1}^{n}\left(\frac{sd\_value_i}{mean\_value_i}\right)$$

where:

n	is the number of mean_value - sd_value pairs
$mean\_value_i$	is the observed mean value of a body composition component
sd_value_i	is the observed standard deviation value of a body composition component

3. Results

3.1. Model evaluation and calibration methods

Table 3 shows that, when comparing all models across species, overall, BC3 had lower calibration errors than the other models tested. However, all models performed similarly for crude protein predictions with a MAPE of approximately 7%, in contrast to crude lipids (MAPE: 9 - 40%), ash (MAPE: 9 - 20%) and phosphorus (MAPE: 10 – 55%), which displayed higher calibration errors. In particular, for Nile tilapia, the BC3 model generally performed better than the other models for all body composition components. For salmon, the BC1 model performed better for crude protein (MAPE ≈ 5%) and ash (MAPE ≈ 10%) predictions, while the BC3 model performed better for the remain components. However, there were differences between the model's calibration errors for crude lipids, with BC3 displaying the lowest and BCRA the highest deviations (MAPE ≈ 9% and MAPE ≈ 41%, respectively). For seabream and seabass, BC2 displayed lower calibration errors for crude protein (MAPE ≈ 4% and MAPE ≈ 5%, respectively). As in the other species, there were differences between model calibration errors for crude lipids in seabream and seabass, with BC3 displaying the lowest (MAPE ≈ 9% and MAPE ≈ 10%, respectively) and BCRA the higher calibration errors (MAPE ≈ 31% and MAPE ≈ 34%, respectively). Moreover, for seabream the calibration errors for phosphorus were the highest one with a MAPE ≈ 54%). Regarding rainbow trout, the BC1 model performed better for crude protein predictions (MAPE ≈ 5%), but there were differences between models for crude lipids.

The BCRA model is similar to the BC1 model, since they both rely only on the body weight as input. Comparing the calibration errors of these two models, the BCRA model had lower calibration errors for ash (MAPE ≈ 21%) and phosphorus (MAPE ≈ 35%) in tilapia, and for ash in trout (MAPE ≈ 15%). For gilthead seabream and seabass, the BCRA model had similar errors to BC1 for most nutrients, except for crude lipids and energy. Overall, the BC2 and BC3 models had similar errors and both were different from BC1 and BCRA.

Overall, the species that exhibited lower calibration errors was European seabass, whereas Nile tilapia had the highest errors.

3.2. Model validation

Overall, the BC3 model displayed lower validation errors for all body composition components (Figure 1 – 5). For Nile tilapia, the BC3 model was the best in predicting all body composition components. However, the same was not observed for the other species, such as salmon, where the BC3 model displayed lower errors for crude lipids (MAPE ≈ 10%), phosphorus (MAPE ≈ 13%) and energy (MAPE ≈ 4%), while the BC1 model showed lower validation errors for protein (MAPE ≈ 5%). Likewise for gilthead seabream, the BC3 model showed lower validation errors for crude lipids (MAPE ≈ 9%), phosphorus (MAPE ≈ 47%) and energy (MAPE ≈ 5%), whereas the BC2 model showed lower validation errors for crude protein predictions (MAPE ≈ 4%). In European seabass, crude protein was more accurately predicted with the BC2 model (MAPE ≈ 5%), while crude lipids and energy was better predicted with the BC3 model (MAPE ≈ 10% and MAPE ≈ 5%), respectively) and phosphorus with the BCRA model (MAPE ≈ 11%). For rainbow trout, BC3 only performed better in predicting crude lipids (MAPE ≈ 9%), whereas for crude proteins and phosphorus, BC1 displayed lower validation errors (MAPE ≈ 5% and MAPE ≈ 13%, respectively), and for energy, the model with the best performance was BC2 (MAPE ≈ 5%). Regardless of model type, the validation errors were generally higher for Nile tilapia, specifically when predicting crude protein (MAPE ≈ 8%), crude lipids (MAPE ≈ 44%) and energy (MAPE ≈ 19%). On the other hand, gilthead seabream had the lowest validations errors for crude protein and lipids (MAPE ≈ 4% and MAPE ≈ 9%, respectively), but the highest for phosphorus (MAPE ≈ 53%). In turn, salmon showed the lowest validations errors for energy predictions with a MAPE ≈ 4%.

Analysing the model performance for each body component, all models exhibited similar validation errors for predicting crude protein (MAPE between 5 and 8%) across all species. Generally, crude protein was the body component with the lowest validation errors. Phosphorus predictions had the highest validation errors, with a MAPE ranging from 11 to 53% in BC1 and BCRA models, from 13 to 52% for BC2, and from 12 to 47% for BC3, which had the lowest range. In terms of crude lipids, the BC3 model displayed the lowest range for validation errors with a MAPE between 9 and 20%, while for BC2 the MAPE ranged from 10 and 24%, for BC1 between 21 and 34%, and for the BCRA model between 31 and 44%, the latter being the highest range. Lastly, for energy predictions, BC3 had the lowest validation errors with a MAPE ranging from 4 to 8%, followed by BC2 (4 to 8%), BC1 (8 to 13%), and BCRA (12 and 19%).

Overall, validation errors were greater for Nile tilapia, when compared to other species, particularly for crude lipids (MAPE 20-44%) and energy (MAPE 7-19%). In contrast, gilthead seabream had in general lower validation errors then the other species for crude lipids (MAPE 9-21%) and energy (MAPE 4-12%).

In general, the mean percentage error (PE) was lower than the prediction errors, except for European seabass and rainbow trout (Figure 1-5). Among all the body composition components, crude lipids and ash showed higher PE values, particularly for Nile tilapia (12% and 11%, respectively), gilthead seabream (6% and 8%, respectively) and rainbow trout (7% and 15%, respectively). For European seabass and Atlantic salmon, phosphorus exhibited the highest PE values (26% and 8%, respectively).

4. Discussion

Accurately measuring or estimating the body composition of fish is of extreme importance for both nutritionists and fish farmers. To achieve this, it is important to have access to low-cost and fast methods for obtaining information on fish body composition. The FicoEst tool, developed by SPAROS, was evidenced to be a promising alternative to conventional analytical methods. These tool offers several options for estimating body composition based on available information. For example, if data on water and ash content are available, the tool can estimate, based on the relationship between crude protein and water and ash content, the crude lipids by difference (i.e., ficoEst BC3 model). If this information is not available, the tool will estimate the body composition based only on fish body weight (ficoEst model BC1, likewise the BCRA model from Raposo et al., 2023) or based on fish body weight and water (ficoEst model BC2). Among all models provided by FicoEst, the BC3 displayed the best performance, whilst BC1 and BC2 models displayed higher prediction errors, meaning that providing information on water and ash content improve model performance.

In order to develop robust models, it is of great importance to use good quality datasets to calibrate the models. In this study, Nile tilapia had higher mean absolute percentage errors (MAPEs) compared to other fish species, possibly due to the quality of the dataset. In some publications, incomplete or inaccurate measurements data can introduce errors in the model predictions and lead to higher MAPEs. These may explain why the correlations between body weight and body composition components were poor for this specie when compared to the other ones, especially the correlation between body weight and crude protein, and water and crude lipids. Additionally, species strain variability can also impact body composition predictions. Different Nile tilapia strains can have different body composition profiles and models that do not account for this variability can lead to less accurate predictions and, thus, higher MAPEs. It is therefore important to visualize and analyse the data, for example using statistical methods (e.g., diagnostic plots), in order to identify outliers and improve the accuracy of body composition predictions. It is also recommended to authors to mention the specie strain in publications.

Overall, the BCRA model demonstrated the poorest prediction capability among the tested models for all species, displaying higher validation errors. In fact, both BC1 and BCRA had higher validation errors for predicting body composition for all species. However, when predicting crude lipids, BC1 exhibited lower validation errors compared to BCRA. When comparing the validation errors of the BCRA model for Nile tilapia with those reported in Raposo et al. (2023) for the same species (see Chapter 2), the errors were found to be similar. This suggests that the performance of the BCRA model in predicting Nile tilapia body composition was not impacted by the different data used in this study. In turn, comparing the higher MAPEs obtained using the BCRA model with the lower ones for the BC3 model highlights the importance of considering water and ash content as inputs to improve model accuracy. On the other hand, when comparing the performance of BC1 and BCRA models (i.e., models with same type of inputs), the validation errors were similar except for crude lipids estimations, which in this case may be related to differences in the datasets used to calibrate both models.

Regardless of the type of model used in this study, higher mean absolute percentage errors (MAPEs) were observed for phosphorus and ash predictions. It is noteworthy that nutritionists typically do not report phosphorus values for body composition. Phosphorus is primarily a mineral that plays a crucial role in many physiological processes in the body (e.g., bone formation and maintenance). However, authors often prioritize discussing broader aspects of nutrient management and environmental impact in fish farming studies. Therefore, the lack of data in the literature on phosphorus levels may have contributed to the overall higher MAPEs observed for this component. However, phosphorus intake can indirectly affect body composition through its role in supporting overall health and energy metabolism. For example, a deficiency in phosphorus can lead to muscle weakness and decreased physical performance, which can ultimately affect body composition (Sugiura et al., 2004; Uyan et al., 2007). Moreover, recent studies have shown that when fishmeal is replaced by vegetable sources, the availability of phosphorus is compromised (Lund et al., 2011; Daniel, 2018). This implies that the body composition of the fish is affected in terms of phosphorus levels. It is therefore important that studies take phosphorus into account in the body composition of fish. In turn, the overall higher MAPEs for ash predictions may be related to laboratory errors. This is also valid for the higher PE for ash. Ash is the least abundant component of fish whole body and is thus difficult to determine with precision, especially during sample homogenization and manipulation (e.g., if samples are nor well homogenized, some may have more scales or bones, which will affect the final ash content). Another factor that can impact body composition predictions, particularly for ash, is the problem of renormalization of data. Renormalization can be used to adjust data so that it conforms to a particular distribution or standard (e.g., body components to sum up to 100%). In the context of fish body composition data, renormalization can be a problem because it can introduce bias into the data if it is not done properly. For example, renormalization in relative terms involves adjusting the body composition data by a factor that is based on the proportion of each component in relation to the total body weight, while renormalization in absolute terms involves adjusting the body composition data by a fixed amount. When renormalization is performed in absolute terms, components with lower abundance in the body composition data, such as ash, will be more affected than components with higher abundance. Thus, renormalization in relative terms may be more appropriate when the goal is to compare body composition between individuals or populations, while renormalization in absolute terms may be more appropriate when the goal is to correct for analytical or sampling differences.

Laboratory errors and the sensitivity of chemical measurements can both have a significant impact on the accuracy of crude lipids measurements in fish samples and thus, may explain the overall higher PE for crude lipids. Different extraction methods (e.g., different solvents, extraction times and temperatures) may yield different results, and some methods may be more sensitive than others for detecting certain types of lipids. Additionally, laboratory errors such as, contamination, improper sample handling or storage, and errors in equipment calibration or data analysis, can also affect lipid measurements.

5. Conclusion

In general, the BC3 model was more accurate in estimating fish body composition. This suggests that, besides fish body weight, considering water and ash whole body content may improve model performance. Therefore, model BC3 in ficoEst seems to be a useful tool to estimate fish body composition of Nile tilapia, Atlantic salmon, gilthead seabream, European seabass and rainbow trout.

There are still some challenges in modelling body composition in fish, such as the lack of representative and high-quality data for certain species and growth stages. Also, the complex nature of fish growth and development makes it difficult to develop models that accurately capture all the variables involved (e.g., changes in fish metabolism during maturation, nutrient flux such as fatty acids in fish body). Therefore, there is still space for further research to overcome these challenges and continue to improve aquaculture practices.

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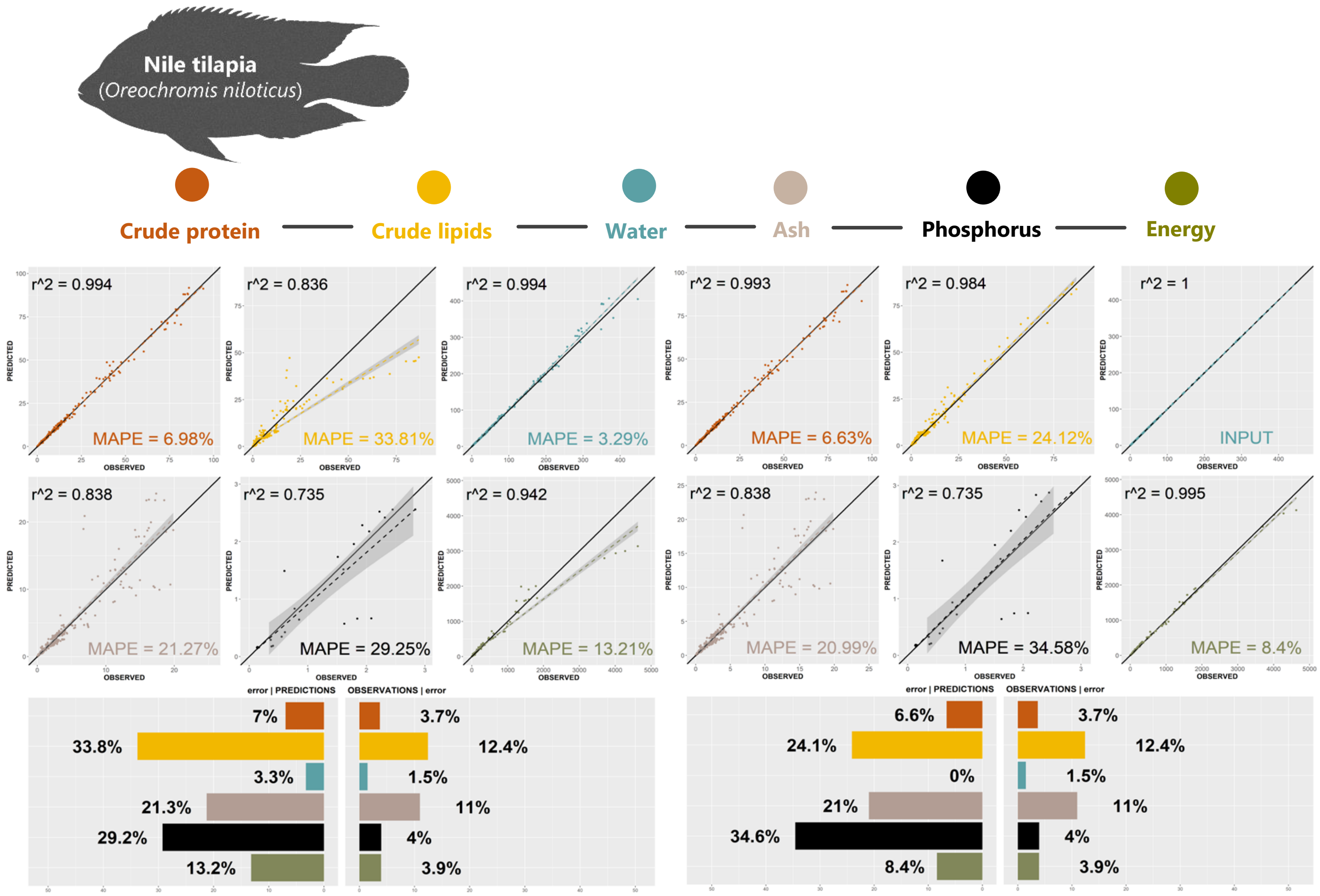
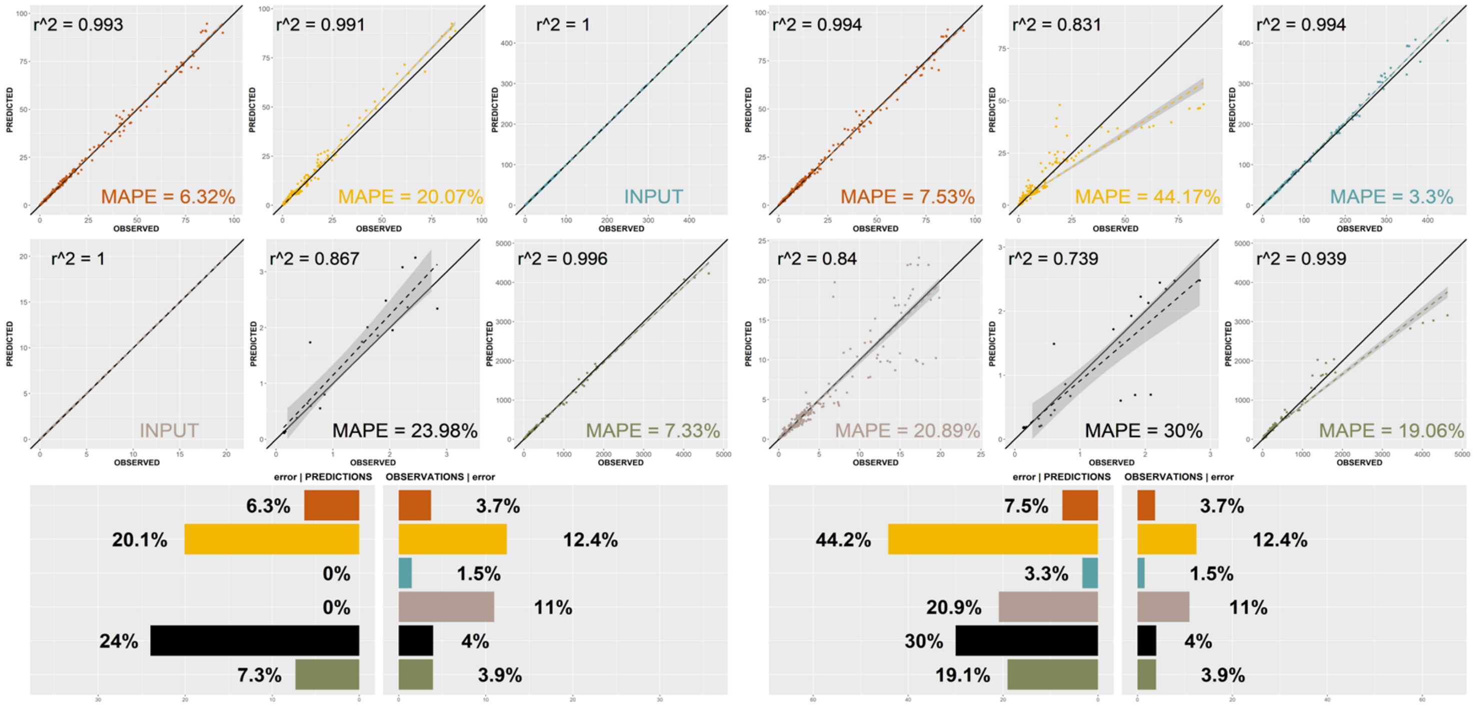


Body composition | model validation | BC1 | tilapia

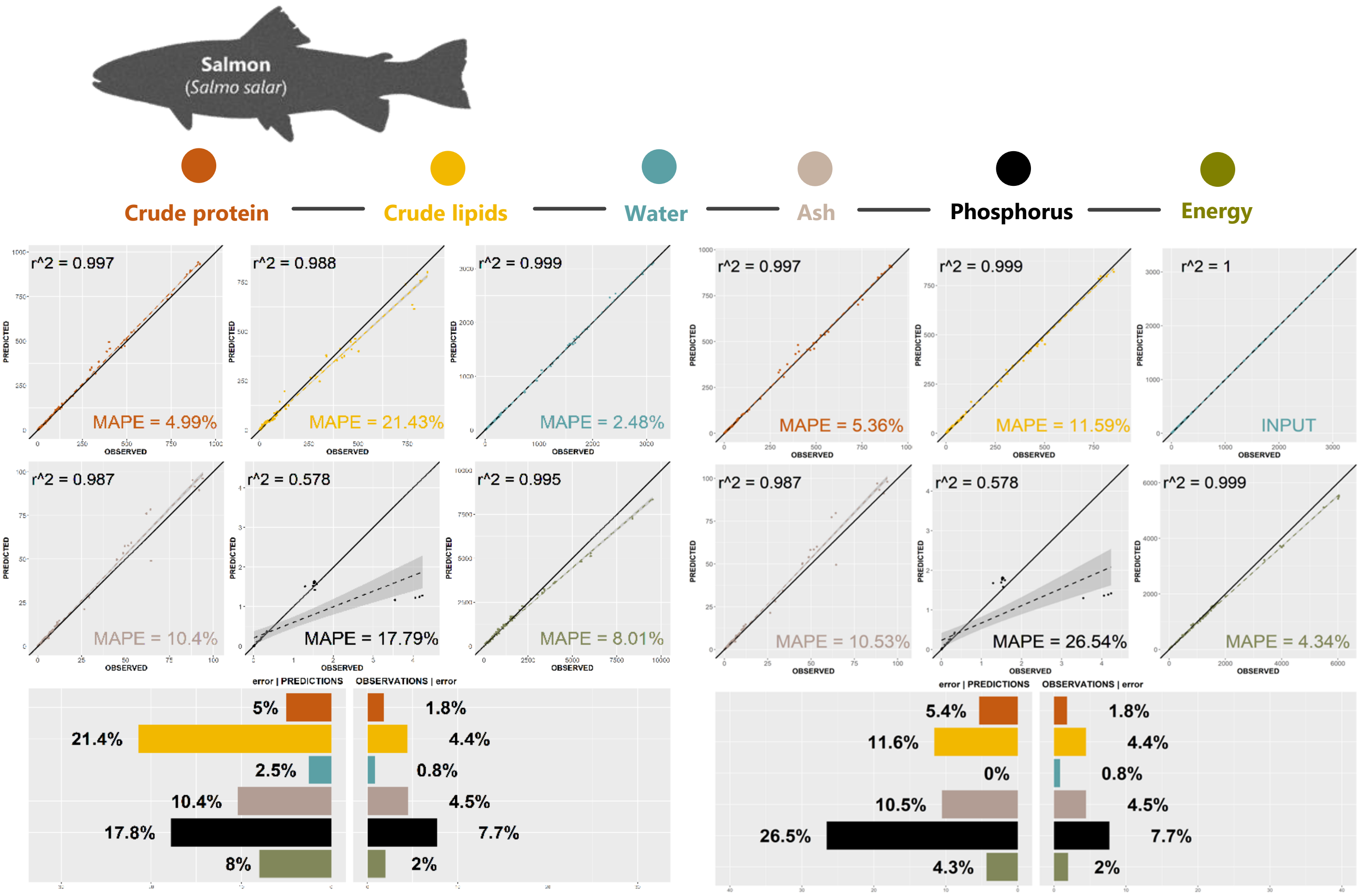
Body composition | model validation | BC2 | tilapia



Body composition | model validation | BC3 | tilapia

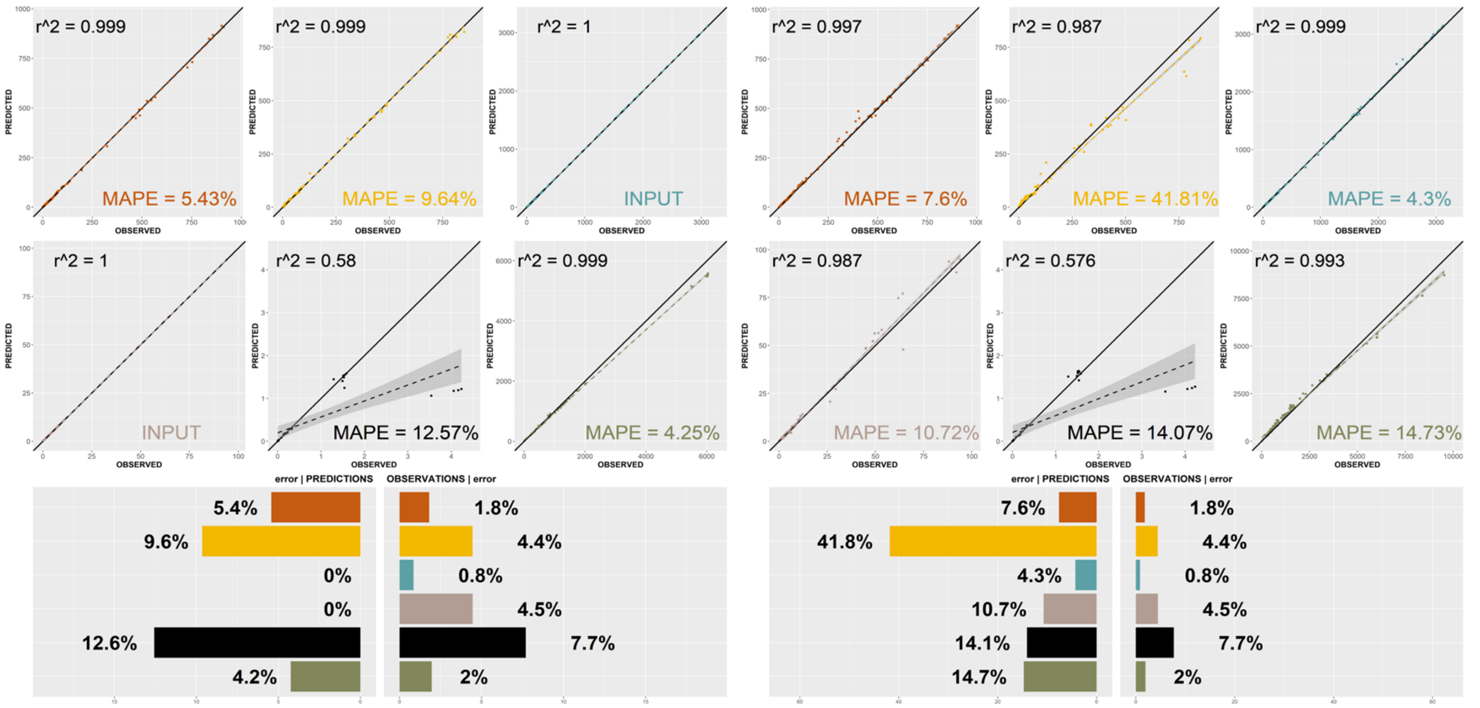
Body composition | model validation | BCRA | tilapia

Figure 1 - Prediction errors (MAPE) from model validation (scatterplots) and mean percentage error (PE) for observations (bar chart) for Nile tilapia.



Body composition | model validation | BC1 | salmon

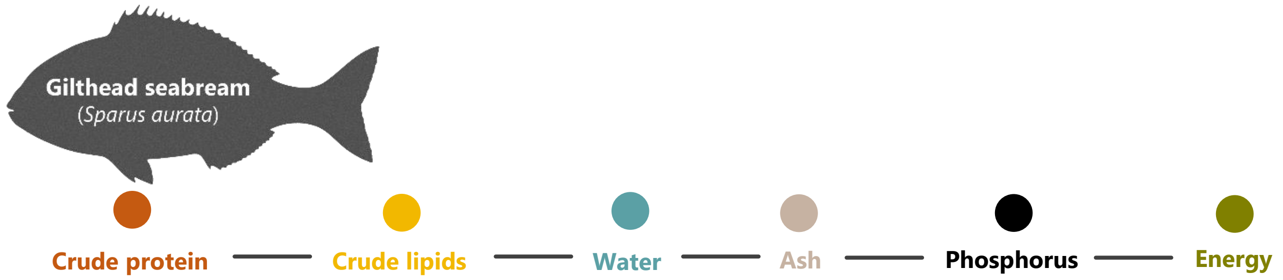
Body composition | model validation | BC2| salmon

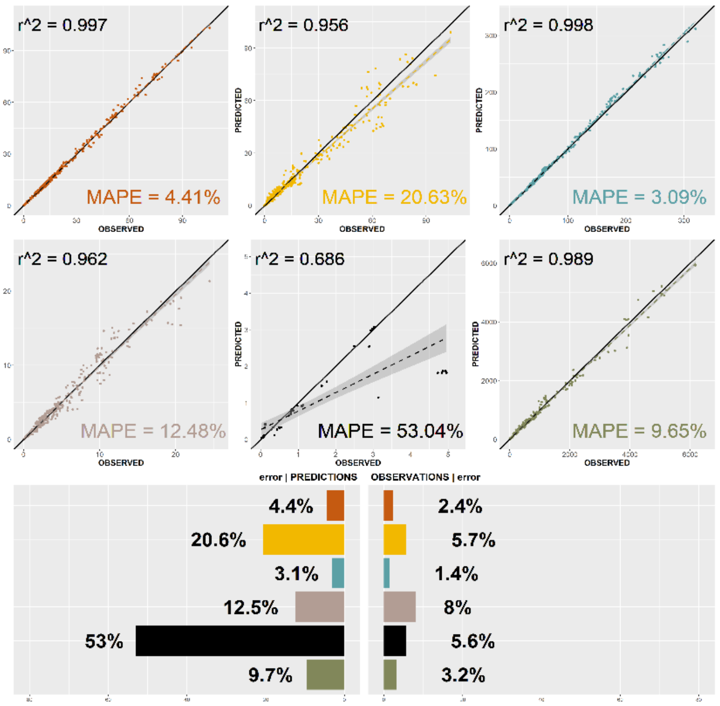


Body composition | model validation | BC3 | salmon

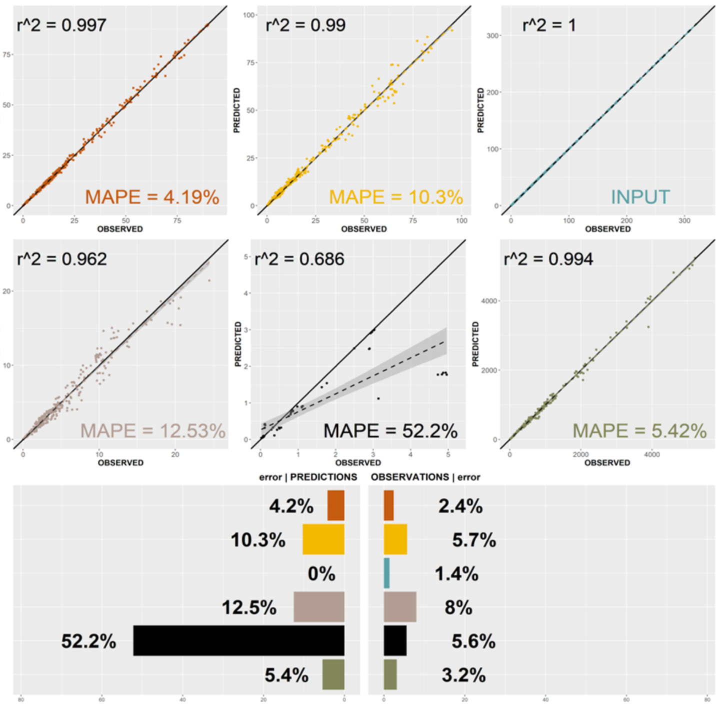
Body composition | model validation | BCRA | salmon

Figure 2 - Prediction errors (MAPE) from model validation (scatterplots) and mean percentage error (PE) for observations (bar chart) for Atlantic salmon.

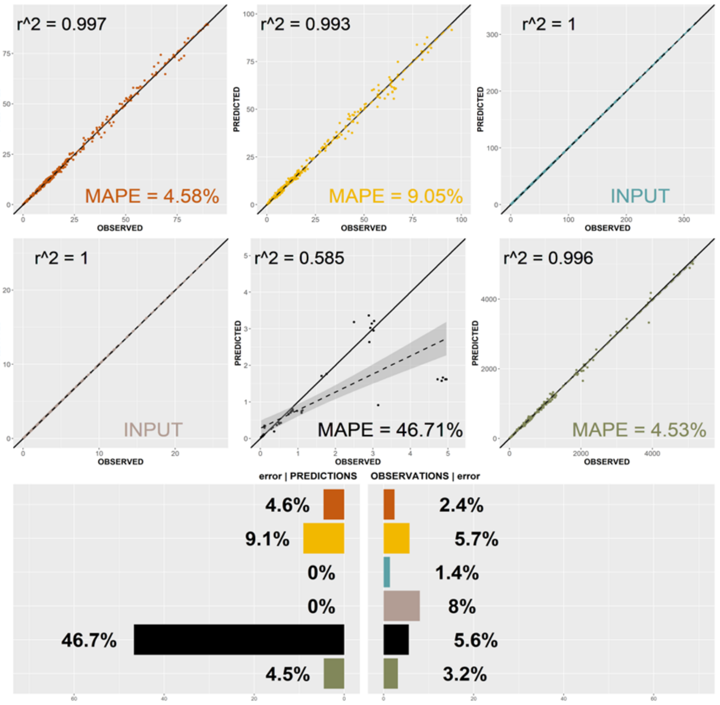




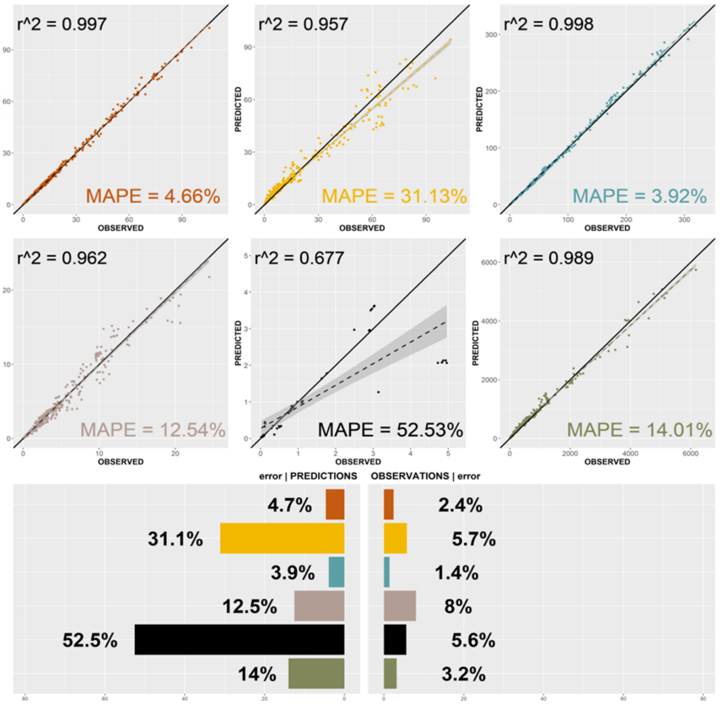
Body composition | model validation | BC1 | seabream



Body composition | model validation | BC2 | seabream

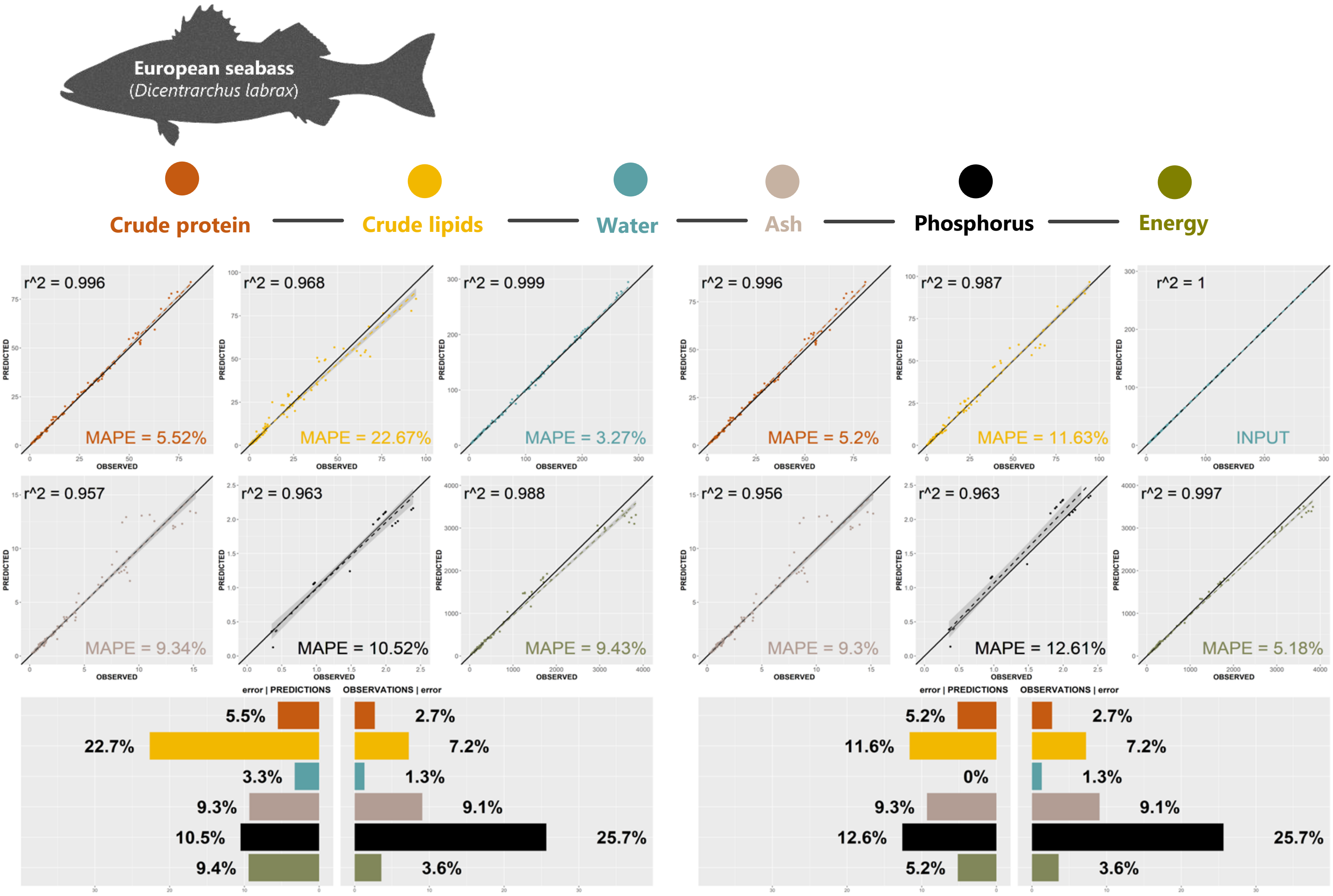


Body composition | model validation | BC3 | seabream



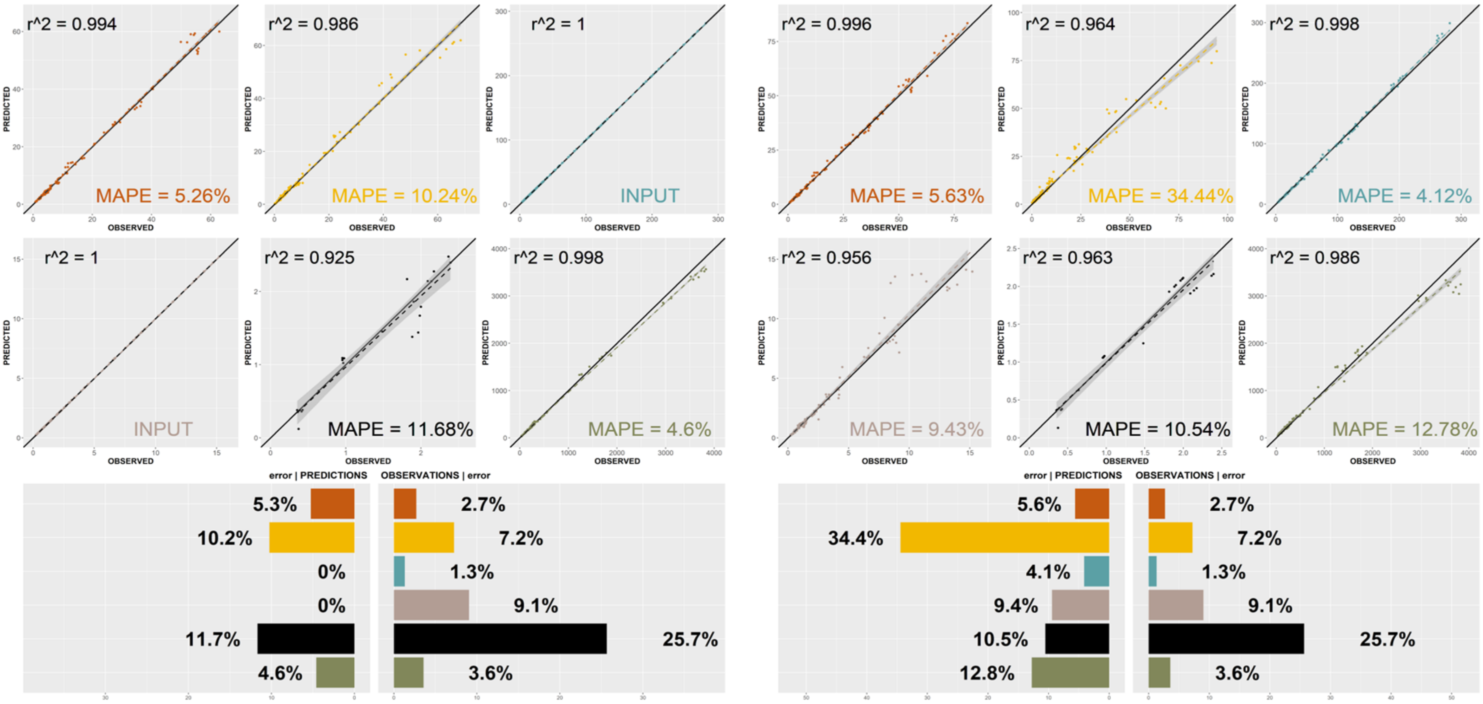
Body composition | model validation | BCRA | seabream

Figure 3 - Prediction errors (MAPE) from validation (scatterplots) and mean percentage error (PE) for observations (bar chart) for gilthead seabream.



Body composition | model validation | BC1 | seabass

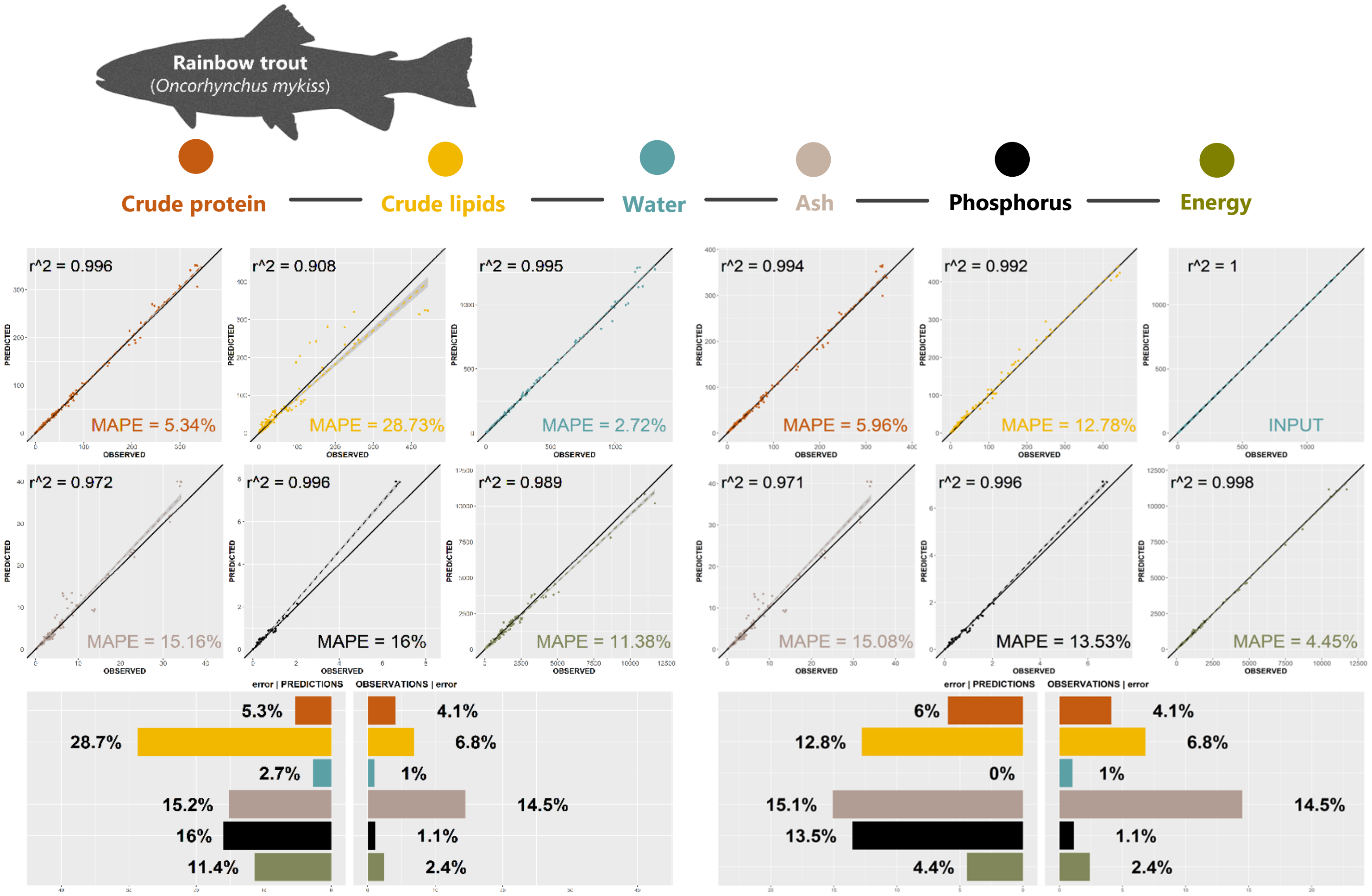
Body composition | model validation | BC2 | seabass



Body composition | model validation | BC3 | seabass

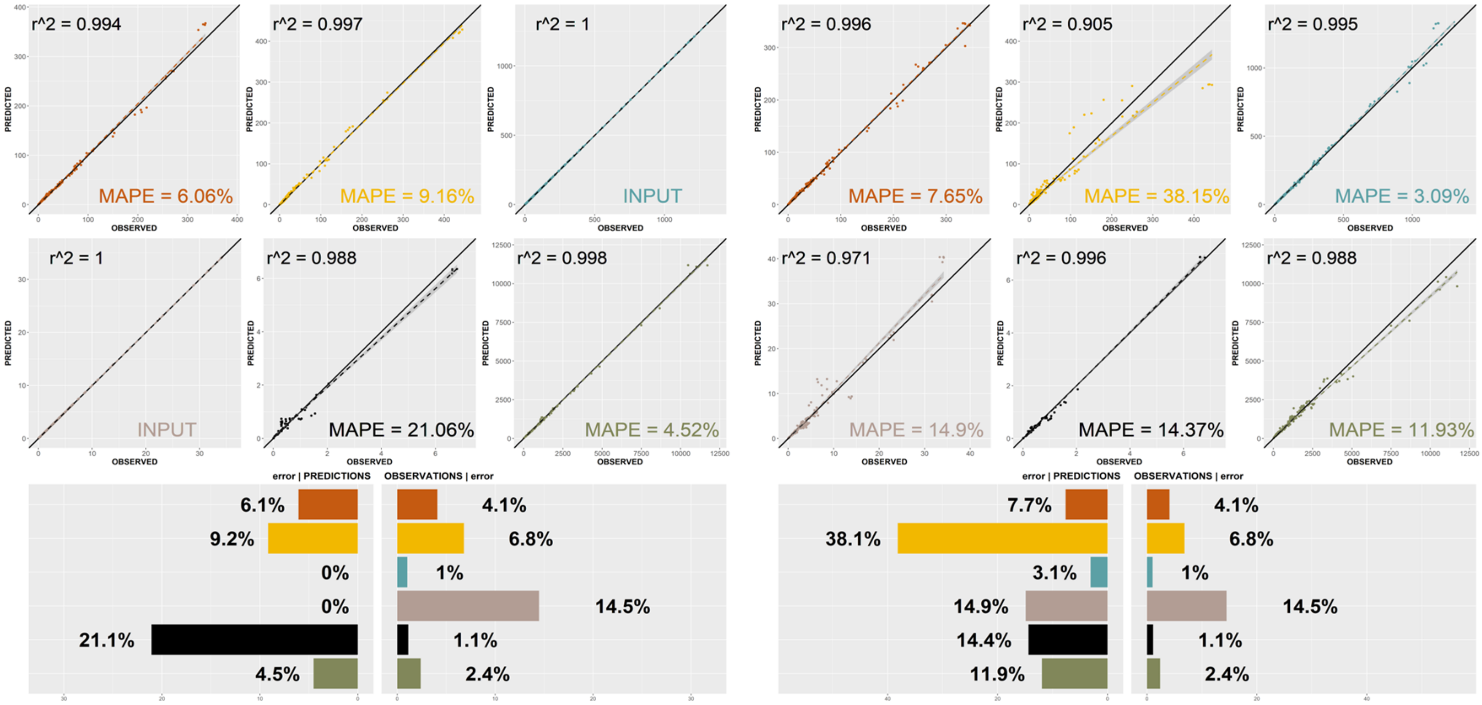
Body composition | model validation | BCRA | seabass

Figure 4 - Prediction errors (MAPE) from validation (scatterplots) and mean percentage error (PE) for observations (bar chart) for European seabass.



Body composition | model validation | BC1 | trout

Body composition | model validation | BC2 | trout



Body composition | model validation | BC3 | trout

Body composition | model validation | BCRA | trout

Figure 5 - Prediction errors (MAPE) from model validation (scatterplots) and mean percentage error (PE) for observations (bar chart) for rainbow trout.

Table 1 – Summary of the data used to calibrate and validate the models for each species.

Attributes	**Unit**	**Nile tilapia**	**Atlantic salmon**	**Gilthead seabream**	**European seabass**	**Rainbow trout**
Nr. of data sources	-	52	50	58	29	41
Nr. of observational units	-	377	368	442	161	331
Body weight range	g	1 – 571	1 - 4950	1 – 582	5 - 482	2 - 2008
Temperature range	°C	21 – 32	2– 23	18 – 27	13 - 29	6 - 19
Body composition						
Water	%	62 – 82	60 – 82	58 - 80	58 – 76	59 – 82
Crude protein	%	10 – 22	12 – 20	14 – 22	14 – 21	13 - 20
Crude lipids	%	1 – 17	2 – 20	2 – 22	1 – 22	2 – 22
Ash	%	1 – 8	1 – 2	3 – 7	3 – 7	1 – 4
Diet composition						
Crude protein	% as fed	18 – 56	31 – 61	36 – 68	33 – 57	16 – 65
Crude lipids	% as fed	2 – 18	10 – 39	7 – 34	8 – 29	3– 35
Gross energy	g/MJ	13 – 21	18 – 26	17 - 24	17 – 25	17 - 25

Table 2 - Overview and description of models.

Model	Crude protein (P)	Crude lipids (L)	Water (W)	Ash (A)	Phosphorus (Ph)	Energy (E)
*BC1	Allometric *f(BW)*	Allometric *f(BW)*	Allometric *f(BW)*	Allometric *f(BW)*	Isometric *f(A)*	Energetic *f(P, L)* (based on energetic coefficients)
*BC2	Ensemble averaging *f(BW, W, A)* (allometric and partition model)	Difference *f(BW, P, W, A)*	*input*	Allometric *f(BW)*	Isometric *f(A)*	Energetic *f(P, L)* (based on energetic coefficients)
*BC3	Ensemble averaging *f(BW, W, A)* (allometric and partition model)	Difference *f(BW, P, W, A)*	*input*	*input*	Isometric *f(A)*	Energetic *f(P, L)* (based on energetic coefficients)
**BCRA	Ensemble averaging *f(BW)* (allometric and isometric)	Ensemble averaging *f(BW)* (allometric and isometric)	Ensemble averaging *f(BW)* (allometric and isometric)	Ensemble averaging *f(BW)* (allometric and isometric)	Ensemble averaging *f(BW)* (allometric and isometric)	Energetic *f(P, L)* (based on energetic coefficients)

* ficoEst models

****** Raposo et al. (2023) model

Table 3 - Mean absolute percentage error (MAPE) for calibration with 5-fold cross-validation (10 repetitions) for tested models for each species.

	Crude protein		**Crude lipids**		**Water**		**Ash**		**Phosphorus**		**Energy**	
Nile tilapia	*Train MAPE*	*Test MAPE*	*Train MAPE*	*Test MAPE*	*Train MAPE*	*Test MAPE*	*Train MAPE*	*Test MAPE*	*Train MAPE*	*Test MAPE*	*Train MAPE*	*Test MAPE*
BC1	6.97	7.07	33.78	33,96	3.28	3.32	21.24	21.48	29.57	29.79	13.19	13.16
BC2	6.62	6.68	24.10	24,27	-	-	20.97	21.20	34.55	38.23	8.40	8.45
BC3	6.31	6.39	20.07	20,15	-	-	-	-	23.75	28.19	7.33	7.35
BCRA	7.53	7.59	44.12	44,24	3.30	3.33	20.88	21.01	30.03	34.58	19.03	19.05
Atlantic salmon												
BC1	4.99	5.03	21.42	21,61	2.48	2.49	10.39	10.49	18.25	18.02	8.00	8.02
BC2	5.35	5.40	11.58	11,65	-	-	10.52	10.62	26.54	27.30	4.33	4.35
BC3	5.43	5.47	9.63	9,69	-	-	-	-	12.57	12.76	4.25	4.27
BCRA	7.63	7.66	41.49	41,52	4.26	4.27	10.83	10.91	14.13	14.72	14.63	14.67
Gilthead seabream												
BC1	4.41	4.43	20.61	20,74	3.09	3.11	12.47	12.56	53.30	54.38	9.65	9.72
BC2	4.19	4.22	10.29	10,34	-	-	12.51	12.61	52.38	54.85	5.42	5.42
BC3	4.58	4.61	9.05	9,09	-	-	-	-	46.97	49.04	4.53	4.53
BCRA	4.66	4.67	31.13	31,21	3.92	3.93	12.54	12.58	52.28	53.92	14.02	14.09
European seabass												
BC1	5.51	5.59	22.63	22,95	3.27	3.30	9.31	9.56	10.57	12.32	9.42	9.49
BC2	5.19	5.28	11.59	11,82	-	-	9.28	9.54	12.63	13.49	5.18	5.21
BC3	5.25	5.34	10.21	10,41	-	-	-	-	11.68	12.62	4.60	4.62
BCRA	5.63	5.71	34.37	34,71	4.11	4.16	9.42	9.57	10.49	11.64	12.75	12.83
Rainbow trout												
BC1	5.33	5.38	28.72	28,97	2.72	2.73	15.12	15.37	16.00	15.97	11.37	11.40
BC2	5.96	6.02	12.76	12,89	-	-	15.05	15.30	13.51	13.89	4.45	4.45
BC3	6.05	6.11	9.15	9,23	-	-	-	-	20.97	20.99	4.52	4.52
BCRA	7.63	7.66	38.30	38,51	3.10	3.12	14.89	15.03	14.30	14.57	11.95	11.99

Appendix 1

Appendix 1.A –This table provides a comprehensive list of sources referenced for data utilized within the study.

Specie	Source
Nile tilapia	Abdel-Tawwab, M., Abdel-Rahman, A. M., & Ismael, N. E. (2008). Evaluation of commercial live bakers' yeast, Saccharomyces cerevisiae as a growth and immunity promoter for Fry Nile tilapia, *Oreochromis niloticus* (L.) challenged in situ with Aeromonas hydrophila. *Aquaculture*, 280(1-4), 185-189. Abdel-Tawwab, M., Ahmad, M. H., Khattab, Y. A., & Shalaby, A. M. (2010). Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture*, 298(3-4), 267-274. Abdel-Tawwab, M., El-Sayed, G. O., & Shady, S. H. (2012). Effects of dietary protein levels and environmental zinc exposure on the growth, feed utilization, and biochemical variables of Nile tilapia, *Oreochromis niloticus* (L.). *Toxicological & Environmental Chemistry*, 94(7), 1368-1382. Abdelghany, A. E., & Ahmad, M. H. (2002). Effects of feeding rates on growth and production of Nile tilapia, common carp and silver carp polycultured in fertilized ponds. *Aquaculture Research*, 33(6), 415-423. Afuang, W., Siddhuraju, P., & Becker, K. (2003). Comparative nutritional evaluation of raw, methanol extracted residues and methanol extracts of moringa (*Moringa oleifera* Lam.) leaves on growth performance and feed utilization in Nile tilapia (*Oreochromis niloticus* L.). *Aquaculture Research*, 34(13), 1147-1159. Ahmad, M. H., & Abdel-Tawwab, M. (2011). The use of caraway seed meal as a feed additive in fish diets: Growth performance, feed utilization, and whole-body composition of Nile tilapia, *Oreochromis niloticus* (L.) fingerlings. *Aquaculture*, 314(1-4), 110-114. Ahmad, M. H., El Mesallamy, A. M., Samir, F., & Zahran, F. (2011). Effect of cinnamon (*Cinnamomum zeylanicum*) on growth performance, feed utilization, whole-body composition, and resistance to *Aeromonas hydrophila* in nile tilapia. Journal of Applied *Aquaculture*, 23(4), 289-298. Al-Asgah, N. A., & Ali, A. (1997). Growth performance and body composition of *Oreochromis niloticus* reared at different water temperatures. In Annales de zootechnie (Vol. 46, No. 4, pp. 331-338). Ali, A. (2003). Effect of feeding different levels of alfalfa meal on the growth performance and body composition of Nile tilapia (Oreochromis niloticus) fingerlings. *Asian Fish. Sci*., 16, 59-67.

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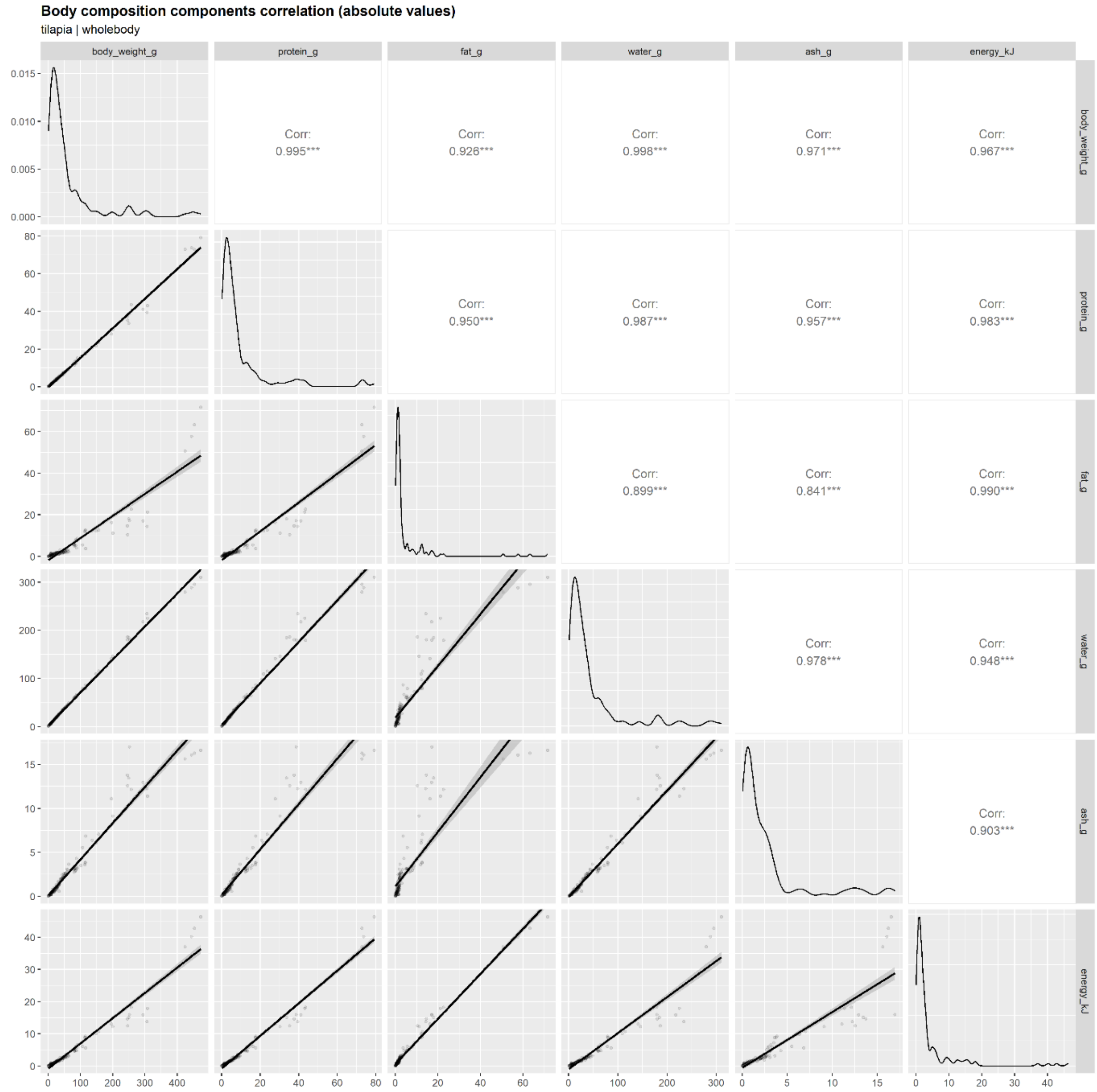
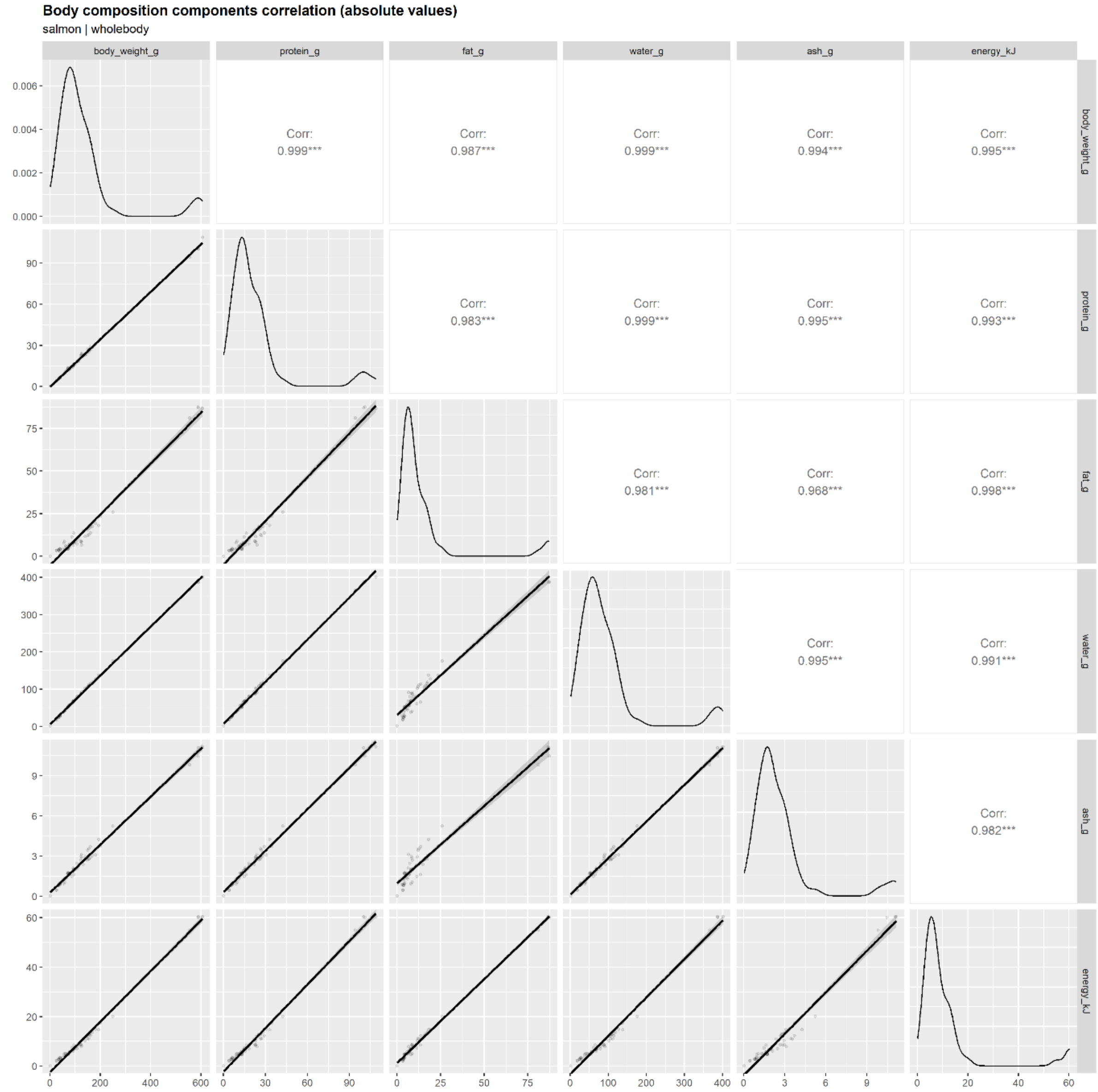
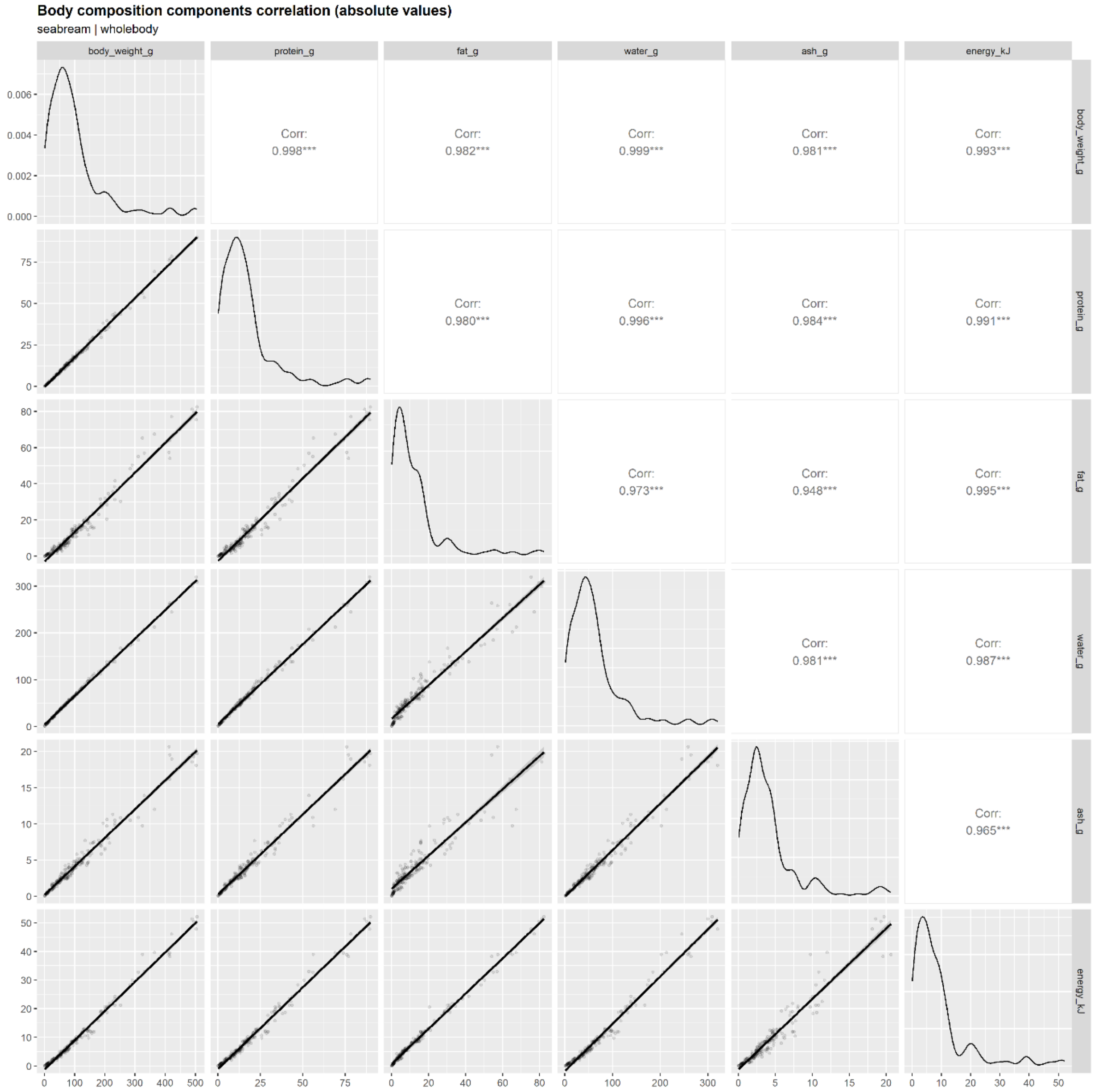
Appendix 2



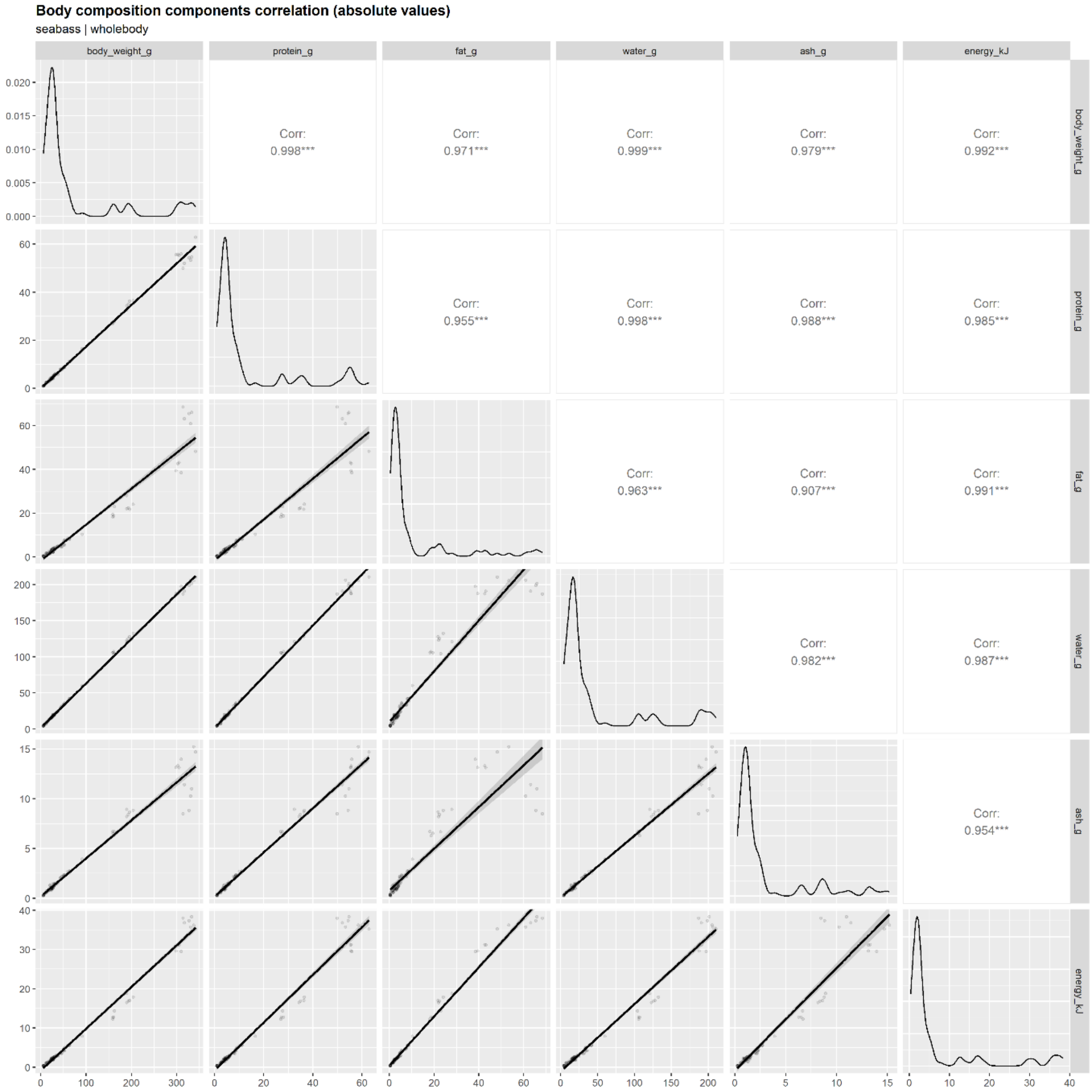
Appendices 2.A – Correlation matrix for body composition components when expressed in absolute values (g or kJ), for Nile tilapia.



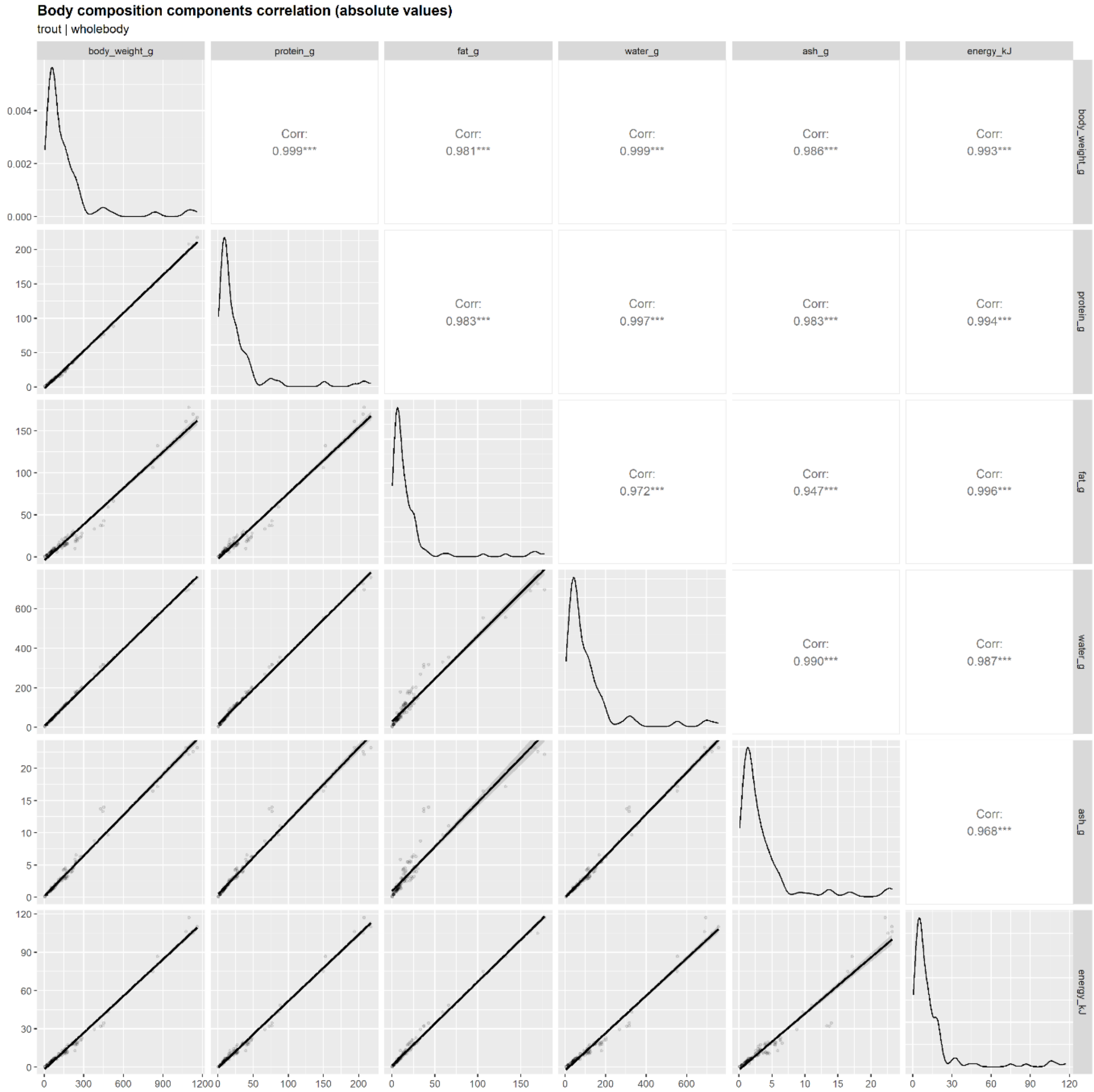
Appendices 2.B - Correlation matrix for body composition components when expressed in absolute values (g or kJ), for Atlantic salmon.



Appendices 2.C - Correlation matrix for body composition components when expressed in absolute values (g or kJ), for gilthead seabream.



Appendices 2.D - Correlation matrix for body composition components when expressed in absolute values (g or kJ), for European seabass.



Body composition components correlation (absolute values)

trout | wholebody

Appendices 2.E - Correlation matrix for body composition components when expressed in absolute values (g or kJ), for rainbow trout.

Chapter 5

Development and evaluation of Nile tilapia dynamic growth and body composition models

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Development of dynamic growth and body composition models for Nile Tilapia (*Oreochromis niloticus*): an exploratory approach to protein and energy metabolism

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Abstract

The aquaculture sector has been steadily growing and thus there is an increasing need to develop mathematical models that allow the estimation of production-related parameters. Prediction of fish growth, feed requirements and waste outputs, are essential in order to ensure the profitability and sustainability of the production activities. Bioenergetic models have been widely used to estimate growth based on energy budgets, but they have some limitations by not explicitly considering the mass-balance of the main macronutrients (e.g., protein). In turn, nutrient-based models are more explanatory, as they consider both energy and nutrient inputs, and estimate fish growth by simulating nutrient accumulation in the fish body. Although some bioenergetic and nutrient-base models for predicting Nile tilapia growth exist in the literature, their suitability is not entirely clear, since their development is often based on uncertain or suboptimal criteria (e.g., relying solely on calibration goodness-of-fit measures).

In this work, Nile tilapia growth datasets covering a wide range of rearing conditions and feed compositions were collected from the scientific literature. An exploratory analysis of the collected data was performed to clarify the relationships between energy/protein intake and gain. In this analysis, a direct relationship was observed between digestible energy intake and energy gain, as well as between digestible protein intake and protein gain. Protein gain showed better efficiency than energy gain, even at higher intake levels and without clear evidence of a saturation effect. While digestible energy intake negatively affects energy retention efficiency, digestible protein intake does not significantly impact protein retention efficiency. Furthermore, while energy retention efficiency varies with fish body weight, the same effect was not observed for protein retention efficiency. Finally, though DP/DE ratio has no apparent effect on energy retention efficiency, it seems to negatively affect protein retention efficiency. Considering these observations, plausible growth models with different levels of complexity were developed and calibrated under a diverse set of assumptions. Additionally, two growth models already published for Nile tilapia were calibrated using the same datasets, and their performance was compared with the models developed in the present study. Furthermore, fish body weight seems to affect the energy retention efficiency. The results of model evaluation showed that energy-protein flux models (*EP* models) have lower errors in predicting fish growth than pure bioenergetic models (MAPE_{bw} ~9% against ~13%, respectively), showing the importance of considering protein intake when estimating Nile tilapia growth. Furthermore, assuming the fixed standard metabolic body weight exponents of 0.80 and 0.70 (for energy and protein, respectively), rather than estimating them from the data, seemingly improved the predictive ability of the models.

This approach demonstrates the benefits of coupling bioenergetics with nutrient-based models to predict growth and body composition of Nile tilapia along time.

Keywords: Energy-protein flux models, Bioenergetic models, Prediction tools, Precision aquaculture, Model selection

Highlights

- Digestible energy intake has a negative effect on energy retention efficiency.
- The DP/DE ratio has a negative effect on protein retention efficiency.
- Protein intake data is useful for estimation of tilapia growth and body composition.
- Using standard body weight exponents results in the best models.

1. Introduction

To achieve optimal performance in aquaculture, it is important to have good control of feeding by adapting it to the current state of production, in order to ensure optimal growth at reduced costs and low environmental impact. Therefore, feeding strategies should be optimized, among other considerations, through a careful daily estimation of the standing biomass. Due to the impracticality of daily measurement of fish biomass, it is crucial to understand in an objective and practical way, the growth patterns, and their underlying mechanisms, to allow (for example) biomass estimation between measurements. Mathematical models are a good tool to use in such cases, where it is impracticable to directly measure the system response, and also assist in data interpretation and decision support (Føre et al., 2018).

Traditionally, empirical mathematical models (e.g., *SGR, TGC, FCR*) have been used in aquaculture to predict growth due to their simplicity and ease of parameter determination. However, such models often ignore important aspects, such as feeding information (e.g., feed quantities or properties), and/or lack clear physical/biological support, being inadequate as general context-independent prediction models. In academic contexts, other alternatives have been suggested, such as the use of bioenergetic and nutrient-based models, which are based on the principles of energy and nutrient balance:

$$\text{net balance} = \text{intake} - \text{losses}$$

These types of models take into consideration feed quantities and (at least some) properties, which allows them to provide more precise growth predictions. A common bioenergetic modelling approach is to describe the partitioning of food energy into excretory energy (FE, UE and ZE), heat production/increment (HiE) and recovered/retained energy (RE) (Brett & Groves, 1979; Xie et al., 2011). The RE can therefore be calculated as:

$$\mathrm{RE} = \mathrm{IE} - \mathrm{FE} - (\mathrm{UE} + \mathrm{ZE}) - \mathrm{HiE},$$ (Xie et al., 1997, 2011)

where IE represents the intake component, while FE+(UE+ZE) +HiE represents the losses component. However, this type of model can be converted to a simpler model where the losses are subdivided into two components: intake-dependent losses (*COG*DE*) and intake-independent losses (*FM*) – the advantage of this approach is that it is simpler (i.e., less parameters) and thus more identifiable and easier to calibrate:

$$\mathrm{RE} = (1 - \mathrm{COG}) \times \mathrm{DE} - \mathrm{FM}$$

where *COG* represents the costs of growth – in this case costs of energy deposition (Bureau et al., 2000; Conceição et al., 1998) – usually equated with the specific dynamic action (SDA), DE is the digestible energy, used for growing and maintenance, and *FM* represents the fasting maintenance losses, which are usually assumed to depend on body weight and temperature:

$$FM(BW,T) = a \times BW^{b} \times e^{c \times T}$$

where *BW* is the body weight, *T* is temperature and *a*, *b* and *c* are estimated parameters. The "b" parameter is often called the "body weight exponent" and has biological significance as it represents how costs scale with fish size.

Bioenergetic models often have significant limitations in describing the chemical composition (i.e., moisture, protein, lipid, and ash) of biomass gain (Dumas et al., 2010). Additionally, they can present several drawbacks when applied under the overly simplistic assumption that the energy density of fish is constant (Canale & Breck, 2013; Dumas et al., 2010). In turn, nutrient-based models, such as energy-protein flux models (*EP* models), besides energy, also consider protein intake to predict growth and body composition based on protein retention equations along with fish weight and temperature (Nobre et al., 2019). In these models, maintenance requirements are influenced by body size and temperature, whereas growth requirements are influenced by the magnitude and composition of weight gain (Bureau et al., 2003; Lupatsch et al., 1998, 2003b, 2003a; Lupatsch & Kissil, 2005). Furthermore, the metabolic expenditure (protein and energy losses) for maintenance can be characterized by an exponent that determines the change in metabolic rate as a function of body weight (Lupatsch & Kissil, 2005).Thus, in this work, we consider an alternative parametrization of the model mentioned above, where an explicit "retention efficiency coefficient" (k) replaces the COG parameter, applying it both to energy and protein retention:

$$\mathrm{RE} = k_{E} \times \mathrm{DE} - \mathrm{FM_{E}}$$

$$\mathrm{RP} = k_{P} \times \mathrm{DP} - \mathrm{FM_{P}}$$

The estimation of these exponents is already documented for different fish families (Clarke & Johnston, 1999) and species, such as barramundi *Lates calcarifer* (Glencross, 2008), gilthead seabream *Sparus aurata* (Lupatsch et al., 1998), European seabass *Dicentrarchus labrax*, white grouper *Epinephelus aeneus* (Lupatsch et al., 2003a; Lupatsch, Kissil, & Sklan, 2001) and yellowtail kingfish *Seriola lalandi* (Booth et al., 2010), and generally similar metabolic weight exponents of energy and protein have been reported. Therefore, it is broadly accepted to assume standard metabolic body weight exponents of 0.80 and 0.70 for energy and protein, respectively. Though it is possible to estimate the metabolic body weight exponents for energy and protein using calibration data related to experiments performed either with fed and unfed fish, it is not clear whether it is preferable or more correct to do so over assuming fixed standard exponents.

There are still some scientific and technical questions regarding the implementation of nutrient-based models, which perhaps hampers their more widespread adoption in commercial settings. One could be the lack of transparency regarding the way models are developed and the assumptions that are made during calibration, which often makes them impossible or very difficult to replicate. During the model development process, it is important to assess both calibration and validation errors. First, one should calibrate models under different assumptions (i.e., using different calibration methods) and estimate the performance of the calibration process (e.g., through cross-validation) in generating models that effectively predict new data. This evaluation should be carried out as objectively as possible, considering not only quantitative criteria (e.g., error metrics), but also qualitative ones (e.g., diagnostic plots), in order to consistently ensure an acceptable predictive ability of the models. Ultimately, to ensure that the model can effectively predict new data (i.e., data not used during the calibration process), and to fairly quantify expected prediction errors, it is necessary to perform a validation with an independent data set and assess validation errors.

There is a need to explore and develop models that can be fairly easy to use by fish farmers and still have the ability to predict fish growth and composition based on fundamental properties of fish metabolism. Therefore, there must be an effort by the scientific community to overcome some scientific and technical issues regarding the implementation of these models by the industry and thus bridge the gap between both.

Herein, Nile tilapia was chosen as a model fish due to its aquaculture importance, since it is one of the most economically important fish species in aquaculture. Moreover, it is a widespread distribution species - which in part is linked to its remarkable ability to tolerate diverse environmental condition (e.g., water temperatures, salinities and oxygen levels). Additionally, it is omnivorous species and thus can thrive on a variety of diets. These makes Nile tilapia an ideal species for investigating responses not only to environmental, but also to different nutritional stressors. In this work, Nile tilapia growth data were collected and used to compare different combinations of models and calibration methods, using objective criteria based on cross-validation, to determine the best combination for this species. Additionally, we also sought to clarify the relative merit of different biological and technical hypotheses by calibrating models under these different assumptions and comparing them in terms of their capacity to generalize for new data.

2. Materials and methods

2.1 Data collection and analysis

Nile tilapia growth data were collected from the literature to be further used for model calibration and validation, covering a wide range of rearing conditions and feed composition (Table 1 and Table 2, respectively). All datasets were converted into a standard format where information on the growth, body composition, feed conversion ratio (FCR), water temperature and diet properties are stored on a daily resolution basis. Missing data was handled by using default values (e.g., apparent digestibility coefficients) or by applying interpolation methods (e.g., daily feed intake was estimated based on FCR and growth, when not reported in literature.

An exploratory analysis of calibration data was performed to see how relevant responses were affected by different factors (e.g., body weight, feed intake, digestible protein intake, digestible energy intake) and to obtain information that could be relevant for model development. This analysis consisted mainly of scatter plot visualization and trend line evaluation (linear vs. non-linear) of ingested against retained protein and energy fractions, using the F-test to assess the statistical significance of results.

2.2 Model evaluation and calibration methods

Growth models with diverse levels of complexity (FCR, bioenergetic, energy-protein flux) were developed. In addition, 2 models already published by other authors (Chowdhury et al., 2013; van Trung et al., 2011) were recalibrated with our dataset in order to fairly evaluate their performance. Different calibration methods were implemented: by considering either least squares or Huber loss; by considering either fixed standard or estimated body weight exponents for the fasting maintenance and body composition sub-models; and by considering either only fixed effects or both fixed and random (mixed model) effects during model calibration. Body weight exponents were estimated by considering that protein/energy loss in fasted fish is proportional to body weight (BW) raised to a certain exponent. Thus, by applying multilinear regressions of log(loss) as a function of log(BW) and temperature, the BW exponents are recovered as one of the coefficients. To complement the limited data available for Nile tilapia under fasting conditions, the exponent estimates also considered (with low weight) fasting loss estimates obtained for growing fish by extrapolation of the trial-wise gain/intake relationships to 'zero intake'. Therefore, by calibrating the different models under the different sets of assumptions (see Figure 1), 73 combinations of "models + calibration methods" were obtained and their performance was evaluated. The combinations of "model + calibration method" were evaluated qualitatively by visual examination of model behaviour (e.g., observed vs. predicted plots) and residuals (e.g., Q-Q plots), and quantitatively by calculating the mean absolute percentage error (MAPE) and root mean square error (RMSE), as follows:

$$MAPE = \frac{1}{n}\, \Sigma_{i=1}^{n} \left|\frac{y_i - \hat{y}_i}{y_i}\right| \times 100$$

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

where, $\hat{y}_i$ is the predicted value, y_i is the observed value and n the number of predicted-observed value pairs.

Moreover, in addition to calibration error metrics, focus was given on cross-validation errors obtained through different cross-validation methods (k-fold cross-validation with k=10, k=5 and k=2, with 5, 10 and 25 repetitions, respectively), to ensure that the chosen models display the best performance when predicting outside their calibration set. To determine the risk of overfitting and to decrease the degree of subjectivity, cross-validation was performed with different k values in order to subject the combinations to different levels of "difficulty", since, as the k value decreases, less data is available to calibrate the model.

All analyses were performed using R version 4.1.2 (R Core Team, 2021), where the *rlm* function from the MASS package (Venables & Ripley, 2002) was used to calibrate the robust models and *nlme* function from the nlme package (Pinheiro & Bates, 2000) was used to calibrate mixed-effects models.

2.3 Validation of calibrated models

From the 73 “model + calibration methods” combinations, a representative sample of 12 models of different types and with different levels of performance were chosen to be validated. In addition to the models from Chowdhury et al. (2013) and van Trung et al. (2011) that were recalibrated with our data, the same models were also validated using their original parametrization (BE_Chow_par model from Chowdhury et al., 2013; EPG1_par model from van Trung et al., 2011).

3. Results

3.1. Data analysis

Exploratory data analysis suggests a direct relationship between digestible energy intake and energy gain, and between digestible protein intake and protein gain

(Figure 2). It appears that there is better efficiency in protein gain, since the data tends to be closer to the $y = x$ line than the energy data. Moreover, since both linear and quadratic models fit the data accurately, there is no strong evidence for a saturation effect at higher intake levels, on either energy or protein gain. On the other hand, though digestible protein intake does not appear to affect protein retention efficiency (F-test $P > 0.05$), which is consistent with the lack of a saturation effect, the same was not observed for digestible energy intake, where a negative effect on energy retention efficiency (F-test, $P < 0.001$) was observed (Figure 3).

Regarding the effect of body weight, while there is no evidence of an effect on protein retention efficiency (F-test $P > 0.05$), a positive effect of fish body weight on energy retention efficiency (F-test, P < 0.001) was observed (Figure 4). Furthermore, no effect was found for DP/DE ratio on energy retention efficiency (F-test, $P > 0.05$) (Figure 5 A). However, it seems that an increased DP/DE ratio has a negative effect on protein retention efficiency (F-test, $P < 0.001$) (Figure 5 B).

3.2. Model calibration and evaluation

Considering the results of cross-validation, nutrient-based models, like the EP model (energy-protein flux), generally appear to provide better predictions than the simpler bioenergetic models. Overall, the results of the different cross-validations were consistent, in the sense that the best and worst performing models were generally the same regardless of the type of cross-validation used. However, it is important to mention that the overall agreement was not entirely exact, since each cross-validation method presented a slightly different order/classification for models (Table 3).

In particular, EP models displayed calibration and cross-validation errors between 7 and 11% for body weight, and between 12 and 14% for body composition. In turn, bioenergetic models had calibration and cross-validation errors between 9 and 26% for body weight, and ≈13% for body composition predictions (Table 3). Additionally, models had better prediction capabilities when calibrated under the assumption of fixed standard body weight exponents for fasting maintenance (0.80 and 0.70 for energy and protein, respectively) or with isometric ones (exponents=1). By visually analysing the models' behaviour, it was evidenced that the same model can display distinct behaviours when calibrated with different methods (see, for example, Figure 6).

The model's complexity seemed to affect its predictive capability (Table 3). Overall, models that only considered energy (i.e., BE models) to predict growth had higher prediction errors than models that also consider protein besides energy (i.e., EP models). Furthermore, bioenergetic models, like BE, displayed poor growth predictions and additionally presented large discrepancies in the initial predictions of body composition

(Figure 6). This was further supported by analysis of the calculated metrics (e.g., MAPE), in which cross-validation errors were higher than calibration ones. The bioenergetic model obtained from Chowdhury et al. (2013) (BE_Chow) was the one with the highest calibration and cross-validation errors, with a MAPE of 26% for body weight and ≈13% for body composition. In turn, the BECOG_iso model was the bioenergetic model with the best performance, showing calibration and cross-validation errors between 9 and 12% for body weight, and ≈14% for body composition.

The van Trung et al. (2011) model (EPG1) performed better than the BE_Chow, obtaining calibration errors with a MAPE 9% for body weight and ≈15% for body composition; cross-validation errors with a MAPE of 12% for body weight and ≈16% for body composition was also obtained (Table 3). Interestingly, the FCR model had better predictions than the bioenergetic models in general and the EPG1 model, in general, showing calibration errors with a MAPE of ≈11% for body weight and ≈13% for body composition, and cross-validation errors with a MAPE≈10% for body weight and ≈12% for body composition (Table 3).

The metabolic body weight exponents were estimated for the calibration dataset (Table 1) by different methods and varied between 0.71-0.77 for energy and 0.76-0.78 for protein. However, models in which the standard metabolic body weight exponents (0.80 and 0.70, for energy and protein, respectively) were assumed showed a better predictive capability than methods that considered the estimated exponents (Table 3).

3.3. Model validation

Out of all tested models, the EP5_mixed (energy-protein model calibrated assuming mixed effects) and the EP5_u_rlm models (energy-protein model calibrated with Huber loss linear regression and with fixed body weight exponents for maintenance costs) displayed the best performance: both models presented similar validation errors, with a MAPE of 9 % for body weight (Table 4). In qualitative terms, both models predicted the validation data well, demonstrating a reasonably capacity to predict Nile tilapia growth and composition (Figure 6). Although both EP5_mixed and EP5_u_rlm displayed similar validation errors in predicting body weight, the EP5_mixed presented slightly lower validation errors when predicting body composition (MAPE of 7% vs. 8%), which may indicate more accurate body composition predictions (Figure 7).

Overall, the bioenergetic models have worse performance in predicting Nile tilapia growth than EP models, though BECOG_iso seems to be the best tested bioenergetic model (MAPE$_{bw}$ ≈13% and MAPE$_{bc}$ ≈8%). Nevertheless, the tested models show fair predictions for Nile tilapia growth, except for the BE_Chow (MAPE$_{bw}$ = 28% and

$MAPE_{bc}$ = 7%), EPG1_par ($MAPE_{bw}$ = 34% and $MAPE_{bc}$ = 116%) and BE_Chow_par ($MAPE_{bw}$ = 178 % and $MAPE_{bc}$ = 10%).

The FCR model displayed the lowest RMSE validation error (16.81 g) of all tested models and one of the lowest MAPE values for both weight and body composition ($MAPE_{bw}$ = 11% and a $MAPE_{bc}$ = 7%).

The Chowdhury et al. (2013) and van Trung et al. (2011) models seem to perform better when parameterised with the dataset used in this study (BE_Chow $MAPE_{bw}$ = 27 % *vs.* BE_Chow_par $MAPE_{bw}$ = 178 %; EPG1 $MAPE_{bw}$ = 10% *vs.* EPG1_par $MAPE_{bw}$ = 34%). However, regardless of the parameterization used, the model retrieved from Chowdhury et al. (2013) performed better when body composition is updated based on the composition of the body weight gained (*BE_Chow2* and *BE_Chow2_par*), rather than considering the whole-body weight of the fish (BE_Chow and BE_Chow_par) (Table 3).

4. Discussion

Mathematical models can be useful tools to describe the various processes involved in fish growth. It is necessary to understand the processes to be modelled, and careful and thorough data collection and analysis is of utmost importance. This will help describe and clarify the relationships inherent to the processes, before starting developing the model. In this context, the relationship between protein and energy intake, and deposition is one of the key aspects underlying fish growth. The data collected in this work pointed towards a linear relationship between digestible energy intake and energy gain for Nile tilapia. This is consistent with what has been previously demonstrated by Lupatsch et al. (2010) for Nile tilapia and by Lupatsch et al. (2001, 2003a, b) for gilthead seabream, but does not support the quadratic relationship model that Van Trung et al. (2011) reported for Nile tilapia. Likewise, the relationship between digestible protein intake and protein gain is linear, confirming previous observations (Lupatsch et al., 1998, 2010). Nevertheless, other studies reported a quadratic relationship in Nile tilapia (Van Trung et al., 2011) and other species (Booth et al., 2010; Glencross et al., 2011; Glencross, 2008; Lupatsch, Kissil, Sklan, et al., 2001). Konnert et al. (2022) have recently stated that protein gain in Nile tilapia follows a two-phased response to increasing protein intake, being described by a linear–plateau model. Their model indicates that protein deposition is linear until 8.4 g $kg^{0.8}$ day^{-1} and then levels-off. In the present study instead of a level-off pattern, a linear relationship was observed, possibly because of data scarcity above 8 g $kg^{0.8}$ day^{-1}. This is mainly because most of the trials in the literature do not overfeed tilapia with fat or protein, making it impossible to obtain data showing the effect of saturation on energy or protein gain. Thus, what the results show is that the linear model works better than the quadratic model to show the relationship between intake and gain when Nile tilapia is fed diets that are not extremely dense in terms of protein or fat composition. Furthermore, our analysis shows that the energy retention efficiency is body weight-dependent. Likewise, Breck (2011), showed that fish energy density varies with body size and should be considered to enhance the performance of bioenergetic models. Moreover, DP/DE was shown to negatively affect protein retention efficiency, supporting previous studies by Haidar et al. (2018) and Konnert et al. (2022) in Nile tilapia. But this does not apply to energy retention efficiency, as Konnert et al. (2022) had already reported for Nile tilapia.

During model evaluation, the quantitative and qualitative analyses were combined with different cross-validation methods to objectively identify the best models and calibration methods. Though generally consistent, the results of these were not always entirely in agreement due to how each method penalizes models according to their complexity: methods with lower k-values are more restrictive and therefore exert a higher penalty on more complex models, than methods with higher k-values (Hastie et al., 2001).

After the calibration and cross-validation process, the models were validated with an independent Nile tilapia dataset, where EP5_u_rlm and EP5_mixed displayed the lowest validation errors and thus, were considered the best models to be used to predict Nile tilapia growth. However, it should be noted that the EP5_mixed model has slightly lower validation errors for predicting body composition than EP5_u_rlm. This difference may be associated with the fact that the EP5_mixed model uses a calibration method where body composition is predicted with mixed-effect models. One advantage of using this type of approach is that it allows to overcome problems related to data heterogeneity (Schielzeth et al., 2020). The dataset used in this study is composed by data collected from different sources with distinct number of data/points, and each one with different conditions (e.g., types of culture, feeds, temperature, strains) that somehow can affect fish growth and body composition. Moreover, mixed-effect models are more flexible and thus overcome violations, when present, in distributional assumptions (Gelman & Hill, 2006; Schielzeth et al., 2020). Furthermore, such models, can determine the appropriate shrinkage (i.e., partial pooling) for low-sample groups, pushing extreme values towards the mean (Schielzeth et al., 2020) and therefore, no matter how many points each source has, all will have balanced weights when performing regression analysis. The use of mixed effects models is therefore strongly advised when using heterogeneous datasets for model calibration.

In this study, different assumptions were made when developing bioenergetic models, which were based on the preliminary data analysis. For instance, for the BE model it was assumed that the fish energy density was body weight-dependent, but costs of growth (COG) were constant, whereas in BECOG both the costs of growth and fish energy density were body weight-dependent. The results showed that assuming that fish energy density and cost of growth are both body weight-dependent seems to be the most correct assumption, as the BECOG model proved to be the best performing bioenergetic model. Moreover, these results emphasize that the energy retention efficiency of Nile tilapia is seemingly body weight-dependent and should be taken into consideration when modelling growth (Breck, 2011), as also suggested by the preliminary data analysis.

The models that were developed in this study presented lower validation errors when compared to those previously reported by van Trung et al. (2011) and Chowdhury et al. (2013) for Nile tilapia. In fact, when these models are parameterized with the dataset used in this study (BE_Chow and EPG1), they display better performance than when using the original parametrization obtained by the authors (BE_Chow_par and EPG1_par), highlighting the relevance of the dataset used to calibrate fish growth models as they can affect their prediction quality. Other factors may explain the poor performance of BE_Chow and BE_Chow_par models, such as the fact that protein intake is not considered and that the update of body composition is forced to follow the body composition model in Chowdhury et al. (2013).

Another important factor that affects models' performance is how the different body components (protein, lipids, water and ash) are updated and predicted. In this work, two different approaches were considered: a) predict body composition with a static model, directly as a function of fish body weight (e.g., BE, FCR, BE_Chow models); or b) use a dynamic model that updates water and ash according to protein retention, which in turn is calculated based on feed intake and feed composition. The first approach displayed large discrepancies in the initial predictions of body composition when compared to the observed ones, besides the poor predictions for growth. In turn, when the body composition is predicted based on protein retention equations (e.g., EP models), models displayed accurate body composition and growth predictions. However, the EP models when considering energy do not include the distinction between fat and carbohydrates. Therefore, this type of models may perform better for carnivorous species because the energy input comes mainly from fat and protein, while omnivorous species such as Nile tilapia use fat, protein and carbohydrates as energy sources (Boonanuntanasarn et al., 2018; Stone, 2010). Interestingly, the FCR model, although one of the simplest tested models, displayed better performance than the bioenergetic ones. Although FCR had shown lower absolute errors (RMSE), they translate into higher relative errors for small fish, which explains the suboptimal MAPE displayed by this method. The overall good performance displayed by this method can be explained, at least in part, by the fact that the daily feed intake in the dataset used in this study was often estimated based on FCR values reported in each source, which may provide the FCR model an unfair advantage. In addition, the good performance of FCR may also be related to tilapia dietary requirements when compared to other finfish species. Nile tilapia can digest carbohydrates well (Maas et al., 2020; NRC, 1993) and thus grow well when fed carbohydrate-rich and protein-poor diets (Bomfim et al., 2008; Botaro et al., 2007; Furuya et al., 2005). Therefore, since FCR ignores variations in diet properties, it makes the model perform somewhat reasonably well, even under extreme variations of these factors. However, the EP models had ≈ 3% more prediction capability compared to FCR, which shows that it is however still important to take protein input into account when modelling tilapia growth.

Furthermore, the type of relationship between energy/protein intake and gain is also important to be considered. In fact, this is the main difference between the van Trung et al. (2011) models (EPG1 and EPG1_par) and the EP models developed in this study: the EPG1 uses quadratic relationships, while the EP5 model assumes linear relationships. This does not mean that the van Trung et al. (2011) approach was necessarily incorrect, but rather that the assumptions made in model development are highly dependent on dataset selection. As previously mentioned, the data collected in this study do not describe any kind of quadratic but linear relationship between energy and protein. Therefore, linear equations were considered when developing the models. It is also for this reason that the *EPG1* model calibrated with our data performed slightly worse than the *EP* models developed in present study.

There is a lack of agreement between authors regarding the exponents for metabolic weight of fish. Clarke & Johnston (1999) reported that a metabolic body weight exponent for energy of 0.75 can be used for several fish species. Ye et al. (2021) also stated that the scaling exponent for Nile tilapia is not significantly different from 0.75, as described by “Kleiber’s law” (Kleiber, 1932). Some authors (Lupatsch et al., 1998, 2003b) have used measurements obtained under fasting conditions to help examine the relationship between changes in energy or protein and body weight. Ultimately, their findings indicate that protein and energy loss cannot be described by the same metabolic body weight. Others, like van Trung et al. (2011) and Glencross et al. (2011) reported for Nile tilapia and Pangasius catfish (respectively) a metabolic body weight exponent of 0.80 for both energy and protein. Both argue that it may have to do with the fact that tilapia is a low trophic level species, which results in different protein utilisation parameters compared to carnivorous species. In this study, we developed models that assumed either fixed/standard exponents for energy and protein (0.80 and 0.70, respectively) according to Lupatsch et al (1998, 2003b, 2010) and Glencross (2008) reports, and models in which these exponents were estimated. Although the estimates of body weight exponents (0.71-0.77 for energy and 0.76-0.78 for protein) were close to standard, models in which the standard metabolic body weight exponents (0.80 and 0.70, for energy and protein, respectively) were used showed a better predictive capability than methods that considered the estimated exponents. This shows that, apparently, it is possible to use the standard values of metabolic body weight exponents (0.80 and 0.70, for energy and protein, respectively), even for omnivorous fish species.

5. Conclusion

The results of this study contributed to a better modelling of the processes involved in Nile tilapia growth and body composition regulation and underline the importance of having a dataset covering a wide range of rearing and feeding conditions for model development. Moreover, the evaluation of models with different calibration assumptions and cross-validation methods, allowed an exploration of different possibilities and contributed to the clarification of important scientific (e.g., best model to predict growth) and technical questions (e.g., best calibration method). This study also highlighted that using information on protein intake is important to improve the estimations of Nile tilapia’s growth and body composition models. Additionally, the standard body weight exponents for maintenance costs for energy and protein (0.80 and 0.70, respectively) seem to be applicable also to Nile tilapia. It can, therefore, be speculated that omnivorous fish species use energy and protein in a similar way to carnivores. Ultimately, in some cases, the use of mixed-effects modelling for model calibration can be a good approach to overcome problems related to data heterogeneity. Thus, the developed work provides a meaningful contribution to a more widespread adoption of highly predictive nutrient-based fish growth models for Nile tilapia, along with best practices in their development and evaluation, which can also be applied to other fish species.

Nevertheless, despite the good prediction quality demonstrated by the EP models developed in this work, they have some limitations. For example, the inability to predict the impact of diets with imbalanced amino acid profiles on fish growth. Therefore, in the future it is important to incorporate other mechanistic features in such growth models, besides the addition of energy and protein intake. Additionally, modelling of amino acid metabolism is likely to increase the predictive accuracy of growth models for Nile tilapia and other fish species, providing a useful tool for the aquafeed sector.

Author statement

All authors significantly contributed to the content of this manuscript. Conceptualization: T. S. Silva, F. Soares, A. Nobre, L. E. C. Conceição; Data curation: T. S. Silva, F. Soares, and A. I. G. Raposo; Formal analysis: T. S. Silva and A. I. G. Raposo; Funding acquisition: L. E. C. Conceição and L.M.P. Valente; Investigation: A. I. G. Raposo, T. S. Silva, F. Soares; Methodology: A. I. G. Raposo, T. S. Silva, F. Soares; Project administration: L. E. C. Conceição and L.M.P. Valente; Resources: A. I. G. Raposo, T. S. Silva, L. E. C. Conceição and L.M.P. Valente; Software: A. I. G. Raposo and T. S. Silva; Supervision: T. S. Silva, L. E. C. Conceição and L.M.P. Valente; Validation: A. I. G. Raposo, T. S. Silva and F. Soares; Visualisation: A. I. G. Raposo, T. S. Silva and F. Soares; Writing – original draft: A. I. G. Raposo; Writing – review & editing: A. I. G. Raposo, T. S. Silva, F. Soares, A. Nobre, L. E. C. Conceição and L.M.P. Valente.

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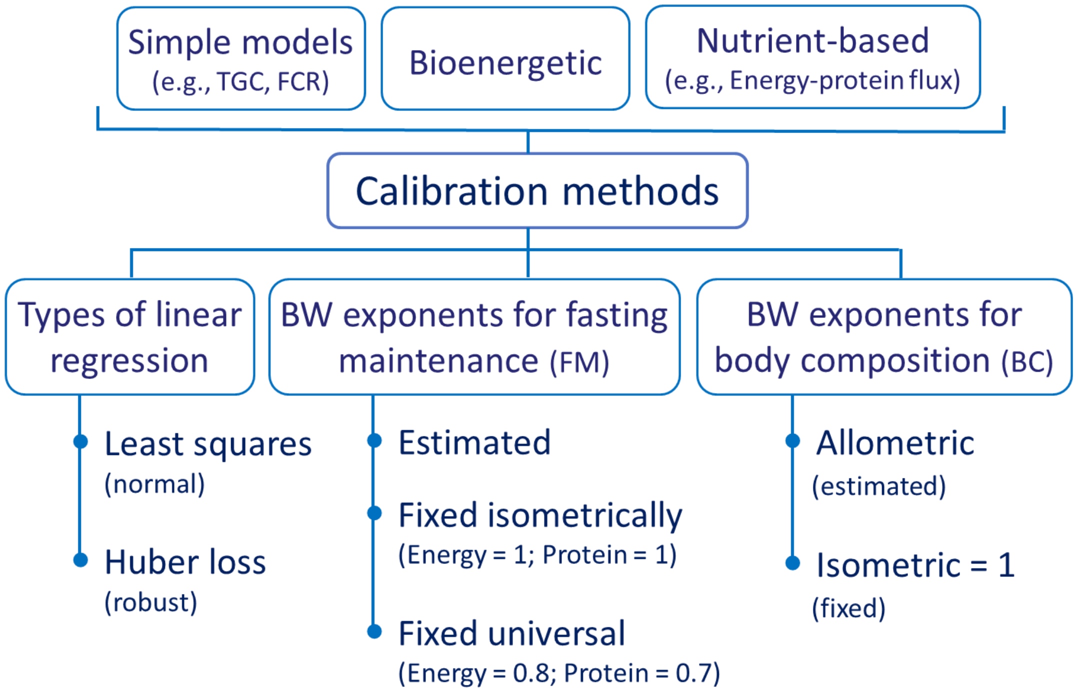

Figure 1 - Diagram explaining the different types of models and calibration methods that have been developed based on different assumptions.

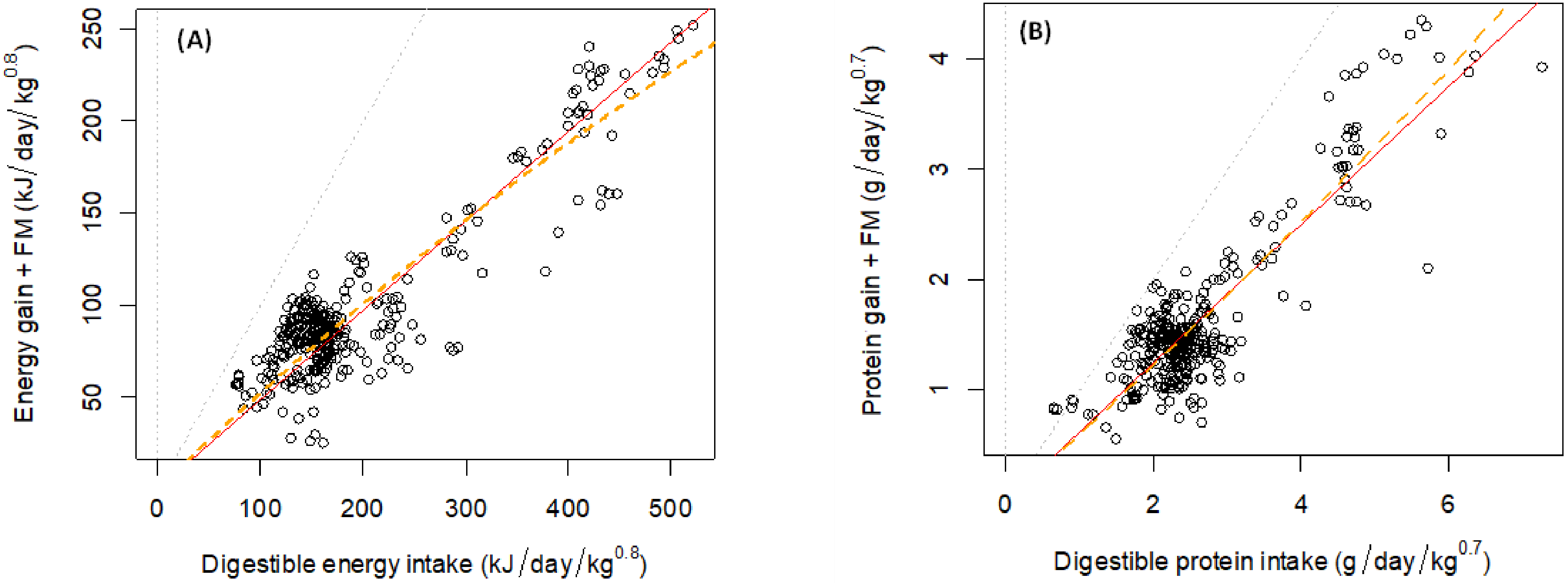

Figure 2 - Scatter plot showing the relation between digestible energy intake (kJ/day/kg$^{0.8}$) and energy gain (kJ/day/kg$^{0.8}$) (A), and the relation between digestible protein intake (g/day/kg$^{0.7}$) and protein gain (g/day/kg$^{0.7}$) (B), corrected by adding the fasting maintenance (FM) costs (to ensure a zero intercept). Black points represent measured values. Red line represents linear regression. Dashed orange line represents quadratic regression. Grey dotted line represents the "y = x" line (theoretical maximal response).

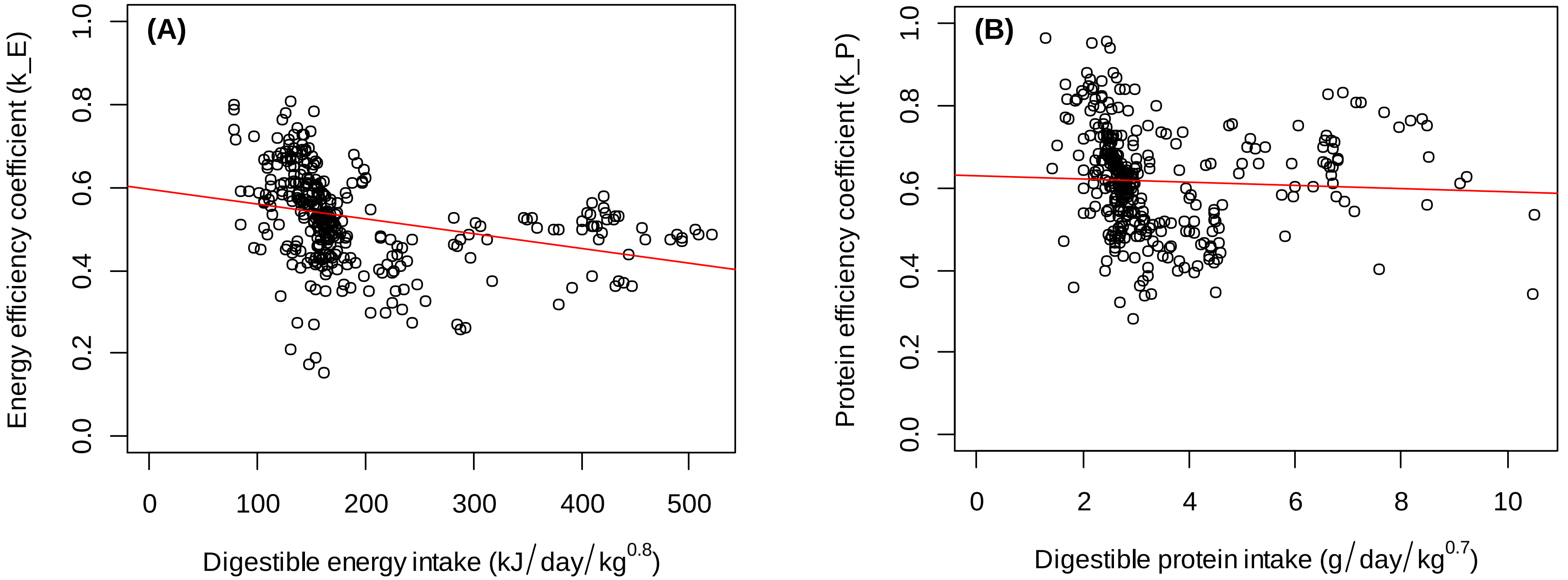

Figure 3 - Scatter plot showing the effect of digestible energy intake (kJ/day/kg$^{0.8}$) on energy retention efficiency (A) and the effect of digestible protein intake (g/day/kg$^{0.7}$) on protein retention efficiency (B). Black points represent measured values. Red line represents linear regression.

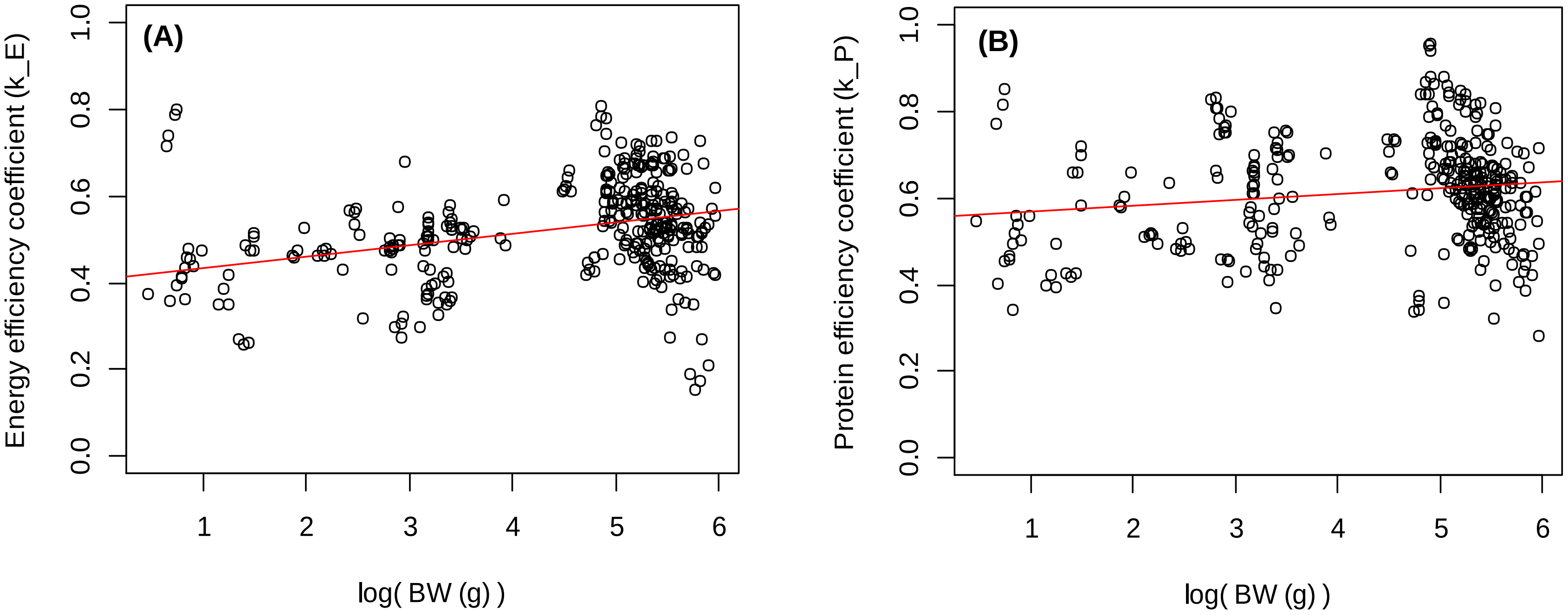

Figure 4 - Scatter plot showing the effect of fish body weight (g) on energy (A) and protein (B) retention efficiency. Black points represent measured values. Red line represents linear regression.

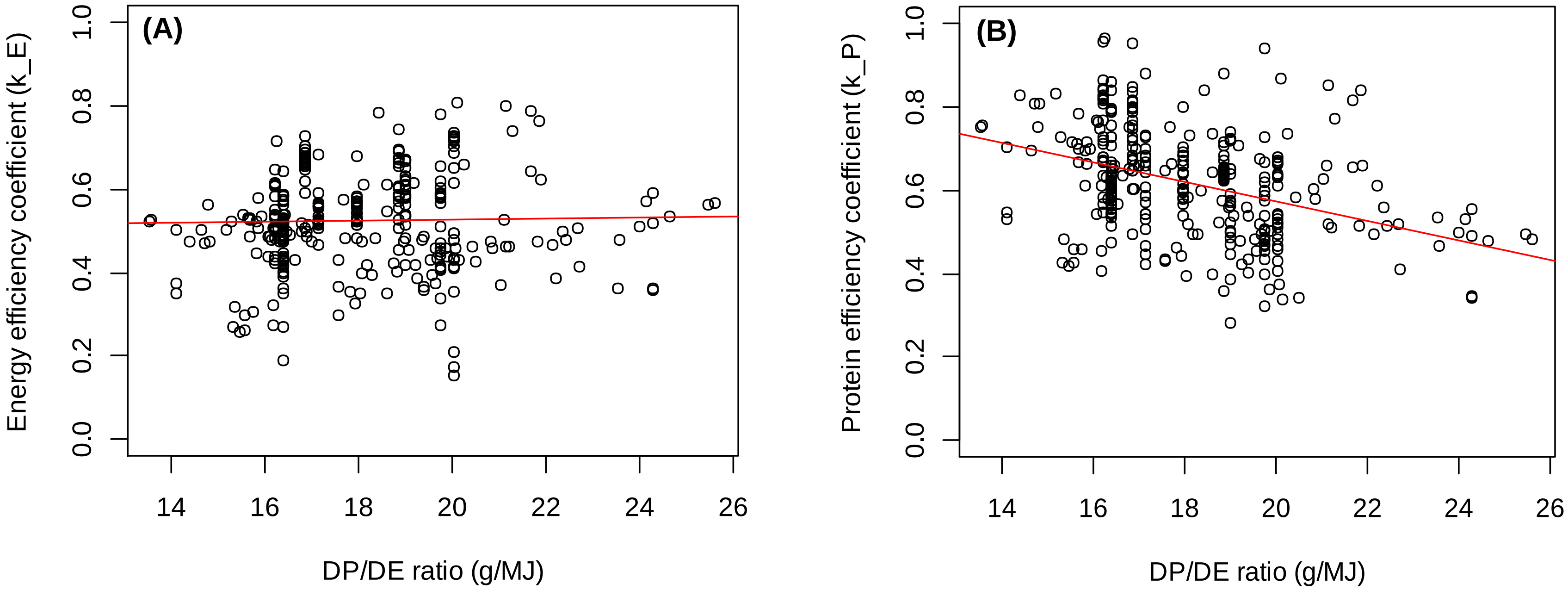

Figure 5 - Scatter plot showing the effect of digestible protein and digestible energy ratio (DP/DE) (g/MJ) on energy (A) and protein (B) retention efficiency. Black points represent measured values. Dashed orange line represents linear regression.

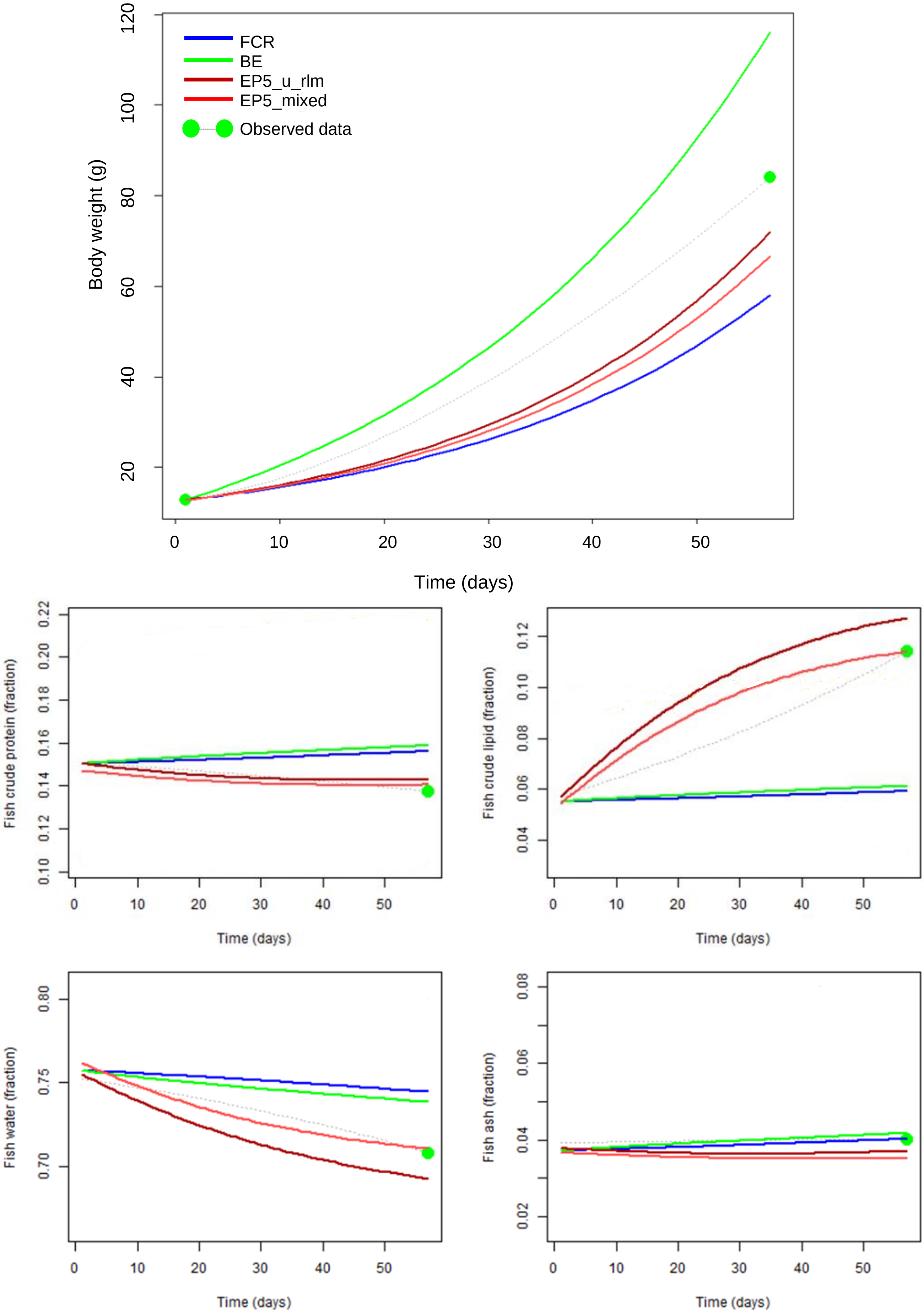

Figure 6 - Line plots showing predicted growth and body composition along time for different models, taking one of the calibration/validation datasets as prediction target. Green points represent measured values. Lines represent either an interpolated body composition value (dashed grey line) or a model prediction (full coloured lines).

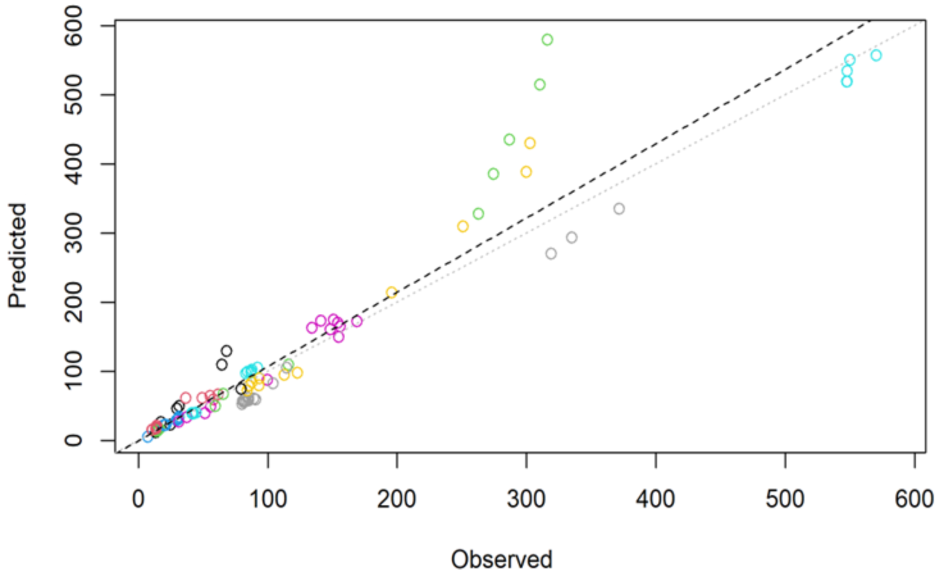

Figure 7 - Scatter plot showing the differences between the observed and the predicted body weight points of EP5_fast_u_rlm model. Segmented black line represents the linear regression of y as a function of x and the dotted grey line represents the y = x line.

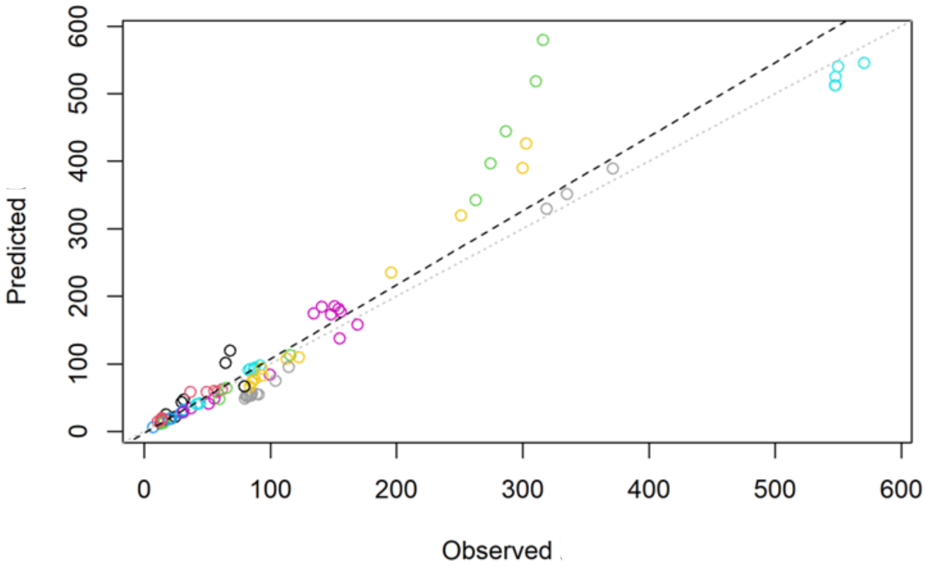

Figure 8 - Scatter plot showing the differences between the observed and the predicted body weight points of EP5_fast_mixed model. Segmented black line represents the linear regression of y as a function of x and the dotted grey line represents the y = x line.

Table 1 - Summary of collected data used to calibrate models.

Attributes	Unit	Nile tilapia
Nr. of data sources	-	27
Nr. of diets	-	150
Body weight range	*g*	0.51 – 457
Temperature range	*°C*	23 – 29
FCR	-	0.9 – 3.9
Diet composition range		
Crude protein	*% as fed*	23 – 46
Crude lipid	*% as fed*	4 – 15
Gross energy	*MJ/kg*	14 – 20

Table 2 - Summary of the independent dataset used to validate models.

Attributes	Unit	Nile tilapia
Nr. of data sources	-	16
Nr. of diets	-	103
Body weight range	*g*	0.51 – 571
Temperature range	*°C*	21.4 – 30
FCR	-	0.9 – 3.5
Diet composition range		
Crude protein	*% as fed*	22 – 45
Crude lipid	*% as fed*	2 – 15
Gross energy	*MJ/kg*	13 – 21

Table 3 – Representative sample of the developed models. Model's error metrics calculated for each method of cross-validation. The greener cells indicate lower estimates of the generalization errors, and the reddish ones indicate higher ones. $MAPE_{bw}$ = Mean absolute percentage error for body weight; $MAPE_{bc}$ = Mean absolute percentage error for body composition. FM = fasting maintenance; BC = body composition; BWexp = body weight exponents. COG = costs of growth.

		CALIBRATION			**CROSS-VALIDATION**		
2-Fold CV	***Model acronym***	**$MAPE_{bw}$(%)**	**RMSE (g)**	**$MAPE_{bc}$(%)**	**$MAPE_{bw}$(%)**	**RMSE (g)**	**$MAPE_{bc}$(%)**
Energy-protein linear regression**,** BWexp = fixed	*EP5_u_iso*	**7,53**	**10,59**	**12,27**	**10,49**	**16,39**	**13,96**
Energy-protein linear regression, mixed effects to calculate all parameters	*EP5_mixed*	**8,00**	**10,49**	**12,13**	**10,94**	**16,41**	**13,15**
Energy-protein robust regression**,** BWexp = fixed	*EP5_u_rlm _iso*	**7,47**	**10,58**	**13,09**	**10,08**	**15,89**	**14,99**
Hybrid Energy-protein allometric/isometric ensemble average, linear regression, FM BWexp = fixed, BC BWexp = estimated	*EP5hyb_u*	**7,55**	**10,20**	**12,43**	**10,56**	**16,26**	**14,20**
Energy-protein linear regression**,** FM BWexp = fixed	*EP5_u*	**7,63**	**10,04**	**12,54**	**10,65**	**16,20**	**14,33**
Energy-protein linear regression**,** BC BWexp = fixed	*EP5_iso*	**7,30**	**9,92**	**12,23**	**11,43**	**16,89**	**13,72**
Energy-protein robust regression**,** FM BWexp = fixed	*EP5_u_rlm*	**7,58**	**10,11**	**13,48**	**10,23**	**15,64**	**15,49**
Energy-protein linear regression, mixed effects only to calculate FM	*EP5_mixed_fm*	**8,63**	**10,78**	**12,66**	**10,84**	**15,50**	**13,42**
FCR, BWexp = estimated	*FCR*	**9,77**	**16,57**	**13,40**	**11,32**	**17,00**	**13,41**
Bioenergetic model, linear regression FM BWexp = estimated, BC BWexp = fixed Fish energy density body weight-dependent, COG body weight-dependent	*BECOG_iso*	**9,19**	**21,39**	**13,85**	**11,81**	**33,89**	**13,96**
***Energy-protein quadratic**, BWexp = estimated	*EPG1*	**8,55**	**10,44**	**14,82**	**11,97**	**15,57**	**19,70**

****Bioenergetic**, linear regression, FM BWexp = estimated, BC BWexp = fixed, Fish energy density constant. Body composition is updated based on the composition of body weight gain.	*BE_Chow2*	13,53	36,94	12,73	14,64	37,40	12,72
Bioenergetic model, linear regression BWexp = estimated, Fish energy density body weight-dependent, COG constant	*BE*	16,25	22,14	13,39	18,55	36,62	13,42
****Bioenergetic**, linear regression, FM BWexp = estimated, BC BWexp = fixed, Fish energy density constant. Body composition is update based on whole-body weight	*BE_Chow*	25,68	57,47	13,11	25,89	57,68	13,10
5-Fold CV							
Energy-protein robust regression, BWexp = fixed	*EP5_u_rlm_iso*	7,81	10,92	12,46	9,58	11,10	12,98
Hybrid Energy-protein allometric/isometric ensemble average, linear regression, FM BWexp = fixed, BC BWexp = estimated	*EP5hyb _u*	8,18	10,89	12,00	10,38	11,53	12,58
Energy-protein linear regression, BWexp = fixed	*EP5_u_iso*	8,13	11,55	11,82	10,31	11,86	12,36
Hybrid Energy-protein allometric/isometric ensemble average, robust regression**,** FM BWexp = fixed, BC BWexp = estimated	*EP5hyb_u_rlm*	7,87	10,38	12,63	9,64	10,76	13,17
Energy-protein linear regression, BWexp = isometric	*EP5_iiso*	9,02	11,92	11,78	10,67	12,29	12,39
Energy-protein linear regression, FM BWexp = fixed	*EP5_u*	8,26	10,54	12,12	10,48	11,37	12,74
Energy-protein robust regression, FM BWexp = fixed	*EP5_u_rlm*	7,96	10,01	12,90	9,72	10,52	13,45
Energy-protein linear regression, mixed effects for all parameters	*EP5_mixed*	8,65	12,91	11,95	10,61	12,09	12,41

FCR, BWexp = estimated	*FCR*	10,49	16,76	12,96	10,05	13,81	12,39
Bioenergetic model, linear regression FM BWexp = estimated, BC BWexp = fixed Fish energy density body weight-dependent, COG body weight-dependent	*BECOG_iso*	8,93	18,79	13,33	10,27	17,35	12,68
****Bioenergetic**, linear regression, FM BWexp = estimated, BC BWexp = fixed, Fish energy density constant. Body composition is updated based on the composition of body weight gain.	*BE_Chow2*	14,49	39,80	12,30	14,04	32,07	11,63
Bioenergetic model, linear regression BWexp = estimated, Fish energy density body weight-dependent, COG constant	*BE*	17,86	19,85	13,02	20,40	23,27	12,34
***Energy-protein quadratic**, BWexp = estimated	*EPG1*	9,01	10,59	14,86	11,46	12,24	15,76
****Bioenergetic**, robust regression, BWexp = estimated, BC BWexp = fixed. Fish energy density constant. Body composition is update based on whole-body weight	*BE_Chow*	26,30	58,74	12,70	25,86	48,83	12,12
10-Fold CV							
Energy-protein linear regression**,** BWexp = isometric	*EP5_iiso*	9,12	11,43	11,78	9,90	9,53	12,93
Energy-protein linear regression, mixed effects for all parameters	*EP5_mixed*	8,81	12,87	12,06	9,77	9,80	13,31
Hybrid Energy-protein allometric/isometric ensemble average, robust regression**,** FM BWexp = fixed, BC BWexp = estimated	*EP5hyb_u_rlm*	8,06	10,42	12,61	9,26	9,25	14,40
Energy-protein robust regression**,** FM BWexp = fixed	*EP5_u_rlm*	8,16	10,04	12,88	9,31	9,03	14,70

Energy-protein robust regression**,** BWexp = fixed	*EP5_u_rlm_iso*	7,99	10,98	12,44	9,22	9,56	14,20
Energy-protein linear regression**,** BWexp = fixed	*EP5_u_iso*	8,36	11,55	11,83	9,88	9,99	13,41
Hybrid Energy-protein allometric/isometric ensemble average, linear regression**,** FM BWexp = fixed, BC BWexp = estimated	*EP5hyb_u*	8,42	10,87	12,01	9,93	9,76	13,63
FCR, BWexp = estimated	*FCR*	10,55	16,88	13,02	9,88	12,26	12,24
Bioenergetic model, linear regression FM BWexp = estimated, BC BWexp = fixed, Fish energy density body weight-dependent, COG body weight-dependent	*BECOG_iso*	8,97	16,91	13,37	10,17	14,28	12,63
****Bioenergetic**, linear regression, FM BWexp = estimated, BC BWexp = fixed, Fish energy density constant. Body composition is updated based on the composition of body weight gain.	*BE_Chow2*	14,70	41,42	12,37	14,12	28,58	11,45
Bioenergetic model, linear regression BWexp = estimated, Fish energy density body weight-dependent, COG constant	*BE*	20,87	18,86	13,11	23,26	19,70	12,29
***Energy-protein quadratic**, BWexp = estimated	*EPG1*	9,34	10,72	14,97	10,98	10,33	15,89
Energy-protein linear regression**,** FM BWexp = fixed	*EP5_u*	9,24	14,08	12,94	10,35	12,50	14,52
****Bioenergetic**, robust regression, FM BWexp = estimated, BC BWexp = fixed. Fish energy density constant. Body composition is update based on whole-body weight	*BE_Chow*	26,43	59,92	12,75	25,96	43,75	11,94

*Van Trung et al. 2011
**Chowdhury et al. 2013

Table 4 – Validation errors metrics for each model after validation with an independent dataset. The greener cells indicate lower estimates of the generalization errors, and the reddish ones indicate higher ones. MAPE_{bw} = Mean absolute percentage error for body weight; MAPE_{bc} = Mean absolute percentage error for body composition. FM = fasting maintenance; BC = body composition; BWexp = body weight exponents. COG = costs of growth.

Model	***Model acronym***	**MAPE_{bw}(%)**	**RMSE (g)**	**MAPE_{bc}(%)**
Energy-protein robust regression**,** FM BWexp = fixed	*EP5_u_rlm*	**9,65**	**31,64**	**7,71**
Hybrid Energy-protein allometric/isometric ensemble average, robust regression**,** FM BWexp = fixed, BC BWexp = estimated	*EP5hyb_u*	**9,74**	**33,40**	**7,71**
Energy-protein linear regression, mixed effects for all parameters	*EP5_mixed*	**9,87**	**32,49**	**6,81**
Energy-protein robust regression**,** BWexp = fixed	*EP5_u_rlm _iso*	**9,88**	**35,41**	**8,01**
Energy-protein linear regression**,** FM BWexp = fixed	*EP5_u*	**10,00**	**33,67**	**7,26**
***Energy-protein quadratic**, BWexp = estimated	*EPG1*	**10,13**	**32,13**	**9,13**
Energy-protein linear regression**,** BWexp = fixed	*EP5_u_iso*	**10,19**	**37,24**	**7,63**
FCR, BWexp = estimated	*FCR*	**11,40**	**16,81**	**6,47**
Bioenergetic model, linear regression FM BWexp = estimated, BC BWexp = fixed Fish energy density body weight-dependent, COG body weight-dependent	*BECOG_iso*	**12,74**	**49,11**	**7,98**
****Bioenergetic**, robust regression, FM BWexp = estimated, BC BWexp = fixed, Fish energy density body weight-dependent, COG constant	*BE_Chow2*	**16,60**	**57,20**	**6,67**
Bioenergetic model, linear regression BWexp = estimated, Fish energy density body weight-dependent, COG constant	*BE*	**19,37**	**22,46**	**6,37**
****Bioenergetic**, robust regression, Fish energy density body weight-dependent, COG constant, with Chowdhury et al. (2013) parameterisation	*BE_Chow2_par*	**21,18**	**57,14**	**9,51**
****Bioenergetic**, robust regression, FM BWexp = estimated, BC BWexp = fixed	*BE_Chow*	**27,38**	**81,77**	**6,47**
***Energy-protein quadratic** with van Trung et al. (2011) parameterisation	*EPG1_par*	**33,73**	**59,68**	**115,78**
****Bioenergetic**, robust regression, with Chowdhury et al. (2013) parameterisation	*BE_Chow_par*	**178,07**	**917,40**	**9,73**

*Van Trung et al. 2011

**Chowdhury et al. 2013

Chapter 6

Modelling growth and body composition: a comparative analysis of seven farmed fish species

Modelling growth and body composition: a comparative analysis of seven farmed fish species

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Abstract

There are a large number of species being produced in aquaculture, and this great variability is a challenge. Therefore, characterising the similarity between species is important because it makes it possible to generalise and transfer knowledge between similar species. A rigorous comparison between species should consider different aspects, such as physiological characteristics, morphology, metabolism, ecology, and behaviour, giving special importance to criteria that can be assessed objectively. In this study, the relationship of similarity between commercial species in terms of parameters and predictions of body composition and growth models was evaluated. Different models (e.g., isometric and allometric, in the case of body composition models) and calibration methods (e.g., least squares, Huber loss regression and quantile regression) were used. Species were compared based on the distance between model parametrizations and between predictions. Results suggest that the similarities within salmonid species are strong and consistent across parameters and thus clearer in the PCA projections. Despite having some differences in growth, European seabass and gilthead seabream also show similarities, especially in the early life stage. However, flatfish species do not group clearly. The variability observed between species in terms of model parameters and predictions may be related to taxonomy, physiological stages, ecological features, fish activity, body mass, as stated in previous studies. Results also suggest the metabolic body weight exponents for energy and protein are likely to be species-specific, ranging from 0.60 to 0.90, which agrees with previous studies that challenge the theory of universal metabolic allometry. This study provides important insights about body composition and growth patterns of different species. Finally, we argue that this research methodology can have a wide range of practical application for both aquaculture and fisheries industries by supporting more accurate data quality control, model calibration synthetic data generation, and assessment of species similarity for effective resource management.

Keywords: Aquaculture, Energy-protein flux model, Regression analysis, Fish nutrition Metabolic allometry

Highlights

- Body composition similarity between species decreases as fish grow.
- Salmonids are the group that show the most consistent similarity.
- The metabolic body weight exponent is species-specific.
- Exploiting species similarities can assist in model calibration for new species.

1. Introduction

Livestock and other animal-source foods are responsible for supplying proteins and essential nutrients to the global population. In 2020, 337.3 million tonnes of land animal protein were produced (FAO, 2022a), distributed between 12 domesticated land animal species (Sandhage-Hofmann, 2016). In turn, production of aquatic animals in 2020 reached a total of 178 million tonnes, in which 313 species of finfish were produced (FAO, 2022b). This suggests that there is significantly more diversity among fish species produced compared to land animals, encompassing various aspects such as physiology, morphology, metabolism, ecology, and behaviour. Consequently, this heterogeneity presents challenges when it comes to, for instance, developing nutritional or growth models for every newly proposed species intended for commercial production, since the research required for each additional species incurs both time and costs. In this context, an important consideration is the generalizability of research findings: can the knowledge gained from studying one species be extended to other closely related species? For example, if a nutritional or growth model is validated for one species, can it reasonably be applied to a similar species? Evaluating generalizability helps determine the broader applicability of research findings. This leads to the transferability of knowledge: how can the knowledge obtained from studying one species be effectively employed to enhance the understanding of other species? By transferring knowledge, it is possible to contribute to practical advances in commercial production, conservation, and resource management. Thus, the study of the similarity relationships between species through the use of objective criteria, such as, genetic (Alarcón et al., 2004; Angienda et al., 2011; Ryman, 1983; Yokogawa & Seki, 1995), ecological (Mablouké et al., 2013; Parrino et al., 2018; Sazima, 1986) or metabolic/nutritional (Azevedo et al., 2005; Bowyer et al., 2013; Breck, 2014; Clarke & Johnston, 1999; Lupatsch et al., 2003a) can assist in the development of nutritional models for farmed fish species. Through such modelling methodologies, it is possible to answer a range of technical and practical questions related to different production aspects, such as, nutritional requirements, optimal feed formulation, optimal feeding/rearing practices, expected growth performance, environmental impact of rearing activities, among others.

In particular, body composition and/or nutrient/energy budget models can be used as objective criteria to explore the similarity relationships between species. To do so, species-specific models are calibrated and then compared either in terms of parametrization (for models with the same structure) or in terms of their predictions, with the assumption that the distances/similarities between species-specific models reflect the distances/similarities between the underlying species. The usual approach described in the literature to predict the body composition of fish is the use of isometric (*a x body weight*) or allometric (*a x body weight* ^{b}) models (Dumas et al., 2010).The difference between isometric and allometric models is that the former assumes that the absolute content of each fish body component is proportional to body weight, whereas the latter assumes a non-proportional relationship between the content of each component and fish body weight. In nutrition studies, it is typically not feasible to conduct comprehensive evaluations of each parameter related to body composition due to the considerable time and effort required for data analysis. As a result, researchers usually make certain assumptions, such as “protein and/or ash follows an isometric pattern in fish” (Shearer, 1994; Dumas et al., 2007; Chowdhury & Bureau, 2009; Chowdhury et al., 2013; Shizari, 2020), often based on limited information or arbitrary/undeclared criteria, without considering the available evidence from other fish species. However, these assumptions may not always be accurate, particularly when assessing body composition in dynamic terms. Unfortunately, the lack of extensive comparisons between species hinders the establishment of global patterns and the ability to make conclusive statements about the effectiveness of these models in different contexts. Therefore, it is necessary to assess the relative merits of isometric vs. allometric models for each component to ensure a more accurate understanding of body composition and its relationship to nutrition in diverse fish species.

To estimate fish growth accurately, models that consider the most important biological and physiological aspects of fish are often used, such as nutrient-based models, in which the fundamentals of energy and nutrient partitioning are considered. The energy-protein flux (EP) model, in particular, in addition to fish weight and temperature, considers both energy and protein intake to predict growth, while also estimating the body composition of fish based on protein retention equations (Nobre et al., 2019). The model explicitly assumes that energy requirements comprehend the energy for maintenance and growth (Booth et al., 2010), where maintenance metabolic expenditure (protein and energy losses) can be defined by exponents that determine the change in metabolic rate as a function of body weight (Lupatsch & Kissil, 2005). However, there is a disagreement between authors regarding the definition of metabolic body weight exponents. Some authors advocate that an exponent of 0.75 can be used for several fish species (Clarke & Johnston, 1999; Kleiber, 1932; Ye et al., 2021), while others recommend the use of species-specific exponents (Lupatsch et al., 1998, 2003b; Lupatsch & Kissil, 2005). Furthermore, while some authors suggest an exponent of 0.80 for energy and 0.70 for protein (Beck & Gropp, 1995; Booth et al., 2010; Glencross, 2008; Lupatsch et al., 1998, 2003a), others argue that, for some species, the same exponent should be used for both energy and protein (Glencross et al., 2011; van Trung et al., 2011). Therefore, it is unclear whether one should assume the same exponents regardless of fish species or whether there are some similarities across a group of species that would justify using the same exponents and, ultimately, whether they should be different for energy and protein.

In this work, commercial fish species, such as Atlantic salmon (*Salmo salar*), gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*), Nile tilapia (*Oreochromis niloticus*), rainbow trout (*Oncorhynchus mykiss*), turbot (*Scophthalmus maximus*) and Senegalese sole (*Solea senegalensis*), were compared on the basis of their body composition and growth performance characteristics. The goal was to assess certain assumptions and hypotheses present in the literature, such as whether isometric or allometric models should be used to describe body composition, the application of "universal" or species-specific metabolic body weight exponents, the use of constant or non-constant protein/energy efficiency ratios, and the assumption of compositional and metabolic similarity between species with similar genetic, morphological, ecological and physiological traits.

2. Materials and methods

2.1. Comparison of body composition models

2.1.1. Data collection

Data on whole-body composition and weight were collected from the literature for seven species (Atlantic salmon, gilthead seabream, European seabass, Nile tilapia, rainbow trout, turbot and Senegalese sole) as shown in Table 1 (see Appendix 1 for list of sources). Carbohydrates are usually unreported, as they represent less than 0.14% of fish (Breck, 2014), and were thus left out of this analysis. Data were collected only from studies in which the whole fish content was determined according to the standard analytical methods of the Association of Official Analytical Chemists (AOAC). The data initially collected in dry matter units were recalculated to reflect wet weight measurements, following Shearer’s (1994) guidance. Each dataset was standardized to contain details about growth, body composition, feed conversion ratio (FCR), water temperature, and dietary properties, all organized on a daily basis.

2.1.2. Model calibration and comparison

Body composition parameters between species were compared based on a set of estimates obtained from two different models (isometric and allometric), both calibrated with three different calibration methods (least squares linear regression, Huber loss linear regression and quantile regression). Additionally, within this process, we also assessed how different data sources affected parameter estimates through leave-one-out (LOO) resampling or jackknife resampling (Efron, 1982). This procedure provided a measure of the variability of parameter estimates while considering the diverse datasets employed for model calibration.

The equations and analysed parameters used in the isometric model were the following:

$$\begin{aligned} protein &= a_{prot} \times BW \\ water &= a_{water} \times BW \\ fat &= a_{fat} \times BW \\ ash &= a_{ash} \times BW, \end{aligned}$$

where *a* gives information about the relative content of each component (assumes proportionality with body weight).

In the allometric model, the following equations and analysed parameters were used:

$$\begin{aligned} protein &= a_{prot} \times BW^{b_{prot}} \\ water &= a_{water} \times BW^{b_{water}} \\ fat &= a_{fat} \times BW^{b_{fat}} \\ ash &= a_{ash} \times BW^{b_{ash}}, \end{aligned}$$

where *a* gives information about the relative content of each component at a reference weight and *b* represents the effect of body weight on each component (assumes non-proportionality with body weight).

For species displaying an isometric relationship between components and fish size, we expect the b parameter (i.e., body weight exponent) estimates for all components to be close to 1 (i.e., their logarithm to be close to zero). Thus, for each species, a (multiplicative) “distance from isometry” was calculated using Euclidian distance, as:

$$d_{iso} = \sqrt{\sum_i \log{(b_i)}^2}$$

where the sum is performed over the square of the logarithm of the body weight exponents of protein, water, fat and ash components. This distance is zero only when the species displays perfect isometry. Using the calculated distance, an “isometry index” was obtained in order to evaluate which species display a more stable body composition as they grow:

$$isometry\ index = e^{-d_{iso}}$$

This index is equal to 1 for species displaying perfect isometry (perfectly stable composition throughout the body weight range), and the lower value, the farther the species is from isometry (body composition changes throughout the body weight range).

The similarity relationships between species in terms of body composition were evaluated through Principal component analysis (PCA), by plotting their respective parameter estimates along the first two principal components. Furthermore, species were compared based on the predictions of isometric and allometric models for their body composition components. This comparison was made through visual observation of line plots. Additionally, the results of both models were assessed for agreement by comparing them through scatterplots. All analyses were performed using R version 4.1.2 (R Core Team, 2021), where the *lm* function from the ‘stats' package (R Core Team, 2021) was used for least squares regression, the *rlm* function from the ‘MASS’ package (Venables & Ripley, 2002) was used for Huber loss linear regression (robust regression), and the *rq* function from the ‘quantreg’ package (Koenker, 2005) was used for quantile regression.

2.2. Comparison of energy/nutrient budget models

2.2.1. Data collection

Data on growth trials covering a wide range of rearing conditions and feed properties were collected from the literature for the selected species (see Table 2 for an overview of the collected data). Senegalese sole growth data were too heterogeneous and patchy (with regards to the joint “body weight and temperature” distribution) and, thus, were left out of the growth comparison to avoid effect confounding and a biased analysis. All datasets were converted into a standard format, where information on the growth, body composition, feed conversion ratio (FCR), water temperature, feed intake and diet properties are stored on a daily resolution basis. Missing data were handled by using default values (e.g., apparent digestibility coefficients) or by applying interpolation methods (e.g., daily feed intake was estimated based on FCR and growth, when not explicitly reported in the data source).

2.2.2. Model calibration and comparison

For each species, an energy-protein flux (EP) model was developed in which, for both energy and protein, a balance between a feed-dependent gain and a feed-independent loss was calculated:

$$Energy\ balance = Energy\ gain - Energy\ loss$$

$$Energy\ gain = (k_E \times DI_E)$$

$$Energy\ loss = \left(fme_a \times BW^{(exp_e)}\right) \times e^{(fme_b \times T)}$$

$$Protein\ balance = Protein\ gain - Protein\ loss$$

$$Protein\ gain = \left(k_p \times DI_P\right)$$

$$Protein\ loss = \left(fmp_a \times BW^{(exp_p)}\right) \times e^{(fmp_b \times T)}$$

Where:

exp_e	Metabolic body weight exponent for energy
exp_p	Metabolic body weight exponent for protein
T	Temperature (°C)
fme_a	Fasting maintenance costs for energy under reference conditions (BW = 1 g; T = 0 °C)
fme_b	Effect of temperature on fasting maintenance costs for energy
fmp_a	Fasting maintenance costs for protein under reference conditions (BW = 1 g; T = 0 °C)
fmp_b	Effect of temperature on fasting maintenance costs for protein
k_E	Efficiency of energy retention (applied before subtracting the fasting maintenance costs)
k_P	Efficiency of protein retention (applied before subtracting the fasting maintenance costs)
DI_E	Digestible energy intake
DI_p	Digestible protein intake

The feed-dependent *gain* depends on an efficiency coefficient (*k)*, and the feed-independent *loss* in turn depends on the effect of the body weight and temperature. The models were calibrated based on different assumptions, which resulted in four different calibration methods (see Table 3). For a better interpretation of the results of the effect of temperature on fasting maintenance costs, the *fme*$_b$ and *fmp*$_b$ parameters were converted and reported as Q10 (temperature coefficient) values (i.e., $e^{(10\times fme_b)}$).

Due to the occasional presence of extreme outlying parameter estimates for some of the leave-one-out folds, which prevents a meaningful PCA analysis, a filtering step was added using a simple univariate rule for outlier detection and removal (|z-score| > 3). This rule is based on the property of the standard Gaussian distribution that 99.7% of its values lie between -3 and 3, thus any z-score greater than +3 or less than -3 can be considered as outlier if we assume parameter distributions to be approximately Gaussian and the number of observations is relatively low (Sarmad, 2006; Shiffler, 1988).

The similarity relationships between species in terms of protein/energy budgets were initially evaluated through PCA analysis, by plotting their respective parameter estimates along the first two principal components. Additionally, the predictions from models calibrated with different methods were assessed through scatter plots to determine if the predictions agreed or not, when fixed universal or estimated parameters were used in the calibration process. All analyses were performed using the regression functions mentioned in section *2.1.2 Model calibration and comparison*.

3. Results

3.1. General similarities between species body composition and growth parameters

The similarity between species in terms of model parameters was evaluated using a PCA analysis (Figure 1). In this analysis, it can be assumed that the distances between points for each species reflect the differences in parameter estimates (e.g., when the points are closer, it indicates that the parameter estimates are more similar) Results suggest that similarities within salmonids are the strongest, consistent across parameters and thus clearer in both PCA projections (Figure 1. a and b). Despite having some differences in growth (Figure 1. B and Appendix 2), seabream and seabass show strong similarities in terms of body composition parameters (Figure 1. a). Flatfish species, however, do not group as clearly. In fact, turbot and sole have distinct body composition parameters (Figure 1. a).

Overall, PCA analysis suggests that water and fat parameters are negatively correlated, and that isometry index is more related with these than with the other components (Figure 1. a).

3.2. Body composition similarities

Figure 2 shows estimates for the relative content of each body component and how they are affected by body weight, for each species, based on the species-specific allometric models (see Appendix 3 for details). Overall, the differences between species in terms of whole-body composition become more pronounced as they grow. This implies that the body composition of small fish (e.g., approximately 1 g) may not be species-dependent (or only weakly so). For instance, when comparing species at a reference body weight of 1 g, fat tends to vary between 2.5 – 7.5 %, whereas when comparing species at reference body weight of 1 kg, the variations are between 5 – 30 % approximately. Ranking fish species in terms of relative fat content at a reference weight of 1kg, seabass and seabream are the fattest species with approximately 20%, followed by salmonids (salmon and rainbow trout) with about 15%, sole with 10%, and tilapia and turbot as the leanest species with approximately 5% fat relative content. For water, the opposite pattern is observed: turbot is the species with highest water relative content (≈ 75% of its body weight), followed by tilapia and sole (≈ 70%), salmonids (≈ 65%), and seabass and seabream with the lowest values (≈ 60% water). In terms of protein, sole is the species with the highest relative content (≈ 20%), followed by salmon and seabream (≈ 18%), and then seabass, rainbow trout, tilapia and turbot (less than 18% of protein). The ash relative content varies greatly even within species and is therefore more difficult to analyse. However, tilapia, turbot, seabass and seabream are the species with the highest ash relative content, while salmonids and sole are the ones with the lowest values.

In terms of how body components are affected by body weight (parameter b), relative water and fat content show high variation, but with clear patterns: water tends to decrease with body weight, whereas fat tends to increase (see Figure 2). Despite the variation, the water slopes seem to be constant and generally negative ($b_{water} < 1$) for all species (see Appendix 3.C for details). For fat, slopes are positive ($b_{fat} > 1$) and variable for all species, being higher for seabream and seabass (highest accumulation of fat as they grow) and lower for tilapia and turbot (lowest accumulation of fat as they grow) (see Appendix 3.B for details). Protein increase as the body weight increases, except for seabass, where the protein content in relative terms seems to be less affected by the body weight, and thus more constant. In fact, the slope tends to be close to zero and b_{prot} much closer to 1 for seabass, when compared with other species (see Appendix 3.A for details). In turn, the effect of body weight on relative ash levels does not present a clear and consistent pattern across species. The relationship between ash relative content and body weight is very variable between and within species (sometimes b_{ash} is above 1, other times below 1), making it unclear whether there is a consistent effect of body weight on ash relative content (see Appendix 3.D for details). Moreover, comparing the isometry index between species (Figure 3), tilapia and turbot seem to be the species closest to isometry, meaning that the effect of the body weight on body components is less strong. In fact, tilapia and turbot BW exponent parameters are in general closer to 1 (isometry) when compared to other species in this study (see Appendix 3).In contrast, seabass and seabream seem to be the ones furthest from isometry, which is consistent with what is shown in Figure 2 where the effects of body weight on body components are generally noticeable.

Regarding the parameter a estimates, there was a general agreement between the isometric and allometric model for each body component of the different species (Figure 4). However, in the specific case of protein, the a_{prot} estimates between the two types of models seem to display lower correlation. Despite this, it is possible to see the same pattern in both isometric and allometric predictions: tilapia, turbot and rainbow trout seem to have lower a_{prot} values than the other species (i.e., lower protein relative content). In terms of fat, the a_{fat} parameter is higher for seabass/seabream and lower for turbot. In turn, the a_{ash} parameter is to be lower for salmonids and sole, and higher for the other species.

Consistent with the PCA analysis, there is a clear negative correlation between fat and water relative content (F-test, $p\text{-}value < 0.001$), according to both allometric and isometric models (Figure 5). Between water and the other two components (protein and ash) there is no such clear pattern. Although a statistically significant negative correlation is present for the isometric model (F-test, $p\text{-}value < 0.001$), these effects are not as clear for the allometric model and are no longer significant for the ash component (F-test, $p\text{-}value > 0.05$).

In general, estimates of the b parameter were also consistent between calibration methods (see Appendix 3), except for b_{ash}.

3.3. Energy/nutrient budget similarities

3.3.1. Metabolic body weight exponent

Overall, the estimated metabolic body weight exponents for energy (exp_e) and protein (exp_p) were not far from what is reported in the literature (0.80 and 0.70 for energy and protein, respectively (Clarke & Johnston, 1999; Lupatsch et al., 2003ab)). The estimate ranges for exp_e were slightly different between species: salmon 0.82 – 0.87; seabass 0.71 – 0.89; seabream 0.51 – 0.72; tilapia 0.69 – 0.85; rainbow trout 0.62 – 0.80; turbot 0.70 – 0.85 (Figure 6). For some species, such as salmon and seabream, the estimated range do not include the standard value of 0.80.

Moreover, for tilapia and trout, most estimates were below 0.80. Seabass and turbot were the only species for which the putative universal exponent (0.80) was found within the estimated range for the parameter exp_e.

For protein, exp_p estimates also seem to be quite different between species: salmon 0.77 – 0.83; seabass 0.78 – 0.97; seabream 0.54 – 0.88; tilapia 0.73 – 0.85; rainbow trout 0.71 – 0.78; turbot 0.61 – 0.74 (Figure 6). In this case, the standard value of 0.70 is not found within the estimated ranges for salmon, seabass, tilapia and trout. Seabream and turbot are the only species for which the estimated range for exp_p include the assumed universal value of 0.70.

3.3.2. Energy fasting maintenance

When converting the estimates of the *fme_b* parameter (which gives information on the effect of temperature on the maintenance costs for energy) to Q10 values, almost all species exhibited values ranging between 1 and 2, with the exception of turbot (Figure 7). Turbot is the species where fasting maintenance costs for energy are most affected by temperature, exhibiting the highest Q10 estimates (approximately 3.5). In turn, rainbow trout appears to be the species in which the energy costs are less influenced by temperature, displaying the lowest Q10 values estimates (approximately 1). The *fme_b* estimates do not seem to be affected by the calibration methods used (see Appendix 4.A for more details).

The estimates for relative energy fasting maintenance costs generally remain consistent when using universal or estimated body weight exponents (see Appendix 3.B for details). Species demonstrate differences in relative energy fasting maintenance costs, with turbot having the lowest costs under reference body weight and temperature conditions, followed by tilapia, seabass, and seabream (see Appendix 4.B). Tilapia is particularly close to seabass. Salmon and rainbow trout, on the other hand, exhibit higher fasting maintenance costs in comparison (*fme_a*).

3.3.3. Protein fasting maintenance

When converting the estimates of the *fmp_b* parameter into Q10 values, most species exhibited values ranging between 1 and 2, apart from turbot and rainbow trout, where the estimated Q10 values were > 2. Turbot and rainbow trout seem to be the species where the fasting maintenance costs for protein are more affected by temperature (see Appendix 4.C). Species such as tilapia, salmon and seabass seem to be those where the relative fasting maintenance costs for protein are least affected by temperature, followed by seabream (see Appendix 4.C).

Tilapia is the species with higher relative fasting maintenance costs for protein under reference conditions (*fmp_a*) (see Appendix 4.D). Other species like salmon, seabass and rainbow trout seem to have lower relative fasting maintenance costs for protein when compared with tilapia.

In general, the estimates for *fmp_b* and *fmp_a* remain consistent regardless of the calibration methods employed, except for tilapia, where some differences are observed between the least squares linear regression and Huber loss linear regression approaches (i.e., lm and rlm, respectively) (see Appendix 4.C and 4.D for more details).

3.3.4. Retention efficiency

Figure 8 shows the estimated values for the energy retention efficiency coefficients. In terms of energy retention, species can be separated into two groups: species with coefficients between 0.45 and 0.50 (e.g., salmon, seabream and trout) and species with coefficients between 0.50 and 0.60 (e.g., seabass, tilapia and turbot). When considering protein retention coefficients, salmon, tilapia, trout, and turbot exhibited higher values (0.55 – 0.65), while seabass and seabream displayed lower values (0.40 – 0.50).

Estimates for energy and protein retention efficiency are insensitive to the use of either universal or estimated metabolic body weight exponents, but there are some differences in terms of the type of linear regression used (e.g., *lm* and *rlm*) (see Appendix 4.F for details).

Overall, salmonid species (salmon and rainbow trout) can be grouped together according to their highest retention efficiency for protein and energy, as illustrated previously in the PCA analysis (Figure 1. b). Seabream and seabass present differences in energy retention coefficients (see Appendix 3.E for details). In turn, tilapia and turbot display closer estimates for both energy and protein retention efficiencies.

4. Discussion

4.1. General similarities between species body composition and growth parameters

It is usually assumed that there should be similarity in terms of body composition and growth between species with similar phylogeny, morphology, ecology and/or physiology. In this study, species were grouped based on similarities in body composition and growth models. In particular: 1) salmonids, with strong and consistent similarities across parameters; and 2) Seabream and seabass with body composition similarities but differences in growth parameters. Flatfish species, in turn, did not group as clearly.

The body composition of the fish is determined by the capacity of species to retain the different macronutrients. Here we found similarities between species in both body composition and growth analyses. Furthermore, the distances in space between species in relation to body composition parameters are, in some cases, similar to those found for the parameters of the growth model. While salmonids are generally close in both analyses, there is not such a clear homogeneity between seabream and seabass and flatfish species. The distance between tilapia and turbot was also different in the two analyses. Tilapia showed similar body composition to turbot, although these similarities were not reflected in the analysis of growth parameters, which may be related to differences in feeding habits (carnivorous vs. omnivorous) and metabolism.

4.2. Body composition similarities

When analysing the similarities in terms of body composition among different species, it was noticeable that species exhibited a higher degree of similarity at very low body weights. Results suggest that, as they grow, fish species tend to diverge in terms of body composition. Seabream and seabass fish species have higher relative fat content than salmonids, when measured at similar body weights. Moreover, salmon tends to have higher protein and lower fat relative content than rainbow trout, as reported by previous authors (Azevedo et al., 2004; Krogdahl et al., 2004). It is important to note, however, that the distribution of data collected for each species varies significantly. For example, for salmon, the database mostly contains information on fish weighing around 51.8 g (median), while for seabass and seabream, the data mostly corresponds to fish weighing approximately 90 g (median). Additionally, the data for salmon covers fish weighing up to 4950 g, while it does not exceed 1000 g for seabream and seabass. Thus, despite these results, some caution should be taken when extrapolating to values outside the range of those used to perform the regression. In flatfish species (i.e., sole and turbot), this divergence may be related to differences in their trophic level (sole 3.2 *vs.* turbot 4.4) (Froese & Pauly, 2023), metabolism or to other factors that may be determined by the different geographic distributions of the two species, such as temperature or depth (Killen et al., 2010). Seabream and seabass are the species where the fat relative content is most affected by body weight (i.e., furthest from isometry), which implies that these species tend to have more variations of fat levels throughout their growth. In turn, tilapia and turbot seem to be the species where the body composition is less affected by body weight (i.e., closest to isometry). In fact, Raposo et al. (2023) reported that, though the body composition of Nile tilapia seems to be allometric, the body weight exponent (*b* parameter) is close to 1 and that, in some cases, it may be reasonable to use isometric models or intermediate models between a purely isometric and allometric models (e.g., ensemble models) to predict Nile tilapia body composition.

The estimates for ash, in this study, turn out to be very variable between and within species, especially for the *b* parameter (b_{ash}), making it difficult to conclude if there are some differences between compared species, and whether b_{ash} is generally different from (i.e., above or below) 1. Generally, ash is the less abundant body composition component in fish and is thus difficult to be determined with precision, due to sample homogenization and manipulation (e.g., if fish samples are not well homogenized, some may contain more scales or bones, which may affect the measurement of ash content by analytical methods). Thus, this may in part explain the variability for b_{ash} estimates. Furthermore, the variability in b_{ash} estimates may also be related to the Leave-One-Out (LOO) resample method used. This is because, when a source related to fish that are either too big or too small is excluded from the estimation, the estimation of the *b* parameter is performed without this information, leading to variable estimates. Additionally, the use of the LOO resampling method, which involves using different datasets in each fold, significantly impacts the estimation of these specific parameters compared to others. Thus, when estimating exponents (e.g., *b* parameter or metabolic body weight exponent), the obtained estimates tend to be highly sensitive to the chosen dataset, contributing significantly to the general variability observed in the estimates. Overall, it seems that the general assumption of isometry for ash ($b_{ash} = 1$) is consistent with our results and probably will not significantly compromise the accuracy of predictions.

4.3. Energy/nutrient budget similarities

4.3.1. Metabolic body weight exponent

Fish growth is a result of several metabolic processes that require energy and thus, may interactively affect metabolic scaling (Glazier, 2005). However, there is a great controversy among authors regarding the exponent of metabolic body weight, which complicates the modelling of fish metabolism and growth. The primary point of contention lies in determining the appropriate and accurate value for the exponent that describes the scaling relationship between metabolic rate and body weight in fish. This controversy makes it difficult to establish a consensus or standardized approach for estimating metabolic rates in fish and understanding their energetics, growth and ecological interactions. In this study, results suggest that the metabolic body weight exponent is different for energy and protein, as reported in previous studies (Beck & Gropp, 1995; Booth et al., 2010; Glencross, 2008; Lupatsch et al., 1998; Lupatsch et al., 2001). Moreover, both energy and protein metabolic body weight exponents may be species-specific, ranging from 0.51 to 0.89, challenging the concept of universal metabolic allometry (Bokma, 2004; Glazier, 2005, 2009; Killen et al., 2010; White et al., 2007). The metabolic body weights for energy estimated in the present study are either within or near the ranges reported for the analysed species: salmon 0.82 – 0.87 *vs*. 0.85 – 1.06 reported by (Cook et al., 2000) and (Kazakov & Khalyapina, 1981); seabass 0.71 – 0.89 *vs.* 0.8 (Lupatsch, Kissil, & Sklan, 2001); tilapia 0.69 – 0.85 *vs.* 0.75 and 0.8 reported by (Ye et al., (2021) and (van Trung et al., (2011), respectively; rainbow trout 0.62 – 0.80 *vs.* 0.8 (Beck & Gropp, 1995); turbot 0.70 – 0.85 *vs*. 0.7 (Waller, 1992). However, for seabream we obtained a range of 0.51 – 0.72, which does not include the value 0.80 reported by (Lupatsch et al., (2003a,b). Although comparable data concerning the metabolic body weight for protein is scarce, some reference values exist in the literature for seabass, seabream, tilapia and rainbow trout. For seabass, the estimated metabolic exponent for protein ranges from 0.78 to 0.97, which does not include the value 0.70 reported by (Lupatsch, Kissil, & Sklan, 2001); for seabream, it is between 0.54 and 0.88 *vs*. 0.70 (Lupatsch et al., 2003a,b); for Nile tilapia 0.73 to 0.85 *vs.* 0.80, close to the value previously reported by Van Trung et al. (2011); for rainbow trout 0.71 to 0.78, similar to the 0.70 value reported by (Beck & Gropp, 1995). The variability of metabolic scaling values between species in this study may be related to taxonomy (Bochdansky & Leggett, 2001; Clarke & Johnston, 1999; Dosdat et al., 1996; Glazier, 2005, 2009), physiological stages (Bochdansky & Leggett, 2001; de Silva et al., 1986; Post & Lee, 1996), ecological features (Killen et al., 2010, 2016), fish activity (Brett & Groves, 1979; Claireaux et al., 2006; Glazier, 2009; Killen et al., 2010, 2016), fish metabolism (Azevedo et al., 2004, 2005; Grisdale-Helland et al., 2007), typical and maximum body mass (Glazier, 2005; Urbina & Glover, 2013), as stated in previous studies.

4.3.2. Energy and protein fasting maintenance

Fish fasting maintenance costs are closely correlated with their size and temperature. It is therefore usual to evaluate the effect of temperature on fasting maintenance costs using the Q10 factor, as it allows to describe and quantify the influence of temperature on the kinetics of the maintenance process in fish. In this study, the Q10 values for energy and protein fasting maintenance costs were, in general, in agreement with what has been reported for teleost fish species (Clarke & Johnston, 1999; Lefevre, 2016; Requena et al., 1997). However, when we compared the values between species, we found that the Q10 values for the turbot were higher. Moreover, turbot seem to have lower maintenance costs energy and protein, when compared to other fish species. The difference in maintenance cost parameters for energy between turbot and the other species may be related to differences in swimming mode, which consequently relates to the fish body form (Webb, 1984). Additionally, fish body form is often closely related to the specific habitats that fish inhabit (Rincon-Sandoval et al., 2020; Schluter, 1993; Townsed & Hildrew, 1994). Turbot is a benthic flatfish species that alternates its swimming form between a balistiform mode, which is used to swim slowly on the bottom, or anguilliform when it needs to swim faster (Gibson, 1995). Additionally, it is a sit-and-wait/slow forage (Gibson, 1995; Killen et al., 2010, 2016). Thus, it is a species with lower activity compared to pelagic (e.g., seabass and seabream) or benthopelagic species (e.g., salmon and rainbow trout), which are more active foragers and have different swimming modes. Turbot, therefore, has less metabolic expenditures compared to seabream, seabass, salmon and rainbow trout (Gibson, 1995; Griffiths, 2020; Killen et al., 2010, 2016), which may partly explain the large differences in their fasting maintenance costs parameters for energy compared to other species. Regarding protein maintenance cost parameters, rainbow trout, salmon and seabass turn out to be similar. Others (e.g., seabream, tilapia and turbot) showed species-specific parameters, which may be reflected in different amino acid requirements between species (Fournier et al., 2002; Tibaldi & Kaushik, 2005).

4.3.3. Energy and protein retention efficiency

Marine fish live in an environment where the concentration of salts and minerals is higher than in freshwater, which can lead to differences in how they process dietary energy and protein (de Silva & Perera, 1985; Krogdahl et al., 2004). Some studies have suggested that differences between fresh and marine water species, in addition to osmoregulation differences, may be in part due to variations in the types microorganisms in their digestive systems (Sullam et al., 2012; Wang et al., 2018). In the present study, seabream displayed higher energy retention efficiency than the other species, especially compared to seabass, which contradicts the similarity that Lupatsch et al. (2003b) reported between these two species. Furthermore, salmon presented higher energy retention efficiencies than rainbow trout as reported by Azevedo et al. (2004, 2005). Seabass and tilapia were the species with lower energy retention efficiency, but no characteristic between the two species was found to justify this similarity. In terms of protein retention efficiency, freshwater (i.e., Nile tilapia) and anadromous species (i.e., Atlantic salmon and rainbow trout) are more efficient than marine species (i.e., seabass and seabream). This is in line with Tibaldi & Kaushik (2005) previous report that seabream and seabass were less efficient at converting protein compared to salmonids. This may be at least partially attributed to the extensive history of breeding programs for salmonids (over 11 generations), while species like sea bass or sea bream have undergone fewer than nine generations of breeding, as noted by Chavanne et al. (2016). However, as reported by de Verdal et al. (2018), enhancing feed efficiency in fish through selective breeding poses numerous challenges. It appears that the response to selection often remains inconclusive due to the limited genetic variation observed in traits associated with feed efficiency. While some authors reported that differences in digestibility may partly explain the variation in observed protein retention ability among species (Garzón, 1995; Hidalgo et al., 1999; López-Ângulo et al., 2014), our findings suggest that the discrepancy in protein retention between the studied freshwater, anadromous, and marine species may be primarily attributed to metabolic differences rather than digestibility. Furthermore, the efficient protein retention displayed by Nile tilapia, despite belonging to a lower trophic level (2) as an omnivorous species, could be attributed to its ability to utilize not only fat and protein, but also carbohydrates as an energy source (Boonanuntanasarn et al., 2018; Stone, 2010). By utilizing carbohydrates as an additional energy source, Nile tilapia can reduce the reliance on protein for energy production (i.e., protein sparing effect). This efficient allocation of protein helps in optimizing growth and overall energy utilization. Thus, it enables tilapia to allocate more protein towards muscle development and growth rather than using a larger proportion for energy production (Boonanuntanasarn et al., 2018; Cheng et al., 2017; Shiau & Peng, 1993; Stone, 2010). Although there is some controversy regarding the comparative protein retention efficiency of carnivorous and omnivorous fish species (Bowyer et al., 2013; NRC, 2011; Tacon et al., 2010; Teles et al., 2020), it is clear that protein digestibility and retention rates vary depending on fish species, metabolism, diet composition, trophic level and other factors. Thus, it is difficult to make broad generalizations about carnivorous *versus* omnivorous fish and the same applies to freshwater versus marine fish.

4.3.4. Practical applications and insights from body composition and growth analysis in fish species

This work has several potential practical applications beyond contributing to the understanding of nutritional status and growth of commercially-relevant fish species. For instance, based on the data obtained from body composition analysis, it is possible to develop a software tool that can identify anomalies (i.e., gross errors) in the results obtained from the chemical analysis. This would be useful to determine if the body composition results from the chemical analysis are in the correct range or if there are any samples that present some anomaly, providing quality control measures that are essential in ensuring the accuracy and reliability of the data generated from this analysis. The intent is for this type of automated quality control analysis to become more widely adopted as practical, user-friendly tools for predicting body composition become available (Soares et al., 2023). A software tool like this can also help farmers to track the quality of their stock and make feed adjustments, if necessary, to assure fish health and growth.

Since, in aquaculture, there is a large number of species being produced, having the ability to calibrate models (e.g., body composition and growth models) for new species with a limited number of samples can useful when new species are proposed for production. This means that, if a farmer wanted to start farming a new species of fish, this method can be used to predict the growth patterns and body composition of the new species, helping to plan feeding and growth strategies. Furthermore, if two species have similar growth patterns, they might be able to be farmed together, saving space and resources. One possible way to achieve this is to predict the samples using the model for each known species and use the prediction error to estimate a distance from each reference species. By using a suitable scheme (e.g., inverse distance weighting) to aggregate parameter or prediction estimates from the reference models, predictions for the new species can be obtained. This type of approach can enable the prediction of body composition or growth patterns for new species based on a set of reference species and potentially assist in the calibration of models for these new species with limited data availability (e.g., through the generation of synthetic data). Additionally, the information about the similarity between species in terms of body composition can be used to estimate the similarity in terms of growth between the same species or vice versa. An example of this type of approach is described in Appendix 5, which shows how body composition information could be used to predict the parametrization of growth models for different species.

5. Conclusion

The findings of this study indicate that, although some species may have some particularities in common (e.g., same family, geographical area, similar diets or body shapes), it does not necessarily mean that they are necessarily similar with regards to other aspects. In the particular case of the two salmonids studied, our analysis showed that they are indeed similar both in terms of body composition and growth. In turn, while the Mediterranean species, seabream and seabass, seem to have similarities regarding body composition, but display some differences in growth parameters. Furthermore, we observed that, while there is high similarity in terms of body composition among all species in their early juvenile stages, this similarity decreases as they grow. Additionally, the evaluation of growth parameters suggests that the metabolic body weight exponent is species-specific, contradicting the theory of using a universal value. Differences in growth parameters among fish species can be related to their distinct retention efficiencies for energy and protein as well as to unique fasting maintenance costs, encompassing energy and protein requirements. The fasting maintenance costs are intricately linked to factors such as the fish's swimming behaviour, body morphology, and other aspects, including ecology and trophic levels, that may contribute to the diversity in maintenance expenses. The differences in fasting maintenance cost consequently manifest as distinct metabolic expenditures among species. Thus, understanding these multifaceted relationships helps understanding the intricate interplay between fish biology, behaviour, and ecological dynamics in influencing maintenance costs and metabolic processes.

The methodology used in this research can have several practical applications, including data quality control, model calibration, synthetic data generation and assessment of similarity between species. These can be particularly useful not only for the aquaculture industry but also for the fisheries industry, where accurate knowledge of body composition and growth patterns is essential for effective management and optimal resource utilization.

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Table 1 – Overall data used for species comparison in terms of body weight and whole-body composition.

	Atlantic Salmon	***Gilthead Seabream***	***European Seabass***	***Senegalese sole***	***Nile tilapia***	***Rainbow trout***	***Turbot***
Body weight (g)	0.17 – 4950.00	0.98 – 582.10	0.51 – 700.00	3.00 – 354.92	0.02 – 316.30	1.40 – 2080.00	2.55 – 880.60
Mean body weight (g)	269.21	133.11	133.48	55.63	65.73	187.78	89.37
Median body weight (g)	51.80	91.70	94.62	30.78	34.10	76.36	54.90
Body composition (% wet weight)							
Protein	12 - 21	14 - 24	15 – 19	12 – 19	9 – 19	6 – 19	11 – 23
Fat	1 - 18	2 - 22	2 - 22	2 – 10	1 – 13	1 – 22	1 - 9
Water	83 - 60	56 - 80	58 - 76	67 – 83	66 – 83	59 – 91	68 – 84
Ash	1 - 5	3 - 17	3 - 6	2 – 4	1 – 8	1 – 3	1 - 11
Nº of observations	506	743	202	121	198	328	469
Sources	53	79	23	22	34	28	64

Table 2 – Overall data used for species comparison in terms of growth parameters.

	Atlantic Salmon	***Gilthead Seabream***	***European Seabass***	***Senegalese sole***	***Nile tilapia***	***Rainbow trout***
Body weight (g)	0.79 – 5787.00	0.72 – 478.00	4.65 – 482.00	0.51 – 457.00	1.80 – 2080.00	2.01 – 1025.90
Temperature (ºC)	4.0 – 22.0	7.6 – 28.8	3.0 – 27.9	22.8 – 28.6	4.0 – 19.4	8.0 – 23.6
Diet Gross Energy (MJ/kg)	18.7 – 29.4	18.7 – 22.8	17.5 – 24.6	14.0 – 19.8	16.8 – 25.5	16.2 – 23.2
Diet Crude Protein (% ww)	29.1 – 53.6	36.5 – 57.8	36.7 – 56.1	22.9 – 45.6	26.2 – 58.2	26.5 – 60.4
Diet Crude Lipids (% ww)	9.7 – 47.0	8.6 – 23.1	7.8 – 30.9	3.5 – 15.1	6.2 – 30.9	5.7 – 25.9
Ratio DP/DE (g/MJ)	12.0 – 25.7	20.8 – 25.7	19.0 – 29.9	13.5 – 25.6	11.3 – 27.6	16.0 – 32.7
FCR	0.56 – 2.61	0.95 – 5.18	0.69 – 3.79	0.90 – 3.89	0.73 – 1.83	0.54 – 2.23
Nº of observations	291	116	144	150	146	238
Sources	52	15	25	27	23	52

ww- wet weight;
DP/DE – digestible protein and energy ratio

Table 3 – Definition of growth model and calibration methods used for species comparison.

Model acronym	Model description
EP_E_lm	Energy-protein flux model calibrated with the assumption of estimated body weight parameters with least squares linear regression.
EP_E_rlm	Energy-protein flux model calibrated with the assumption of estimated body weight parameters with Huber loss linear regression.
EP_F_lm	Energy-protein flux model calibrated with the assumption of fixed universal body weight parameters (0.8 for energy and 0.7 for protein, consistently with Clarke & Johnston (1999), Glencross (2008), Lupatsch et al. (1998, 2003)
EP_F_rlm	Energy-protein flux model calibrated with the assumption of fixed universal body weight parameters (0.8 for energy and 0.7 for protein, consistently with Clarke & Johnston (1999), Glencross (2008), Lupatsch et al. (1998, 2003) with Huber loss linear regression.

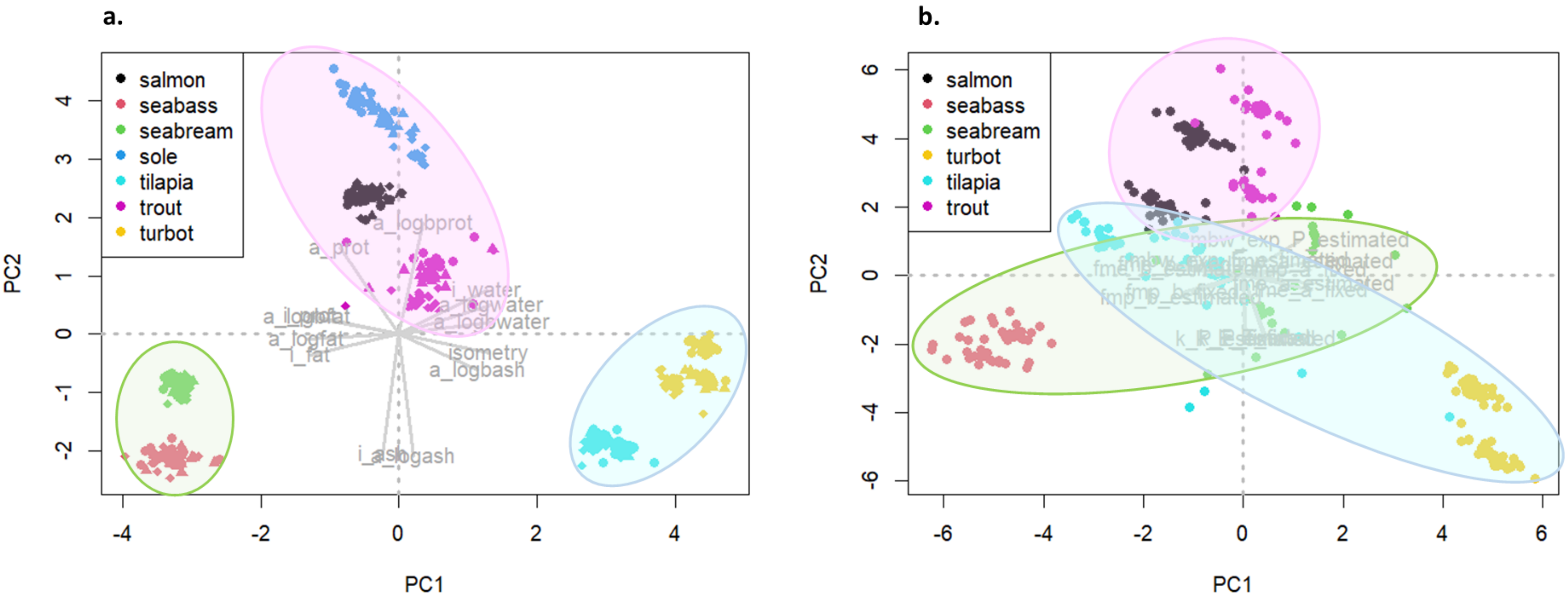


Figure 1 – a. Principal component analysis (PCA) with estimates of parameter a for each component according to an isometric and an allometric model for each species, parameter b and with the isometry index. ai prefix correspond to parameter a estimated by isometric model; aa and b prefix corresponds to parameter a or b estimated by allometric model. Circles with different colour indicates the species clusters: green circle seabream and seabass species (seabream and seabass); pink circle salmonids (salmon and trout) and sole; blue circle tilapia and turbot. b. Principal component analysis (PCA) displaying the overall distance between species, considering the estimates or the use of fixed universal parameters to predict growth. Circles with different colours indicate the species clusters. The figure shows an overlapping of species clusters and some distance between species within some cluster, due to differences in parameters estimations.

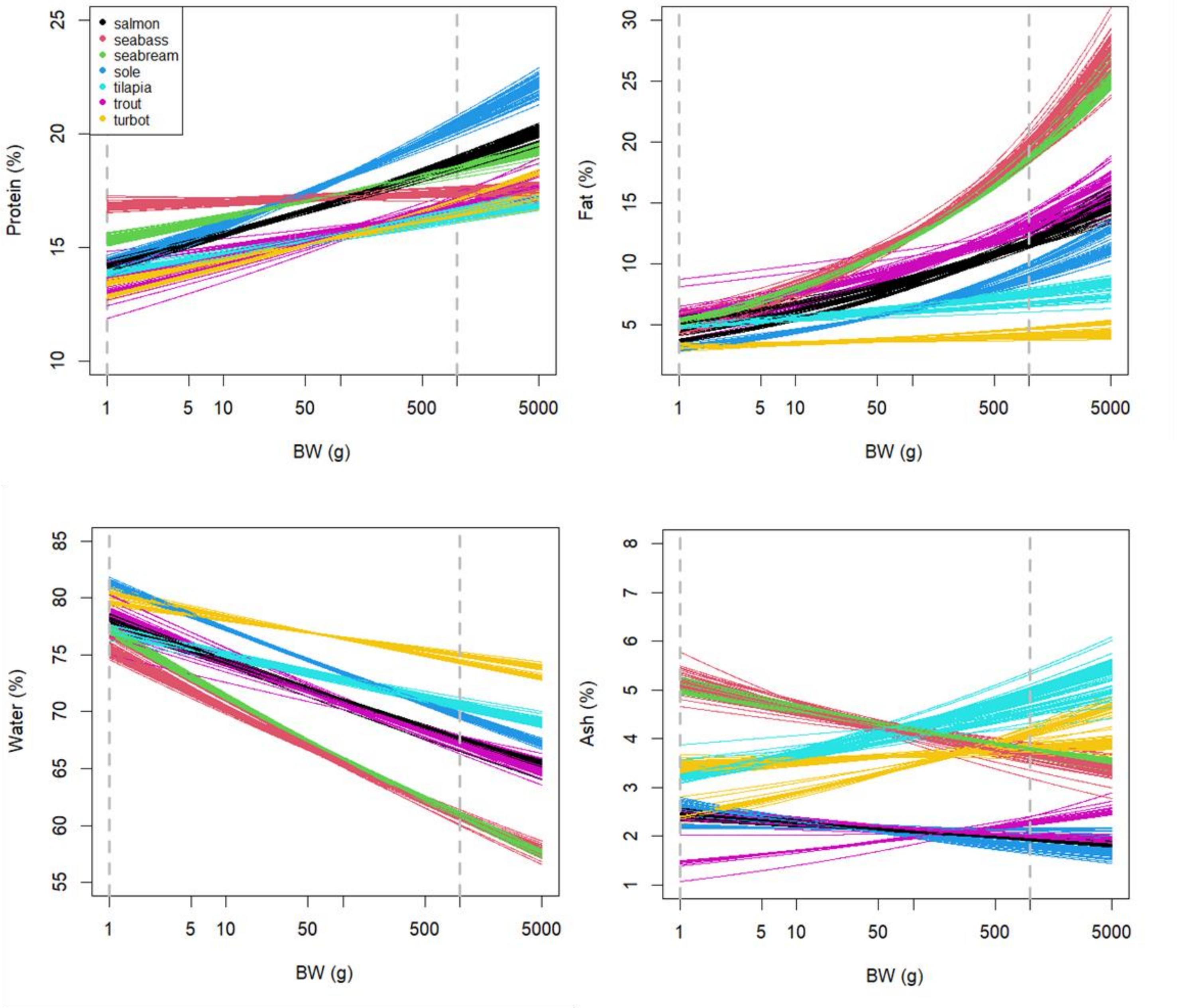


Figure 2 – Line plots showing predictions of body composition components as a function of body weight. Solid lines represent the predictions obtained for each model (calibrated on a LOO sample). Different colour lines denote different species. Dashed grey lines indicates body weight = 1 g and body weight = 5000 g.

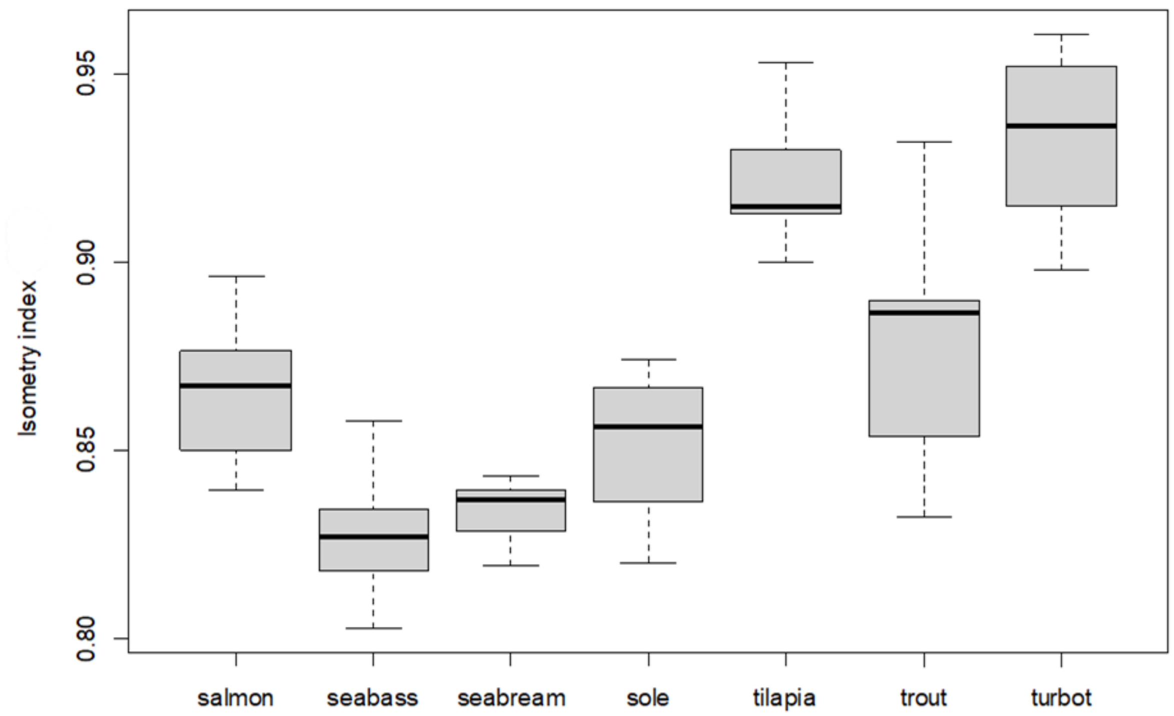


Figure 3 – Boxplot of the isometry index, demonstrating how each species body composition is affected by body weight (closer to value 1, means that the overall species body composition is not dependent/affected by body weight). The middle line in the box is the median. The box, divided into two parts shows the first quartile and the third quartile (Q1, at the bottom of the box, represents the lowest 25% of the data and the Q3, the upper part of the box, represents the highest 25% of the data). The whiskers show the minimum and maximum values within the data that are not considered outliers. Outliers are shown as individual points beyond the whiskers.

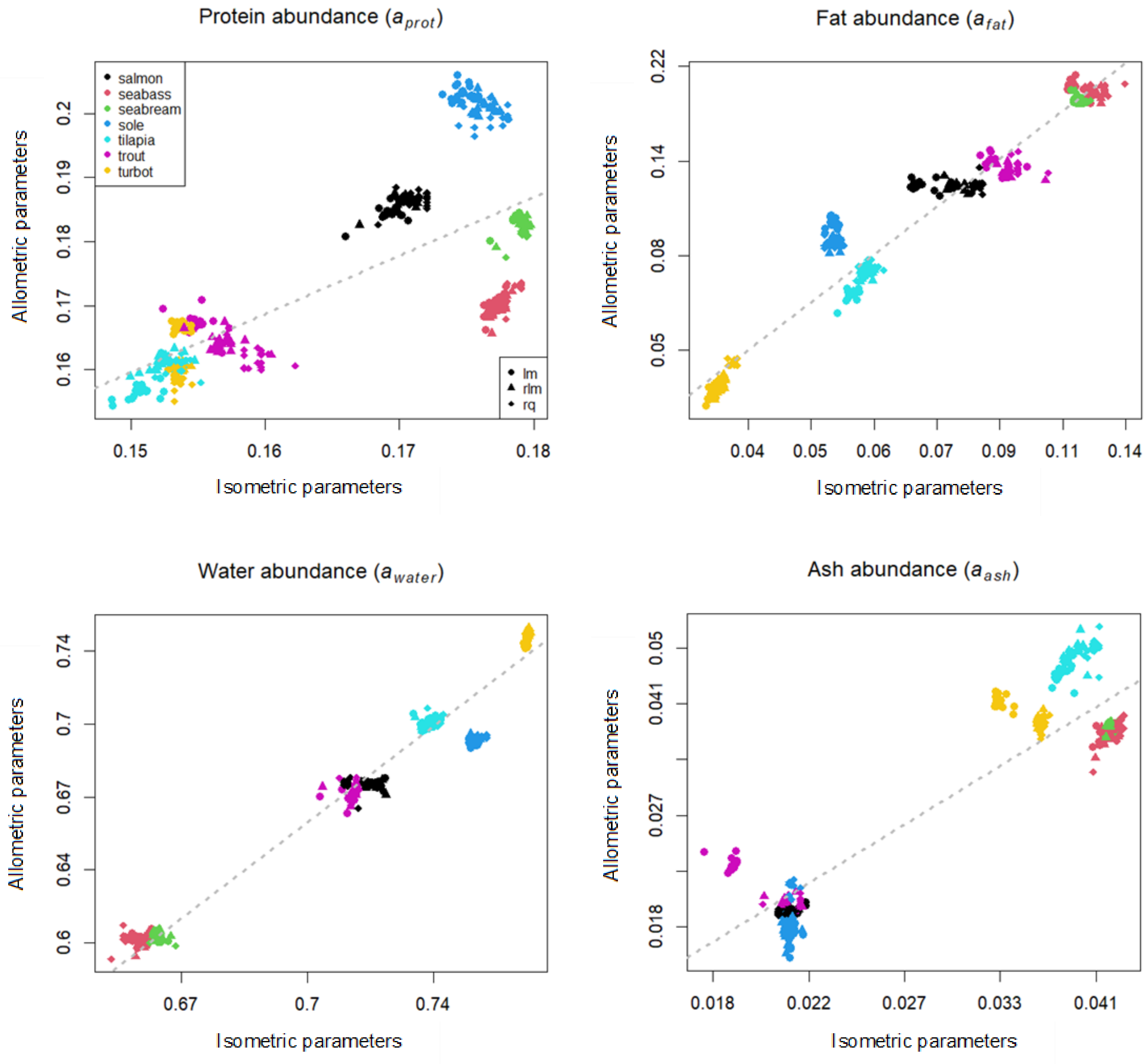


Figure 4 – Scatterplots showing allometric and isometric estimates for parameter a, for each body composition component. Points represent parameter a estimates according to each calibration method. Different colours indicate different species. Grey dashed line is the linear regression between allometric and isometric estimates.

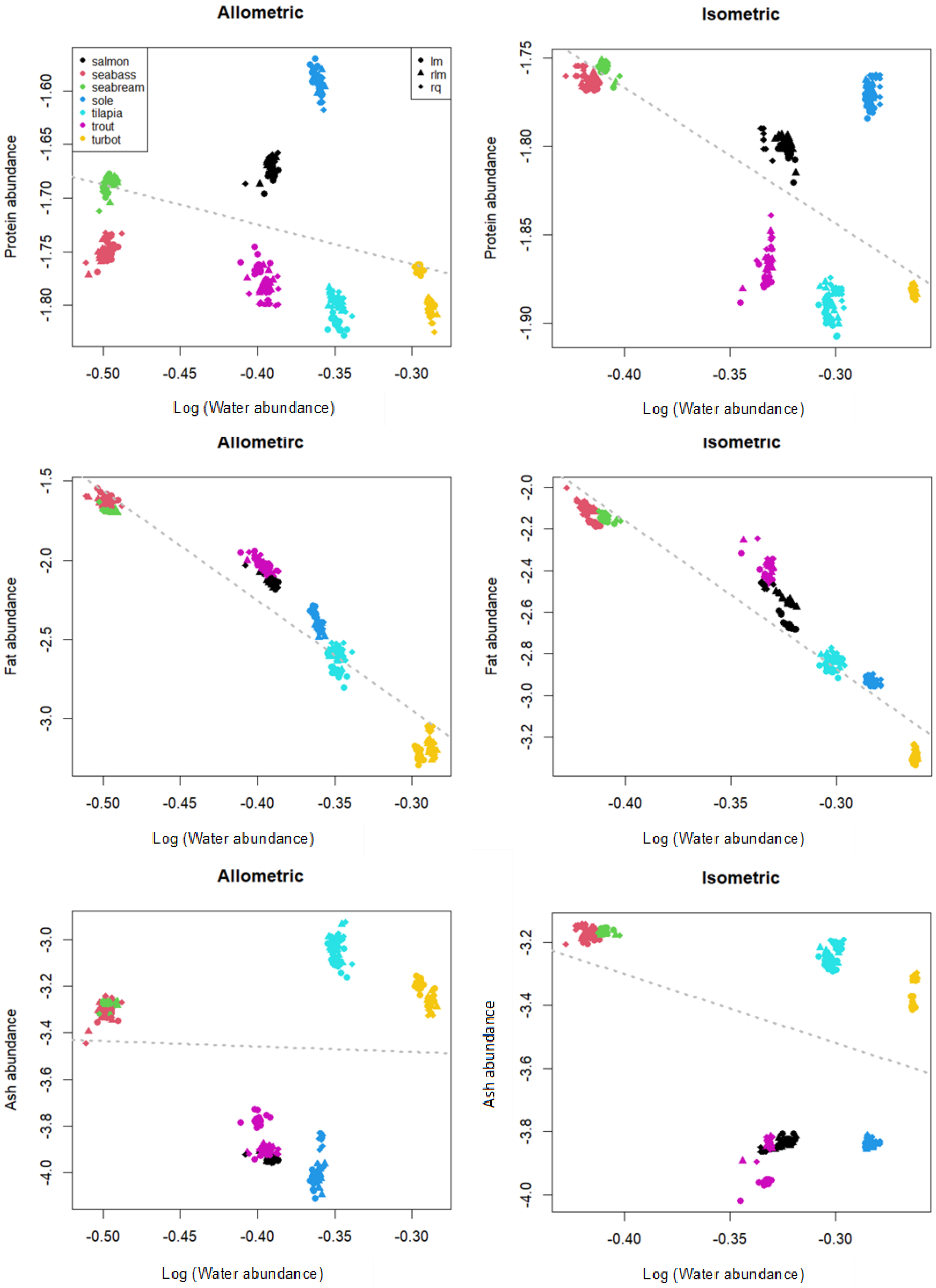


Figure 5 – Scatterplots showing the relation between water and other body composition components, according to allometric and isometric models. Points represent parameter a estimates according to each calibration method. Different colour point denotes each species. Grey dashed line is the linear regression between components.

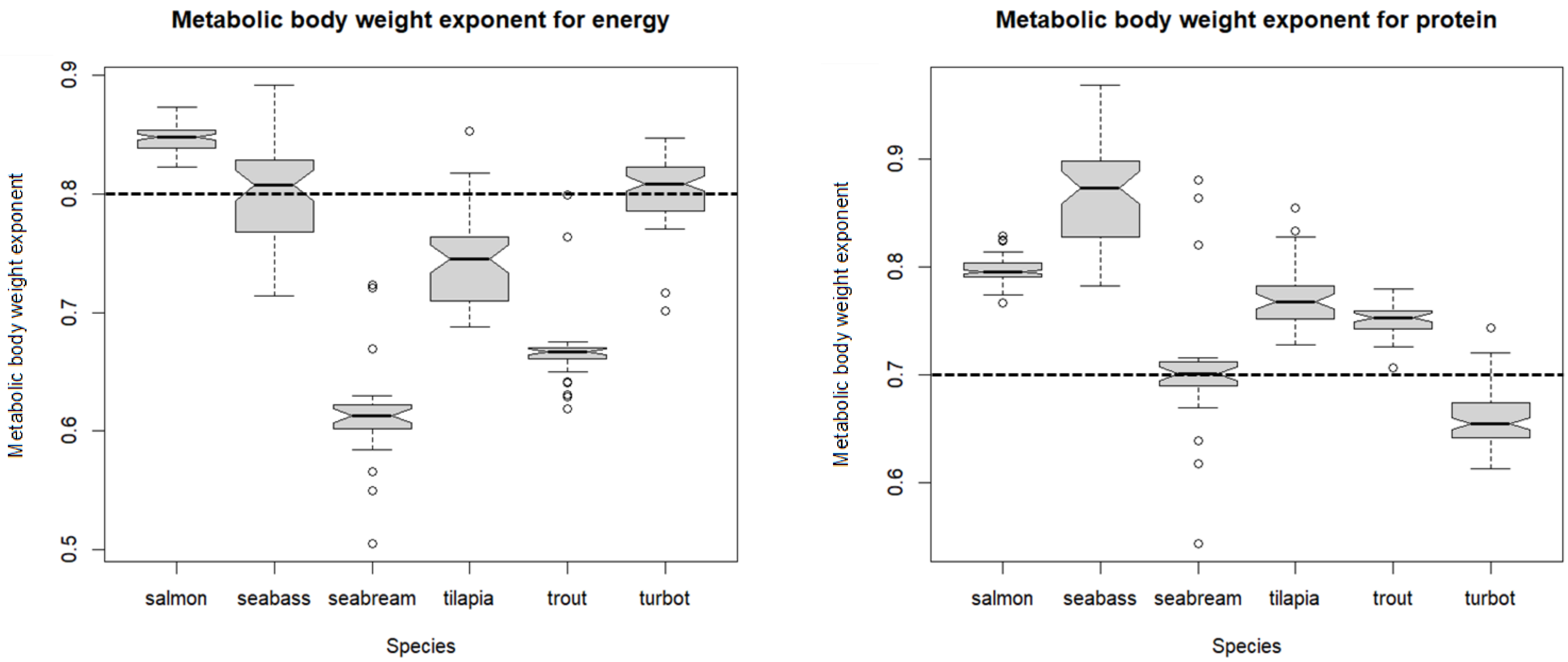
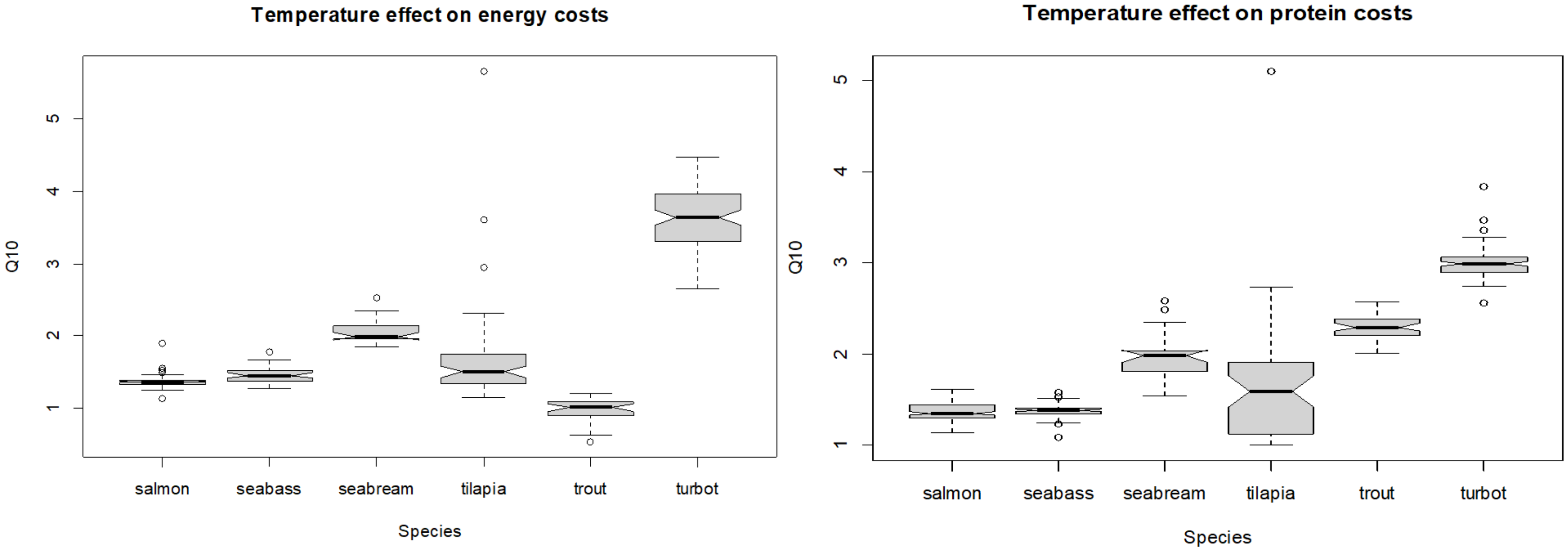


Figure 6 – Boxplot showing the estimates of metabolic body weight exponents for energy (exp_e) on the left, and for protein (exp_p) on the right, according to each species. The middle line in the box is the median. The box, divided into two parts shows the first quartile and the third quartile (Q1, at the bottom of the box, represents the lowest 25% of the data and the Q3, the upper part of the box, represents the highest 25% of the data). The whiskers show the minimum and maximum values within the data that are not considered outliers. Outliers are shown as individual points beyond the whiskers. The boxplot notches indicate an approximate 95% confidence interval for the median. Dashed line represents the so-called universal value for metabolic body weight exponent for energy (left) and protein (right).


Temperature effect on energy costs

Temperature effect on protein costs

Figure 7 – Box plots displaying Q10 value estimates for temperature effects on energy (left) and protein (right) fasting maintenance costs. The middle line in the box is the median. The box, divided into two parts shows the first quartile and the third quartile (Q1, at the bottom of the box, represents the lowest 25% of the data and the Q3, the upper part of the box, represents the highest 25% of the data). The whiskers show the minimum and maximum values within the data that are not considered outliers. Outliers are shown as individual points beyond the whiskers. The boxplot notches indicate an approximate 95% confidence interval for the median.

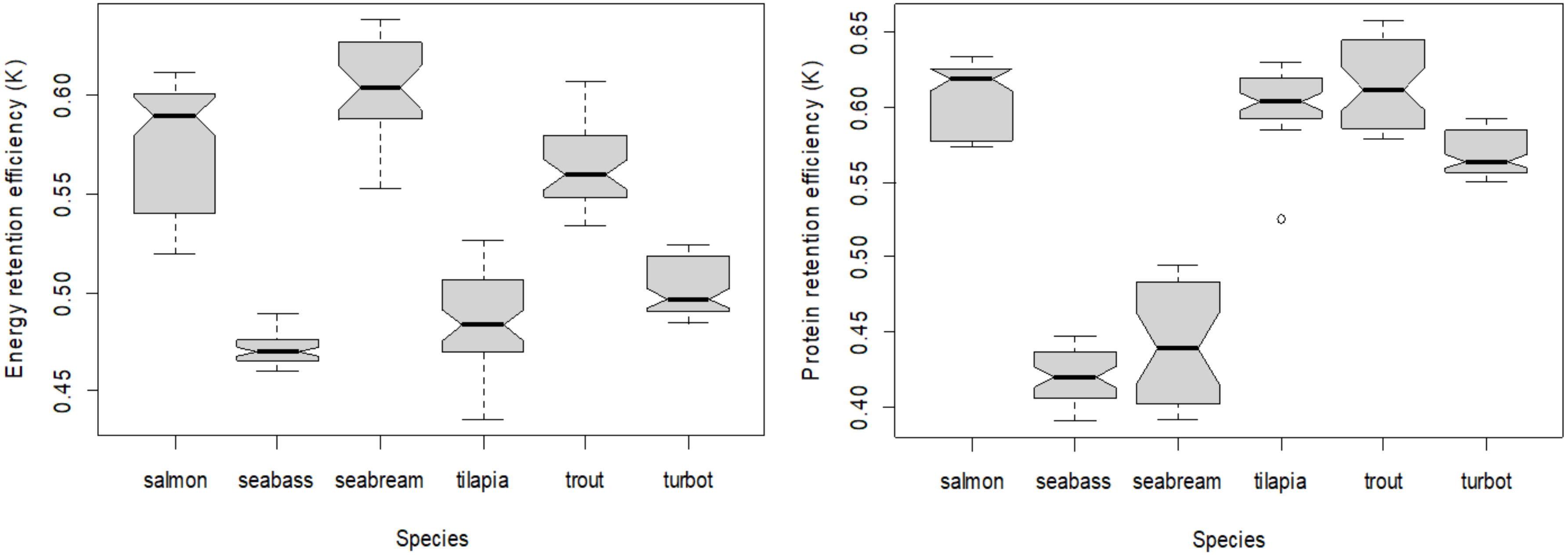


Figure 8 – Box plot showing the estimates range for energy (left) and protein (right) retention efficiency. The middle line in the box is the median. The box, divided into two parts shows the first quartile and the third quartile (Q1, at the bottom of the box, represents the lowest 25% of the data and the Q3, the upper part of the box, represents the highest 25% of the data). The whiskers show the minimum and maximum values within the data that are not considered outliers. Outliers are shown as individual points beyond the whiskers. The boxplot notches indicate an approximate 95% confidence interval for the median.

Appendix 1

Appendix 1.A –This table provides a comprehensive list of sources referenced for data utilized within the study.

Specie	Source
Nile tilapia	Abdel-Tawwab, M., Abdel-Rahman, A. M., & Ismael, N. E. (2008). Evaluation of commercial live bakers' yeast, Saccharomyces cerevisiae as a growth and immunity promoter for Fry Nile tilapia, *Oreochromis niloticus* (L.) challenged in situ with Aeromonas hydrophila. *Aquaculture*, 280(1-4), 185-189. Abdel-Tawwab, M., Ahmad, M. H., Khattab, Y. A., & Shalaby, A. M. (2010). Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture*, 298(3-4), 267-274. Abdel-Tawwab, M., El-Sayed, G. O., & Shady, S. H. (2012). Effects of dietary protein levels and environmental zinc exposure on the growth, feed utilization, and biochemical variables of Nile tilapia, *Oreochromis niloticus* (L.). *Toxicological & Environmental Chemistry*, 94(7), 1368-1382. Abdelghany, A. E., & Ahmad, M. H. (2002). Effects of feeding rates on growth and production of Nile tilapia, common carp and silver carp polycultured in fertilized ponds. *Aquaculture Research*, 33(6), 415-423. Afuang, W., Siddhuraju, P., & Becker, K. (2003). Comparative nutritional evaluation of raw, methanol extracted residues and methanol extracts of moringa (*Moringa oleifera* Lam.) leaves on growth performance and feed utilization in Nile tilapia (*Oreochromis niloticus* L.). *Aquaculture Research*, 34(13), 1147-1159. Ahmad, M. H., & Abdel-Tawwab, M. (2011). The use of caraway seed meal as a feed additive in fish diets: Growth performance, feed utilization, and whole-body composition of Nile tilapia, *Oreochromis niloticus* (L.) fingerlings. *Aquaculture*, 314(1-4), 110-114. Ahmad, M. H., El Mesallamy, A. M., Samir, F., & Zahran, F. (2011). Effect of cinnamon (*Cinnamomum zeylanicum*) on growth performance, feed utilization, whole-body composition, and resistance to *Aeromonas hydrophila* in nile tilapia. Journal of Applied *Aquaculture*, 23(4), 289-298. Al-Asgah, N. A., & Ali, A. (1997). Growth performance and body composition of *Oreochromis niloticus* reared at different water temperatures. In Annales de zootechnie (Vol. 46, No. 4, pp. 331-338). Ali, A. (2003). Effect of feeding different levels of alfalfa meal on the growth performance and body composition of Nile tilapia (Oreochromis niloticus) fingerlings. *Asian Fish. Sci*., 16, 59-67.

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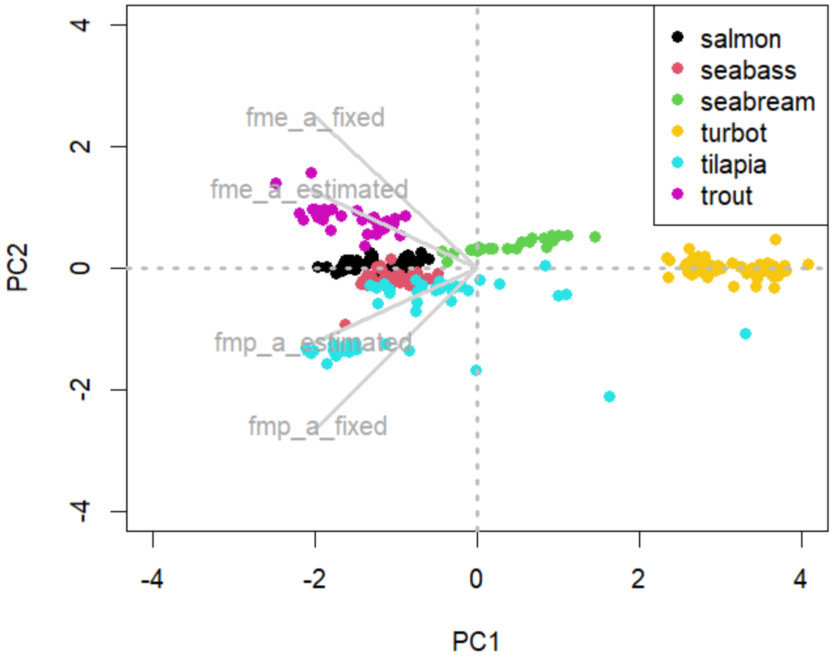
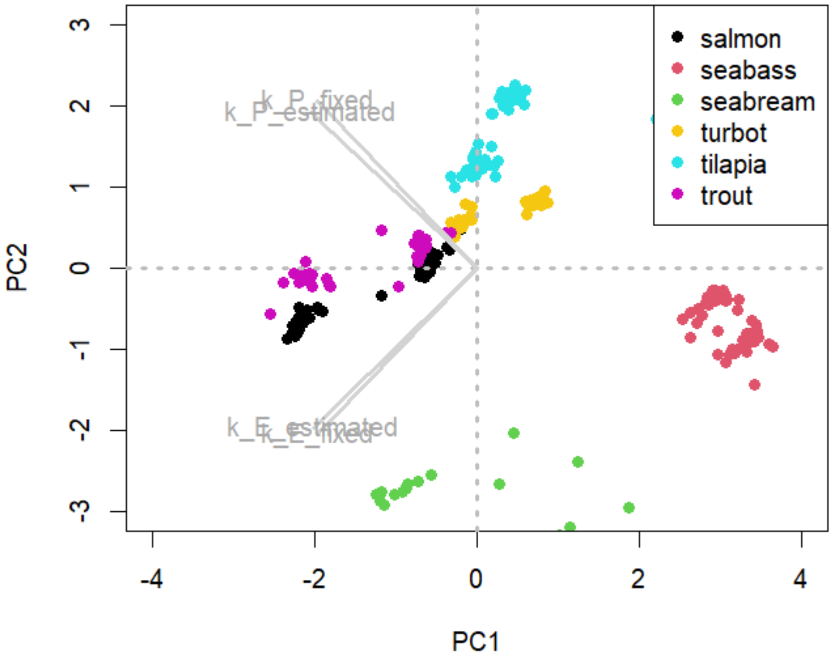
Appendix 2

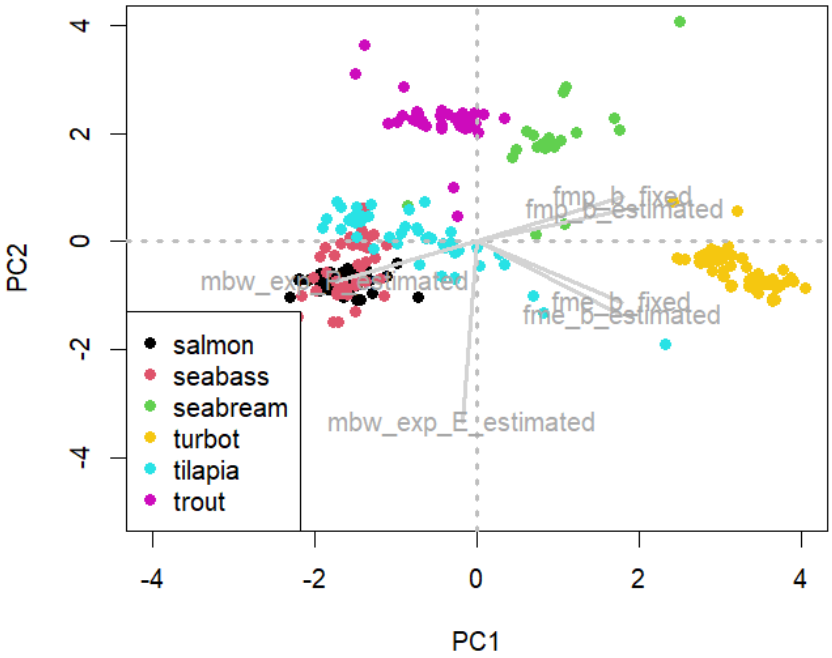
Here several PCA analyses based on the different parameters of the growth models are given. These analyses provide insight into which parameters are similar across species, and whether they remain consistent when body weight exponents are either universal or estimated.



Appendix 2.A - Principal component analysis (PCA) displaying species distance in terms of maintenance costs parameters.



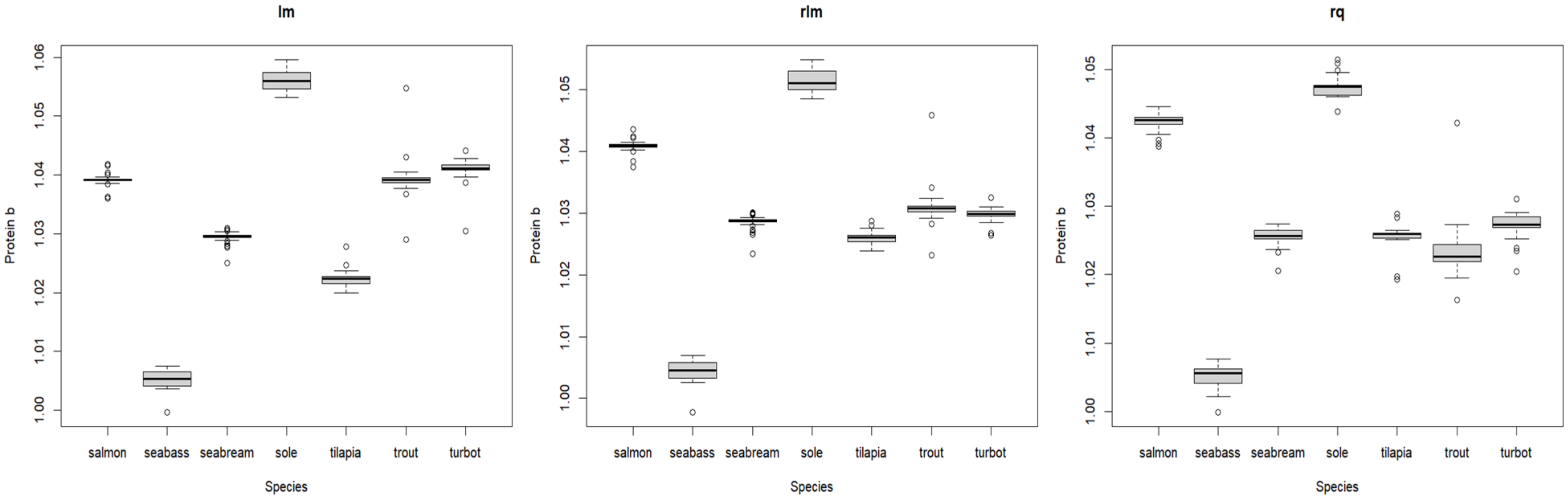
Appendix 2.B - Principal component analysis (PCA) showing species distance in terms of retention efficiency parameters.



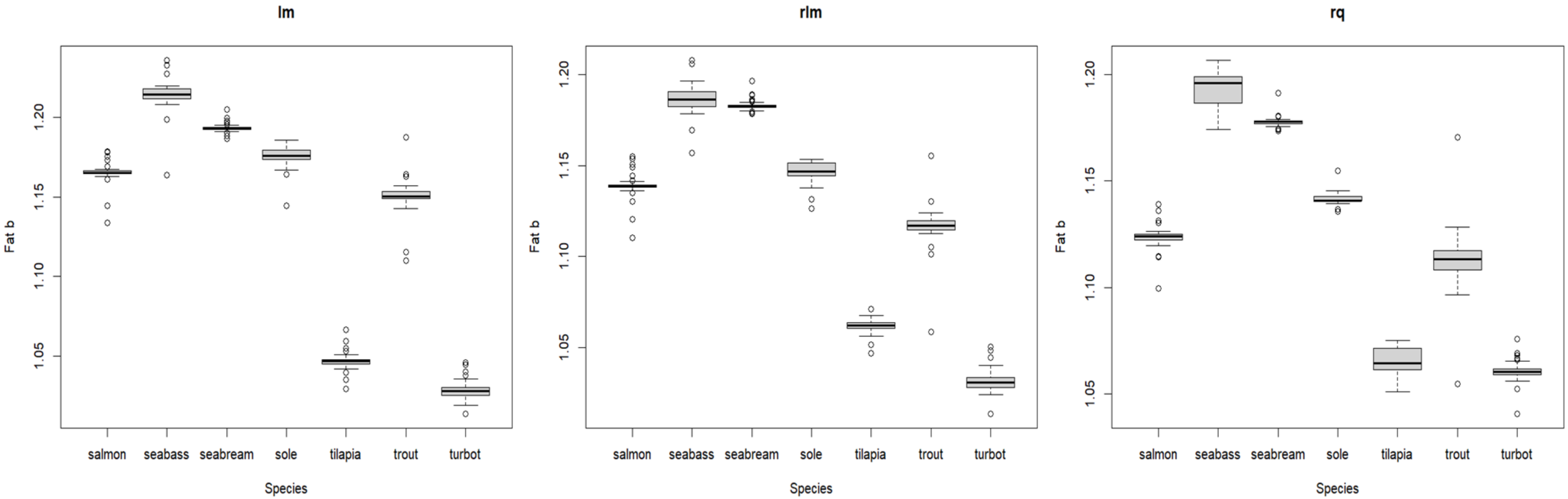
Appendix 2.C- Principal component analysis (PCA) showing species distance considering the metabolic body weight and temperature effect.

Appendix 3

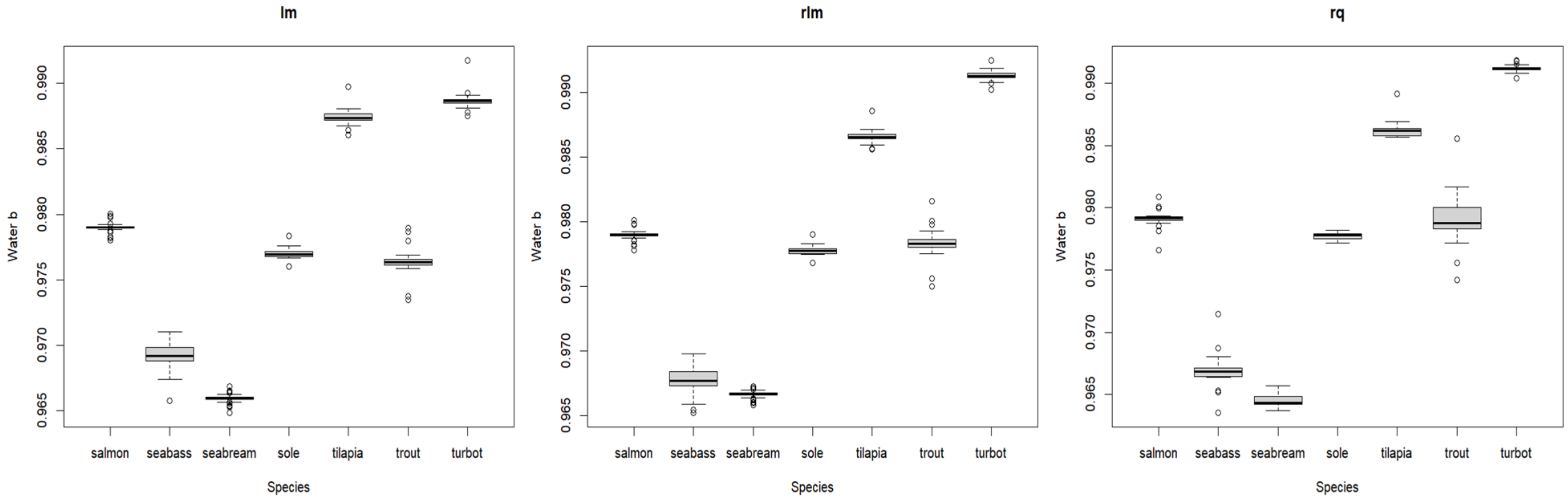
This appendix includes a supplementary analysis of the *b* parameter estimates for each component of the body composition models, using various regression methods. The boxplots below provide information on whether the value of b parameter of each body composition component is closer or further away from 1 (isometry). Additionally, it also shows whether there is agreement between the different types of regression used in predicting the b parameter.



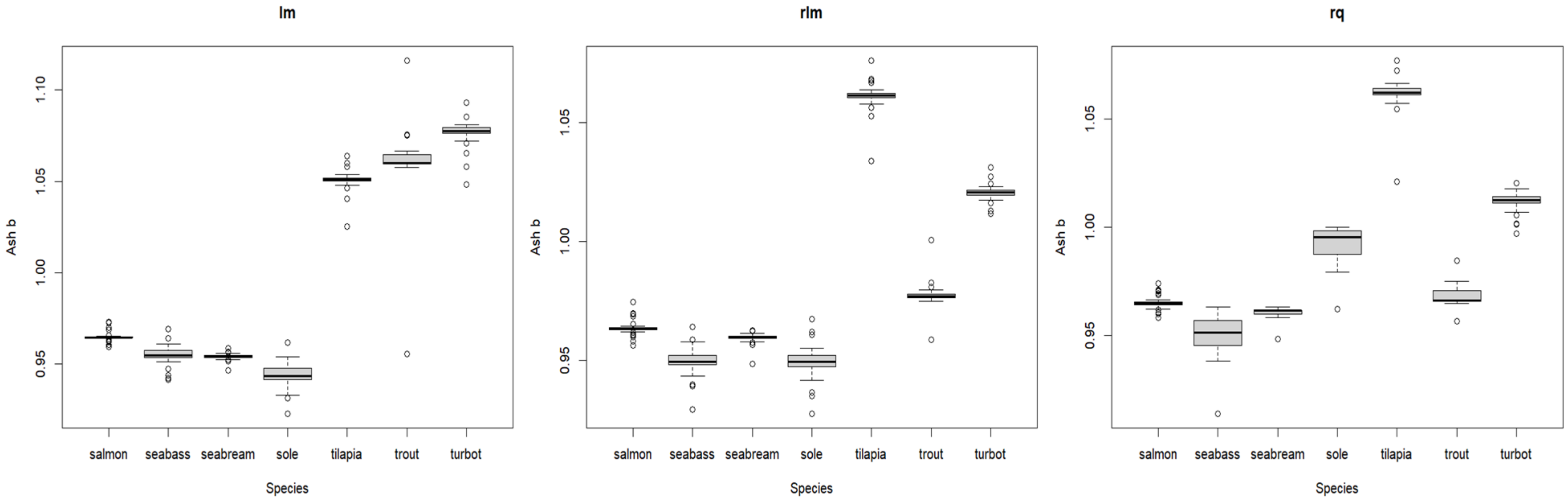
Appendix 3.A – Box plot displaying parameter b estimates for protein with different types of regressions for the compared species. lm = least squares linear regression; rlm = Huber loss linear regression; rq = quantile regression. The middle line in the box is the median. The box, divided into two parts shows the first quartile and the third quartile (Q1, at the bottom of the box, represents the lowest 25% of the data and the Q3, the upper part of the box, represents the highest 25% of the data). The whiskers show the minimum and maximum values within the data that are not considered outliers. Outliers are shown as individual points beyond the whiskers.



Appendix 3.B – Box plot displaying parameter b estimates for fat with different types of regressions for the compared species. lm = least squares linear regression; rlm = Huber loss linear regression; rq = quantile regression. The middle line in the box is the median. The box, divided into two parts shows the first quartile and the third quartile (Q1, at the bottom of the box, represents the lowest 25% of the data and the Q3, the upper part of the box, represents the highest 25% of the data). The whiskers show the minimum and maximum values within the data that are not considered outliers. Outliers are shown as individual points beyond the whiskers.



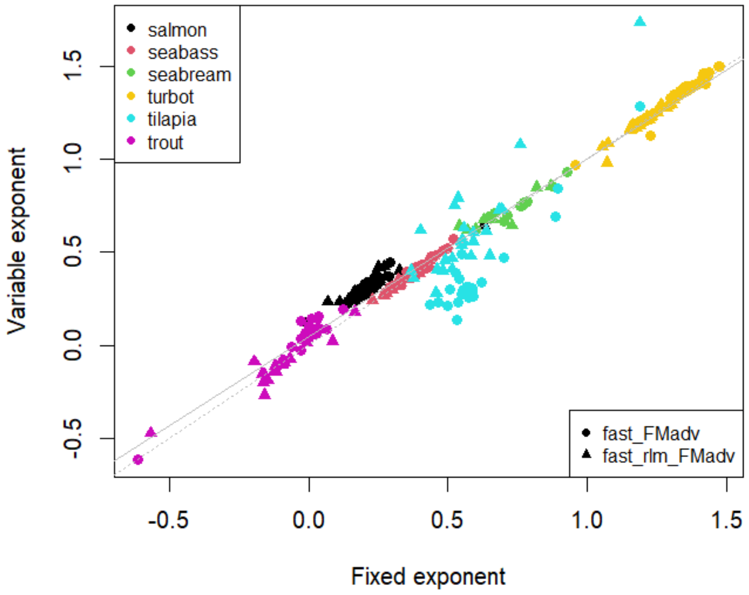
Appendix 3.C – Box plot displaying parameter b estimates for water with different types of regressions for the compared species. lm = least squares linear regression; rlm = Huber loss linear regression; rq = quantile regression. The middle line in the box is the median. The box, divided into two parts shows the first quartile and the third quartile (Q1, at the bottom of the box, represents the lowest 25% of the data and the Q3, the upper part of the box, represents the highest 25% of the data). The whiskers show the minimum and maximum values within the data that are not considered outliers. Outliers are shown as individual points beyond the whiskers.



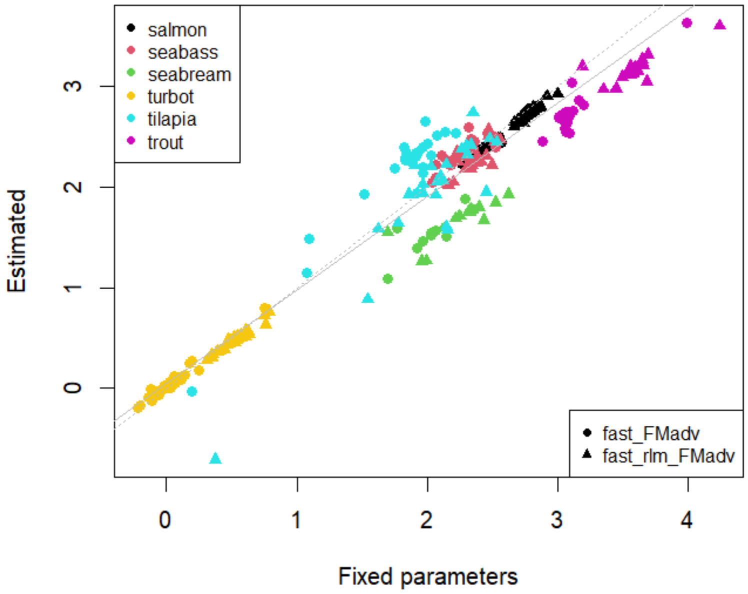
Appendix 3.D – Box plot displaying parameter b estimates for ash with different types of regressions for the compared species. lm = least squares linear regression; rlm = Huber loss linear regression; rq = quantile regression. The middle line in the box is the median. The box, divided into two parts shows the first quartile and the third quartile (Q1, at the bottom of the box, represents the lowest 25% of the data and the Q3, the upper part of the box, represents the highest 25% of the data). The whiskers show the minimum and maximum values within the data that are not considered outliers. Outliers are shown as individual points beyond the whiskers.

Appendix 4

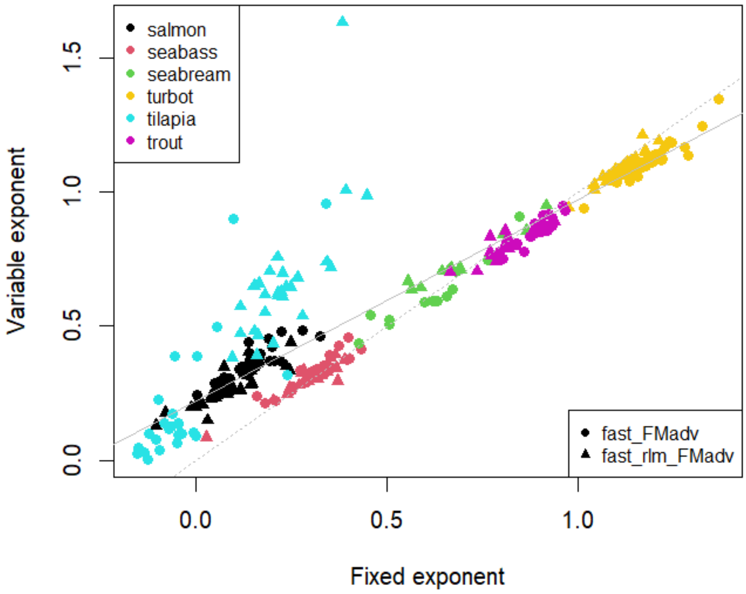
This appendix contains the results of a comparison made between the estimates of the different parameters of the growth models, using fixed universal and estimated parameters, and with different calibration methods. The scatterplots below are useful to understand which species tend to have higher or lower parameters and how similar or different they are between each other.



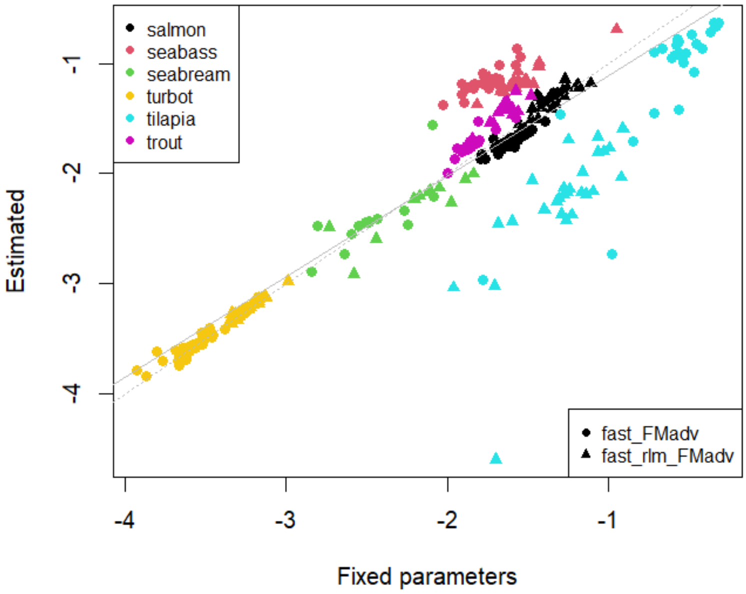
Appendix 4.A – Scatterplot showing estimates of the effect of temperature on fasting maintenance costs for energy using fixed universal or estimated parameters. Different points represent the type of linear regression used. Different colour points represent each species. Grey dashed line denotes y=x. Solid grey line represents the linear regression between estimates.



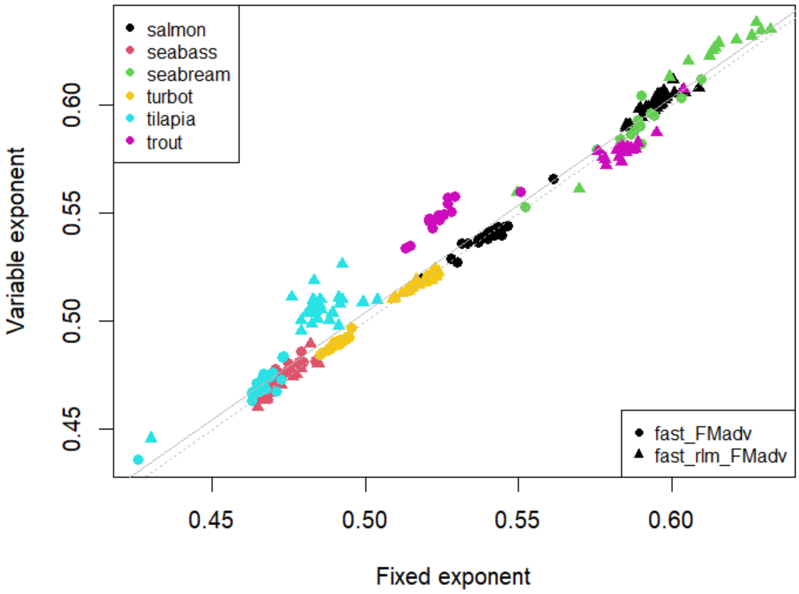
Appendix 4.B – Scatterplot showing estimates of fasting maintenance costs for energy using fixed universal or estimated parameters in log scale. Different points represent the type of linear regression used. Different colour points represent each species. Grey dashed line denotes y=x. Solid grey line represents the linear regression between estimates.



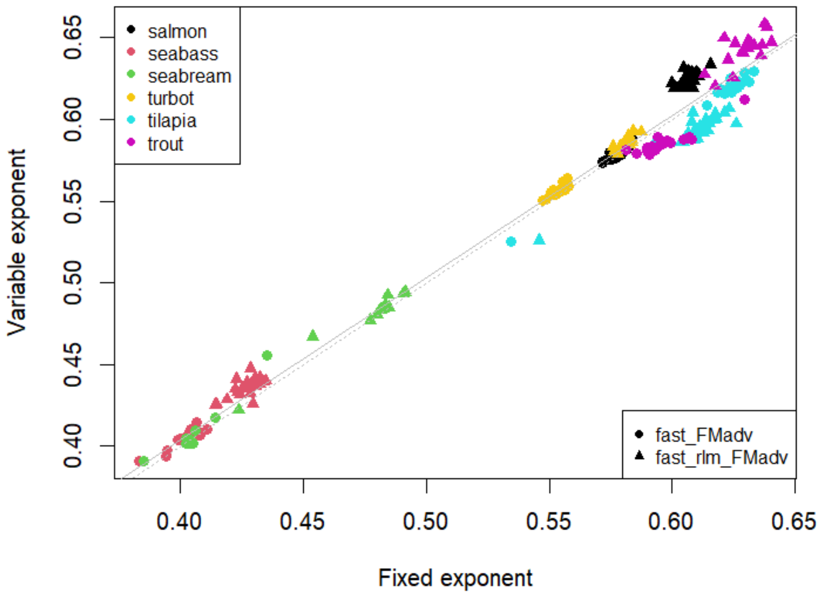
Appendix 4.C – Scatterplot showing estimates of temperature effect on fasting maintenance costs for protein using fixed universal or estimated parameters. Different points represent the type of linear regression used. Different colour points represent each species. Grey dashed line denotes y=x. Solid grey line represents the linear regression between estimates.



Appendix 4.D - Scatterplot showing estimates of fasting maintenance costs for protein using fixed universal or estimated parameters in log scale. Different points represent the type of linear regression used. Different colour points represent each species. Grey dashed line denotes y=x. Solid grey line represents the linear regression between estimates.



Appendix 4.E - Scatterplot showing estimates of energy retention efficiency using fixed universal or estimated parameters. Different points represent the type of linear regression used. Different colour points represent each species. Grey dashed line denotes y=x. Solid grey line represents the linear regression between estimates.



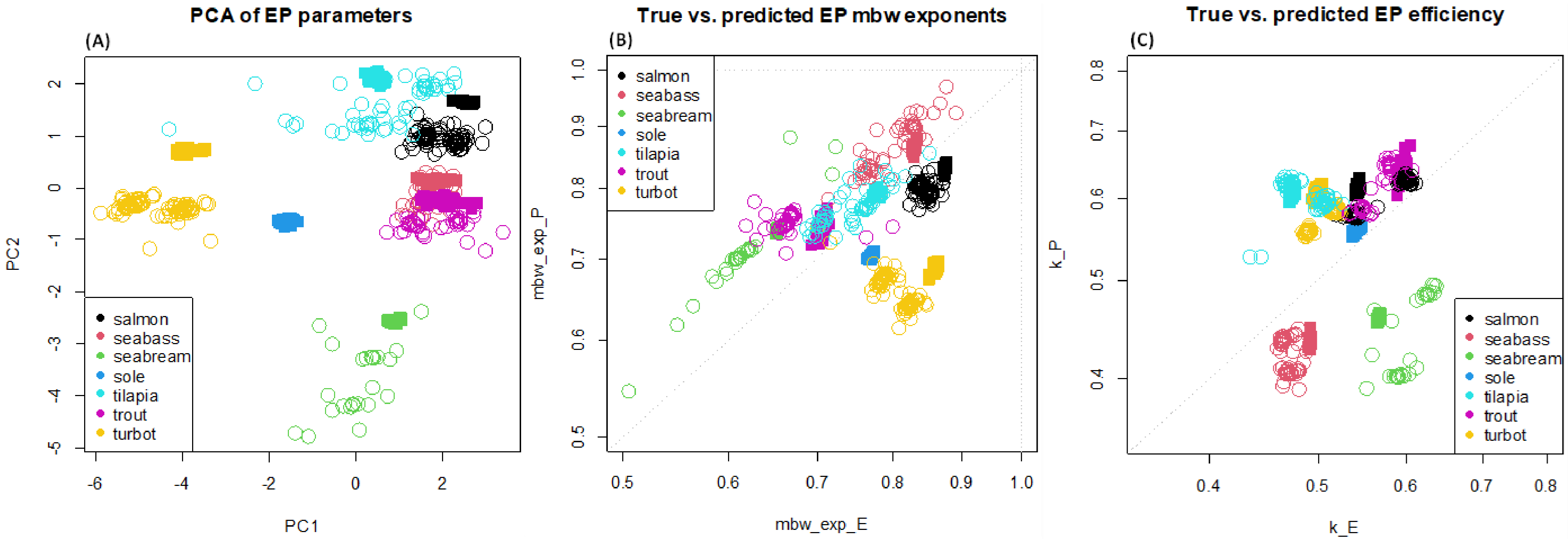
Appendix 4.F – Scatterplot showing estimates of protein retention efficiency using fixed universal or estimated parameters. Different points represent the type of linear regression used. Different colour points represent each species. Grey dashed line denotes y=x. Solid grey line represents the linear regression between estimates.

Appendix 5

In this appendix, a practical example is given of how the obtained and processed information in this study can be used. Herein, we assumed that the distance between species estimates, for both body composition and growth, characterize the distance in similarity between species. Moreover, in growth comparison analysis, as we stated previously, Senegalese sole was left out due to scarcity of data. However, we suggest that it may be possible through the method of Partial Least Squares regression (PLS) to predict its growth similarity with other species, based on the data of body composition parameters (i.e., distance between sole and other species on body composition predictions).

After using the PLS method for prediction of growth parameters based on body composition parameters, we performed a PCA analysis to evaluate the overall differences between true and the predicted general growth parameters of species [Appendix 5.A1(A)]. The results shown that in general, the EP parameter predictions obtained from the body composition parameters are close to the true values. Taking these estimates as accurate, Senegalese sole seems to have some similarities with turbot. However, regarding the metabolic body weight for energy, Senegalese sole displays exponents closer to Nile tilapia, while the exponents for protein are more alike turbot and rainbow trout [Appendix 5.A1(B)]. In terms of retention efficiency, sole seems to have energy retention efficiency similar to the Atlantic salmon and protein retention efficiency similar to that of salmonids, Nile tilapia and turbot [Appendix 5.A1(C)].

This method allows us to get an estimate of the growth patterns of different species when data (e.g., growth trials) are scarce.



Appendix 5.A1 – Results obtained by using PLS method to predict the distance/similarity of Senegalese sole in relation to other species. Scatterplot (A) shows the PCA analysis of general growth parameters predictions of sole and other species. Scatterplots (B) e (C) shows the true and predicted metabolic body weight exponents for protein (mbw_exp_P) and energy (mbw_exp_E), and protein (k_P) and energy (k_E) efficiency, respectively. Open circles are the true values and full squares represent the estimates of each species.

Chapter 7

General discussion and conclusion

Given the current situation of overexploitation of fisheries and the growth of the world population, it is essential to explore and develop the aquaculture sector in a sustainable way. Sustainability has grown in importance within the social and economic context, with a crucial focus on ensuring the profitability of production. To enhance efficiency and profitability, it is necessary to closely monitor and control many aspects of fish production in aquaculture (e.g., temperature, feed intake, fish density, fish waste). In this regard, the development of tools that assist producers and enable well informed decision-making during critical periods is imperative. Mathematical models have been employed in this sector for several years, proving their applicability and effectiveness in optimizing farm management operations (Cacho, 1997; Cuenco et al., 1985; Dumas et al., 2010; Ernst et al., 2000; Føre et al., 2018; Lupatsch, 2003; Lupatsch et al., 2003a, 2010; Lupatsch & Kissil, 1998, 2005; McDonald et al., 1996; Mei et al., 2022; Nobre et al., 2019; O'Donncha et al., 2021; Santos et al., 2019; Soares et al., 2023). Nonetheless, there is still potential for refining these models, which can advance our understanding of fish metabolism. Understanding fish metabolism plays an important role in determining the suitable feed, feeding method, and nutrient utilization for fish growth and body composition. These aspects are crucial not only for improving production management, efficiency and sustainability, but also for ensuring the quality of the end product.

In recent years, there has been a tendency to improve the efficiency of aquaculture through technologically advanced methodologies, e.g., the use of sensors, models and increased automation (Bownik & Wlodkowic, 2021; Hu et al., 2020; Kaur et al., 2023; Mei et al., 2022; O'Donncha et al., 2021; Wang et al., 2021). In the particular case of Nile tilapia, considering the prevailing socioeconomic conditions in which it is typically cultivated, the implementation of advanced precision farming solutions involving sensors and real-time control is often impractical. This underlines the importance of having reliable mathematical models for production control that can bridge the gap between fish samplings.

This work contributes with advances in mathematic modelling for fish aquaculture, specifically regarding the methodology involved in process of model development and validation. It also provides insights regarding the body composition and growth patterns of Nile tilapia and other economically important fish species, contributing as well for the understanding of the differences in terms of metabolism in fish species. Finally, and most importantly from a practical point-of-view, robust models of body composition and growth for tilapia tested against independent data were obtained as a result of this work.

7.1. Model development

As mentioned in Chapter 1, modelling is a complex process that involves creating simplified representations of complex systems to gain insight and predict their behaviour. However, achieving accuracy in modelling requires careful consideration and attention to detail. The selection of an appropriate modelling approach and methodology should consider the specific context and requirements of the model. Therefore, when developing models, several options and assumptions have to be taken into consideration. These include the choice of the model type (e.g., allometric or isometric), the nature of error (e.g., additive or multiplicative), and the specific calibration methods to be used (e.g., linear or Huber loss regression), which can exert a strong impact on the quality of the model. This implies that the choices regarding model construction and calibration should be made with a strong reliance on objective criteria. To achieve this, it is important to evaluate not only the calibration errors, but also consider cross-validation errors and diagnostic (e.g., residual) plots, to allow the selection of a model and calibration method combination that best generalizes the data. This is critical because complex models can overfit the data, resulting in overconfidence. Estimations of error scale made on the calibration dataset can underestimate the scale of future prediction errors. Thus, to ensure the development of high-quality models, it is imperative to validate the model construction process using a rigorous and objective process.

In this work, a wide range of plausible models, calibrated using different sensitive calibration methods, were tested through *k*-fold cross-validation, with different values of *k* (i.e., number of folds utilized to split the explored data to estimate the error of each algorithm). In Chapter 2 and 5, it becomes evident that the outcomes of cross-validation can be influenced by the number of folds used, with more complex models being less penalized at higher values of *k* (Hastie et al., 2001). This suggests that a more robust analysis can be achieved by considering multiple values of *k*, to ensure that the chosen process is not overly sensitive to the amount of calibration data available. Additionally, the quantity and quality of the dataset used for model calibration are of paramount importance. The use of datasets that encompass a broad range of weights and various growing conditions in model calibration allowed us to develop body composition (Chapter 2, 3 and 4) and growth models (Chapter 5) with stronger predictive capabilities than some models already published in literature (Chowdhury et al., 2013; Chowdhury & Bureau, 2009; Shizari, 2020; Van Trung et al., 2011). Ultimately, once the final model has been calibrated with the complete training dataset using the optimal process determined by cross-validation, it is crucial to validate it with an independent dataset that was not part of the cross-validation and calibration processes. This step guarantees an unbiased estimate of the expected error when the model is applied to future, unseen data, just as it was done in this work. In contrast, in many prior studies, it can be challenging to ascertain how the model development process was performed - specifically, whether cross-validation or another objective process was employed in the selection of the model and calibration method, and whether the final model was validated using independent data (Breck, 2014; Chowdhury et al., 2013; Chowdhury & Bureau, 2009; de Castro Silva et al., 2015; Konnert et al., 2022ab; Shizari, 2020; Van Trung et al., 2011). This uncertainty poses a challenge when attempting to apply such models in practical scenarios. Therefore, in this work, an effort was made to maintain transparency throughout the model development process, in order to enhance comprehension and offer a description of best practices in modelling, contributing to the scientific community.

7.2. Fish body composition

The body components of Nile tilapia do not seem to exhibit strict proportionality to body weight (referred to as isometry), where a horizontal trend would be expected when plotting the relative abundance of components against fish body weight. Instead, these components appear to be better described by power relationships between the amount of the component and the weight of the fish (known as allometry) (Gayon, 2000; Karachle & Stergiou, 2012). This involves either a positive or negative trend when plotting the relative abundance of components as a function of the fish body weight. However, these relationships appear to be stronger for some components than for others. For example, a strong relationship was observed between Nile tilapia body weight and the relative content of water and lipids (Chapter 2). These findings align with the results reported by Chowdhury and Bureau (2009) concerning the relationship between body weight and lipids. However, there is a disagreement regarding the relationship between body weight and water. The relationship was comparatively weaker for relative protein and ash content. This implies that as fish weight increases, there is not a significant variation in the relative protein and ash content, in contrast to the variation observed in the content of water and lipids. In the case of protein, although the trend is usually slightly positive, it often does not differ significantly from zero or exhibit a horizontal (isometric) pattern (Chapter 6). In the case of ash, the relationship sometimes seems positive, while at other times it appears negative. This variability makes it challenging to assert whether there is a consistent horizontal pattern (isometric) (Chapter 6), or if it deviates significantly from zero, as Chowdhury et al. (2013) and Chowdhury & Bureau (2009) reported for Nile tilapia. Consequently, it may be acceptable to employ isometric models to estimate the relative protein and ash contents, as already reported by some authors (Chowdhury et al., 2013; Chowdhury & Bureau, 2009; Dumas et al., 2007; Lupatsch et al., 2001). As demonstrated in Chapters 2, 3, and 4, using ensemble averaging models that combine predictions from both allometric and isometric models could be a practical approach, particularly when it is difficult to assert whether a trend of certain body component is significantly different from zero, as an alternative to employing different models to predict distinct body components (Chowdhury et al., 2013; Chowdhury & Bureau, 2009; Shizari, 2020; Van Trung et al., 2011). This not only applies to Nile tilapia (Chapter 2), but also to the other species analysed in this work (Chapter 3, 4 and 6). Furthermore, in Chapter 3, a strong relationship (in absolute terms) was identified between water and lipids, as well as between water and protein in fish, both for Nile tilapia and the other species analysed in this study. Similarly, Breck (2011, 2014) reported a strong relation for protein and ash with water mass, showing that these components are constrained by each other. Such relationships between components show less variation when expressed on a lipid-free mass basis and thus could potentially be used to enhance the predictive capabilities of body composition models (Chapter 3). For example, the BC2 model developed in Chapter 3 uses fish body weight and water as inputs, harnessing these associations to improve its accuracy. Therefore, by fixing the water component, in addition to ensuring that the error in the most abundant component (water) is zero, it also helps to ensure that the other body components (e.g., fat, protein and ash) are not overestimated. As a result, the BC2 model demonstrated superior predictive capability compared to models that solely considered fish body weight as an input. Furthermore, the relationship between body weight and ash was weak, with significant variation observed in this component (Chapter 3). However, by incorporating the amount of ash as an input (as demonstrated in model BC3 developed in Chapter 3 and applied in Chapter 4), the variability associated with ash was effectively mitigated, resulting in improved predictions of the other components. These findings allowed the development of a valuable tool for researchers in fish nutrition and fish farmers, enabling them to estimate the body composition of fish in terms of crude protein, crude lipids, water, ash, phosphorus, and energy, as detailed in Chapter 3. These new models exhibit superior predictive capabilities compared to previously published ones (Chowdhury et al., 2013; Chowdhury & Bureau, 2009; Shizari, 2020), as elaborated in Chapter 4. Consequently, their use may be advisable, especially when partial body composition information, such as water and/or ash, is available.

The estimation of fish body composition can be achieved through the utilization of static models (Chapter 2, 3, and 4) or dynamic models (Chapter 5). Static models directly estimate the body composition as a function of fish body weight and/or other observables (e.g., partial body composition information) (Chowdhury & Bureau, 2009;

Dumas et al., 2007). On the other hand, dynamic models consider intrinsic (e.g., body weight) and extrinsic factors (e.g., environmental conditions and nutrition) over time (Chowdhury et al., 2013; Cuenco, 1989; Shizari, 2020; Van Trung et al., 2011). In addition, these models also account for the life history of the fish. This implies that the estimation of future body composition typically considers the current body composition, and other influencing factors, resulting in an accumulation of various effects over time. Thus, by incorporating these dynamic elements, it becomes possible to estimate both the growth and body composition of fish over time. In fact, the results of the combination of static body composition and dynamic growth models (Chapter 5) showed that updating water and ash retention rates in EP models as a function of protein retention rates improves the model accuracy. This integration of dynamic growth models with body composition models proves to be highly valuable for researchers and fish farmers aiming to gain insights into the evolving physiological traits of fish as they growth, and how these characteristics may be affected by factors such as environment and nutrition.

7.3. Fish growth performance

In many fish growth models, energy intake is often the central focus and driving variable (Breck, 2011; Cho & Bureau, 1998; Cuenco et al., 1985; Nisbet et al., 2012; Strand, 2005). However, protein intake should also be considered given that it is a vital nutrient for growth and fish development. It plays a central role in promoting muscle growth, maintaining organ function, and supporting overall metabolic activities (Cowey, 2013; Kaushik & Seiliez, 2010; Lupatsch, 2003; Lupatsch et al., 2010; Lupatsch & Kissil, 2005; Nemova et al., 2021). Protein requirements vary among fish species (Lupatsch et al., 2003a; Tacon & Cowey, 1985; Teles et al., 2020), life stages (Radhakrishnan et al., 2020) and environmental conditions (Carter et al., 2010), emphasizing the need for tailored models that consider these specific factors. By incorporating protein into fish growth models, researchers and practitioners gain a more comprehensive understanding of the factors influencing growth dynamics. Moreover, the interaction between protein and energy metabolism is crucial for growth modelling (Lupatsch et al., 2003a, 2003b; Nobre et al., 2019; Soares et al., 2023). Protein and energy are interconnected in metabolic pathways, where both protein synthesis and turnover require energy expenditure (Hawkins, 1991). Thus, the balance between protein and energy utilization affects growth efficiency and nutrient partitioning in fish. Hence, comprehensive growth models that incorporate both protein and energy factors provide a more accurate representation of growth processes, as demonstrated in Chapter 5. Additionally, accounting for protein in growth models enables the identification of potential limitations or deficiencies in protein intake (Conceição et al., 1998; Konnert et al., 2022; Li et al., 2022; Lupatsch, 2003; Lupatsch et al., 1998, 2003b; Nobre et al., 2019; Soares et al., 2023; Van Trung et al., 2011).

In the analysis of Nile tilapia data, a linear relationship between digestible energy intake and energy gain, and between digestible protein intake and protein gain was observed (Chapter 5), consistent with the observations of Lupatsch et al. (2010). However, it is important to note that this linear relationship may be influenced by the range of diet compositions used in the trials, as pointed out by Konnert et al. (2022ab)., but also by the fish's weight and its genetic growth potential (Halver & Hardy, 2003).Typically, tilapia diets do not contain high levels of protein or fat, which means that a saturation point in terms of protein and fat intake, as reported by Konnert et al., 2022 for Nile tilapia, may have not been reached in the current study due to the reliance on literature reported in the literature. Nevertheless, in the case of carnivorous species or those with high protein requirements, when data on their maximum energy and protein retention efficiency capacity is available, it could be advisable to utilize linear-plateau models or other saturating models (Konnert et al., 2022ab).

In addition, a comparative analysis regrading growth predictions and model parameters between Nile tilapia and other commercially relevant species, was performed in Chapter 6. The results of this comparison question the applicability of universal metabolic body weight exponents across species, as reported by some authors (Clarke & Johnston, 1999; Kleiber, 1932; Ye et al., 2021). However, in Chapter 5, where various growth models were explored for Nile tilapia, we found that the predictive power of the models increased significantly when using fixed universal exponents instead of estimated ones. In this particular case (Chapter 5), the preference for using fixed universal exponents as the best approach may be attributed to the sensitivity of the models, since small changes in the input data can lead to large changes in the estimates of the exponents. Therefore, it is not entirely clear which assumption should be considered valid, and the most prudent approach is to thoroughly analyse the data and objectively explore various hypotheses for model development on a case-by-case basis. In fact, the results presented in Chapter 6 indicate that even though certain species share some characteristics, it does not necessarily imply that it is advisable to apply the same assumptions, such as metabolic body weight or other parameters related to body composition or growth modelling.

7.4. Work limitations and future perspectives

Throughout the development of this thesis, some challenges and limitations were identified, which may be addressed in future studies. Although the models developed in this work for Nile tilapia showed better performance than others in literature, upon comparison with those for other species (Chapter 3 and 4), the models for Nile tilapia were relatively poorer. This discrepancy may be partially caused by the fact that the data collected is from different strains of tilapia. Thus, one solution would be to calibrate the models according to the strains of tilapia or introduce the strain as a factor. Differences between predictions and observations can also be attributed to the quality of available data in the literature, primarily because Nile tilapia is predominantly produced in extensive production systems characterized by limited control and monitoring, which leads to a higher amount of irreducible error. If the calibration and validation data exhibit significant noise, this will be reflected in the predictions errors, making it difficult to precisely evaluate the magnitude of these prediction errors. In order to minimize this issue, it is essential to employ data filters or develop algorithms designed to detect outliers and subsequently exclude them from the analysis. Another alternative would be to obtain higher resolution data with new technologies (e.g., individually tagged fish, to measure individual fish weight on a regular basis) (Mei et al., 2022; Wang et al., 2021). Furthermore, being an omnivorous fish species, Nile tilapia can efficiently utilize carbohydrates as a source of energy, a capability that sets it apart from carnivorous fish. Carbohydrates play a significant role in the nutritional requirements and metabolic processes of tilapia, impacting their growth and body composition (Maas et al., 2020; Schrama et al., 2012, 2018). The fact that carbohydrates were not considered in the models developed in this work may also contribute to explain the higher prediction errors for tilapia. Thus, in the case of omnivorous fish, such as tilapia, it may be worth considering the incorporation of carbohydrates into body composition and growth models to enhance their accuracy. This approach can help in the formulation of balanced diets that meet the specific energy requirements of Nile tilapia, thereby promoting their growth and overall health.

Although the model construction process was developed and validated through a rigorous and objective process, there is no absolute assurance that the choices made during the construction process were optimal for achieving the best possible models. This could potentially be attributed to the selection of a modelling approach and methodology that may not have been ideally suited to the specific context and requirements of the model, thereby not resulting in the best model. The alternative would be to test more alternative models, such as DEB models (Breck, 2011; Nisbet et al., 2012), EP models considering a saturating relationship (Konnert et al., 2022ab), or complex nutrient-based models (Soares et al., 2023), in the case of growth models. Additionally, test alternative calibration methods and carry out parameter recovery tests (i.e., verify whether the calibration method can recover the true parameters when provided with data generated according to the model). Finally, one should also consider that the limitations of the datasets used, which do not cover all possible factor combinations, such as the full range of possible values for body weight, temperature and diet composition, and may contain inherent biases. Thus, further work in this sense should include an effort to obtain a more complete database.

During the development and validation of growth models, in Chapter 5, it is highlighted that protein is an essential component of fish nutrition, directly influencing growth, development, and body composition. Nevertheless, fish also have specific requirements for essential amino acids, which serve as the building blocks of proteins and other critical biomolecules (Furuya et al., 2023). Since fish cannot synthesize these amino acids, they must acquire them through their diet. The metabolism of amino acids in fish is complex and plays a vital role in various physiological processes (e.g., protein synthesis, energy metabolism, and immune function). By incorporating fish amino acid metabolism into growth and body composition models, as previously demonstrated (Soares et al., 2023), it becomes possible to obtain a more comprehensive understanding of how dietary protein is utilized and its implications for fish growth. Moreover, this approach would provide valuable insights into the efficiency of protein utilization, enable the identification of potential limitations, and help determine the optimal dietary balance of amino acids to promote growth. Ultimately, the integration of fish amino acid metabolism into these models would enhance their predictive capabilities, leading to more accurate predictions and a deeper understanding of the factors influencing fish growth and body composition. Fatty acids play also an important role in fish body composition, due to their influence in fat storage/deposition, which can affect unequally all the compartments of the fish body (Soares et al., 2023; Weil et al., 2013). Some fatty acids are also essential nutrients, e.g., n-3 polyunsaturated fatty acids, with key roles in cell membranes and eicosanoid metabolism (Calder, 2012; Tocher, 2003). Other micronutrients, such as, vitamins and minerals, are also important for fish, especially during their development (Halver, 2003; Lall & Kaushik, 2021). Thus, considering dietary fatty acids, vitamins and minerals would also be an important step towards a complete modelling of fish metabolism and growth. Finally, it would also be interesting to conduct a similarity assessment in terms of body composition and growth model predictions and parameters between fish species using these models that account for these specifications (e.g., micronutrients), besides protein and energy, and to contrast it with other auxiliary information (e.g., phylogenetic distances between species).

7.5. Conclusion

This study highlighted the importance of considering both energy and protein when modelling fish growth and body composition. It demonstrated and applied advances in mathematical modelling to achieve accurate predictions of fish body composition and growth. Moreover, it provides valuable reference models for precision farming of Nile tilapia, encompassing both a static body composition model and a dynamic growth model, which were rigorously validated with independent data. Both Nile tilapia body composition and growth models showed lower validation errors compared to some previously published models, demonstrating the importance of developing and calibrating models using objective methods and exploring different assumptions to ensure models that generalize effectively.

Furthermore, this research highlighted the relevance of assessing the similarity in terms of body composition and growth between species to better understand their metabolic differences, which in turn can be relevant for modelling the metabolism of other economically-relevant species like meagre, perch, tuna, cod, and eels.

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