João Miguel Gonçalves Cruz

Development of an empirical growth model for feeding optimization in European sea bass, *Dicentrachus labrax* (Linnaeus, 1758) grown in earthen ponds

Universidade do Algarve

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Declaração de autoria de trabalho

Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

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To my personal and old friends, specially Dinis, Hugo, Tiago and Diogo. Thanks for your support and for your pure friendship.

ABSTRACT

The main objective of this work was to develop a model to predict the biomass of European sea bass in aquaculture earth ponds. Data from several years of sea bass production from the company Aqualvor were compiled to estimate daily biomass as the product of individual average weight (growth model) and number of individuals (mortality model), using an empirical approach to choose the models and estimate their parameters.

Two situations were considered: (1) mean biomass production along the life of the batches, and (2) biomass estimation for short periods (days) based on a bioenergetic model. This bioenergetic model consists of a generalized linear model to predict growth (weight increment) as a function of the explanatory variables water temperature, feed properties and phase of the maturity cycle.

For the mean biomass production, the numbers of the batch were considered to follow a decaying exponential, with age as explanatory variable, and a parameter M, the instantaneous mortality rate. Since mortality rates changes along the production, a model for M as a function of age is also proposed (inverted sigmoid model). For the mean weight as a function of age, a power function was chosen.

The bioenergetic model, to estimate growth was not significant and therefore no estimation of biomass was attempted using this approach. This happened due to a large variation in the response variable (growth increment), resulting from uncontrolled factors, a situation typical of an open system.

To improve fitting of a bioenergetic model, precision on estimation of individual weight and numbers present is necessary. One of the conclusions of this work is the need to develop appropriate sampling methodologies to access growth and mortality that do not induce stress in the fish.

All calculations and data processing were done in R (version R-4.0.3, implemented by R-studio).

Keywords: Sea bass, *Dicentrachus labrax*, aquaculture, feeding optimization, growth model, Aqualvor

RESUMO

Os dados de vários anos de produção de robalo (*Dicentrachus labrax*) da empresa de aquacultura Aqualvor foram compilados para construir um modelo empírico de estimativa da biomassa diária em cada tanque, com o objetivo de otimizar a quantidade de alimento fornecido. Este problema é de grande importância pois a ração é a componente mais cara dos custos de produção aquícola marinha. Dada em excesso, para além do desperdício económico, pode ter consequências negativas na qualidade da água e saúde dos peixes e se insuficiente traduz-se em taxas de crescimento inferiores às ótimas, representando perdas de valor.

Foram utilizados nove lotes (conjunto de indivíduos da mesma idade e origem) de robalo. Cada lote pode corresponder de um a três tanques. Quando a carga no tanque inicial (tanque primário) se torna elevada, parte do peixe é transportado para um tanque vazio (tanque secundário), podendo ainda haver novas subdivisões para outros tanques (tanques terciários). Os lotes nunca foram misturados. No total, os nove lotes corresponderam a 16 tanques. Neste trabalho os dados foram tratados por lote, tanque ou fase da produção, conforme os objetivos.

A estimação da biomassa foi abordada de duas formas diferentes: (1) estimação da biomassa média em função da idade para toda a vida do lote e (2) biomassa estimada para um período de tempo curto (alguns dias), baseada num modelo bioenergético. Estes dois modelos têm aplicações práticas diferentes. No primeiro caso pretende-se estimar a biomassa média global do lote em diferentes pontos (dias de produção). No segundo, utilizar a informação sobre a biomassa para, em cada tanque, fornecer a quantidade de alimento correta para a otimização da produção.

Para o cálculo da biomassa média dos lotes em função da idade, a biomassa foi decomposta em dois componentes, o número de indivíduos no tanque e o peso médio dos mesmos. Ambos os modelos foram desenvolvidos empiricamente com base nos dados dos nove lotes disponíveis.

Considerou-se que o número de indivíduos de um lote segue uma exponencial decrescente, com uma taxa de mortalidade M. A taxa de mortalidade M não foi considerada fixa, mas dependente da idade, de acordo com um modelo de sigmoide invertida. Neste modelo, as taxas de mortalidade são altas nas idades mais jovens, até

um ano de idade, e decaem para valores baixos até aos dois anos de idade. Os valores utilizados para estimar os parâmetros do modelo de mortalidade (sigmoide invertida) foram obtidos a partir das fases da produção em que a contagem de indivíduos é feita com mais rigor. O ciclo de produção dos peixes foi dividido em quatro fases: pré-engorda (onde a mortalidade é bem avaliada), primeiro ano de produção (mortalidade não é bem avaliada devido ao elevado de peixes que morrem sem ser contabilizados), indivíduos com mais do que um ano de idade (mortalidade é bem avaliada, pois os peixes mortos flutuam e podem ser contados) e pesca (nesta fase a mortalidade é bastante baixa e a fase de pescas curta, pelo que não é uma fase relevante para o cálculo da mortalidade). O modelo de mortalidade foi baseado em dados de fases da produção em que a contagem de indivíduos é feita com mais rigor, as fases de pré-engorda e adulta (até se iniciarem as operações de pesca) e foi depois extrapolado para toda a extensão da produção.

Relativamente ao crescimento, e uma vez que a fase de desaceleração do crescimento e aproximação de um valor assimptótico nunca é observada, foi escolhida uma função de potência com expoente livre (parâmetro do modelo) para expressar o peso médio em função da idade. Também para este modelo foram secionados dados em que a taxa de crescimento fosse associada a menor erro, tendo sido escolhidos períodos de crescimento (entre amostragens biológicas do peso dos indivíduos) com um máximo de 10 dias. A biomassa foi obtida multiplicando o peso médio dos indivíduos pelo número de indivíduos no tanque em cada dia da vida do lote. Este modelo de biomassa permitiu calcular a produção média global dos lotes ao longo do tempo.

Para o cálculo da biomassa a curto prazo, foio desenvolvido um modelo bioenergético, que tinha como objetivo final determinar a quantidade de ração a fornecer em cada tanque. Este modelo foi baseado nos modelos bioenergéticos que determinam o aumento de peso diário em função de fatores que influenciam o crescimento. A variável resposta escolhida foi o aumento de peso diário, que se considerou ser dependente da idade, temperatura da água, características do alimento (energia bruta, energia digestível e rácio proteína digestível / energia digestível) e a fase do ciclo reprodutivo (considerou-que o período de maturação, para indivíduos com mais do que um ano de idade, se estende desde 1 de janeiro a 31 de março). Foi usado um modelo linear e o método *stepwise* para identificar as variáveis significativas. Apenas a idade foi retida pelo modelo, o que inviabilizou a definição de uma metodologia para a

estimação da biomassa baseada em fatores ambientais e bioenergéticos e a fase do ciclo reprodutivo.

Todos os cálculos necessários à adequação da base de dados, extração da informação necessário à estimação de parâmetros dos modelos e sua aplicação e produção de gráficos, foram implementado utilizando o software R implementado através de R.

Os resultados evidenciam a complexidade em estimar as taxas de mortalidade em tanques de aquacultura, com grandes variações de fatores não controlados, e dificuldade em obter informação precisa sobre o número de indivíduos nos tanques. No máximo, apenas 13% do total de indivíduos mortos foi retirado o que dificulta a estimação regular do número de indivíduos no tanque. Para melhorar o desempenho do modelo é necessário introduzir com frequência correções do peso individual e números presentes que têm de ser estimadas com maior precisão. Uma das conclusões do trabalho é a necessidade de desenvolver metodologias de amostragem adequadas para avaliar o crescimento e mortalidade, que não induzam stress no peixe.

Palavras-chave: Robalo, *Dicentrachus labrax*, aquacultura, otimização da alimentação, modelo de crescimento, Aqualvor

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1 Introduction

1.1 Importance of marine aquaculture

The gradual increase in demand for fish has led to overexploitation of many stocks. It is estimated that, in 2013, globally, 31.4% of the stocks were overexploited, 58.1% at maximum levels of exploitation and only 10.5% underexploited (FAO, 2016).

The catch of fish in the marine environment reached a maximum in the mid-1990's and since then has remained with slight fluctuations, never surpassing 90 million tons (Figure 1.1). The growing needs of fish have been met by the aquaculture industry, and this activity can be considered as a way of releasing the pressure on marine resources, thus contributing to their conservation.

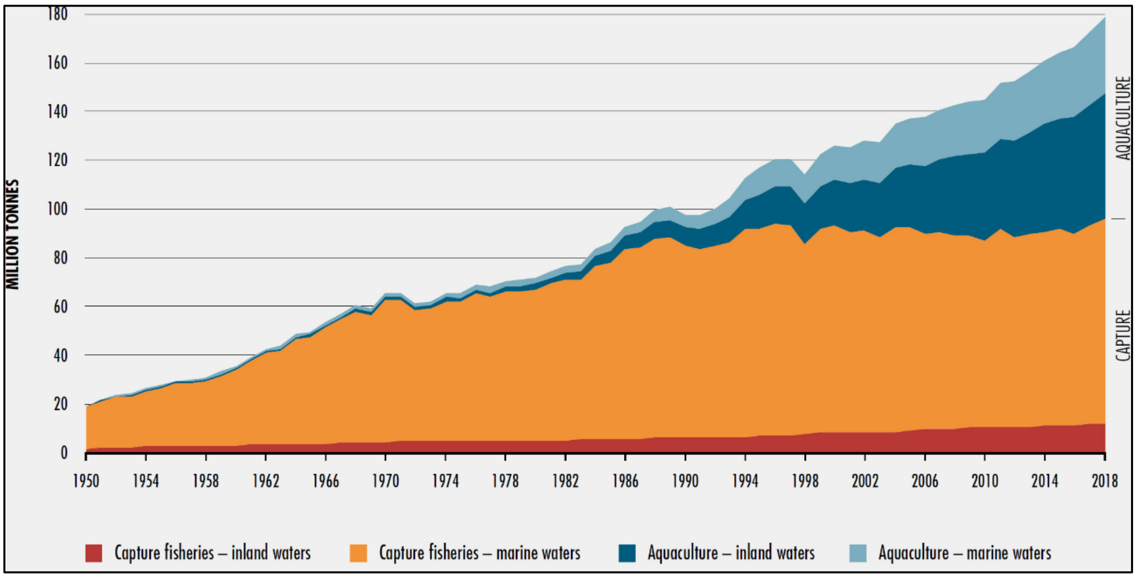

Figure 1.1 - World capture fisheries and aquaculture production (Million tonnes) between 1950 and 2018 (excludes reptiles and seaweeds). Source: SOFIA, FAO, 2020.

Unlike capture in the wild, aquaculture production has increased substantially and, in 2018, aquaculture accounted for 52% of fish consumed by humans (FAO, 2020). This increase was mainly due to the production of species in inland waters (Figure 1.2), species which are eminently herbivorous (light blue bar in Figure 1.2). The main contribution for freshwater fish production comes from Asian countries representing, in 2018, 89% of world´s production by weight. China is the main responsible for this dominance, alone producing 57.9% of the global aquaculture products.

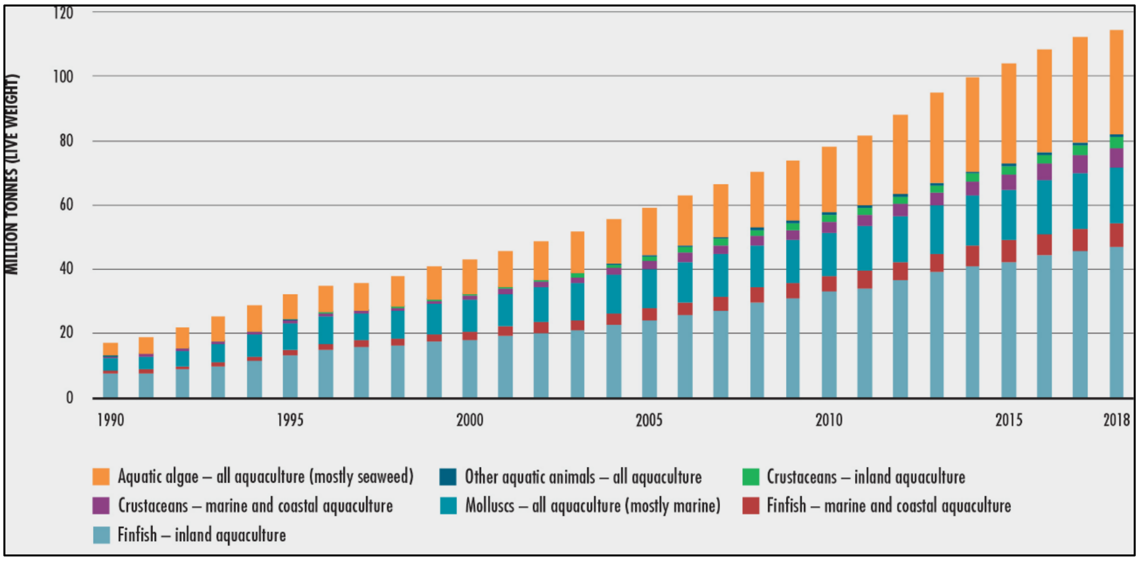

Figure 1.2 - World aquaculture production of aquatic animals and algae, between 1990 and 2018. Source: SOFIA, FAO, 2020.

Aquaculture of marine fish has not increased substantially (red bar in Figure 1.2). This segment, related to the production of carnivorous species, such as salmon, sea bream or sea bass, dominates fish aquaculture in Europe. Despite higher value per weight produced when compared with inland production, low prices related to market competitiveness for mass-produced species have brought great challenges to producers, who continuously seek for solutions to optimize their businesses (FAO, 2020).

1.2 Production of sea bass and sea bream in Southern Europe

Aquaculture has a long history in the Mediterranean, with evidence of fish capture and fattening dating back more than 2000 years. However, it is since the beginning of the 1980's, when the production of sea bass (*Dicentrachus labrax*) and sea bream (*Sparus aurata*) started, that Mediterranean aquaculture has become a success in terms of biomass produced (Department of Marketing & Institute of Aquaculture (University of Stirling), 2004). Nowadays, these two species, which become predominant over other finfish species due to the ease with which they acclimate to captivity, continue to be the two biggest references in the region among produced saltwater species (FEAP, 2019).

In economic terms, these two species have become aquaculture species (Vandeputte *et al*., 2019). In the case of European sea bass, aquaculture production is higher than wild capture since 1992 and in 2016 accounted for 96% of the species' total production, of which 94% was produced in the Mediterranean area (FAO, 2018). In the Mediterranean Sea, farming of sea bass and sea bream is mainly carried out in ponds and cages (Lupatsch, 2003). Greece and Turkey are the main producers, accounting for about 77% of the total production of sea bass and 82% of sea bream in 2019, according to data published by the Federation of European Aquaculture Producers (FEAP, 2019).

The production of these two species has had an exponential growth since its beginning, however, in recent years there seems to have been a stagnation in their production, despite the increase between 2018 and 2019 observed for both species. Indeed, sea bass production increased from an estimated 108 thousand tons in 1998 (Department of Marketing & Institute of Aquaculture (University of Stirling), 2004) to 158 thousand tons in 2015. Production remained relatively constant reaching until around 160 thousand tons in 2019 (FEAP, 2019). Due the difference in temperature between Mediterranean regions, the time required to reach harvest weight, and therefore, costs associated with fish farming vary across regions (Gasca-Leyva *et al.*, 2002).

Portugal, due to its geographical location and characteristics of its coastal area has a unique potential for aquaculture activity (Castelo Branco, 2003). Nevertheless, it was only in the 1990’s that the aquaculture sector in Portugal took off (INE, 2019), with the establishment of many small fish farms. Turbot is currently the most produced species in Portugal, followed by sea bass and sea bream with a total production of 200 and 898 tons, respectively (INE, 2019). These production estimations differ from data presented by (FEAP, 2019), which reports for 2019 the production of sea bass and sea bream to be 450 and 1200 tons, respectively. Both species were mainly cultured under intensive regime in offshore cages (INE, 2019).

In terms of sea bass and sea bream production, Aqualvor is among the most representative companies in the country. It is a 20-hectare fish farm located in Ria de Alvor nature reserve, on the Algarve coast (Figure 2.1). The production system of the farm is semi-intensive, in earthen ponds. The beginning of its activity originated in 1989 with the production of oysters, sea bass and sea bream. Currently, only the last two species are produced, although species such as eel, mullet, common bream and sole, emerge naturally inside the ponds. The maximum capacity of production is around 500 tons/year (https://www.aqualvor.pt/) for the two species.

1.3 Production of sea bass and sea bream at Aqualvor

Aqualvor´s facilities are located in the Ria de Alvor nature reserve (Figure 2.1), a small meso-tidal lagoon with tides between 2-4 m in amplitude, situated on the western Algarve. Besides its role for local tourism and fisheries, the Ria present some interesting features for the culture of marine species as sea bass or sea bream. Water quality is favoured by a highly dynamic water circulation within the lagoon with low residence times (Picado *et al.,* 2020).

Optimal growth for sea bass is reached around 24ºC (Person-Le Ruyet *et al.,* 2004), while for sea bream is around 26ºC (Lupatsch, 2003). Since the average annual temperature in the Ria is around 18ºC, water temperature is the major constraints for the production of sea bass. Water temperature presents also a strong seasonal pattern, varying from approximately 14ºC in winter to around 22ºC in the summer (Picado *et al.,* 2020).

The intensification and extension of sea bass and sea bream production across the Mediterranean and the low prices related, have brought additional challenges to companies such as Aqualvor, which are continuously seeking for solutions to optimize and differentiate their production.

1.4 Optimization of production

The optimization of a fish culture towards attractive economic profits involves maximizing animal´s growth, without compromising its survival, and reducing associated costs. The growth of fish in farms is influenced by several variables: feeding rates, diet composition, water temperature, stocking densities, fish weight, quality of fingerlings, water quality and all environmental conditions. The interaction of all these variables makes fish growth a complex process, difficult to control or predict (Hernández *et al.,* 2003). Nevertheless, this is generally approached by controlling the variables that most influence fish growth.

1.4.1 Feeding

Among all the operations and resources to be managed within a fish farm, feeding represents the largest portion of expenses (30-60% of total operating costs) and is therefore the most important process to optimize (Soares *et al.*, 2020).

However, feeding fish is a complex task when fish are raised in natural environments. Insufficient feeding will lead to lower growth rates and excessive feeding generates waste that is quickly degraded by bacteria in the water, with loss of the feed nutritional properties in a few hours (Cacho *et al.,* 1991). In addition, the degradation of non-consumed feed may cause medium degradation, directly affecting the water quality and the bottom sediments. Therefore, inadequate feeding practices and strategies can result in significant economic losses and/or environmental contamination.

The amount of feed to provide to the fish is an important decision producers face daily. Feeding *ad libitum* is a strategy which is not advised at an industrial scale (Lupatsch, 2003) and farmers are generally guided by tables provided by feed suppliers. These tables, created for each species, determine the amount of food per unit of biomass as a function of water temperature, the factor that has the greatest influence on the amount of food ingested by poikilotherms (Brett & Groves, 1979; Jobling, 1994). Nevertheless, the estimation of the biomass is a complex process, requiring estimates of the number of fish in the tank and average individual weight daily, one of the biggest challenges for farmers (Li *et al.,* 2020). In addition, the variability of temperature adds a risk component to technical farming decisions everyday (Léon *et al.,* 2006).

In recent years, extensive progress has been made in the study of nutritional requirements of fishes. The qualitative requirements of fish are similar to those of other vertebrates (energy, essential amino acids, fatty acids, vitamins and minerals) (Sargent *et al.*, 1999). However, there seem to be differences in quantitative requirements mainly for energy and protein. The actual requirement for dietary gross energy (GE) and protein must take into account the partial efficiency of utilization of these nutrients for maintenance and growth. Ideally, a compound diet should be able to increase its nutritional value based on its digestible energy (DE) and digestible crude protein (DP). However, energy and protein requirements are very complex as they are closely linked. As protein can function as an energy source in addition to its essential role for growth, the efficiency for growth is dependent on the balance between the supply of dietary nonprotein energy and protein (Lupatsch, 2003). The ratio DP/DE is widely used to evaluate this relationship (Haidar *et al.*, 2018). From the perspective of an aquaculture company, the value of these variables is often unknown, given the high variability of raw materials used and the variation in their digestibility coefficients.

In recent years, several improvements have been made at Aqualvor towards optimizing feeding, as shown in Figure 1.3, including:

- Programable automatic feeders allow to extend the daily feeding period and adjust the feeding rhythms to the metabolic needs and appetite of the fish (Figure 1.3-A).
- Floating rations force fish to come to the surface to feed and pellets are well visible if not consumed, providing greater feeding control (Figure 1.3-B).
- Specifically formulated rations, based on a species dietary requirments, were developed in colaboration with feed manufacters (Figure 1.3-C).
- Feeding monitoring was improved with the instalation of video cameras (Figure 1.3-D).

Figure 1.3 - Improvements towards optimization of feeding: automatic feeders (A), fish feeding on floating ration (B), example of sac with specifically formulated ration (C) and feeding monitoring with video cameras (D).

1.4.2 Relevance of water temperature

Water temperature has been pointed out as being the variable with the greatest impact on the success of a fish farm (Hernández *et al.,* 2003, 2007; León *et al.,* 2006). Temperature has significant effects on fish growth by affecting variables such as feed consumption, metabolic rate and energy expenditure (Brett, 1979; Elliott, 1982; Dutta, 1994; Bhikajee & Gobin, 1997). For most warm-water fish species (e.g., sea bass and sea bream), the growth rate increases with increasing temperature to an optimal value for which growth is optimized (Corey *et al.,* 1983; Heap & Thorpe, 1987; Talbot, 1993). However, it should be noted that this optimal temperature is a few degrees lower than the temperature at which feed intake is greatest (Jobling, 1994). The consciousness of this fact is crucial for better feeding management and optimization, especially at warmer temperatures. Over a certain critical temperature, the rates of ingestion decrease drastically, reaching a point where fish stops feeding (Jobling, 1993). Warmer waters generally hold less dissolved oxygen, which is critical for fish appetite and consequently for growth (Thetmeyer *et al.,* 1999; Pichavant *et al.,* 2001). Furthermore, when the critical temperature is reached, the active metabolism of fish increases to levels above the standard ones (Brett, 1964), resulting in a reduction in the growth rate (Calderer Reig, 2001).

In open production systems, whether inshore or offshore, the geographic location of a fish farm is essential to optimize a culture, as the thermal profile of the location will have a great influence on fish growth (Hernández *et al.,* 2003, 2007). Indeed, although some strategies could aid to improve thermal conditions in pond cultures (e.g. manipulation of water renewal/retention in production tanks or reservoir tanks at certain times of the day), producers are always very dependent on the natural conditions of the site. The possibility of having a higher annual average temperature will generate faster growth and enable farmers to either produce more batches or larger fish.

Another important factor is temperature seasonal oscillation which implies that fish growth will not always occur at maximum rate. This fact raises questions regarding, for example, the date of introduction of new fingerlings into production, implying the management of a fish farm is strictly linked to the temperature profile of its location.

1.4.3 Biomass estimation

One of the major constraints affecting feeding optimization is the correct estimation of the fish biomass in a tank. This is a very important task in aquaculture as it is used to decide the amount of feed provided daily to avoid under- or overfeeding (Alver *et al.*, 2005). However, this is a complex process, as fish farming generally involves the culture of thousands of fish inside a tank, and the biomass is influenced by variable daily growth and mortality rates. The most common biomass estimation procedures involve periodic sampling to obtain the fish mean weight (Chan *et al.,* 1998), with the number of existing fish calculated by the difference between the initial number and counted dead fish (Rodríguez-Sánchez *et al.*, 2018). Biomass is then estimated by multiplying the average weight by the number (Costa *et al.,* 2006). However, manual sampling can induce stress on fish and cause physical damage, affecting feeding and consequently growth (Ashley, 2007). Moreover, the number of losses cannot be quantified in the case of extensive deaths, theft, or predators (Li *et al.,* 2020).

Another method used to estimate biomass is based on the daily feed intake that is converted into biomass using the expected feed conversion ratio (Aunsmo *et al.,* 2013). This is however a process which may not be very accurate.

Biomass estimations can also be affected by fish reproduction events. This occurs as the gonad´s weight, during some periods of the reproductive cycle, is a significantly part of the fish weight. The European sea bass, under cultured conditions, mainly males, show a high incidence of early sexual maturation when compared to individuals from wild populations (Graziano *et al*., 2018). In the wild, males normally mature at two to three years of age and females at three to four years of age, between December and March in the Mediterranean (Pérez-Ruzafa & Marcos, 2014). In sea bass aquaculture 20% to 30% of males reach sexual maturity during the first year of life, depending on environmental stimuli and fish metabolism. Male *Dicentrachus labrax* are predominant in cultured stocks (~70%) (Graziano *et al*., 2018).

1.5 Application of growth models to fish production

Fish growth in aquaculture has long been estimated by means of mathematical modelling. Models are useful tools as they have the ability to represent complex phenomenon (e.g. fish growth) in a relatively simple way (e.g. weight increment as a function of temperature/protein intake) (Dumas *et al.,* 2010). In fish farming, growth models can be constructed from the information accumulated over the years of activity. Although these types of tools have been used by the scientific community for several years (e.g. Lupatsch & Kissil, 1998; Hernández *et al.,* 2003; Mayer *et al.,* 2008), the importance of its application for producers has only recently emerged.

The development of suitable growth prediction models could be essential to reduce operating costs by optimizing the daily food amount, the organization of management operations and the production plan. Several tasks could be improved within a fish farm depending on a good estimation: calculation of daily feeding rates, planning of feed purchases, control over biomass, unloading of new batches and harvesting schedule.

Nevertheless, the various environmental factors that influence fish growth and the necessity to develop adequate growth models, show the need to develop production models adapted to local conditions and species.

1.6 Proposed work

Over the past 30 years, Aqualvor produced a volume of data regarding sea bass and sea bream culture, that constitutes an important know-how, with the potential to be explored to optimize feeding. In this work, these historical data will be used to develop an empirical production model adapted to the company's conditions in order to guide feeding decisions in the future. The final objective is to produce a tool that, based on daily information on water temperature, biomass, and feed type, outputs the amount of feed to supply to each tank. This should require minimal human involvement, relying on data which is automatically collected.

It is expected that the information necessary to the creation of the tool will be provided, using as a model the sea bass *Dicentrachus labrax*.

2 Materials and Methods

2.1 *Aqualvor modus operandi*

Aqualvor's production system currently comprises twenty production tanks (earthen ponds in a semi-intensive regime), and two pre-fattening indoor facilities (cement tanks in semi-closed system) with three tanks each. The fish production process begins with the entrance of new fingerlings obtained from external hatcheries. The fingerlings arrive with an average weight between 2g and 15g and are placed in the pre-fattening tanks. Often, when one of the earthen ponds is available, the fingerlings do not go through the pre-fattening facility and go directly to the outer tanks. Information about the initial number of individuals and average weight from each batch are provided by the hatcheries.

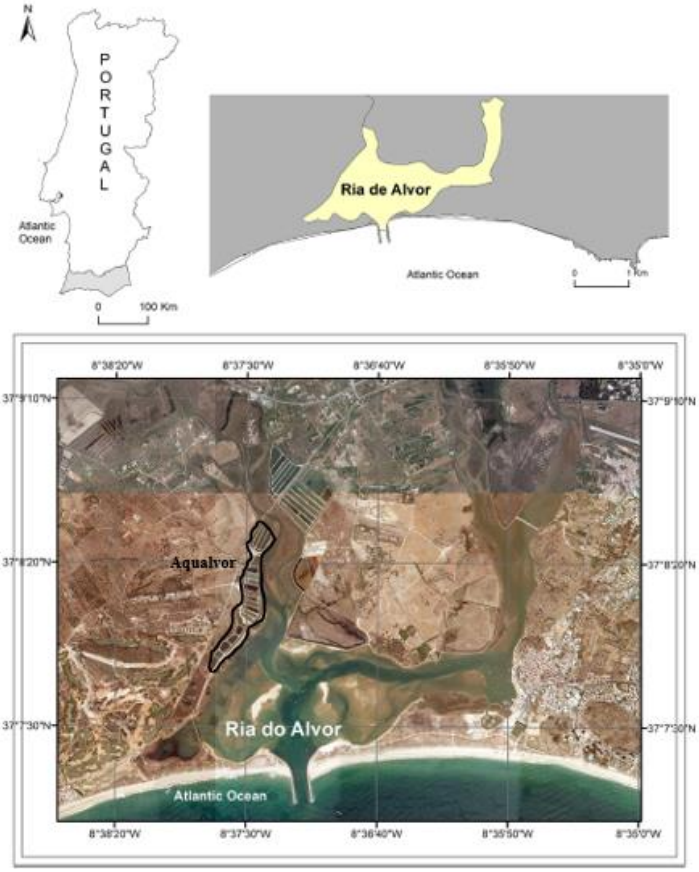

Figure 2.1 - Location of Aqualvor. Original figure from Mateus et al., (2016)*, modified by introducing the geographic delimitation of Aqualvor (with permission from the authors).*

During pre-fattening, fish culture conditions differ from earthen ponds by a higher control over environmental variables, feeding and biomass. The pre-fattening circuits are semi-closed with permanent water recirculation among the tanks, and a reservoir tank with water pumped from the outside. The physicochemical parameters are regularly assessed and adjusted to optimal values. Feeding is closely monitored and carried out with automatic feeders with manual feed supplementation (important to reduce fish size variation). Feed provided is around optimal values as the biomass in pre-fattening tanks is regularly estimated. The number of individuals is estimated daily by subtracting the number of dead fish observed from the number in the previous day. The mortality in the pre-fattening tanks is checked daily through a collector located in the central area of the tank, where all dead fish are retained (Figure 2.2-A). This method allows the exact counting of daily losses, a different situation from earthen ponds where daily mortality is very hard to access at this life stage of the fish. During the pre-fattening phase, fish sampling is periodically carried at least every 2 weeks. Sampling in indoor installations (done via dip-nets) is less laborious than sampling carried in the outside tanks (done with purse seine, Figure 2.2-B) and introduces less stress to the fish.

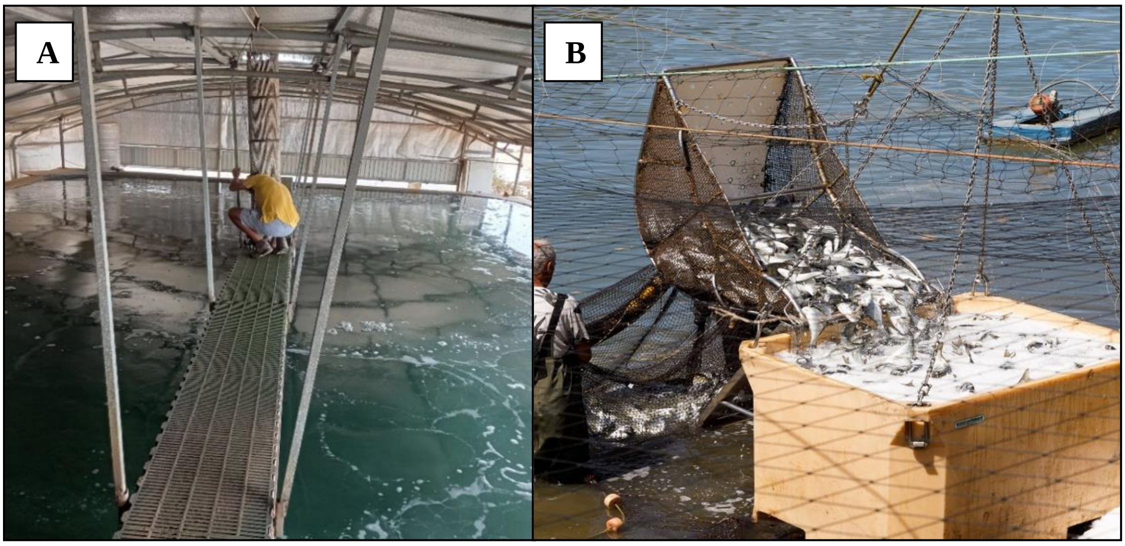

Figure 2.2 - (A) Mortality is checked every day throughout pre-fattening phase. The total number of dead fish is easily accessible as the cadaver are retained in a central collector (photo by João Cruz, 2021). (B) At the end of the production cycle, fishing takes place two or three times a week. A tank is fished over several weeks until it is completely empty. Fishing periods in a tank can be continuous or interrupted with during weeks or months. (Source: adapted from https://www.aqualvor.pt/)

Fish are moved to earthen tanks with an average weight of between 30-60g or as soon as a tank is available. In these tanks, control over biomass is more complex. The collection and quantification of the number of losses occurs only when dead fish are observed floating. Thus, mortality is underestimated, as many losses accumulate on the bottom and are not counted (in small fish the decomposition process is especially accelerated). In addition, in the case of extensive deaths, mortality cannot be well quantified as fish get lost on the tank´ margins where they are exposed to the action of predators (mostly crabs).

Sampling strategies are not consistent along the life of a batch or among batches. During the pre-fattening phase, each sample consists of 300 individuals that are weighed, and, from a sub-sample of 30 individuals, the individual total length, body depth and thickness and head width are measured. Other aspects such as physical condition or color are evaluated according to qualitative scales. In production tanks, biomass estimation is biased by the low number of samplings carried out. In this phase, the period between samples increases to about three months, to minimize the impact on fish feeding. In extreme cases, after a sampling event, the fish may take 2 weeks to recover and regain its regular appetite. Despite this, in some batches, the sampling frequency was maintained high throughout the production. In all tanks the fish is sampled frequently during fishing operations, but these data are not useful to estimate growth because of the stress induced and eventual choices (intentional or not) of weight categories along the fishing phase.

Along the production phase, and depending on space availability, part of the biomass of a tank can be transported to another tank. Transports take place to reduce the density of individuals in a tank and speed up the production cycle. Fish are only transported to tanks that are empty to avoid mixing fish from different batches. The source tank, where all the fish from a batch started production, is identified as primary tank. After transport. the batch of fish continues its production in the primary and secondary tank. This process may be repeated, and a tertiary tank may become associated to the same batch.

At Aqualvor, the production process ends about 2 years after the arrival of the fingerlings, when the fish reaches around 400g. Certain batches of sea bass reach about 1000g, with an extended production period that is never less than 3 years. The fishing process takes several weeks until the tank is completely empty (Figure 2.2-B).

In recent years, Aqualvor has made considerable investments in technology to automate processes and reduce labor. At various locations throughout the system, oxygen, temperature, and level sensors, with instantaneous readings, were installed. The water renovation process is currently carried out automatically through the opening and closing of gates.

Water temperature was the only environmental variable used in this work. Temperature data were collected from sensors positioned at a depth of about 1m (considering minimum level of the tank). These sensors are present in every land-based and pre-fattening tank and are also placed in strategic locations outside the company's facilities close to water catchment and discharge. Temperature sensors are subject to regular maintenance and calibration and temperature readings are stored in a database that include daily average, minimum and maximum temperature. For this work, the average daily temperature was used.

Feeding was optimized with the implementation of automatic feeders, programmed to work in specific periods according to the feeding strategies defined for each tank and species. Feeding is monitored *in situ* by observers who make regular rounds and verify the best use of the feed. The company uses a fish appetite scale (0 to 5) to evaluate the feeding behaviour (0 being no appetite and 5 highly voracious appetite). Changes to quantity of feed provided are made based on these observations to avoid food waste.

Since 2012, data collection procedures were standardized (biological sampling, feeding and physical-chemical variables) and databases were created. In this work, only sea bass production data from 2012 to 2020 were used.

2.2 Database setup

This work includes data from 9 batches of sea bass that have grown in Aqualvor's facilities since 2012. The batches were identified by an ID, which results from the concatenation of the primary tank number and the date of arrival of the fingerlings at Aqualvor (for example T07_20140711 means the fingerlings arrived the 11 of July of 2014 and were placed in tank 7) (Table 2.1).

Table 2.1- Summary table for the 9 batches of sea bass (2012-2020).

Batch ID	**Tank**	**Estimated initial n individuals (thousands)**
T09_20120713	9	155
T15_20120713	15	155
T18_20130314	18	420
T12_20140516	12	240
	13	162
	14	66
T07_20140711	7	150
	9	162
T16S_20150506	16S	361
	17	154
T07_20160407	7	182
	8	179
T12_20160713	12	307
	11	135
T15_20170413	15	229
	14	167

Other variables in the database included age of the fish, information related to the number of individuals in the tank, type and amount of feed, number of individuals transported between tanks, average weight of sampled fish, reproductive period (in reproduction or not), fishing operations and temperature.

Table 2.2- List of variables included in the initial database.

NAME	TYPE	DESCRIPTION
ID	Factor	Batch identification
Data	Date	Date
PreEngorda	Factor (Boolean)	Pre-fattening (0=no, 1=yes)
Tanque	Factor	Tank
TanqueOrdem	Numerical	Tank Order (1=primary, 2= secondary, 3=tertiary)
Especie	Factor	Species (sea bass or sea bream)
Idade	Numerical	Age in days
Reproduz	Factor (Boolean)	Jan-Mar = 1, other months =0 (sea bass)
N_Estimado	Numerical	Number at date estimated (based on food consumption)
N_Observado	Numerical	Number at date observed (based on discount of counted dead fish)
N_Mortos	Numerical	Number dead fish removed from tank
Fase	Numerical	Stages of the fish production cycle.
Temperatura	Numerical	Average daily temperature (ºC)
TipoRacao	Factor	Numerical code for feed type
QtdRacao	Numerical	Number of sacs of feed
Transportes	Numerical	Number of individuals transported to another tank
PescaDourada	Numerical	Number of fished individuals - sea bream
PescasRobalo	Numerical	Number of fished individuals - sea bass
Amostragens	Numerical	Average weight of 300 sampled individuals (g)
Pontos_modelo	Factor (Boolean)	Samples to include in the growth model (0=no, 1=yes)
TempLag	Numerical	Average temperature for 7 days.

The average daily temperature (from the temperature sensors) was used to generate a new temperature variable by applying a running average of 7 days (prior to the date of interest). The choice of 7 days was guided by the present practices at Aqualvor. Presently, the biomass in each tank is calculated from the food consumed, assumed to correspond to optimal feeding, and the conversion rate provided by the feed manufacturer. The prediction of biomass for the following day is based on this estimate and the water temperature from the previous 7 days.

The database includes daily records of 20 variables (Table 2.2). All data manipulation in the database were conducted using R software, version R-4.0.3, implemented by R-studio (R Core Team, 2020).

2.2.1 Variables related to feed properties

Feed type was represented in the database using a numerical code (TipoRacao). This code is associated with specific feed data, such as manufacturer and feed properties. Other properties of the feed composition were included such as gross energy (GE) (MJ/kg), digestible energy (DE) (MJ/kg) and the ratio between digestible protein and digestible energy (DP/DE ratio) (g/MJ). The information on feed nutritional properties was obtained from the technical feed sheets. In cases where the values were not available, the following equations for gross energy (Equation 1), digestible energy (Equation 3) and digestible protein (Equation 4) were used:

$$\textbf{Gross Energy (MJ/kg)} = \begin{array}{l} \text{Crude Protein (\%) x 23.6 MJ/kg +} \\ \text{Lipids (\%) x 39.5 MJ/kg +} \\ \text{Total Carbohydrate (\%) x 17.2 MJ/kg} \end{array} \qquad \text{(Equation 1)}$$

Mean values of crude protein (CP), lipids and carbohydrates (23.6 MJ/kg, 39.5 MJ/kg and 17.2 MJ/kg, respectively) were used based on Blaxter (1989). The percentage values of CP and fat were obtained from the technical feed sheets. Aproximate percentage of total carbohydrate was estimated using the Association of Official Analytical Chemists (AOAC, 2000) method:

$$\textbf{Total Carbohydrate (\%)} = 100 - (\text{Protein} + \text{Fat} + \text{Moisture} + \text{Ash}) \qquad \text{(Equation 2)}$$

Digestible energy (DE) was calculated from gross energy (GE) and $ADC_{GrossEnergy}$ (Apparent Digestibility Coefficient of gross energy), assumed to be 88%:

$$\textbf{Digestible Energy (MJ/kg)} = \text{Gross Energy (MJ/kg) x } ADC_{Gross\ Energy}\ (\%) \quad \text{(Equation 3)}$$

Digestible protein (DP) was estimated in a similar manner, based on crude protein (CP) and an ADC value of 89%:

$$\textbf{Digestible protein (g/MJ)} = \text{Crude Protein (MJ/kg) x } ADC_{Crude\ Protein}\ (\%) \quad \text{(Equation 4)}$$

The values for the ADC coefficients were obtained by calculating the ratios DE/GE and DP/CP for cases where the values of the variables were known.

For lack of better information, a 7-day running average (same number of days as for temperature) was applied to the feed properties.

The variables related to food properties included in the database are listed in Table 2.3. They were merged with in initial database using the numerical code for feed type, common to both databases.

Table 2.3 - List of variables related with feed properties, added to the database.

NAME	TYPE	DESCRIPTION
TipoRacao	Factor	Numerical code for feed type
NomeRacao	Factor	Feed name
Peso_unidade	Numerical	Weight (kg) of each bag of feed
EnergiaBruta	Numerical	Feed raw energy (MJ/kg)
EnergiaDigestivel	Numerical	Feed digestible energy (MJ/kg)
RacioPD_ED	Numerical	Ratio between digestible protein and digestible energy (g/MJ)
EBLag	Numerical	Feed raw energy 7-day running average (MJ/kg)
EDLag	Numerical	Feed digestible energy 7-day running average (MJ/kg)
RPDLag	Numerical	Ratio between digestible protein and digestible energy 7-day running average (g/MJ)

2.3 Biomass estimation

Two situations were considered: (1) mean biomass production along the life of the batches, and (2) biomass estimation for short periods (days) based on a bioenergetic model. The two models differ in the way growth is considered. While in the mean biomass model growth was expressed by a function relating average weight and age (growth model), in the bioenergetic model growth increment, for a short period of time, was based on a general linear model with the explanatory variables: water temperature, feed properties and phase of the maturation cycle.

With respect to mortality, the number of fish along time was considered to depend on age and mortality rate, also dependent on age (mortality model). Such a model could be used to predict numbers either along the life of a batch or over short periods of time.

2.3.1 Estimation of the number of individuals (mortality model)

The number of individuals was built with two components: (1) a simple decaying exponential function with one parameter, the instantaneous mortality rate M, and (2) a model for the instantaneous mortality rate M based on the age of the fish. The first part of the model is expressed by equation 5:

$$N_{t+1} = N_t \, e^{-Mt} \qquad \text{(Equation 5)}$$

where N_t is the number of individuals at the beginning of time interval t, N_{t+1} is the number of individuals at the beginning of time interval t+1 (the same as the number of individuals at the end time interval t), M is the instantaneous mortality rate and t is age. The instantaneous mortality rate results from the invention of equation 5:

$$M = - [\ln(N_{t+1}/N_t)]/t \qquad \text{(Equation 6)}$$

Since t in days, the values of M refer to instantaneous daily mortality rate. Experience obtained during years of production at Aqualvor showed that mortality is higher at early stages, reducing to low values towards the end of the production process. For this reason, M was not considered constant, and a model to express the change in M with age was empirically developed, based on mortality rates obtained from individual tanks for phases of the production where the number of individuals at the beginning and end of the phase could be estimated with acceptable accuracy.

The production cycle was divided into four phases assuming that, at the end of the production process, all survivors of the batch were removed during the fishing operations. The following phases were defined:

- Phase 0 – Pre-fattening – The initial number is well estimated (provided by the fingerlings supplier), and number of dead individuals are counted and are removed daily (see Figure 2.2-A). A mortality rate for this period of the life of the fish can be estimated, based on equation 6.
- Phase 1 – Fish in earthen ponds until day 365 of production - Initial number is well estimated (either provided by the fingerlings supplier or number out of the pre-fattening tanks) but final number is not (unaccounted mortality during this phase), therefore M cannot be estimated.
- Phase 2 – Fish more than 1 year in production, but before fishing operations starts - It is assumed that from this point on mortality can be assessed with acceptable accuracy. The numbers at the beginning of phase 2 were estimated by adding all fish dead or removed (fished) from the tank until the end of production. The final number of phase 2 can be estimated by adding fish dead and removed from the tanks during the fishing phase (phase 3).
- Phase 3 – Fishing – This phase starts with the first fishing operation and lasts until all fish are removed from the tanks. This phase is short and was not considered for mortality estimation.

The phases were defined in the initial database (Table 2.2). Only data from phases 0 (pre-fattening) or 2 (from tanks where dead fish were regularly collected) were used to obtain M. The mortality rates were studied as a function of the age of the fish, assumed to be the average age of the phase.

Two models were considered: (1) a simple linear model where M decays linearly as a function of age and (2) an inverted sigmoid model (Chen & Chang, 1991) where mortalities have a high plateau at early ages and decay to a lower plateau at older ages. The inverted sigmoid model is:

$$M\ (t) = P1 + \frac{P2-P1}{1+exp\{k(t-P3)\}} \qquad \text{(Equation 7)}$$

where t (independent variable) is age, P1 is the asymptote for high values of M observed at early ages, P2 the is the asymptote for low values of M observed at older ages, P3 inflection point of the inverted sigmoid model and k is a constant related with the slope between asymptotes. The parameters of the models were obtained using, the linear and non-linear fitting function available in R (R Core Team, 2020).

2.3.2 Estimation of the average weight (growth model)

For the average biomass estimation, a power function was used to predict weight at age:

$$W = a\ t^{b} \qquad \text{(Equation 8)}$$

where W is the weight of a fish (grams) at age t (years), and “a” and “b” are parameters. This simple model was chosen because an earlier evaluation of the evolution of the weight did not show deceleration or trend towards an asymptotic value.

2.3.3 Bioenergetic model

In the bioenergetic model, growth was based on the biological samplings done regularly. Periods between samples were considered and the daily weight gain (DWG) was estimated by:

$$DWG = \Delta w / \Delta t \qquad \text{(Equation 9)}$$

where DWG is weight gain in grams and Δt the duration of the period between samplings in days. Only periods of 10 days or less were used.

The dependence of DWG on several variables was assessed with the general linear model:

$$DWG = R + age + T_{7days} + GE_{7days} + DE_{7days} + DP\_DE_{7days} + error$$

(Equation 10)

where R is a strig variable (0 –gonads not maturing, 1 –gonads in maturation), age is the average age of the period in days, T is the water temperature, GE is the gross energy in MJ/kg, DE is digestible energy in MJ/kg and DP_DE the ratio digestible protein, digestible energy. For the variables T, GE, DE and DP_DE, a running average of 7 days was applied to each day of the period. The fitting was done using the *lm* function of the package {olsrr} from R (R Core Team, 2020).

3 Results

3.1 Mortality

Global analysis of mortality is presented for the different batches and tanks in % (Table 3.1).

Table 3.1- Data for the 9 batches of sea bass farmed between 2012-2020 (in %). Total number of individuals at the beginning of the batch or tank was distributed in 3 groups, fished, dead (removed and counted) and unaccounted (fish assumed to have died). Total mortality was the summation of dead and unaccounted fish.

Batch ID	Tank	% Fished	% Dead counted	% Unaccounted
T09_20120713	9	68	11	21
T15_20120713	15	66	1	33
T18_20130314	18	63	1	36
T12_20140516	total	63	3	34
	12	43	1	56
	13	82	4	14
	14	90	9	1
T07_20140711	total	75	8	17
	7	68	9	23
	9	82	8	10
T16S_20150506	total	53	5	42
	16S	40	4	56
	17	85	7	8
T07_20160407	total	60	6	34
	7	63	12	25
	8	56	0	44
T12_20160713	total	52	2	46
	12	32	2	66
	11	98	2	0
T15_20170413	total	59	13	28
	15	37	22	41
	14	89	0	11

It is observed that, at most, only 13% of the total number of dead individuals is accounted for in each batch (T15_20170413). Unaccounted mortality varied between 17% and 46% for the different batches. The low number of dead fish counted is due to two causes: (1) high proportion of fish that dies and either degrades or sinks or (2) inconsistencies in the registration of dead fish. In fact, for some of the tanks, there were long periods where no dead individuals were counted. For this reason, when using values from phase 2, for the mortality model, only tanks with regular dead counts were considered. The low number dead fish accounted for is a problem if the number of fish in the tank is estimated by deducting the number of deaths. This error will lead to significant overestimation of the biomass in the tank and consequently overfeeding.

The use of average values of total mortalities (including counted and unaccounted dead fish) would also be a problem due to large variability among tanks, particularly at earlier stages of the production process. The observed variation in total mortality is presented in Figure 3.1, for primary tanks (tank type=1) and secondary and tertiary tanks (tank type=2). Primary tanks, including phases with younger individuals, have global mortality values ranging from of 32% to 68% while in secondary and tertiary thanks the mortality ranges between 2% and 44%.

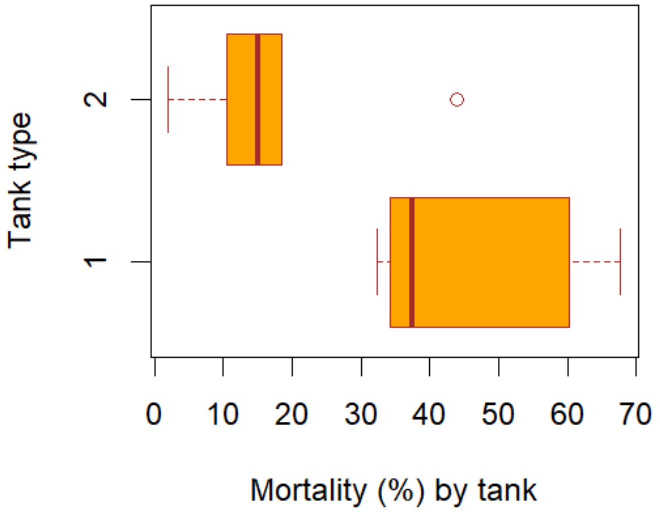

Figure 3.1 - Boxplots representing the percentage of mortality in each tank individuals by primary tanks (initial tank of the batch) and secondary and tertiary tanks (opened later when biomass density becomes high in the primary tanks).

The results obtained for instantaneous mortality rates (M) in phases 0 and 2 are shown in Figure 3.2 (mortality rates converted to year by multuplying daily rates by 365). The values of M ranged between 0.0890 and 0.1604 for phase 0 and between 0.0058 and 0.0275 for fase 2 (excluding outliers). These results confirm the assumption that mortality rates decay over time.

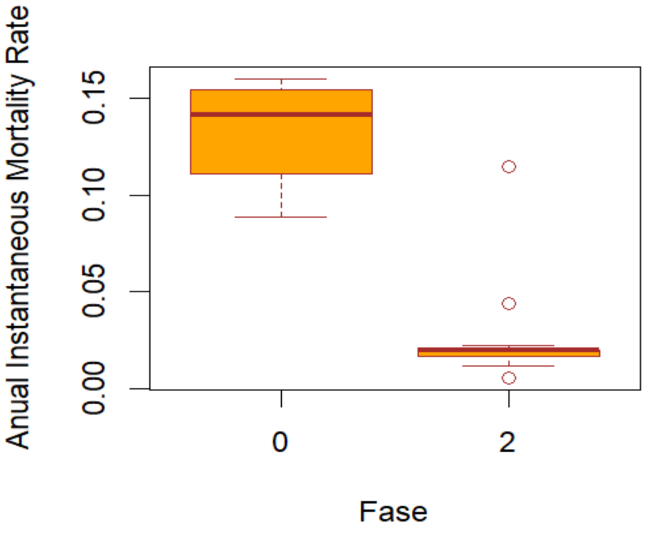

Figure 3.2 – Graphical representation (boxplots) of the range of estimated mortality rate values for phases 0 and 2.

Two models were used to represent the change in mortality as a function of age (average age values for the phase were used), linear and inverted sigmoid.

The fitting of the linear model is presented in Figure 3.3 (residual standard error (RSE) = 0.02932). The estimated parameters were intercept a= 0.1874 and slope b=-0.0820 (p-value for the test $\beta=0$ was <0.001).

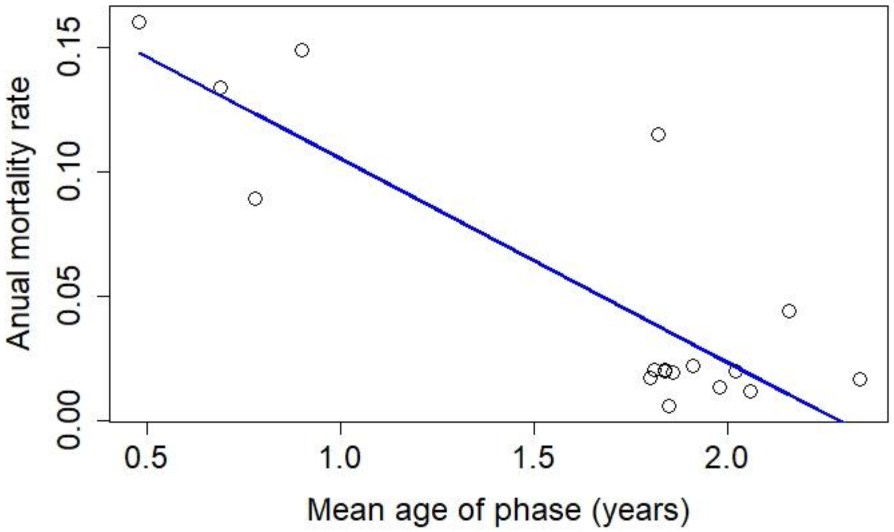

Figure 3.3 – Linear model for instantaneous mortality rate as a function of age: M=0.1874-0.0820age.

The fitting of the inverted sigmoid model is presented in Figure 3.4. This model represents well high mortality at young ages (phase 0 lasts until one year in production), followed by a drop in mortality between phase 0 and the beginning of phase 2 and a stabilization of low mortality at older ages. The estimated parameters were P1=0.0112, P2=0.1691, P3=1.1475 and k=2.8769. The results showed a good fit of the curve to the data (RSE = 0.0301, and convergence criteria met).

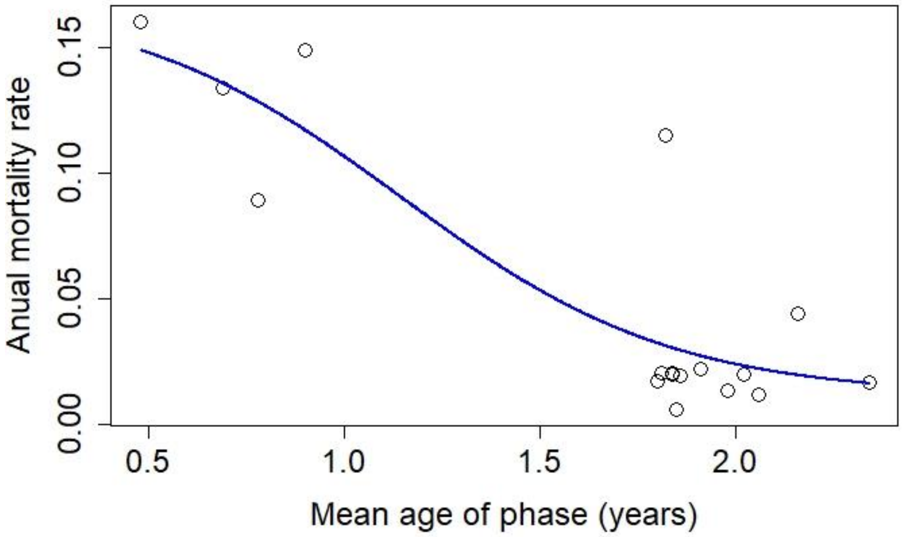

Figure 3.4 – Inverted sigmoid model for instantaneous mortality rate as a function of age: $M = 0.0112 + (0.1691-0.0112) / [1+e^{2.8769\,(age-1.1475)}]$.

The fitness of the models (evaluated here by RSE) shows that both functions could be used to model the sea bass mortality. Although the RSE was slightly lower for the regression line, the sigmoidal model makes more sense from the biological point of view, because the evolution of mortality is unlikely to be linear and the sigmoid model does not generate negative values for M at older ages. Therefore, this function was used in this work to estimate mortality and the number of individuals over time.

3.2 Growth

Data for initial and final weights obtained in each of the nine batches and tanks are presented in Table 3.2. Production started in each of the primary tanks with estimated individual average weight between 1.9g and 4.4g. Initial ages were comprised between 128 and 175 days after hatching. After division, six of the nine batches continued its production in secondary/tertiary tanks with initial weights ranging between 36.1g and 269.3g. The final individual average weight for each batch varied between 365g for tank 12 (T12_20140516) and 1112g for tank 17 (T16S_20150506). The production time (period between arrival and last fishing) ranged between 750 days for tank 12 (T12_20140516) and 1500 days for tank 17 (T16S_20150506).

Table 3.2- Growth data for the 9 batches of sea bass farmed between 2012-2020. The final weight is the average of weights obtained in the last 5 fishing operations.

Batch ID	Tank	Initial weight (g)	Final weight (g)
T09_20120713	9	4.4	522
T15_20120713	15	4.2	384
T18_20130314	18	3.2	448
T12_20140516	12	4.2	365
	13	40.3	861
	14	196.6	855
T07_20140711	7	3.3	521
	9	36.1	543
T16S_20150506	16S	3.7	1037
	17	67.7	1112
T07_20160407	7	3.3	594
	8	269.3	516
T12_20160713	12	1.9	551
	11	193	716
T15_20170413	15	1.9	530
	14	207	666

3.2.1 Weight as a function of age

In total, 933 growth periods (time between samplings) were available for the 9 baches in the study. From the analysis of growth profiles for individual tanks (appendix I) it was clear that sampling events during fishing operations could indicate a decrease in average weight, likely due to removal of larger fish first. Another potential source of error was the duration of the periods, ranging from a few days to several months. Long periods could contain differences in variables, such as temperature and feed properties, used as explanatory variables for DWG, that would introduce considerable bias. Two filters were applied to exclude data: (1) points during fishing operations with negative DWG and (2) points corresponding to periods greater than 10 days. Extreme outliers for DWG, assumed to be the result of sampling errors were also removed (23 points). The final database used to model growth had 434 observations.

Mean weight as a function of average age of the period is presented in Figure 3.5.

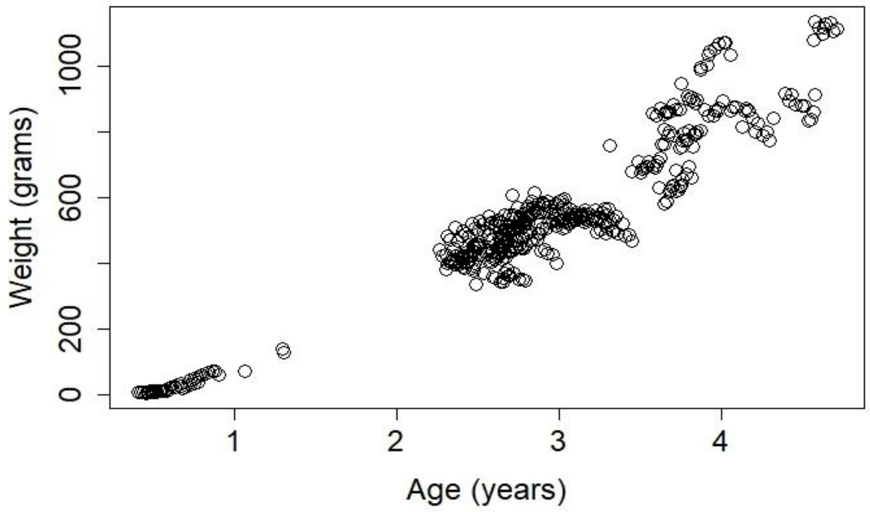

Figure 3.5- Relationship of average weight (g) with average age (years) of the periods, n=434.

Due to the variability in the data, and unbalance in sampling effort for different age, mean weight values for half year periods were obtained and the model was fitted to these averages (Figure 3.6): $W = 0.3721\ t^{1.4322}$ (p-value < 0.001 for both parameters). The non-linear function *nls* from R{base} was used to fit the model.

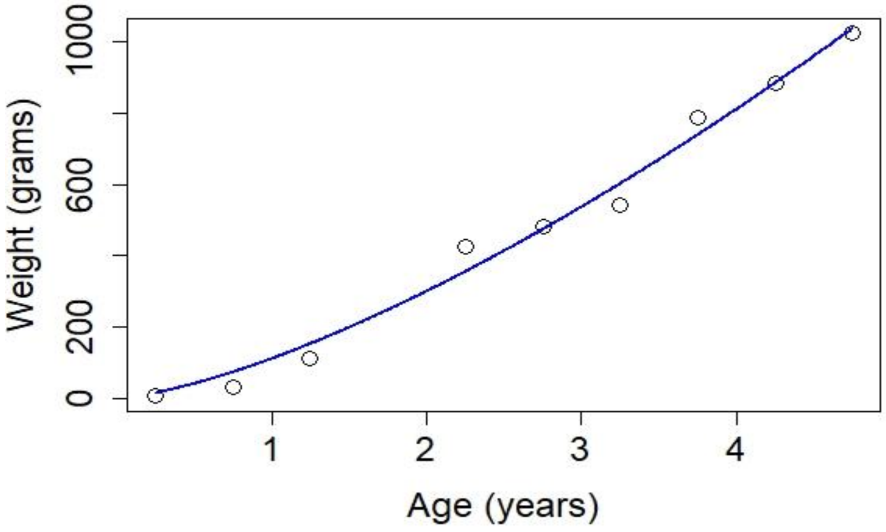

Figure 3.6- Power model obtained for weight (g) as a function of age (years): $W = 0.3721\ t^{1.4322}$

3.2.2 Bioenergetic model for daily weight gain

A first evaluation the relationship of DWG with age, temperature, food properties and reproductive period was done with scatter plots (Figure 3.7).

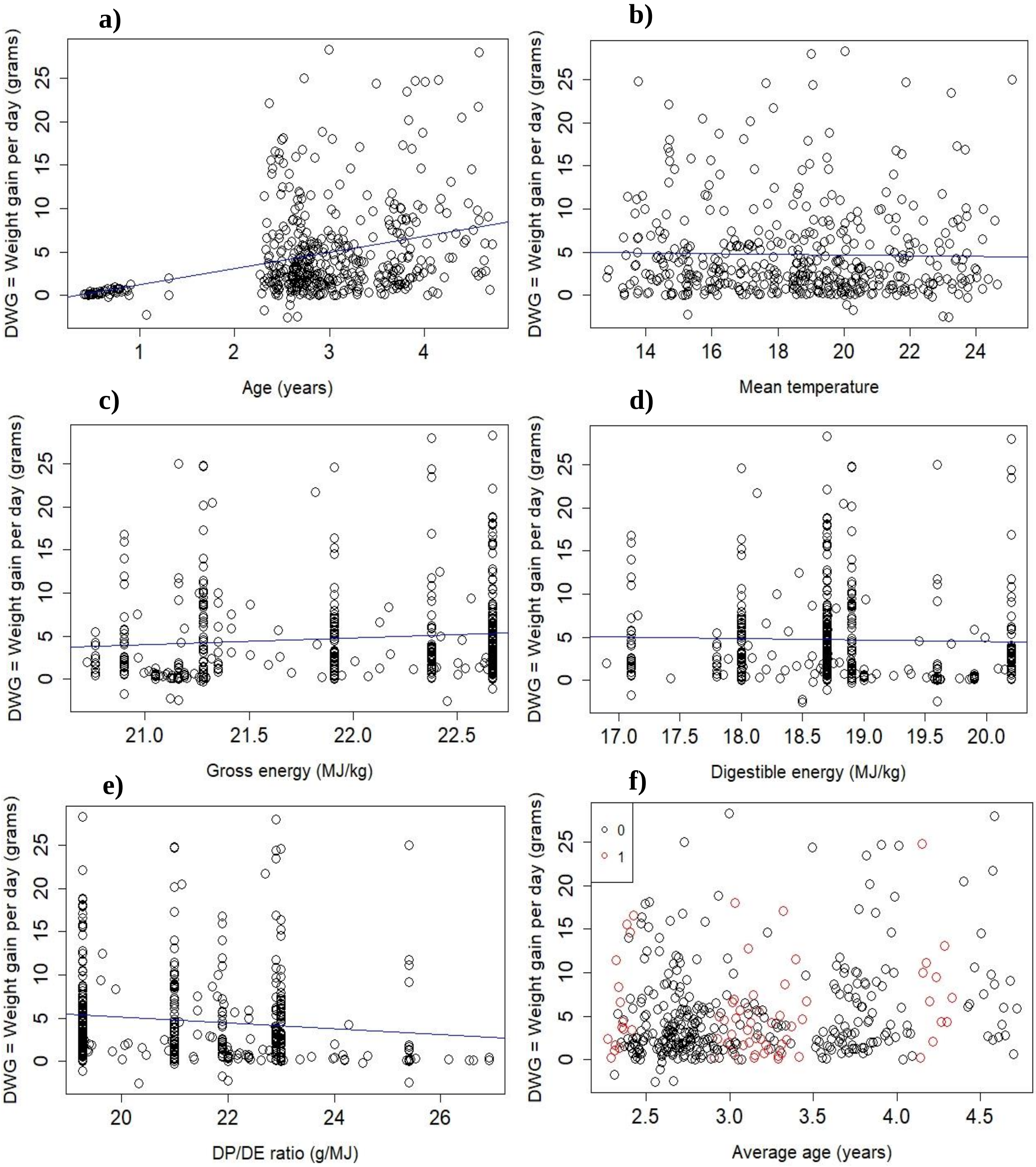

Figure 3.7- Scatter plots of DWG with trend lines (images a–e). Image f, in (red) or out (black) of the reproduction period for ages ≥2.

The variable DWG displays a large vertical dispersion over the explanatory variables. The correlations of DWG with the other numeric variables and the correlations among the feed property variables, and respective p-values, are shown in Table 3.3. For the correlations involving DWG, only age is significant. As expected, the correlations among feed properties are all highly significant.

Table 3.3- a) correlations of DWG with other numeric variables (blue) and correlations among feed variables (yellow). b) corresponding p-values.

a) Correlation

	DWG	Mean GE	Mean DE
MeanAge	0.16		
MeanTemperature	-0.07		
MeanGE	0.01		
MeanDE	-0.02	0.39	
MeanRatioDP/DE	-0.07	-0.53	0.14

b) p-values

	DWG	Mean GE	Mean DE
MeanAge	**0.0023**		
MeanTemperature	0.2037		
MeanGE	0.7905		
MeanDE	0.7224	<0.0001	
MeanRatioDP/DE	0.1803	<0.0001	0.0067

All variables were considered for the linear model because they may have significant partial correlations as they enter the model later. Eventual redundancy associated with the feed properties will be delt with by the stepwise regression model. The results of the general linear model are presented in Table 3.4. Only the variable age was retained for the final model.

Table 3.4- Results of the generalized linear model for Daily Weight Gain.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
MeanAge	1	221,1	221,08	9,42	**0,0023**
MeanTemperature	1	30,0	30,04	1,28	0,2586
MeanGE	1	0,1	0,08	0,00	0,9545
MeanDE	1	45,9	45,90	1,96	0,1628
MeanRatioDP/DE	1	34,1	34,09	1,45	0,2289
InReproductivePeriod	1	0,0	0,01	0,00	0,9824
Residuals	377	8847,5	23,47		

An alternative model for DWG (as a function of initial weight of the period) was considered, and a scatter plot of the points of the model is presented in Figure 3.8.

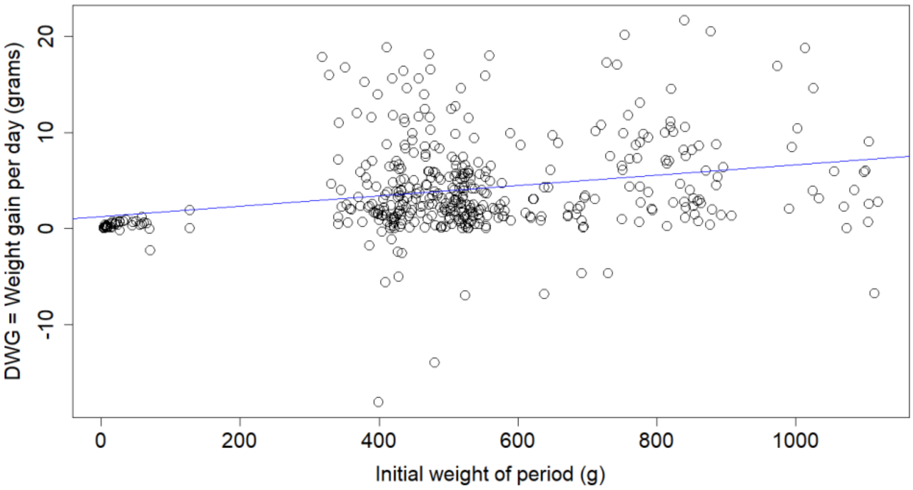

Figure 3.8- Scatter plot of DWG as a function of initial weight of the period.

The p-value of the linear trend represented in Figure 3.8 (null hypothesis for b=0) is 0.0022. The plot is very similar to Figure 3.7-a) (DWG as a function of average age of the period) and initial weight of the period could be an alternative to age to predict DWG.

3.3 Biomass estimation

The estimation of global biomass was obtained by multiplying, day by day, the weight (red curve in Figure 3.9) and the numbers (blue curve in Figure 3.9). The numbers are relative to 100 000 individuals and the fish production was simulated up to 1500 days (4.1 years). The resulting biomass is presented in Figure 3.10.

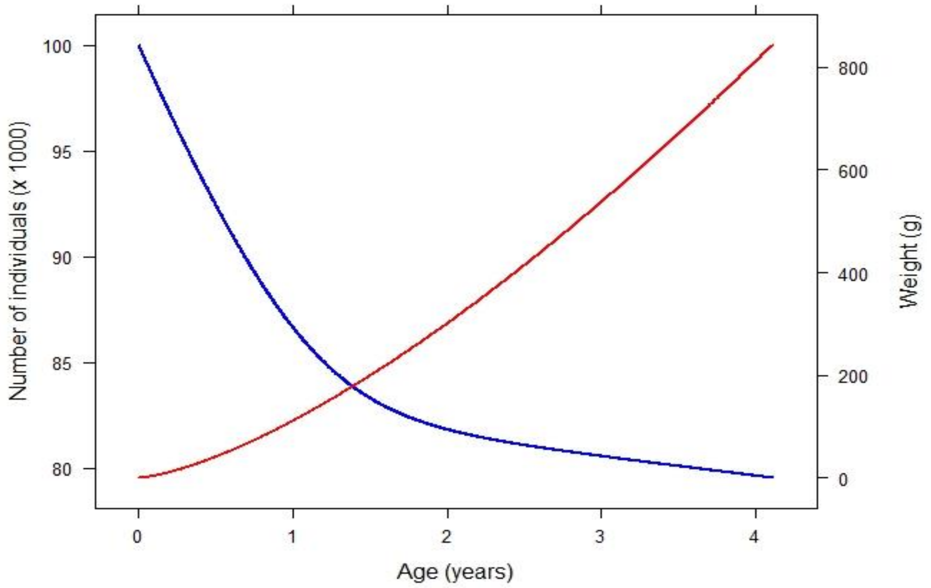

Figure 3.9- Numbers (blue curve) and weight (red curve) generated based on 100 000 individuals over 4.1 years of age.

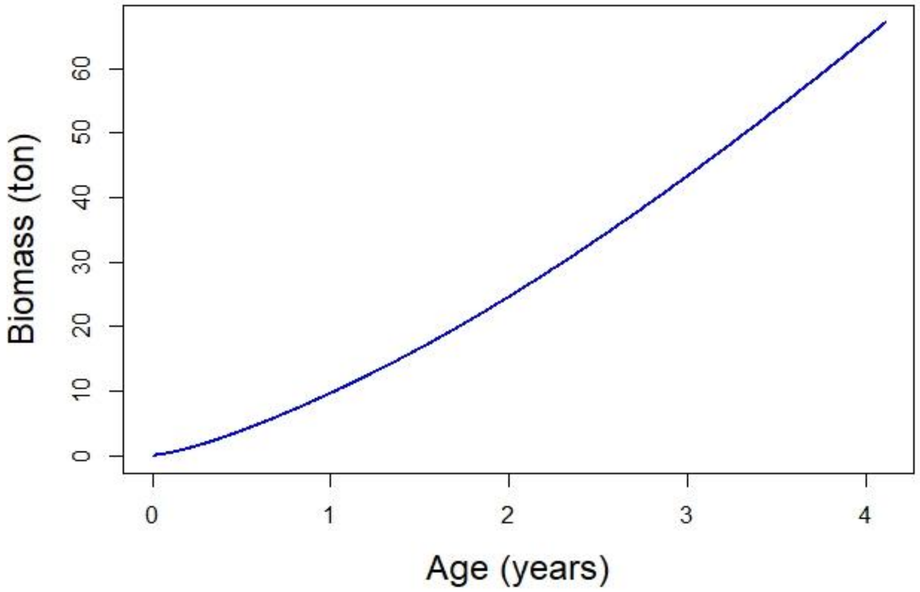

Figure 3.10- Global values of biomass obtained from a simulation generated with 100 000 initial individuals over 4.1 years.

Table 3.5 shows some biomass values at steps of half a year. This type of information can be valuable for long-term planning.

Table 3.5- Tons of biomass expected every half year of age.

Age (years)	Biomass (ton)
0.0	0.0
0.5	3.9
1.0	9.7
1.5	16.6
2.0	24.6
2.5	33.6
3.0	43.3
3.5	53.6
4.0	64.6

4 Discussion

In the production of fish in aquaculture, the amount of feed provided is very important. From the economic point of view, feed represents the most important portion of production costs and it is, therefore, very important to optimize feeding, seeking efficiency in fish growth rates. Excess feeding translates into economic loss and can have negative effects on water quality and fish health. Insufficient feed will result in suboptimal growth rates, also implying economic loss.

The main purpose of this thesis was to find a method to estimate biomass in order to provide the optimal amount of feed in each earth pond (tank) at Aqualvor. The desired model should be based on existing data from the production at Aqualvor, identifying the environmental components (such as temperature), feed properties and biological condition (weight of the fish and influence of the maturation stage on growth) that are relevant for predicting biomass. Another component of biomass estimation is the number of fish present in each tank, so a mortality model was developed to obtain number of fish. Biomass is estimated by multiplying the number of fish by the average weight of the fish. These two components of biomass, numbers (mortality model) and fish weight (growth model), were treated separately.

4.1 Mortality component

Global analysis of mortality data showed the complexity of estimating mortality rates in aquaculture. Unaccounted fish, for each batch, ranged from 17% to 46% (Table 3.1). If individual tanks are considered, the range of values is even wider (Figure 3.1) with primary tanks having higher values of fish unaccounted for, likely because they include earlier stages of the production, where fish mortality is higher and more difficult to quantify.

The possibility of estimating the number of fish present in a tank, at a given point in time, by deducting the number of dead fish from the initial number previously determined, is unrealistic, due to the large numbers of unaccounted dead fish. This is a problem for estimating biomass to provide adequate amounts of feed. Despite this, global mortality values can be estimated for the full duration of the production, since we can assume that the total number of dead fish is the difference between the number initially stocked and all the fish captures (fished) at the end of the production.

Survival percentages (defined as % of total individuals fished in relation to total number stocked) ranged between 52 and 75%, meaning there were batches where about half of the production was lost. These results are markedly distinct from the ones presented in the survey from Muniesa *et al.,* (2020) which reported an average survival rate of 85% for sea bass Dic*entrachus labrax* produced throughout the Mediterranean (the survey included 50 companies from 10 Mediterranean countries). Nevertheless, the majority of companies in this survey were from intensive producers in open sea cages. According to the authors from this survey, the causes that most contribute to mortality are disease-related in a proportion of 10% to 5% compared to other causes (non-pathogens infections). One of the biggest disadvantages from earthen ponds compared to cages in open-sea is the lower renewal capacity (mostly in low tides) which can contribute to greater accumulation of pathogens in the tanks. Also, earth ponds favour predation from bottom invertebrates such as crabs and/or birds. Nevertheless, Lorenzen (1996) did not observe significant differences in mortality between fish cultured in earthen ponds and cages in a study that included several species from different regions.

Detailed information on the mortality rates, by tank and production phase, was used to estimate the number of fish (dependent variable) as a function of mortality rate. It was necessary to obtain a model for the evolution of mortality, as it was clear that mortality rates could not be considered constant. Data from mortality at the early stages, was obtained from the batches that went through the pre-fattening facility, where the dead fish are effectively collected and counted. The adult phase (from around 2 years of age) provided the information for later stages. Here the numbers were estimated by going backwards, building the numbers at the beginning of this phase by adding counted dead fish and captured fish after that point. The data confirmed the decrease in mortality over time. This observation is in line with the practical knowledge acquired at Aqualvor, that the mortality rate is higher in early stages of the fish life, a fact also confirmed by other studies (Lorenzen, 1996, Muniesa *et al.,* 2020).

Lorenzen (1996) described a relationship between mortality and weight. The author observed that survival increases rapidly with weight in small fish, but changes little once fish have reached a weight of about 200 g. Muniesa *et al.,* (2020) also reported a relationship between fish mortality and the production phase, considering the effect of the diseases that most affect fish in the Mediterranean. Higher mortality rates were observed in hatchery and pre-growing fish affected by tenacibaculosis (also known as flexibacteriosis) compared to on-growing individuals. Flexibacteriousis is one of the most common pathogens present in Aqualvor.

Two choices for the model of mortality rate (M) as a function of time (age) were considered: (1) a linear model with negative slope, and (2) an inverted sigmoid model. From the mathematical point of view both models performed well, with very similar error terms but, since there are no datapoints from 1 to 1.5 years, there is no information to decide which one is the best model. The inverted sigmoid was chosen because it is more in agreement with existing information that indicates there is a plateau of low mortality after the early life stages (Lorenzen, 1996). In addition, the inverted sigmoid does not produce negative mortality values at older ages.

The low survival rate and the high percentage of individuals that disappear draw attention to the importance of properly estimating mortality and identity its causes. Finding non-invasive and accurate methodologies to estimate the number of fish in the tanks, is one of the main challenges for the future. Such methodologies should permit the counting of fish frequently and independently from human intervention and should be effective for all stages of the production, in particular late juvenile to early adult stages, when individuals are small and are already in earth ponds where mortality is greatly underestimated.

4.2 Growth component

From the two approaches used to study growth, (1) mean individual weight as a function of age and (2) daily weight gain based on the bioenergetic model, only the first approach produced growth estimates. The model obtained for the mean weight as a function of average age (Figure 3.6) allows a global description of the fish growth at Aqualvor (average of all batches). To model weight at age a power function with free exponent was used. This simple model was preferred to popular growth models such as von Bertalanffy and Gompertz curves. These are commonly used to study weight or length-at-age and consider asymptotic fish growth (Ricker, 1979). They are not applicable in aquaculture, as fish are overfed and are caught before the asymptotic growth phase is reached (Mayer *et al.*, 2008).

The weight-at-age model used in this work integrates information from all the batches analysed (regular samples of 300 fish) and is not useful to estimate daily growth rates for specific tanks. For prediction of tank biomass, a bioenergetic model was used, similarly to the model developed by Lupatsch (2003), where daily weight gain (DWG) was the response variable and the explanatory variables were age, water temperature, gross energy, digestible energy, ratio digestible protein/digestible energy and sexual maturation. The only significant variable was age, but it explained only 2.4% of the total variation, making the model useless for practical applications. An alternative model with log(DWG) as response produced similar results.

The results of the bioenergetic model obtained in this work do not mean the variables considered are not affecting growth, they just mean that the base data has too much error (variability of DWG) and the effects could not be detected. Temperature has clear effects on fish growth and has been considered the variable with the greatest impact on the growth of cultured Mediterranean species (Hernández *et al.*, 2003, Person-Le Ruyet *et al.*, 2004; 2007; León *et al.*, 2006). Nevertheless, these authors observed the effect of temperature under controlled conditions, where temperature had fixed levels and fish were sampled regularly. To observe significant correlatins at Aqualvor facilities, the number of samplings would have to be more regular and, above all, coincide with periods of temperature inversion. As for temperature, the detection of the effect of sexual maturation, (when fish lose appetite and weight), requires the sampling effort includes before, during and after maturation phases, from beginning of December to end of April (Pérez-Ruzafa & Marcos, 2014).

For feed variables, the absence of significance was expected since the ranges of values along the variable x (Figure 3.7-c,d,e) were too short to observe significant associations and the composition of feed components was not determined with analytical methods. Feed properties were simply taken form the labels provided by the manufacturers and ranges, not single values, are provided for each one of the feed components. The formulas presented in the Material and Methods section are an approximate method of calculation that can be used by fish producers for superficial conclusions about the composition of the feed. In most studies of the influence of feed composition on growth, these variables enter the models with fixed levels and the observations are made in a controlled setting. This allows the expression of the effects of dietary composition on growth (Lupatsch, 2003). In addition, the composition of similar feeds is variable, as feed manufacturers depend on raw materials with different energy profiles available to produce the feed.

4.3 Critical assessment of the assumptions implicit in this work

The data used in this work comes from the monitoring of aquaculture production in an open system where many factors may affect the response variables of the different models and be a source of confounding. Some of the uncontrolled factors are listed bellow:

(1) **Water quality.** In Aqualvor, water quality is optimized through adequate strategies of control and renovation. Inside the facilities, water quality is regularly assessed by measuring pH and nitrite–ammonia parameters, which are maintained around optimal values through suitable renovation strategies. For this reason, water quality parameters were not considered.

(2) **Stocking densities**. Aqualvor's production strategy is similar regarding the number of individuals assigned to each tank. It is assumed that fish grows from fingerlings until reach commercial size at non-restrictive densities. Therefore, for these facilities, stocking density is a variable that was not considered.

(3) **Fish satiety.** Fish is fed according to the theoretical feeding rate given by feed manufacturers for each temperature and estimated biomass. The amount is adjusted according to the fish feeding behaviour and it is stopped when the fish shows signs of satiety.

(4) **Dissolved oxygen concentration.** Oxygen concentration is constantly monitored by permanent readings from automatic sensors in each tank (including pre-fattening). Depending on the value, aerators inject air or oxygen in water, not allowing oxygen values to fall below 50%.

(5) **Fingerling's quality.** Origin of fingerlings may be different and different batches may have qualities that lead to differentiated mortality and growth rates but, in the absence indicators of fingerling condition, it is assumed that the growth potential of fish from different batches is the same.

(6) **Pathogens effects.** The fish´s health is assessed by regular analysis of fish gills. In addition, histological analysis of the spleen, liver and intestine are periodically performed. Prophylaxis is ensured by renovation strategies that prevent the deposition of pathogens in the system. For these reasons it is assumed that pathogens effects are residual and moderate.

(7) **Stress from fish handling.** Fish handling during sampling procedures (part of the fish is released after being caught by seine net) can be a source of stress for the fish caught or in the vicinity of the net. The effects of these procedures are only significant during short periods of time, although feeding may be affected. Stress effects due to sampling cannot be isolated given the high variability in amount of stress induced or number of fish affected. It is expected that these effects are randomly distributed over tanks and time. The same reasoning can be applied to transports between tanks. The effect of fishing is also not an issue since this phase is short and feeding is not provided. In the absence of indicators of the stress effect on fish, and the assumption that all fish have the same probability of suffering these effects, fish handling was not considered as a factor affecting growth.

(8) **Counting of dead fish in outer tanks.** The estimation of mortality rates in earthen ponds is difficult due to the deficient counting of dead fish. The collection of dead fish is not carried out systematically as in the pre-fattening facility, and extensive mortality events may not be detected. The percentage of unobserved losses is high due to the rapid decomposition process of the fish and the action of predators in the pond-tanks.

4.4 Limitations and future work

The main purposes of this work, to develop a model that could be used to predict biomass in the earth ponds, could not be achieved because the data available, not collected for this purpose, had too much variability. Correlation between the three main variables affecting fish growth at Aqualvor, water temperature, type of feed and sexual maturation, and growth rates, could not be found. A possible explanation may be linked to the effect of other variables on growth, not controlled and contributing to the error term, a normal situation in an open system.

One of the biggest drawbacks from the dataset was the infrequent and not adequately spaced samples. Sometimes, the period between samplings was too long.

About half of the sampling points were reduced after using filters on the data. When only the points with a sampling interval of 10 days were considered significant for the model, a large reduction of points was observed, mainly between 1 and 2 years of age (Figure 3.5). This creates a data gap at a crucial stage in the fish's life.

In the future, we expect to improve the data for the models by introducing information of other batches already available at Aqualvor. Furthermore, it is important to introduce confidence bands for the models that have age as an independent variable and were used to produce the global biomass estimation. This could be done using resampling strategies and/or simulations, for example with Monte Carlo estimation. This approach would also allow parameter sensitivity studies.

With respect to the bioenergetic model, it is worth considering a completely different approach to biomass estimation, for example, with sampling devices based on cameras and image analysis. Fish lengths (easily translated to fish weight) and numbers in the tank could be directly estimated. Such a technology, combined with information of feed properties, could be linked to the automatic feeders achieving the desired objectives of optimizing feed quantities.

5 Conclusions

This work provided important insights into what could be a future biomass estimation model for the sea bass *Dicentrachus labrax* farmed at Aqualvor. This model will be essential not only for optimizing the company's feeding process, but also for other procedures of planning such as the entry of new batches and harvest schedules. The mortality model presented here considers an inverted sigmoid function with significant fitting. The model predicts higher mortality rates in the early stages of the fish's life and a stabilization in later stages which agrees with known facts from the company's empirical knowledge. This model can, however, be better adjusted with the introduction of more batches, mostly with more data in phase 0 (pre-fattening). For the growth model, no significant correlations were found between fish growth and the variables temperature, feed properties and reproduction period. Furthermore, the periods between samplings were long, which did not allow for an accurate assessment of the variables. The power function allowed to estimate biomass reasonably.

From this thesis, it is concluded that the company should invest in more regular sampling methods and to find non-invasive ways to estimate biomass. In this study, real historical data from the company were used, which is an advantage over commercialized bioenergetic models that do not integrate a mortality model and are developed based on the growth of a single fish.

The programs in R were developed so that, in the future, more batches can be integrated, and the parameters of the models automatically updated. The programs are also prepared to repeat the same methodologies on the sea bream *Sparus aurata*.

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7 Appendix A

Growth profiles for individual tanks and batches.

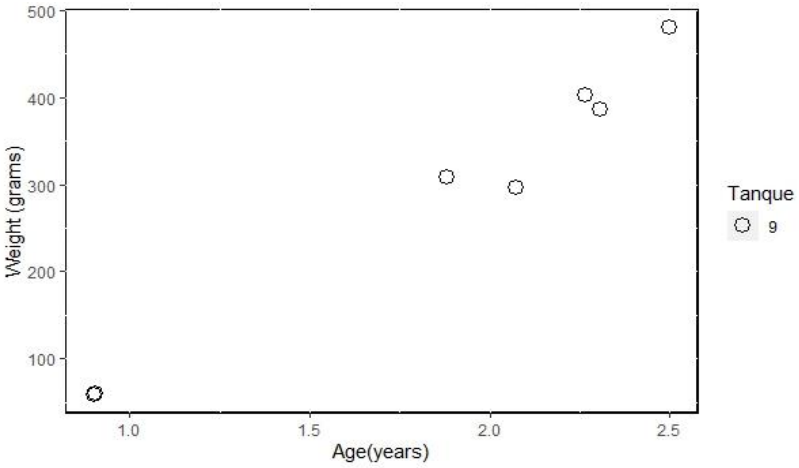

Figure A1 - Average weight (g) sampled over the production time for tank 9 (T09_20120713).

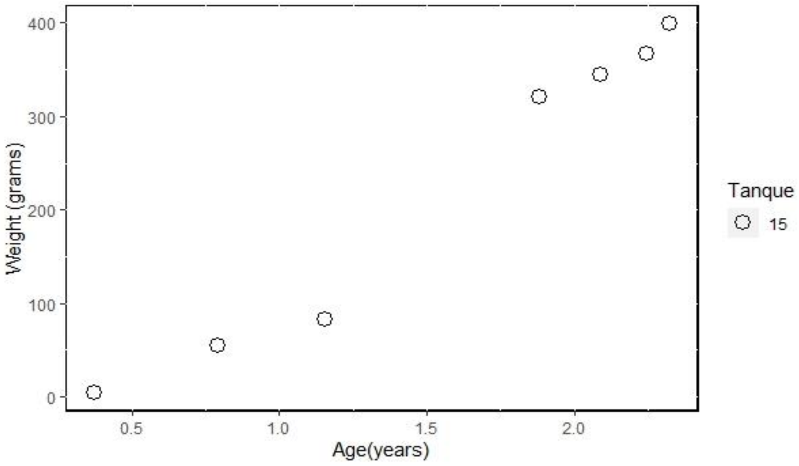

Figure A2 - Average weight (g) sampled over the production time for tank 15 (T15_20120713).

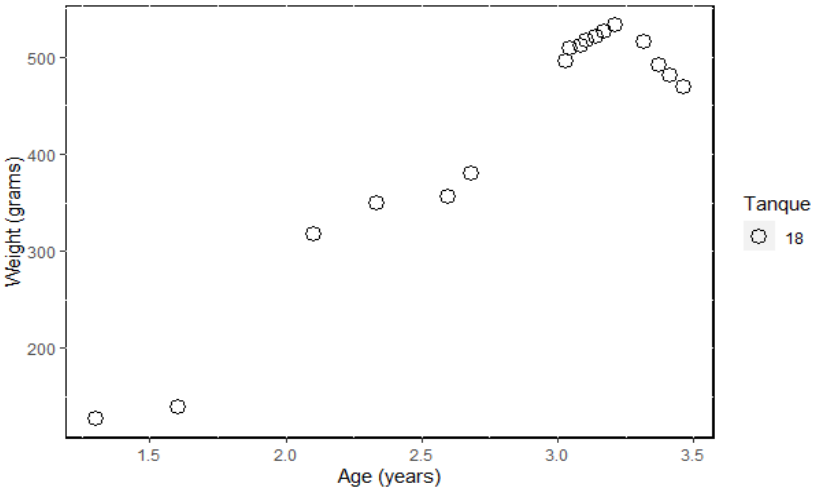

Figure A3 - Average weight (g) sampled over the production time for tank 18 (T18_20130314).

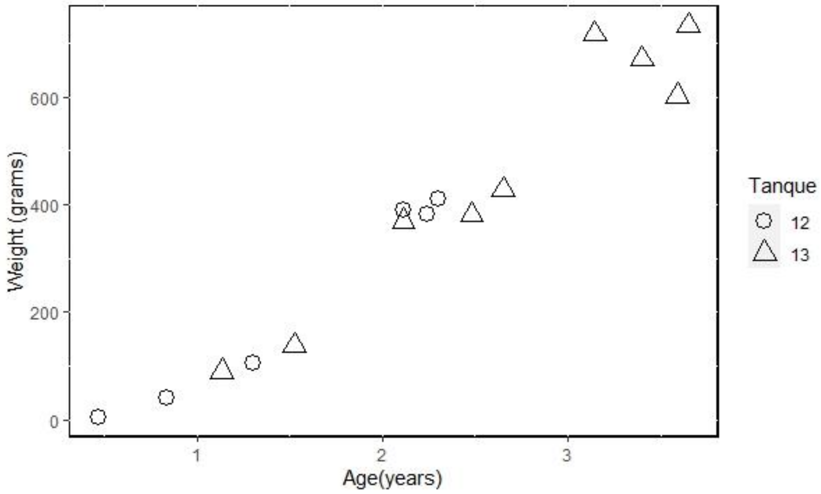

Figure A4 - Average weight (g) sampled over the production time for tanks 12 and 13 (T12_20140516).

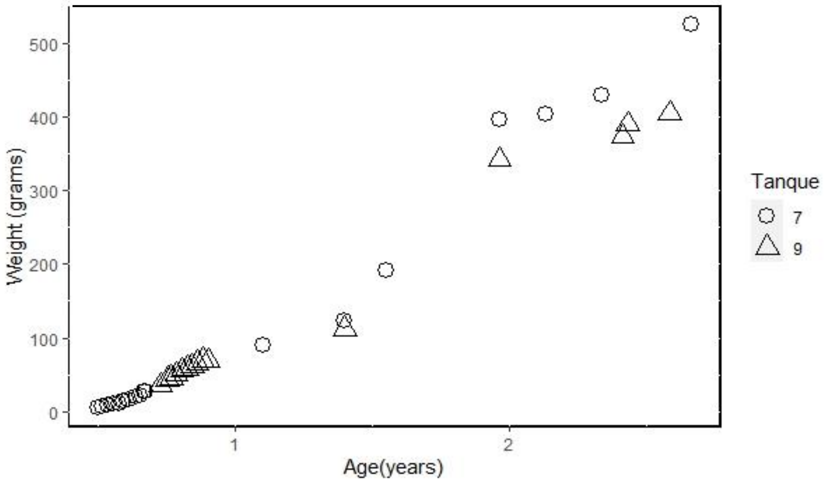

Figure A5 - Average weight (g) sampled over the production time for tanks 7 and 9 (T07_20140711).

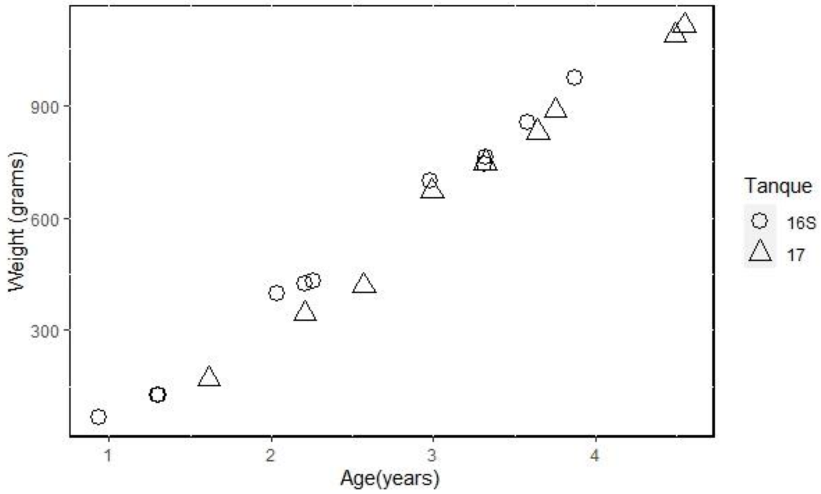

Figure A6 - Average weight (g) sampled over the production time for tanks 16S and 17 (T16S_20150506).

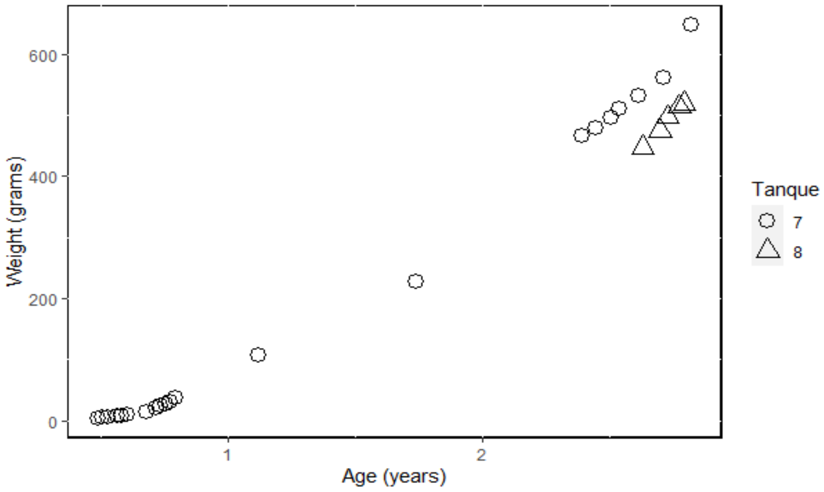

Figure A7 - Average weight (g) sampled over the production time for tanks 7 and 8 (T07_20160407).

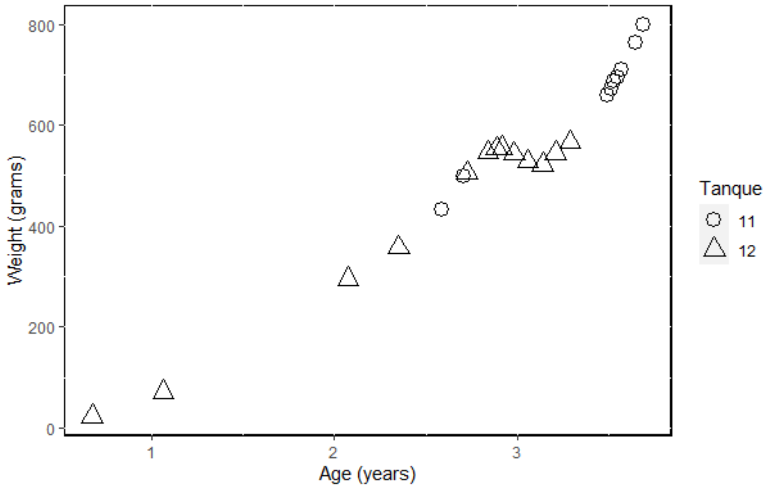

Figure A8 - Average weight (g) sampled over the production time for tanks 12 and 11 (T12_20160713).

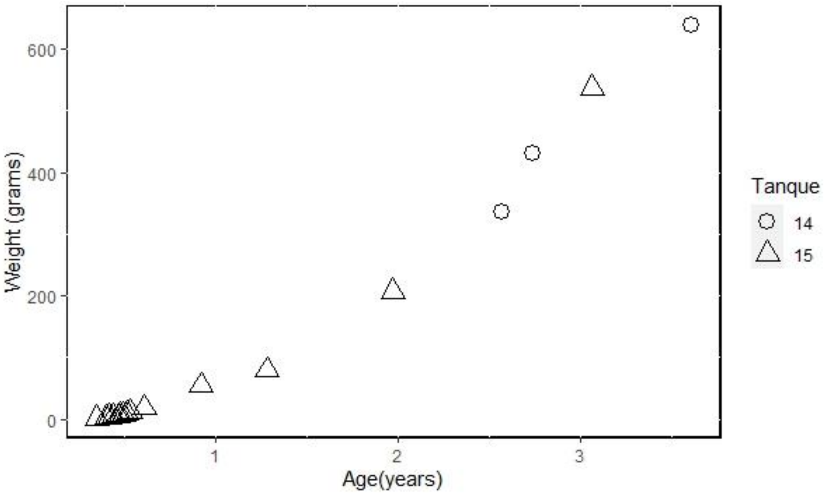

Figure A9 - Average weight (g) sampled over the production time for tanks 15 and 14 (T15_20170413).