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Individual feed efficiency in fishes: direct measurement methods and indirect predictors to develop selective breeding programs in two major aquaculture species: European sea bass *Dicentrarchus labrax* and Nile tilapia *Oreochromis niloticus*

Charles Rodde

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THÈSE POUR OBTENIR LE GRADE DE DOCTEUR DE L'UNIVERSITÉ DE MONTPELLIER

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Individual feed efficiency in fishes: direct measurement methods and indirect predictors to develop selective breeding programs in two major aquaculture species: European sea bass *Dicentrarchus labrax* and Nile tilapia *Oreochromis niloticus*.

Présentée par Charles RODDE

Le 10 Décembre 2020

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et l'encadrement de Hugues DE VERDAL

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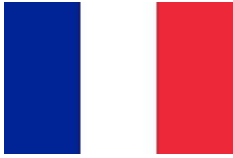
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LIST OF ABBREVIATIONS AND SYMBOLS

AT Atlantic population of European sea bass

BW Body weight

BWG Body weight gain

Cirad French Agricultural Research Centre for International Development

CGIAR Consortium of International Agricultural Research Centers

DADA-EAT Développement d'une aquaculture durable par l'amélioration de l'efficacité alimentaire chez le tilapia

DNA Deoxyribonucleic acid

EL Energy loss

EM East Mediterranean population of European sea bass

FAO Food and Agriculture Organization of the United Nations

FCR Feed conversion ratio

FEAMP Fonds européen pour les affaires maritimes et la pêche

FER Feed efficiency ratio

FI Feed intake

FIGIS Fisheries Global Information System

FISH CGIAR Research Program on Fish Agrifood Systems

GIFT Genetically improved farmed tilapia

h² Heritability

HLPE High Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security

H2020 AQUAEXCEL²⁰²⁰ Aquaculture infrastructures for excellence in European fish research towards 2020

IFAD International Fund for Agricultural Development

Ifremer French Research Institute for Exploitation of the Sea

ISEM Institute of evolution sciences of Montpellier

MARBEC Marine biodiversity, exploitation and conservation

MBW Metabolic body weight

NRC National Research Council of the National Academies

OECD Organisation for Economic Co-operation and Development

PIT Passive integrated transponder

PW Production weight

r Correlation coefficient

RBWG Residual body weight gain

RFI Residual feed intake

RMR Routine metabolic rate

SE Standard error

SMR Standard metabolic rate

SNP Single nucleotide polymorphism

STECF Scientific, Technical and Economic Committee for Fisheries

US United States of America

WM West Mediterranean population of European sea bass

$\delta^{13}\text{C}$ Stable carbon isotope values

$\delta^{15}\text{N}$ Stable nitrogen isotope values

FOREWORD

Experiments presented in the present thesis were made possible through support provided by Cirad, WorldFish, the CGIAR Research Program on Fish Agrifood Systems (FISH) and the International Fund for Agricultural Development (IFAD), the European project H2020 AQUAEXCEL²⁰²⁰ (“Aquaculture infrastructures for excellence in European fish research towards 2020”), and the project DADA-EAT (“Développement d’une aquaculture durable par l’amélioration de l’efficacité alimentaire chez le tilapia”) funded by the “Fonds européen pour les affaires maritimes et la pêche” (FEAMP). The Agreenium Institute, the MARBEC (“Marine biodiversity, exploitation and conservation”) UMR, the ISEM (“Institute of evolution sciences of Montpellier”) UMR and the University of Montpellier also allocated funds to support missions and attendance to conferences abroad (Malaysia and Germany) and in France (Lyon, Dijon, Perpignan).

The experiments I conducted on the GIFT strain of Nile tilapia were made at WorldFish Aquaculture Extension Center in Jitra (Kedah State, Malaysia). I set up and led these experiments during two missions in May-June 2018 and June-July 2019. I managed a team of about ten persons working at Jitra, in co-operation with WorldFish Center in Bayan Lepas (Penang State, Malaysia). In the case of the first experiment, it lasted more than 30 weeks, so I initiated it during my mission and taught the team a clear protocol to delegate the experiment that I then supervised from France.

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I analysed stable isotopes of European sea bass scales in partnership with the CEFREM (“Centre of education and research on Mediterranean environments”) UMR at Perpignan University Via Domitia (Perpignan, Pyrénées-Orientales, France) over a few weeks during the first half of 2019.

Finally, Hugues de Verdal conducted the experiment on the Cirad strain of Nile tilapia at the Palavas Experimental Aquaculture Research Station, from October 2019 to January 2020, with a co-designed protocol.

To date (September 2020), results collected in the present thesis project were reported in three accepted articles, two being already published, in a fourth article under review and a fifth one still in preparation. Results were also presented during two conferences (at Montpellier and at Berlin). For complete references and details, please refer to the “Publications and communications” section.

INTRODUCTION

1. The aquaculture sector is growing fast but must address sustainability issues

The world human population is over 7 billion in 2020 and, according to projections, it will reach 8 to 10 billion towards 2050 (United Nations, 2011). The increasing world population is accompanied by a growing demand for food. Regarding the world consumption of animal proteins per capita, the relative contribution of products from aquatic origins has kept increasing over the last 50 years, at the expense of livestock except poultry (Béné et al., 2015; OECD-FAO, 2018). Besides, shifting future diets away from terrestrial protein towards aquatic proteins is expected to spare lands and feed crops (Froehlich et al., 2018). Thus, aquatic products will become ever more central to human food security in the future (Béné et al., 2015; OECD-FAO, 2018). This need in aquatic products has been partially satisfied by fisheries. However, their production has stagnated over the last 20 years around 90 million tons annually, and many fish stocks dedicated to human consumption are currently overfished, suggesting a future decrease of landings (FAO, 2018). In contrast, aquaculture has kept increasing to reach 82 million tons (excluding aquatic plants) in 2018, with an average annual growth in production of +7.6% since 1950 (Fig. 1; FAO-FIGIS, 2020). Consequently, meeting the increasing global demand for aquatic products will depend almost exclusively upon aquaculture. Currently, aquaculture production is concentrated in Asia (88.8% of world production), and more specifically in China (58.8% of world production; FAO-FIGIS, 2020, Fig. 2).

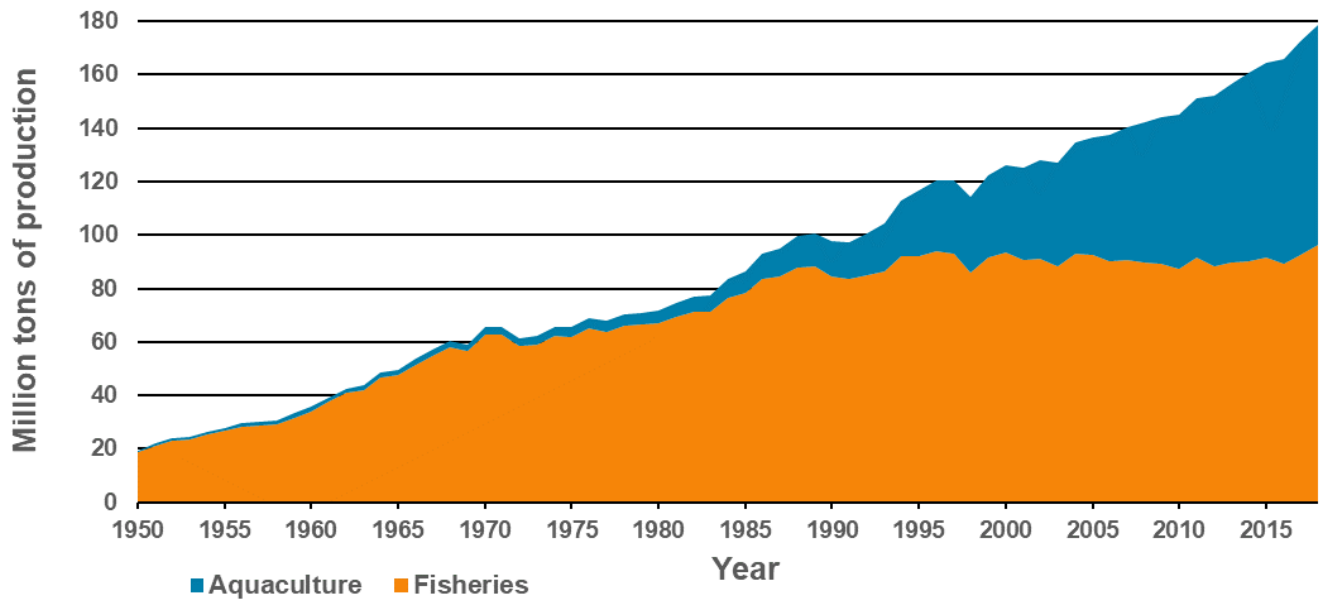


Figure 1. World aquaculture and fisheries production (using data from FAO-FIGIS, 2020; excluding aquatic plants).

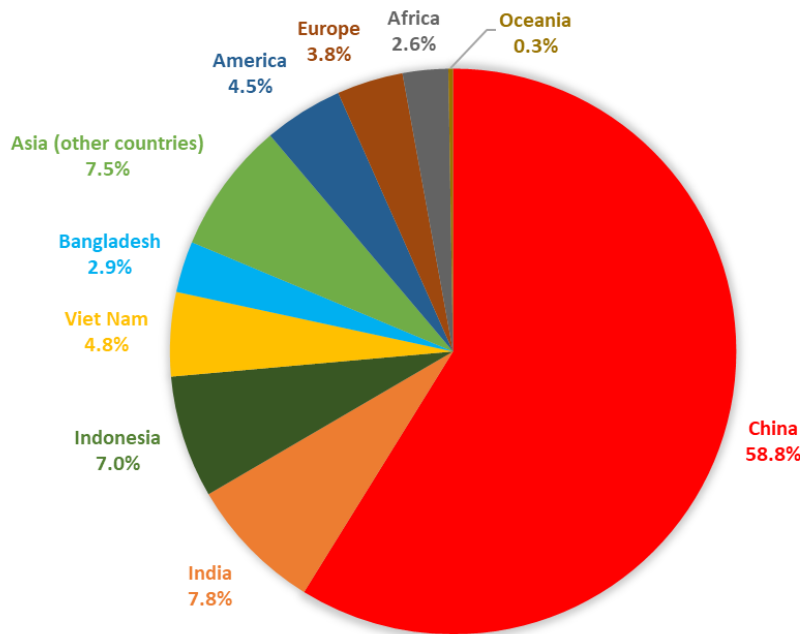


Figure 2. World aquaculture production share (in %) by geographical areas (countries were separated from continents when their production was over two billion tons, using data from FAO-FIGIS, 2020; excluding aquatic plants).

Moreover, aquaculture does not fulfil the same objectives in advanced economies compared to emerging ones. Although developed countries consume much more fish per capita than less developed countries (24.9 kg/year *versus* 12.6 kg/year in 2015, respectively; FAO, 2018), fish protein represents a lower proportion of total animal protein intake (11.4% *versus* 26.0% in

2015; FAO, 2018). Thus, fishes are a crucial source of animal protein for less developed countries. In particular, fish protein contains several essential amino acids such as lysine and methionine, which are rather scarce in these countries' diets (HLPE, 2014). Beyond proteins, fish products contain several other key essential nutrients. Fishes provide long-chain polyunsaturated fatty acids, with beneficial effects for child development and adult health. Moreover, fishes are an important source of essential micronutrients, such as vitamins B and D, and of minerals, such as calcium and iron (HLPE, 2014). Including aquatic products in the diet can improve nutrition balance and avoid malnutrition in least developed countries (Kawarazuka and Béné, 2010).

In spite of rapid development, aquaculture still needs to overcome sustainability issues. Sustainability is defined as “meeting the needs of the present without compromising the ability of future generations to meet their own needs” (Brundtland, 1987). This concept involves three axes: economy, environmental impact and society. One major obstacle to a sustainable development of aquaculture is linked to fish feed. Fishes generally use feed more efficiently than terrestrial livestock (Fig. 3) but improvements are still necessary. Typical commercial fish feed is composed of fish meal and fish oil from marine capture fisheries, plus land animal protein meal and lipid (e.g. meat by-products, feathers and blood) where this is permitted by legislation (banned in European countries), completed by plant meals and oils (mostly cereals, oilseeds and pulses; Tacon et al., 2011). The proportion of each ingredient in the diet depends on the fish species. For carnivorous fishes such as salmonids or marine finfish, fish meal and fish oil used to be the main ingredients until the early 2000's but nowadays represent less than 25% of the diet, having been replaced by plant-based ingredients (Médale et al., 2013). In omnivorous or herbivorous freshwater species such as cyprinids, pangassids or cichlids, fish-based ingredients barely represent 5% of the diet (Médale et al., 2013). Moreover, in these latter species, rearing practices do not necessarily involve commercial feed because several farmers

rather use fresh or farm-made feeds, or even let fish forage for themselves (Tacon et al., 2011).

Commercial feeds, however, are increasingly used globally (Tacon et al., 2011).

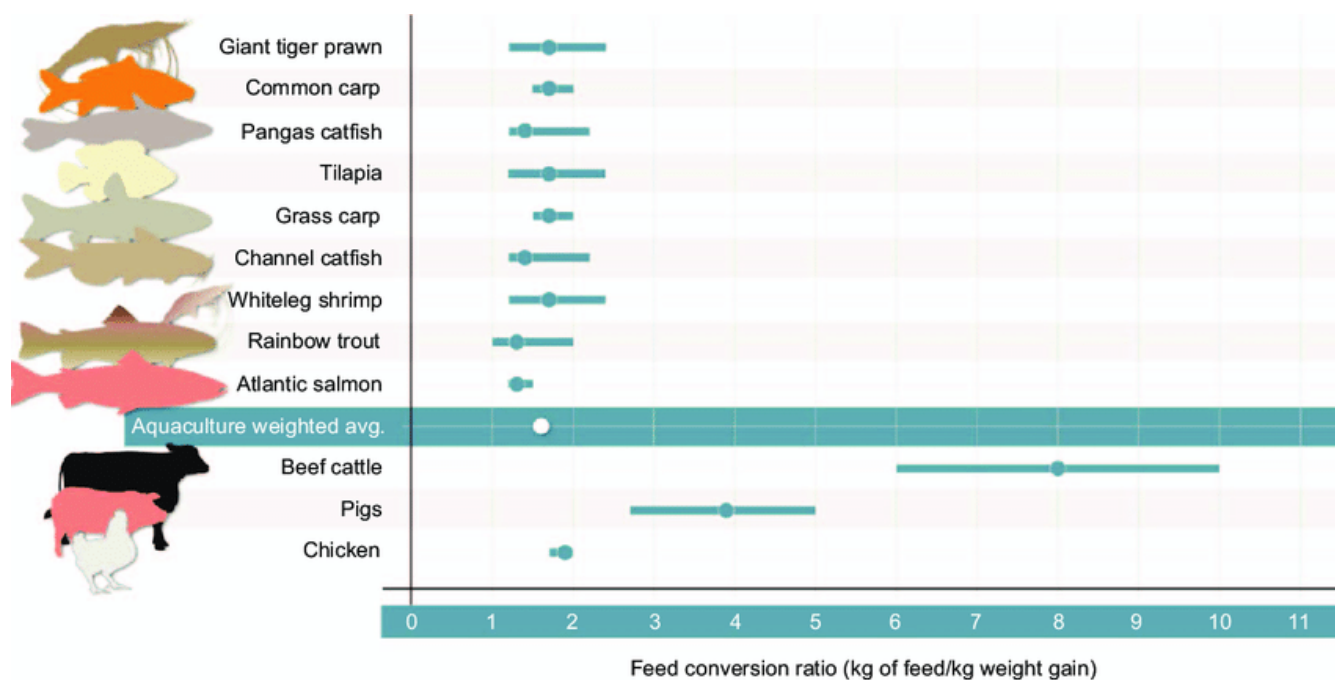


Figure 3. Feed conversion ratio, i.e. the ratio between feed intake and body weight gain, for selected aquatic and terrestrial farmed animal species. Dots represent means and bars indicate range. Lower values signify better feed use. Figure extracted from Fry et al., 2018. Giant tiger prawn: *Penaeus monodon*; common carp: *Cyprinus carpio*; pangas catfish: *Pangasius pangasius*; tilapia: *Oreochromis niloticus*, *O. mossambicus*, *O. aureus*, *O. andersonii*, *O. spilurus*; grass carp: *Ctenopharyngodon idella*; channel catfish: *Ictalurus punctatus*; whiteleg shrimp: *Litopenaeus vannamei*; rainbow trout: *Oncorhynchus mykiss*; Atlantic salmon: *Salmo salar*.

Feed is expensive, costs range from 30% to 70% of total costs in intensive fish farms (Goddard, 1996; Rana Sunil Siriwardena and Hasan, 2009; STECF, 2018). Moreover, ingredients used in fish feeds have undergone major increases in price since 2000, whether derived from wild fish or plants (Rana Sunil Siriwardena and Hasan, 2009; OECD-FAO, 2018). For instance, European sea bass *Dicentrarchus labrax* production was 83 000 tons in Europe in 2018 (FAO-FIGIS, 2020) and it is estimated that this species consumes 1.38 kilogram of feed to gain one kilogram of body weight (Besson et al., 2019). If we consider that feed cost price is 1.5 € per kilogram, then an improvement of feed use by 5% (from 1.38 to 1.31 kilogram feed to gain one kilogram of body weight) would save 8 715 000 € for European sea bass production in 2018.

Regarding environmental impact, improving feed use could reduce global warming, acidification and eutrophication impacts linked to both fish feed production and consumption (Aubin et al., 2009; Besson et al., 2016). Aubin et al. (2009) estimated that feed alone (production and then consumption) accounted for 96 to 100% of the eutrophication potential, 32 to 86% of the climate change potential and 29 to 80% of the acidification potential of a fish farm. These impacts were assessed for rainbow trout *Oncorhynchus mykiss* in freshwater raceways, for European sea bass in sea cages and turbot *Scophthalmus maximus* in an inland recirculating system (Aubin et al., 2009).

Finally, from a social aspect, competition between humans and farmed fish for access to food sources raises questions about the true impact of aquaculture on food security (Troell et al., 2014). For instance, anchoveta *Engraulis ringens* stocks along the Peruvian coast are exploited both for direct human consumption and fish feed production (Fréon et al., 2008). In conclusion, there are economic, environmental and societal reasons to improve feed use, and this is of major importance for finfish aquaculture sustainability and resilience.

2. “Feed efficiency”: definition, estimation, underlying factors and improvement strategies

2.1. Definition

The concept of “feed efficiency” can be defined as how an animal uses its feed, i.e. how much it eats and how much it grows. Improving feed efficiency produces the same amount of fish on less feed, or more fish with the same amount of feed. Thus, feed efficiency is defined by two key factors: feed intake of an animal (FI), and its subsequent body weight gain (BWG).

2.2. Estimation

Feed efficiency can be estimated in various ways. The most common indicators in the literature are feed conversion ratio (FCR), defined as $FCR = FI/BWG$, and its reciprocal, feed efficiency

ratio (FER), defined as $FER = BWG/FI$ (reviewed by de Verdal et al., 2018a). These two indicators provide different viewpoints on efficiency; FCR indicates how much feed is required for a fish to gain one unit of body weight, whereas FER indicates how much body weight gain can be expected when feeding a fish with one unit of feed. An efficient animal is an animal with a low FCR or a high FER. These two indicators have the major advantage of being very tangible, they do not require any expertise in biology to be understood. However, the fact that they are ratios can raise issues. It is difficult to predict how FI and/or BWG will change when FCR or FER are improved (Aggrey et al., 2010). For instance, FCR can be improved if BWG increases while FI remains constant. The FCR can also be improved, however, if BWG decreases, but proportionally less than FI. The former is interesting from a farmer's point of view, the latter can be undesirable.

Other indicators have been formulated which do not use ratios but are based on linear relationships, such as residual feed intake (RFI; Koch et al., 1963) or residual body weight gain (RBWG; Koch et al., 1963). In contrast with FCR and FER, these indicators can distinguish between dietary energy allocated to growth or to body maintenance, by considering the metabolic body weight of the fish (MBW). The MBW is defined as $MBW = BW^b$, where BW is the body weight and b is a constant exponent which reflects energy loss linked to body maintenance. This exponent is defined as $EL = a * BW^b$ (Lupatsch et al., 2003) where EL is the energy loss during fasting in kJ.day^{-1} , and a and b are two species-dependent constants determined using statistical modelling. In fish, b is commonly reported around 0.8 (Johnston et al., 1991; Lemarié et al., 1992; Clarke and Johnston, 1999; Lupatsch et al., 2003).

From a biological point of view, the RFI of an animal is the difference between its actual FI and its expected FI based on its MBW and BWG. Similarly, the RBWG of an animal is the difference between its actual BWG and its expected BWG based on its MBW and FI. The RFI and RBWG of each animal are calculated by linear models on the experimental population.

Thus, RFI and RBWG estimate whether each animal is rather efficient or inefficient relatively to the whole group. The equation of the linear regression used to calculate RFI is expressed as: $RFI = FI - \mu + \alpha * MBW + \beta * BWG$ with μ the intercept of the linear regression, α the regression coefficient for MBW (i.e. body maintenance) of the animals, β the regression coefficient for BWG (i.e. growth) of the animals (Fig. 4). An efficient animal, relative to the whole group, has a negative RFI. Similarly, the equation used to calculate RBWG is expressed as: $RBWG = BWG - \mu + \alpha * MBW + \beta * FI$. An efficient animal, relative to the whole group, has a positive RBWG.

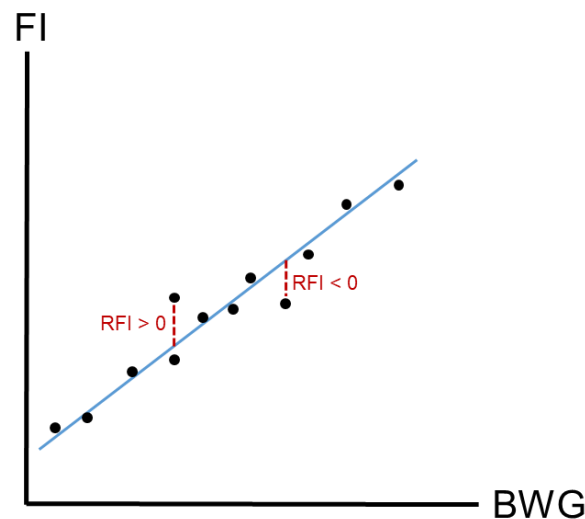


Figure 4. Illustration of the estimation of residual feed intake (RFI) from the linear relationship between feed intake (FI) and body weight gain (BWG). Each black dot represents an individual data and the blue line is the linear regression line between FI and BWG. In the present example, metabolic body weight is considered as similar among all individuals and thus not taken into account.

These linear regressions can be extended to include extra variables that can have an impact on energy allocation, such as egg production in poultry (Luiting and Urff, 1991) or milk production in cows (Connor et al., 2013). The equation of the extended linear regression is expressed as: $RFI = FI - \mu + \alpha * MBW + \beta * BWG + \gamma * PW$ where PW is the production weight (of eggs or milk) and γ the regression coefficient for PW. To our knowledge, such model including production traits was never used in fish, but could be relevant, for instance, when studying fish grown to produce eggs (e.g. Atlantic salmon *Salmo salar* whose eggs are consumed).

2.3. *Factors underlying feed efficiency*

2.3.1. *Intrinsic factors*

When estimating feed efficiency traits, the processes that convert FI into BWG are treated as a “black box”: it is not required to understand them to estimate feed efficiency. Nonetheless, feed efficiency must be influenced by mechanisms that convert feed into physiologically useful energy (Warren and Davis; 1967; Bureau et al., 2003), namely energy stored as proteins and lipids that is available for physiological functions. These mechanisms include digestion of feed in the alimentary canal, assimilation of nutrients into the blood and their distribution within the animal (Warren and Davis; 1967; Bureau et al., 2003). Once energy is made available, it is allocated to various biological functions, such as basal metabolism, locomotion or growth (Warren and Davis, 1967; Bureau et al., 2003). The higher the proportion of energy allocated to growth, the better the feed efficiency. Feed efficiency traits are variable among species (reviewed by de Verdal et al., 2018a), among strains (Overturf et al., 2003), among development stages (Bureau and Hua, 2008; Robinson and Li, 2010) and among individuals (Kause et al., 2006b; Quinton et al., 2007a; Grima et al., 2008; de Verdal et al., 2018b; Besson et al., 2019).

2.3.2. *Extrinsic factors*

Feed efficiency is variable among rearing environments (NRC, 2011). For instance, fish feed efficiency is impacted by feeding rate (Huisman, 1976; Brett, 1979), feed composition (Guillaume et al., 2001), water temperature (Azevedo et al., 1998; Árnason et al., 2009; Yoo and Lee, 2016), photoperiod (Biswas et al., 2005), and salinity (Imsland et al., 2008). For example, an optimal feeding rate (e.g. 2% of body weight per day in rainbow trout *Oncorhynchus mykiss*; Huisman, 1976) and an optimal temperature (e.g. 9°C in rainbow trout; Azevedo et al., 1998) can be identified to optimize feed efficiency, deviations from this result in reduced feed efficiency. However, the different environmental factors can interact: the

optimal feeding rate may vary with rearing temperature (Brett, 1979). Consequently, investigation of feed efficiency requires careful control of experimental conditions.

2.4. *Strategies developed to improve feed efficiency*

2.4.1. *At group level*

Improving feed efficiency in fish can involve nutrition (Huisman, 1976; Brett, 1979; De Silva and Anderson, 1995; Guillaume et al., 2001; NRC, 2011) as well as husbandry (Brett, 1979; Azevedo et al., 1998; Biswas et al., 2005; Imsland et al., 2008; Árnason et al., 2009; Yoo and Lee, 2016). These two strategies have already been widely investigated, using protocols on groups of fish reared in tanks with FI of the whole group used to calculate FCR or FER. One method commonly used to determine FI at group level is to trap, remove and count uneaten pellets, to calculate FI as the difference between the weight of feed given and the weight of feed wasted by fish (Jobling et al., 2001).

2.4.2. *At individual level*

A promising avenue to improve feed efficiency in fishes is the use of genetics and selective breeding programs. This is an emerging field, considering that selective breeding itself only started in the 1990's in fish (reviewed by Vandeputte et al., 2019). Selective breeding requires, however, that feed efficiency is a heritable trait, namely that phenotypic variation among individuals is partly explained by genetic variation and is not determined exclusively by environmental variables. Heritability (h^2 ; bounded between 0 and 1) is defined as the ratio of additive genetic variance over phenotypic variance, and a trait is heritable when $h^2 > 0$ (Falconer and Mackay, 1996). In order to accurately determine heritability and identify fish to be used as broodstock, feed efficiency must be estimated at individual level. That is, developing a selective breeding program for feed efficiency requires individual phenotyping for BWG and FI. In classical rearing systems, individual BWG is easy to measure by individual identification with

passive integrated transponder (PIT) tags (Roussel et al., 2000). In contrast, individual FI is much more difficult to measure in fish that are reared in large groups and eat simultaneously when feed is supplied. Thus, phenotyping fish for their individual FI requires specific approaches.

3. Methods used to measure individual feed intake in fish

Several techniques have been developed to measure individual FI in fish, which each has advantages and drawbacks.

3.1. Use of dyed feed

This method is suitable for fish reared in a group, coloured pellets are provided and, after feeding, stomach contents are collected and weighed to assess individual FI (Johnston et al., 1994; Unprasert et al., 1999). If the stomach contains the remains of several meals, pellets colour is changed between each meal to reveal this (Johnston et al., 1994; Unprasert et al., 1999). To our knowledge, however, this method has only been used to investigate feeding activity, not individual feed efficiency.

It is however technically difficult to collect and accurately identify meals in the stomach content so that this method may not be reliable to estimate individual feed efficiency (Jobling et al., 2001). Moreover, individuals must be sacrificed to dissect the gastrointestinal tract and collect stomach content (Johnston et al., 1994; Unprasert et al., 1999), so the method is not suitable for continuous monitoring of individual FI. Furthermore, sacrificing fish to assess individual FI is not compatible with current legislation for the protection of animals used for scientific purposes (European Union, 2010; US Government, 2015). Even if non-lethal methods such as gastric lavage could be used (Bromley, 1994), dyed feed is not used to estimate individual FI anymore.

3.2. Use of X-radiography

A second method for fish reared in a group is X-radiography. Pellets are produced with radio-opaque markers that are visible by X-radiography of the gastrointestinal tract (Talbot and Higgins, 1983; Fig. 5). The number of ingested pellets can be counted to measure FI (Jobling et al., 2001). Commonly used markers are iron powder (Talbot and Higgins, 1983) or ballotini glass beads (McCarthy et al., 1993; Silverstein et al., 2001; Boujard et al., 2006; Kause et al., 2006a; 2006b; Quinton et al., 2007a; 2007b; Grima et al., 2008).



Figure 5. X-radiography of fish feed intake. Yellow marks within fish gastrointestinal tract represent ballotini glass beads ingested with the feed. © L. Grima

The X-radiography must be conducted within a few hours of feeding to avoid a loss of markers by defecation, under anaesthesia (Jobling et al., 2001). This latter is problematic because fish need to recover from handling and anaesthesia and so frequent measurements may bias measurements of FI (Jobling et al., 2001). Indeed, full recovery of feeding behaviour after handling may require several weeks, for example two weeks for European whitefish *Coregonus lavaretus* (Quinton et al., 2007a) and three weeks for rainbow trout *Oncorhynchus mykiss* (Grima et al., 2008). Thus, individual FI is measured on a sub-sample of the total number of meals. Feed intake is, however, highly variable from one meal to another in fish (Smagula and Adelman, 1982; Tackett et al., 1988) so FI measurements with this method have low

repeatability, between 0.09 and 0.32 in rainbow trout (Kause et al., 2006a; Grima et al., 2008). That is, correlation between two FI measurements on a same fish is low. Thus, X-radiography is a “one-shot” method and not suitable for continuous monitoring of individual FI (Jobling et al., 2001).

3.3. *Use of isogenic clonal lines*

Using isogenic clonal lines of fish is not a measurement method of individual FI by itself, but rather a methodological tool to distinguish between the amount of variation in FI and feed efficiency that can be attributed to genetics or the environment. Genetic variation in a clonal line is non-existent so it can be used to increase the number of measurements per genotype within a given environment, such as a tank. Therefore, the same genotype can also be reared in different environments. Clonal lines are, consequently, useful experimental tools to estimate accurately genetic parameters in traits such as feed efficiency.

Grima et al. (2008) estimated individual FI of isogenic clonal rainbow trout with the X-radiography method, but clonal lines could of course be used with other techniques to measure individual FI. The clones were heterozygous, obtained by mating females and males from different homozygous clonal lines, themselves developed in rainbow trout by chromosome set manipulation methods using gynogenesis techniques (Quillet et al., 2007; Grima et al., 2008). Ten different clonal lines were reared in six tanks to have a balanced factorial design with seven fish of each line per tank. This increased the accuracy of estimates of genetic parameters for FI and feed efficiency, these two traits being highly sensitive to environmental variation (Grima et al., 2008). Clonal lines cannot, however, be used in commercial conditions or to develop selective breeding programs for feed efficiency.

3.4. *Use of external coloured tags and video-recording*

The use of video-recording has been widely used to investigate fish feeding behaviour (e.g. Kadri et al., 1991; Juell et al., 1994; Damsgård and Dill, 1998; Benhaïm et al., 2017). Very few

studies have, however, focused on measurement of individual FI with this method. The method is only feasible on small groups of fish, up to 15 fish in Nile tilapia *Oreochromis niloticus* (de Verdal et al., 2017). Thus, rearing conditions differ from commercial farms but, nonetheless, fish can still interact with conspecifics and therefore social structures are at least partly maintained.

Individual FI and feed efficiency were assessed in Atlantic halibut *Hippoglossus hippoglossus* using this method (Tuene and Nortvedt; 1995), using large numbered disc tags and direct observation. The first study to use this method from a viewpoint of selection, however, was by de Verdal et al. (2017) in Nile tilapia, using coloured T-bar tags on groups of up to 15 individuals. Each meal was video-recorded, with feed supplied pellet by pellet to facilitate measurement of individual FI (Fig. 6). This method has the major advantage of being exhaustive because it measures individual FI for each meal. Moreover, repeatability of individual FI with this method is very good, with $r = 0.95$ after analysis of 11 meals (de Verdal et al., 2017). The major constraint in the application of this method is the time required, firstly for feeding pellets one by one, and then to analyse video-recordings.



Figure 6. Nile tilapia *Oreochromis niloticus* tagged with external coloured tags. © C. Rodde

3.5. Use of individual rearing

One final method to measure individual FI is to rear fish in isolation (Silverstein et al., 2005; Silverstein, 2006; Martins et al., 2006; 2011; Besson et al., 2019; Fig. 7). Each fish is given a known amount of feed, and, several hours after feeding, waste pellets are collected and counted to calculate FI as the difference between feed given and feed wasted.

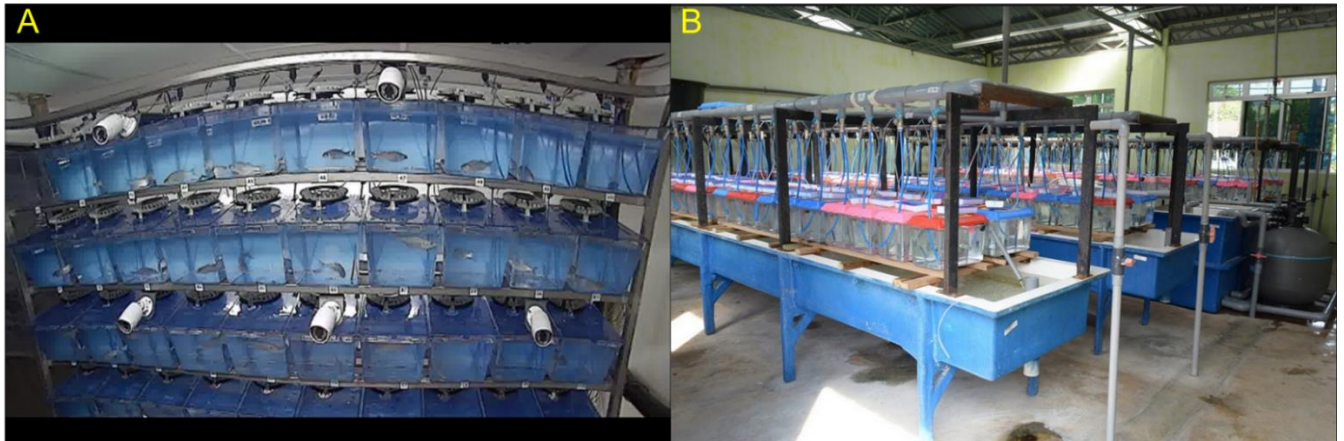


Figure 7. Two individual rearing setups. A) A system of aquaria for European sea bass *Dicentrarchus labrax* at the Ifremer Experimental Aquaculture Research Station (Palavas-les-Flots, France). An automatic feeder, comprising several small feed compartments (one per meal), is set on the cover of each aquarium. Cameras are used to monitor the room remotely. © F. Allal B) A system for Nile tilapia *Oreochromis niloticus* at WorldFish Aquaculture Extension Center (Jitra, Kedah State, Malaysia). A pill organiser, disposed on the cover of each aquarium, is used to hold and distribute each ration manually. © T. Quoc Trinh

The major advantage of this method is being exhaustive, FI can be measured for each meal over several months to account for temporal variability (Besson et al., 2019). Furthermore, the FI can be determined a few hours after feeding. Besson et al. (2019) assessed feed efficiency of 588 European sea bass *Dicentrarchus labrax* over 194 days using this methodology. It is, nonetheless, rather tedious to collect all the uneaten pellets in all the individual aquaria. Besson et al. (2019) restricted feeding to 50% of the optimal rate. The method has demonstrated that selecting faster-growing individuals under restricted ration improved feed efficiency of progeny in pigs (Nguyen et al., 2005) and rabbits (Drouilhet et al., 2016). This was true whether progeny was then fed at a restricted rate or *ad libitum*. The restricted feeding reduces, of course, the workload of collecting and counting wasted pellets.

It still remains to be demonstrated, however, that the most efficient fish under a restricted feeding rate in individual aquaria are also the most efficient when fed at *ad libitum*. Another drawback of this method is that it prevents fish from having normal interactions with conspecifics; isolation may impact individual FI and feed efficiency. Thus, more research is needed to establish whether individual FI and feed efficiency measured in isolation are accurate predictors of what would be observed in classical rearing systems.

To summarize, various methods have been developed to measure individual FI but none of them are “perfect”; all have flaws from a technical and/or a biological perspective (Table 1).

Table 1. Summary of the advantages and drawbacks of the various individual FI measurement methods.

Individual FI measurement method	Advantages	Drawbacks
Dyed feed	Group rearing	Not accurate, "one-shot" method
X-radiography	Group rearing, accurate	"One-shot" method, low repeatability
Isogenic clonal lines (in association with another method)	Increase the accuracy of genetic parameters estimation	Cannot be used for selective breeding
External coloured tags and video-recording	Group rearing, accurate, exhaustive, high repeatability	Time-consuming, groups are rather small
Individual rearing design	Accurate, exhaustive, data immediately available	Loss of social interactions between fish

Nonetheless, these methods have established that individual feed efficiency is heritable in a few fish species (Table 2). Therefore, individual feed efficiency can be improved by selective breeding. However, the various estimations of heritability (h^2) vary among species and between methods in the same species (Table 2). In particular, the low heritability estimated by Quinton et al. (2007a) and Kause et al. (2016) may be due to low repeatability of the X-ray method. This underscores that selective breeding programs must be based upon phenotyping methods with high repeatability.

Table 2. Heritabilities reported in literature for feed efficiency (including exclusively studies measuring feed intake at individual level).

$h^2 \pm SE$	Species	Trait(s)	Individual feed intake measurement method	Reference
0.06 ± 0.10^a 0.07 ± 0.11^b	European white fish <i>Coregonus lavaretus</i>	FER	X-ray	Quinton et al., 2007a
0.23 ± 0.09	Rainbow trout <i>Oncorhynchus mykiss</i>	RFI	X-ray with clonal lines	Grima et al., 2008
0.10 ± 0.05 0.11 ± 0.06 0.13 ± 0.07 0.14 ± 0.08	Rainbow trout <i>Oncorhynchus mykiss</i>	FCR ^{c,d} RFI ^d FCR ^e RFI ^e	X-ray	Kause et al., 2016
0.32 ± 0.11 0.50 ± 0.10	Nile tilapia <i>Oreochromis niloticus</i>	FCR RFI	External coloured tags	de Verdal et al., 2018b
0.25 ± 0.10^f 0.47 ± 0.07^g	European sea bass <i>Dicentrarchus labrax</i>	FCR	Individual rearing (restricted feeding rate)	Besson et al., 2019

SE: standard error; ^awith fishmeal diet; ^bwith soybean meal diet; ^ccorrected by body weight; ^drecorded at 27 months post hatching exclusively; ^erecorded at 11, 16 and 27 months post hatching; ^fwith pedigree information exclusively; ^gwith pedigree and genomic information.

All methods have, to date, only been applied for brief periods, and mostly to juveniles. For instance, individual feed efficiency was estimated over ten days in juvenile Nile tilapia by de Verdal et al. (2017) and over 28 days in juvenile European sea bass by Besson et al. (2019). To our knowledge, only Kause et al. (2006a; 2016) estimated individual feed efficiency up to commercial size (at 140, 750 and 2000 g in the first study and at 11, 16 and 27 months post hatching in the second one) in rainbow trout using the X-ray method. Nevertheless, feed efficiency was only estimated over three weeks at each stage, with one FI measurement per week. These studies did not report correlations among estimates at the three stages. Due to rearing infrastructures needed to measure individual FI on large fish and to rearing costs, in particular regarding feed (most feed is consumed during the later stages of growth; Alanärä et al., 2001), it is much more convenient to select for feed efficiency at juvenile stage. The aim of a selective breeding program would be to improve feed efficiency over the whole rearing period, not just over the juvenile stage, so it is essential to assess how individual feed efficiency of juveniles reflects individual feed efficiency up to commercial size.

Individual feed efficiency (or any other heritable phenotypic trait) can be improved genetically with two strategies: direct and indirect selection. Direct selection uses direct estimation of individual feed efficiency, which remains technically challenging and/or possibly inaccurate. Indirect selection uses measurement of traits whose variation is heritable and closely genetically correlated with feed efficiency. Selecting fish for the trait will, therefore, also select for feed efficiency. The goal, therefore, is to find traits that meet these criteria but that are also easier to measure than individual feed efficiency to be included in a selective breeding program.

4. Potential traits for indirect selection on feed efficiency

Traits presented below were chosen by two criteria: 1) a genetic correlation with feed efficiency that has already been reported in fish and 2) ease of measurement on individuals.

4.1. Growth

Individual growth is easy to measure on tagged fish and is already a selection criterion in almost all the commercial breeding programs in fishes, due to its economic importance. Thus, it is crucial to investigate any correlation with feed efficiency, in particular that selection for rapid growth does not degrade feed efficiency due to a negative genetic correlation.

Reported correlations between growth and feed efficiency traits are high at a phenotypic level, being positive between growth and FER, and negative between growth and FCR. That is, faster-grower fish are more efficient (reviewed by de Verdal et al., 2018a). Most studies have, however, focused on correlations at group level, and not at individual level, which may bias estimations. At individual level, de Verdal et al. (2017; 2018b) estimated a phenotypic correlation from -0.46 to -0.62 between growth and FCR in Nile tilapia *Oreochromis niloticus* using the video-recording method. Besson et al. (2019) reported $r = -0.78$ between growth and FCR in European sea bass *Dicentrarchus labrax* using the individual rearing method. However, it is essential to determine the contribution of genetics *versus* environment to any such

phenotypic correlations. At genetic level, Henryon et al. (2002) found a correlation between FER and body weight from 0.44 to 0.99 in rainbow trout *Oncorhynchus mykiss*, however they estimated FER at group (family) level. In Nile tilapia, de Verdal et al. (2018b) found no significant genetic correlation between individual growth and FCR whereas, in European sea bass, Besson et al. (2019) reported an extremely strong and significant genetic correlation ranging from -0.95 (without genomic information) to -0.98 (with genomic information). Beyond species differences, these contrasting results may also be methodological because de Verdal et al. (2018b) used an optimal feeding rate whereas Besson et al. (2019) restricted fish to 50% of optimal. The results reported by Besson et al. (2019) are, however, consistent with observations on pigs (Nguyen et al., 2005) and rabbits (Drouilhet et al., 2016) where there is a genetic correlation between growth and feed efficiency on restricted feeding rates. That is, when feeding rate is restricted, mechanistically faster-growing fish are the most efficient whereas, when feed is not restricted, some fish can also grow rapidly simply by consuming greater quantities of feed than others. Kause et al. (2006b) and Quinton et al. (2007a) demonstrated in rainbow trout and European whitefish *Coregonus lavaretus* that selecting for growth would improve individual FER even if it also increased individual FI. Indeed, according to their estimations, selecting for growth would increase BWG proportionally more than FI, resulting in improved FER.

Regarding RFI, no significant phenotypic correlation with growth was reported by de Verdal et al. (2017; 2018b) for Nile tilapia. In contrast, Silverstein (2006) reported significant phenotypic correlations of -0.31 and -0.57, when feeding rainbow trout to satiety or on a restricted ration, respectively. There was no genetic correlation between growth and RFI in Nile tilapia (de Verdal et al., 2018b) or rainbow trout (Grima et al., 2008). Such lack of correlation may reflect the statistical construction of RFI itself. That is, because RFI is the residual of the linear relationship of FI as a function of BWG and MBW, this creates a statistical independence

between BWG and RFI, even if not complete because of the simultaneous inclusion of MBW in the model. In the case of Grima et al. (2008), MBW was not even included in the linear model, resulting in almost zero correlation. In contrast, the significant correlation found by Silverstein (2006) may be due to the fact the study did not predict FI according to a model calibrated on its data, but according to a bioenergetics model developed for adult fish (despite using juvenile fish). This bioenergetics model included several factors such as water temperature and energy content of the feed.

No clear conclusions can be drawn about the potential to improve feed efficiency indirectly by selecting for growth traits. Any genetic correlations between these traits seem to be both species-dependent and feeding rate-dependent.

4.2. *Energy requirements for body maintenance and swimming activity*

4.2.1. *Weight loss during fasting*

Weight loss during fasting could be a predictor of individual feed efficiency because individuals with higher energy costs for body maintenance and routine activities would lose weight more when fasting. Similarly, under a restricted ration, fish with higher costs for maintenance and activity would allocate less energy to growth, thus being less efficient. When, however, fish are fed *ad libitum*, individuals may be able to compensate for higher costs by consuming more feed, thus appearing to be not less efficient.

Daulé et al. (2014) selected European sea bass for one generation according to weight loss during fasting and produced two divergent lines: fasting-tolerant and fasting-sensitive. These two divergent lines did not, however, differ significantly for RFI although the authors suggested that a second generation of selection might result in a difference between the two lines. In Nile tilapia, de Verdal et al. (2018b) reported a genetic correlation of 0.80 between weight loss during fasting and FCR, and of 0.70 between weight loss during fasting and RFI: the most efficient fish were fasting-sensitive (i.e. losing weight more rapidly). In contrast, Besson et al.

(2019) reported that fasting-tolerant European sea bass (i.e. losing weight less rapidly) were the most efficient.

Grima et al. (2008) considered both weight loss during fasting and compensatory growth during subsequent refeeding in rainbow trout. After feed deprivation, fish exhibit a phase of compensatory growth due to hyperphagia, which permits them to achieve a growth trajectory that converges with what would have been observed without feed deprivation (Ali et al., 2003). Grima et al. (2008) found no genetic correlation between RFI and weight loss during fasting or weight gain during refeeding. However, they found a genetic correlation between RFI and various linear indexes combining both weight loss at fasting and weight gain during refeeding ($r^2 = 0.44-0.59$).

Therefore, any potential link between feed efficiency and weight loss during fasting requires confirmation. Moreover, BWG during refeeding may also provide relevant information to predict feed efficiency. Finally, results appear to differ among species.

4.2.2. Metabolic rate

The metabolic rate of an animal, to meet energy demands of maintenance and activity, can be quantified with two methods: directly as heat produced and indirectly by respirometry measuring rates of O₂ consumed or CO₂ produced (Speakman, 2013). Both methods are commonly used in terrestrial animals but, for fishes, only the second method is technically easy to perform. That is, the high thermal capacitance of water makes it very difficult to detect any heat produced by metabolism. Measuring metabolic rate as oxygen consumption requires the isolation of individuals but is technically easier and shorter (less than two days) to perform than measuring individual FI (McKenzie et al., 2014). Large numbers of animals can be measured simultaneously and no feed is handled (fish are fasting). Oxygen consumption of undisturbed fish has reasonable repeatability over the short to medium term, with $r = 0.48$ in European sea bass for measurements separated by 20 minutes (Marras et al., 2010). Repeatability can decline

over time, with estimates ranging from $r = 0.68$ for two measurements separated by 17 weeks in Atlantic salmon *Salmo salar* (McCarthy, 2000) to as low as $r = 0.09$ for two measurements separated by 15 weeks in brown trout *Salmo trutta* (Norin and Malte, 2011), possibly due to context-dependent phenotypic flexibility.

Routine metabolic rate (RMR) is defined as the metabolic rate (i.e. energy expenditure) of post-absorptive, undisturbed, resting animals that also includes the costs of random activity and the maintenance of posture and equilibrium, at their acclimation temperature (Killen et al., 2011). The standard metabolic rate (SMR) is defined as the minimal energy cost of living of an ectotherm at its acclimation temperature (Hulbert and Else, 2004). This does not include the costs of random activity contrary to RMR. Killen et al. (2011) reported that individual European sea bass with high RMR had greater weight loss during fasting ($r = 0.53$ with 39 fish), suggesting that rates of oxygen consumption reflect allocation of energy to meet costs of maintenance and activity.

In livestock the most efficient individuals emit less heat and consume less oxygen, being true of cattle (Nkrumah et al., 2006; Arndt et al., 2015; Chaves et al., 2015), poultry (Luiting et al., 1991), and sheep (Paganoni et al., 2017). In sheep, Paganoni et al. (2017) measured oxygen concentrations after animals had been held in a closed respirometry chamber, which is a rough indicator of RMR. This measure was, nonetheless, genetically correlated with RFI ($r = -0.62$) at the hogget stage.

In fishes, a link between individual feed efficiency and oxygen consumption has, to our knowledge, never been investigated. In rainbow trout Kinghorn (1983) estimated that the phenotypic and genetic correlations between FER and oxygen consumption were -0.42 and close to -1 , respectively, at a family level. However, fish FI was estimated from oxygen consumption at a family level, which questions the accuracy of feed efficiency estimations.

Also, correlations estimated at family level can bias results as no intra-family variability is taken into account.

Furthermore, the feeding rate used to estimate feed efficiency (restricted or *ad libitum*) may have a strong influence on the extent to which oxygen consumption reflects feed efficiency. Zeng et al. (2017) reported that groups of crucian carp *Carassius auratus* comprising individuals with low oxygen consumption lost less weight during fasting and were more efficient under a restricted feeding rate. Conversely, groups of individuals with high oxygen consumption were more efficient when feed was supplied to satiation.

Beyond overall weight loss at fasting or oxygen consumption, it is also important to consider which substrates are used to provide energy, in particular protein *versus* lipid. Lipid reserves contain around twice as much energy per unit weight as proteins. Thus, if individual fish support metabolism with protein rather than with lipids during fasting, they can exhibit a stronger rate of weight loss than fish that rely more heavily on lipids, even if their overall rate of energy expenditure is lower (McKenzie et al., 2014).

4.3. Whole body, muscle and visceral fat content

Due to the high energy content of lipid reserves, the amount of fat contained in the whole fish body, or more specifically in its muscle or viscera, may influence apparent feed efficiency. Fat content within fish muscle can be measured non-invasively with a “fatmeter” (Quillet et al., 2005). After four generations of divergent genetic selection of rainbow trout based on intramuscular fat content, Kamalam et al. (2012) demonstrated that a lean muscle line had an improved FCR at group level than a fat muscle line. In coho salmon *Oncorhynchus kisutch*, Neely et al. (2008) demonstrated that selection for growth over 16 generations resulted in fish with a higher FER at group-level and a lower whole-body lipid content (measured in slaughtered fish). In European white fish, Quinton et al. (2007b) concluded that whole-body lipid content was not significantly genetically correlated with individual FER. However, the

same study found that selecting fish for growth and against lipid content led to a higher genetic gain (i.e. a greater improvement) for FER than selection exclusively for growth (0.73% *versus* 0.49% of genetic gain, respectively). Kause et al. (2016) demonstrated that whole-fish lipid content was genetically correlated with individual FCR ($r = 0.58$) and RFI ($r = 0.48$) in rainbow trout. Similarly, muscle lipid content was also genetically correlated with FCR (0.68) and RFI (0.57), but no correlation was found for visceral lipid.

From these different studies, it appears that a low fat content is linked with improved feed efficiency in fish. This observation has also been reported in terrestrial species such as pigs (Knap and Kause, 2018). Neely et al. (2008) argued that leaner animals may be sparing dietary protein for growth and utilizing dietary lipids for energy expenditure. As lipids contain more energy per unit weight, this is probably an optimal use of each type of reserve to improve feed efficiency. Moreover, as detailed by Knap and Kause (2018), deposition of 1 g of lipid leads to 1.1 g of weight gain, including 0.1 g of water in the associated adipose tissue. In contrast, deposition of 1 g of protein leads to 4-5 g of weight gain, including 3-4 g of water. Protein deposition is energetically more expensive than lipid deposition (59.9 kJ/g *vs.* 43.5-55.3 kJ/g), but this higher energetic cost is outweighed by the four to fivefold increase in weight gain associated with protein deposition (Knap and Kause, 2018).

There are, however, some exceptions to this line of reasoning. Grima et al. (2010) found no significant phenotypic correlation between RFI and muscular fat content or perivisceral fat in rainbow trout, and Besson et al. (2019) concluded that selecting European sea bass with fatter muscles could actually improve FCR. Besson et al. (2019), however, used the restricted feeding rate, which might influence how fish allocate dietary fat between energy expenditure and growth. To conclude, the link between fat deposition and individual feed efficiency in fish is not yet fully understood, and may depend on the feeding rate, the tissue (muscle, viscera or whole body) and the species.

4.4. Stable isotope values of fish tissues

Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values of a sample (e.g. animal or plant tissue) are defined as the ratio of heavy to light isotope ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$). When an animal starts feeding on a new diet, i.e. with stable isotope values that differ from the previous one, the stable isotope values of its tissues will progressively change (dynamic state) towards a new equilibrium (steady state), as illustrated in Fig. 8.

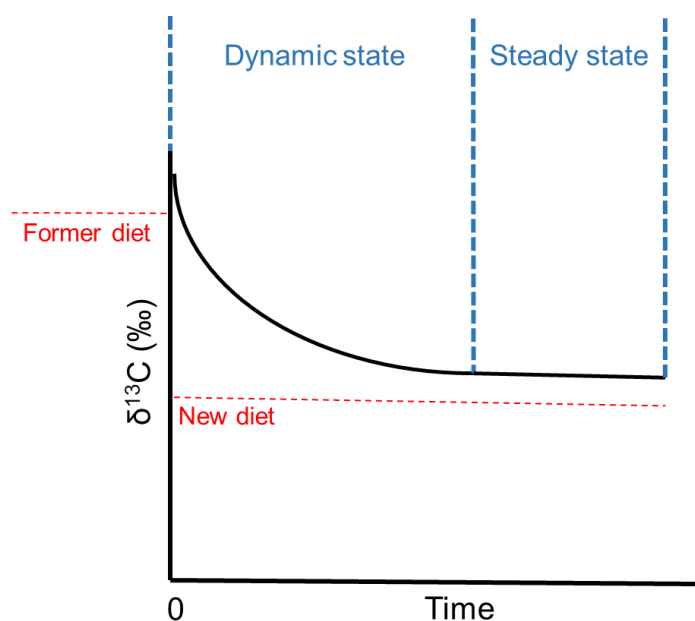


Figure 8. Illustration of the change in carbon stable isotope values within an animal tissue following a diet change. At the beginning, a dynamic state is observed, then a steady state is reached. The same type of curve would be observed in the case of nitrogen stable isotope values.

Stable isotope values at steady state have proven to predict individual feed efficiency in livestock at a phenotypic level. That is, a phenotypic correlation from -0.59 to -0.66 was reported between stable nitrogen values of plasma and FER in beef cattle (Wheadon et al., 2014; Cantalapiedra-Hijar et al., 2015) and a correlation of -0.73 between stable nitrogen values of muscle and FER was observed in lambs (Cantalapiedra-Hijar et al., 2016).

In fishes, only Dvergedal et al. (2019a; 2019b) have, to my knowledge, investigated the link between individual stable isotope values and group feed efficiency, focussing on stable isotope

values at dynamic state in Atlantic salmon. The study design involved sacrificing fish 12 days after a diet change, to determine carbon and nitrogen stable isotope values in liver, muscle and mid-intestine. Stable isotope values of the tissues were genetically correlated with FCR, from -0.43 to -0.90. Using a ratio of stable isotope signature over BWG, these authors found a genetic correlation of 1 with FCR, whereas BWG alone had a genetic correlation of “only” -0.74 with FCR (Dvergedal et al., 2019a; 2019b). Thus, these studies indicate that stable isotope values are promising candidates for indirect selection on feed efficiency in fish. However, fish FCR was estimated at group (family) level and not individual level in Dvergedal et al. (2019a; 2019b), in contrast with stable isotope values which were determined for each fish. This could bias genetic estimation of parameters because intra-familial variation in FCR is not taken into account.

Any tissue can be sampled to determine stable isotope values of an animal. Thus, sampling a tissue whose ablation is not lethal for fish, such as scales, could be more useful as a strategy to apply stable isotope determinations in selective breeding programs. Selective breeding would lead to a higher genetic gain if done directly on the fish whose stable isotope values are determined rather than on collaterals.

Finally, whether one should focus on stable isotope values at dynamic state, at steady state, or at both to predict individual feed efficiency is still unknown. According to existing literature, all the options might lead to a successful prediction of individual feed efficiency. From a biological point of view, the mechanisms underlying stable isotope dynamics are still not thoroughly understood. The dynamic changes in values are due to both growth (i.e. adjunction of new tissues) and catabolism (i.e. replacement of tissues; Hesslein et al., 1993). At steady state, values appears to depend on the balance between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the feed consumed, faeces and excretory products (Minagawa and Wada, 1984; Ponsard and Averbuch, 1999; Olive

et al., 2003). To date, therefore, stable isotope values are treated as “black box” predictors of individual feed efficiency. This is not, however, an obstacle for selective breeding.

5. European sea bass and Nile tilapia as model species

The European sea bass and Nile tilapia are both major aquaculture species but they differ widely in their biology and farming (Table 3). They have both been the subject of studies of individual feed efficiency (de Verdal et al., 2017; de Verdal et al., 2018b; Besson et al., 2019) within ongoing research projects that aim to develop selective breeding programs. Thus, many technical aspects of measuring individual FI have already been resolved for both species. Information on their individual feed efficiency is of interest in itself while the comparison of the two species can provide added value, notably whether methods available to initiate selective breeding programs can be generalized across biological models and rearing conditions.

Table 3. Main characteristics of biology and farming of European sea bass and Nile tilapia.

	European sea bass <i>Dicentrarchus labrax</i>	Nile tilapia <i>Oreochromis niloticus</i>
Geographic distribution	Marine European waters: North-East Atlantic Ocean (from Scotland and Norway to Morocco), Mediterranean Sea and Black Sea (Pickett and Pawson, 1994).	Originally African freshwaters: Nile river from Uganda to Egypt, Western rift lakes down to Lake Tanganyika, Chad and Niger basins and West Africa (up to Senegal and Mauritania). Introduced to many other areas of Africa (e.g. Madagascar), to South-East Asia and the Americas (Philippart and Ruwet, 1982).
Temperature tolerance	Eurythermal (2-32°C as critical limits) but cannot grow below 10°C. Optimal temperature range for growth and feed efficiency from 22°C to 25°C (Barnabé, 1980; Person-Le Ruyet et al., 2004).	Temperature preferendum around 28-30°C, reduction of activity and feeding below 20°C. Upper temperature limit: 42°C (Philippart and Ruwet, 1982).
Natural diet composition	Carnivorous (high trophic level), natural diet is mainly finfish and crustaceans (Rogdakis et al., 2010).	Omnivorous (low trophic level), natural diet includes phytoplankton, macrophytes, zooplankton, insects and nematodes (Teshahun and Temesgen, 2018).
Rearing methods	Mainly grown in sea cages in natural waters, fed with commercial feed. Larval production is typically in inland intensive hatcheries in temperature-controlled water (Chatain and Chavanne, 2009; Vandeputte et al., 2019).	Very wide range of farming systems extensive or intensive (e.g. cages within lakes, land-based ponds or concrete tanks), with or without supply of commercial feed, in monoculture or polyculture (e.g. with rice, vegetables, other fishes or livestock; Modadugu and Acosta, 2004). Can tolerate poor water quality (hypoxia) and brackish salinities up to 30‰ (Philippart and Ruwet, 1982).

Behaviour in captivity	Shoaling behaviour from larval stage onwards (Barnabé, 1980). Cannibalism if individuals have heterogeneous sizes and feed is scarce (Barnabé, 1980).	Frequent aggressive interactions to establish dominance hierarchies can cause physical injuries and mortality, chronic stress, increased energy expenditure, reduced growth in subordinate fish (reviewed by Gonçalves-de-Freitas et al., 2019).
Sexual dimorphism	Females grow faster than males (Chatain et al., 1997; Gardeur et al., 2001; Saillant et al., 2001).	Males grow faster than females (Hulata et al., 1986; Lind et al., 2015).
Importance in aquaculture	Mainly reared in Turkey, Greece and Spain (FAO-FIGIS, 2020). Production around 235 000 tons. This is a small proportion of global finfish aquaculture but it is a major species for European aquaculture, with a high commercial value (STECEF, 2018).	Mainly reared in Africa (27.9%) and Asia (62.5%; FAO-FIGIS, 2020). Production is huge, around 4.5 million tons. Third most reared fish in the world after grass carp <i>Ctenopharyngodon idellus</i> and silver carp <i>Hypophthalmichthys molitrix</i> .

The two species however have a common asset: their genetic resources are wide. Three genetically distinct populations of European sea bass exist in the wild: Atlantic (AT), West Mediterranean (WM) and East Mediterranean (EM; Guinand et al., 2017), as a result of evolutionary processes that include selection by thermal regime (Duranton et al., 2018; Duranton et al., 2020). There may be an interaction of population by temperature on feed efficiency, as this has been reported for growth (Vandeputte et al., 2014). Consequently, it is interesting to compare individual feed efficiency of European sea bass populations at different temperatures. The European sea bass is already the focus of research into individual feed efficiency (Besson et al., 2019) at the Ifremer Aquaculture Research Station in Palavas-les-Flots (Hérault, France).

Wild tilapia genetic resources have permitted selection of high performance strains for aquaculture (Lind et al., 2019). The “Genetically improved farmed tilapia” (GIFT) strain is currently the most advanced in terms of genetic improvement. It was produced in the 1990’s from eight different strains taken from wild or recently domesticated populations (Eknath et al., 1993). Since 2002, the GIFT strain has been reared at the WorldFish Aquaculture Extension Center at Jitra (Kedah State, Malaysia), with a breeding program based on growth that is in its 18th generation (Ponzoni et al., 2010; Ponzoni et al., 2011). A genetic gain of 64% was achieved over the first nine generations (7.1% per generation; Ponzoni et al., 2011). In parallel with selection on growth, WorldFish disseminated the GIFT strain to hatcheries in several countries,

such as Malaysia, China, Bangladesh, Vietnam or Brazil (Ponzoni et al., 2010; Ponzoni et al., 2011). A current objective at WorldFish is to improve individual feed efficiency in GIFT strain (J. Benzie, personal communication, 2020).

6. Moving forward to estimate and select for individual feed efficiency in fishes

The literature seems to offer promising opportunities to improve feed efficiency. Firstly, by selecting directly for feed efficiency, by measuring individual FI accurately. Secondly, by selecting indirectly on traits that are genetically correlated with feed efficiency but easier to measure at the individual level. However, the emergence of research on genetic improvement of feed efficiency is recent and many questions remain to be answered. Many critical issues need to be addressed to develop selective breeding programs for individual feed efficiency. In this thesis project, I aimed to study several major methodological issues and to identify an indirect selection criterion for feed efficiency. The Results section therefore comprises five research articles (three accepted, one under review and one still in preparation), briefly outlined here.

6.1. Methodological issues in estimating individual feed efficiency

The objective of a breeding program must be to improve feed efficiency from juvenile stage to commercial size but experiments have only been conducted over short periods. There is no evidence that estimations of individual feed efficiency over a few days or weeks are correlated with estimations of individual feed efficiency over the whole production cycle. This question will be addressed in the first chapter of the Results, an article entitled “Can individual feed conversion ratio at commercial size be predicted from juvenile performance in individually reared Nile tilapia *Oreochromis niloticus*?” published in Aquaculture Reports.

Secondly, as described above, existing methods to measure individual FI have advantages and drawbacks. No study has ever directly compared these methods on the same fish, to determine whether they provide equivalent estimations of individual feed efficiency. This issue is

considered in the second chapter of the Results, an article entitled “The effects of feed restriction and isolated or group rearing on the measurement of individual feed intake and estimation of feed conversion ratio in juvenile Nile tilapia (*Oreochromis niloticus*) for selective breeding purposes” submitted to Frontiers in Genetics.

Another unknown issue is the influence of feeding rate on individual feed efficiency. That is, as detailed above, whether the most efficient fish at a restricted feeding rate are also the most efficient at *ad libitum* feeding. This question is investigated in the third chapter, an article entitled “Population, temperature and feeding rate effects on individual feed efficiency in European sea bass (*Dicentrarchus labrax*)” accepted in Frontiers in Marine Science.

6.2. Evaluation of a criterion for indirect selection

I have focussed upon two particularly promising traits: metabolic rate as oxygen consumption, and tissues stable isotope values.

The relevance of oxygen consumption was studied in the fourth chapter of the Results, an article entitled “An investigation of links between metabolic rate and feed efficiency in European sea bass *Dicentrarchus labrax*” in preparation for submission.

Regarding stable isotope values, they have potential as an indirect criterion and can be determined without slaughtering fish by sampling scales. Nonetheless, whether one should focus rather on the dynamic state of stable isotope values, on the steady state, or on both after a diet change needs investigation. As a preliminary step, how long stable isotope values take to reach the steady state must be determined. Then, it may be possible to design protocols studying carbon and nitrogen stable isotope values, both at dynamic and steady states, as an indirect selection criterion for individual feed efficiency. This preliminary question was studied in the fifth chapter of the Results, an article entitled “Variations in incorporation rates and trophic discrimination factors of carbon and nitrogen stable isotopes in scales from three European sea

bass (*Dicentrarchus labrax*) populations” published in the Journal of Experimental Marine Biology and Ecology.

CHAPTER I

Can individual feed conversion ratio at commercial size be predicted from juvenile performance in individually reared Nile tilapia *Oreochromis niloticus*?



Can individual feed conversion ratio at commercial size be predicted from juvenile performance in individually reared Nile tilapia *Oreochromis niloticus*?

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ABSTRACT

Feed conversion ratio (FCR), the ratio between feed intake and body weight gain, is of major interest for improving aquaculture sustainability through reduced feed costs and environmental impacts. Demonstrating whether FCR measured in juvenile fish is an accurate predictor of their performance during the whole rearing period is critical to developing genetic improvement programs for this trait. This is especially true for estimates obtained in individually reared fish, for which this has high implications regarding the size of the necessary rearing structures. We obtained individual FCR from 30 male Nile tilapia *Oreochromis niloticus* from the GIFT strain individually reared in a recirculating system, from 36 to 260 g mean weight. They were fed twice a day and uneaten pellets were counted every day to determine the feed intake of each fish. Individual growth was monitored every week. Feed conversion ratio was estimated over two-week periods and over the whole rearing period (210 days). Phenotypic correlations between the two-week FCRs and global FCR estimations were mostly significant (ranged from 0.38 to 0.64). A significant phenotypic correlation between growth and FCR was also found: faster-growing fish had a better (lower) FCR. Individual breeding values for global FCR were estimated using FCR phenotypes from the present study and previously published heritabilities for FCR in Nile tilapia. Potential estimated genetic gain for global FCR was 2.2% per generation with 50% selection intensity. When selecting fish on their FCR from only a two-week period, approximately 50% of the reference genetic gain could be obtained with the same selection intensity. FCR measured during a two-week period at juvenile stage could be a moderately accurate approximation of the whole rearing period FCR, and could be used as a lower cost criterion to select for FCR in future genetic improvement programs using individual rearing of fish.

1. Introduction

Continuing to feed the increasing world's human population while reducing food production pressure on the environment is a major challenge. Fish is seen as a key component of sustainable future diets (Froehlich et al., 2018). Since fisheries production stagnates, meeting the future demand for products of aquatic origin will rely on aquaculture (FAO, 2016). However, increasing aquaculture production will require an increase in fish feed production which will compete for

access to ingredients with agriculture and direct human consumption (Troell et al., 2014). Improving the ability of cultured fish to convert feed intake into biomass could play a significant role in reducing feed use in aquaculture and improving its sustainability through reduced costs and environmental impacts (Besson et al., 2014, 2016; de Verdal et al., 2018a). The ability to convert feed intake into body weight gain can be measured by the feed conversion ratio (FCR) which is the ratio between feed intake (FI) and body weight gain (BWG) over a given time period. Feed conversion ratio can be improved through changes in feed

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composition and husbandry (NRC, 2011) and through selective breeding (de Verdal et al., 2018a). The main challenge to improving FCR in breeding programs is the capacity to accurately measure FCR at the individual level on a large number of fish.

Measuring the individual FI of a large number of fish is particularly difficult as fish are reared in groups, and the share of a meal eaten by each individual is not easily recorded. Various methods have been proposed to measure individual FI, such as X-radiography with radio-opaque pellets (Kause et al., 2006; Grima et al., 2008) or using video recording of small groups of fish distinguished by colored T-bar tags (de Verdal et al., 2017, 2018b). Another option is the rearing of individual fish in aquaria with collection of all uneaten pellets (Silverstein, 2006; Martins et al., 2011; Besson et al., 2019). This method is tedious, but has potential to be used for selective breeding through the identification of Quantitative Trait Loci (QTLs) or the use of genomic selection (Lu et al., 2017; Besson et al., 2019).

Estimating individual fish FCR beyond juvenile stages is particularly important as the amount of feed consumed during the later stages of growth is higher than during the younger stages (Alanärä et al., 2001). In broiler chicken, de Verdal et al. (2013) made a long-term FCR evaluation and showed that selection for FCR undertaken at a given age improves offspring FCR much more at that selection age than at other ages. That work demonstrated it is essential to estimate the correlations between FCRs measured at different development stages, in order to assess the ability to use data from one given stage to select efficiently for FCR over the whole rearing period. Due to the rearing infrastructures needed and to rearing costs, it would be much more convenient to select for juvenile fish than for fish at commercial size, especially when individual rearing is used. Whether FCR estimated at early stages gives a reliable picture of FCR at older stages is thus critical information in this respect.

The objective of the present study was to assess the changes over time of three key performance traits (i.e. BWG, FI and FCR), to estimate whether fish with the best (lowest) FCR at juvenile stage also had the best FCR during the whole rearing period. Nile tilapia (*Oreochromis niloticus*) was used as this is the second most farmed aquaculture species in the world (FAO-FIGIS, 2019). We used the GIFT (Genetically Improved Farmed Tilapia) strain (Ponzoni et al., 2010), for which phenotypic and genetic data on individual FCR are available (de Verdal et al., 2018b). In the present study, male Nile tilapia were reared individually in aquaria, in order to measure individual BWG, FI and FCR from the juvenile stage (36 g) up to commercial size (250–300 g), and to evaluate the relevance of FCR estimated over short periods to predict FCR over the whole grow-out period.

2. Material and methods

2.1. Ethics statement

This study utilised phenotypic data collected as part of the GIFT selective breeding program managed by WorldFish at Jitra, Kedah State, Malaysia (6°15'32" N; 100°25'47" E). All fish in the GIFT breeding population are managed in accordance with the Guiding Principles of the Animal Care, Welfare and Ethics Policy of the WorldFish.

2.2. Biological material

Forty individual Nile tilapia were used in the experiment, taken from two families (20 full-sibs from each family) from the 17th generation of GIFT produced on the 27th of December 2017 at WorldFish Aquaculture Extension Centre in Jitra. Fish were reared in two distinct hapas in the same pond and transferred to 1500 L holding tanks (3 × 1 × 0.5 m) at 110 days post hatching (dph). During this period, PIT-tags were injected to identify each fish individually. These 40 fish were initially sorted from a larger group at 131 dph to have a similar body weight at the beginning of the experiment, allowing easier comparisons

between individuals.

At 145 dph, fish were too young to be sexed. Fish were sexable only after seven weeks of experiment (at 201 dph). Among these 40 fish, nine were females and 31 were males, one of which jumped and died after the beginning of the experiment. Although females were kept in the rearing system, the study focused on the 30 remaining males (18 coming from the first family and 12 from the second one). The first objective was to study both sexes, but the number of females was too small to ensure a reliable statistical analysis including both sexes.

2.3. Rearing system

The rearing system consisted of two recirculating water systems, in the same room, each including 20 aquaria, a sand and a biological filter. Each fish was placed into a 60 L (61 × 30 × 33 cm) single plastic aquarium at 145 dph and left for one week of acclimation time. The experiment started at 152 dph with males weighing 36.3 ± 5.9 g (mean \pm standard deviation). The initial coefficient of variation ($CV = 100 * (\text{Standard deviation} / \text{Mean})$) of body weight was thus 16.3%. The 30 males were shared equally (15 and 15) between both recirculating water systems even if fish were distributed by a random draw.

Water renewal rate was 240% per hour and each aquarium included a constant aeration system. Water temperature was 29.1 ± 1.2 °C, water oxygen saturation rate was on average 7.1 mg/L (92.1% of saturation), water pH was 7.0 and photoperiod was natural, around 12 h light/12 h dark. The feed used was the same during all the experiment: a commercial tilapia feed (Cargill®, “Starter tilapia 6113”) with 34.0% crude protein, 5.0% crude fat, 5.0% crude fibre and 12.0% moisture, with constant pellet size (2 mm diameter). The 100% daily feed ration (DFR; in percentage of body weight) was calculated based on the formula published by Mélard et al. (1997):

$$DFR = 14.23 * BW^{-0.322} \text{ with BW the body weight of each fish (in g).}$$

Throughout the experiment, fish were fed 90% of the calculated DFR, shared equally in two meals. Fish were fed by hand twice a day at 9 a.m. and 2 p.m. (all fish were fed in less than 10 min), except on days of body weight measurements where fish were fed only at 2 p.m. The fish were fed 90% rather than 100% of the DFR in order to reduce the amount of uneaten feed and thus the time needed for counting uneaten pellets. With this feeding rate, fish were generally wasting a few pellets at each meal, indicating that they were close to *ad libitum*. Furthermore, while the equation developed by Mélard et al. (1997) was not developed on the same feed and on the same tilapia strain, a calculated ration was preferred to an “*ad libitum*” feed ration. Several people were involved in the management of the experiment, and from one experimenter to another, the amount of feed given to a fish as “*ad libitum*” can fluctuate widely, reducing repeatability of the FI measurement.

2.4. Feed intake measurement and FCR calculation

Each fish was anaesthetized with clove oil (0.5 mL per litre of water) and weighed once a week. The DFR was updated every week for each fish. Every day, feed given to the fish was weighed and the uneaten pellets were counted and removed from the aquaria at least two h after the last meal of the day. The uneaten feed weight was estimated every day, considering that all pellets had the same weight (16.2 ± 1.8 mg). Daily feed intake (DFI) was calculated for each fish as the difference between daily feed weight given and daily feed weight uneaten.

The FI, BWG and FCR for individual fish were calculated on two-week time steps. Two-week periods were chosen instead of one-week periods to smooth the strong weekly variation of individual BWG (Supplementary Material 1). Bi-weekly FI values were calculated for each fish as the sum of the DFI during two full consecutive weeks. Bi-weekly BWG was calculated for each fish as $BWG = BW_f - BW_i$, with BW_i and BW_f the body weights at the beginning and at the end of the two-week period, respectively. Each fish was measured for FI and BWG

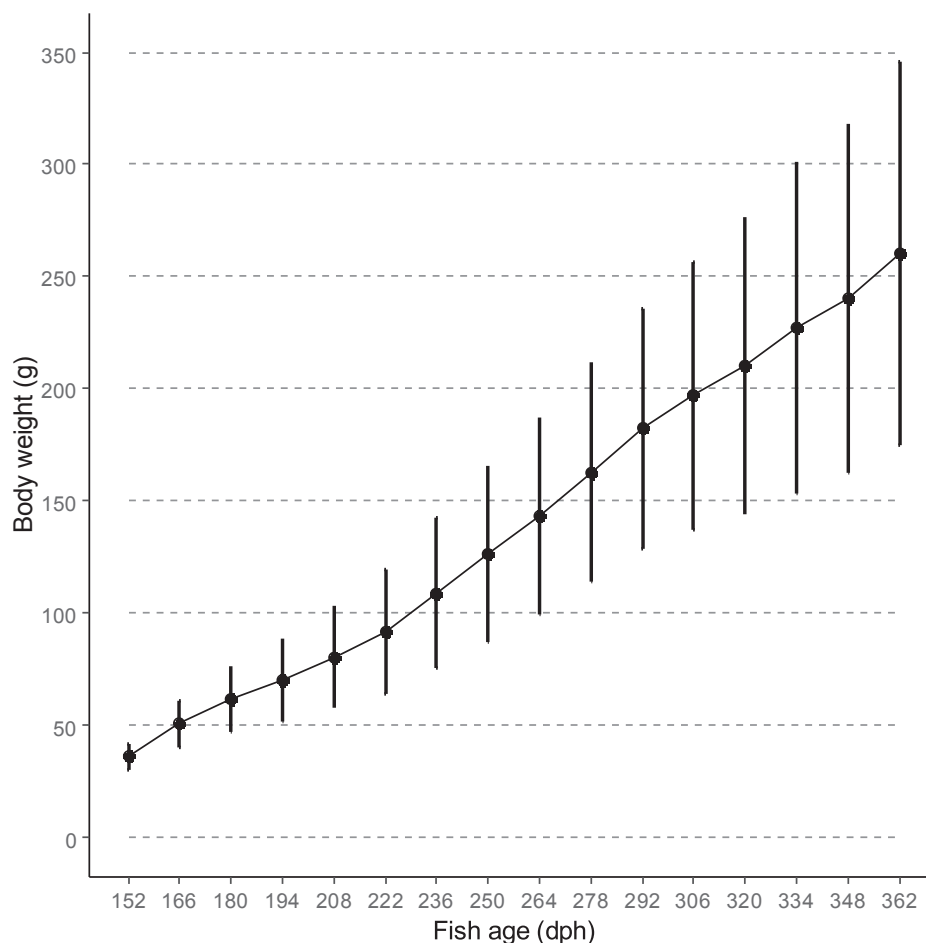


Fig. 1. Mean body weight (g) over the duration of the experiment (error bars represent standard deviation).

over 15 consecutive two-week periods (30 weeks of experiment in total), from 152 to 362 dph. Global FI (FIg) and BWG (BWGg) were calculated for each fish over the whole experimental period, as the sum of all DFI values and as the difference between body weights at the end and at the beginning of the experiment, respectively, in order to estimate global FCR ($FCRg = FIg \cdot BWGg^{-1}$).

2.5. Statistical analysis

All statistical analyses were performed using R software (R Core Team, 2018). Negative and outlier bi-weekly FCR values (10 data points out of 450) were not included in the statistical analysis. Over each period, FCR values were considered outliers when not between $M - 3 \cdot Sd$ and $M + 3 \cdot Sd$, with M the mean FCR and Sd the standard deviation of FCR over the period. Negative FCR were due to fish losing weight and outlier (high) FCR to fish gaining very little weight. The family and the recirculating water system effects were not significant for any trait in any period, and are thus not reported in the analyses.

2.5.1. Linear mixed models

The aim was to determine how fish performance traits (FI, BWG and FCR) changed through time. Firstly, whether they could be modelled as a function of time with only one linear regression through the whole experiment was tested. Otherwise, a segmented regression was used in the case of performance with sequences of increase and decrease. Potential breakpoints and segments in fish performance were detected using Chow test with the R package “strucchange” (Zeileis et al., 2002) that can handle repeated measures on the same individuals. Then, performance traits were analyzed on each separate segment with the

following repeated measures linear mixed model:

$$Y_{ij} = \mu + \beta \cdot T_i + A_j + \varepsilon_{ij}$$

where Y_{ij} is the phenotype (FI, BWG, FCR) of individual j measured for the two-week measurement period i (i between 1 and 15), μ is the general mean, β is the fixed effect of time T for every period i , A_j the random effect of the animal j with $A_j \sim N(0; \sigma_a^2)$, and ε_{ij} the residual ($\varepsilon_{ij} \sim N(0; \sigma_e^2)$). The normality of residuals was checked using the quantile-quantile method (comparing residuals quantiles with theoretical normal quantiles), and their homoscedasticity and independence by comparing residuals with the model fitted values. Linear mixed models and Student tests associated to these models were realized using R packages “lme4” (Bates et al., 2015) and “lmerTest” (Kuznetsova et al., 2017).

2.5.2. Correlation estimates and correlation temporal patterns

Individual values of FI, BWG and FCR were log-transformed (lnFI, lnBWG, lnFCR) to achieve normal distribution, allowing Pearson correlation analyses. Correlations between lnFIg, lnBWGg and lnFCRg allowed the estimation of phenotypic links between the three traits over the whole rearing period. Then, for each trait, the correlation between each two-week period and the whole rearing period was estimated. For each trait, pairwise correlations between the different two-week periods were submitted to a Mantel test (R “ape” package; Paradis and Schliep, 2018) to assess whether they were significantly structured along a temporal gradient. The Mantel test was performed between the matrix of between-periods correlations and the matrix of time lapse between periods.

The relevance of measuring FCR during a two-week period rather than during the whole rearing period was then assessed. To this end, the

potential genetic gain on FCRg using direct mass selection on FCRg was compared to the potential genetic gain on FCRg using mass selection on FCR measured during the two-week periods which showed 1) the highest and 2) the lowest correlation with FCRg. For each fish, an estimated breeding value for FCRg was obtained with the following equation (Falconer and Mackay, 1996):

$$EBV_i = h^2 * (FCRg_i - \bar{FCRg})$$

with EBV_i the estimated breeding value of fish i for FCRg, h^2 the heritability of FCRg, $FCRg_i$ the FCRg of fish i and \bar{FCRg} the mean FCRg of the 30 fish. Heritability was set to 0.32, the estimate for juvenile FCR in GIFT Nile tilapia from de Verdal et al. (2018b).

In mass selection, the best fish are selected based on their own phenotypes. So, the fish were ranked with three alternative methods: with FCRg (reference method), with FCR on the two-week period having the best correlation with FCRg, and with FCR on the two-week period having the worst correlation with FCRg. In each case, the fifteen best fish were identified, corresponding to a selection intensity of 50%. These best fish were the ones that would be selected in a mass selection program. Thus, the mean EBVs for FCRg of the fifteen best fish obtained with each of the three methods were estimated.

3. Results

3.1. Temporal patterns of growth, BWG, FI and FCR

Fish reached commercial size (260.5 ± 85.4 g) at 362 dph and the variability of body weight increased through time (Fig. 1). The mean BWGg over the full experiment was 224.5 ± 84.4 g. The corresponding mean FIg was 385.0 ± 128.6 g, resulting in a mean FCRg of 1.76 ± 0.19 .

Performance traits were modelled with segmented linear mixed regressions as, according to the Chow test, the changes in FI, BWG and FCR over time were best modelled with breakpoints (Fig. 2). The CV of FCR was ranged from 11.3 to 36.2% with an average of $23.7 \pm 7.7\%$ (Fig. 3).

3.2. Correlation among traits and time periods

3.2.1. Correlation among traits

Over the whole experiment, the correlation between \ln BWGg and \ln FIg was high and significant ($r = 0.98$). The correlation between \ln BWGg and \ln FCRg was significant and negative ($r = -0.63$). Finally, the correlation between \ln FIg and \ln FCRg was also significant and negative ($r = -0.44$).

3.2.2. Correlation among time periods within traits

All two-week \ln BWG were significantly and moderately to highly correlated with \ln BWGg ($r = 0.55-0.94$). The same results were observed for \ln FI ($r = 0.67-0.97$ with \ln FIg). Global FCR (\ln FCRg) was significantly and positively correlated with \ln FCR recorded in 11 out of the 15 two-week periods, with correlations ranged from 0.38 to 0.64 (Fig. 4). Significant and higher correlations were mainly seen during the first half of the experiment (between 152 and 250 dph).

For each trait, the period to period correlation matrix was significantly structured along a temporal gradient, with higher correlations for closer periods (Mantel test, $P < 0.001$ for \ln BWG and \ln FI and $P < 0.05$ for \ln FCR). However, only 19 out of 105 pairwise correlations were significant for \ln FCR (only 7 out of 14 considering exclusively consecutive periods pairs).

3.3. Potential genetic gain for FCR

Estimated improvement in FCRg was 2.2% per generation with 50% of selection intensity on FCRg itself. This reference genetic gain for

FCRg was compared with that projected using FCR from two-week periods to rank the fish. When using FCR from 152 to 166 dph to rank the fish (the period for which FCR was best correlated with FCRg, $r = 0.64$), the estimated genetic gain was 1.0%. When using FCR from 334 to 348 dph to rank the fish ($r = 0.38$ with FCRg, the worst period) the estimated genetic gain was 1.2%. Globally, when using a two-week period to rank the fish, approximately 50% of the reference genetic gain can be obtained with 50% selection intensity.

4. Discussion

4.1. Temporal variation in parameters

The aim of the present study was to determine whether FCR measured in young fish would reflect their performance during the whole rearing period. Feed intake, BWG and FCR globally increased with time but also fluctuated through time. Two major fluctuations in the measured performance occurred, which might result from physiological changes in the fish, since abiotic parameters were constant over time.

First, the decrease in BWG and FI between 152 and 194 dph might be explained by sexual maturation. The mean weights during this period (36.0 g at 152 dph and 70.3 g at 194 dph) correspond to the weight at onset of maturity in Nile tilapia reported in the literature (30–60 g, Galemoni de Graaf and Huisman, 1999; Gómez-Márquez et al., 2003; Hussain, 2004). Decrease in FI linked with male maturation has been demonstrated in several fish species (Kelly and Peter, 2006; Leal et al., 2009; Nishiguchi et al., 2012).

Until 292 dph, FI and BWG changes through time were simultaneous and in similar proportions, FCR did not change strongly during that period. However, BWG decreased between 292 and 348 dph, without related FI decrease, leading to a significant increase in FCR during this time frame. Even though fish were reared individually, pheromones from the few females kept in the same water system could be transmitted through the water exchange between tanks (Stacey and Sorensen, 2002). Female pheromones may induce an increased allocation of energy to gonad development in male fish (Miranda et al., 2005) and aggressive behavior (Giaquinto and Volpato, 1997), reducing investment in growth. Reports that male tilapia in a monosex group grew faster than in a mixed-sex group may provide indirect evidence to support this hypothesis (Macintosh and Little, 1995; Green et al., 1997; Dan and Little, 2000; Hafeez-ur-Rehman et al., 2008). However, individual rearing may have impeded behavioral aspects of tilapia reproductive functions, and present observations may not be completely comparable to large populations with mixed-sex rearing systems.

4.2. Correlations among traits and time periods

For \ln FI and \ln BWG, the closer two two-week periods were in time, the higher the correlation between them, meaning that a measurement at a given period would better predict performance at adjacent periods. A similar result was observed for body weight in a GIFT-derived strain of Nile tilapia when reared in mixed-sex groups (He et al., 2017). For \ln FCR, the correlation was also greater between closer measurements in time, but these correlations were generally low and not significant, showing that FCR measured at a given two-week period is a poor predictor of FCR at any other two-week period. However, \ln FCRg for 11 out of 15 two-week periods were significantly correlated with the global FCRg measured over the whole experiment, suggesting that a two-week FCR assessment may efficiently predict global FCR. Among the four two-week periods that were not significantly correlated with FCRg, three occurred just before or during the second BWG decrease.

A significant but moderate correlation was observed between \ln FCRg and \ln BWGg ($r = 0.63$), showing that faster-growing fish had a better (lower) FCR. This is in accordance with phenotypic correlations found in the literature between FCR and growth traits of fish reared in groups, whose values are ranged between -0.6 and -0.9 (de Verdal

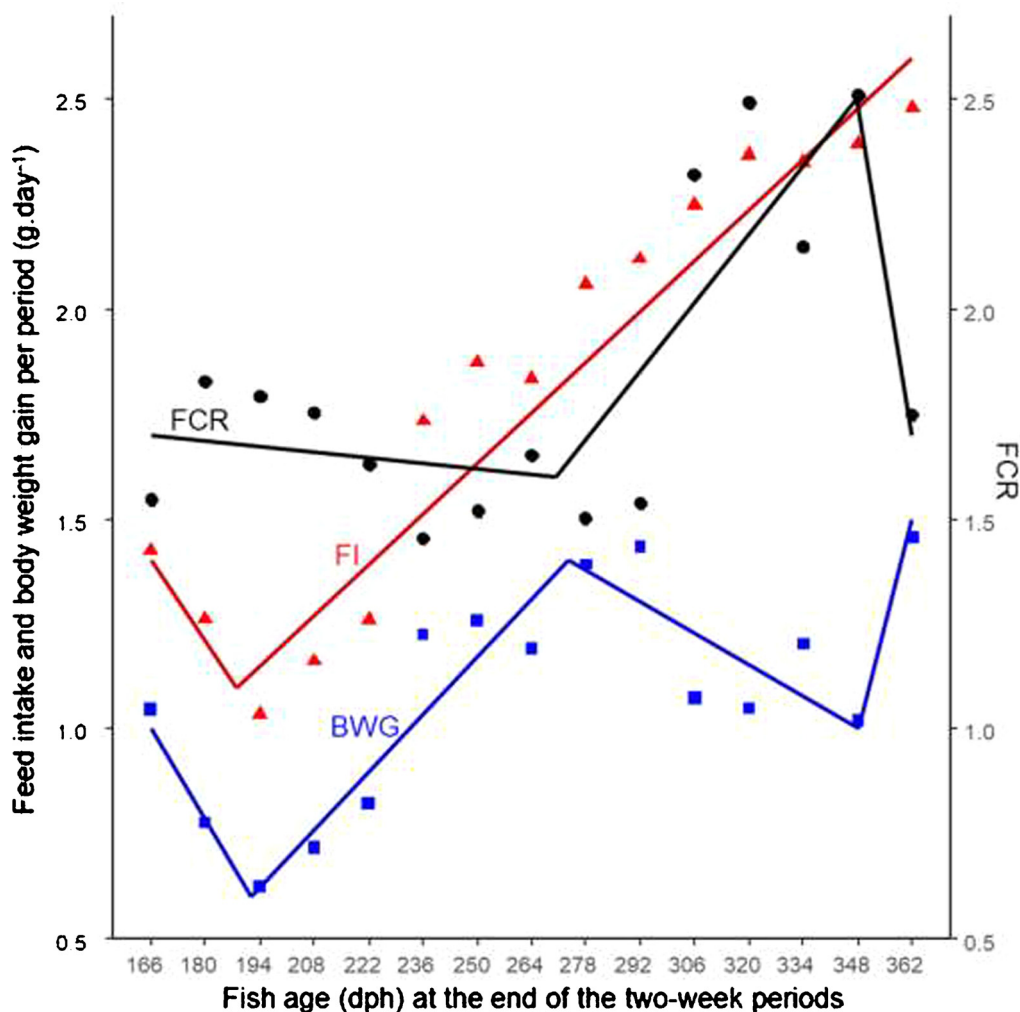


Fig. 2. Feed intake (FI, g day^{-1}), body weight gain (BWG, g day^{-1}) and feed conversion ratio (FCR) measured over the course of the experiment (dots), with segmented linear regressions associated (regression lines were extended until intersection).

et al., 2018a). However, at genetic level, de Verdal et al. (2018b) did not find a significant correlation between FCR and BWG (0.07 ± 0.24) with fish around 20–30 g. The negative phenotypic correlation observed here should thus be interpreted with care in a selective breeding context as this is a phenotypic but not a genetic correlation: selection for BWG may improve FCR, or not, depending on the (unknown) value of the genetic correlation.

4.3. Implications for genetic improvement programs

The genetic gain estimated for FCRg when ranking fish based on a two-week FCR provided a substantial proportion (around 50%) of that estimated using FCRg itself to rank the fish. The estimated genetic gain in FCRg, when selecting fish with two-week FCR values, ranged between 1.0% and 1.2% per generation with a selection intensity of 50%. Since FCR is tedious and expensive to estimate, applying such a low selection intensity would allow a sufficient number of breeders for the next generation to be obtained with the evaluation of a relatively small number of fish, reducing the number of fish to phenotype.

As first FCR measurements (before 250 dph) were the most correlated with FCRg, early measurements between 36 and 70 g (between 152 and 194 dph) would be appropriate. This would save 24–28 weeks of fish maintenance compared to the measurement of FCRg. The benefit of saving in time and money would need to be balanced against the reduced selection gain in comparison with using FCRg directly to rank

the fish.

Large phenotypic variability contributes to genetic gain in a breeding program. In the present study, the average CV of FCR (23.7%) is in line with literature estimates for GIFT tilapia, ranging from 22.1%–23.4% (de Verdal et al., 2017, 2018b), and for other species like European sea bass *Dicentrarchus labrax* (21.9%, Besson et al., 2019). The CV of FCR was above average (between 27.5% and 36.2%) during the three periods between 152 and 194 dph, suggesting a potentially higher genetic gain if selection was done at that stage, provided a constant level of heritability.

The present results suggest it could be relevant to record FCR before 250 dph, as it is more variable and better correlated to FCRg than in later periods, thus increasing the likely response to selection. This will need to be confirmed in additional experiments. Further work is also needed to increase the accuracy of the approach, especially regarding heritability estimates at the different periods, which were considered constant and equal to the one estimated on a one-week period by de Verdal et al. (2018b).

The need to obtain individual information to enable selection for FCR led us to use individual rearing in the present experiment. This method has the major advantage to allow recording individual FI every consecutive day for several months. However, in aquaculture, fish are always held in social groups. Studies on several species has suggested group rearing affects FI and FCR, e.g. bluegill sunfish (*Lepomis macrochirus*, McComish, 1971); Atlantic salmon (*Salmo salar*, Nicieza and

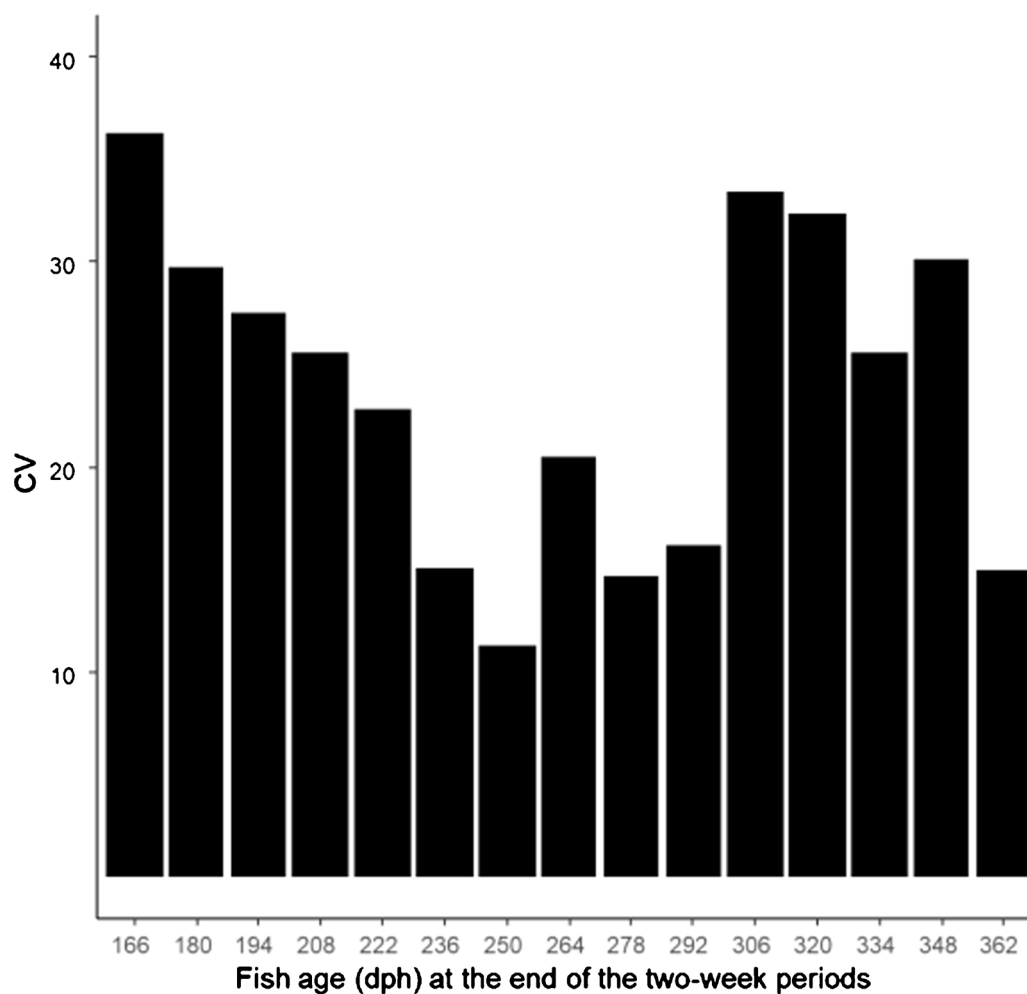


Fig. 3. The coefficients of variation (CV) of individual feed conversion ratio over time.

Metcalfe, 1999) and Nile tilapia (Schreiber et al., 1998). In the case of Nile tilapia, Schreiber et al. (1998) suggested that individual rearing led to better access to feed and to better growth performance. Still, using the same GIFT strain as the present experiment, de Verdal et al. (2019) found that agonistic behaviors were not phenotypically correlated with growth or FCR. Even if group rearing can create competition for feed among fish, individual rearing may induce stress, and thus be even more detrimental to fish performance. Here, fish could not come in contact with fellows and were disturbed every day when the uneaten pellets were removed, and every week to be weighed. Nevertheless, other evidence may suggest little difference between group and individual rearing. In group rearing, phenotypic correlations between BWG and FCR or between BWG and FI were rather similar to the ones observed in the present study (Kolstad et al., 2004; Doupé and Lymbery, 2004; de Verdal et al., 2017).

The impact of individual rearing on fish performance remains debatable and probably dependent on the species, the rearing conditions and the measurement methodology used. To our knowledge, no experiment has compared individual FCRs of the same fish successively reared as a group (but assessed individually) and isolated. Such an experiment would be very relevant for the evaluation of the reliability of assessing individual FCR with an individual rearing design. Some clues were provided by Besson et al. (2019) who have shown that the average individual FCR of European sea bass was partly reflected in subsequent group FCR differences. Beyond biological aspects, Besson et al. (2019) also demonstrated that individual rearing is a method that permits phenotyping several hundreds of juvenile fish in very short periods (two weeks) with a favorable cost-benefit ratio, and is therefore

potentially promising for large-scale commercial practice.

5. Conclusion

Our results suggest that the use of FCR estimates of juveniles over short time periods should be adequate to perform selection for FCR until commercial size in male tilapia. Despite fluctuations of FI, BWG and FCR over time, most of the FCR values obtained over two-week periods were positively correlated with FCR_g calculated over the whole rearing period. This was especially true for measurements performed at juvenile stage (around 152–194 dph, 36–70 g). Under the hypotheses made, potential genetic improvement of FCR of approximately 1% per generation, with 50% selection intensity, could be within reach.

Author statement

CR, BC, MV, JAHB and HV conceptualized the experiment, CR and TQT conducted the experiment; CR, MV and HV analyzed the data and CR wrote the manuscript and all the co-authors worked on the writing and editing process; JAHB, BC and HV acquired the financial support for the experiment and the publication.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

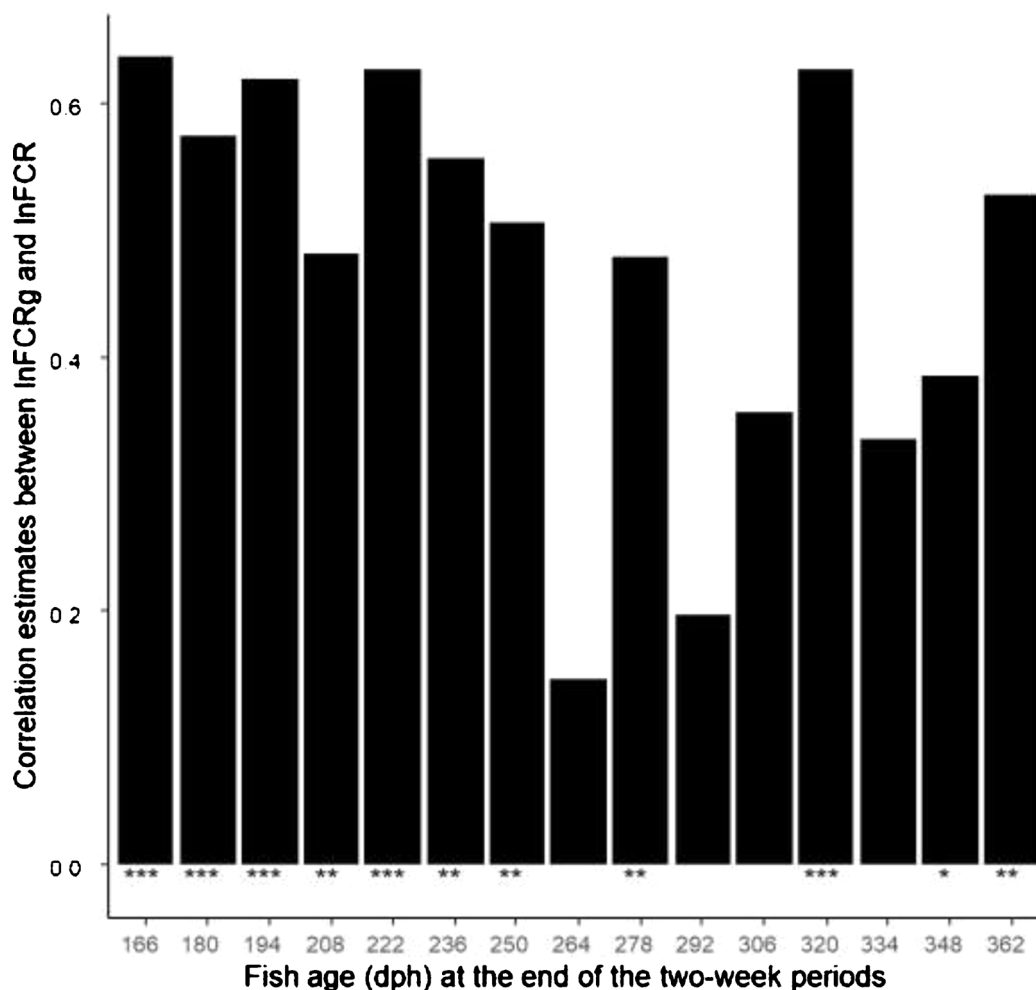


Fig. 4. Correlation estimates between log-transformed feed conversion ratio for the whole rearing period (lnFCRg) and log-transformed feed conversion ratio for each two-week period (lnFCR) with significant correlations indicated as follows (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.aqrep.2020.100349>.

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CHAPTER II

The effects of feed restriction and isolated or group rearing on the measurement of individual feed intake and estimation of feed conversion ratio in juvenile Nile tilapia (*Oreochromis niloticus*) for selective breeding purposes

The effects of feed restriction and isolated or group rearing on the measurement of individual feed intake and estimation of feed conversion ratio in juvenile Nile tilapia (*Oreochromis niloticus*) for selective breeding purposes

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Abstract

Measuring accurately the phenotype at the individual level is critical to the success of selective breeding programs. Feed efficiency, a key sustainability trait, is approached through feed conversion ratio (FCR = feed intake / body weight gain), which requires measurements of feed intake, a technical challenge in fish. We assessed two principal methods to measure feed intake over several consecutive days and estimating FCR in fish: a) individual rearing of fish fed with a restricted feed ration and b) group rearing (10 fish per group) and video-records of meals given to the fish. Juvenile Nile tilapia (*Oreochromis niloticus*) from the Genetically Improved Farmed Tilapia (GIFT) strain were reared over three time periods of seven days each: i) in groups and fed an optimal (g100) or ii) a 50% restricted (g50) feed ration with video-records of

all the meals and iii) in isolation and fed with a 50% restricted ration. A second strain, the Cirad strain, was tested in conditions i and iii. Fish were on average 10.3 g (GIFT) and 11.2g (Cirad) at the beginning of the experiment and were fed twice a day during all the experiment with ration calculated according to their body weight. In total, 237 fish were assessed in this study (128 and 109 fish from the GIFT and Cirad strain, respectively). Correlations were positive and significant between BWG.g100 and BWG.g50 (0.49), FI.g100 and FI.g50 (0.63) and between FI.g50 and FI.it (0.50) but not between BWG.g50 and BWG.it (0.29, NS). The phenotypic correlation estimated for FCR between group periods (i and ii) with fish fed an optimal or a 50% restricted ration was low and not significant (0.22). Feed Conversion Ratio for GIFT fish reared in groups or in isolation and fed with a restricted ration (ii and iii) were not correlated either (correlations ranged from -0.13 to -0.08).

Social interactions between fish, potentially impacting their efficiency, may explain the results. Therefore, selective breeding programs seeking to improve feed efficiency will need to carefully plan the feeding rate and the rearing system used to estimate FCR in order to optimise selection for the targeted production system.

1. Introduction

In aquaculture, feed represents 30 to 70% of farm costs and is the primary expenditure of intensive fish farming (Rana et al., 2009). Several ways have been investigated to improve the use of feed by fish, including nutrition (Reigh and Ellis, 1992; Gaylord and Gatlin III, 2001; Yao et al., 2014), husbandry (Alanärä, 1996; Imsland et al., 2005; Yilmaz and Arabaci, 2010) and genetics (Kause et al., 2006b; de Verdal et al., 2018b; Knap and Kause, 2018; Besson et al., 2020). While nutrition and husbandry have been widely studied and applied in production, genetic approaches need more investigation to enable practical implementation. A selective breeding program to improve feed efficiency typically involves recording of feed conversion ratio (FCR), which has to be accurately measured at the individual level. Feed conversion ratio is the ratio between the feed consumed by a fish and its growth during the same period of time ($FCR = \text{feed intake} / \text{body weight gain}$). Measurement of individual FCR requires accurate measurement of individual feed intake (FI). This is particularly complex for fish as they are reared in water and generally in large groups. The most commonly used method of the few developed to date has been the X-ray method. This uses radio-opaque glass beads included in the feed pellets allowing an assessment of how much feed the fish have ingested (Talbot and Higgins, 1983; McCarthy et al., 1993; Jobling et al., 2001; Silverstein et al., 2001; Kause et al., 2006a; Grima et al., 2008). However, while this method is accurate to monitor feed intake in a one specific meal, the repeatability of FI measurement is relatively low (Kause et al., 2006a; Grima et al., 2008) and it is not possible to measure FI of several consecutive meals due to the recovery time needed between two measurements. In genetic studies, even with repeated measurements (five measurements at two weeks intervals), heritability of FCR remains low (<0.07) in whitefish, suggesting the existence of significant residual environmental variance (Quinton et al., 2007).

As the FI of an individual fish in consecutive days is highly variable (Jobling and Koskela, 1996; de Verdal et al., 2017), the ideal method to measure individual feed efficiency should allow the measurement of FI for each meal over several consecutive days, so that amount of feed eaten by a fish over a period where it achieves significant growth is known with a high precision.

Two methods have been recently upscaled to meet these specific constraints in settings where several hundreds of fish were measured, to be able to study the genetic variation of feed efficiency. The first one is individual rearing of fish in aquaria fed a known restricted feed ration, combined with precise daily counting of uneaten pellets (Besson et al., 2019). Using this

method, fish can be reared for a few weeks or months, and FI can be measured accurately over a long period of time. An important aspect of this method is that fish are fed under a restricted ration, leading to a strong correlation of FCR with growth as individuals cannot express their own variability for satiety level (Henryon et al., 2002). This can be an advantage, as simple selection for growth under restricted feeding can lead to improvement in feed efficiency, which are suggested in fish (Besson et al., 2019) and well proven in rabbits and pigs (Nguyen et al., 2005; Drouilhet et al., 2016). Another advantage of restricted feeding is that the amount of uneaten pellets to be removed and counted every day is reduced compared to what would happen under satiation feeding, making the workload more compatible with the evaluation of hundreds of fish (Besson et al., 2019). However, restricted feeding may be problematic because the FCR expressed in this condition may differ from that under satiation feeding. Also, as fish are reared in isolation, they lose all the social interactions between each other, and this can have a high impact on performance.

The second method, developed some time ago (see review by Jobling et al., 2001) and adapted to genetic studies by de Verdal et al. (2017) consists of rearing small groups of fish in aquaria (ten to 15 fish together) and to video-record all the meals, pellets being provided one by one in several different places in the aquarium to reduce competition between fish. Using this methodology and having a visible identification of all the fish in the aquarium, it is possible to count the number of pellets eaten by each individual fish, and consequently, to estimate their feed intake. Measurement of FI using this method is accurate, the feed ration can be optimal (no need for any restriction), it permits social interactions between the fish, but it is time-consuming, as it is necessary to analyse all the videos of all the meals.

When used with family designs in fish, both methods produced comparable heritability estimates: 0.47 for FCR in European sea bass (*Dicentrarchus labrax*) with the isolation method using restricted ration and genomic information (Besson et al., 2019), and 0.32 for FCR in Nile tilapia (*Oreochromis niloticus*) with the video method using pedigree information de Verdal et al., 2018b). However, these two methods (isolation with a restricted feed ration vs. in groups with an optimal feed ration) have very different approaches. There is presently no evidence of correlations between feed efficiency traits measured on the same fish with these two methods, which although tedious, have the potential to be used for selective breeding of more efficient fish. As an example, using another feed efficiency trait, the residual feed intake (RFI), Silverstein (2006) found a significant correlation at family level between RFI of rainbow trout (*Oncorhynchus mykiss*) reared individually and RFI of fish reared in groups. He also detected differences among families for FI, growth and RFI when fish were fed *ad-libitum* but not when

fed a restricted ration. Besson et al. (2019) found a moderate but non-significant correlation between individual growth of European sea bass under restricted ration measured in isolated fish with growth of the same fish reared in groups under satiation. However, they found a relationship between individual FCR in fish reared in isolation with a restricted ration and subsequent *ad libitum* FCR in groups formed of the same fish. Given these variable results, it is important to determine whether both methods lead to similar FCR estimations or not, in order to help choose the most relevant methodology to set up selective breeding programs to improve feed efficiency.

The aim of the present study was to perform a comparison of data for traits relating to feed efficiency collected from Nile tilapia fed under different regimes, and to assess whether or not correlations were significant using different approaches. Growth, FI and FCR of individual Nile tilapia were compared when the same fish were held in small groups and fed either an optimal or restricted ration (half of the optimal ration), with FI being monitored using video-recording. Data collected from group-reared fish were also compared with those from the same fish reared in isolation on the same restricted ration, thereby testing the effects of group- and individual-rearing. These comparisons were carried out on the Genetically Improved Farmed Tilapia (GIFT) strain, selected for 18 generations on growth by WorldFish (Ponzoni et al., 2011). The data from groups fed an optimal ration and from fish reared in isolation on a restricted ration were compared also in a second tilapia strain named “Cirad strain”. This additional test of the Cirad strain, which to our knowledge has not been selected for growth, provided a replication study to better assess the generality of the observations with the combination of a different strain (GIFT vs. Cirad), a different feed (Cargill vs. le Gouessant) and a different experimental site (Malaysia vs. France).

2. Materials and Methods

2.1 Ethics statement

This study utilised phenotypic data collected as part of the GIFT selective breeding program managed by WorldFish at Jitra, Kedah State, Malaysia (6°15'32"N; 100°25'47"E). All fish in the GIFT breeding population are managed in accordance with the Guiding Principles of the Animal Care, Welfare and Ethics Policy of the WorldFish including the “3-Rs” rule. Regarding the Cirad strain, this part of the study was carried out in accordance with the recommendations of Directive 2010-63-EU on the protection of animals used for scientific purposes. The

protocols were approved by C2EA-36 (“Comité d'éthique en expérimentation animale Languedoc-Roussillon”) under authorization APAFiS n° 2017112215278675 #12552 v4.

2.2 Origin and rearing of the fish

The study was carried out on two distinct populations (GIFT and Cirad) in two different areas (Malaysia and France). The GIFT strain of Nile tilapia was selected for growth using a fully pedigreed design for 18 generations (Ponzoni et al., 2011). The families were produced by natural spawning from the 4th March to the 4th of April 2019 at the WorldFish Research station in Jitra, Kedah State, Malaysia (6°15'32°N; 100°25'47°E). The experiment was performed on 200 individuals from five families (40 fish per family) from the 10th of June to the 22nd of July 2019. After hatching, each family was reared in different hapas in the same pond and transferred to 1500 L holding tanks (3 × 1 × 0.5 m) at 110 days post hatching (dph). All the fish were injected with a Passive Integrated Transponder tag (PIT-tag, Trovan®) between 53 and 84 dph (around 10g of BW). Fish from each family were sorted according to their body weight to make four homogeneous groups of ten fish which were randomly put into four plastic aquaria of 60 L (61x30x33 cm). In total, 20 aquariums with ten fish in each were used. After anaesthesia with clove oil (0.5 mL per litre of water), each fish was tagged in the dorsal muscle with two coloured T-bar tags (Avery Dennison tags, 25 mm), one tag on each side of the body, using an Avery Dennison Mark III pistol Grip tool. This allowed each fish to be uniquely and individually identified by one colour of tag within an aquarium regardless of which side of the body was shown and video recorded. Commercial pelleted feed (Cargill®, “Starter tilapia 6113”) with 34% of crude proteins, 5% of crude fat, 5% of crude fiber and 12% of moisture was used to feed the fish during the whole experiment. Daily water temperature ranged from 28 to 30°C depending on the hour of measurement.

The Cirad strain of Nile tilapia was derived from a cross between Cirad-IRD females, originally from Egypt, kept in Cirad-IRD facility (Montpellier, France) for several generations and from males sold by FishGen (UK) in 2018 and kept in Cirad facilities in Palavas-les-Flots (France). This new cross was called “Cirad strain” to simplify the nomenclature for the present study. For this experiment, 320 fish from 16 families (20 fish per family) hatched from the 5th to the 26th of July 2019 were used. After hatching, each family was kept isolated until the end of the experiment. When fish reached on average 10g of BW, fish from each family were spread into two 38 L aquaria (10 fish per aquarium). After anaesthesia with clove oil, each fish was tagged into the dorsal muscle with two coloured T-bar tags (Avery Dennison tags, 25 mm), one tag on

each side of the body, using an Avery Dennison Mark III pistol Grip tool. Each fish within an aquarium was tagged with an exclusive colour to identify each fish individually regardless of which side of the body was shown and video recorded. Fish were fed a commercial pelleted feed (Le Gouessant, “Tilapia Starter Flot 1” and “Tilapia Starter Flot 2”) with 38% of crude proteins, 8% of crude fat, 3.9% of crude fiber and 7% of moisture during the whole experiment. Water temperature was maintained at 28°C during the whole experiment.

2.3 Experimental design and trait measurements

The experimental design is summarized in Figure 1. The experiment consisted of three periods of FI measurement, and consequently, three FCR measurement periods: i) individual FI measured in groups (ten fish per group) with an optimal feed ration (coded g100), ii) individual FI measured in groups (ten fish per group) on the same fish as i) with half of the optimal feed ration (coded g50), iii) individual FI measured in isolation on the same fish as i) and ii) with half of the optimal feed ration (coded i1, i2 and it for the first week of this period, the second week of this period and both weeks of this period together, respectively). Fish were not measured in isolation with the optimal ration as they may waste too many pellets to allow precise counting, and the accuracy of the exact FI would thus be questionable. All fish were anaesthetized with clove oil (0.5 mL per litre of water) when weighed during the course of the experiment. No sign of stress or abnormal behaviour was seen during the experiment except the stress due to the normal fish interactions.

After seven days of adaptation to group aquaria, all the individual fish were anaesthetized and weighed (BW_{i.g100}). In the first period of FI measurement fish were fed twice a day with a 100% daily feed ration (DFR, in percentage of body weight) except the weighing day when they were not fed. The DFR was calculated based on the formula published by Mélard et al. (1997): $DFR = 14.23 \times BW^{-0.322}$ with BW the body weight of each fish (in g) at the beginning of each period (BW_{i.g100}, BW_{i.g50}, BW_{i.i1} and BW_{i.i2} were used to calculate the DFR used during the g100, g50, i1 and i2 periods, respectively, Figure 1). As different experimenters were involved in the feeding process, a calculated ration was preferred to an “*ad-libitum*” ration, which is less repeatable from one experimenter to another. This calculated ration was also useful to ensure that the same maximal feed ration was given at every meal. The DFR was equally shared for each of the two daily meals. Feed was given using two pipes going to the aquarium, allowing a reduction of stress since the fish did not see the experimenter when given the feed. Frequently, fish did not eat the entire DFR and the choice was made to stop the meal

when a few pellets remained uneaten after approximately one minute (corresponding actually to an *ad-libitum* ration). Uneaten pellets were removed from the aquarium using a small net.

All the meals were recorded by video for FI.g100 and FCR.g100 estimations. At the end of this first period of seven days (12 meals), fish were anaesthetised and weighed (BWi.g50) and the individual growth during that period ($BWG.g100 = BWi.g50 - BWi.g100$) was calculated.

In the second period of FI measurement of seven days (12 meals) in groups, fish were fed a restricted ration (calculated as 50% of the DFR using the previously mentioned equation) to estimate the impact of a restricted ration compared to an optimal ration on FCR. As during the 100% DFR period, all the meals were video-recorded to count the number of pellets eaten by each individual fish and estimate FI.g50. At the end of this second period of seven days (12 meals), fish were anaesthetized and weighed, allowing calculation of BWG.g50 and estimation of FCR.g50.

Before the beginning of the third period, the 200 fish were randomly distributed into two hundred 10L isolated aquariums and adapted for seven days to this new individual rearing system. Each fish was able to see the fish in neighbouring tanks. The third period consisted of two consecutive weeks with the same experimental protocol. All the fish were anaesthetized and weighed at the beginning and the end of each week (BWi.i1, BWi.i2 and BWend), allowing calculation of BWG.i1 and BWG.i2. Fish were fed twice a day (except on the day of weighing) with 50% DFR, as in the second period. The DFR was updated every week for each fish. Feed for each individual fish was weighed accurately every day and the uneaten pellets were counted and removed from the aquaria at least two hours after the last meal of the day. The uneaten feed weight was estimated assuming that all pellets had the same weight (16.2 ± 1.8 mg), and FI.i1 and FI.i2 were calculated for each week. Knowing the BWG and FI for both periods, it was possible to estimate FCR.i1 and FCR.i2 for the first and the second weeks of this third period of the experiment. To reduce the effects of FI fluctuations from one week to another, both weeks were combined and global estimations were done for BWG.it, FI.it and FCR.it.

The same measurements were performed on the Cirad strain, except the measurement of FI in groups with restricted ration which was not performed due to logistical reasons (i.e. limited infrastructure availability), with the experiment undertaken from the 8th of October 2019 to the 16th of December 2019. The experiment was performed as described for the GIFT strain except that fish were fed 13 meals during the group period (an extra-meal was given the afternoon after weighing the fish). From the 320 fish measured in groups, a total of 133 randomly drawn fish were kept and measured for FI in isolation and were included in the analyses. Due to the limited number of aquariums available, fish were measured in three distinct batches (around 50 fish per

batch). The experimental protocol for each batch was similar and the batch effect was not significant and consequently, was not included in the present analyses. In the meantime, fish were identified with a passive integrated transponder tag (PIT-tag, Biolog-id[®]) and reared in a common garden environment in four 300 L tanks for five to six weeks.

In both experiments, the weekly FI was the sum of all the daily FI of the week. Mortality was recorded daily and the feed ration changed accordingly during the group rearing periods. Body weight gain (BWG) was calculated as the difference between the body weight of each fish at the end and at the beginning of the week. The feed conversion ratio (FCR) was calculated as the ratio between FI and BWG ($FCR = FI / BWG$), the most efficient fish being the fish showing the smallest FCR values.

The Kinovea 0.8.15 software (Copyright © 2006–2011– Joan Charmant & Contrib.) was used to analyse the videos of the meals and to count for the number of pellets eaten by each fish when reared in groups.

2.4 Statistical analyses

All statistical analyses were performed using the R software (R Development Core Team, 2018). Negative FCR (35 out of the 1187 FCR measurements in total) values were not included in the statistical analysis. Outliers were highlighted using the `boxplot.stats` function of the R package “stats” (R Development Core Team, 2018) and were not included in the analyses. After checking with the Shapiro-Wilk test, data for several traits (mainly FCR) were not normally distributed and consequently, non-parametric tests were preferred for the data analyses. Wilcoxon tests were used to analyse the block effects (including the strain, experimental protocol and feed used) when the same traits were measured in both conditions, to assess the consistency of the results. Spearman correlations between traits were estimated using the R package “psych” (Revelle, 2015).

3. Results

3.1. Basic statistics

The Nile tilapia used in this study were at the juvenile stage (Table 1), with initial BW (BW_i.g100) on average of 10.3 ± 2.6 g and 11.2 ± 3.3 g for the GIFT and Cirad strain, respectively. The Cirad fish at the beginning of the isolation period were heavier than the GIFT fish (on average a difference of 4.2 g between both strains). The coefficient of variation of body weight was slightly higher for the Cirad strain (ranged from 27.5 to 41.0 %) than for the GIFT

strain (ranged from 23.0 to 26.9 %, Table 1). The number of individuals in each family (from one to 28) and the number of families (five and 16 for the GIFT and the Cirad strain, respectively) were too small to consider this family level as relevant for the present analyses.

3.1.1. The GIFT strain

During the restricted feeding period in groups, the BWG of GIFT fish was reduced and was to 37.3 % of that of the same fish fed an optimal ration (Table 1). Feed intake during this restricted period was only reduced to 58.6% of the value observed with 100%DFR (from 3.48 to 2.04 g). Thus, FCR was lower in fish fed 100% DFR than in fish fed with 50% DFR. Interestingly, the coefficient of variation of BWG and FCR was higher when fish were fed under restriction than with an optimal ration (Table 1). Isolated GIFT fish showed similar growth, BWG, FI and FCR during the first and the second week of measurement (Table 1). The coefficient of variation of BWG, FI and FCR was lower when fish were reared in isolation (ranged from 15.9 to 22.0 %) than when they were reared in groups (from 26.5 to 52.7 %).

3.1.2. The Cirad strain

Because of the limited time infrastructure was available with the Cirad strain, it was only possible to compare FCR measured in groups with 100% DFR and in isolation. Therefore we could not assess the specific effects of social interactions and feed ration on FCR but the comparison of the main results can be used to assess the replicability of some results with another strain and a different rearing protocol. Cirad strain fish reared in groups (on the optimal ration) had a lower FCR than in isolation (Table 1). It is interesting to note that whatever the trait, similar to the GIFT fish, coefficients of variation were higher when fish were reared in groups than they were in isolation.

3.1.3. Block effect

The block effect (including the strain, site, experimental protocol and feed used) was always significant. Fish from Cirad strain were 8.7 % bigger at the beginning of the group rearing period, and 19.9 % heavier at the beginning of the isolated period than those of the GIFT strain (Table 1). The coefficients of variation of BWG and FI were higher for the Cirad strain than for the GIFT strain (Table 1).

3.2. Phenotypic correlations

The details of the phenotypic correlations between the traits measured at all periods are presented in Table 2. The first question raised in this study was the impact of feed restriction on FCR in groups, which could only be estimated on the GIFT strain, as only those fish were subjected to a restricted feeding period in group rearing (Figure 2). The correlation between FCR.g100 and FCR.g50 was low and not significant (0.22) as illustrated in Figure 2. However, correlations were positive and significant, although not very high, between BWG.g100 and BWG.g50 (0.49) and between FI.g100 and FI.g50 (0.63).

The second question raised was whether FCR measured in groups was correlated with FCR measured on isolated fish. This was done on the GIFT strain only with restricted ration (Table 2 and Figure 3). The correlations between FCRs measured in groups with restricted feeding (FCR.g50) and FCRs measured in isolation (FCR.i1, FCR.i2 and FCR.it) were low, negative (from -0.13 to -0.08) and not significant (Table 2). Here again, positive and significant correlations were seen between FI.g50 and FI.it (0.50) but this time not between BWG.g50 and BWG.it (0.29, NS). Comparison between fish reared in groups fed with an optimal ration (video method) and fish reared in isolation and fed with a 50% restricted ration (isolation method) was possible both for the Cirad and for the GIFT strain (Figure 4). In both strains, BWG.g100 and BWG.it were significantly correlated (0.54 in the GIFT strain, 0.36 in the Cirad strain), as well as FI.g100 and FI.it (0.71 in the GIFT strain, 0.50 in the Cirad strain). Additionally, BWG was significantly correlated to FI in both periods, with higher correlations for the GIFT strain (0.85) than for the Cirad strain (0.52, Table 2). Here again, FCRs measured in groups with optimal ration and in isolation with restricted ration were not significantly correlated (correlations of -0.17 and -0.18 for GIFT and Cirad strain, respectively).

4. Discussion

In selective breeding programs, it is essential to measure accurately the trait under selection. Furthermore, due to potential genotype by environment interactions, the environment in which the selective breeding program is performed needs to be as close as possible to the production rearing environment. No method is available to accurately measure the individual FI of fish reared in large groups (in tanks or ponds) during several consecutive days. Individual FI can be measured precisely in a single meal with the X-ray methodology, but although repeated measurements at several days intervals are possible, the day to day variability in individual FI cannot be fully taken into account (Kause et al., 2006a; Grima et al., 2008). The only two methods employed to date for genetic studies to precisely measure the individual FI of many fish during several consecutive days are group rearing with video-recording of all the meals and

a posteriori analysis of all the videos (de Verdal et al., 2017) or individual rearing with a restricted ration (Besson et al., 2019). In this last method, the variability of FI cannot be fully expressed due to restricted feeding, and as a consequence, FCR and BWG are strongly correlated (Henryon et al., 2002). The objectives of the present study were to assess the impact of ration level (100% DFR or 50% DFR) and of the rearing system (group rearing or isolation) on FCR, trait commonly used to assess feed efficiency.

4.1. Impact of feed restriction

Growth and FI were significantly and positively correlated when measured in groups with an optimal or a restricted ration. However, FCRs measured in both conditions were poorly to moderately correlated (0.22), and the correlation was not significantly different from zero. Consequently, in groups, the most efficient fish fed with an optimal ration were not always the most efficient fish when fed with a restricted ration. Using group measurements in rainbow trout, Azevedo et al. (1998) and Rasmussen and Ostefeld (2000) found a restricted feed ration had a significant effect on growth (fish under the restricted ration growing less than under high ration) but did not impact feed efficiency. In the present experiment, BWG and FCR in group reared fish were more variable when fish were fed with 50% DFR (CV = 52.3 and 52.7 %, respectively) than when fish were fed with 100% DFR (CV = 37.0 and 28.7 %, respectively) but the CV of FI did not change between these two periods. Using X-Ray methodology, Jobling and Koskela (1996) showed a similar increase in the CV of BWG under restricted feeding in rainbow trout, which in their case was also accompanied by an increase in the CV of FI. They attributed this to an increase of the social interaction when feed is restricted, which could also partly be the case here, although no increased variability in FI was seen. With fish fed an optimal ration, de Verdal et al. (2019) did not find any correlations between agonistic behaviour and growth and feed efficiency. However, it can be hypothesized that agonistic behaviours were exacerbated under restricted diets, and consequently some fish will lose more energy to deal with these social interactions than others.

In rabbits and pigs selection for growth using a restricted feed ration was shown to improve feed efficiency of their progenies even when they were held in conditions where they were fed to satiety (Nguyen et al., 2005; Drouilhet et al., 2016). The proposed explanation of these results is that higher growth under a restricted diet is due to lower maintenance requirements, which is also beneficial for animals fed to satiety. The maintenance requirements of fish, as poikilotherms, cannot be easily compared to those of warm blooded livestock species, which may explain some differences observed between fish and livestock. In the present study, the

phenotypic correlations were high (as in livestock species) between BWG.g50 and FCR.g50, but not between BWG.g50 and FCR.g100, indicating that selection for growth under restricted feeding in groups was unlikely to improve feed efficiency in fish fed to satiation. In rainbow trout, it was shown that feeding a restricted ration created social hierarchies in the tanks, leading to some fish consistently eating a larger or smaller share of the ration given, which was less the case under satiation (McCarthy et al., 1992; Jobling and Koskela, 1996). Then, it seems reasonable not to select fish for growth under restricted feeding in groups to improve feed efficiency. Nevertheless, our results were based only on phenotypic correlations, which can influence conclusions considerably. Firm conclusions on this issue will require the estimation of genetic correlations.

4.2. Impact of the social interactions

Individual rearing systems remove all the direct social interactions between fish whereas clear social interactions were seen in videos of group reared fish, including an extensive repertoire of agonistic behaviours between fish (de Verdal et al., 2019). There were no significant correlations between FCR of GIFT strain reared under restricted ration when measured in groups (g50) or in isolation (i1, i2 or it). Using the video analyses method, de Verdal et al. (2019) noted that neither the amount of agonistic behaviours nor the hierarchy in Nile tilapia were significantly correlated with feed efficiency when fish were fed with 100% DFR. These results would suggest there should be limited or no effects of social interactions. However, the present experiment shows a clear effect of group rearing on the FCR estimations. The experiment of de Verdal et al. (2019) only measured agonistic behaviour but social interactions are broader and the present results suggest more complex interactions are involved. A number of studies have reported that fish reared in isolation were more efficient (Jackson et al., 2003; Silverstein, 2006) as a result of stress reduction. It is known that stress, by increasing the maintenance requirements, reduces the efficiency of the fish to convert feed (Martins et al., 2006, 2011). From the present data, GIFT fish reared under restricted feeding in groups (g50) showed a FCR 37.9% higher than when reared in isolation (it). It is important to note that under our feed ration conditions, the coefficient of variation of FCR of fish reared in groups was almost twice that of the same fish reared in isolation. Group rearing could induce stress at the individual level, with a probable high variation between dominant and subordinate fish (Martins et al., 2005, 2006). This social impact, leading to an increased energy expenditure, could explain the differences in CVs of FCR between fish reared in groups or in isolation and why the most efficient fish were not the same when the rearing conditions changed. An interesting aspect of

our study is also the fact that the correlation between FI in isolation under restricted feeding and FI in groups is higher when group results are obtained under satiation than when they are obtained under restricted feeding ($r=0.71$ vs 0.50). Similar observations are made with BWG ($r=0.54$ vs 0.29). This probably highlights, as discussed before, that social hierarchies are very high in groups under restricted feeding, and that social interactions are less intense both in individual rearing and in groups fed to satiation, in accordance with the results of de Verdal et al. (2019) estimating non-significant phenotypic correlations between FCR and agonistic behaviours in juvenile Nile tilapia reared in groups and fed to satiation. Still, although both BWG and FI are more correlated between group satiation and isolation than between groups under restricted feeding and isolation, this does not lead to significant correlations of FCR between both methods.

These results are probably dependent of the fish species under consideration. Nile tilapia is known to be a social species, with behavioural interaction between fish, which is not the case for all fish species. As a consequence, the difference of stress experienced by a Nile tilapia reared in groups or in isolation will not be comparable with other species, which may explain the different results found in the literature. Strand et al. (2007) indicated that juvenile perch (*Perca fluviatilis*) were much more efficient in large groups (FCR of around 1.1 when reared in groups of 12 fish) than in isolation (FCR of around 4.5) probably due to reduced stress when fish were reared in groups. Besson et al. (2019) also showed that FCR of European sea bass reared in individual aquaria was higher (1.38) than that of the same fish held in groups (approximately 1.23). Taken as a whole all these results tend to show that the individual efficiency of fish reared in groups or in isolation differs, depending probably on the differences in stress levels experienced by the fish according to the rearing conditions and species.

4.3. Choice of method for use in selective breeding programs

The final aim of the present work was to assess which methodology might be best in a selective breeding program targeting feed efficiency (through FCR) as one of the breeding objectives. To succeed in a selective breeding program, it is essential to have an accurate measure of the phenotype of interest, and the trait should also ideally be measured in conditions similar to commercial production to reduce the risk of genotype by environment interactions. Nile tilapia is produced in large groups in ponds/cages/tanks/raceways where social interactions occur. As the measure of FCR in groups and in isolation are not significantly correlated in the present study, selecting fish in groups seems more relevant in the case of the tilapia than measuring fish in isolation. As discussed in the preceding section this is likely not true for all fish species. As

an example, Besson et al. (2019) showed that the most efficient European sea bass measured in isolated aquaria tended to stay the most efficient later in life when reared in groups. One of the main advantages of the isolation method compared to the video method is the fact that the phenotypes are known immediately, whereas using the video method requires time-consuming video analysis in order to estimate the phenotypes. However, both methods involve a large amount of phenotyping work which may restrict the number of individuals and families that can realistically be evaluated.

The high CV of FCR when fish were fed under restriction could be seen as an interesting feature for a selective breeding program, as the level of phenotypic variance is one of the criteria to take into consideration when choosing the best trait for which to select, with higher variances being preferred (Falconer and MacKay, 1996). However, we discussed that selection under a restricted ration may increase agonistic behaviour between fish, which would not be favourable in production systems, and could increase mortality in the farms. Those effects could be enough to outweigh the benefit of selecting from a higher observed variance. Furthermore, it was previously shown that agonistic behaviours were negatively correlated with growth when fish were reared in an environment where the level of social interactions was high (Ruzzante and Doyle, 1991). Thus, selecting fish for feed efficiency in groups under restricted feeding is likely not a valuable option.

There is no perfect method to measure FI accurately over several days and to estimate FCR robustly. However, rearing different tilapia strains in different conditions (experimental protocols and feed) gave similar results, suggesting some level of generality of the observations done. The aim of the present study was to compare two methods used to estimate accurately individual feed efficiency in Nile tilapia during several consecutive days and to highlight the most relevant method to use in selective breeding programs. The most favourable outcome would have been to see good correlations between FCR measurements done with the group or with the isolation method, which would have given more opportunities for designing breeding programs for feed efficiency. This was not the case, and then there is no simple answer to guide the choice of the method. What is relatively clear is that the group method under restricted feeding is not adequate, as it exacerbates social hierarchies, and it is not representative either of the *ad libitum* group method or of the isolation restricted method. As the question is complex, selection experiments will be needed to ascertain which are more efficient and economically viable phenotyping methods for selective breeding for feed efficiency.

Conflict of interest

The authors declare that they have no conflict of interest.

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Author Contributions

CR and HdV designed the experiment; CR, TQT, VD, MC and HdV performed the experiment; CR, MV and HdV analysed the data; CR, MV, TQT, JAHB and HdV wrote the paper. All authors read and approved the final manuscript.

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Table Caption

Table 1. Basic statistics: mean \pm standard deviation, minimum, maximum and coefficient of variation (CV) of all the traits measured during the experiment for the GIFT and the Cirad strain, and the p-value of the block effect calculated using Wilcoxon tests.

Table 2. Phenotypic correlation between all the measured traits. GIFT strain correlations are above the diagonal, and Cirad strain correlations are below the diagonal. Bold values are significantly different from zero.

Figure Caption

Figure 1. Scheme of the different periods designed in the experimental protocol and corresponding traits measured in each period for the GIFT strain (on the top of the figure and in blue) and the Cirad strain (on the bottom of the figure and in green). BW_i: individual body weight; BW_{end}: individual body weight at the end of the experiment; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; .g100: fish reared in groups with 100% DFR ration; .g50: fish reared in groups with 50% DFR ration; .i1, .i2 and .it: fish reared in isolated aquaria during the first, the second and the total of the first and second weeks of isolation period. The main measured traits were highlighted and the background of the frame was coloured.

Figure 2. Relations between FCR when fish were reared in groups and fed with either an optimal feed ration (g100) or a 50% restricted ration (g50). Each point is representing data for one fish.

Figure 3. Relations between FCR when fish were fed a restricted feed and reared in groups (g50) or in isolation (it). Data for GIFT only. Each point is representing data for one fish.

Figure 4. Relations between fish reared in groups and fed with an optimal feed ration (g100) and fish reared in isolation with a restricted ration (it) for BWG (A), FI (B) and FCR (C). Black and grey circles corresponded to individuals of the GIFT and Cirad strains, respectively. The equation of the linear regression and the coefficient of determination R² are surrounded in black and grey for the GIFT and the Cirad strain, respectively.

Table 1

Basic statistics: mean \pm standard deviation, minimum, maximum and coefficient of variation (CV) of all the traits measured during the experiment for the GIFT and the Cirad strain, and the p-value of the block effect calculated using Wilcoxon tests.

	GIFT Strain				CIRAD Strain				Block effect
	Mean \pm SD	Min	Max	CV	Mean \pm SD	Min	Max	CV	
BWi.g100	10.3 \pm 2.60	5.20	16.5	24.7	11.2 \pm 3.31	6.04	18.70	27.5	0.040
BWi.g50	14.6 \pm 4.18	6.30	23.7	26.9
BWi.i1	21.1 \pm 5.84	10.7	33.3	24.6	25.3 \pm 10.6	9.98	54.9	4.00	0.007
BWi.i2	23.7 \pm 6.01	12.5	36.3	23.0	29.1 \pm 11.3	12.6	60.8	38.1	0.0004
BWG.g100	4.29 \pm 1.64	1.10	8.30	37.0	3.03 \pm 1.28	0.80	6.26	41.9	<0.0001
BWG.g50	1.60 \pm 0.87	0.30	4.00	52.3
BWG.i1	2.97 \pm 0.68	1.55	4.67	22.0	3.80 \pm 0.99	1.69	6.47	25.7	<0.0001
BWG.i2	2.58 \pm 0.54	1.30	3.95	19.6	4.22 \pm 1.25	1.72	7.50	29.5	<0.0001
BWG.it	5.55 \pm 1.07	2.85	7.91	18.4	8.02 \pm 1.98	3.84	12.5	24.6	<0.0001
FI.g100	3.48 \pm 0.97	1.50	5.87	26.5	2.27 \pm 0.76	1.04	3.90	32.6	<0.0001
FI.g50	2.04 \pm 0.61	0.91	3.64	28.2
FI.i1	3.02 \pm 0.62	1.80	4.16	18.0	3.75 \pm 1.07	2.03	6.45	28.0	<0.0001
FI.i2	3.35 \pm 0.62	2.12	4.60	16.8	4.13 \pm 1.10	2.37	6.92	25.9	<0.0001
FI.it	6.37 \pm 1.22	3.92	8.76	17.4	7.87 \pm 2.15	4.40	13.4	26.9	<0.0001
FCR.g100	0.87 \pm 0.26	0.39	1.88	28.7	0.81 \pm 0.22	0.40	1.44	27.5	0.041
FCR.g50	1.60 \pm 0.86	0.48	4.00	52.7
FCR.i1	1.04 \pm 0.20	0.68	1.59	18.9	1.00 \pm 0.23	0.61	1.65	22.1	0.050
FCR.i2	1.33 \pm 0.26	0.90	2.15	19.8	1.01 \pm 0.18	0.67	1.48	17.8	<0.0001
FCR.it	1.16 \pm 0.19	0.81	1.75	15.9	0.99 \pm 0.14	0.73	1.32	13.9	<0.0001

BWi: individual body weight; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; .g100: fish reared in groups with 100% DFR ration; .g50: fish reared in groups with 50% DFR ration; .i1, .i2 and .it: fish reared in isolated aquaria during the first, the second and the total of the first and second week of isolation period; ne: non estimable.

Table 2

Phenotypic correlation between all the measured traits. GIFT strain correlations are above the diagonal, and Cirad strain correlations are below the diagonal. Bold values are significantly different from zero.

		BWG					FI					FCR				
		g100	g50	i1	i2	it	g100	g50	i1	i2	it	g100	g50	i1	i2	it
BWG	g100		0.49	0.56	0.34	0.54	0.73	0.54	0.85	0.85	0.85	-0.65	-0.25	0.19	0.42	0.35
	g50	.		0.30	0.18	0.29	0.40	0.59	0.57	0.56	0.56	-0.26	-0.85	0.17	0.28	0.25
	i1	0.40	.		0.54	0.91	0.49	0.24	0.61	0.69	0.65	-0.29	-0.23	-0.55	0.06	-0.32
	i2	0.25	.	0.56		0.83	0.27	0.08	0.39	0.43	0.41	-0.25	-0.15	-0.24	-0.60	-0.48
	it	0.36	.	0.85	0.90		0.44	0.21	0.59	0.66	0.63	-0.33	-0.23	-0.47	-0.24	-0.44
FI	g100	0.79	.	0.42	0.25	0.35		0.63	0.70	0.71	0.71	0.00	-0.10	0.11	0.36	0.26
	g50		0.50	0.49	0.50	-0.11	-0.11	0.16	0.33	0.28
	i1	0.51	.	0.70	0.81	0.86	0.49	.		0.99	1.00	-0.46	-0.37	0.27	0.44	0.41
	i2	0.52	.	0.74	0.81	0.87	0.50	.	1.00		1.00	-0.46	-0.37	0.18	0.42	0.33
	it	0.52	.	0.72	0.81	0.86	0.50	.	1.00	1.00		-0.46	-0.37	0.22	0.43	0.37
FCR	g100	-0.66	.	-0.09	-0.08	-0.11	-0.10	.	-0.22	-0.22	-0.22		0.22	-0.17	-0.20	-0.22
	g50		-0.08	-0.13	-0.12
	i1	0.20	.	-0.22	0.39	0.14	0.16	.	0.50	0.45	0.47	-0.18	.		0.41	0.86
	i2	0.31	.	0.12	-0.49	-0.25	0.30	.	0.07	0.08	0.08	-0.15	.	0.00		0.80
	it	0.35	.	-0.11	-0.05	-0.08	0.29	.	0.41	0.37	0.39	-0.24	.	0.73	0.65	

BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; .g100: fish reared in groups with 100% DFR ration; .g50: fish reared in groups with 50% DFR ration; .i1, .i2 and .it: fish reared in isolated aquaria during the first, the second and the total of the first and second week of isolation period.

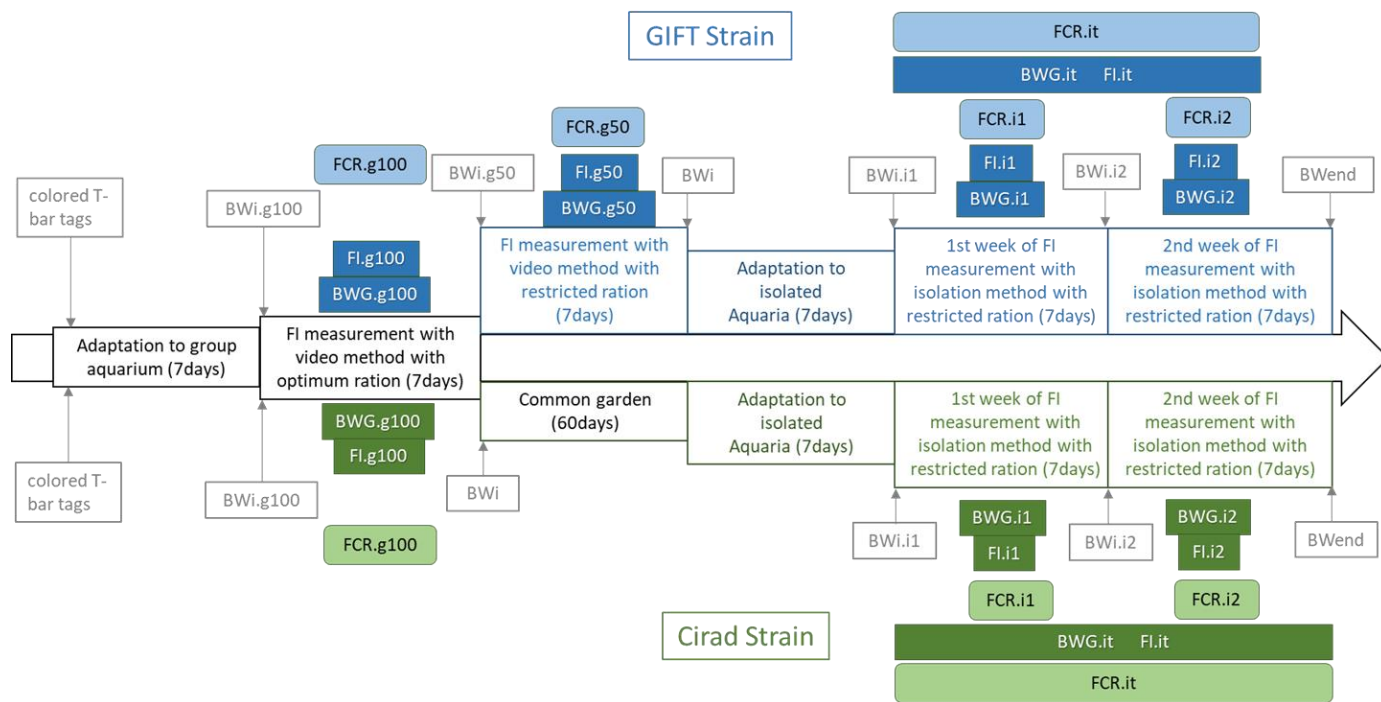


Figure 1. Scheme of the different periods designed in the experimental protocol and corresponding traits measured in each period for the GIFT strain (on the top of the figure and in blue) and the Cirad strain (on the bottom of the figure and in green). BWi: individual body weight; BWend: individual body weight at the end of the experiment; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; .g100: fish reared in groups with 100% DFR ration; .g50: fish reared in groups with 50% DFR ration; .i1, .i2 and .it: fish reared in isolated aquaria during the first, the second and the total of the first and second weeks of isolation period. The main measured traits were highlighted and the background of the frame was coloured.

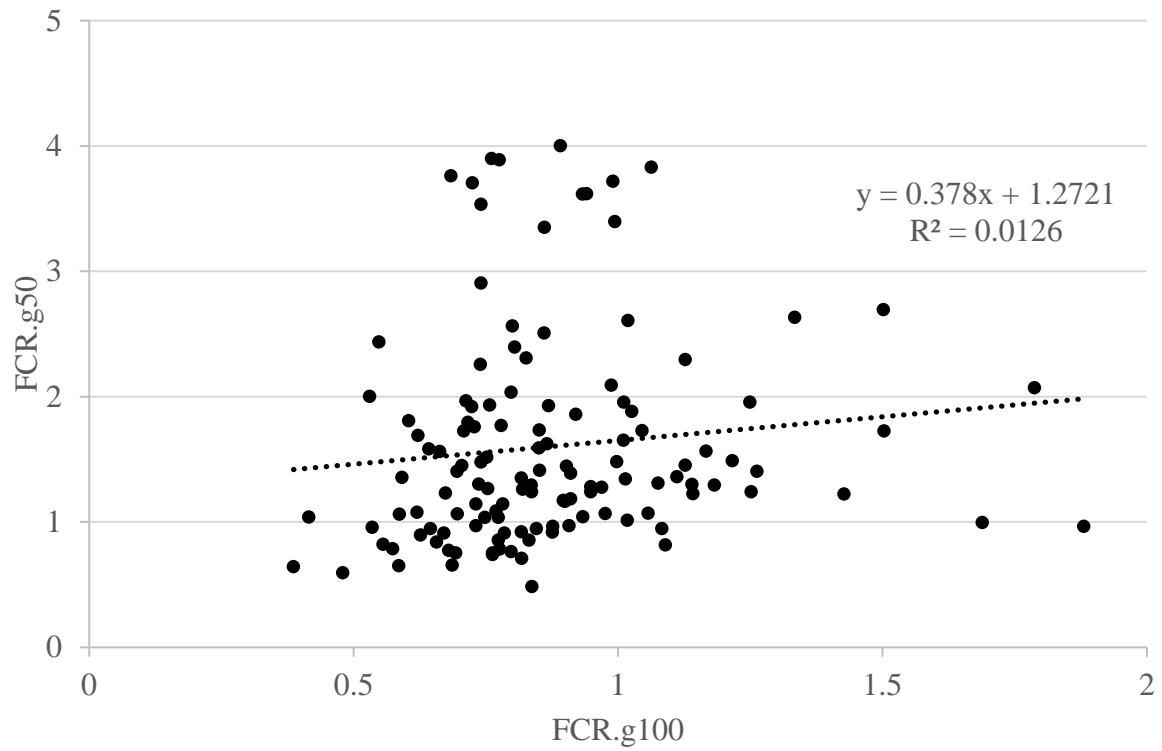


Figure 2. Relations between FCR when fish were reared in groups and fed with either an optimal feed ration (g100) or a 50% restricted ration (g50). Each point is representing data for one fish.

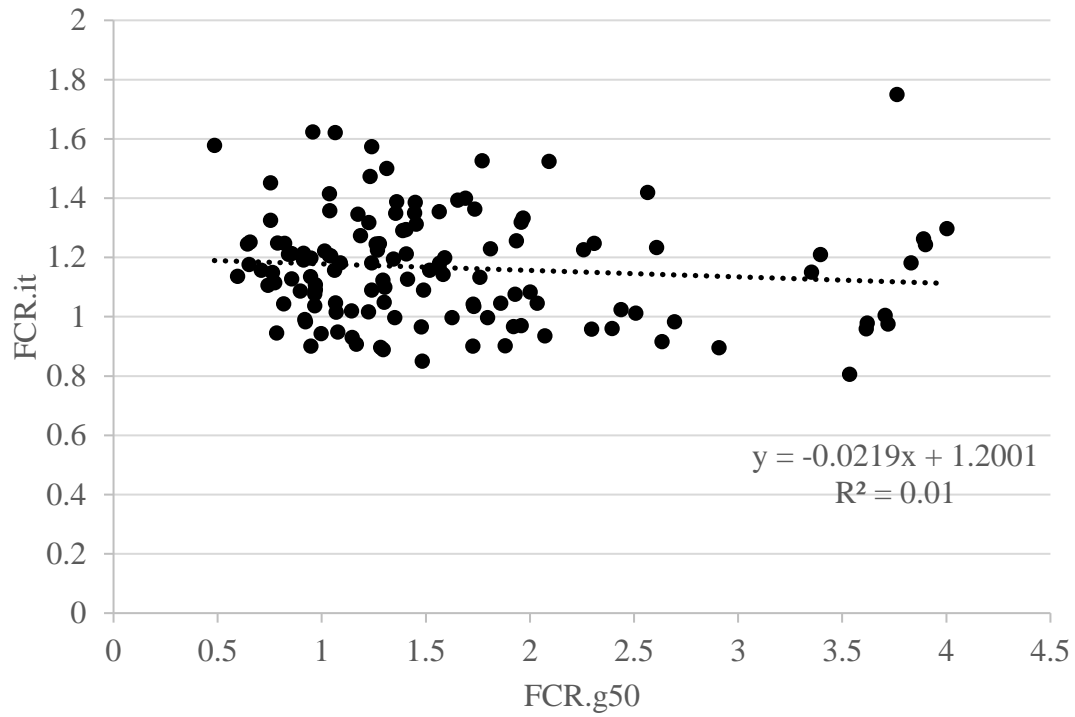


Figure 3. Relations between FCR when fish were fed a restricted feed and reared in groups (g50) or in isolation (it). Data for GIFT only. Each point is representing data for one fish.

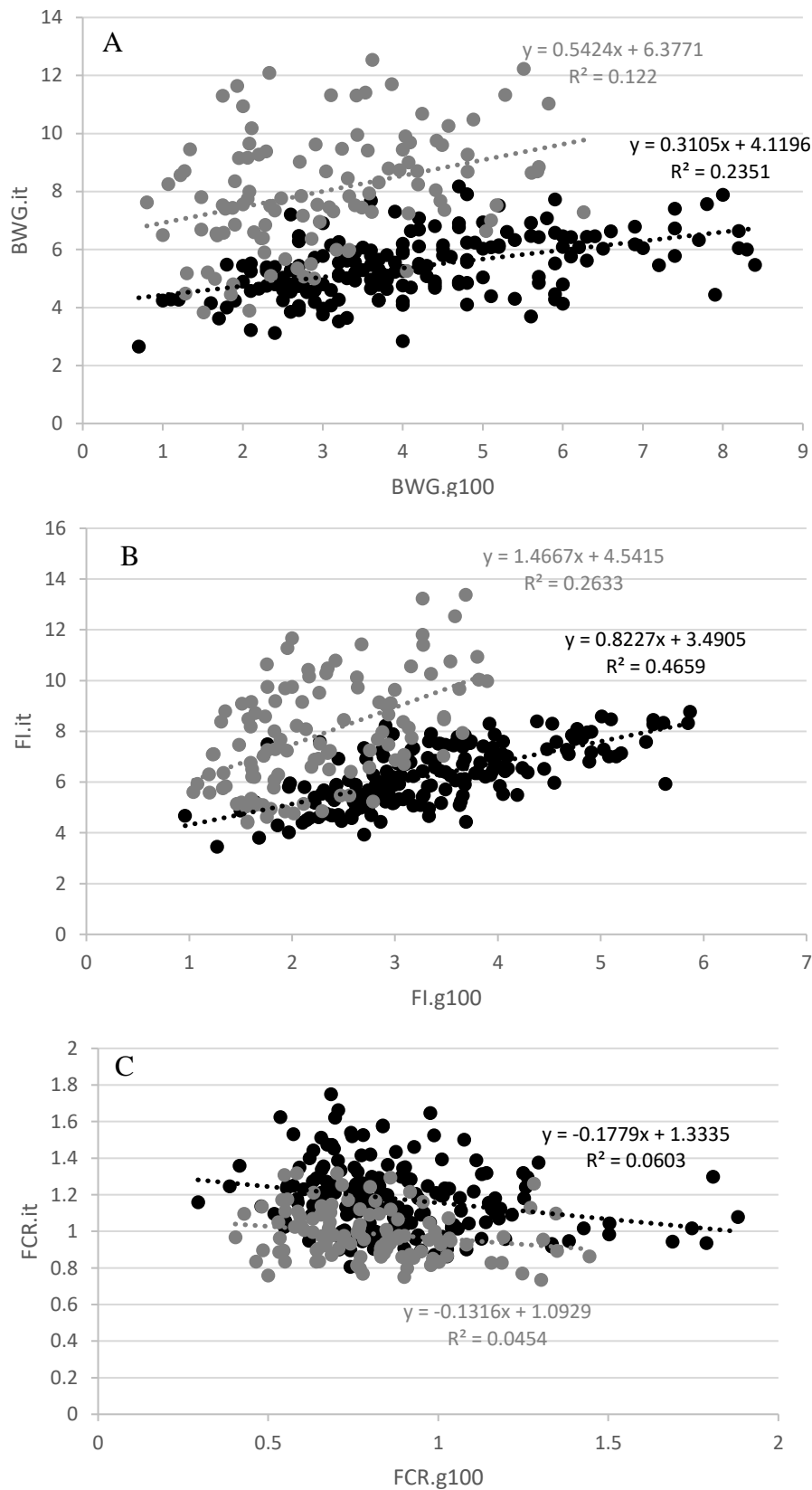


Figure 4. Relations between fish reared in groups and fed with an optimal feed ration (g100) and fish reared in isolation with a restricted ration (it) for BWG (A), FI (B) and FCR (C). Black and grey circles corresponded to individuals of the GIFT and Cirad strains, respectively. The

equation of the linear regression and the coefficient of determination R^2 are surrounded in black and grey for the GIFT and the Cirad strain, respectively.

CHAPTER III

Population, temperature and feeding rate effects on individual feed efficiency in European sea bass (*Dicentrarchus labrax*)

Population, temperature and feeding rate effects on individual feed efficiency in European sea bass (*Dicentrarchus labrax*)

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Abstract

Using breeding programs to improve feed efficiency, the ratio between fish body weight gain (BWG) and feed intake (FI), could increase aquaculture sustainability through reduced feed costs and environmental impact. To this end, individual phenotypic information is required. Individual FI can be measured by isolating each fish. Under these conditions, restricting the feeding rate has proved relevant to improve feed efficiency indirectly by selecting faster-growing animals. Moreover, a restricted feeding rate reduces the work load of collecting uneaten pellets after each meal. The approach assumes the most efficient fish at high and low feeding rates are the same, but this assumption remains untested. In European sea bass (*Dicentrarchus labrax*), feed efficiency is likely to be impacted also by population, temperature, and their interaction, as already demonstrated for growth in this species. To investigate these issues, 200 European sea bass from three wild populations, Atlantic (AT), West Mediterranean (WM) and East Mediterranean (EM), were reared individually at two temperatures, 18°C and 24°C. Their BWG and FI were measured at six different feeding rates, from *ad libitum* (100%

ADL) down to fasting. A trade-off between performance at 100% *ADL* and at fasting was observed: more efficient fish at 100% *ADL* showed a stronger decrease in BWG (standardized to metabolic weight) when the feeding rate was progressively lowered and lost more weight at fasting. The most efficient fish were not the same depending on the feeding rate, suggesting the feeding rate used to phenotype fish in selective breeding programs must be the same as that used in commercial practices. The slope in the linear relationship between BWG and FI (both standardized to metabolic weight) was similar among populations and temperatures. However, EM fish had a higher intercept than others, suggesting this population grew more and thus was more efficient for an equal feeding rate. Similarly, fish reared at 18°C were more efficient for an equal feeding rate. When feed efficiency was studied in fish fed at 100% *ADL*, the temperature effect disappeared but the population effect remained. This highlights the complex interplay between population, temperature and feeding rate when evaluating individual feed efficiency.

1 Introduction

In order to feed the increasing world's population, including more fish in future diets could help provide a solution because it would spare lands and feed crops when comparing with livestock production (Froehlich et al., 2018). Fisheries production has not increased over the last two decades, thus meeting the future demand for aquatic products will rely on aquaculture (FAO, 2018). However, to reach the fish production required, aquaculture needs to address some sustainability issues. The main issue is linked to fish feed which accounts for 30% to 60% of total costs in an intensive fish farm (Goddard, 1996). Furthermore, feed production will have to compete with both agriculture and human consumption for access to ingredients (Troell et al., 2014), and is responsible for a high proportion of the environmental impact of aquaculture (Aubin et al., 2009; Besson et al., 2016a).

Improved use of feed by fish may involve fish nutrition (NRC, 2011), husbandry (De Silva and Anderson, 1995) and genetics (de Verdal et al., 2018a; 2018b; Besson et al., 2019). Nutrition and husbandry have already been widely addressed, but genetic studies are scarce. Selective breeding could reduce feed use in aquaculture and improve sustainability by improving feed efficiency of farmed fish, i.e. the ratio between fish body weight gain (BWG) and feed intake (FI) (Besson et al., 2014; Besson et al., 2016a). Improving feed efficiency means using less feed to produce the same amount of fish, or producing more fish with the same amount of feed. However, to perform a selective breeding program, accurate individual phenotypic information is required. Measuring individual BWG is easy when fish are individually tagged, but measuring the individual FI of a large number of fish is challenging as fish are reared in large groups. Individual FI can be measured using individual rearing (Silverstein, 2006; Martins et al., 2011; Besson et al., 2019). This implies managing the exact number of pellets eaten by each individual. This method has the major advantage of being exhaustive: FI can be measured for each meal over several months and thus the temporal variability of FI is fully considered (Rodde et al., 2020). Moreover, the method gives immediate results (FI can be determined only a few hours after feeding). Besson et al. (2019) already managed to assess the feed efficiency of 588 European sea bass *Dicentrarchus labrax* in 194 days using this methodology. However, this method is tedious because of the need to collect all the uneaten pellets in all the individual aquariums. In their study, Besson et al. (2019) restricted the feeding rate to 50% of the optimal feeding rate. Using this methodology, it was demonstrated that selecting faster-growing individuals under a restricted feeding rate improved feed efficiency of the progenies in pigs (Nguyen et al., 2005) and in rabbits (Drouilhet et al., 2016), no matter if those progenies were

then fed at a restricted or an *ad libitum* (abbreviated as “100% *ADL*” in the present study) feeding rate. Advantageously, using a restricted feeding rate reduces the workload. The reduced labour costs from lowering fish feeding rate make the transfer of this phenotyping method to practical selective breeding programs more likely and economically viable (Besson et al., 2019).

However, to our knowledge, there is no existing evidence that the most efficient fish at high feeding rates are also the most efficient at low feeding rates, and this issue needs to be addressed prior to starting breeding programs. As changing the feeding rate may impact the accuracy of feed efficiency estimates, it is useful to study individual variation in feed efficiency according to the feeding rate. For this study, three populations of European sea bass identified in the wild were used, Atlantic (AT), West Mediterranean (WM) and East Mediterranean (EM) (Guinand et al., 2017). The Atlantic and Mediterranean lineages of European sea bass started to diverge around 300,000 years before present following spatial separation during this glacial period. While the differentiation between the EM and the AT European sea bass was maintained, the secondary contact between these two lineages led to an admixed population in West Mediterranean area (Duranton et al., 2018; Duranton et al., 2020). This evolutionary process occurred in environments whose average temperatures differed, since a North-West to South-East temperature gradient exists in European waters (Lindgren and Håkanson, 2011). Feed efficiency performance in European sea bass may thus be impacted by population, rearing temperature, and their interaction. Population by rearing temperature interactions on growth have been demonstrated already in this species (Vandeputte et al., 2014) suggesting similar effects could be found in feed efficiency. If such differences exist, there is a potential to choose more efficient source populations to start a selective breeding program, and to favour some specific rearing sites according to the temperature gradient existing in European waters.

In the present study, individual fish BWG and FI were measured at six different feeding rates, from 100% *ADL* down to fasting (0% *ADL*) to test whether feed efficiency at low feeding rates reflected feed efficiency at high feeding rates. A total of 200 European sea bass from AT, WM and EM were reared individually at two temperatures: 18°C and 24°C, corresponding to the average and optimal temperatures, respectively, for European sea bass growth in the West Mediterranean area (Person-Le Ruyet et al., 2004; Besson et al., 2016b). Moreover, 18°C and 24°C reflect well the coldest and warmest average temperatures at which European sea bass is reared across Europe (Vandeputte et al., 2014). The objectives of the present study were 1) to assess the impact of the feed ration on the relationship between BWG and FI at individual level,

and 2) to determine whether the relationship between BWG and FI varies with population and/or temperature.

2 Material and methods

2.1 Ethics statement

This study was carried out in accordance with the recommendations of Directive 2010-63-EU on the protection of animals used for scientific purposes. The protocols were approved by C2EA-36 (“Comité d'éthique en expérimentation animale Languedoc-Roussillon”) under authorization APAFiS n° 2018032109435819 (version 2).

2.2 Biological material

The 200 European sea bass used in the present study were produced by artificial fertilization on the 5th of February 2018 at the Ifremer Experimental Aquaculture Research Station (Palavas-les-Flots, France, 43°31'13"N; 3°54'37"E), with a similar protocol to Doan et al. (2017). The Atlantic (AT) and West Mediterranean (WM) groups were produced by mating wild sires and wild dams from each of the populations in a full factorial mating design, thus producing pure AT and WM offspring. Wild AT and WM fish were initially captured in the English Channel and the Gulf of Lions (France), respectively. Equal numbers of eggs from 22 WM dams, fertilized with sperm from 40 WM sires, and eggs from 9 AT dams, fertilized by 26 AT sires, were used. The East Mediterranean (EM) group was produced by mating 39 wild EM sires and 13 F1 EM x WM dams thus producing 75% EM-25% WM backcross progenies, hereafter called EM. Only wild EM males were available, which were initially captured in Turkey and Egypt (Vandeputte et al., 2014), and this is why only 75%EM-25%WM progenies could be produced. Hatching occurred on the 9th of February 2018. Each fish population was reared in separated tanks until 188 days post-hatching (dph). Fish (62 AT, 66 WM and 72 EM) were then gathered in a single 1000 L holding tank after the injection of PIT-tags (Biolog-id, France). At 213 dph, fish were fasted until the beginning of the experiment at 221 dph to stimulate their appetite.

2.3 Rearing system

The rearing system consisted of two independent recirculating water systems placed in the same room. Each rearing system was comprised of 100 aquariums (10 L each), a sand filter, a biological filter and a UV filter. Water renewal rate was 300% per hour in each aquarium. Water temperature was set at 18 and 24°C in first and second rearing systems, respectively. For the

first and second systems, mean oxygen saturation was respectively 114.1% (8.64 mg O₂/L) and 107.1% (7.28 mg O₂/L), mean water salinity was respectively 37.2‰ and 37.4‰ and mean water pH was 8.3 in both cases. Photoperiod was artificial: 12 hours light/12 hours dark.

2.4 Experimental design

The experimental procedure consisted of three distinct periods: acclimation, reaching 100% *ADL* and phenotyping (Fig. 1). Acclimation lasted four weeks. During the first two weeks, five fish were reared per aquarium. Groups were then split to acclimate one fish per aquarium for another two weeks, as described by Besson et al. (2019). Feed, manufactured by Le Gouessant Aquaculture, was from the commercial diet called “Neo Start 3” with: 47% of crude protein, 18% of crude fat, 1.5% of crude fibre, 8% of ash, 1% of phosphorus, 19 MJ/kg for digestible energy content, 23 g/MJ for digestible protein/digestible energy ratio. The diet used remained the same over the whole experiment. From the beginning of acclimation onwards, feed was supplied once a day in the morning (9 a.m.) by automatic feeders. During acclimation, fish were fed 50% of the feeding ration recommended by the feed provider with a single daily meal, and the number of uneaten pellets per aquarium was recorded as soon as fish were reared individually.

During the second period, three rounds of one week were needed to reach 100% *ADL* for each fish (Fig. 1). Over each round, the number of pellets uneaten by each fish was manually counted two hours after each meal. The last week of acclimation was used as a starting point to estimate which fish were already fed at 100% *ADL*. At the end of each round, different choices were made according to the number of pellets wasted by each fish over the week:

- a) if the fish left a relatively low number of uneaten pellets (less than 30 pellets per day), that fish was considered to have reached 100% *ADL* and the FI of the fish was considered equal to its 100% *ADL*;
- b) if the fish left a large number of uneaten pellets (higher than 30 pellets per day), feed ration was decreased the week after by 10% of the feeding ration recommended by the feed provider to ease the wasted pellets counting;
- c) if the fish did not waste any pellets, its feed ration was increased the week after by 20% of the feeding ration recommended by the feed provider to reach 100% *ADL*.

At 270 dph, the end of round 3, there were 99 fish reared at 18°C (28 AT, 34 WM and 37 EM) and 95 fish reared at 24°C (28 AT, 32 WM and 35 EM). Six fish died before 270 dph because they jumped out of their aquarium (five AT at 18°C and one AT at 24°C).

The third period of the experiment was the phenotyping period, fish were first fed for 22 days (from 270 to 292 dph) with an individual feeding rate taking into account the various choices previously made to reach 100% *ADL* (Fig. 1). Fish were weighed at 270, 281 and 292 dph (beginning, half and end of the 22 days) to update the individual feeding ration according to their body weight (BW) and to determine individual BWG (final BW – initial BW). Fish were anaesthetized with benzocaine (37.5 g per m³ of seawater) before weight measurements. Uneaten pellets were counted in each aquarium and removed daily. The uneaten feed weight was estimated every day, considering that all the pellets had the same weight (14.6 ± 1.6 mg with CV = 11.0 %) and cumulated over the 100% *ADL* step. Fish were fasted the day of weight measurements and the day before. Feed intake of each fish during this 100% *ADL* step was calculated as: weight of feed given – weight of feed uneaten, and converted to a % of BW per day to estimate 100% *ADL*.

After estimation of 100% *ADL*, fish were successively fed 80%, 60%, 40%, 20% and 0% *ADL* for ten to 11 days at each step (Fig. 1). Individual BWG and FI were measured over each step as previously defined, with fish being weighed on the days indicated in Fig. 1. For ethical reasons, if a fish had lost weight both between 270 and 281 dph and between 281 and 292 dph when fed at 100% *ADL* (Fig. 1), or if a fish had lost weight when fed at 80%, 60% or 40% *ADL*, the next step was directly 0% *ADL* and then the fish was removed from the experiment. Moreover, five fish (two AT fish at 18°C, two AT fish at 24°C and one WM fish at 24°C) did not eat at all at 100% *ADL* (< 1% of their BW over the 22 days) and were directly removed from the experiment, without going through a 0% *ADL* step.

2.5 Statistical analysis

2.5.1 General data treatment

All statistical analyses were done using R software (R Core Team, 2018). The normality of residuals was checked using the quantile-quantile method (comparing residuals quantiles with theoretical normal quantiles). The homoscedasticity and independence of the residuals were checked by comparing the residuals with the fitted values from the models. Linear mixed models and tests associated to these models were performed using R packages “lme4” (Bates et al., 2015), “lmerTest” (Kuznetsova et al., 2017) and “lsmeans” (Lenth, 2016).

Individual metabolic weight (MBW) was calculated for each step (100% *ADL* to 0% *ADL*) as $MBW = \sqrt{(W_i \times W_f)^{0.8}}$ with W_i and W_f the initial and final BW of each specific feeding rate step (Lupatsch et al., 2003; Saravanan et al., 2012). In order to allow a more accurate

comparison between fish with heterogeneous BW, individual BWG and FI were standardized to MBW (respectively named StdBWG and StdFI) at each feeding rate step as $StdBWG = 100 * BWG/MBW$ and $StdFI = 100 * FI/MBW$ and expressed in % of $MBW \cdot day^{-1}$. Metabolic body weight was used instead of BW as BWG and FI in fish are more closely related to MBW than to BW (Paloheimo and Dickie, 1966; Warren and Davis, 1967; Fonds et al., 1992).

2.5.2 Temperature and population effects at 100% ADL

To study individual feed efficiency at 100% ADL, feed efficiency ratio (FER) was calculated as $FER = BWG/FI$. The impact of temperature and population on StdBWG, StdFI and FER during the 100% ADL step was determined using the following linear model:

$$Y_{ijk} = \mu + T_i + P_j + TP_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the phenotype (StdBWG, StdFI or FER) at temperature i (18 or 24°C), for population j (AT, WM or EM) and animal k ; μ is the general mean; T is the fixed effect of temperature i (18 or 24°C); P is the fixed effect of population j (AT, WM or EM); TP is the interaction of these two effects, and ε_{ijk} is the residual ($\varepsilon_{ijk} \sim N(0; \sigma_e^2)$). Fixed effects significance was determined with Fisher test and then pairwise differences between temperature by population combinations were determined with Tukey post-hoc test.

2.5.3 Relationship between StdBWG and StdFI at population and temperature levels

The individual StdBWG and StdFI data of every step (100% ADL to 0% ADL) were then analyzed all together. Focus was firstly made on the variability between temperatures and populations in the relationship between StdBWG and StdFI. The following repeated measures linear mixed model was used to analyse this relationship:

$$\begin{aligned} StdBWG_{ijkl} = & [\mu + T_i + P_j + TP_{ij} + A_l] \\ & + [\beta + \beta T_i + \beta P_j + \beta TP_{ij} + B_l] * StdFI_{ijkl} \\ & + \varepsilon_{ijkl} \end{aligned}$$

where $StdBWG_{ijkl}$ and $StdFI_{ijkl}$ are respectively StdBWG and StdFI at temperature i (18 or 24°C) for population j (AT, WM or EM), step k (k between 1 for 100% ADL and 6 for 0% ADL) and animal l ; μ is the general mean, T is the fixed effect of temperature i (18 or 24°C), P is the fixed effect of population j (AT, WM or EM), and TP is the interaction of temperature by population: all those effects are the “intercept” part of the relationship since they do not depend on StdFI; β is the fixed effect of StdFI, βT and βP are the interactions of StdFI by temperature and

population, respectively, and β_{TP} is the triple interaction of StdFI by temperature and population: all those effects are the “slope” part of the relationship since they depend on StdFI. Finally, A_l and B_l are the random effects of the animal l respectively associated to intercept and slope, with $A_l \sim N(0; \sigma_a^2)$ and $B_l \sim N(0; \sigma_b^2)$, and ε_{ijkl} is the residual ($\varepsilon_{ijkl} \sim N(0; \sigma_e^2)$). Fixed effects significance was determined with Fisher test and then pairwise differences between populations or temperatures were determined with Tukey post-hoc test. Data were considered as outliers and discarded from the analyses when their Cook’s distance (i.e. their influence) in the model linear mixed model was higher than $4/n$ (Algur and Biradar, 2017), with n the total number of StdBWG and StdFI measurements. The residuals of the model, i.e. ε_{ijkl} , were extracted for data collected at 100% ADL (res100%ADL) and 0% ADL (res0%ADL). From a biological point of view, the higher the individual res100%ADL, the more efficient the fish at 100% ADL, and the lower the individual res0%ADL, the higher the body weight loss at fasting.

2.5.4 Relationship between StdBWG and StdFI at individual level

To assess individual variability within each temperature by population combination, parameters of the linear relationship between StdBWG and StdFI, i.e. intercept and slope, were calculated for each individual using the following model:

$$StdBWG_k = intercept + slope * StdFI_k + \varepsilon_k$$

where $StdBWG_k$ and $StdFI_k$ are respectively the StdBWG and StdFI of the individual at step k (k between 1 for 100% ADL and 6 for 0% ADL) and ε_k the residual ($\varepsilon_k \sim N(0; \sigma_e^2)$). This calculation was done exclusively for individuals with data available for at least 100% ADL, 80% ADL, 60% ADL and 0% ADL (114 individuals out of 194). The coefficient of variation ($CV = 100 * standard\ deviation / mean$, expressed in %) was then estimated for intercepts and slopes within each population by temperature combination.

Finally, Pearson’s correlations were estimated between res100%ADL, res0%ADL, intercepts and slopes. Since the number of data for individual intercepts and slopes within each combination of temperature by population was too low (from nine to 28) to ensure robust correlation analysis at combination level, it was decided to merge the data from the different combinations to estimate Pearson’s correlation. To avoid a bias in correlation estimations due to potential population and temperature effects on individual intercepts and slopes, intercepts and slopes were corrected by these fixed effects before merging the data. To correct for these effects, the following linear model was used:

$$Y_{ijk} = \mu + T_i + P_j + TP_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the intercept or slope at temperature i (18 or 24°C), for population j (AT, WM or EM) and animal k ; μ is the general mean; T is the fixed effect of temperature i (18 or 24°C); P is the fixed effect of population j (AT, WM or EM); TP is the interaction of these two effects, and ε_{ijk} is the residual ($\varepsilon_{ijk} \sim N(0; \sigma_e^2)$). In the present model, residuals are the individual intercepts and slopes corrected for potential temperature, population, and interaction effects. Thus, these residuals were extracted to estimate the correlations.

3 Results

3.1 Performance at 100% ADL

All fish left some pellets uneaten and thus reached 100% ADL during the 22-day period: 95.9% of the fish left feed uneaten over at least nine meals out of 18 and 83.5% of the fish did so over at least 15 meals out of 18. Among the 194 fish that were successfully evaluated at 100% ADL, 46 (23.7%) lost weight and were consequently discarded from the analyses. Over the 22 days, fish grew from 26.5 ± 10.1 g (CV = 38.1%) to 29.6 ± 11.4 g (CV = 38.4%). Individual BWG, StdBWG, FI and StdFI were all significantly different according to temperature. This effect was driven by WM and EM fish which grew faster and consumed more feed at 24°C than at 18°C (Table 1). Individual FI was also significantly different according to population (Table 1), with EM fish eating on average 15 and 17% less than WM and AT fish, respectively. Feed efficiency ratio was significantly different according to population but not according to temperature (Table 1), with EM fish being on average 18 and 41% more efficient than WM and AT fish, respectively.

3.2 Relationship between StdBWG and StdFI at temperature and population levels

The data from 100% ADL to 0% ADL were merged. Using Cook's distance to detect outlier data, 30 data out of 729 were rejected (4.1% of the total dataset). The proportion of variance explained by the models with StdBWG as a linear function of StdFI ranged from $R^2 = 0.80$ to $R^2 = 0.90$ according to the population by temperature combination (Fig. 2). No difference was seen in slopes between temperatures or populations, as well as no temperature by population interaction ($P > 0.05$ in all cases with Fisher test). The intercept, i.e. the part of StdBWG variance that does not depend on StdFI, was significantly different among populations and temperatures ($P < 0.001$ in both cases with Fisher test), but the interaction was not significant ($P > 0.05$, Fisher test). The intercept was higher for EM than for WM ($P < 0.001$, Tukey test) which was higher than for AT ($P < 0.001$, Tukey test). The intercept was also significantly

higher at 18°C than at 24°C ($P < 0.001$, Tukey test). This result means that for an equal StdFI, StdBWG was higher for EM fish than for WM and AT fish, and thus EM fish were the most efficient. Similarly, fish reared at 18°C were more efficient than fish reared at 24°C for an equal StdFI.

3.3 Individual variability in the relationship between StdBWG and StdFI

Modelling the individual relationship between StdBWG and StdFI with a linear function appeared suitable since 101 individuals out of 114 had a corresponding R^2 higher than 0.80. The coefficients of variation were between 14.5% and 38.8% for intercepts and between 14.4% and 34.0% for slopes among the six different temperature by population combinations (Fig. 3). No significant correlation was found between *res100%ADL* and *res0%ADL*. A significant and positive correlation was found between intercept and *res0%ADL* as well as between *res100%ADL* and slope. A significant and negative correlation was found between intercept and *res100%ADL*, between intercept and slope, as well as between *res0%ADL* and slope (Table 2).

4 Discussion

The objective of the present study was firstly to determine whether individual feed efficiency at low feeding rates reflected feed efficiency at high feeding rate. This aspect is key to give new insights on the potential development of selective breeding programs for feed efficiency in European sea bass. Secondly, the present study aimed at providing a better understanding of the variations in the relationship between BWG and FI at population and temperature levels, across a range of feeding rates. Such variations are of major interest to determine the impact of temperature and population on feed efficiency.

4.1 Variation in individual feed efficiency at different feeding rates

At the individual level, there appeared to be a trade-off between performance observed at 100% *ADL* and at fasting. Due to the high and significant correlation between *res100%ADL* and the slope of the linear relationship between StdBWG and StdFI, it seemed that more efficient fish at 100% *ADL* were showing a stronger decrease in StdBWG when the feeding rate was progressively lowered. Moreover, these fish were losing more weight at fasting (lower intercept). Surprisingly, no significant correlation was found between *res100%ADL* and *res0%ADL*. Actually, both *res0%ADL* and intercept are an estimation of body weight loss at

fasting. These two parameters are not perfectly equivalent ($r = 0.47$) and the intercept of the linear relationship may better reflect body weight loss at fasting. Indeed, the intercept integrates all data from 100% down to 0% *ADL* (four to six measurement periods) whereas *res0%ADL* is based only on one measurement period.

Trade-offs in growth performance between high and low feeding rates have been reported in European sea bass (Grima et al., 2010; Dupont-Prinet et al., 2010), but not specifically for feed efficiency. These authors identified two profiles of fish: those exploiting the available feed as much as possible, growing faster and losing more weight during a fasting period (“boom and bust”) *versus* those with less capacity to exploit the available feed, growing slower and losing less weight during feed deprivation (Dupont-Prinet et al., 2010). McKenzie et al. (2014) provided evidence that such variation in weight loss at fasting was linked to metabolic costs, but also to the composition of the reserves used. They concluded that fish tolerant to feed deprivation rely more on lipids whereas fish sensitive to feed deprivation rely more on proteins. Variation in feed efficiency at 100% *ADL* might be linked to the same factors as variation in weight loss at fasting, i.e. differences in metabolic costs or in the nature of the energy reserves used, but also to differences in the digestive process as observed by Dupont-Prinet et al. (2010). Further investigation is required to determine the physiological processes underlying these observations, but present results strengthen the previously noted hypothesis that some individuals are more adapted to feed abundance whereas others are more adapted to feed deprivation. In contrast, measuring feed efficiency both in isolated aquariums and in groups, Besson et al. (2019) highlighted that fish with lower weight loss at fasting were more efficient. This difference might be due to the fact that Besson et al. (2019) used genetic information whereas present data are only phenotypic. For instance, de Verdal et al. (2018b) found no phenotypic correlation between feed efficiency and weight loss at fasting in Nile tilapia *Oreochromis niloticus* but found a strong genetic correlation between the same traits.

At the individual level, the most efficient fish at high feeding rates are not the most efficient at low feeding rates. Present results suggest that the feeding rate used to phenotype fish in selective breeding programs for feed efficiency must be the same as that used in commercial practices. However, the present results are only phenotypic and it is required to describe the genetic correlations between these traits before adding such characters in selective breeding programs.

4.2 Variability between temperatures and populations

In this study, WM and EM fish grew more at 24°C than at 18°C when fed at 100% *ADL*, but no population effect was significant. This contrasts with previous results (Vandeputte et al.,

2014) showing EM had higher growth rates compared to other populations when reared at an average of 24.4°C. This difference may be due to the fact that Vandeputte et al. (2014) used EMxAT and EMxWM hybrids to extract additive effects of the EM population, whereas in the present study EM fish were in fact 75% EM-25% WM. Furthermore, in the present study, WM and EM fish showed an increase of FI and StdFI with temperature, similar to evidence of increase of FI with temperature reported in larger European sea bass (Lanari et al., 2002). The fact that AT fish did not exhibit a lower BWG and FI at 18°C than at 24°C could be explained by evolutionary differences between AT and the two Mediterranean populations (WM and EM). In particular, one possible explanation is a potential specific adaptation of AT population to lower temperatures, since the Atlantic Ocean is colder than the Mediterranean sea. The differentiation between the populations has been widely reported at the genomic level (Duranton et al., 2018) and associated with phenotypic variation in sex ratio, muscle fat or resistance to viral nervous necrosis (Guinand et al., 2017; Doan et al., 2017). Duranton et al. (2020) demonstrated that the maintenance of the genomic differentiation between the AT and Mediterranean populations was due to reproductive isolation barriers established after the ancient admixture of the Atlantic European sea bass with the closely related *Dicentrarchus punctatus*. In addition, the subsequent rapid fixation of some *D. punctatus* alleles in the Atlantic *D. labrax* could have provided a selective advantage in the Atlantic environment compared to ancestral *D. labrax* alleles (Duranton et al. 2020).

Results from the six different feeding rates (from 100% *ADL* to 0% *ADL*) showed that for an equal value of StdFI, fish from the EM population and fish reared at 18°C had the highest StdBWG, and thus were the most efficient. To explain these differences, it can be hypothesized that metabolic costs are not similar between the different temperatures and populations. Fish obtain energy from feed and invest this energy both into metabolism (to ensure routine requirements) and growth (Warren and Davis, 1967; Bureau et al., 2003). The use of energy could be differently balanced between metabolism and growth among the various populations and temperatures. In the case of temperature, the fact that higher temperatures increase fish metabolic costs is well known in various species (meta-analysis by Clarke and Johnston, 1999), including European sea bass (Claireaux and Lagardère, 1999). This supports the idea that fish reared at 24°C in the present study had higher metabolic costs and so were less efficient than fish reared at 18°C for an equal value of StdFI. Similarly, AT fish might have higher metabolic costs because their StdBWG was lower than other populations for an equal value of StdFI. However, differences in metabolic costs among populations have never been studied in European sea bass to our knowledge. Further investigation is required to assess these aspects.

Alternatively, the observation that AT fish were less efficient may be linked to their muscle fat content, higher than in the Mediterranean populations (Vandeputte et al., 2014; F. Allal, personal communication, 2020). Indeed, it was demonstrated in several terrestrial and aquatic species that the most efficient animals had the lowest muscle fat content (Knap and Kause, 2018). As detailed by Knap and Kause (2018), deposition of 1 g of lipid leads to 1.1 g of weight gain, including 0.1 g of water in the associated adipose tissue. Conversely, deposition of 1 g of protein leads to 4-5 g of weight gain, including 3-4 g of water. Even if protein deposition is energetically more expensive than lipid deposition (59.9 kJ/g vs. 43.5-55.3 kJ/g), this higher energetic cost is small compared to the four to fivefold increase in weight gain associated to protein deposition (Knap and Kause, 2018). Considering an equal value of StdFI, fish reared at 18°C were more efficient than fish reared at 24°C, which was not the case when fish were fed at 100% *ADL*, i.e. with different values of StdFI. When fed at 100% *ADL*, fish reared at 24°C had a higher FI, permitting them to compensate for probably higher metabolic costs, and increasing the proportion of dietary energy allocated to growth.

Present results indicate that whatever the rearing temperature (18°C or 24°C), and for an equal feeding rate, the EM population had better individual feed efficiency than the AT and WM populations. This population effect remained when fish were fed at 100% *ADL*. In contrast, the impact of temperature on growth and feed efficiency was different whether fish were restricted or fed at 100% *ADL*. However, investigating a broader range of temperatures could give more generic results. Furthermore, these results still need to be validated in group rearing systems.

4.3 Impact of individual rearing on fish performance

The need to obtain individual data regarding European sea bass FI implied the use of an individual rearing system. However, whether performance exhibited in individual rearing reflects what would be observed in group rearing is debatable. The level of 100% *ADL* ranged from 0.53 to 0.73% and from 0.85 to 1.12% of BW.day⁻¹ at 18°C and 24°C, respectively, which is low compared to group rearing. With the model developed by Lanari et al. (2002) for European sea bass, 100% *ADL* was estimated to be around 1.1% and 1.7% of BW.day⁻¹ at 18°C and 24°C, respectively, for fish weighing 26.5 g (mean weight at the beginning of the 100% *ADL* step). Feed provider tables were advising an even higher feeding rate: about 1.90% and 2.75% of BW.day⁻¹ at 18°C and 24°C, respectively. It can be hypothesized that fish performance was degraded because of stress due to isolation, as it has been demonstrated that chronic stress lowers BWG and FI performance in European sea bass (Leal et al., 2011). Present results may also reflect individual variation in stress resistance because stress reaction is known to be highly

variable among individuals. For instance, Volckaert et al. (2012) estimated a CV of 44.4% in plasma cortisol (stress marker) when an acute stress is applied in European sea bass. It is important to note that almost one quarter of the fish lost weight at 100% *ADL* in the present study, which can be considered as a non-adaptation to this isolation rearing system. In particular, at 24°C, more AT and WM than EM fish lost weight at 100% *ADL*. It suggests an interaction between population and temperature may exist in the ability to adapt to the isolation rearing system. This ability to adapt to the individual rearing system, whatever the temperature, may also be linked to the coping style of European sea bass. Indeed, we showed in a preliminary (unpublished) experiment that shy (reactive) fish adapted better to the individual rearing system than bold (proactive) fish. Moreover, the proportion of bold and shy fish might be different from a population to another, as a genetic basis to coping style has been evidenced in European sea bass (Ferrari et al., 2016). Further investigation is required to confirm this hypothesis. Nevertheless, it is still unknown whether stress or coping style affect the ranking of the fish based on the feed efficiency performance. Although individual rearing certainly has lowered fish performance, its relevance for selective breeding was supported by Besson et al. (2019) who demonstrated a link between individual feed efficiency, measured using the same isolated rearing system, and subsequent group feed efficiency. Thus, it can be suggested that the most efficient fish in the present study would still be the most efficient in “classical” group rearing.

5 Conclusion

At the individual level, the phenotypic variability reported here in the relationship between StdBWG and StdFI suggests opportunities to develop genetic breeding programs for feed efficiency. Whether this variability is genetically determined or not still needs to be addressed. However, the most efficient fish at high feeding rates were not the most efficient at low feeding rates, stressing the fact that feeding rate must be chosen carefully before phenotyping fish for selective breeding. Besides, it was observed that for an equal feeding rate, whatever the temperature, EM fish were the most efficient. Furthermore, for an equal feeding rate and whatever the population, fish were more efficient when reared at 18°C than at 24°C, but this effect disappeared when fish were fed at 100% *ADL* while the population effect remained. These results were measured on fish reared in isolation and need to be validated in group rearing, as well as at other development stages. Nevertheless, investigating the interactions between populations and temperatures seems a promising pathway to improve on-farm feed efficiency.

Conflict of interest

The authors declare that they have no conflict of interest.

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Table Caption

Table 1. Mean \pm standard deviation of body weight gain (BWG), body weight gain standardized to metabolic body weight (StdBWG), feed intake (FI), feed intake standardized to metabolic body weight (StdFI) and feed efficiency ratio (FER) recorded for 22 days with fish fed at 100% of *ad libitum* feeding rate. Results are presented for Atlantic (AT), West Mediterranean (WM) and East Mediterranean (EM) populations reared at 18°C or 24°C.

Table 2. Correlation matrix with phenotypic correlations above diagonal and statistical significance (given as p-values) of the correlations below diagonal. Correlations with associated p-values lower than 0.05 are considered as significant and presented in bold. *res100%ADL*: residual body weight gain at 100% *ad libitum* feeding rate; *res0%ADL*: residual body weight loss at fasting; intercept and slope are the intercept and slope of the linear relationship between body weight gain and feed intake (standardized to metabolic body weight). All parameters were previously corrected for potential population and temperature effects.

Figure Caption

Figure 1. Experimental schedule applied to each fish. Fish age (in days post hatching) at each step is indicated by the numbers. *ADL*: *ad libitum* feeding rate; *BW*: body weight measurement.

Figure 2. Linear relationships between body weight gain and feed intake, standardized to metabolic body weight (MBW), for the different population by temperature combinations. The three populations are from Atlantic Ocean (AT), West Mediterranean sea (WM) and East Mediterranean sea (EM). Standardized body weight gain and feed intake are expressed in % of MBW per day.

Figure 3. Linear relationships between body weight gain and feed intake, standardized to metabolic body weight (MBW), at individual level within the different population by temperature combinations. The three populations are from Atlantic Ocean (AT), West Mediterranean sea (WM) and East Mediterranean sea (EM). Standardized body weight gain and feed intake are expressed in % of MBW per day. Parameters presented within each combination are the minimum, maximum and average of the R-squared (R^2) of the various linear relationships, the number of individuals (n) and the coefficients of variation of intercept (CV_{int}) and slope (CV_{slope}).

Table 1

Mean \pm standard deviation of body weight gain (BWG), body weight gain standardized to metabolic body weight (StdBWG), feed intake (FI), feed intake standardized to metabolic body weight (StdFI) and feed efficiency ratio (FER) recorded for 22 days with fish fed at 100% of *ad libitum* feeding rate. Results are presented for Atlantic (AT), West Mediterranean (WM) and East Mediterranean (EM) populations reared at 18°C or 24°C.

	BWG* (% of BW.day ⁻¹)	StdBWG** (% of MBW.day ⁻¹)	FI* (% of BW.day ⁻¹)	StdFI** (% of MBW.day ⁻¹)	FER***	Proportion of fish that lost weight (%)
<i>Combinations</i>						
AT x 18°C	0.43 \pm 0.22 a,b	0.84 \pm 0.42 a,b	0.73 \pm 0.16 b,c,d	1.43 \pm 0.30 b,c	0.57 \pm 0.22 a,b	7/28 - 25.0
WM x 18°C	0.35 \pm 0.21 b	0.67 \pm 0.40 b	0.61 \pm 0.35 c,d	1.15 \pm 0.64 c	0.60 \pm 0.34 a,b	5/34 - 14.7
EM x 18°C	0.39 \pm 0.20 b	0.78 \pm 0.42 b	0.53 \pm 0.16 d	1.05 \pm 0.37 c	0.71 \pm 0.25 a	6/37 - 16.2
AT x 24°C	0.47 \pm 0.41 a,b	0.91 \pm 0.81 a,b	0.95 \pm 0.47 a,b	1.81 \pm 0.96 a,b	0.44 \pm 0.27 b	11/28 - 39.3
WM x 24°C	0.69 \pm 0.37 a	1.29 \pm 0.71 a	1.12 \pm 0.58 a	2.08 \pm 1.08 a	0.64 \pm 0.26 a,b	13/32 - 40.6
EM x 24°C	0.65 \pm 0.34 a	1.29 \pm 0.69 a	0.85 \pm 0.31 a,b,c	1.68 \pm 0.63 a,b	0.72 \pm 0.21 a	4/35 - 11.4
<i>Populations</i>						
AT	0.45 \pm 0.31	0.88 \pm 0.62	0.83 \pm 0.35	1.60 \pm 0.70	0.51 \pm 0.25	18/56 - 32.1
WM	0.49 \pm 0.33	0.92 \pm 0.62	0.81 \pm 0.51	1.52 \pm 0.95	0.61 \pm 0.31	18/66 - 27.3
EM	0.52 \pm 0.31	1.03 \pm 0.62	0.69 \pm 0.29	1.37 \pm 0.60	0.72 \pm 0.23	10/72 - 13.9
<i>Temperatures</i>						
18°C	0.39 \pm 0.21	0.76 \pm 0.41	0.61 \pm 0.25	1.18 \pm 0.49	0.63 \pm 0.28	18/99 - 18.2
24°C	0.61 \pm 0.37	1.19 \pm 0.73	0.95 \pm 0.45	1.83 \pm 0.87	0.63 \pm 0.26	28/95 - 29.5
<i>Significance of the effects (p-values)</i>						
Pop. x Temp.	F _{2,142} = 2.86, P = 0.061	F _{2,142} = 2.59, P = 0.079	F _{2,142} = 1.93, P = 0.148	F _{2,142} = 1.66, P = 0.194	F _{2,142} = 1.22, P = 0.299	
Population	F _{2,144} = 0.54, P = 0.581	F _{2,144} = 0.65, P = 0.523	F_{2,144} = 3.70, P = 0.027	F _{2,144} = 2.32, P = 0.102	F_{2,144} = 7.47, P = 8.2×10⁻⁴	
Temperature	F_{1,144} = 20.72, P = 1.1×10⁻⁵	F_{1,144} = 19.67, P = 1.8×10⁻⁵	F_{1,144} = 37.05, P = 9.9×10⁻⁹	F_{1,144} = 34.64, P = 2.7×10⁻⁸	F _{1,144} = 0.15, P = 0.701	

Letters indicate significant differences among population by temperature combinations (Tukey test, P < 0.05).

* Considering BW_i and BW_f are respectively the initial and final body weights over the period, bodyweight (BW) is expressed as: $BW = \sqrt{(BW_i \times BW_f)}$.

** Considering BW_i and BW_f are respectively the initial and final body weights over the period, metabolic body weight (MBW) is expressed as: $MBW = \sqrt{(BW_i \times BW_f)^{0.8}}$.

*** $FER = BWG/FI$

Table 2

Correlation matrix with phenotypic correlations above diagonal and statistical significance (given as p-values) of the correlations below diagonal. Correlations with associated p-values lower than 0.05 are considered as significant and presented in bold. *res100%ADL*: residual body weight gain at 100% *ad libitum* feeding rate; *res0%ADL*: residual body weight loss at fasting; intercept and slope are the intercept and slope of the linear relationship between body weight gain and feed intake (standardized to metabolic body weight). All parameters were previously corrected for potential population and temperature effects.

	<i>res100%ADL</i>	<i>res0%ADL</i>	Intercept	Slope
<i>res100%ADL</i>	1	-0.08	-0.32	0.67
<i>res0%ADL</i>	0.39	1	0.46	-0.35
Intercept	2.5×10^{-3}	$< 1 \times 10^{-4}$	1	-0.37
Slope	$< 1 \times 10^{-4}$	3×10^{-4}	$< 1 \times 10^{-4}$	1

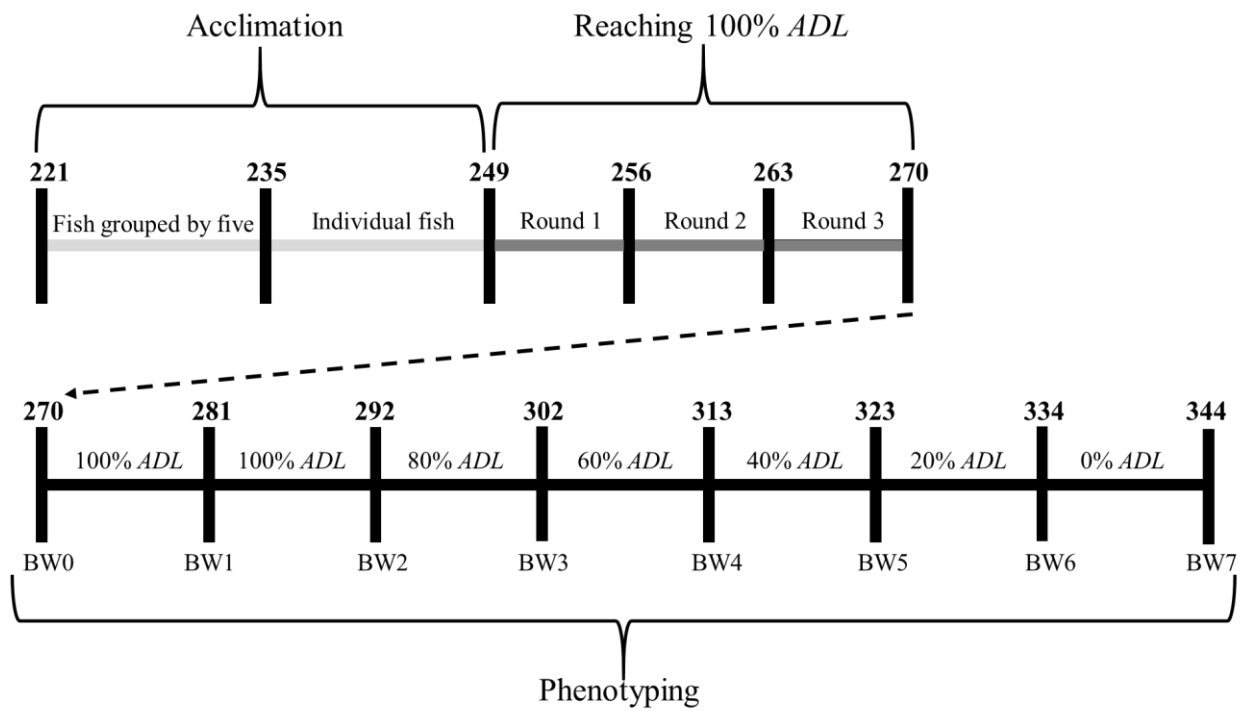


Fig. 1. Experimental schedule applied to each fish. Fish age (in days post hatching) at each step is indicated by the numbers. *ADL*: *ad libitum* feeding rate; *BW*: body weight measurement.

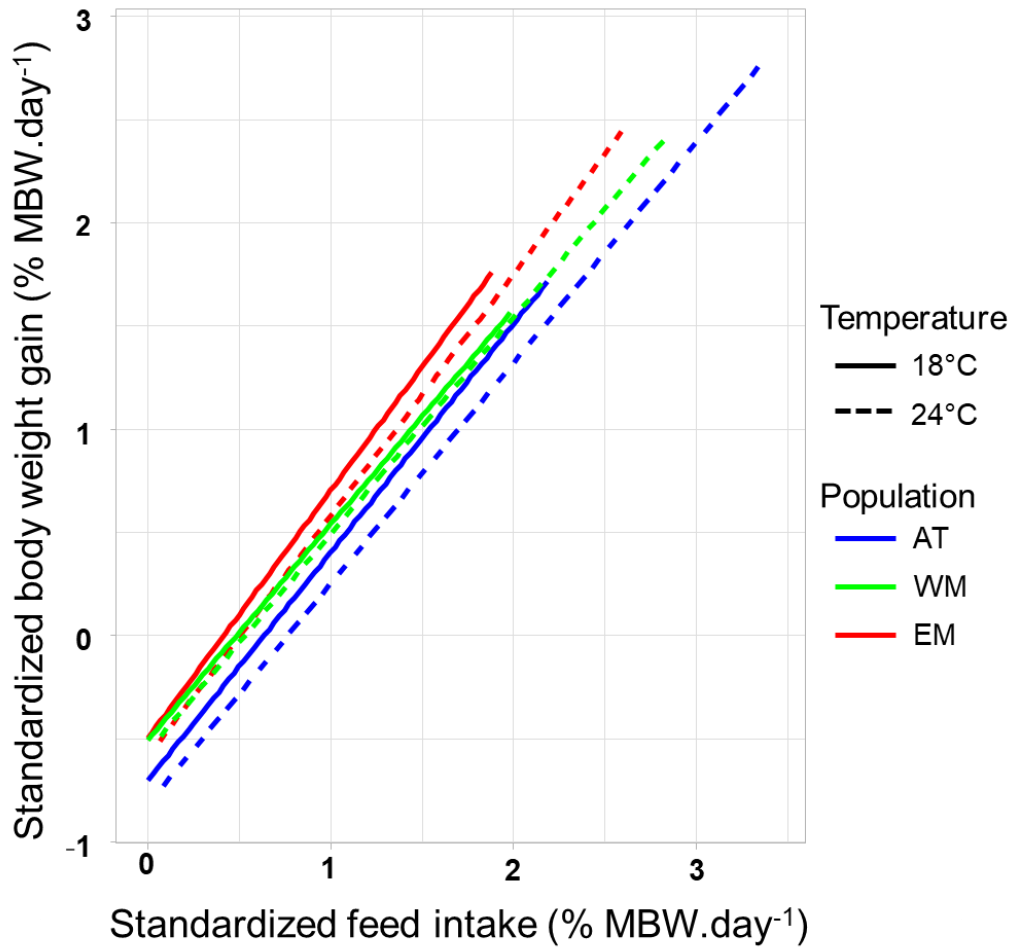


Fig. 2. Linear relationships between body weight gain and feed intake, standardized to metabolic body weight (MBW), for the different population by temperature combinations. The three populations are from Atlantic Ocean (AT), West Mediterranean sea (WM) and East Mediterranean sea (EM). Standardized body weight gain and feed intake are expressed in % of MBW per day.

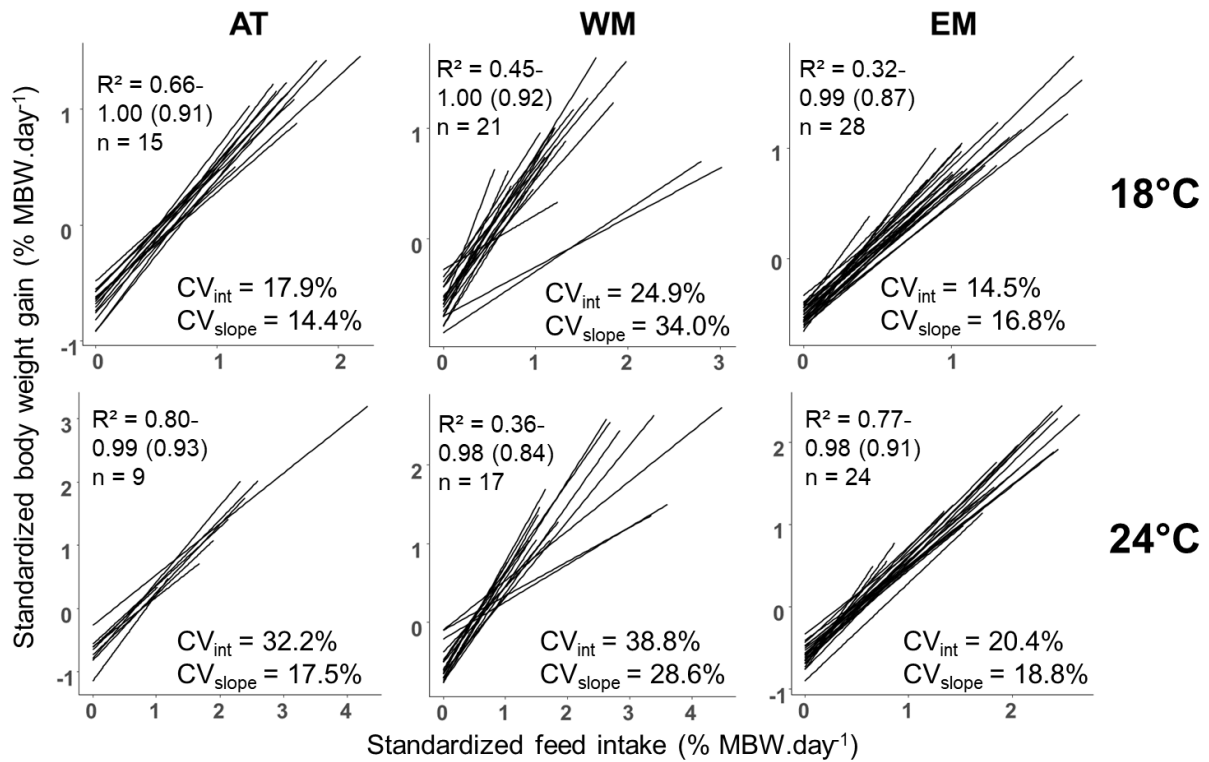


Fig. 3. Linear relationships between body weight gain and feed intake, standardized to metabolic body weight (MBW), at individual level within the different population by temperature combinations. The three populations are from Atlantic Ocean (AT), West Mediterranean sea (WM) and East Mediterranean sea (EM). Standardized body weight gain and feed intake are expressed in % of MBW per day. Parameters presented within each combination are the minimum, maximum and average of the R-squared (R^2) of the various linear relationships, the number of individuals (n) and the coefficients of variation of intercept (CV_{int}) and slope (CV_{slope}).

CHAPTER IV

An investigation of links between metabolic rate and feed efficiency in European sea bass *Dicentrarchus labrax*

An investigation of links between metabolic rate and feed efficiency in European sea bass *Dicentrarchus labrax*

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Abstract

Feed efficiency is the amount of body weight gain (BWG) for a given feed intake (FI). Improving feed efficiency through selective breeding is key for sustainable aquaculture but its evaluation at individual level is technically challenging. We investigated whether individual routine metabolic rate (RMR) was a predictor of individual feed efficiency. The European sea bass, a major species in European mariculture, has three genetically distinct populations across its geographical range, in Atlantic Ocean (AT), West Mediterranean (WM) and East Mediterranean (EM). We compared feed efficiency and RMR of fish from these three populations at 18 °C or 24 °C. We held 200 fish (62 AT, 66 WM and 72 EM) in individual aquaria and fed them from *ad libitum* down to fasting. Feed efficiency was assessed for an *ad libitum* feeding rate and for a restricted feeding rate (1% of metabolic body weight.day⁻¹). After being refed 12 weeks in a common tank, individual RMR was measured over 36h by intermittent flow respirometry. There were significant and consistent differences in mean feed efficiency and RMR between temperatures and among populations, whatever the feeding rate applied. Fish at 18 °C were more efficient and had lower RMR. Similarly, AT fish were less efficient and had a higher RMR than WM and EM. No temperature by population interaction was found. However, individual feed efficiency and RMR were not correlated, no matter if fish were fed at *ad libitum* or restricted. Therefore, although the results provide evidence of an association between metabolic rate and efficiency, RMR was not a predictor of individual feed efficiency, for reasons that require further investigation.

Keywords: Aquaculture; genetic populations; individual rearing; oxygen consumption; respirometry

Introduction

Including more fish in future diets can be a solution to feed the world increasing population more sustainably (Godfray et al., 2010; Froehlich et al., 2018). This will depend on aquaculture sector whose growth is particularly strong (FAO, 2018). However, feed use in aquaculture results in high costs (Goddard, 1996) and environmental impact (Besson et al., 2016a).

Selective breeding to improve feed efficiency of farmed fish, i.e. the ratio between fish body weight gain (BWG) and feed intake (FI), is promising to make aquaculture more sustainable (Besson et al., 2014; Besson et al., 2016a). However, a crucial requirement is to be able to phenotype fish individually. Measuring the individual FI of a large number of farmed fish is technically challenging because they are usually reared in large groups. This problem can be circumvented by individual rearing in aquaria (Silverstein, 2006; Martins et al., 2011; Besson et al., 2019). This method has been applied to European sea bass (*Dicentrarchus labrax*), a major species for finfish aquaculture, by Besson et al. (2019). They found evidence that individual rearing provides a reliable estimation of individual feed efficiency for this species, because individual efficiency in aquaria was linked to subsequent efficiency of groups reared in tanks. Still, the method is time-consuming and tedious because the fish need to be kept in isolation at least six weeks and all uneaten food must be carefully collected daily (Besson et al., 2019), which may impede its application on a large scale for selective breeding programs.

It would be much easier if there were an accurate indirect predictor of individual feed efficiency that could be measured more easily and rapidly. Bioenergetics is promising because it is assumed that energy intake from feed in fish is allocated among several processes, most notably maintenance metabolism, activity and growth (Warren and Davis, 1967; Bureau et al., 2003). It is conceivable, therefore, that for a given feed intake, the most efficient fish will be those that allocate the least energy to maintenance and activity, and the most to growth. In terrestrial livestock such as cattle, sheep and poultry, there is clear evidence of a negative correlation between individual metabolic rate and feed efficiency (Luiting et al., 1991; Nkrumah et al., 2006; Arndt et al., 2015; Chaves et al., 2015; Paganoni et al., 2017). Although there is some evidence of a link between metabolic rate and feed efficiency in groups of fishes (Kinghorn, 1983; Zeng et al., 2017), this remains to be demonstrated at an individual level.

This study investigated whether individual metabolic rate, measured indirectly as rates of oxygen uptake, was a predictor of individual feed efficiency in the European sea bass. In this species, individual metabolic rate is negatively correlated with mass loss during fasting (Killen et al., 2011; McKenzie et al., 2014), indicating a link between metabolism and non-growth

energy requirements, which could also relate to individual bioenergetics when feeding. Measuring individual oxygen consumption by respirometry on fasted (post-prandial) fish is technically quite simple and takes less than 2 days per animal (McKenzie et al., 2014).

Three genetically distinct populations of European sea bass have been identified across its natural geographical range: Atlantic (AT), West Mediterranean (WM) and East Mediterranean (EM; Guinand et al., 2017). These populations started to diverge 300,000 years ago (Duranton et al., 2018; Duranton et al., 2020) in environments whose temperatures differed along a North-West to South-East temperature gradient (Lindgren and Håkanson, 2011). European sea bass farming is by mariculture in coastal cages so it is necessary to understand whether the populations may differ in their bioenergetics at different water temperatures. We therefore investigated this, as it could have important implications for selection programs for fish to be reared in different areas of Europe.

In a previous study (Rodde et al., 2020), we reared 200 European sea bass from AT, WM and EM populations, at two temperatures: 18°C and 24°C. These correspond to the average and optimal temperatures for European sea bass growth in the West Mediterranean area, respectively (Besson et al., 2016b; Person-Le Ruyet et al., 2004). Furthermore, 18°C and 24°C are two temperatures reflecting well the coldest and warmest sites where European sea bass is reared across Europe (Vandeputte et al., 2014). We measured individual BWG and FI at six different feeding rates, from *ad libitum* down to fasting. In the present study, we evaluated the metabolic rate of these individuals, at their acclimation temperature, to investigate how this related to the feed efficiency at individual and population levels.

Material and methods

Ethics statement

Experimental procedures were approved by C2EA-36 (“Comité d'éthique en expérimentation animale Languedoc-Roussillon”) under authorisations APAFiS n° 2018032109435819 and n° 2018100910598940.

Animals

Complete details of how the fish were produced, reared and evaluated for their individual feed efficiency are provided in Rodde et al. (2020). Briefly, 200 European sea bass from the three populations (62 AT, 66 WM and 72 EM) were produced by controlled breeding at the Ifremer Experimental Aquaculture Research Station (Palavas-les-Flots, France, 43°31'13"N;

3°54'37"E). When fish reached 221 days post hatching (23.4 ± 8.4 g), they were isolated to be able to record their individual FI. This was the starting point of the acclimation of 100 of them to 18°C, and of 100 others to 24°C (Rodde et al., 2020).

Measurement of individual BWG and FI

Fish were firstly acclimated to individual aquaria by groups of five (two weeks), and then individually (two other weeks). Once acclimated, three weeks were spent to determine each fish individual *ad libitum* feeding rate. After this step, 99 fish were remaining at 18°C (28 AT, 34 WM and 37 EM) and 95 fish at 24°C (28 AT, 32 WM and 35 EM). Six fish died before because they jumped out of their aquarium (five AT at 18°C and one AT at 24°C).

Fish were then fed gradually from *ad libitum* feeding rate down to fasting over six sequential steps (100%, 80%, 60%, 40%, 20% and 0% of *ad libitum* feeding rate). The 100% *ad libitum* step lasted 22 days and the other steps 10 to 11 days. Fish individual BWG and FI were measured at each step. In total, fish remained under isolation for 123 days (Rodde et al., 2020). After fasting, fish were grouped into two common tanks supplied with water at either 18°C or 24°C and fed *ad libitum* for 12 weeks, leading to a fourfold increase in body mass. This period ensured that fish were in a steady nutritional state and that physiology and behaviour were not directly influenced by the feed deprivation or any stress linked to individual rearing (Dupont-Prinet et al., 2010; Rubio et al., 2010; McKenzie et al., 2014). When respirometry was performed, the mean mass was 99.0 ± 29.7 g and 146.2 ± 40.4 g for fish at 18 and 24°C, respectively.

Metabolic rate estimation

Metabolic rate was estimated using a system of 32 individual semi-transparent respirometry chambers immersed in trays supplied with aerated biofiltered seawater at either 18°C or 24°C. The trays were shielded behind opaque black plastic to avoid any visual disturbance, with the fishes in dim light following an artificial photoperiod of 12 hours light/12 hours dark. The system was similar to the feed efficiency aquaria, in that individuals will have been aware of their conspecifics in adjacent chambers.

Fish were distributed among three holding tanks per temperature, with a tank fasted 24h prior to respirometry then, in the afternoon, fish were netted at random from it, identified by a PIT-tag and weighed. They were then placed into chambers (volume either 1.8 or 3.0 L) according to their size, such that they were free to move easily, and left for 12h to recover from handling.

Measurements of oxygen uptake (MO_2) were made by intermittent stopped-flow respirometry (Steffensen, 1989) as described in McKenzie et al. (2014), but with a 15 minute cycle comprising eight minutes stopped flow and seven minutes flushing with aerated water. Water oxygen levels in the chambers were measured and recorded every ten seconds by optodes (Oxy10 mini; PreSens Precision Sensing GmbH) and associated software (Pre-Sens Oxy 4v2). During stopped flow, oxygen saturation in the chambers declined due to consumption by the fish, MO_2 was calculated as $mg\ O_2 \cdot kg^{-0.80} \cdot h^{-1}$ considering the volume of the chamber and the solubility of oxygen in seawater at 18°C or 24°C and a salinity of 37‰ (Steffensen, 1989; Dupont-Prinet et al., 2010). We corrected to metabolic body weight (MBW) rather than by body weight (BW; $MBW = BW^{0.8}$) based upon a mass exponent of 0.8 for resting metabolism in European sea bass (Lemarié et al., 1992; Lupatsch et al., 2003). Following the 12h recovery period, measurements were then made for a further 24h. Upon removal of a batch from their chambers, background oxygen consumption due to bacterial respiration was measured (McKenzie et al., 2014). It represented about 2% of the total oxygen consumption of fish per cycle, and thus no correction was applied.

Routine metabolic rate (RMR), defined as the metabolic rate of post-absorptive, undisturbed, resting animals that also includes the costs of random activity and the maintenance of posture and equilibrium (Killen et al., 2011), was taken as the mean rate of MO_2 over 24h. Standard metabolic rate (SMR), defined as the minimal energy cost of living for an organism (Hulbert and Else, 2004), was estimated as the 0.25 quantile of MO_2 values over the 24h period (Chabot et al., 2016).

Statistical analysis

The BWG and FI measured throughout the individual rearing experiment were standardized to MBW and named “StdBWG” and “StdFI”, respectively (Rodde et al., 2020). All statistical analyses were done using R software (R Core Team, 2018). The normality of residuals was checked using the quantile-quantile method (comparing residuals quantiles with theoretical normal quantiles). The homoscedasticity and independence of the residuals were checked by comparing the residuals with the fitted values from the models. Linear mixed models and tests associated to these models were performed using R packages “lme4” (Bates et al., 2015) and “lmerTest” (Kuznetsova et al., 2017).

Relationship between StdBWG and StdFI

All StdBWG and StdFI data obtained from *ad libitum* down to fasting were merged. Among the 194 fish that were successfully evaluated at *ad libitum* feeding rate, 46 (23.7%) lost weight and were consequently discarded from the analyses.

The following repeated measures linear mixed model was used:

$$StdBWG_{ij} = intercept + slope * StdFI_{ij} + A_j + B_j * StdFI_{ij} + \varepsilon_{ij}$$

where $StdBWG_{ij}$ and $StdFI_{ij}$ are respectively the StdBWG and StdFI at step i (i between 1 for *ad libitum* and 6 for fasting) for animal j , A_j and B_j are the random effects of the animal j respectively associated to intercept and slope, with $A_j \sim N(0; \sigma_a^2)$, with $B_j \sim N(0; \sigma_b^2)$ and ε_{ij} the residual ($\varepsilon_{ij} \sim N(0; \sigma_e^2)$). The residuals of the model were extracted for data collected *ad libitum* and named “resBWG”. From a biological point of view, the higher the individual resBWG is and the more efficient the fish are when fed *ad libitum*.

Then, the following linear model was used for each fish:

$$StdBWG_i = intercept + slope * StdFI_i + \varepsilon_i$$

where $StdBWG_i$ and $StdFI_i$ are respectively the StdBWG and StdFI at step i (i between 1 for *ad libitum* and 6 for fasting) for each fish, and ε_i the residual ($\varepsilon_i \sim N(0; \sigma_e^2)$). The intercept and slope of this relationship permitted to predict for each fish its StdBWG for a restricted StdFI set to 1% of MBW.day⁻¹, named “PredStdBWG”. This linear model was applied only to fish successfully phenotyped on at least four steps out of six from *ad libitum* down to fasting.

Variation in resBWG, PredStdBWG and RMR among populations and temperatures

The following linear model was used to determine the variation of each trait at temperature and population levels:

$$Y_{ijk} = \mu + T_i + P_j + TP_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the phenotypic trait considered (resBWG, PredStdBWG or RMR) at temperature i (18°C or 24°C), for genetic population j (AT, WM or EM) and animal k ; μ is the general mean, T is the fixed effect of temperature, P is the fixed effect of population, TP the interaction of these two effects, and ε_{ijk} the residuals ($\varepsilon_{ijk} \sim N(0; \sigma_e^2)$). Regarding the three populations, their pairwise differences were further explored using Tukey post-hoc test.

Link between resBWG, PredStdBWG and RMR at individual level

The link between resBWG, PredStdBWG and RMR at individual level was investigated using Pearson’s correlation, for each single population by temperature combination.

Results

Variation in resBWG, PredStdBWG and RMR among populations and between temperatures

Firstly, resBWG (*ad libitum* feeding rate) as well as PredStdBWG (1% of MBW.day⁻¹ feeding rate) were significantly different between temperatures ($F_{1,126} = 4.75$, $P = 3.11 \times 10^{-2}$ and $F_{1,103} = 8.88$, $P = 3.59 \times 10^{-3}$, respectively) and among populations ($F_{2,126} = 7.25$, $P = 1.05 \times 10^{-3}$ and $F_{2,103} = 12.32$, $P = 1.59 \times 10^{-5}$, respectively). Both resBWG and PredStdBWG were higher at 18°C than at 24°C. Using Tukey post-hoc test, it was found that AT fish had significantly lower resBWG and PredStdBWG than WM fish ($P = 2.30 \times 10^{-2}$ and 6.73×10^{-3} , respectively) and EM fish ($P = 1.02 \times 10^{-3}$ and $P = 2.79 \times 10^{-5}$, respectively), but these traits were not significantly different between WM and EM fish ($P = 0.70$ and $P = 0.31$, respectively). However, no temperature by population interaction was found ($F_{2,124} = 0.58$, $P = 0.56$ and $F_{2,101} = 0.21$, $P = 0.81$, respectively).

Regarding RMR and SMR, mean individual values obtained within each temperature by population combination are presented in Table 1 and RMR values are shown individually in Figure 1. Firstly RMR was positively and strongly correlated with SMR, whatever the population by temperature combination ($r = 0.60-0.97$). Thus, we chose to focus only on RMR results since SMR has very similar variations. Within each population by temperature combination, individual RMR was moderately variable with a CV (coefficient of variation = $100 \times \text{standard deviation}/\text{mean}$) between 9.8 and 14.8%. Moreover, RMR differed significantly by temperature, with fish reared at 18°C having lower RMR ($F_{1,116} = 130.89$, $P < 2.2 \times 10^{-16}$), but also by population ($F_{2,116} = 14.02$, $P = 3.52 \times 10^{-6}$), whereby AT fish had a significantly higher RMR than WM and EM fish ($P = 1.95 \times 10^{-4}$ and $P = 1.81 \times 10^{-4}$ respectively using Tukey post-hoc test). In contrast, the RMR of WM and EM fish was not significantly different ($P = 0.94$). Moreover, there was no interaction effect between temperature and population on RMR ($F_{2,114} = 1.67$, $P = 0.19$).

Correlation between performance and RMR

As illustrated in Figure 2, a link between RMR and resBWG as well as PredStdBWG appears at temperature and populations levels. Indeed, fish at 18°C have a lower RMR and higher resBWG and PredStdBWG than 24°C. Similarly, AT fish have a higher RMR and lower resBWG and PredStdBWG than WM and EM fish.

At individual level, the correlations between RMR and resBWG ranged from -0.33 to 0.39 among the various population by temperature combinations (Figure 3), but none of them was significant ($P > 0.05$ in all cases). Similarly, the correlations between RMR and PredStdBWG

ranged from -0.43 to 0.15 among the various combinations (Figure 4) and none of them was either significant ($P > 0.05$ in all cases).

Discussion

This study is the first to attempt to relate individual variation in feed efficiency to metabolic rate in European sea bass. The results indicated that fish reared at 18°C were more efficient, both at *ad libitum* and 1% of MBW.day⁻¹ feeding rates, and had lower RMR than fish at 24°C. Similarly, AT fish were less efficient at both feeding rates and had higher RMR than WM and EM fish. However, within temperature by population combinations, no clear relationship appeared between individual feed efficiency and RMR.

Link between feed efficiency and RMR among populations and between temperatures

Both resBWG and PredStdBWG results, corresponding respectively to an *ad libitum* and a restricted (1% of MBW.day⁻¹) feeding rates, suggest fish reared at 18°C are more efficient. Similarly, whatever the feeding rate, WM and EM fish were more efficient than AT ones. The initial hypothesis made was that the most efficient fish, for a given feeding rate (1% of MBW.day⁻¹ in the present study), were those allocating the least energy to maintenance and activity, resulting in more available energy for growth. Present RMR results tend to validate this hypothesis among populations and between temperatures. Indeed, RMR differed in a consistent way with PredStdBWG: fish at 18°C were more efficient for 1% of MBW.day⁻¹ and had a lower RMR than at 24°C, AT fish were less efficient for 1% of MBW.day⁻¹ and had a higher RMR than WM and EM fish.

The same consistence between RMR and resBWG results was observed, which is more surprising since resBWG is estimated for fish fed *ad libitum*. It might have been suggested that fish with higher metabolic costs (i.e. RMR) would have compensated by increasing their *ad libitum* energy intake. However, present resBWG data suggest metabolic costs outweighed any potential compensation through an increased energy intake. This contrasts with Zeng et al. (2017) results showing that Chinese crucian carps (*Carassius auratus*) with higher RMR were less efficient under a restricted feeding rate but more efficient when fed at *ad libitum*. It may be explained by the fact that fish were reared as a group by Zeng et al. (2017) but individually in the present study. Indeed, *ad libitum* FI was much lower than expected in the present study (Rodde et al., 2020), suggesting individual rearing certainly prevented the fish from reaching their full feed consumption potential.

The fact that RMR was higher in fish reared at 24°C than in those reared at 18°C is not surprising: oxygen consumption is known to increase with temperature in every fish species (meta-analysis by Clarke and Johnston, 1999), including European sea bass (Claireaux and Lagardère, 1999). In contrast, it is interesting that AT fish had a higher RMR than the two Mediterranean populations. This has, to our knowledge, never been reported before, although they are known to differ at the genomic level (Duranton et al., 2018) and in other traits such as growth, sex ratio, muscle fat or resistance to viral nervous necrosis (Guinand et al., 2017; Doan et al., 2017; Vandeputte et al., 2019). In particular, an ancient admixture occurred between the Atlantic European sea bass and the closely related *Dicentrarchus punctatus* (Duranton et al., 2020). This led to the subsequent rapid fixation of some *D. punctatus* alleles in the Atlantic *D. labrax* and to the establishment of reproductive isolation barriers between Atlantic and Mediterranean populations (Duranton et al., 2020). This event may have provided a genetic basis for differences in metabolic rate between the AT population and Mediterranean ones.

The phenotypic traits underlying such differences among populations in metabolic rate still need to be determined. However, it seems unlikely that variation is due to behavioural differences. Indeed, SMR was strongly correlated with RMR within each population by temperature combination. This can be explained by the fact that fish exhibited little swimming activity while in the individual respirometry chamber. Even if the experimental set-up permitted to avoid any disturbance from the outside, such little activity appears surprising. This may be due to the fact these fish had already experienced 123 days in isolated aquaria before being evaluated for RMR. Thus, the fish used here were probably much more acclimated to isolation than usual, resulting in a low swimming activity. Other factors may be accountable for the RMR differences among populations. For instance, higher RMR might be associated to bigger sizes of metabolically expensive organs such as heart, liver or brain (Konarzewski and Książek, 2013), higher mitochondrial density (i.e. energy consumption per unit mass of tissue), higher activity of mitochondrial enzymes or lower ATP production efficiency (i.e. ATP produced per unit consumption of oxygen; Norin and Metcalfe, 2018). Investigating these various hypotheses could provide a better understanding of the factors underlying RMR variation among populations.

Link between feed efficiency and RMR at individual level

Differences observed among populations and between temperatures revealed a consistent link between high feed efficiency performance and low RMR, whatever the feeding rate. In contrast, no correlation appeared at individual level, no matter if the feeding rate was restricted (1% of

MWB.day⁻¹) or not (*ad libitum*). Nevertheless, there is a need for further investigation before concluding that RMR is of no use to improve feed efficiency in a selective breeding program. Firstly, only genetic correlations permit to conclude if a trait can be selected indirectly using another trait. At individual level, a CV of 9.8 to 14.8% was found for RMR. Similarly, Killen et al. (2011) found a CV of 13% for European sea bass RMR, measured with the same experimental set-up and corrected for metabolic body weight as well. There is a need to determine whether this phenotypic variation in RMR is due (at least partly) to a genetic basis. However, setting up an experimental design permitting to estimate genetic correlations is technically challenging. The number of fish phenotyped for both feed efficiency and RMR would need to be multiplied by at least four or five in comparison with the present study. Moreover, fish BWG and FI performance were measured before RMR, and not simultaneously. The time lapse between these measurements was 12 weeks and fish had their weight multiplied by four, so their development stage was not similar, and this may explain why not correlation is found. Metabolic rate estimation is known to have a moderate short term-repeatability in European sea bass ($r = 0.48$ for measurements separated by 20 minutes; Marras et al., 2010), but its longer term repeatability is, to our knowledge, unknown in this species. This is problematic because metabolic rate long-term repeatability is species-dependant. For instance, it was reported as high ($r = 0.68$ for measurements separated by 17 weeks) in Atlantic salmon *Salmo salar* (McCarthy, 2000) but as very low ($r = 0.093$ for measurements separated by 15 weeks) in brown trout *Salmo trutta* (Norin and Malte, 2011). Similarly, the long-term repeatability of individual feed efficiency estimation was, to our knowledge, never reported in the case of European sea bass. Since it is not technically feasible to estimate simultaneously individual feed efficiency and RMR, further investigation of both traits long-term repeatability is required specifically for this species.

Another information that is unknown is the type of reserves, i.e. proteins or lipids, on which each fish relies the most to produce its energy. Indeed, lipids provide twice as much energy as proteins do for an equal weight. Thus, to ensure equal maintenance costs, fish degrading lipids will consume a lower mass of reserves than fish degrading proteins. For instance, McKenzie et al. (2014) reported that European sea bass relying on proteins rather than on lipids to produce energy while fasting were losing more weight. Consequently, a link between the main type of reserves used and individual feed efficiency may exist. In particular, AT fish muscle fat content is higher than in the Mediterranean populations (Vandeputte et al., 2014; F. Allal, personal communication, 2020). Thus, AT fish may use their lipid reserves less than Mediterranean populations (and so they tend to accumulate them), degrading their protein reserves instead.

This could explain why AT fish are ultimately less efficient. This hypothesis is supported by results reported in several species such as pig or rainbow trout showing the most efficient animals had the lowest muscle fat content (Kamalam et al., 2012; Kause et al., 2016; Knap and Kause, 2018).

To conclude, presents results demonstrated a variability among European sea bass populations regarding oxygen consumption, in addition to the well-known effect of temperature on this trait. Among populations and between temperatures, fish with a lower oxygen consumption were more efficient. However, at individual level, no significant correlation was found. Further investigation is still required to fully understand the link between individual feed efficiency and oxygen consumption in fish.

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Table Caption

Table 1. Mean \pm standard deviation ($100 \times \text{standard deviation}/\text{mean}$) of routine metabolic rate (RMR), standard metabolic rate (SMR) and weight at respirometry. Results are presented for Atlantic (AT), West Mediterranean (WM) and East Mediterranean (EM) populations reared at 18°C or 24°C. Within each combination of population by temperature, the correlation between RMR and SMR is given with p-value, as well as the number of fish.

Figure Caption

Figure 1. Routine metabolic rate (RMR) values observed for each combination of population by temperature. Results are presented for Atlantic (AT), West Mediterranean (WM) and East Mediterranean (EM) populations reared at 18°C or 24°. In the box and whisker plots presented, the box lower and upper limits are respectively the 0.25 and 0.75 quantiles of the RMR data and the box is divided by the median of the values. The whiskers lower and upper ends are respectively the lowest and highest RMR values. Dots represent each fish RMR.

Figure 2. A) Residual body weight gain at *ad libitum* feeding rate as a function of routine metabolic rate (RMR) among population by temperature combinations. B) Predicted body weight gain as a function of RMR among population by temperature combinations. Predicted body weight gain is expressed in % of metabolic body weight (MBW) and is corresponding a level of feed intake set to 1% of MBW.day⁻¹. Results are presented for Atlantic (AT), West Mediterranean (WM) and East Mediterranean (EM) populations reared at 18°C or 24°. Horizontal and vertical bars associated to each point are corresponding to standard errors.

Figure 3. Individual residual body weight gain at *ad libitum* feeding rate as a function of individual routine metabolic rate (RMR). Results are presented for Atlantic (AT), West Mediterranean (WM) and East Mediterranean (EM) populations reared at 18°C or 24°C. The straight lines represent the linear regressions of individual residual body weight gain as a function of individual RMR in each case.

Figure 4. Individual predicted body weight gain as a function of individual routine metabolic rate (RMR). Predicted body weight gain is expressed in % of metabolic body weight (MBW) and is corresponding a level of feed intake set to 1% of MBW.day⁻¹. Results are presented for Atlantic (AT), West Mediterranean (WM) and East Mediterranean (EM) populations reared at

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Table 1

Mean \pm standard deviation (100*standard deviation/mean) of routine metabolic rate (RMR), standard metabolic rate (SMR) and weight at respirometry. Results are presented for Atlantic (AT), West Mediterranean (WM) and East Mediterranean (EM) populations reared at 18°C or 24°C. Within each combination of population by temperature, the correlation between RMR and SMR is given with p-value, as well as the number of fish.

	RMR (in mg O ₂ .kg ^{-0.8} .h ⁻¹)	SMR (in mg O ₂ .kg ^{-0.8} .h ⁻¹)	Weight at respirometry (in g)	Correlation between RMR and SMR	Number of fish
<i>Combinations</i>					
AT x 18°C	78.7 \pm 8.0 (10.2)	67.8 \pm 4.7 (7.0)	107.6 \pm 34.9 (32.5)	0.60 (P < 0.01)	18
WM x 18°C	70.1 \pm 6.9 (9.8)	63.8 \pm 5.8 (9.1)	80.0 \pm 20.8 (25.9)	0.88 (P < 0.001)	19
EM x 18°C	70.3 \pm 9.0 (12.9)	65.4 \pm 7.2 (11.0)	107.2 \pm 25.5 (23.8)	0.92 (P < 0.001)	25
AT x 24°C	108.5 \pm 15.7 (14.5)	86.8 \pm 13.9 (16.0)	142.2 \pm 39.8 (28.0)	0.83 (P < 0.001)	14
WM x 24°C	91.9 \pm 12.1 (13.2)	80.7 \pm 10.4 (12.8)	125.5 \pm 26.9 (21.4)	0.91 (P < 0.001)	17
EM x 24°C	91.3 \pm 13.6 (14.8)	80.8 \pm 13.1 (16.2)	161.3 \pm 42.7 (26.5)	0.97 (P < 0.001)	27

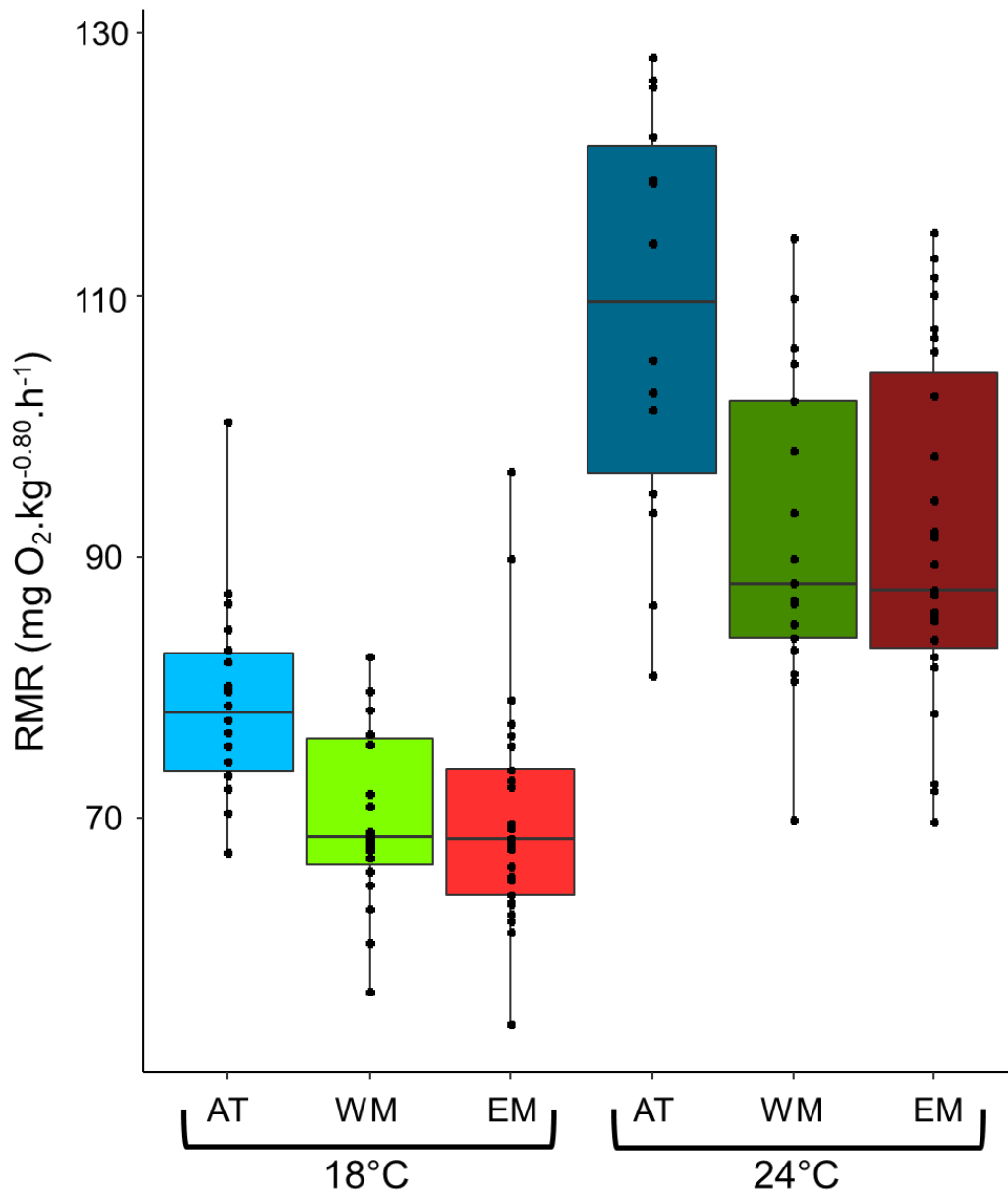


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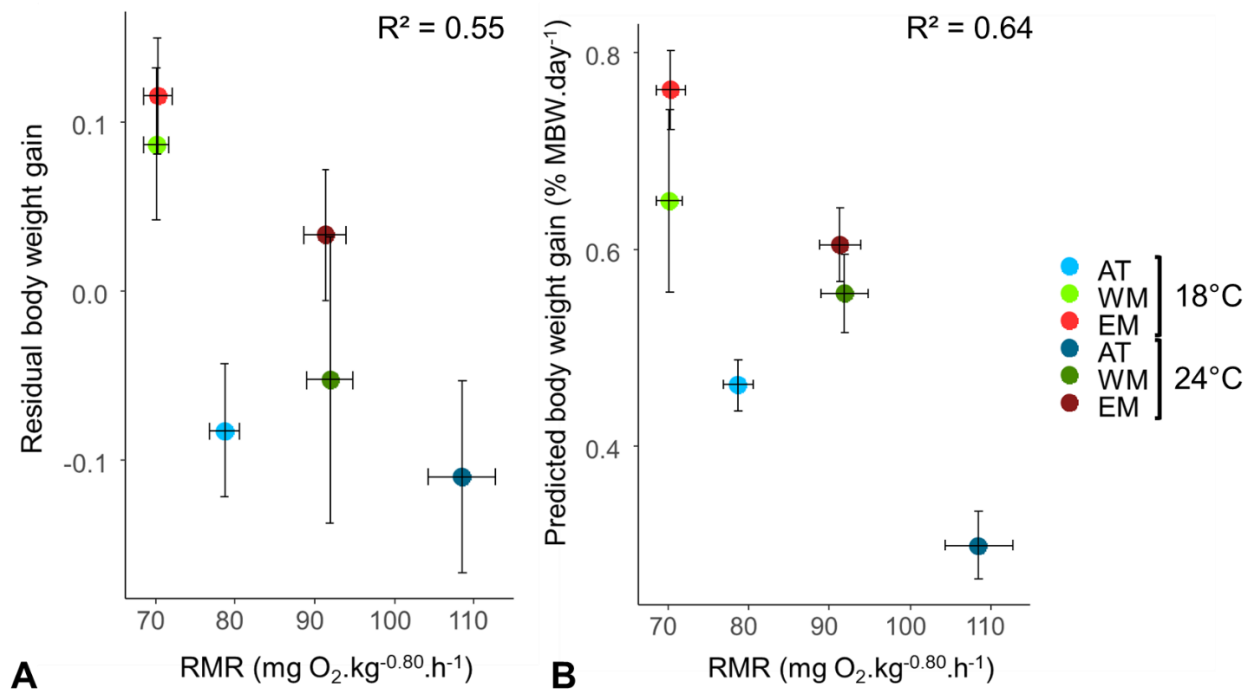


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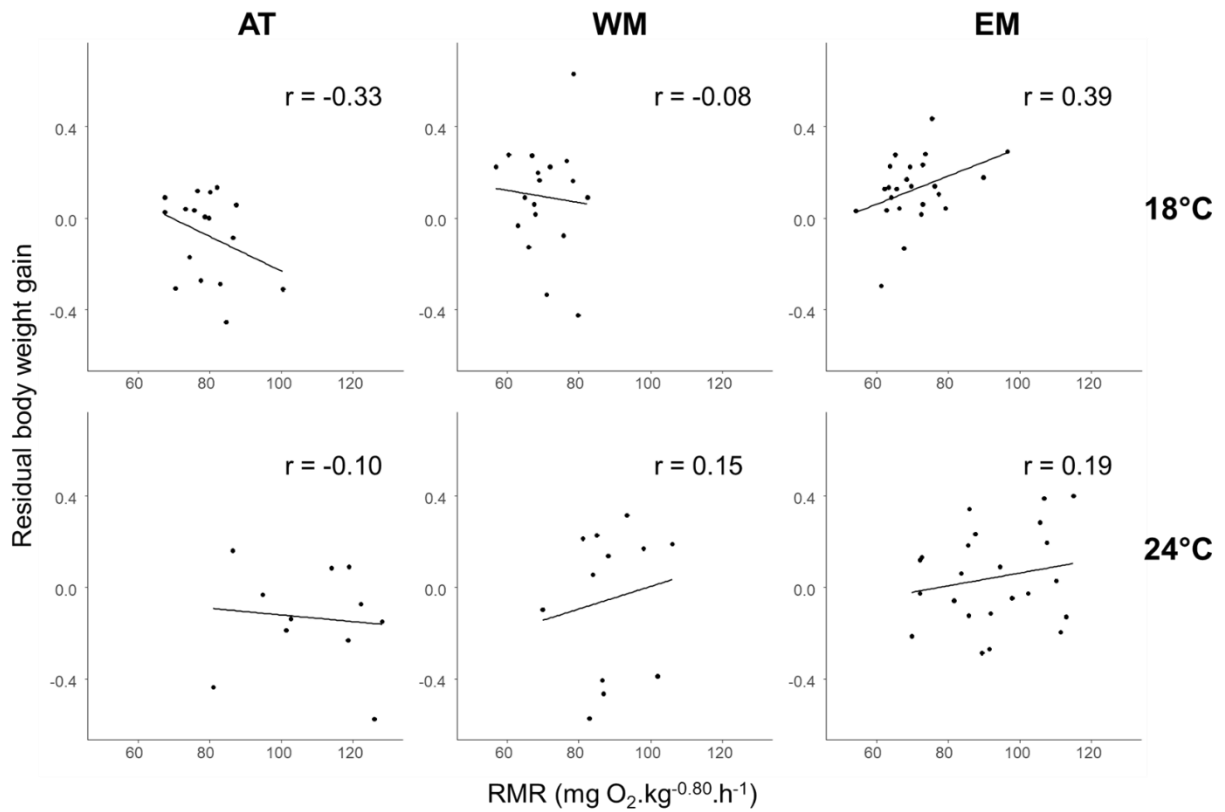


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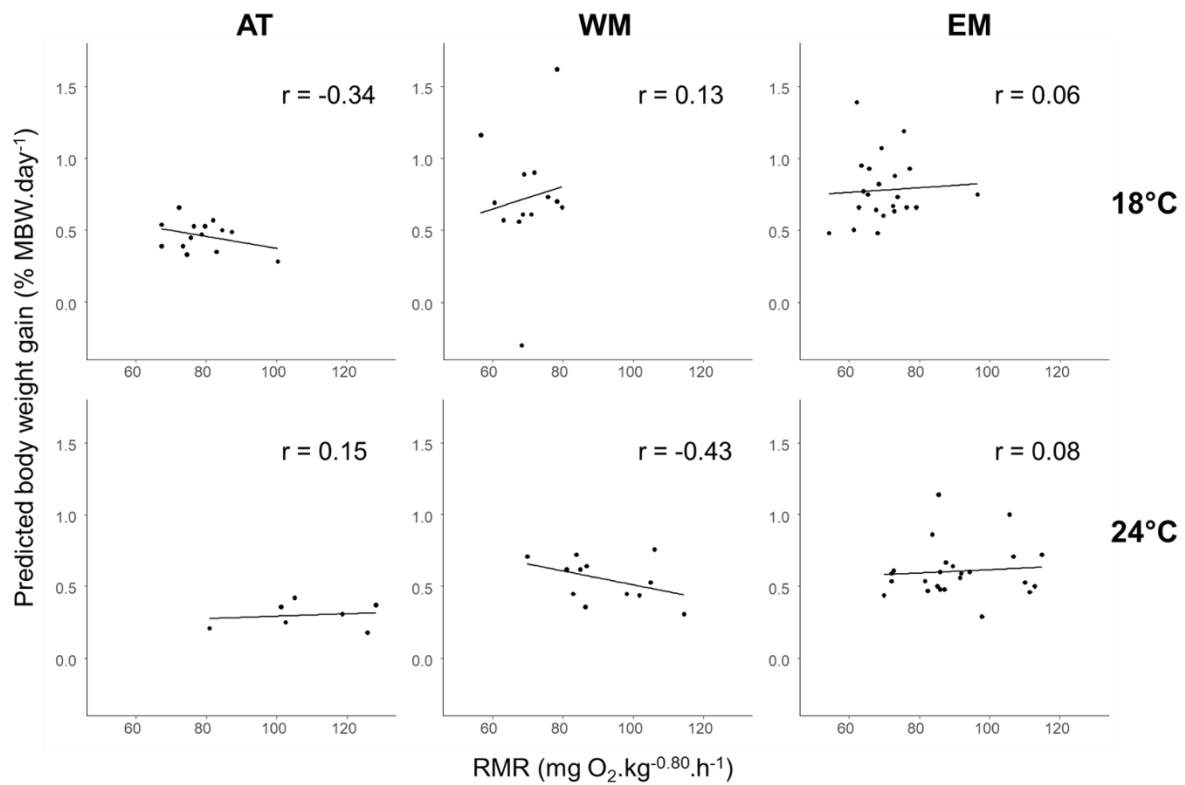
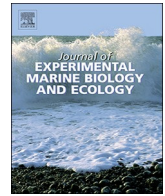


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CHAPTER V

Variations in isotope incorporation rates and trophic discrimination factors of carbon and nitrogen stable isotopes in scales from three European seabass (*Dicentrarchus labrax*) populations



Variations in isotope incorporation rates and trophic discrimination factors of carbon and nitrogen stable isotopes in scales from three European sea bass (*Dicentrarchus labrax*) populations

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ABSTRACT

Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope analyses are used in marine ecology to study trophic relationships and migrations of species since they reflect dietary sources consumed which may vary geographically. However, better estimations of isotope incorporation rates and trophic discrimination factors (TDF) under controlled conditions are required. Moreover, variability of isotope incorporation rates and TDF among and within populations has been poorly described, especially in fish scales, whereas the use of non-lethal method is becoming a standard. This study aimed to experimentally assess whether carbon and nitrogen isotope incorporation rates (λC and λN , respectively) and TDF of scales vary in the European sea bass (*Dicentrarchus labrax*) among (1) Atlantic, West Mediterranean and East Mediterranean populations, (2) sexes and (3) individuals. Fish were reared under controlled conditions and switched from a diet 1 to a diet 2 with different $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Scales were sampled repeatedly on 16 fish within the three populations, from the day of diet change (day 0) to the end of the experiment (day 217). Isotope incorporation rates of scales and TDF were determined using a time-dependent model. Isotopic carbon and nitrogen half-lives ($t_{50}\text{C}$ and $t_{50}\text{N}$) were similar among the three populations but males had significantly lower $t_{50}\text{C}$ and $t_{50}\text{N}$ than females (29 ± 2 and 35 ± 2 days vs. 53 ± 7 and 80 ± 11 days, respectively). Females had higher growth rates but lower catabolic rates than males. Variability of λC and λN was large within sexes: $t_{50}\text{C}$ ranged from 17 to 159 days and $t_{50}\text{N}$ ranged from 18 to 342 days among individuals. Thus, variability between sexes and among individuals must be considered to avoid misinterpretation in field-based studies. For the 48 fish, TDF were 4.91 ± 0.03 and $2.46 \pm 0.06\text{‰}$ for carbon and nitrogen, respectively, and similar between sexes and among populations. Besides, TDF varied among individuals from 2.95 to 5.59‰ and from 0.93 to 3.55‰ for carbon and nitrogen, respectively. Empirical mixing models were run to estimate how different TDF influenced estimation of the contributions of food sources to diet of their consumer. The output differed considerably when using TDF from fish literature or those estimated herein, which confirms that a tissue-specific TDF must be used to avoid misinterpretation in field-based studies. Individual variation in TDF did not, however, influence estimation of the contributions of food sources, confirming that scales are a valid tissue for non-lethal sampling.

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1. Introduction

Analysis of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotopes has proven to be a powerful tool in marine ecology, to study trophic relationships and migrations of various species through time and space (Hansson et al., 1997; Perga and Gerdeaux, 2003; Dempson et al., 2010; Sweeting, 2010). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of organisms reflect those of assimilated dietary sources as it is generally accepted that consumers are enriched by 1.0 and 3.5‰ in ^{13}C and ^{15}N , respectively, relative to their diets (Fry and Arnold, 1982; Minagawa and Wada, 1984). However, these assumptions are not universal and reconstruction of diet history as well as quantification of trophic relationships of organisms in their environment require better estimations of both carbon and nitrogen isotope incorporation rates and trophic discrimination factors (TDF) under controlled conditions (Wolf et al., 2009; Martínez del Río and Carleton, 2012).

Isotope incorporation rate is defined as the time required by an organism to acquire the isotopic composition of its new diet (Martínez del Río and Carleton, 2012). This variable is essential to determine the temporal window in which stable isotope data can be used to elucidate the diet of an animal (Perga and Gerdeaux, 2005; Wolf et al., 2009). Stable isotope values of an organism in a situation of disequilibrium, after a change in diet, do not represent either the past or the present diet (Sweeting, 2010). It is well established that isotope incorporation rates are higher in metabolically active species, organisms or tissues, depending on both growth rate (i.e., anabolic rate or adjunction of new tissues) and catabolic rate (i.e., replacement of tissues; Hesslein et al., 1993; MacNeil et al., 2006). Generally, isotope incorporation rates are higher in liver and plasma than in muscle and red blood cells of fishes (Carleton and Martínez del Río, 2010). Moreover, isotope incorporation rates vary with environmental conditions such as temperature, food quantity and quality as well as the physiological state of the animal, such as ontogenetic stage or level of stress (Witting et al., 2004; Carleton and Martínez del Río, 2010; Bloomfield et al., 2011; Carter et al., 2019).

Trophic discrimination factor, the difference between stable isotope values of a consumer and its diet when at isotopic equilibrium, also fluctuates considerably. For fishes, carbon and nitrogen TDF vary from 0.2 to 4.0‰ and from -0.4 to 5.5‰, respectively (Sweeting et al., 2007a, 2007b). Accurate values are required to interpret relationships across trophic levels. A robust estimation of TDF is also a fundamental requirement for mixing models that predict the proportional composition of consumers' diets from stable isotope data (Phillips et al., 2014). Physiological mechanisms underlying TDF are not thoroughly understood but result from the balance between processes of assimilation and excretion of light versus heavy elements acquired in the food (Minagawa and Wada, 1984; Ponsard and Averbuch, 1999; Olive et al., 2003). Thus, TDF is influenced by both dietary and non-dietary factors (Trueman et al., 2005; Barnes et al., 2007; Matley et al., 2016; Nuche-Pascual et al., 2018).

In isotope-based studies, variation among individuals has been evaluated as the variance of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. For fishes, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of tissues have been shown to vary within and among populations (Barnes et al., 2008), and especially with sex (Kim et al., 2012; Marcus et al., 2019). To date, however, studies of variation in isotope incorporation rates and TDF within and among populations are scarce. When distinct isotope values appear among individuals, the assumption commonly made is that they must have been feeding on distinct food sources (e.g. Grey, 2001). However, several studies revealed an inherent variability between individuals fed with a same diet (Matthews and Mazumder, 2004; Araújo et al., 2007; Barnes et al., 2008). These studies underscored the need to take this variability into account in field-studies before concluding that individuals have distinct feeding habits. Differences in individual physiology are suggested to be the cause of this inherent individual variability (Bearhop et al., 2004). Isotope incorporation rates are estimated by changing from one

isotopically distinct experimental diet to another, and then sampling tissues over time. In fishes, most isotope incorporation rate studies have used dorsal white muscle, so individuals must be sacrificed at each sampling point to monitor stable isotope values (Hesslein et al., 1993; German and Miles, 2010; Madigan et al., 2012). This precludes the study of individual variation in the kinetics of isotopic incorporation. Using non-lethal methods, such as sampling of red blood cells, plasma, fins and scales, would permit multiple sequential samplings on the same individuals, to study variation in isotope incorporation rates. Moreover, the development of non-lethal methods is desirable from a perspective of animal welfare (European Union, 2010; Australian Government, 2013; US Government, 2015). To our knowledge, the few studies that have sampled the same individuals over time after diet change have shown marked variation in carbon and nitrogen isotope incorporation rates (Hilderbrand et al., 1996; Voigt et al., 2003; Evans Ogden et al., 2004; Kim et al., 2012). Similarly, individual variation in TDF was revealed within different species (Lecomte et al., 2011; Kurle et al., 2014; Galván et al., 2016).

In the present study, we estimated isotope incorporation rates and TDF of carbon and nitrogen stable isotopes in the scales of 48 European sea bass (*Dicentrarchus labrax*) from three distinct populations (Atlantic AT, West Mediterranean WM and East Mediterranean EM; Guinand et al., 2017) reared under controlled conditions. European sea bass is highly prized by both commercial and sports fishermen, but a severe decline in stocks has recently raised concern about the conservation status of the species (de Pontual et al., 2019). The use of carbon and nitrogen stable isotope analyses is a good tool to improve the management of this species because it can reveal its feeding habitats and migrations (Cambiè et al., 2016). We assessed whether there were differences in isotope incorporation rates and TDF among (1) the three populations, (2) sexes and (3) individuals. Finally, empirical stable isotope mixing models were run with different carbon and nitrogen TDF to assess their influence on dietary predictions in field-based studies. Scales are a superposition of an organic layer mainly composed of proteins (mostly collagen) and an inorganic layer which is a carbonate salt (Hutchinson and Trueman, 2006). However, scales are not an inalterable record: several studies support the existence of a catabolic activity in scales with the destruction and the renewal of collagen by cells within the scale structure, respectively named osteoclasts and osteoblasts (Sire et al., 1990; Suzuki et al., 2000). Thus, it can be hypothesized that isotope incorporation rate in scale might not be only driven by growth.

2. Material and methods

2.1. Ethics statement

This study was carried out in accordance with the recommendations of Directive 2010-63-EU on the protection of animals used for scientific purposes. Protocols were approved by C2EA – 36 (“Comité d'éthique en expérimentation animale Languedoc-Roussillon”) under the authorization APAFiS n° 2,018,081,714,549,886 (version 2).

2.2. Animals and rearing conditions

A controlled feeding experiment was conducted on the three populations of European sea bass: AT, WM and EM. Fish were produced at the Ifremer Experimental Aquaculture Research Station in Palavas-les-Flots, France (43°31'13"N, 3°54'37"E). Fish were produced on the same day by artificial fertilization and each population reared in triplicate in nine separate tanks from birth to 188 days of age. At that stage, 51 AT, 46 WM and 51 EM fish were randomly selected from the different tanks, individually identified by injecting a passive integrated transponder tag (PIT-tag, Biolog-id®), then grouped in a 1500 L tank 21 days before the beginning of the diet change experiment. The tank was supplied with recirculated water treated by UV, sand filter and biological filter,

renewal rate was 100% per hour. Water temperature was 21.1 ± 0.9 °C and oxygen saturation rate was on average 11.9 mg L^{-1} . An artificial photoperiod was set up to provide a light-dark ratio of 12:12 h.

2.3. Diet change experiment

For 100 days until the beginning of the diet change experiment (at 209 days post hatching), fish were fed ad libitum (approximately 2.5% of their body weight per day) with a commercial diet (diet 1, "Neo Supra-S", Le Gouessant Aquaculture®, Lamballe, France). Then, fish were switched to a diet (diet 2) manufactured at the experimental fish farm of Donzacq (INRAE, France, $43^{\circ}39'20''\text{N } 0^{\circ}47'24''\text{W}$). Each diet was taken from a single bag of feed, to avoid any potential variability in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values among feed batches. Diet 2 was formulated with a similar proximate composition to diet 1 (58% of crude protein, 13% of fat, 8.4% of carbohydrates, 10% of ash and 0.5% of fiber) in order to minimize nutritional stress, but it was formulated to have markedly different $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between the two diets were achieved by inclusion of ingredients from different origins. The values of $\delta^{13}\text{C}$ were $-22.24 \pm 0.08\text{‰}$ in diet 1 vs. $-25.30 \pm 0.07\text{‰}$ in diet 2, while $\delta^{15}\text{N}$ was $7.98 \pm 0.12\text{‰}$ in diet 1 vs. $6.39 \pm 0.15\text{‰}$ in diet 2. Diets were supplied by an automatic self-feeder that fish were able to activate as desired (Covès et al., 2006).

2.4. Growth and scale sampling

At the beginning of the diet change experiment (day 0, i.e. at 209 days post hatching), 16 tagged fish per population (48 fish from the 148 fish reared in total) were randomly selected. Before any handling, all the fish were anaesthetized with benzocaine (37.5 g of ethyl 4-aminobenzoate per m^3 of seawater). The fish were weighed once a week from day 0 to day 63, once every three weeks from day 63 to day 126 and then at days 154, 161, 175 and 217. For each individual fish, growth rate (Kg) was estimated by fitting an exponential growth model to fish weight data as following:

$$W_t = W_0 e^{Kg \times t} \quad (1)$$

where W_t and W_0 are the fish weights at time t and at the beginning of the experiment respectively, Kg expressed in day^{-1} .

At each weighing time, five to ten fully formed scales were sampled from the dorsal area behind the head of each of the 48 selected fish, without taking the new scales that had regenerated after the previous samplings. Scales were obtained gently with curved pliers, taking great care not to cause any deep wounds. The fish were then treated with a povidone-iodine gel to promote the healing process. Scales were carefully rinsed with ultra-pure water (milli-Q®, Merck-Millipore, Molsheim, France), dried at 60 °C for 48 h and stored in a cool and dry place pending analysis. To assess whether the sampling protocol was stressful or not, the weights of the 48 fish were compared to those of the other fish reared in the same tank and treated identically without any scale sampling. At the end of the experiment, fish were dissected to be sexed.

2.5. Carbon and nitrogen stable isotope analysis

Preliminary isotope analyses were performed to test the influence of carbonate on $\delta^{13}\text{C}$ values of scales (Perga and Gerdeaux, 2003). Several scales were rinsed with hydrochloric acid (HCl, 2 mol L^{-1}), rinsed three times with ultra-pure water and finally dried for 12 h at 45 °C. The differences between $\delta^{13}\text{C}$ values of untreated and acid-washed scales was $-0.25 \pm 0.15\text{‰}$ and thus inferior to analytical error as previously reported by Sinnatamby et al. (2008). Consequently, scales samples were used in their raw form without treatment. Different scales from the same part of a same fish, sampled a same day, had similar carbon and nitrogen stable isotope values ($-19.81 \pm 0.18\text{‰}$ and

$9.95 \pm 0.09\text{‰}$, respectively). Furthermore, as C:N ratio of scales was 3.0 ± 0.1 , lipid extraction was not necessary (Skinner et al., 2016). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were independent of the C:N ratio ($R^2 = 0.1102$ between $\delta^{13}\text{C}$ values and C:N ratio; $R^2 = 0.0015$ between $\delta^{15}\text{N}$ values and C:N ratio). Between 0.3 and 2 mg of whole dried scales per sample were packed into a tin capsule to determine $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ simultaneously (scales were never cut or ground). Moreover, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of diets 1 and 2 were determined using pellets from different areas of each feed bag. Diets 1 and 2 were ground into a fine and homogeneous powder using a mortar and a pestle. Then, approximately 0.5 mg of powder was packed and also analysed.

Continuous-flow elemental analyzer/isotope ratio mass spectrometry (EA/IRMS) was used to analyze $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of all samples using an Isoprime GVI IRMS (Elementar, Langensfeld, Germany) interfaced with an EuroEA 3000 elemental analyzer (Eurovector, Pavia, Italia). The $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios were expressed in conventional delta (δ) notation in *per mille* (‰) relative to the levels of ^{13}C in Vienna Pee Dee Belemnite and ^{15}N in atmospheric air, according to the following equation:

$$\delta x = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}}$$

where x is ^{13}C or ^{15}N and R is the ratio of heavy to light isotope ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$). Repeated measurements on alanine exhibited a precision of $\pm 0.11\text{‰}$ and $\pm 0.12\text{‰}$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively. Commercial standards, alanine, wheat flour and corn flour from IsoAnalytical Lab (Crew, United Kingdom), IAEA-N-1, IAEA-N-2, IAEA-CH3 cellulose and USGS24 graphite from National Institute of Standard and Technology (Gaithersburg, USA) were used for a multi-point calibration.

2.6. Estimation of isotope incorporation rates, catabolic rates and trophic discrimination factors

For each fish, isotope incorporation rates of carbon and nitrogen were estimated using a single-compartment and first-order kinetic time-dependent model (Hobson and Clark, 1992):

$$\delta x_t = \delta x_{\infty} + (\delta x_0 - \delta x_{\infty}) e^{-\lambda x t} \quad (2)$$

where x is carbon or nitrogen, δx_t is the isotopic value of fish scale at time t , δx_{∞} is the estimated asymptotic stable isotope value that fish scales reach at the steady state with their new diet, δx_0 is the isotopic value of fish scale at the beginning of the diet change experiment, and λx is the isotope incorporation rate (expressed in day^{-1}). A one-compartment model was chosen as this is more relevant than a multi-compartment model for scales (Heady and Moore, 2013).

Isotopic half-life, i.e. the time needed for half of the carbon or nitrogen in the scales to be replaced by atoms from a new diet, was calculated as:

$$t_{50x} = \frac{\ln(2)}{\lambda x} \quad (3)$$

where t_{50} is the isotopic half-life (expressed in days), x is carbon or nitrogen, and λx is the estimated value of isotope incorporation rate.

In order to estimate how long it takes to reach an equilibrium state, the time needed for 95% of the scale carbon or nitrogen to be replaced by atoms from a new diet was calculated as:

$$t_{95x} = \frac{\ln(20)}{\lambda x} \quad (4)$$

The relative contribution of growth and catabolic rates to change in carbon and nitrogen stable isotope values were estimated using a time-dependent model. Isotope incorporation rate (λ) is the result of joint contribution of growth rate (Kg) and catabolic rate (Kc, Hesslein et al., 1993). For each individual, catabolic rates of carbon and nitrogen were determined as:

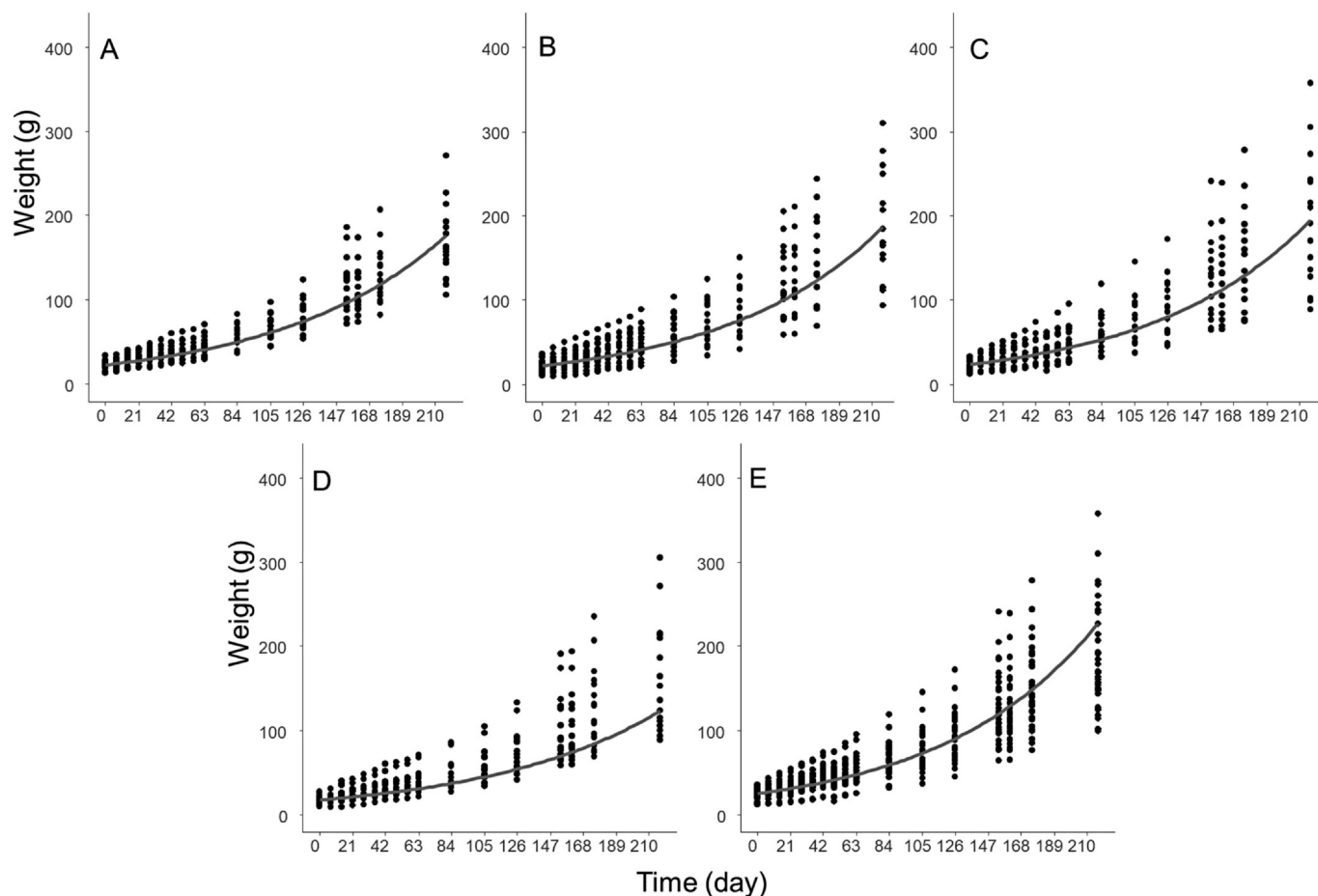


Fig. 1. Fish growth according to the population: Atlantic (A, $n = 16$), West Mediterranean (B, $n = 16$) and East Mediterranean (C, $n = 16$) populations as well as males (D, $n = 17$) and females (E, $n = 31$). Curved lines indicate the mean experimental growth model fitted to measured data using iterative nonlinear regression. Points indicate the weight measured for each fish.

$$\lambda x = Kg + Kcx \quad (5)$$

where x is carbon or nitrogen, Kg is estimated using eq. 1 and Kcx is the catabolic rate (expressed in day^{-1}).

Finally, carbon and nitrogen diet-to-fish trophic discrimination factors (TDF) was calculated for each fish as:

$$\Delta x = \delta x - \delta x_{\text{diet}} \quad (6)$$

where Δx is the TDF (expressed in ‰), x is carbon or nitrogen, δx is the stable isotope value measured in fish scales at the end of the experiment (δx at day 217) or at the steady-state (δx_{∞} estimated by eq. 2) and δx_{diet} is the stable isotope value of the diet.

2.7. Empirical mixing models

To further explore the importance of using an accurate TDF, empirical mixing models were run with six different sets of carbon and nitrogen TDF. Sets chosen were: (1) 1.5 and 2.75‰, (2) 5.01 and 2.50‰, (3) 4.83 and 2.56‰, (4) 4.90 and 2.36‰, (5) 2.95 and 0.93‰, (6) 5.59 and 3.55‰, for carbon and nitrogen respectively. Set (1) is from literature for fish tissues (Sweeting et al., 2007a, 2007b). Sets (2), (3) and (4) are corresponding to the average values estimated in this study for scales from AT, WM and EM, respectively. Sets (5) and (6) are corresponding to minimal and maximal values estimated in this study. These five sets reflect the variation in scale TDF at population and individual levels. The relevance of empirical mixing models was to determine (1) whether it was really necessary to use TDF of scales rather than those of others tissues from fish literature and (2) whether the variability measured among populations or individuals influenced the

predictions of the mixing model. The contributions of the two hypothetical diets (source 1 and source 2) were determined using *simmr* package (“Stable Isotope Mixing Models in R”, Parnell et al., 2013; Parnell, 2019). Empirical $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of fish scales, source 1 and source 2 were -15 and 8‰ ; -25 and 11‰ and -15 and 2‰ , respectively. The carbon and nitrogen stable isotope values of sources 1 and 2 were chosen to have almost an equal contribution of 50%/50% to the diet when estimated with carbon and nitrogen TDFs obtained on the 48 fish.

2.8. Statistical analysis and modelling

The exponential growth model and time-dependent incorporation models were firstly applied to the combined data from all 48 fish, using iterative nonlinear regression with the “nlme()” function from the *nlme* package (Pinheiro et al., 2018) in R (version 3.5.2., R Core Team, 2018) assuming that individual effect was a random effect with a normal distribution. Growth rate (Kg), as carbon and nitrogen isotope incorporation rates (λx), catabolic rates (Kcx) and TDF (Δx) were thus estimated for the whole fish group while taking into account variability among individuals. Secondly, each parameter was estimated for each population and sex, and values were compared among populations and sexes (considered as covariates) using an analysis of variance (ANOVA; Pinheiro and Bates, 2000). Pairwise differences were then explored using post hoc Student tests. The standard error (SE) of the parameters, i.e. the accuracy of the estimations made by the models, was calculated for the whole group, the populations and sexes. We then estimated one value of each parameter for each individual, using the same models.

Table 1

Estimated parameters from eqs. 1 to 6 using iterative nonlinear regression: growth rate (Kg), carbon and nitrogen isotope incorporation rates (λC and λN), carbon and nitrogen half-lives (t_{50C} and t_{50N}), time to reach carbon and nitrogen isotopic equilibria with the new diet (t_{95C} and t_{95N}), carbon and nitrogen catabolic rates (KcC and KcN), $KcC/\lambda C \times 100$ and $KcN/\lambda N \times 100$, asymptotic values $\delta^{13}C_{\infty}$ and $\delta^{15}N_{\infty}$ as well as carbon and nitrogen asymptotic trophic discrimination factors (ΔC_{∞} , ΔN_{∞}). Measured $\delta^{13}C$ and $\delta^{15}N$ values ($\delta^{13}C_{217}$ and $\delta^{15}N_{217}$) as well as carbon and nitrogen trophic discrimination factor (ΔC_{217} , ΔN_{217}) at day 217. Mean values are given with standard error ($\pm SE$, $n = 48$ for all fish, $n = 18$ for AT, WM and EM populations, $n = 17$ and 31 for males and females, respectively). Standard error reflects the accuracy of the estimations provided by the models fitted at whole group level as well as at population and sex levels.

	All fish	AT	WM	EM	Males	Females
Kg (day^{-1})	0.0097 \pm 0.0003	0.0095 \pm 0.0005	0.0098 \pm 0.0005	0.0098 \pm 0.0005	0.0090 \pm 0.0004b	0.0101 \pm 0.0003a
Carbon						
λC (day^{-1})	0.0210 \pm 0.0010	0.0231 \pm 0.0019	0.0196 \pm 0.0018	0.0208 \pm 0.0018	0.0235 \pm 0.0017a	0.0198 \pm 0.0012b
t_{50C} (day)	33 \pm 2	30 \pm 2	35 \pm 3	33 \pm 3	29 \pm 2a	35 \pm 2b
t_{95C} (day)	143 \pm 7	130 \pm 11	153 \pm 14	144 \pm 13	127 \pm 9a	151 \pm 9b
KcC (day^{-1})	0.0115 \pm 0.0011	0.0140 \pm 0.0019	0.0098 \pm 0.0018	0.0110 \pm 0.0018	0.0146 \pm 0.0018a	0.0098 \pm 0.0013b
$KcC/\lambda C \times 100$ (%)	53.3	60.6	50.0	52.9	62.1	49.5
$\delta^{13}C_{\infty}$ (‰)	-20.39 \pm 0.03	-20.29 \pm 0.05A	-20.47 \pm 0.06B	-20.40 \pm 0.05A,B	-20.42 \pm 0.05	-20.36 \pm 0.04
ΔC_{∞} (‰)	4.91 \pm 0.03	5.01 \pm 0.05A	4.83 \pm 0.06B	4.90 \pm 0.05A,B	4.88 \pm 0.05	4.94 \pm 0.04
$\delta^{13}C_{217}$ (‰)	-20.25 \pm 0.04	-20.17 \pm 0.09	-20.29 \pm 0.06	-20.29 \pm 0.08	-20.31 \pm 0.08	-20.21 \pm 0.05
ΔC_{217} (‰)	5.05 \pm 0.04	5.13 \pm 0.09	5.01 \pm 0.06	5.01 \pm 0.08	4.99 \pm 0.08	5.09 \pm 0.05
Nitrogen						
λN (day^{-1})	0.0103 \pm 0.0010	0.0105 \pm 0.0017	0.0108 \pm 0.0017	0.0094 \pm 0.0017	0.0130 \pm 0.0017a	0.0087 \pm 0.0012b
t_{50N} (day)	67 \pm 7	66 \pm 11	64 \pm 10	74 \pm 14	53 \pm 7a	80 \pm 11b
t_{95N} (day)	291 \pm 29	285 \pm 47	277 \pm 45	319 \pm 60	230 \pm 31a	344 \pm 48b
KcN (day^{-1})	0.0006 \pm 0.0010	0.0010 \pm 0.0017	0.0010 \pm 0.0017	-0.0004 \pm 0.0017	0.0040 \pm 0.0017a	-0.0014 \pm 0.0012b
$KcN/\lambda N \times 100$ (%)	5.8	9.5	9.3	0	30.8	0
$\delta^{15}N_{\infty}$ (‰)	8.85 \pm 0.06	8.89 \pm 0.09	8.95 \pm 0.09	8.75 \pm 0.10	8.86 \pm 0.08	8.82 \pm 0.08
ΔN_{∞} (‰)	2.46 \pm 0.06	2.50 \pm 0.09	2.56 \pm 0.09	2.36 \pm 0.10	2.47 \pm 0.08	2.43 \pm 0.08
$\delta^{15}N_{217}$ (‰)	9.15 \pm 0.05	9.18 \pm 0.08	9.22 \pm 0.08	9.06 \pm 0.11	9.09 \pm 0.08	9.19 \pm 0.07
ΔN_{217} (‰)	2.76 \pm 0.05	2.79 \pm 0.08	2.83 \pm 0.08	2.67 \pm 0.11	2.70 \pm 0.08	2.80 \pm 0.07

Upper and lower case letters indicate significant differences among populations and sexes, respectively (ANOVA, $p < 0.05$).

The mean and the standard deviation (i.e. the variability) of each parameter were then calculated combining all individual estimations. The $\delta^{13}C$ and $\delta^{15}N$ values measured at day 217 were compared among populations and sexes with an ANOVA applied to a linear model, considering population or sex as a fixed effect. The assumptions of normality and homoscedasticity of residuals were tested with Shapiro-Wilk and Bartlett tests, respectively, for both nonlinear and linear models.

3. Results

3.1. Fish growth rates and survival

Fish survival was 100% throughout the experiment and no significant difference appeared when comparing growth rates between sampled and non-sampled fish. Among the sampled fish, the numbers of males and females were respectively 6 and 10 for AT, 5 and 11 for WM and 6 and 10 for EM.

During the experiment, fish grew exponentially from 22.5 ± 5.5 g to 167.2 ± 45.3 g for AT, 22.3 ± 7.4 g to 183.6 ± 64.6 g for WM and 23.2 ± 7.2 g to 188.2 ± 80.7 g for EM (Fig. 1). Growth rates (Kg) were similar among populations (Table 1, $p > 0.05$, ANOVA). In contrast, Kg differed significantly according to sex (Fig. 1, Table 1, $p < 0.001$, ANOVA) with the 31 females having a higher Kg than the 17 males. Females grew from 25.5 ± 5.6 g to 188.9 ± 62.4 g (0.75 g day^{-1}) whereas males grew from 17.6 ± 4.8 g to 162.4 ± 61.8 g (0.67 g day^{-1}). Individual growth rate varied from 0.0061 to 0.0126 (mean \pm standard deviation: 0.0090 ± 0.0019) and from 0.0066 to 0.0136 day^{-1} (0.0101 ± 0.0016) for males and females, respectively.

3.2. Isotope incorporation rates

To reach a new equilibrium with diet 2, $\delta^{13}C$ and $\delta^{15}N$ values in the scales of the 48 fish rapidly changed with time from -17.72 ± 0.60 to -20.25 ± 0.28 ‰ and from 10.85 ± 0.36 to 9.15 ± 0.36 ‰, respectively (Fig. 2 and Fig. 3). Carbon and nitrogen isotope incorporation rates (λC and λN , respectively) were accurately estimated using a

single compartment first-order kinetic time-dependent model. Neither λC nor λN were significantly different among the three populations ($p > 0.05$, ANOVA, Table 1). The mean time necessary for half of the carbon and nitrogen in the 48 fish scales to be replaced by new atoms following the diet change was 33 days for carbon and 67 days for nitrogen. A diet steady state would be reached after 143 days and 291 days for carbon and nitrogen, respectively (Table 1).

Both λC and λN were significantly different between sexes ($p < 0.01$, ANOVA, Table 1) with males having higher λC and λN than females. Carbon and nitrogen half-lives differed markedly among individuals. Carbon half-life varied from 17 to 159 days (mean \pm standard deviation: 34 ± 32 days) and from 15 to 143 days (45 ± 29 days), for males and females, respectively. Nitrogen half-life varied from 18 to 107 days (54 ± 26 days) and from 34 to 342 days (87 ± 66 days) for males and females, respectively.

3.3. Contribution of growth and catabolic rates to isotopic incorporation

Carbon and nitrogen Kc of fish scales were similar among the three populations ($p > 0.05$, ANOVA, Table 1). In contrast, KcC and KcN differed significantly between sexes, with males having higher Kc than females ($p < 0.05$, ANOVA, Table 1). Regarding KcN , it was never significantly different from zero, except for males ($p < 0.05$, Student test, Table 1). Carbon Kc varied from -0.0028 to 0.0318 day^{-1} (mean \pm standard deviation: 0.0189 ± 0.0093 day^{-1}) and from -0.0060 to 0.0337 day^{-1} (0.0107 ± 0.0102 day^{-1}) for males and females, respectively. Nitrogen Kc varied from -0.0058 to 0.0315 day^{-1} (0.0074 ± 0.0094 day^{-1}) and from -0.0076 to 0.0078 day^{-1} (0.0008 ± 0.0047 day^{-1}) for males and females, respectively.

3.4. Sensitivity of empirical mixing models to TDF

At day 217, scales from the 48 fish reached carbon and nitrogen steady-state with the new diet. Fish scale $\delta^{13}C$ and $\delta^{15}N$ values measured at day 217 were close to the asymptotic values estimated by the model. Indeed, the differences between $\delta^{13}C$ and $\delta^{15}N$ values measured

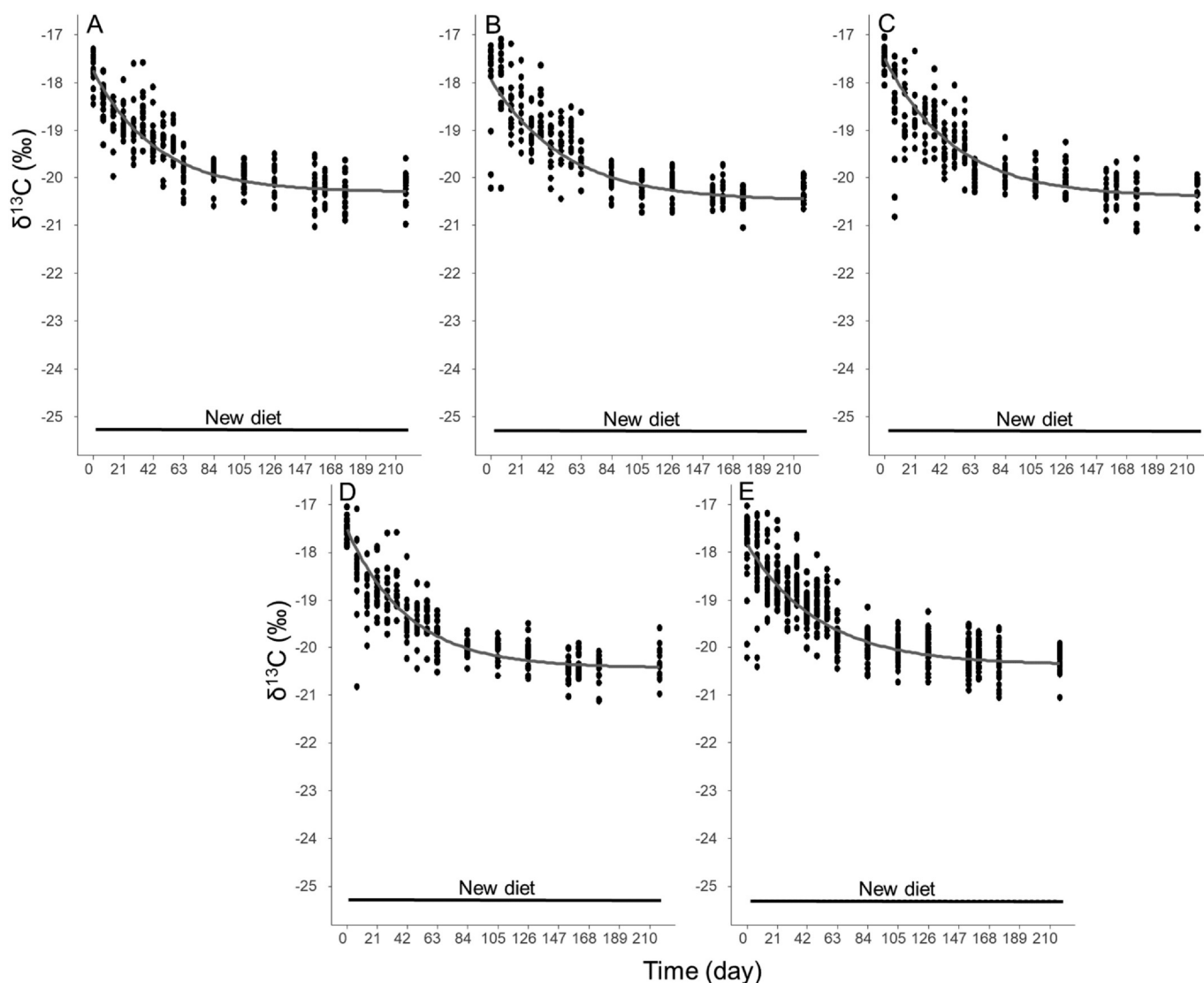


Fig. 2. Change in $\delta^{13}\text{C}$ values of fish scales following a diet change according to the population: Atlantic (A, $n = 16$), West Mediterranean (B, $n = 16$) and East Mediterranean (C, $n = 16$) populations as well as males (D, $n = 17$) and females (E, $n = 31$). Straight lines represent the $\delta^{13}\text{C}$ value for the new diet (diet 2). Curved lines represent the mean single-compartment first-order kinetic model fitted to measured data using iterative nonlinear regression. Points indicate $\delta^{13}\text{C}$ values of scales measured for each fish.

at day 217 and asymptotic values were 0.14‰ and 0.30‰, respectively (eq. 2, Table 1). Based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values measured at day 217, carbon and nitrogen TDF were not significantly different among populations or sexes ($p > 0.05$, ANOVA, Table 1). However, when based on the estimated asymptotic values, carbon TDF was significantly different among populations ($p < 0.05$, ANOVA, Table 1). This difference was, however, inferior to the analytical error (less than 0.2‰ between minimal and maximal TDF among populations). Carbon and nitrogen TDF varied among individuals from 2.95 to 5.59‰ (mean \pm standard deviation: 4.83 ± 0.44 ‰) and from 0.93 to 3.55‰ (2.63 ± 0.56 ‰), respectively.

In empirical mixing models, the use of average carbon and nitrogen TDF estimated for each population, as well as minimal and maximal TDF, had no significant influence on the relative contributions of source 1 and source 2 to fish diet (Fig. 4). Indeed, the different scale TDF led to a predicted contribution of source 1 between 41.5 and 45.2% and a predicted contribution of source 2 between 54.8 and 58.5%. On the other hand, the use of carbon and nitrogen TDF estimates for fish tissues from the literature caused a large under-estimation of the source 1 (24.4%) and over-estimation of the source 2 (75.6%), compared to the

use of specific fish scale carbon and nitrogen TDF.

4. Discussion

In the present study, non-lethal sampling of scales permitted estimation of how carbon and nitrogen isotope incorporation rates, and TDF, differed among populations, sexes and individuals of European sea bass. This revealed variation between sexes in both carbon and nitrogen isotope incorporation rates, among populations in carbon TDF, and was particularly marked among individuals. Empirical mixing models showed that (1) the specific TDF of scales is needed to obtain accurate predictions, literature values for other tissues are not satisfactory, and (2) the variability that existed among populations or individuals did not influence mixing model predictions.

4.1. Variation in growth rate

Our results highlighted that growth rates of fish (Kg) were equivalent between fish from AT, WM and EM populations. These results were not in accordance with Vandeputte et al. (2014). The differences

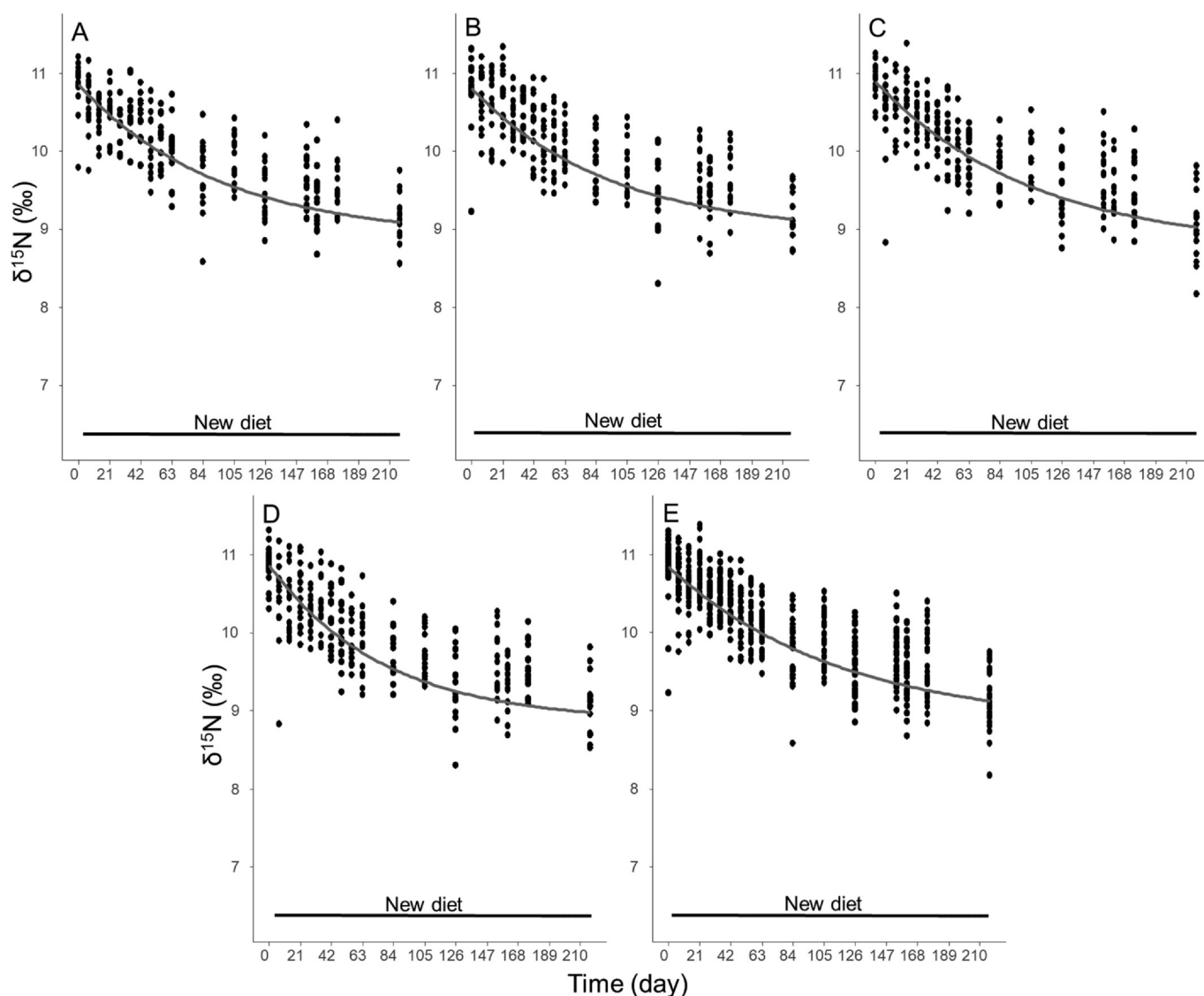


Fig. 3. Change in $\delta^{15}\text{N}$ values of fish scales following a diet change according to the population: Atlantic (A, $n = 16$), West Mediterranean (B, $n = 16$) and East Mediterranean (C, $n = 16$) populations as well as males (D, $n = 17$) and females (E, $n = 31$). Straight lines represent the $\delta^{15}\text{N}$ value for the new diet (diet 2). Curved lines represent the mean single-compartment first-order kinetic model fitted to measured data using iterative nonlinear regression. Points indicate $\delta^{15}\text{N}$ values measured of scales for each fish.

between both studies may be explained by the design of the experimental system such as feed, temperature, rearing density, anaesthesia frequencies or other uncontrolled effects. Fish multiplied their weight by eight after 217 days of experimentation. The 48 fish sampled for stable isotope analyses had similar final weights to non-sampled fish present in the rearing system indicating that frequent scale sampling does not markedly impact fish welfare.

Our study confirmed that Kg of European sea bass is influenced by sex with the 31 females having 12% higher Kg than the 17 males. Previous studies have also shown that females are larger than males at a given age, with differences ranging between 20 and 40% (Chatain et al., 1997; Gardeur et al., 2001; Saillant et al., 2001).

4.2. Variation in isotope incorporation rates

Isotopic carbon and nitrogen half-lives (t_{50C} and t_{50N}) of fish scales were similar among the three fish populations. Carbon t_{50} was estimated to be 33 days whereas nitrogen t_{50} was estimated to be 67 days for whole scales of fish. It is important to note this result was obtained in juvenile fish and would probably be different in other development

stages. Values estimated for nitrogen were relatively close to those reported in scales of *Oncorhynchus mykiss* (27.7 days for nitrogen, Heady and Moore, 2013) but different from those reported for *Barbus barbus* (145 days, Busst and Britton, 2018). To our knowledge, no estimation of t_{50C} is available in literature for fish scales. In the present study, t_{50C} was half the t_{50N} . Depending on species, tissues and environmental conditions, t_{50C} and t_{50N} have almost any relationship: they can be closely linked (Herzka and Holt, 2000; Vander Zanden et al., 2015), t_{50C} can be higher than t_{50N} (Church et al., 2009; Lefebvre and Dubois, 2016) or lower (Carleton and Martínez del Río, 2005). Such differences between t_{50C} and t_{50N} indicate a decoupling of carbon and nitrogen metabolic pathways and are dependent upon the sources of carbon and nitrogen used for de novo synthesis of proteins in scales. Fish scale is composed of an organic layer (mainly collagen) and an inorganic layer (carbonate salt). However, carbonate content in the scales is very low so the measured $\delta^{13}\text{C}$ value of scales only represents the organic layer (Hutchinson and Trueman, 2006). Proteins are synthesized from dietary and non-dietary sources including proteins, lipids and carbohydrates. Firstly, the use of dietary carbohydrates and lipids rather than dietary proteins to build proteins of fish scales could explain such carbon and

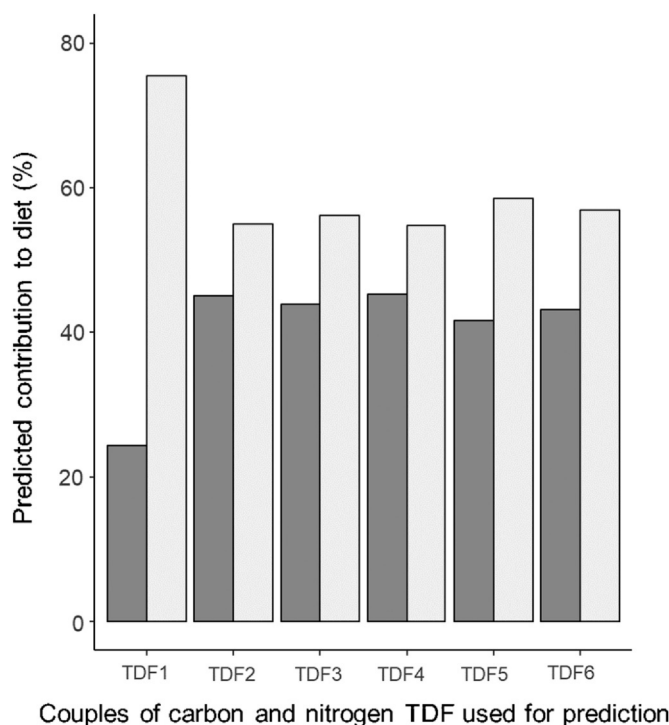


Fig. 4. Estimation of the contributions of two food sources to fish diet using mixing models with different couples of carbon and nitrogen trophic discrimination factors (TDF). TDF 1 is 1.5 and 2.75‰ estimated for fish tissues by Sweeting et al. (2007a, 2007b). TDF 2, 3 and 4 are: 5.01 and 2.50‰, 4.83 and 2.56‰, 4.90 and 2.36‰, corresponding to the average values estimated in this study for scales from respectively Atlantic, West Mediterranean and East Mediterranean fish. TDF 5 and 6 are: 2.95 and 0.93‰, 5.59 and 3.55‰, corresponding to the minimal and maximal values estimated in this study. Grey and white bars are the percentages of source 1 and source 2, respectively, estimated by the empirical mixing models.

nitrogen decoupling (Hobson and Bairlein, 2003). Since neither carbohydrates nor lipids can provide the nitrogen in proteins, lower value of t_{50C} than t_{50N} can be explained by an increase of the contribution of endogenous nitrogen to synthesize amino acids, leading to a decrease of dietary nitrogen incorporation. Conversely, it can be hypothesized that dietary vs. non dietary contributions of carbon remain constant (Carleton and Martínez del Río, 2005). Secondly, Carleton and Martínez del Río (2005) hypothesized that dietary nitrogen incorporation is reduced compared to dietary carbon incorporation when nitrogen is used to synthesize non-essential amino acids. Endogenous nitrogen is reused after amino acid degradation to synthesize new non-essential amino acids, instead of being excreted. In contrast, this process does not exist with essential amino acids because they cannot be synthesized and are obtained exclusively from feed. As scale collagen is mainly composed of non-essential amino acids (i.e. glycine, alanine and proline) with essentials estimated to comprise less than 20% of scale collagen (Kimura et al., 1991; Kaushik, 1998), a decoupling between carbon and nitrogen may occur.

Results of the time-dependent model indicated that K_c contributed to 53.3% and 5.8% to λ_C and λ_N , respectively. Regarding nitrogen, previous studies reported that K_cN was close to zero in fish scales, but did not indicate the sex of the sampled fish (Heady and Moore, 2013; Busst and Britton, 2018). Present results are consistent with previous studies, except for males whose K_cN contributed to 30.8% to λ_N . In the case of carbon, isotope incorporation rate seems to be partly driven by catabolic rate. Thus, the hypothesis that isotope incorporation rate in scales was not driven only by growth seems to be validated for carbon, but not for nitrogen (except in the case of males). To estimate K_cC and K_cN , we assumed the change in total weight of fish was a reliable proxy

to estimate K_g of scales. This assumption is supported by Leim (1924) and Heidarsson et al. (2006) who proved that specific growth rate of whole fish was correlated to specific growth rates of scales with a 1:1 ratio. Moreover, the elemental composition of scales (i.e. C:N ratio) was constant throughout the diet change experiment (3.0 ± 0.1 for C:N ratio, $23.29 \pm 2.04\%$ and $7.68 \pm 0.67\%$ for C and N percentages, respectively). Consequently, we concluded that no shift in scale composition occurred over time and thus whole fish K_g could be used without bias to estimate both carbon and nitrogen catabolic rates. However, whole fish K_g likely remains a rough estimation of the true K_g of carbon and nitrogen in scales and other factors such as moisture concentration in whole fish could be taken into account to improve K_g estimation in scales. Thus, further investigation is required to validate our results.

It is interesting that males and females differed in their carbon and nitrogen half-lives. Although females had higher K_g than males, lower K_cC and K_cN were estimated. These results would support that K_g is inversely correlated with K_c in young fish with smaller males having higher K_c (Rossignol et al., 2011). However, the variability of t_{50C} and t_{50N} was high within each sex. For example, some females reached t_{50C} after only 17 days whereas others needed more than 150 days. Similar variations have been measured in muscle of leopard shark with t_{50C} varying from 150 to 792 days among individuals (Kim et al., 2012). As variability of K_g within males and females was low (standard deviation: mean ratio was around 0.15 for each sex), these results suggest that such variability of λ_C and λ_N was due to the high variability of K_cC and K_cN , respectively. This level of variability in growth rate is similar to previous reports for European sea bass (Gardeur et al., 2001; Vandeputte et al., 2014).

The results of our study provide important understanding of the isotopic clock, which is essential to interpret diet and habitat shifts over time in field (Phillips and Eldridge, 2006; Wolf et al., 2009; Sweeting, 2010). In field-based stable isotope studies, the identification of the new food sources using scales of juvenile fish will require at least 143 and 291 days after a diet change for carbon and nitrogen, respectively. Moreover, after a diet change, female European sea bass will need 19% and 50% more time than males to reach an isotopic equilibrium for carbon and nitrogen, respectively. The potential variability among individuals will also be important to consider to avoid misinterpretation of field data. Our results highlight that strong variation in individual stable isotope values can appear even if individuals are fed with a similar diet. Thus, as already mentioned by Barnes et al. (2008), one should be careful before concluding that individual variation in isotopic values is a proof that individuals rely on distinct food sources. Variability in isotope incorporation rates was linked to the intrinsic metabolism of each fish rather than to a measurement bias. Carbon and nitrogen stable isotope values were similar among scales from the same part of a same fish. Furthermore, neither carbonate nor lipid content of the scales could account for the variability. The variability in isotope incorporations rates of scales does not preclude the use of this tissue in field-based studies. Indeed, individual variability in isotope incorporations rates of scales was less than that found for red blood cells and muscle, two tissues frequently used in field-based studies (Kim et al., 2012). The use of scales to elucidate ecology of fish must consider the time required to reach an equilibrium state with diet. As individual variability must be high, interpretation of results needs to take into account this constraint that is true for other tissues.

4.3. Trophic discrimination factor

Our values of carbon and nitrogen TDF of scales (4.91‰ and 2.46‰, respectively) are within the range of TDF reported for fish scales in the literature (Heady and Moore, 2013; Busst and Britton, 2016, 2018). For example, Busst and Britton (2016) reported carbon TDF of 4.7 and 4.9‰ and nitrogen TDF of 2.4 and 2.4‰ for *Barbus barbus* and *Squalius cephalus* scales, respectively. Accurate TDF of a

species can only be estimated when equilibrium with the new diet has been reached. Therefore, if the TDF estimated using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values measured at day 217 included any vestiges of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from the diet 1, prior to the diet change, they would not be valid and reliable. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values measured at day 217 were, however, similar to those estimated by the model at the asymptote, indicating that fish scales were indeed at equilibrium with their new diet; any influence of diet 1 was negligible. The higher value of carbon relative to nitrogen contrasts with values reported for other fish tissues such as white muscle (Sweeting et al., 2007a, 2007b). In fact, carbon and nitrogen TDF of scales reflect the amino acid composition of collagen that differs from that of muscle (Howland et al., 2003). The variability found among individuals for carbon and nitrogen TDF (from 2.95 to 5.59‰ and from 0.93 to 3.55‰, respectively) is broader than that previously reported for scale collagen (Guiry and Hunt, 2020). Individual variability of TDF in scales may vary depending upon fish species and whether scales were analysed in their raw form or if collagen, their main component, was extracted for separate analysis (e.g. Guiry and Hunt, 2020). Our results highlighted that the output of the mixing model considerably differs when using TDF of fish tissues from literature (Sweeting et al., 2007a, 2007b) or TDF estimated from scales. In isotope field-based studies, we recommend to use scale-specific TDF to avoid any bias in the estimation of the contributions to diet of different food sources. Although TDF were variable in scales among populations and individuals, estimation of the contribution of each food source to diet based upon the TDF showed almost no variability. Consequently, variation in scale TDF is not an obstacle in field-based studies to accurately determine the contributions to diet of various food sources.

To conclude, present results highlight the need to take into account individual variation in field-based studies. In particular, individual variation can have a strong impact when scheduling sampling campaigns, to ensure all fish have reached equilibrium after a diet change, and when discussing whether fish rely on distinct or similar food sources.

Author contributions

CR, HdV and SN designed the experiment; CR, HdV and FC performed the experiment on the fish; CR and CM analysed the scale samples; CR, HdV, SL, CM, and SN analysed the data; CR, HdV and SN wrote the paper with the support of MV, FA, DJMK and JAHB. All authors read and approved the final manuscript.

Declaration of Competing Interest

None.

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DISCUSSION

Selective breeding programs offer promise for sustainable improvements to feed efficiency in farmed fishes. Nonetheless, protocols to select for feed efficiency have not yet been developed due to a lack of critical information. In my thesis research, I aimed to address some major methodological issues in estimating individual feed efficiency and to evaluate indirect selection criteria for this trait. I studied two different species, the Nile tilapia *Oreochromis niloticus* and the European sea bass *Dicentrarchus labrax*. In Nile tilapia, I firstly estimated individual feed efficiency from juvenile to commercial size. I then estimated individual feed efficiency with two methods (individual rearing and video-recording on groups), under two feeding rates (optimal and restricted) and in two strains (GIFT strain in Malaysia and Cirad strain in France). In European sea bass, I studied individual feed efficiency in three different populations (AT, WM and EM) reared at two different temperatures (18°C and 24°C), with feeding rates ranging from *ad libitum* down to fasting. I finally focussed upon oxygen consumption and stable isotope values as potential predictors of individual feed efficiency.

In light of my results, I will discuss whether the approaches developed can be generalized across biological models and rearing conditions to set up selective breeding programs. I will then propose some ideas to identify indirect selection criteria for feed efficiency. These ideas will be based upon my results plus the potential for implementing other existing tools.

1. Which method should be used to measure individual feed intake?

Two methods were used to measure individual FI: individual rearing and video-recording on groups. These methods were chosen because individual FI is measured for each meal over several consecutive days, unlike the use of X-radiography.

Focussing on a single method would have advantages, such as that future studies will be easier to compare and efforts could be concentrated to optimise technical aspects of FI measurement.

Technical and biological aspects of both methods must, however, be considered in the light of the results, before concluding that one is better than the other.

1.1. Technical aspects

The goal is to develop methods that are viable for selective breeding programs, so technical aspects cannot be neglected. Table 4 sums up critical steps of both methods based on the experience gained.

Table 4. Main technical aspects of the video-recording and individual rearing methods. Fish are assumed to be juveniles and only one person phenotypes them.

	Video-recording method	Individual rearing method
Feeding trial	<p>Nile tilapia: one week necessary for acclimation and at least another week to estimate individual feed efficiency (to observe growth variability).</p> <p>Not used in European sea bass.</p>	<p>Nile tilapia: one week necessary for acclimation and at least another week to estimate individual feed efficiency.</p> <p>European sea bass: four weeks necessary for acclimation and at least ten days to estimate individual feed efficiency.</p>
Feeding fish	<p>Nile tilapia: pellets are given one after another and by hand. About 8 minutes necessary to feed a group of ten fish (each weighing 10 g) with an optimal ration. For 200 fish: 2 hours and 40 minutes per meal (two meals per day). Probably not feasible in larger Nile tilapia because the onset of sexual maturity increases aggressive behaviour and fish mortality (reviewed by Gonçalves-de-Freitas et al., 2019).</p> <p>European sea bass: not used because fish will probably not eat while a human being is close to their aquarium.</p>	<p>Not species-dependent. Feed rations are prepared on a weekly basis: the amount of feed is weighed and shared equally among meals. This takes about 4 hours per week. About 1 hour per meal for 200 fish fed without automatic feeders (however European sea bass may not eat before the experimenter has left the room). About 15 minutes necessary per meal for 200 fish fed with automatic feeders (to check they have worked properly). However, automatic feeders require frequent cleaning and repair, in particular for use in seawater.</p>
Collecting and counting uneaten pellets	<p>(Step not included: 0 minutes)</p>	<p>Not species-dependent, but strongly feeding rate-dependent. For 200 fish wasting up to 30 pellets per meal under an excess feeding rate, up to three hours are necessary after each meal. For 200 fish under an optimal (but not excessive) or restricted feeding rate, only 30-60 minutes are necessary after each meal. Using this method, all individual FI data are available by the end of the feeding trial.</p>
Analysing video-recordings	<p>Nile tilapia: analysis requires about 20 minutes per meal for ten fish fed an optimal ration, and 10 minutes for a restricted ration (50% of optimal). For 200 fish, 12 meals per week in a two-week feeding trial (one week for each feeding rate), video-analysis requires about 120 hours. For a selective breeding program phenotyping five batches of 200 fish with an optimal feeding rate for one week, 400 hours would be necessary. Individual FI data are thus obtained a long time after the end of the feeding trial. The time needed is, however, experimenter-dependent. This is particularly long, but software to automate video-analysis may soon become available (H. de Verdal, personal communication, 2020).</p>	<p>(Step not included: 0 minutes)</p>

Using the video-recording method on European sea bass seems impossible because, from our own experience, fish stop feeding while a human being is close to their aquarium. However, individual rearing is feasible at medium to high scale. I managed to rear 200 fish simultaneously so phenotyping successive batches for a selective breeding program with about 1000 fish is clearly within reach. Moreover, the infrastructure and labour costs are counterbalanced by future reductions in feed costs due to improved feed efficiency (Besson et al., 2019). In Nile tilapia, individual rearing seems even more feasible because fish acclimate and grow faster than European sea bass when isolated. In Nile tilapia, the video-recording method seems technically feasible at medium scale with 200 fish for experimental purposes. This method would, however, be particularly tedious at higher scale for selective breeding purposes, with five batches of 200 fish, for instance. The analysis of video-recordings would be very long (Table 4) and have high labour costs. This issue may, however, be solved by improvements in video-tracking software.

1.2. *Biological aspects*

In Nile tilapia, I found no correlation between individual FCR estimated by individual rearing compared to video-recording, in both GIFT and Cirad strains, indicating a consistent result whether fish are selected for enhanced growth or not.

Nile tilapia is a social species, with aggressive interactions among conspecifics used to establish a dominance hierarchy (reviewed by Gonçalves-de-Freitas et al., 2019), such that the lack of correlation between rearing individually *versus* in a group is not surprising. However, de Verdal et al. (2019) reported no significant phenotypic correlation between individual feed efficiency and agonistic interactions in this species, using the video-recording method. Nile tilapia behaviour is, nevertheless, quite complex and includes also non-agonistic social communication (reviewed by Gonçalves-de-Freitas et al., 2019).

The video-recording method is probably also not a perfect reflection of commercial rearing practices. Nile tilapia are farmed in large groups in ponds, tanks or cages (Modadugu and

Acosta, 2004) whereas the video-recording method is on small groups of ten to fifteen fish. In large groups, social structures are probably more complex. Furthermore, feed pellets are provided one by one, which may strengthen competition for feed and aggressive interactions and bias individual feed efficiency estimation.

In European sea bass, Besson et al. (2019) demonstrated a link between individual FCR, estimated using individual rearing, and subsequent group FCR. These results suggest individual rearing provides an estimation of feed efficiency that is equivalent with that observed in group rearing. Feed efficiency should, however, be estimated at individual level even when fish are reared in a group, to confirm this conclusion. Moreover, I clearly observed that FI of isolated fish was much lower than expected, about 25% of the 200 isolated European sea bass lost weight even when fed at *ad libitum*. The extent to which isolation influences this species behaviour is not yet clear. On the one hand, European sea bass does not exhibit marked aggressive interactions with conspecifics (Barnabé, 1980) but, on the other hand, juveniles are gregarious and individual rearing prevents the shoaling behaviour that is systematically observed in captivity (Barnabé, 1980). Furthermore, European sea bass behaviour is variable among individuals. Some fish exhibit “shy” behaviour defined as “freezing and hiding” when exposed to an environment with potential threats, whereas others exhibit “bold” behaviour, taking risks and rapidly exploring their environment (Benhaïm et al., 2016). It seems that shy fish adapt better to the individual rearing system (M. Vandeputte, personal communication, 2020), with clear possible implications for FI and feed efficiency.

From my various results, therefore, it is not possible to conclude that an “optimal” method exists to estimate individual feed efficiency across all species. Before initiating a breeding program for feed efficiency in a given species, careful evaluation of its behaviour seems necessary, to gain insight into which method might provide the best estimates of individual feed efficiency. For instance, it can be suggested that individual rearing may bias individual feed

efficiency estimation in species exhibiting dominant-subordinate relationships. Similarly, both methods may bias estimations in species that shoal. It can also be assumed that species which feel threatened by human presence may not feed in the video-recording method. All these aspects need to be addressed on a case-by-case basis.

2. To what extent do developmental stage and rearing practices influence estimates of individual feed efficiency?

Phenotyping fish for individual feed efficiency not only implies being able to measure individual FI, but must also consider their developmental stage and rearing environment, such as feeding rate or water temperature.

2.1. Developmental stage

In isolated Nile tilapia, I found that individual FCR estimated in juveniles was a good predictor of individual FCR up to commercial size. I concluded that selecting fish for FCR over two weeks at juvenile stage would improve the entire rearing FCR by about 1% per generation with a selection intensity of 50%. This result is particularly interesting because it implies selection costs could be reduced, since selecting fish at the juvenile stage avoids maintaining up to commercial size animals that would not be used for broodstock, thus decreasing rearing and labour costs.

These results need to be consolidated by assessing heritability of individual FCR from juvenile stage to commercial size. I calculated genetic gain using the FCR heritability published by de Verdal et al. (2018b), estimated over one week at juvenile stage using the video-recording method. Moreover, the correlations I estimated between individual FCR at juvenile stage and over the whole rearing period were phenotypic. Only genetic correlations will reveal to what extent selecting fish at juvenile stage can improve FCR from juvenile stage to commercial size. Whether the same conclusions would be reached in European sea bass remains an open question. Ideally, this issue should be investigated whenever a selective breeding program for

feed efficiency is initiated in a species, because of the opportunity to reduce selection costs. Many species are, however, much larger than Nile tilapia at commercial size, such as European sea bass or Atlantic salmon *Salmo salar*. Phenotyping them over a whole rearing cycle would not be technically feasible whatever the method used.

2.2. Rearing environment

I aimed to determine whether most efficient fish when fed to satiety were also the most efficient under a restricted feeding rate. I found no significant phenotypic correlation between individual FCR at optimal rate and FCR at restricted (50% of the optimal) rate in Nile tilapia (GIFT strain) using the video-recording method. In European sea bass, I observed that the most efficient fish at *ad libitum* were much more sensitive to progressive feed deprivation and actually ended up losing more weight when fasting. Thus, the most efficient fish differed with feeding rate. Therefore, the feeding rate used to phenotype for individual feed efficiency in selective breeding programs must be the same as that used in commercial practices. These conclusions need, however, to be validated at genetic level in both species. In particular, results reported in European sea bass by Besson et al. (2019) at genetic level contrast with the present results because they suggested that the most efficient fish lost less weight at fasting.

Surprisingly, my results differ greatly from those reported in livestock: selecting the most efficient animals under restricted ration improved *ad libitum* feed efficiency of progeny in pigs (Nguyen et al., 2005) and rabbits (Drouilhet et al., 2016). Feed efficiency seems to be context-dependent in fish: some animals are more efficient at high feeding rate whereas others are more efficient at low feeding rate. The same observation was made by Dupont-Prinet et al. (2010) for growth in European sea bass. Performing better at low feeding rate is not important on the fish farm because animals are fed to satiety. This could, however, be a major advantage in the wild where fish may have to cope with feed deprivation. Since fish domestication and selection is recent, it can be hypothesized that some animals are still specifically adapted to feed

deprivation. In contrast, livestock domestication and selection being much more ancient, it may be that no adaptation to feed deprivation remains and, thus, no trade-off between performance at high and low feeding rates is observed.

Secondly, I investigated the influence of temperature on feed efficiency in European sea bass, finding that EM fish were the most and AT fish the least efficient whether reared at 18 or 24°C. These temperatures reflect the coldest and warmest average temperatures at which European sea bass is reared across Europe (Vandeputte et al., 2014). From a breeder's point of view, EM fish should be used across Europe because there were always more efficient, and breeding programs should focus on this population. This result needs, however, to be confirmed in group rearing and at other temperatures (for instance 21°C).

To conclude, the results suggest rearing conditions can influence fish ranking according to individual feed efficiency. In a context of selective breeding, whatever the species under consideration, the default strategy should be to phenotype fish for individual feed efficiency in conditions as close as possible to rearing practices. Rearing environments are diverse and exploring not only individual variability but also diversity among populations or strains is promising to improve feed efficiency in a wide range of situations.

So far, I have discussed methods to improve individual feed efficiency through direct selection. However, estimating individual feed efficiency remains tedious. This is why finding an indirect selection criterion for feed efficiency is of major importance.

3. Which traits hold promise as indirect selection criteria for feed efficiency?

Indirect selection traits must be variable, heritable and genetically correlated with individual feed efficiency, and technically easier to measure. I focussed upon two candidate traits: metabolic rate and stable isotope values. For stable isotope values, I did not directly assess a correlation with individual feed efficiency but investigated methodological issues that will be critical for implementation in future studies. I will suggest ideas to further investigate the

correlations of these two traits with individual feed efficiency. To conclude, I will consider two other potential approaches to predict individual feed efficiency: cortisol secretion and genomic selection.

3.1. Perspectives provided by the experimental results

3.1.1. From oxygen consumption to body composition

I did not find any phenotypic correlation between oxygen consumption and individual feed efficiency in European sea bass. However, variation in oxygen consumption among the AT, WM and EM populations, and between rearing temperatures of 18 and 24°C, exhibited a clear link with individual feed efficiency. Fish reared at 18°C were more efficient than those reared at 24°C and had a lower oxygen consumption. Similarly, AT fish were the least efficient and had the highest oxygen consumption.

Two main issues must be addressed to potentially use oxygen consumption as an indirect selection criterion. Firstly, it does not seem technically feasible to measure individual oxygen consumption and feed efficiency simultaneously in fish. Thus, individual feed efficiency and individual oxygen consumption have to be estimated at two different development stages. Consequently, long-term repeatability of oxygen consumption must be established before using the trait as an indirect selection criterion in a given species.

Secondly, oxygen consumption estimates how much energy is required for body maintenance and activity, but it does not reveal which substrates are used to provide energy. Lipid contains about twice as much energy as protein per unit of weight. Catabolising proteins rather than lipids to support metabolism leads to a greater loss of weight while fish are fasting (McKenzie et al., 2014). When fish are fed, they store proteins and lipids to grow. However, energy expenditure for metabolism and activity remains, which induces catabolism and partly counterbalances proteins and lipids storage. This suggests that, when fed, fish relying more heavily on proteins to provide energy to metabolism and locomotion may be less efficient. The

type of reserves used as fuel for maintenance and locomotion can be assessed by measuring ammonia excretion while fish are fasting. Ammonia excretion reveals protein catabolism and is determined by sampling water (Kieffer and Wakefield, 2009). It is feasible to determine ammonia excretion while fish are individually reared by making them fast at the end of the feeding trial for feed efficiency. Such information could be complementary to metabolic rate in predicting individual feed efficiency.

More generally, there is a need to determine how proteins and lipids are used by fish, not only for basal maintenance and activity, but also for growth. As explained by Knap and Kause (2018), deposition of 1 g of lipid leads to 1.1 g of weight gain, including 0.1 g of water in the associated adipose tissue. Conversely, deposition of 1 g of protein leads to 4-5 g of weight gain, including 3-4 g of water. Protein deposition is energetically more expensive than lipid deposition (59.9 kJ/g vs. 43.5-55.3 kJ/g), but this higher energetic cost is marginal compared to the four to fivefold increase in weight gain associated with protein deposition (Knap and Kause, 2018). The fact that most efficient animals were reported to have a lower muscle or whole body fat content in several fish species (Neely et al., 2008; Kamalam et al., 2012; Kause et al., 2016) is therefore not surprising.

Consequently, determining fish protein and fat content, as well as its variability over time while fish are fasting or growing, may provide indirect indicators of individual feed efficiency. This would be a complement to non-growth energy expenditure. Measuring fat content frequently is easy and not invasive in the case of muscle (Quillet et al., 2005). However, focussing on whole body composition rather than exclusively on muscles can be more informative because other compartments of the fish have large fat reserves, notably the viscera. Studying the variability of whole body composition over time implies fish must not be slaughtered, which might seem impossible. However, emerging imaging techniques that provide an accurate 3-D representation

of whole fish composition may resolve this issue (Hancz et al., 2003; Ceballos-Francisco et al., 2020).

3.1.2. Carbon and nitrogen stable isotope values

Stable isotope values might predict individual feed efficiency, as already suggested in Atlantic salmon (Dvergedal et al., 2019a; 2019b) and terrestrial livestock (Wheadon et al., 2014; Cantalapiedra-Hijar et al., 2015; Cantalapiedra-Hijar et al., 2016). However, it remains unknown whether stable isotope values should be determined at dynamic state, at steady state, or at both to predict individual feed efficiency. Before setting up a protocol investigating this issue in European sea bass, I aimed to determine the time required by stable isotope values of scales to reach steady state after a diet change. I chose to sample scales because it is non-lethal, about 80-100 fish scales can be analysed for stable isotope values per day at a relatively modest cost per sample of about 1.5-2 €. The results highlight some key issues in developing a protocol to use stable isotope values to predict individual feed efficiency.

In European sea bass, individual feed efficiency is estimated with an individual rearing protocol. My initial hope was to change diet once fish were acclimated to isolation, then keep them isolated until the stable isotope values of scales reached a new equilibrium. The time required was, however, too long to be compatible with individual rearing as it is a tedious method: 143 and 291 days for carbon and nitrogen, respectively. Moreover, the time needed to reach equilibrium depends on growth rate (Hesslein et al., 1993). Consequently, stable isotope values of isolated fish may take more time than expected to reach equilibrium because FI was relatively low in isolation. Individual rearing may, thus, only be used during the beginning of the dynamic state. As no suitable method is currently available to determine individual feed efficiency in larger European sea bass, investigating the link between individual feed efficiency and stable isotope values at steady state seems out of reach.

The same investigation of time required for stable isotope values to reach equilibrium should be performed in any relevant tissue or species before trying to investigate a link with individual feed efficiency. Indeed, the present results for European sea bass cannot be generalized as the time needed to reach a new equilibrium is both tissue and species-dependent (Carleton and Martínez del Rio, 2005; Weidel et al., 2011; Busst and Britton, 2016). In species or strains exhibiting particularly high growth rates, stable isotope values will reach steady state quicker. Consequently, the feeding trial used to estimate individual feed efficiency may be long enough for stable isotope values to reach equilibrium, whether the individual rearing or video-recording method is used.

Scales may, however, not be the best candidate to investigate the link between individual feed efficiency and nitrogen stable isotope values. According to my results, nitrogen stable isotopes incorporation is driven almost exclusively by growth (94.2%) in European sea bass scales. Determining nitrogen stable isotope values at dynamic state in scales may in fact be equivalent to measuring growth rate. In contrast, both growth and catabolism seem to contribute to carbon stable isotopes incorporation in scales. Two alternatives that could be proposed are mucus or blood sampling. None of them are lethal and the incorporation of carbon and nitrogen stable isotopes into these tissues depends on both growth and catabolism (German and Miles, 2010; Winter et al., 2019).

To conclude, our results constitute a preliminary step towards investigating the link between individual feed efficiency and stable isotope values. How long stable isotope values take to reach equilibrium needs, however, to be determined on a case-by-case basis for each species by tissue combination. One critical point is the choice of the tissue used to measure stable isotope values. Its sampling should not be lethal for a selective breeding context. Moreover, if stable isotope values are determined at dynamic state, it should be ensured they are not exclusively influenced by growth rate, but also by catabolism.

3.2. *Future prospects*

Due to time limitation, I did not investigate several strategies that I considered as interesting to predict individual feed efficiency. However, I want to propose two of them that seem particularly promising.

3.2.1. *Cortisol secretion*

Cortisol, a corticosteroid hormone, is the main indicator of the degree of stress experienced by fishes (reviewed by Barton, 2002). Cortisol stimulates energy expenditure for basal maintenance and activity in fish (reviewed by Mommsen et al., 1999; Lawrence et al., 2019). Consequently, it could be hypothesized that individuals which secrete the highest levels of cortisol will allocate less energy to growth and be less efficient. Martins et al. (2006; 2011) investigated this issue at phenotypic level in African catfish *Clarias gariepinus* and Nile tilapia reared individually, but found no significant correlation between RFI and basal plasma cortisol level. Martins et al. (2006) did, however, report correlations ranging from 0.05 to 0.42 between individual plasma cortisol level measured after stress and RFI in African catfish. In Nile tilapia, Martins et al. (2011) found a phenotypic correlation of $r = 0.49$ between stressed cortisol level and individual RFI. There is, however, no information about genetic correlation between these traits.

Measuring basal cortisol level might also be relevant to evaluate impacts on fish of the methods used to estimate individual feed efficiency. That is, if a given phenotyping method induces unusual cortisol levels in a given species, it may indicate that estimations of FI and feed efficiency are biased. For instance, isolated Nile tilapia may secrete less cortisol than when in a group because they do not compete with conspecifics. This could explain why the individual rearing and video-recording methods did not provided correlated FCR estimations in this species.

Cortisol dynamics can be measured in various ways, but two seem particularly interesting to investigate the link with individual feed efficiency. The first one is water, which has the major advantage of being not invasive and adequate to measure both basal and stressed cortisol levels. However, it cannot be measured at individual level without an individual rearing design (reviewed by Sadoul and Geffroy, 2019). The second method is blood (plasma), which enables measurement of individual cortisol levels even if fish are reared in a group. However, if fish are reared in large groups it can be very difficult to measure basal cortisol level because capturing the first fish will induce cortisol secretion in the others (reviewed by Sadoul and Geffroy, 2019).

To conclude, cortisol secretion may be a predictor of individual feed efficiency, but also useful to evaluate the phenotyping methods used for individual feed efficiency. Moreover, cortisol measurement does not require killing the fish, which is advantageous for selective breeding.

3.2.2. *Genomic selection*

Briefly, genomic selection is a method that emerged as DNA sequencing technologies have become progressively cheaper. It has permitted the discovery of many thousands of single nucleotide polymorphism (SNP) markers in livestock and fish genomes. In “traditional” selective breeding, breeding values of animals (i.e. expected phenotypic values of an animal’s offspring) are estimated using phenotypes and family relationships, based on the pedigree of the animals. In genomic selection, breeding values are estimated using phenotypes and genomic relationships, calculated thanks to SNP genotyping. Genomic relationships replace pedigree relationships. For instance, the pedigree relationship between two full sibs is 0.50, which means that two full sibs are expected to have 50% of their genes in common. However, in reality, two fullsibs may not share exactly 50% of their genes. They may share, for example, 60% or only 40% of their genes. These deviations from the pedigree-based expectation are detected by SNP

genotyping, such that genomic relationships are more accurate than pedigree relationships (reviewed by Meuwissen et al., 2016).

To perform genomic selection, a part of the population is phenotyped for the trait of interest and genotyped, and the other part is only genotyped. Then, genomic breeding values of the non-phenotyped individuals are predicted using genomic relationships between them and the phenotyped animals. Thus, breeding values can be estimated without phenotyping all animals (reviewed by Meuwissen et al., 2016).

Genomic selection seems particularly interesting in the case of individual feed efficiency. Indeed, phenotyping fish for individual feed efficiency remains particularly tedious whatever the method used. This is an obstacle to the inclusion of this trait in “traditional” selective breeding programs. Thus, being able to estimate the breeding values of all animals by phenotyping only a sub-sample of them is a major step forward.

This tool has already been used in isolated European sea bass by Besson et al. (2019). Genomic analysis estimated individual feed efficiency breeding values, heritability and genetic correlations more accurately than classical pedigree information. These authors concluded that only 80% of the whole population had to be phenotyped to estimate all breeding values accurately. However, much more investigation is required before starting a genomic selection program for individual feed efficiency in fishes. In the GIFT strain of Nile tilapia, studies led by WorldFish are ongoing to develop genomic selection for individual feed efficiency, phenotyping this trait with the video-recording method (J. Benzie and H. de Verdal, personal communication, 2020). Genomic selection for individual feed efficiency might be initiated in virtually any species provided an adequate phenotyping method is available.

CONCLUSIONS

- In a selective breeding program, the method used to phenotype for individual feed efficiency will depend on the species. No “universal” method exists.
- An in-depth understanding of the species behaviour can offer clues to determining whether a phenotyping method is suitable or not.
- If technically feasible, the opportunity to select fish for individual feed efficiency at the juvenile stage must be investigated, as this can cut selection costs.
- In a selective breeding program for individual feed efficiency, whatever the species, the default strategy should be to rear fish in an environment as close as possible to commercial rearing practices.
- Investigating variability among populations or strains of a given species is promising to improve feed efficiency.
- Metabolic rate might predict individual feed efficiency if complemented with information about ammonia excretion and body composition.
- Investigating the link between individual feed efficiency and carbon and nitrogen stable isotope values shortly after a diet change is technically feasible.
- Nonetheless, the tissues to sample must be carefully chosen. Their ablation must not be lethal. Moreover, their incorporation of stable isotopes must not be explained exclusively by growth.

RÉSUMÉ EN
FRANÇAIS

Plus de 7 milliards de personnes vivent sur Terre en 2020 et la population mondiale devrait atteindre 8 à 10 milliards d'habitants d'ici 2050 selon les projections (United Nations, 2011). La croissance démographique mondiale s'accompagne d'une augmentation de la demande en denrées alimentaires, entre autres d'origine aquatique. Cette demande est partiellement satisfaite par la production halieutique, mais cette dernière stagne autour de 90 millions de tonnes par an depuis environ 20 ans (FAO, 2018). A l'inverse, la production aquacole n'a cessé de croître pour atteindre 82 millions de tonnes (hors végétaux aquatiques) en 2018 (FAO-FIGIS, 2020). Par conséquent, réussir à satisfaire la demande croissante en produits d'origines aquatiques dépendra presque uniquement du secteur aquacole.

Pour améliorer l'aquaculture, une vision durable est nécessaire. La durabilité est définie comme « la capacité à répondre aux besoins du présent sans empêcher les générations futures de répondre à leurs propres besoins » (Brundtland, 1987). Un des obstacles à la durabilité de l'aquaculture est l'aliment utilisé pour nourrir les poissons d'élevage. Ce dernier coûte cher : il représente 30 à 70% des coûts des piscicultures intensives (Goddard, 1996; Rana Sunil Siriwardena and Hasan, 2009; STECF, 2018). De plus, la production et l'utilisation de l'aliment sont responsables de la majeure partie de l'impact environnemental des fermes piscicoles. Entre autres, l'alimentation est responsable de 32 à 86% des émissions de gaz à effet de serre imputables aux fermes. Elle provoque aussi l'eutrophisation (96-100% de l'impact des fermes) et l'acidification (29-80% de l'impact des fermes) du milieu aquatique environnant (Aubin et al., 2009; Besson et al., 2016). Enfin, des interrogations sociales sont soulevées par le fait que l'alimentation aquacole entre en compétition avec l'alimentation humaine pour accéder aux matières premières (Troell et al., 2014). Améliorer l'utilisation de l'aliment à la ferme permettrait donc d'outrepasser ces différents obstacles à la durabilité de l'aquaculture.

L'utilisation faite par un animal de son aliment peut être caractérisée par le concept d'« efficacité alimentaire », c'est-à-dire la relation qui existe entre la quantité d'aliment

consommée et le gain de masse résultant. Concrètement, améliorer l'efficacité alimentaire signifie produire autant de poisson avec moins d'aliment, ou plus de poisson avec autant d'aliment. L'efficacité alimentaire se calcule grâce à deux mesures : la quantité d'aliment consommée, c'est-à-dire la prise alimentaire (PA), et le gain de masse (GM). Différentes manières d'estimer l'efficacité alimentaire existent. Dans la plupart des cas, le ratio entre la PA et le GM (ou son inverse) est calculé (revue par de Verdal et al., 2018a). Dans d'autres cas, la PA est exprimée en fonction du GM (ou inversement) et les résidus du modèle linéaire associé sont utilisés comme des indicateurs de l'efficacité alimentaire (consommation alimentaire résiduelle ou gain de masse résiduel; Koch et al., 1963).

L'efficacité alimentaire peut être améliorée par le biais de la nutrition (Huisman, 1976; Brett, 1979; De Silva and Anderson, 1995; Guillaume et al., 2001; NRC, 2011) et de la zootechnie (Brett, 1979; Azevedo et al., 1998; Biswas et al., 2005; Imsland et al., 2008; Árnason et al., 2009; Yoo and Lee, 2016). Une autre stratégie possible pour améliorer l'efficacité alimentaire est l'utilisation de la génétique par le biais de programmes de sélection. Cependant, afin de mener un programme de sélection génétique, il faut être capable d'estimer l'efficacité alimentaire au niveau individuel, et donc de mesurer la PA et le GM individuellement, ce qui n'est pas le cas dans les études portant sur la nutrition ou la zootechnie. Mesurer le GM individuel est facile grâce à l'utilisation de PIT (« passive integrated transponder ») tags (Roussel et al., 2000), qui permettent d'identifier les poissons individuellement. En revanche, mesurer la PA au niveau individuel est particulièrement difficile car les poissons sont élevés en larges groupes et mangent simultanément lorsque l'aliment est distribué. Mesurer la PA au niveau individuel requiert donc des méthodes spécifiques.

Parmi les méthodes principalement utilisées dans la littérature, une première consiste à élever les poissons en larges groupes et à les nourrir avec un aliment contenant des particules repérables par radiographie aux rayons X une fois dans l'appareil digestif du poisson. Peu après

que les poissons aient été nourris, ils sont anesthésiés et passés aux rayons X (Talbot and Higgins, 1983; McCarthy et al., 1993; Jobling et al., 2001 ; Silverstein et al., 2001; Boujard et al., 2006; Kause et al., 2006a; 2006b; Quinton et al., 2007a; 2007b; Grima et al., 2008). La principale limite de cette méthode est le temps nécessaire aux poissons pour récupérer après anesthésie et manipulation, qui peut être de plusieurs semaines (Jobling et al., 2001; Quinton et al., 2007a; Grima et al., 2008). La PA individuelle n'est donc mesurée que sur un nombre marginal de repas de la période d'évaluation de l'efficacité alimentaire. Cet aspect est particulièrement problématique car la PA des poissons est variable au cours du temps (Smagula and Adelman, 1982; Tackett et al., 1988) et, par conséquent, la répétabilité des mesures de PA obtenues avec cette méthode est faible (entre 0.09 et 0.32; Kause et al., 2006a; Grima et al., 2008). Réussir à mesurer la PA individuelle des poissons à chaque repas est donc nécessaire.

Une seconde méthode consiste à élever les poissons en petits groupes (10 à 15 animaux) et à les filmer lors des repas, l'aliment étant distribué granulé par granulé. Les poissons sont distinguables les uns des autres grâce à des tags externes colorés. La PA des poissons est mesurée ultérieurement, en analysant les enregistrements vidéo des repas (de Verdal et al., 2017). L'atout majeur de cette méthode est que la PA individuelle est déterminée à chaque repas, sans exception, ce qui permet d'obtenir une très bonne répétabilité des mesures de PA (de Verdal et al., 2017). En revanche, analyser les enregistrements vidéo de chaque repas nécessite beaucoup de temps.

Une dernière méthode consiste à élever les poissons individuellement en aquarium. La quantité d'aliment distribuée à chaque poisson est connue, et les granulés non consommés sont collectés et comptés pour estimer la quantité de gaspillage réalisée par chaque poisson. La PA individuelle est alors calculée comme la différence entre la quantité d'aliment distribuée et la quantité d'aliment gaspillée (Silverstein et al., 2005; Silverstein, 2006; Martins et al., 2011; Besson et al., 2019). L'avantage majeur de cette méthode est que la PA est estimée à chaque

repas. Cependant, collecter et compter la quantité de granulés gaspillée à chaque repas est fastidieux. En élevage individuelle, restreindre le taux de nourrissage des animaux a déjà permis d'améliorer l'efficacité alimentaire (à taux de nourrissage restreint et *ad libitum*) chez le porc et chez le lapin (Nguyen et al., 2005; Drouilhet et al., 2016). Lorsque le taux de nourrissage est restreint, les animaux les plus efficaces sont tout simplement ceux qui grossissent le plus vite, car la PA individuelle est constante d'un animal à l'autre. De plus, restreindre le taux de nourrissage diminue la quantité de travail liée au gaspillage. Cependant, il n'existe encore aucune preuve que l'efficacité alimentaire individuelle à taux de nourrissage restreint est corrélée avec l'efficacité alimentaire individuelle *ad libitum* chez le poisson. Par ailleurs, une limite majeure de l'élevage individuel est que les poissons sont isolés et ne peuvent donc plus interagir avec leurs congénères. Cela peut potentiellement induire un biais dans les estimations d'efficacité alimentaire individuelle réalisées grâce à cette méthode.

Pour résumer, toutes ces méthodes ont des atouts et des limites, mais elles ont permis de démontrer que l'efficacité alimentaire individuelle est un trait héritable ($h^2 = 0.06-0.50$), c'est-à-dire améliorable grâce à la sélection génétique (Quinton et al., 2007a; Grima et al., 2008; Kause et al., 2016; de Verdal et al., 2018b; Besson et al., 2019). Cependant, l'héritabilité de l'efficacité alimentaire est bien moindre lorsque la méthode de la radiographie par rayons X est utilisée (Quinton et al., 2007a; Kause et al., 2016), ce qui est probablement lié à la faible répétabilité de la mesure.

En outre, toutes ces méthodes n'ont été utilisées que sur des périodes courtes de la vie du poisson, la plupart du temps au stade juvénile. Cela s'explique par le fait que mesurer la PA individuelle à des stades ultérieurs nécessiterait des infrastructures bien plus volumineuses, et engendrerait des coûts bien plus élevés, notamment d'aliment. Cependant, l'objectif est d'améliorer l'efficacité alimentaire sur toute la période d'élevage, et non uniquement au stade juvénile. Néanmoins, aucune preuve n'existe que l'efficacité alimentaire estimée chez des

juvéniles reflète réellement l'efficacité alimentaire de chaque poisson sur l'ensemble de la période d'élevage.

L'efficacité alimentaire, comme tout trait phénotypique héritable, peut être améliorée par sélection directe ou indirecte. La sélection directe repose sur une estimation individuelle de l'efficacité alimentaire qui, malgré les diverses méthodes présentées, reste complexe. La sélection indirecte, quant à elle, repose sur la mesure individuelle de traits présentant une variabilité et corrélés génétiquement avec l'efficacité alimentaire. En sélectionnant les poissons sur ces traits, on peut indirectement améliorer l'efficacité alimentaire individuelle des poissons. Un objectif majeur est donc d'identifier un trait qui répond aux critères précédents, mais qui soit aussi plus facile à mesurer au niveau individuel que l'efficacité alimentaire.

Plusieurs traits déjà explorés dans la littérature sont la croissance (par exemple Kause et al., 2006b; Quinton et al, 2007a; de Verdal et al., 2018b; Besson et al., 2019), la perte de masse au jeûne (par exemple Grima et al., 2008; Daulé et al., 2014; de Verdal et al., 2018b) ou encore le taux de gras intramusculaire ou total des poissons (par exemple Neely et al., 2008; Kamalam et al., 2012; Kause et al., 2016). Ces différents traits, bien que prometteurs, n'ont pas encore été suffisamment convaincants pour être utilisés comme critères de sélection indirecte pour l'efficacité alimentaire.

D'autres traits phénotypiques, telles que le taux métabolique, estimée chez les poissons par la consommation d'oxygène (Luiting et al., 1991; Nkrumah et al., 2006; Arndt et al., 2015; Chaves et al., 2015; Paganini et al. 2017), ou les signatures isotopiques en ^{15}N des tissus (Wheadon et al., 2014; Cantalapiedra-Hijar et al., 2015; Cantalapiedra-Hijar et al., 2016) ont déjà permis de prédire l'efficacité alimentaire individuelle chez les animaux terrestres. Cependant, ils sont restés très peu étudiés chez le poisson. Dans le cas des signatures isotopiques en ^{13}C et ^{15}N des tissus, à notre connaissance, seuls Dvergedal et al. (2019a ; 2019b) ont étudié leur lien avec

l'efficacité alimentaire. Cependant, ces auteurs ont estimé l'efficacité alimentaire uniquement à l'échelle du groupe.

Des questions méthodologiques se posent. Lorsqu'un animal reçoit un nouvel aliment avec une signature isotopique différente du précédent, alors la signature isotopique de ses tissus va progressivement évoluer jusqu'à atteindre un équilibre. Certaines études se sont concentrées sur l'état transitoire des signatures isotopiques pour prédire l'efficacité alimentaire individuelle (Dvergedal et al., 2019a ; 2019b), alors que d'autres se sont plutôt focalisées sur l'état d'équilibre (Wheadon et al., 2014; Cantalapiedra-Hijar et al., 2015; Cantalapiedra-Hijar et al., 2016). Les deux possibilités ont permis de prédire l'efficacité alimentaire, et aucun élément ne permet de savoir laquelle choisir préférentiellement (les deux possibilités pouvant potentiellement se compléter). Par ailleurs, dans un contexte de sélection génétique, il est préférable de développer des méthodes non létales pour déterminer de la signature isotopique des animaux. En effet, la sélection génétique sera plus efficace si réalisée directement sur les animaux dont la signature isotopique a été déterminée plutôt que sur leur collatéraux.

Dans ce travail de thèse, deux espèces ont été étudiées pour appréhender la problématique de l'efficacité alimentaire individuelle : le bar européen (*Dicentrarchus labrax*) et le tilapia du Nil (*Oreochromis niloticus*). Ces deux espèces diffèrent beaucoup, la première étant endémique de l'Océan Atlantique, de la Mer Méditerranée et de la Mer Noire (Pickett and Pawson, 1994), et la seconde des fleuves et lacs africains (Philippart and Ruwet, 1982). Le bar européen est produit dans des nurseries en production intensive puis en général transféré dans des cages en pleine mer (Chatain and Chavanne, 2009; Vandeputte et al., 2019). En revanche, le tilapia du Nil est produit aussi bien en conditions extensives qu'intensives (dans des cages lacustres, étangs ou bassins à terre; Modadugu and Acosta, 2004). En captivité, les bars européens ont tendance à former des bancs, alors que les tilapias du Nil sont bien plus agressifs entre eux pour établir une hiérarchie avec des dominants et des dominés. Le bar européen représente une faible

part de la production mondiale, mais demeure une espèce clef pour l'aquaculture européenne, avec une forte valeur ajoutée (STCEF, 2018 ; FAO-FIGIS, 2020). En revanche, le tilapia du Nil est la 3^{ème} espèce la plus élevée au monde, principalement en Asie où elle a été introduite, et dans une moindre mesure en Afrique (FAO-FIGIS, 2020). L'efficacité alimentaire individuelle de ces deux espèces a déjà été étudiée (de Verdal, 2017; de Verdal, 2018b; Besson et al., 2019), et donc de nombreux progrès techniques ont été déjà réalisés pour mesurer leur PA individuelle. Comparer ces deux modèles biologiques très différents pourrait permettre d'établir des protocoles de sélection de l'efficacité alimentaire individuelle qui soient le plus génériques possibles.

L'objectif de ce travail de thèse a été de développer des protocoles utilisables au cours de programmes de sélection pour améliorer l'efficacité alimentaire. Dans ce but, j'ai cherché dans un premier temps à résoudre des questions d'ordre méthodologiques. Est-ce que l'efficacité alimentaire individuelle estimée au stade juvénile est un bon prédicteur de l'efficacité alimentaire individuelle sur l'ensemble du cycle d'élevage ? Est-ce que les méthodes de l'élevage individuel et de l'enregistrement vidéo fournissent des estimations équivalentes de l'efficacité alimentaire individuelle ? Est-ce que l'efficacité alimentaire individuelle estimées à un taux de nourrissage restreint reflète l'efficacité alimentaire individuelle *ad libitum* ?

Mes résultats permettent de supposer que sélectionner les tilapias du Nil sur leur efficacité alimentaire au stade juvénile améliorerait leur efficacité alimentaire sur l'ensemble de la période d'élevage. Un gain d'environ 1% par génération avec une intensité de sélection de 50% a été projeté. Ce résultat est particulièrement intéressant car il implique que les poissons pourraient être sélectionnés dès le stade juvénile, réduisant drastiquement les coûts de sélection. En effet, les poissons non sélectionnés n'auraient pas besoin d'être conservés jusqu'à taille commerciale, ce qui réduirait notamment les coûts d'alimentation. Idéalement, le même travail

devrait être mené pour chaque espèce afin de généraliser les résultats obtenus. Cependant, cela est techniquement très difficile dans le cas des espèces trop volumineuses à taille commerciale. Ensuite, j'ai constaté chez le tilapia du Nil que l'efficacité alimentaire individuelle estimée par la méthode de l'élevage individuelle n'était pas corrélée à l'efficacité alimentaire individuelle estimée par analyse vidéo. En revanche, Besson et al. (2019) ont déjà démontré chez le bar européen que l'efficacité alimentaire individuelle obtenue par isolement des poissons se reflétait ensuite dans l'efficacité alimentaire de groupe, une fois les poissons remis ensemble. Ces résultats m'ont permis de conclure qu'au sein d'un programme de sélection, la méthode de phénotypage des poissons doit dépendre de l'espèce considérée. Aucune méthode « universelle » n'existe. Une forte compréhension du comportement de chaque espèce est nécessaire pour décider au mieux de la méthode à utiliser.

Par la suite, j'ai observé chez le bar européen et le tilapia du Nil que les animaux les plus efficaces à taux de rationnement restreint n'étaient pas les plus efficaces à satiété. J'en ai conclu que, dans un programme de sélection génétique, il est préférable de phénotyper les poissons dans des conditions les plus proches possible de celles en élevage commercial.

Chez le bar européen, j'ai observé que les poissons issus de l'Est de la Méditerranée étaient les plus efficaces, et ceux issus de l'Atlantique les moins efficaces, que la température d'élevage soit de 18 ou 24°C (deux températures représentatives des sites d'élevage européens). Du point de vue d'un sélectionneur, cela signifie que la population issue de l'Est de la Méditerranée pourrait être utilisée partout à travers l'Europe car elle est la plus efficace quelle que soit la température. Cela implique aussi qu'un programme de sélection sur l'efficacité alimentaire chez le bar européen devrait utiliser cette population dès le départ. Cela démontre que la diversité des différentes populations ou souches d'une espèce est un atout pour améliorer l'efficacité alimentaire individuelle dans des environnements variés. Mes résultats doivent, cependant, être confirmés en élevage de groupe.

Dans un second temps, j'ai cherché à identifier des critères de sélection indirecte pour prédire l'efficacité alimentaire. Le taux métabolique, estimé grâce à la consommation d'oxygène, a été testé comme potentiel prédicteur de l'efficacité alimentaire. Ensuite, notre attention s'est portée sur la signature isotopique des écailles des poissons. La corrélation avec l'efficacité alimentaire n'a pas été directement estimée car il a fallu au préalable résoudre des questions d'ordre méthodologique. J'ai étudié le temps nécessaire à la signature isotopique des écailles, suite à un changement d'aliment, pour passer de l'état transitoire à l'état d'équilibre. Cela permettra de mettre en place des protocoles étudiant le lien entre efficacité alimentaire individuelle et signature isotopique au cours d'études ultérieures.

Chez le bar européen, l'efficacité alimentaire individuelle n'a pas pu être prédite par le taux métabolique. Cependant, d'autres informations, telles que l'excrétion d'ammoniac ou la composition corporelle, semblent pertinentes pour compléter le taux métabolique afin de potentiellement prédire l'efficacité alimentaire.

Enfin, j'ai observé dans le cas du bar européen que le temps nécessaire à la signature isotopique des écailles pour atteindre un équilibre était trop long pour être compatible avec une estimation en continu de l'efficacité alimentaire individuelle. Cependant, il paraît tout à fait possible d'étudier le lien entre efficacité alimentaire individuelle et signature isotopique au début de la phase transitoire. Ce problème pourrait être potentiellement résolu dans les espèces à croissance plus rapide. En effet, l'incorporation des isotopes stables dans les tissus dépend de leur croissance et de leur catabolisme, donc plus une espèce croît vite, et plus l'équilibre sera atteint tôt. En ce qui concerne le choix du tissu à prélever, deux critères semblent à prendre en compte dans un contexte de sélection. Premièrement, l'ablation du tissu ne doit pas être mortelle. Ensuite, l'incorporation des isotopes stables au sein de ce tissu ne doit pas être due uniquement à la croissance des poissons, mais aussi à leur catabolisme, sinon déterminer les signatures isotopiques lors de la phase transitoire sera équivalent à tout simplement mesurer la croissance.

En conclusion, ce travail de thèse a mis en lumière des points méthodologiques critiques qu'il faut prendre en compte pour développer un programme de sélection génétique sur l'efficacité alimentaire. De plus, il a posé les bases de nouvelles approches afin d'identifier un critère de sélection indirecte de l'efficacité alimentaire.

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Rodde, C., Chatain, B., Vandeputte, M., Quoc Trinh, T., Benzie, J.A.H., de Verdal, H., 2020a. Can individual feed conversion ratio at commercial size be predicted from juvenile performance in individually reared Nile tilapia *Oreochromis niloticus*? *Aquaculture Reports* 17, 100349.

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Abstract - Individual feed efficiency in fishes: direct measurement methods and indirect predictors to develop selective breeding programs in two major aquaculture species: European sea bass *Dicentrarchus labrax* and Nile tilapia *Oreochromis niloticus*.

In finfish aquaculture, feed is expensive and has environmental impacts. Improving feed efficiency (FE) to produce the same amount of fish with less feed is a major objective for sustainable aquaculture. This can be achieved by selective breeding but this requires methods for individual phenotyping, and measuring individual feed intake (FI) is technically challenging for fishes. The two best methods, to date, are either to rear fish individually or to tag them externally so that they can be visually identified while reared in small groups.

I investigated some important issues related to estimation of individual FE, on two major aquaculture species, European sea bass *Dicentrarchus labrax* and Nile tilapia *Oreochromis niloticus*. I demonstrated that estimating individual FE at juvenile stage in Nile tilapia was predictive of FE over the whole production cycle. Then, I compared the two phenotyping methods in Nile tilapia, to discover that they did not provide equivalent estimations. Finally, I also found that the most efficient fish at restricted feeding were not the most efficient at satiety, in both species.

Both phenotyping methods remain, however, tedious. I therefore investigated potential indirect selection criteria that are easier to measure. In European sea bass, there was no correlation of individual FE with individual metabolic rate (as oxygen consumption). Furthermore, I addressed methodological issues for implementation of stable isotope analyses with non-lethal sampling of fish scales as an indirect selection criterion.

To conclude, selecting fish at juvenile stage seems reliable and will cut selection costs. Phenotyping method for individual FE towards selective breeding depends, however, upon the species. In particular, species collective behaviour must be considered when choosing the most suitable method. Furthermore, fish should be phenotyped at holding conditions and feeding levels that are as close as possible to commercial practices. Metabolic rate might be useful as an indirect criterion if coupled with measures of ammonia excretion or body composition. Scale stable isotope analyses are technically feasible and require further investigation.

Résumé - L'efficacité alimentaire individuelle chez le poisson : méthodes de mesure directe et prédicteurs indirects pour développer des programmes de sélection génétique chez deux espèces aquacoles majeures : le bar européen *Dicentrarchus labrax* et le tilapia du Nil *Oreochromis niloticus*.

L'aliment utilisé en pisciculture est onéreux et impacte l'environnement. Améliorer l'efficacité alimentaire (EA) pour produire la même quantité de poisson en utilisant moins d'aliment est un objectif majeur pour rendre l'aquaculture plus durable. Cet objectif pourrait être atteint grâce à la sélection génétique, mais cela nécessite des méthodes de phénotypage individuel, et mesurer la prise alimentaire individuelle est complexe chez le poisson. Les deux meilleures méthodes, à l'heure actuelle, consistent soit à élever les poissons individuellement, soit à les marquer avec un tag externe pour les identifier visuellement au sein de petits groupes.

Je me suis focalisé sur des questions d'importance critique en lien avec l'estimation de l'EA individuelle, chez deux espèces aquacoles majeures, le bar Européen *Dicentrarchus labrax* et le tilapia du Nil *Oreochromis niloticus*. J'ai démontré qu'estimer l'EA individuelle au stade juvénile chez le tilapia du Nil permettait de prédire l'EA sur l'ensemble du cycle de production. Ensuite, j'ai comparé les deux méthodes de phénotypage chez le tilapia du Nil, et observé qu'elles ne fournissent pas des estimations équivalentes. Enfin, j'ai aussi constaté que les poissons les plus efficaces à taux de rationnement restreint n'étaient pas les plus efficaces à satiété, chez les deux espèces.

Les deux méthodes de phénotypage demeurent, cependant, fastidieuses. J'ai, en conséquence, cherché de potentiels critères de sélection indirecte qui soient plus faciles à mesurer. Chez le bar européen, il n'y avait pas de corrélation entre l'EA individuelle et le taux métabolique individuel (estimé par la consommation d'oxygène). Par ailleurs, j'ai résolu des problèmes d'ordre méthodologique afin d'implémenter l'analyse de la signature isotopique, grâce à l'échantillonnage non létal des écailles, en tant que critère de sélection indirecte.

Pour conclure, sélectionner les poissons au stade juvénile semble fiable et permettra de réduire les coûts de sélection. La méthode de phénotypage de l'EA individuelle à utiliser pour faire de la sélection génétique dépend, cependant, de l'espèce. En particulier, la structure sociale de l'espèce doit être prise en compte dans le choix de la méthode la plus appropriée. En outre, les poissons devraient être phénotypés dans des conditions d'élevage et de nourrissage aussi proches que possible de celles en élevage commercial. Le taux métabolique pourrait être utilisable en tant que critère de sélection indirecte si couplé avec des mesures de l'excrétion d'ammoniac ou de la composition corporelle. Quant à l'analyse de la signature isotopique des écailles, elle est techniquement réalisable et nécessite d'être plus amplement étudiée.