AQUAEXCEL

Aquaculture Infrastructures for Excellence in European Fish Research

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Deliverable 3.2

Best practices & cross-applicability of methods to measure phenotypes

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CO Confidential, only for members of the consortium (including the Commission Services)	

INDEX

TABLES INDEX

FIGURES INDEX

Abstract

Taking into account that aquaculture research is an applied science where extrapolation of experimental results at industrial scale is essential, the standardization of interesting traits definitions and its measurement methods and the implementation processes are of great importance in order to generate a perfect harmony between experimental and industrial scale. Moreover, the adequate characterization and definition of phenotypic traits partially determines the validation of biological models and their degree of transfer to industry. Consequently, public and regulatory norms have been increasing over time in many countries to protect the rights of companies and consumers. These quality regulations include aspects like composition and structure of the shell, meat quality, food safety, traceability and animal welfare.

AQUAEXCEL will contribute to the standardization of traits and methods and processes used to measure them in aquaculture by this deliverable titled “Best practices & cross-applicability of methods to measure phenotypes”, such as traits related to quality of fish and meat, growth and biosanitary quality of fish. For this reason and considering that the general objective of this project is to integrate, at a European scale, aquaculture research infrastructures with the goal of promoting their everyday use and development, this task has aimed to harmonize and standardize the way of measuring the phenotypic traits of most relevance (related to growth, performance, diet, reproduction, fish and meat quality and biosanitary safety), as well as experimental procedures taking into account the different culture systems, scales and species.

For this, a questionnaire to select the most relevant traits was created and sent to all partners of AQUAEXCEL. A total number of 354 *children traits* were included in this questionnaire from the ontology AQUAEXCEL-ATOL (Task 3.1 of AQUAEXCEL) and after analysis of the results 62 traits were selected. The measurement method of these traits was described and checked by all partners. This document establishes the first standardized “best practices” manual to measure the most usual phenotypic traits found in fish farming studies, covering traits for growth, productive, reproductive, nutrition, fish quality, meat quality and disease resistance.

The information will be used to create an experimental data repository (Task 3.3 of AQUAEXCEL), proposing a common frame for data organization. Moreover, this manual may be used by different research groups, companies and even consumers, as a reference guide of measurement methods. This will mean a great step towards a larger integration of current infrastructures in aquaculture research and European-scale standardization which will allow study and laboratory comparisons, both in research and industrial contexts.

Background

AQUAEXCEL is an aquaculture infrastructure network of excellence at European scale. Its aim is to coordinate this network to promote the development of aquaculture in Europe through research, development, innovation and knowledge transfer between research institutions and the industrial sector, thus serving better the needs of the market and the consumers. To achieve this, AQUAEXCEL will:

- Create the basis for the development of joint research projects between research institutions and companies by including the available culture systems (cage, flow-through, recirculation, pond and hatchery aquaculture systems, land and sea based, fresh and salt water installations).
- Promote the use, optimization and harmonization of research infrastructures.
- Generate “best practices” guides for the development of work standards in research in order to ease knowledge transfer and integration of specific production systems for each type of enterprise.
- Harmonize the methodology and organize the information flow produced by research and excellence infrastructures that define aquaculture in Europe.
- Promote the use, development and coordination of aquaculture infrastructures which are remotely accessible (e-Infrastructures).
- Develop new methodologies at an experimental level which shall be scalable to industry.
- Create scale correction models, with the purpose of incrementing the application of results to the industrial sector.

Taking into account that aquaculture research is an applied science where extrapolation of experimental results at industrial scale is essential, not only the standardization of interesting traits definitions but also its measurement methods and the implementation processes are of great importance in order to generate a perfect harmony between experimental and industrial scale. Moreover, the adequate characterization and definition of phenotypic traits partially determines the validation of biological models and their degree of transfer to industry. The high number of traits with industrial economic relevance, together with the different measurement methods described for every one of them produces results and phenotypic databases which cannot be compared one another. In this way, the identification and standardization of the measurement methods of the most relevant phenotypic traits in aquaculture research, promotes experiments among institutions This information, as a normalized manual, would facilitate the combination of databases, comparison of results among research groups and companies, and extrapolation of experimental results to industrial scale.

For instance, a simple mismatch in the way of characterizing the traits could result in great economic losses for companies, taking into account the scale factor of thousands of tonnes which are cultivated inside and between species. Therefore, as can be appreciated in scientific

publications, standardization and harmonization are more and more required in science (Araujo, 2009). Consequently, public and regulatory norms have been increasing over time in many countries to protect the rights of companies and consumers. These quality regulations include aspects like composition and structure of the shell, meat quality, food safety, traceability and animal welfare. As a matter of fact, today there are many recognized international organizations which offer guidelines for standardization and validation of measurement methods. Some of them are the Association of Official Analytical Chemists (AOAC), the American Society for Testing and Material (ASTM), the Codex Committee on Methods of Analysis and Sampling (CCMAS), the European Committee for Normalization (CEN), the Cooperation on International Traceability in Analytical Chemistry (CITAC), the European Cooperation for Accreditation (EA), the Food and Agricultural Organization (FAO), the United States Food and Drug Administration (FDA), the International Conference on Harmonization (ICH), the International Laboratory Accreditation Cooperation (ILAC), the World Health Organization (WHO) and the International Organization for Standardization (ISO).

AQUAEXCEL will contribute to the standardization of traits and methods and processes used to measure them in aquaculture by this deliverable titled "*Best practices & cross-applicability of methods to measure phenotypes*", such as traits related to quality of fish and meat, growth and biosanitary quality of fish.

One of the traits with the greatest economic impact in the industrial production of fish is the quality of whole fish due to the presence of morphological abnormalities (Afonso and Roo, 2007). The degree in which these morphological abnormalities affect company viability varies greatly due to the fact that there are discrepancies both between and in the companies themselves regarding malformation types, prevalence, economic repercussion, species and measurement criteria. In species such as European seabass, gilthead seabream and red porgy, the most relevant malformations are the ones which affect the opercular complex, the neurocranium and the spinal column (lordosis and vertebral fusion) (Koumoundouros *et al.,* 1997; Boglione *et al.,* 2001; Roo *et al.,* 2005). To fulfill market requirements, breeding companies are obliged to implant screening processes for their production batches, which adds associated costs. However, there are no normalized criteria between companies to define whether an individual is deformed or not. As a result, companies may reject healthy individuals as deformed fish or may sell deformed individuals as false positives, which could even create technical problems in filleted processes (Gjerde *et al*., 2005). This may generate economic losses for both breeding and ongrowing companies, particularly for species which are commercialized as whole animals such as seabass, gilthead seabream, red porgy or meagre. Moreover, the lack of standardized criteria for trait definition creates distrust between breeding and ongrowing companies to such an extent that business relationships and business expansion or production capacity may be destroyed when scale production is required to attend market necessities. Furthermore, there are other indirect ways where morphological abnormalities are affecting the companies. While fish farming morphological abnormalities are a

problem which has been broadly studied but partially understood (Witten *et al*., 2009; Boglione and Costa, 2011), they can be affected by nutritional, physiological, genetic, environmental and management factors (Thankappan and Thampy, 1990; Chatain, 1994; Astorga *et al*., 2004; Afonso and Roo, 2007; Lall and Lewis-McCrea, 2007; Bæverfjord *et al*., 2009; Roo *et al*., 2009; Izquierdo *et al*., 2010; Boglione and Costa, 2011), which at the same time enables strategy development across these determining factors, thus minimizing malformations in company-owned culture batches. However, to ensure the effectiveness of any developed strategy, it must include standardized criteria for trait definition. For instance, an inadequate characterization of deformed fish at the initial management phase could produce an underlying prevalence of abnormalities in breeding batches, which will manifest later in ongrowing batches.

The lack of standards in measurement and/or traits characterization between companies and/or research centers introduces harmful source variations, as is the case for the implementation of effective genetic strategies, which depend on the correct definition of malformations. In the European seabass (Dupont-Nivet *et al*., 2008 and Karahan *et al.,* 2012), and in the gilthead seabream (PROGENSA®, 2009 project), heritabilities for the absence-presence trait of skeleton malformations at the slaughter age from cultured and measured fishes at four different facilities were estimated. The estimates of heritability from all fish were lower than those estimated from the fishes cultured in each of production stations. This highlights the fact that the lower the variation generated by the way phenotype traits are measured, the more exact the estimations and the economic gains will be (Falconer and Mackay, 1996). The lack of harmonization could even prevent the implementation of genetic strategies. In the common carp (Kocour *et al*., 2006) and in the gilthead seabream (PROGENSA®, 2009 project) the lack of additive genetic variations in head malformations is not exempt from the difficulties to consider this deformity in a homogeneous and objective form.

As in meat industry, quality traits are becoming more relevant in aquaculture industry due to consumer demand (Gjedrem, 1997). Consumers are willing to pay more for high-quality certified meat, so the development and implementation of measures which guarantee quality in the food industry are required today more than ever before (Weeranantanaphan *et al*., 2011). As a matter of fact, it is common to see nutritional profiles included in the products during their commercialization stage as an argument for quality and traceability. In meat quality, the composition of muscle plays a leading role through attributes such as flavor, juiciness, texture and appearance. There are many factors which may alter the appearance of fish. For instance, the skin color and the meat elasticity are parameters which are generally included in the sensorial analysis of gilthead seabream (Huidobro *et al.,* 2000). Flavor and juiciness are highly and positively connected to fat content in the muscle and it is inversely related to moisture (Grigorakis, 2007). Traditionally, meat composition has been analyzed using biochemical methods. However, these methods involve the use of chemical agents which are dangerous for health and the environment, and are, in general, long-lasting, expensive, invasive and show a

relevant variation. This has encouraged the development of standard methods, which can be automated and minimize errors in measurement.

In fish farming, growth and performance traits are the most important ones from an economic point of view, which also happens in other meat species (Navarro *et al.,* 2009). Due to this fact, they have been the most studied ones, although there are many traits which cover these parameters. Nevertheless, traits which may be regarded as simple to measure, such as length, have different measurement methods. When reviewing past and present scientific literature, and even in several fish type catalogues, the length measurement method is neither defined nor specified. The consequences are discrepancies between studies, registered measurements in the original description of the species and the ones done in catalogues (Howe, 2002). In this sense, the lack of standardized methods has obstructed the attempts to synthesize data, especially in comparative studies of fish species because of the lack of information (Gaygusuz *et al.,* 2006). This also happens with fillet performance, a trait that is getting more relevance overtime, taking into account that current society demands more and more processed products. The variations in the fillet system (manual, automatic, including or not including the belly), lead to differences which make data comparison difficult even for the same species (Navarro *et al.,* 2009).

The same can be applied to results concerning disease resistance: the fact that different challenge procedures to pathogens produce differences in resistance makes more difficult the extraction of consistent results and the extrapolation of experimental scale results to industrial scale ones.

According to the facts expressed above, the adequate definition of traits and the standardization of their measurements are important for results comparison, evaluation and regulation. Although there are discrepancies in simple objective traits such as the ones quoted, there are many more in subjective traits as for instance meat quality and animal welfare, which should be defined and described first (Becker, 2002; Ginés *et al.,* 2004; Huntingford *et al.,* 2006).

Objectives

For this reason and considering that the general objective of this project is to integrate, at a European scale, aquaculture research infrastructures with the goal of promoting their everyday use and development, this task has aimed

a) To select the phenotypic traits of most relevance in aquaculture related to growth, performance, diet, reproduction, fish and meat quality and bio-sanitary safety.

b) To harmonize and standardize the measurement methods of these phenotypic traits , as well as experimental procedures taking into account the different culture systems, scales and species.

c) The purpose is to generate with this information a “best practices” manual as a way of harmonizing, normalizing and validating experimental processes and phenotypic characterization in a common language for researchers, companies and even consumers, which will allow study and laboratory comparisons, both in research and industrial contexts.

Methodology

i) Identifying relevant phenotypes to be measured

The spectrum of aquaculture phenotypic traits is very wide. For this reason, the leader of task 3.2 "*Harmonization and standardization of phenotypic and experimental conditions"*, Dr Marisol Izquierdo, suggested preparing a selection of the 50 most relevant traits. This was approved in the AQUAEXCEL Annual Meeting held in Gran Canaria in February 2012. To achieve this task, it started with the ontology AQUAEXCEL-ATOL, developed and led by Dr Pierre-Yves Lebail in the context of task 3.1 "*Development of an ontology for fish traits, measure methods and environment conditions"* (http://www.atol-ontology.com/index.php/en/les-ontologies-en/visualisation-en). It is a semantic network which comprehends a set of related concepts describing a subject in its totality. This ontology is a conceptual representation shared and agreed of fish measurable traits in the aquaculture context. The traits are organized in a hierarchical way, grouped in four branches ("*grandparent traits*"):

- *Animal Welfare traits*
- *Growth and Meat Production traits*
- *Nutrition traits*
- *Reproduction traits*.

These four branches are ramified in *parent traits*, which include several more specific traits until arriving at the most specific ones, named *children traits*. Every one of these has an identification number and its definition, as can be observed in figure 1.

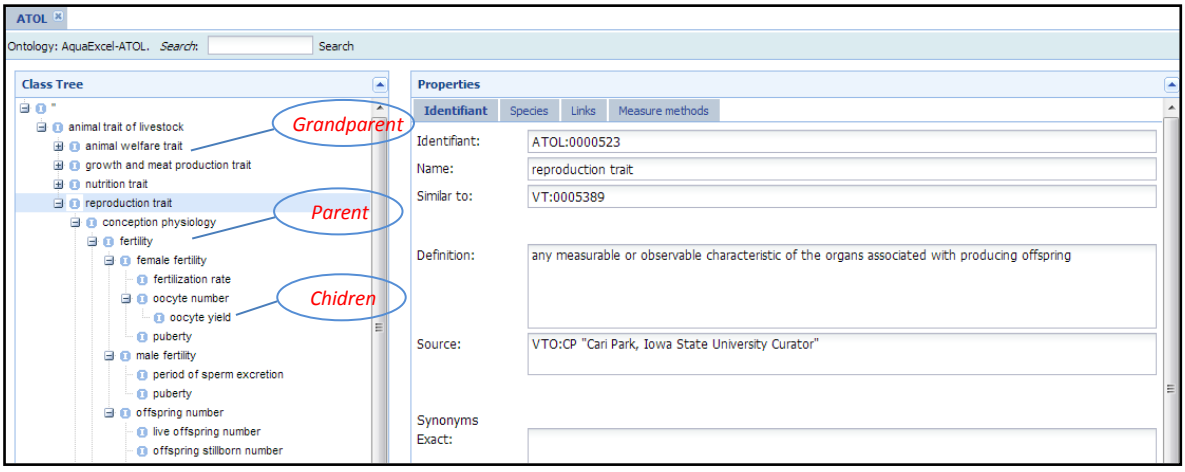

Figure 1. Screen capture of the fish trait ontology for livestock**.**

To select the most relevant traits, a questionnaire between expert partners was created so as to obtain the most objective, coherent and useful possible criteria. The questionnaire used an Excel file structured in an easy and fast way to fill in. All *children traits* of the AQUAEXCEL-

ATOL ontology were included in it grouped in four sheets according to the four big groups they belonged to (*Animal Welfare traits*, *Growth and Meat Production traits*, *Nutrition traits* and *Reproduction traits*). Those *children traits* which were repeated in several branches of AQUAEXCEL-ATOL were included only once in the questionnaire.

An example of the Excel file can be observed in figure 2. Each row contains all the information related to each trait according to the AQUAEXCEL ontology:

- In the first column, the identification number (ID or IDENTIFIANT).
- In the second column, an intermediate trait in the hierarchy (A PARENT TRAIT).
- In the third column, the name of the trait (CHILDREN TRAIT).
- In the fourth column, an optional checkbox where each partner selects the trait (SELECTED).
- In the fifth column, a pull-down menu where each partner selects the importance of the trait (PRIORITY).
- In the sixth column, the definition of the trait (DEFINITION).

Each expert partner has selected what they consider the 50 most relevant traits choosing them from the “SELECTED” checkbox and for each of them they chose their importance using the drop-down list “PRIORITY” (*important*, *very important* or *essential*). To ease the accounting of the selected traits, each sheet has a box which automatically adds up the selected traits from (“ANIMAL WELFARE COUNTING”, “GROWTH AND MEAT COUNTING”, “NUTRITION COUNTING” and “REPRODUCTION COUNTING”) and another box which accounts for all the selected traits (“TOTAL COUNTING”). In figure 2, both boxes are highlighted in yellow. If a partner considered that an essential trait was missing from the list of *children traits*, there is the possibility of including it at the end of the questionnaire. Moreover, the questionnaire supplied a simple user guide with all the necessary information to complete it.

	A	B	C	D	E	F
1	NUTRITION TRAIT			NUTRITION COUNTING		TOTAL COUNTING
2	N=72			14		50
3	ID	A PARENT TRAIT	CHILDREN TRAIT	SELECTED	PRIORITY	DEFINITION
4	ATOL:0000558	gastrointestinal	intestine length	☑	Important	any measurable characteristic rela
5	ATOL:0001079	gastrointestinal	empty intestine relative weight	☐		ratio of empty tissue weight of th
6	ATOL:0001078:	gastrointestinal	empty intestine weight	☐		weight of empty intestine
7	ATOL:0001078	gastrointestinal	full intestine weight	☑	Essential	weight of tissue and content of th
8	ATOL:0001817	gastrointestinal	pyloric cecum morphology	☐		any measurable characteristic rela
9	ATOL:0000553	gastrointestinal	oesophagus length	☑	Very Imp.	e length of the oesophagus
10	ATOL:0001063	gastrointestinal	empty oesophagus relative weight		Essential / Very Imp. / Important	tio of empty tissue weight of th
11	ATOL:0001822:	gastrointestinal	empty oesophagus weight			eight of empty oesophagus
12	ATOL:0001822	gastrointestinal	full oesophagus weight	☐		weight of tissue and content of th

ANIMAL WELFARE | GROWTH AND MEAT | NUTRITION | REPRODUCTION | Hoja3

Figure 2. Excel questionnaire sent to each partner to select the most relevant traits.

These questionnaires were filled in by experts of the following institutions which are part of the project AQUAEXCEL: CSIC, HAKI, HCMR, IFREMER, IMARE, IMR, INRA, NOFIMA, NTNU, SINTEF, ULPGC, VURH and WU (87% of all participant institutions). Once all questionnaires were received, ULPGC added up all the votes obtained by each trait (meaning, how many partners selected that specific trait), and the accounting of points according to their priority (adding 1 point if it was selected as *Important*, 2 points if it was selected as *Very important* and 3 points if it was selected as *Essential*). All the traits that had 3 or less votes were discarded. From those which had 4 votes, the ones with 8 or less points were also discarded.

ii) Standardization and review of measurement methods of selected traits

To standardize measurement methods for the selected traits, we prepared an identification file of each of them using their ID number, name and definition stated in the AQUAEXCEL-ATOL. Moreover, each file contained 8 boxes ready to be filled which were previously agreed with the developers of AQUAEXCEL-ATOL (research team of Dr Pierre Yves-Lebail) and the AQUAEXCEL coordinator (Dr Marc Vandeputte). This file is shown in Table 1.

Table 1. Boxes of the form to fill, in order to standardize measurement methods of the selected traits.

SIMILAR TO: (*If it's appropriate, in connection with other Identifiant number*)
MEASUREMENT METHOD: *(The description has to be enough precise to be sure that it cannot be any difference of application. In this way, all results acquired on phenotypic traits in our various structures will be fully comparable, taking into account the metadata related to the environment).*
MATERIAL (biological, reagents & instrumental): (*If it's appropriate*)
UNIT AND RANGE OF VALUE:
PARAMETERS TO MEASURE:
BIBLIOGRAPHIC REFERENCES:
SYNONYMS EXACT: (*If it's appropriate*)
OTHER ASPECTS TO INCLUDE: (*If it's appropriate*)

Every partner was sent four of these trait files ready to be filled, which had been previously selected by every one of them. During the reply period, we kept in touch with each of the partners when necessary to ensure that the files were completed on time and form.

iii) Developing a best practices manual and harmonizing protocols and measurement methods

The files were reviewed, corrected and completed by ULPGC staff once they had all been received. The new versions of the files were sent to all expert partners, members of the AQUAEXCEL project, so they could all add or change what they deemed necessary. The objective of this step was to ensure that the information was as standardized and objective as possible, taking into account the wide range of measurement conditions and species involved.

To ensure that each expert partner could carry out the revision of the files as precisely and easily as possible, ULPGC sent them a summary table including all the initially selected traits and priorities by every partner. In addition to the trait's name in each file, ULPGC added an internal code as prefix to facilitate its identification. This code consisted on the initials of their *grandparent trait* group (AW, Animal Welfare traits; GM, Growth and Meat production traits; NT, Nutrition traits; RP, Reproduction traits) and a correlative number. E.g.: "AW.1_Aggressive behavior.docx".

Once all reviewed files were received, ULPGC generated a draft version including all suggestions and changes made by every partner, and including the global revision by INRA (Dr. Lebail, Dr. Joret). This draft version was sent to specialists per children trait and partner, in order to be enriched and increased in networking. To do it, previously, each partner supplied a list with specialists to target it adequately. A wide period was opened for answering and interaction. When all reviewed files were received, ULPGC (Dr. Izquierdo, Dr. Navarro, Dr. Afonso) integrated all recommendations made by every specialist, and generated a final version including all suggestions and changes. This final version included a general revision by other specialists per *grandparent trait* group: Animal Welfare traits (Dr. Sitjà-Bobadilla), Growth and Meat production traits (Dr. Pérez-Sánchez), Nutrition traits (Dr. Kaushik), and Reproduction traits (Dr. Mañanós). The final version was then validated by Dr. Fyhn Terjesen and Dr. Vandepute, following the internal process established in the AQUAEXCEL project.

Results

i) Identifying relevant phenotypes to be measured

Regarding AQUAEXCEL-ATOL, a total number of 354 *children traits* were included in the questionnaire:

- 103 *Animal welfare traits*
- 127 *Growth and Meat production traits*
- 72 *Nutrition traits*
- 52 *Reproduction traits*

Once all the questionnaires completed by the expert partners were received, the results obtained showed that 214 traits were voted at least once (60.4% of the total) and 5 new traits were added ("*ovary weight*", "*body weight*", "*carcass weight*", "*carcass yield*" and "*visceral adipose tissue weight*"). Although these traits are in AQUAEXCEL-ATOL, they were not part of the *children traits*. In Figure 3, the distribution of those 214 traits according to the number of votes can be observed. As it is reported, 72% of the traits were voted 1, 2 or 3 times and only 28% had 4 or more votes (60 traits). This showed a division and turning point between the traits voted 4 or more times and those voted less than 4 times. All these results were presented to the Executive Committee of AQUAEXCEL in the meeting held in Norway in September 2012. In the light of these results, the Executive Committee, based on the suggestion of the WP3 package leader (ULPGC), decided to consider only the traits voted 4 or more times and the new 5 traits due to their relevance.

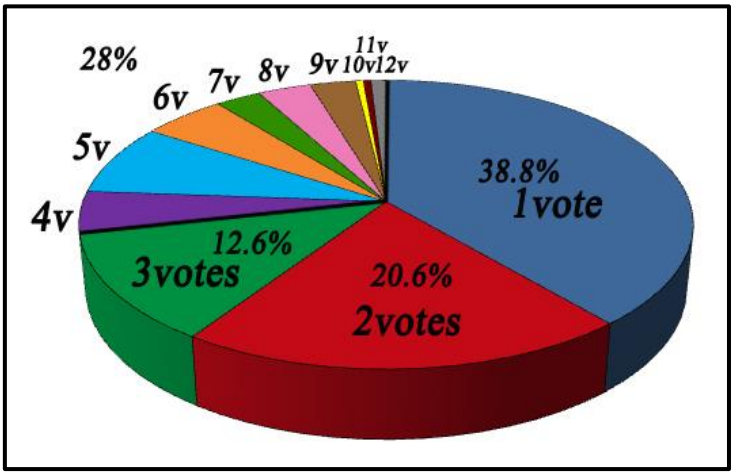

Figure 3. Distribution of the number of traits based on the number of votes.

Figure 4 shows the votes obtained for the 60 most voted 4 or more times, grouped by their *grandparent trait*. These were:

- 15 *Animal Welfare traits*
- 18 *Growth and Meat production traits*
- 17 *Nutrition traits*
- 10 *Reproduction traits*

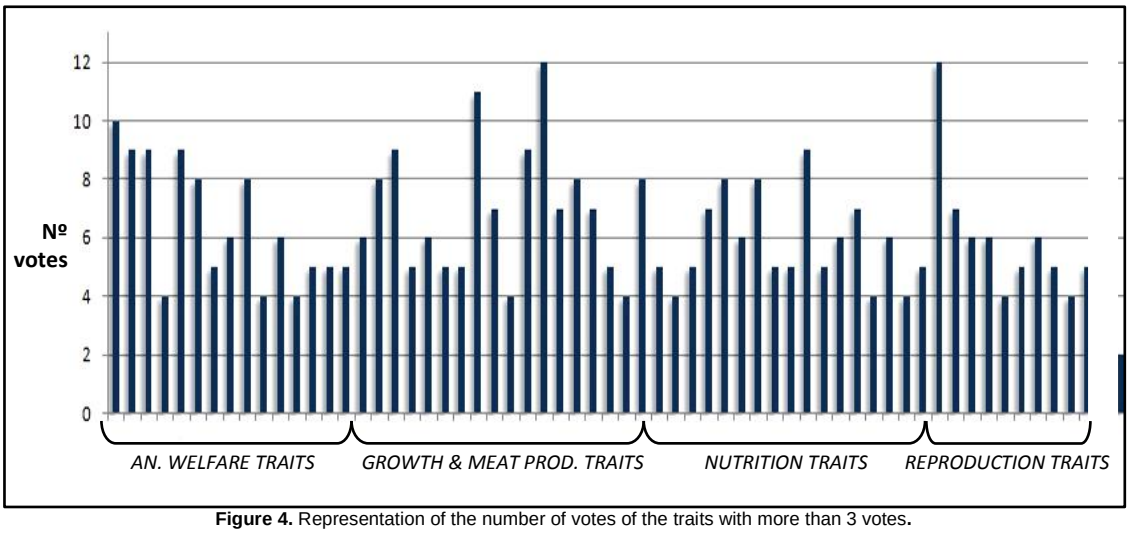

Figure 4. Representation of the number of votes of the traits with more than 3 votes**.**

Since the 65 traits selected for the standardization of measure methods (60 with 4 or more votes and the 5 new ones included by the partners) exceeded the number originally included in WP3 (50 traits), it was decided to take into account the priority score established by each of the expert partners on top of the number of votes. In Figure 5, the traits ordered by the number of votes and by priority score can be observed.

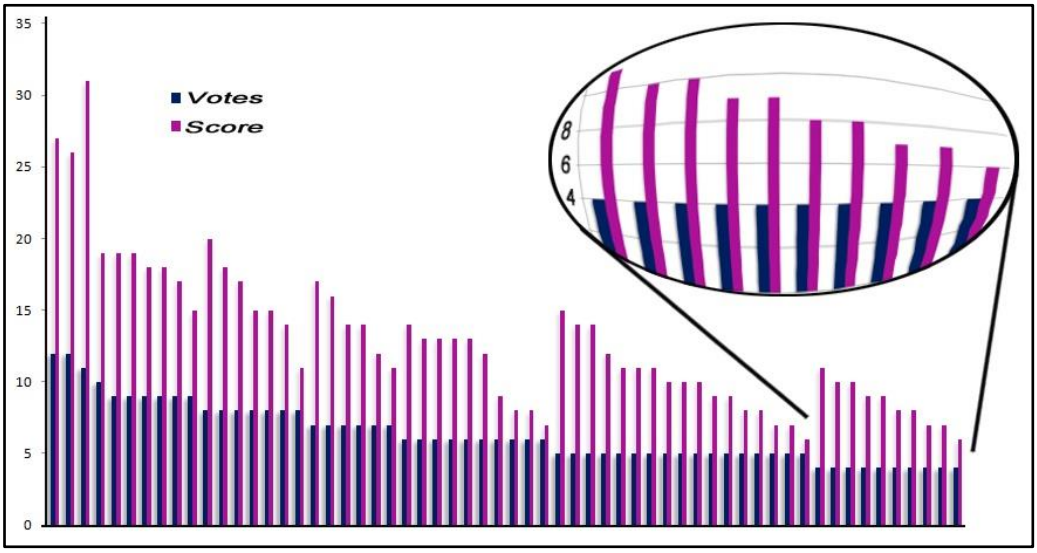

Figure 5. Traits ordered by the number of votes and the priority score.
The oval is a magnification of the traits with only 4 votes.

From the traits with 4 votes it was decided that the ones with least score (< 8) should be rejected (3 traits). Finally, 62 traits were selected as relevant phenotypes for the standardization of the measurement method (Table 2):

- 15 *Animal Welfare traits*
- 21 *Growth and Meat production traits*
- 15 *Nutrition traits*
- 11 *Reproduction traits*

Table 2. List of all selected relevant traits grouped by the four big branches of AQUAEXCEL-ATOL and arranged alphabetically. Within each group, the first column is an internal code and is linked to the "best practices" manual generated in this Task (Annex-1).

ANIMAL WELFARE		GROWTH AND MEAT		NUTRITION		REPRODUCTION	
AW1	Aggressive behavior	GM1	Age at slaughter	NT1	Average daily feed intake	RP1	Fertilization rate
AW2	Body integrity	GM2	Body compartment yield	NT2	Eating rate	RP2	Live offspring number
AW3	Carbohydrate metabolism in liver	GM3	Body weight	NT3	Energy requirement	RP3	Oocyte yield
AW4	Feeding learning	GM4	Carcass weight	NT4	Feces gross energy content	RP4	Oogenesis
AW5	Food preference	GM5	Carcass yield	NT5	Feces total lipid content	RP5	Ovarian follicle morphology
AW6	Cortisol level	GM6	Condition factor	NT6	Feces major mineral content	RP6	Ovary weight
AW7	Glucocorticoid level	GM7	Exterior defects	NT7	Feed apparent digestible nutrient	RP7	Puberty
AW8	Lipid metabolism	GM8	Fork length	NT8	Feed component apparent digestibility	RP8	Sperm mobility
AW9	Protein efficiency	GM9	Meat colour	NT9	Feed component true digestibility	RP9	Sperm motility
AW10	Respiratory rate	GM10	Meat cooking loss	NT10	Feed efficiency	RP10	Spermatogenesis
AW11	Social dominance	GM11	Meat flavour	NT11	Hepato-somatic-index	RP11	Testes weight
AW12	Stress HPA sensitivity	GM12	Meat lipid content	NT12	Nutrient absorption		
AW13	Susceptibility to bacterial infection	GM13	Meat ω6 to ω3 fatty acid ratio	NT13	Nutrient balance		
AW14	Susceptibility to viral infection	GM14	Meat pH	NT14	Trace mineral element requirement		
AW15	Swimming behavior	GM15	Meat protein content	NT15	Voluntary feed intake		
		GM16	Muscle to body weight ratio				
		GM17	Skin colour trait				
		GM18	Specific growth rate				
		GM19	Thermal growth coefficient				
		GM20	Total length				
		GM21	Visceral adipose tissue weight trait				

ii) Standardization and review of measurement methods of selected traits and development of a best practices manual

Once the file card for the standardization of measurement methods of the 62 selected traits was generated, it was sent to all partners and all the information was received, the latter was reviewed, corrected and the vocabulary homogenized. The files were completed with information from the literature and other European projects such as FAIR, RAFOA, PUFAFEED, LARVANET, AQUAFIRST or ARRAINA, as well as from national projects such as PROGENSA® in order to comprise all the species included in this project (seabream, seabass, red porgy, meagre, rainbow trout, zebrafish, cod, Atlantic salmon).

With all this information, a draft document was created and sent to all expert partners, so they could check all the contents in each of the trait files, taking into account the species and work conditions. Once suggestions and comments were received, a final improved version was produced which is included in Annex-I. Thus, this final version incorporated a very important networking in terms of institutions and specialists, with a 72.2% of answers from 255 required revisions (institutions-researchers-children traits). An average of 3.39 specialists contributed in each trait file (figure 6).

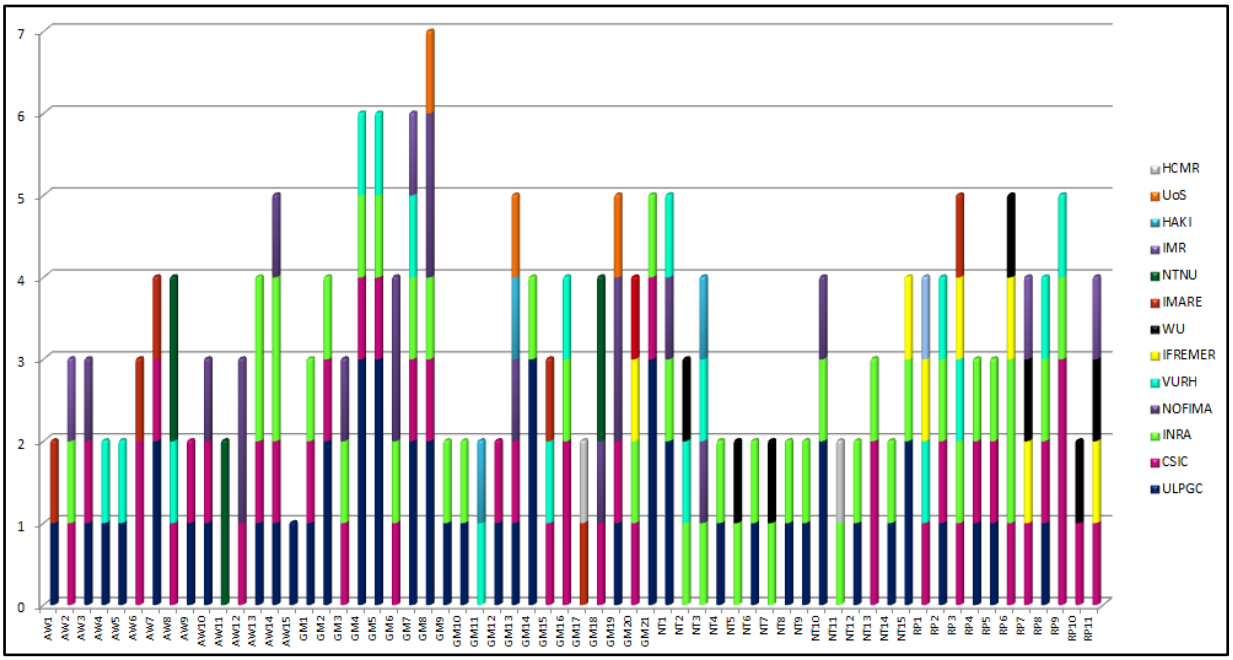

Figure 6. Number of specialists per children trait.

This version establishes the first standardized “best practices” manual, named “Deliverable 3.2-*Best practices & cross-applicability of methods to measure phenotypes*”, to measure the most usual phenotypic traits found in fish farming studies, covering traits for growth, productive, reproductive, nutrition, fish quality, meat quality and disease resistance. This document will be used to create an experimental data repository (Task 3.3 of AQUAEXCEL), proposing a common frame for data organization. Moreover, this manual includes a documented and normalized explanation of measurement methods for the 62 phenotypic traits, which may be

used by different research groups, companies and even consumers, as a reference guide. This will mean a great step towards a larger integration of current infrastructures in aquaculture research and European-scale standardization which will allow study and laboratory comparisons, both in research and industrial contexts.

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ANNEX-1

Deliverable 3.2-BEST PRACTICES

& CROSS-APPLICABILITY OF METHODS

TO MEASURE PHENOTYPES

Identifiant	ATOL:0000813	**Code:** AW1
Name of Trait:	Aggressive behavior	
Definition:	Any measurable or observable characteristic related to behavioural display or sign performed by an individual leading to threat and/or physical attack.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Aggressive behaviour is classified in three levels; aim, face and chase. *Aim*: an action that targets another fish from behind for 1 s or more while the fish bends the body in an L-shape. *Face*: an action by which fish face each another in a threatening manner for 1 s or more while both fish bend their bodies in an L-shape. *Chase*: an action involving pecking, chasing and/or biting another fish from the side and behind.

The forms of aggressive behaviour in each tank are counted by 1-4 observers for 5-30 min at 5-6 defined times in a period of 24 h, according to the hypothesis and objectives of the trial. Averages for the different progressive time-periods are converted to frequency per fish per hour. The frequencies of the three forms of aggressive behaviour are used separately, or summed.

MATERIAL (biological, reagents & instrumental):

Video recording, analyzed by computer software (example; Noldus software).

UNIT AND RANGE OF VALUE:

Number of aggressive actions/ hour.

PARAMETERS TO MEASURE:

Number of aggressive actions per fish per hour.

Additional criteria.

BIBLIOGRAPHIC REFERENCES:

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SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

There are various parameters that influence aggressive behaviour of fish in aquaculture settings and these should be considered when aggressive behaviour is monitored. These include the density of the fish, the feeding regime, the fish size, the size dispersion of the fish in the tanks. Moreover, aggressive behaviour is highly species specific, different species react differently to the conditions that the fish encounter

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Link Table 2

Identifiant	ATOL:0000931	**Code:** AW2
Name of Trait:	Body integrity	
Definition:	Any measurable or observable external characteristic of the whole body related to any damage, such as scratches, abrasions, inflammation, wounds, hemorrhages, or fin amputations.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Abrasions: A scraped area on the skin or on a mucous membrane. Must be described by what part of the body is inflicted and size (total area) and severity.

Scratches: A small shallow wound, but the skin or surface is broken. Must be described by its size (total area), severity (number and size of scratches), and its location.

Wounds and ulcers: Damage to skin or membrane where the surface is broken (total area and degree of severity). Wounds can be very small (i.e. scratches) or big deep and fatal to the organism. Wounds can turn into ulcers when colonized by bacteria.

Inflammation: A response of a tissue to injury. It is normally characterized by redness and swelling. It must be described by its size (total area), severity (degree of severity), and its location.

Haemorrhage: Bleeding from open wounds in e.g. skin or gills or from the vent or mouth. It must be described by its severity (degree of severity), and where the haemorrhage is found.

Fin damage: Scratches, wounds, inflammation and full or partial loss of fins with or without haemorrhage.

Eye abnormalities: such as absence of eye balls, opacity of the crystalline or exophthalmia (a marked protrusion of the eyeballs) should be recorded.

Tumours: irregular growths on fins or body. They must be described by their sizes, affected area, affected tissue layers (skin, muscle), texture (lumpy or solid), color (white, red,etc.). Tissue samples for histology and virology should be taken to classify the tumour and determine the possible cause.

Abdominal swelling: increased abnormal size of the abdomen out of the spawning season. It is an external sign of some infections (virus, bacteria or parasites) or osmorregulatory imbalances (dropsy). The bulk of the swelling and its texture (soft or hard) has to be described. When possible, samples of the ascitic liquid accumulated in the abdomen can be taken with a fine needle-syringe, without killing the animal under anaesthesia.

MATERIAL (biological, reagents & instrumental):

Digital caliper, ruler, automatic image processing software, digital camera

UNIT AND RANGE OF VALUE:

PARAMETERS TO MEASURE:

Abrasions

Scratches

Wounds

Inflammation

Haemorrhage

Fin damage

Eye abnormalities

Tumours

Abdominal swelling

BIBLIOGRAPHIC REFERENCES:

Ellis, T., Hoyle, I., Oidtmann, B., Turnbull, J.F. Jacklin, T.E. Knowles, T.G., 2009. Further development of the “Fin Index” method for quantifying fin erosion in rainbow trout. *Aquaculture* 289, 283-288.

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SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

Inflammation, wounds, or hemorrhages are often the symptoms of viral diseases (VHS, IHN) bacterial (*Flavobacterium, Psychrophilum, Yersinia ruckeri*) or consequence of parasitic infections (sea lice, *Gyrodactylus*, *Ichthyophthirius multifiliis*)

This trait is related to “Exterior defects” (GM7) which includes shape, pigmentation, scales, swim bladder and skeletal. In none of them other aspects such as ulcers, white spots, tumours or eye emptiness are included.

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(20/06/13)

Link Table 2

Identifiant	**ATOL:0000___**	**Code:** AW3
Name of Trait:	Carbohydrate metabolism in liver	
Definition:	Any measurable characteristic related to the chemical reactions and pathways involving glucose, the aldohexose gluco-hexose in liver.	

SIMILAR TO:

ATOL:0000827

MEASUREMENT METHOD:

Activity of Glucose-6-phosphate 1-dehydrogenase G6PDH, malic enzyme, Acyl-CoA oxidase and L-3-hydroxyacyl-CoA dehydrogenase.

Liver samples are homogenized in 3 volumes of ice cold buffer (20 mM Tris–HCl, 0.25 M sucrose, 2 mM EDTA, pH 7.4) and centrifuged at 20000g for 40 min at 4°C. Activities of *glucose-6-phosphate dehydrogenase* (G6PDH, EC 1.1.1.49) and *malic enzyme* (ME, EC 1.1.1.40) are assayed using spectrophotometric procedures on the supernatant.

Acyl-CoA oxidase (ACO) is assayed in peroxisome enriched liver fractions prepared by homogenizing in 3 volumes of ice-cold buffer (20 mM Tris-HCl, 0.25M Sucrose, 2 mM EDTA, pH 7.4). The homogenate is centrifuged at 7200g for 10 min at 4ºC and the supernatant fraction collected. The resulting pellet is washed once with 500 µl of the same buffer, centrifuged as above and the supernatant collected and combined with the first one. Combined supernatants are centrifuged at 18000g for 30 min and the resulting pellet resuspended with 600 µl of the buffer prior to sonication bath for 30 min. After sonication, supernatants are centrifuged at 18000g for 45 min and the supernatants collected for analyses. ACO is assayed by the spectrophotometric determination of H_2O_2 production, coupled to the oxidation of 2',7'-dichlorofluorescein diacetate (LDCF) at 502 nm. The reaction mixture contains 2.6 mM LDCF, 1M aminotriazole, 5mg/ml horseradish peroxidase type II, 5% triton X100, 1 M Tris-HCl pH 8.5, 15 mM Flavin adenine dinucleotide, 50mg/ml BSA. The reaction is started by the addition of 1mM Palmitoyl-CoA.

Mitochondrial preparations of liver samples are centrifuged and the activity of *L-3-hydroxyacyl-CoA dehydrogenase* (L3HOAD; EC 1.1.135) is measured on mitochondrial isolates disrupted by sonication in a 1% Triton X-100 solution. All enzyme assays must be performed at least in triplicate.

Soluble protein content of liver homogenates is determined on the supernatant using bovine serum albumin (BSA) as standard. Care must be taken to ensure that initial rates are measured in all assays and that enzymes are stable in the buffer solution used during the time and temperature required to perform the assays.

MATERIAL (biological, reagents & instrumental):

UNIT AND RANGE OF VALUE:

Enzyme activity units (IU), defined as micromoles of substrate converted to product at assay temperature per minute, are expressed per mg of hepatic soluble protein (specific activity).

PARAMETERS TO MEASURE:

Activity of Glucose-6-phosphate 1-dehydrogenase G6PDH, malic enzyme, Acyl-CoA oxidase and L-3-hydroxyacyl-CoA dehydrogenase in liver and sample protein content.

BIBLIOGRAPHIC REFERENCES:

Álvarez, M.J., López-Bote, C.J., Díez, A., Corraze, G., Ariel, J., Días, J., Kaushik, S.J., Bautista, J.M., 1998. Dietary fish oil and digestible protein modify susceptibly to lipid peroxidation in the muscle of rainbow trout (*Oncorhynchus mykiss*) and sea bass (*Dicentrarchus labra*x). *J Nutr.* 80, 281-9.

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Makol, A., Torrecillas, S., Fernández-Vaquero A., Robaina L., Montero, D., Caballero, M.J., Tort, Ll., Izquierdo, M.S., 2009. Effect of conjugated linoleic acid on dietary lipids utilization, liver morphology and selected immune parameters in sea bass juveniles (*Dicentrarchus labrax*). *Comp. Biochem. Physiol*. 154, 179–187.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

Gene expression of:

Glucose-6-phosphate 1-dehydrogenase (G6PDH)

Hexose-6-phosphate 1-dehydrogenase (H6PDH)

6-phosphogluconate dehydrogenase (PGD)

Fructose-1, 6-bisphosphatase 1 (FBP1)

Fructose-bisphosphate aldolase A (ALDOA)

Fructose-bisphosphate aldolase B (ALDOB)

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Link Table 2

Identifiant	ATOL: 0000924	**Code:** AW4
Name of Trait:	Feeding learning	
Definition:	Any measurable or observable characteristic related to the ability of an animal to learn information associated to feeding context or food characteristics.	

SIMILAR TO:

ATOL:0000364

MEASUREMENT METHOD:

Adaptation phase: The fish must be trained to use the self-feeder device during a 15-day adaptation period. In this phase, feeders must contain same diet. At the end of the adaptation, feeder solicitations within each tank are compared in order to analyze feeder preferences, independent of the diet. The preferred feeder is assigned as the one receiving more than 50% of total feed demands over the last 5 days (d5–d0) of the adaptation. The initial preference (P*ini*, %) is calculated as the ratio: 100 * (feed demands in preferred feeder [d5-d0]/total feed demands [d5-d0]). Groups with P*ini*>90% or which did not adapt to the self-feeder device must be excluded from the trials.

Trial phase: In a 15-day test phase (d1–d15), the preferred feeder distributed a test diet and the less preferred diet used in the adaptation phase. Relative preference (RP, %) is calculated as ratio: 100*(P/P*ini*) with preference rate P=100*(feed demands in the initially preferred feeder/total feed demands averaged over the 5-day periods). The RP varies between 100% at the start of the test phase or in case no changes in feeder demands occurred (P=P*ini*), and 0% in case of complete avoidance of the test diet (P=0).

Verification phase: Each test phase is followed by a validation phase in order to verify if changes

in feeder preferences are steered by the diet composition. In the groups which had a change of at least 5% in the RP at the end of the test (RP11 – 15<95%), the diets in the two feeders are exchanged. In the other groups (RP11 –15>95%), the test diet is replaced by a repellent control diet with sulfamerazin. The duration of the validation period must be 15 days. The changes in RP during the validation phase are calculated relative to the RP11 – 15 at the end of the test 100 (P/RP11 – 15) with preference rate P=100*(feed demands in the preferred feeder at the end of the test/total feed demands over a given 5-day period). The RP varies between 100% at the start of the validation phase or in case no changes in feeder demands occurred (P=P11 – 15), and 0% in case of complete avoidance of the previously preferred feeder (P=0).

MATERIAL (biological, reagents & instrumental):

Tank with self-feeder device.

UNIT AND RANGE OF VALUE:

%

PARAMETERS TO MEASURE:

Dynamic of feeder preference according to the diets during the trial phase, measured in percentage of responding animals and training time (time required to respond).

BIBLIOGRAPHIC REFERENCES:

Geurden, I., Cuvier, A., Gondouin, E., Olsen, R.E., Ruohonen, K., Kaushik, S., Boujard, T., 2005. Rainbow trout can discriminate between feeds with different oil sources. *Physiology & Behavior* 85, 107 – 114.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

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(20/06/13)

Link Table 2

Identifiant	ATOL:0000364	Code: AW5
Name of Trait:	Food preference	
Definition:	Any measurable or observable characteristic related to the consumption of one type of food relative to other ones.	

SIMILAR TO:

Feeding learning (ATOL: 0000924)

MEASUREMENT METHOD:

Adaptation phase: The fish must be trained to use the self-feeder device during a 15-day adaptation period. In this phase, feeders must contain same diet. At the end of the adaptation, feeder solicitations within each tank are compared in order to analyze feeder preferences,

independent of the diet. The preferred feeder is assigned as the one receiving more than 50% of total feed demands over the last 5 days (d5–d0) of the adaptation. The initial preference (P*ini*, %) is calculated as 100_* (feed demands in preferred feeder [d5_d0]/total feed demands [d5_d0]). Groups with P*ini*>90% or which did not adapt to the self-feeder device were excluded from the trials.

Trial phase: In a 15-day test phase (d1–d15), the preferred feeder distributed a test diet and the less preferred one the diet used in the adaptation phase. Relative preference (RP, %) is calculated as 100*(P/P*ini*) with preference rate P=100*(feed demands in the initially preferred feeder/total feed demands averaged over the 5-day periods). The RP varies between 100% at the start of the test phase or in case no changes in feeder demands occurred (P=P*ini*), and 0% in case of complete avoidance of the test diet (P=0).

Verification phase: Each test phase is followed by a validation phase in order to verify if changes in feeder preferences are steered by the diet composition. In the groups which had a change of at least 5% in the RP at the end of the test (RP11 – 15<95%), the diets in the two feeders are exchanged. In the other groups (RP11 –15>95%), the test diet is replaced by a repellent control diet with sulfamerazin. The duration of the validation period must be 15 days. The changes in RP during the validation phase are calculated relative to the RP11 – 15 at the end of the test 100(P/RP11 – 15) with preference rate P=100*(feed demands in the preferred feeder at the end of the test/total feed demands over a given 5-day period). The RP varies between 100% at the start of the validation phase or in case no changes in feeder demands occurred (P=P11 – 15), and 0% in case of complete avoidance of the previously preferred feeder (P=0).

MATERIAL (biological, reagents & instrumental):

Tank with self-feeder device

UNIT AND RANGE OF VALUE:

%

PARAMETERS TO MEASURE:

Relative preference by a test diet

BIBLIOGRAPHIC REFERENCES:

Geurden, I, Cuvier, A, Gondouin, E, Olsen, RE, Ruohonen, K, Kaushik, S, Boujard, T., 2005. Rainbow trout can discriminate between feeds with different oil sources. *Physiology & Behavior* 85, 107 – 114.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

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(20/06/13)

Link Table 2

Identifiant ATOL:0000___ **Code:** AW6

Name of Trait:	Cortisol level
Definition:	Any measurable characteristic related to the proportion or amount in a body fluid or tissue of cortisol.

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Immunoassay kits are designed to quantitatively measure cortisol in water extracts, fecal extracts, serum, plasma and tissue culture media samples. A cortisol standard is provided to generate a standard curve and a cortisol-peroxidase conjugate is added to standard and samples in the wells of microtiter plates coated with monoclonal cortisol antibodies. After incubation, a peroxidase substrate is added and cortisol concentration is calculated after absorbance measurements in a microplate reader.

Cortisol can also be determined by radioimmunoassay with commercially available antiserum. Samples are incubated overnight at 4°C with 100 µl first antibody (IgG-F-1; 1:800), 2,000 cpm 125I-cortisol and 100 µl secondary antibody (GARGG; 1:320). All constituents were dissolved in cortisol RIA buffer. Immune complexes are precipitated by addition of 1 ml ice-cold 5% (w/v) polyethylene glycol and 2% (w/v) bovine serum albumin and subsequent centrifugation (20 min, 2,000 x g, 4°C). Pellets are counted in a gamma counter.

MATERIAL (biological, reagents & instrumental):

Frozen extracted samples at -20ºC, colorimetric microplate reader, 96-well microplates, repeater pipet, multichannel pipet, and software for converting optical density reading from the plate reader and carrying out four parameter logistic curve fitting.

UNIT AND RANGE OF VALUE:

ng/ml or nM

Less than 10 ng/ml plasma indicates a non-stressed fish for most fish species.

PARAMETERS TO MEASURE:

Cortisol levels in biological samples.

BIBLIOGRAPHIC REFERENCES:

Fanouraki, E., Mylonas, C.C., Papandroulakis, N., Pavlidis, M., 2011. Species specificity in the magnitude and duration of the acute stress response in Mediterranean marine fish in culture. *Gen Comp Endocri*nol 173, 313-22.

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SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

This measurement is an effective evaluator of stress in fish. It is very important to adequately sample the designated sample. Sampling of fish induces stress, and since the cortisol response is rapid, it is vital to anesthetize or kill the fish within one minute to prevent handling stress that confines with the treatment.

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(20/06/13)

Link Table 2

Identifiant	ATOL: 0000799	**Code:** AW7
Name of Trait:	Glucocorticoid level	
Definition:	Any measurable characteristic related to the proportion or amount in a body fluid or tissue of the corticosteroids (cortisol, corticosterone).	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Corticosteroids level in fish plasma (MacFarlane, 1984):

Simultaneous determination of cortisol, cortisone and corticosterone in fish plasma using high performance liquid chromatography (HPLC).
Corticosteroids are extracted from plasma by methylene chloride and Sep-Pak cartridges for comparative purposes. The Sep-Pak method has proved more convenient and efficient.
Chromatography is accomplished on a nitrile bonded phase using a methanol-water mobile phase. Steroids are identified and quantified by monitoring absorbance at 254 and 280 nm simultaneously. Sensitivity is approximately 5 ng for each steroid.
The HPLC technique provides specific determination of each corticosteroid (thus eliminating cross-reactivity problems inherent in radioimmunoassay methods), resolves corticosteroids from androgens and estrogens and permits the isolation and purification of individual steroids.

Corticosteroids level in fish fecal samples (Turner Jr. J.W. *et al.,* 2003):

a) Fecal samples are extracted and reconstituted for simultaneous, cortisol and corticosterone measurement.
18 ml of dichloromethane is added to the vial containing a 6 ml fecal slurry (1:1, water:solid), and shaken vigorously on a motorized shaker (Burrel, Pittsburgh, PA) for 1 h. The vial is then centrifuged at 2000g for 10 min to separate the fecal, water, and dichloromethane layers. The bottom (dichloromethane) layer (16 ml) is recovered and vortexed for 2 min with 1ml of 0.1N NaOH to remove any remaining water soluble materials. The dichloromethane layer is recovered, mixed with 1ml of 18 MΩ water and vortexed for 2 min. The dichloromethane layer (15 ml) is then pipetted into borosilicate glass culture tubes and filtered (Pall Gelman Acrodisc 0.45 lm mesh membrane filters, Louisville, KY). The filtrate is evaporated to dryness in a vacuum centrifuge, and the samples are covered with parafilm and stored at -37 ºC until reconstitution. The dried samples are brought to room temperature, reconstituted in 100% HPLC-grade acetonitrile (ACN) and diluted with 18MΩ water to yield 500 µl of a 10% ACN solution. A 250 µl aliquot of the clear reconstitute is then analyzed by High Performance Liquid Chromatography (HPLC) for cortisol and corticosterone.

b) HPLC analysis:
HPLC is chosen for hormone measurement because it enabled measurement of all hormones in a single extract.
The hormone measurement system is a Reverse- Phase HPLC (Dionex, Sunnyvale, CA) employing a standard 3.9_300 mm, C-18 column (Waters, Milford, MA) and a variable wavelength UV detector set at 240 nm. Prior to sample analyses a water blank is run until the column is free of major peaks, and a reference standard containing cortisol, corticosterone, and testosterone is run to verify retention times. The flow rate is 1 ml/min, and the elution gradient changed from 10% ACN/90% water to 90% ACN/10% water over a period of 45 min, ensuring complete separation of

sample compounds. The standard curve for each hormone is developed by HPLC runs of duplicate samples of 10 known concentrations of each hormone. The correlation coefficient for actual dilutions vs. the calculated logarithmic curve ($y = ae^x$) averaged 0.995 for cortisol and 0.992 for corticosterone.

c) Verification of hormone identity:
Because fecal extracts contain numerous compounds, it is necessary to verify that the HPLC peaks assign as cortisol and corticosterone contained these hormones and only these hormones. This verification can be made by three methods (Turner *et al.,* 2002): (I) chemical derivitization, (II) radioactive tracer, and (III) mass spectrometry.
Derivitization is performed on cortisol and corticosterone standards and on eluent peaks of presumptive cortisol and corticosterone obtained from fecal extracts, using the derivatizing agent methoxyamine-HCl (MOX) in pyridine (Pierce Chemical, Rockford, IL). The derivitization converted the carbonyl group at the 3 and 20 positions in the steroids to a methoxime group at these positions, resulting in a characteristic shift in HPLC elution time. To determine the derivitization response of presumptive hormone eluent peaks from fecal extracts, for each hormone a pool of eluent peaks is prepared from HPLC analyses of 15 separate fecal extracts. Each of the three pools of peaks (one pool for each hormone) is then subjected to derivitization and injected into the HPLC. Eluent peaks for cortisol and corticosterone standards and presumptive cortisol and corticosterone eluent peaks from fecal extracts are collected for mass spectrometry analysis. Final assessment of hormone identity in HPLC eluents is made by spiking separate fecal extracts with tritiated cortisol or corticosterone (ICN Pharmaceuticals, Costa Mesa, CA), and determining radioactivity in HPLC eluents from these fecal extracts by liquid scintillation. Only eluent peaks coincident with the respective retention times for these hormones show significant radioactivity.

MATERIAL (biological, reagents & instrumental):

High performance liquid chromatography (HPLC.).

UNIT AND RANGE OF VALUE:

In plasma: ng/ml. Less than 10 ng/ml plasma indicates a non-stressed fish for most fish species.

In feces: Hormone values are reported as ng/g feces (wet wt.). The lower limit of hormone detection in plasma is usually 1.8 ng per sample. Hormone levels in extracts rarely approached this limit.

PARAMETERS TO MEASURE:

Level of cortisol, cortisone and corticosterone in fish plasma.

Level of cortisol, cortisone and corticosterone in fish fecal samples.

It is very important to adequately sample the designated sample. Sampling of fish induces stress, and since the glucocorticoid response is rapid, it is vital to anesthetize or kill the fish within one minute to prevent handling stress that confines with the treatment.

BIBLIOGRAPHIC REFERENCES:

MacFarlane, R.B., 1984. Determination of corticosteroids in fish plasma by high performance liquid chromatography: Evaluation of the method using striped bass (*Morone saxatilis*). *Canadian Journal of Fisheries and Aquatic Sciences* 41, 1280-1280.

Turner, Jr. J.W., Nemeth, R., Rogers, C., 2003. Measurement of fecal glucocorticoids in parrotfishes to assess stress. *General and Comparative Endocrinology* 133, 341-352.

Turner, Jr. J.W., Tolson, P., Hamad, N., 2002. Remote assessment of stress in white rhinoceros (*Certotherium simum*) and black rhinoceros (*Dicerosbicornis*) via measurement of adrenal steroid in feces. *J. Zoo Wildlife Med*. 33, 214-221.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

RESEARCHER CONTRIBUTION (and date of the last modification):

Ana Navarro (ULPGC), Juan Manuel Afonso (ULPGC)
Léa Joret (INRA)
Wout Abbink (IMARES)
Jaume Pérez-Sánchez (CSIC)

(20/06/13)

Link Table 2

Identifiant	ATOL:0000956	**Code:** AW8
Name of Trait:	Lipid metabolism	
Definition:	Any measurable characteristic related to the chemical reactions and pathways involving lipids.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Many methods used for studies of lipid metabolism employ advanced chromatographic and mass spectrometric techniques to extract, isolate and characterize lipids (Christie, 2003). Various techniques are available to measure the kinetics of lipid metabolism including stable isotope approaches, radio-isotope approaches, and non-isotopic approaches (Patterson, 2002). Lipid profiling is an area of increasing interest and development (Roberts et al., 2008).

MATERIAL (biological, reagents & instrumental):

General laboratory material, glassware, reagents, chromatographic equipment including Gas Chromatography with a range of detectors.

UNIT AND RANGE OF VALUE:

Data are often reported as weight % and mg g^{-1} tissue (wet weight or dry weight).

PARAMETERS TO MEASURE:

Lipid content, fatty acids, lipid classes, conversion and incorporation rates.

BIBLIOGRAPHIC REFERENCES:

Christie W.W., 2003. Lipid Analysis. Oily Press, Bridgwater 1–289.

Gurr, M.I., Harwood, J.L., 1991. Lipid biochemistry. An Introduction. 4th ed, Chapman and Hall, London. Chapter 4, pp 119-162.

Patterson, B.W., 2002. Assessment of nutritional status and analytical methods. *Current Opinion in Clinical Nutrition & Metabolic Care* 5, 475-479.

Roberts, L.D., McCombie, G., Titman, C.M., Griffin, J., 2008. A matter of fat: An introduction to lipidomic profiling methods. *J. Chromatogr. B* 971, 174-187.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

RESEARCHER CONTRIBUTION (and date of the last modification):

Jan Ove (NTNU) Alexandra Neyts (NTNU) Jan Mraz (VURH) Ariadna Sitjà-Bobadilla (CSIC), JC Navarro (CSIC) (20/06/13)

Link Table 2

Identifiant	ATOL: 0001560	**Code:** AW9
Name of Trait:	Protein efficiency	
Definition:	Any measurable characteristic related to biochemical processes responsible for the synthesis or the breakdown of proteins by catabolism.	

SIMILAR TO:

ATOL: 0001583

MEASUREMENT METHOD:

Under production conditions protein metabolism is addressed to maximize dietary protein utilization, termed as Protein efficiency ratio (PER). PER is defined as the grams of fish wet weight gained per gram of crude protein consumed.

PER = ((final mean weight [g] – initial mean weight [g])/ protein fed [g])

Crude protein content is determined from the nitrogen content of each sample, which assumes that protein contains 16% nitrogen, more often using Kjeldahl analysis according to the standard method (AOAC 2000) and manufactures protocol.

MATERIAL (biological, reagents & instrumental):

A scale (accuracy of 0.001 unit)

Kjeldahl

UNIT AND RANGE OF VALUE:

PER (no unit)

PARAMETERS TO MEASURE:

Fish Initial and Final body weight, and whole fish protein content by Kjeldahl method to calculate PER.

Other parameters used are ammonia-N excretion (mg N/kg/d).

BIBLIOGRAPHIC REFERENCES:

Cho, C.Y., Kaushik, S.J., 1990. Nutritional energetics in fish: Energy and utilization in rainbow trout. *World Rev. Nutr. Diet*. 61, 132–172.

Wilson, R.P., 1979. Aminoacids and proteins In: Halver, J.E. and Tiews, K. (Eds.), *Finfish Nutrition and Fishfood Technology,* Vol. 2, Heenemann GmbH, Berlin, pp. 239–247.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

RESEARCHER CONTRIBUTION (and date of the last modification):

Marisol Izquierdo (ULPGC)
Léa Joret (INRA)
Jaume Pérez-Sánchez (CSIC)
(20/06/13)

Link Table 2

Identifiant	ATOL:0001796	AW10
Name of Trait:	Respiratory rate	
Definition:	Measure of the fish metabolic rate and it differs for different fuels.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Respiratory rate is equivalent to the respiratory quotient (RQ) and refers to the ratio of CO_2 excreted in relation to the O_2 uptake. The respiratory rate is a measure of the fish metabolic rate and it differs for different fuels. For the complete combustion of glucose is 1.0, whereas for the complete combustion of fat is 0.7 and the oxidation of mixtures of different amino acids is 0.80. The respiratory rate is affected by different factors such as stress conditions, cortisol exposure, lipid metabolism, etc.

Ventilation is determined by the opercular displacement in the fish and two different terms are studied: Ventilation amplitude as determined from the range of opercular displacement using impedance electrodes, and Ventilation frequency, as the number of opercular displacement in a given period of time (min).

MATERIAL (biological, reagents & instrumental):

UNIT AND RANGE OF VALUE:

Respiratory rate (no unit).

Ventilation amplitude (opercular displacement)

Ventilation frequency (number of opercular displacement per min).

PARAMETERS TO MEASURE:

Respiratory rate (O_2 comsumption and CO_2 production).

Ventilation amplitude

Ventilation frequency

BIBLIOGRAPHIC REFERENCES:

McKenzie, D.J., 2001. Effects of dietary fatty acids on the respiratory and cardiovascular physiology of fish. *Comp. Biochem. Physiol.* 128A, 607-621.

Perry, S.F, Bernier, N.J., 1999. The acute humoral adrenergic stress response in fish: facts and fiction. *Aquaculture* 177, 285–295.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

Respiratory rate: Measure of the fish metabolic rate and it differs for different fuels.

Ventilation: Number of breaths or gill cover movements taken within a set amount of time.

RESEARCHER CONTRIBUTION (and date of the last modification):

Marisol Izquierdo (ULPGC)
Jaume Pérez-Sánchez (CSIC)
Barb Helland (NOFIMA)

(20/06/13)

Link Table 2

Identifiant	ATOL:0000907	AW11
Name of Trait:	Social dominance	
Definition:	Any measurable or observable characteristic related to the ascendency of an individual over another animal in a social unit.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

"A point source of continuous food resources will be provided, which allows access by only one or a very limited number of individuals at any one time. After a period of food deprivation, generating high feeding motivation, social dominance will be measured as individual priority of access to the food resource in the rank order that the fish are able to feed successfully. The recording duration have to cover all time needed until all fish in the tank have been able to feed for the first time, thereby ensuring that no one individual is food deprived for an excessive or harmful length of time. This process will be repeated at least three times comparing the individual rank orders for consistency. If significantly consistent is found then a composite measure of mean ranks across all trials will be used as the measure of relative social dominance within each tank."

If no consistent results are obtained check the potential impact of the food deprivation time (not adjusted to the animal characteristics) or the access to the food resource (not adjusted to the size of group, density,…).

MATERIAL (biological, reagents & instrumental):

UNIT AND RANGE OF VALUE:

PARAMETERS TO MEASURE:

Time

Rank order of individuals in the first access to the food resource

BIBLIOGRAPHIC REFERENCES:

Drew, C., 1993. The Concept and Definition of Dominance in Animal Behaviour. *Behaviour* 125, 283-313.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE: Note the question marks for the periods of time, etc. will depend on the species and the animal characteristics (age, group size, density,...).
RESEARCHER CONTRIBUTION (and date of the last modification): Elin Kjørsvik (NTNU), Alexandra Neyts (NTNU) Léa Joret (INRA) (28/02/13)

Link Table 2

Identifiant	ATOL:0000948	AW12
Name of Trait:	Stress HPA sensitivity	
Definition:	Any measurable characteristic related to the ability of the HPA (hypothalamic pituitary adrenal) axis to react to stress or ACTH injection by producing and releasing glucocorticoids.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*
MEASUREMENT METHOD: Plasma cortisol concentration measured by Radioimmuno-assay using hot cortisol as tracer and a commercial antiserum validated for a wide range of fish after a stress challenge (Pickering *et al.,* 1987).
MATERIAL (biological, reagents & instrumental): Plasma
UNIT AND RANGE OF VALUE: E.g. nmol/l (e.g. 0 – 1000)
PARAMETERS TO MEASURE: Circulating plasma cortisol concentration.
BIBLIOGRAPHIC REFERENCES: Pickering, A.D., Pottinger, T.G., Sumpter, J.P., 1987. On the use of dexamethasone to block the pituitary-interrenal axis in the brown trout, *Salmo trutta* L. *Gen. Comp. Endocrinol.* 65, 346-353.
SYNONYMS EXACT:
OTHER ASPECTS TO INCLUDE: May also be measured in whole tissue or water.
RESEARCHER CONTRIBUTION (and date of the last modification): Åsa Maria Espmark (NOFIMA), Bendik Fyhn Terjesen (NOFIMA) Léa Joret (INRA) Ariadna Sitjà-Bobadilla (CSIC)

(20/06/13)

Link Table 2

Identifiant	ATOL:0001570	AW13
Name of Trait:	Susceptibility to bacterial infection	
Definition:	Any detectable or measurable disorder or clinical effect reflecting the degree of pathogenicity or lethality induced in animals or animal populations by bacterial invasion, bacterial components or bacterial productions.	

SIMILAR TO:

ATOL:0000408

MEASUREMENT METHOD:

The susceptibility to bacteria is measured in fish exposed to a bacterial challenge. For such purpose a control group (not exposed) and different challenged groups have to be established. Challenged groups have to include different doses of bacteria or bacterial products. The size, strain and rearing conditions of the fish, as well as the bacteria strain or serotype and the infection route have to be defined. The results can be evaluated by three different methods:

Method 1:
Counting of animals showing clinical signs or measurement of biological effects or biochemical parameters related to the bacterial infection, from the onset of the challenge until an endpoint. The duration of the survey will depend on the known effect of the bacteria or bacterial toxin tested. This method requires sufficient knowledge in the studied infection and is not commonly used in fish bacteriology (examples: "furuncle" production in typical salmonid furunculosis; circulating p57 protein in bacterial kidney disease).

Method 2:
The median lethal dose (also named semilethal dose) (LD_{50}), or survival doses fifty (SD50), is calculated as the minimum quantity of bacteria or bacterial toxin that it is necessary to produce the death to the 50% of exposed animals. The lower the LD_{50} dose, the more toxic or pathogenic is the bacteria or toxin.

Effect of the tested product is measured in animal groups exposed to increasing doses. Mathematical analysis is conducted according to interpolating methods, as described in Reed and Muench (1938).

Method 3:

If only one challenged group is established, the relative percentage of survival (RPS) can be calculated as:

RPS=1- [(Mortality (%) in challenged group) / (Mortality (%) in control group)] ×100

The higher the value, the higher the survival.

MATERIAL (biological, reagents & instrumental):

Pathology unit with tanks with containment measures for the bacteria and the water effluents, and bacteria-free income water. Each dose has to be set up in different replicated tanks.

Production of bacteria / bacterial toxin in a laboratory equipped for and used to bacteriological work

Adequate containers for biohazard disposal of material in contact with bacteria and dead fish.

Petri plates with adequate culture medium for the recovery of the bacterial strain from dead animals.

UNIT AND RANGE OF VALUE:

Method 1: Number of animals (range: 0-100%). Clinical signs may be recorded as qualitative traits only, using a +/- notation or coded according to a scale of intensity. For biochemical parameters or tests, laboratory methods are required with their respective reagents and procedures.

Method 2: LD_{50} (a $x10^{x}$) (range: 10^{3}-10^{9}): Bacterial doses are adjusted and expressed as colony forming units (cfu/ml). Toxins or biochemical components doses are ordinary expressed as pure product (in g, mg or µg /ml) and sometimes as functional activity (units /ml; enzymes for instance).

Method 3: RPS (%) (range: 0-100%)

PARAMETERS TO MEASURE:

Presence, number, intensity of lesions

Level of chemical parameters

Median lethal dose (LD50): dead fish

RPS

BIBLIOGRAPHIC REFERENCES:

Amend, D.F., 1981. Potency testing of fish vaccines. In: Anderson, D.P., Hennessen, H. (eds). Fish Biologies: Serodiagnostics and Vaccines. *Development in Biological Standardization* 49, Karger, Basel: 447-454.

El Aamri, F., Padilla, D., Acosta, F., Caballero, M.J., Roo, J., Bravo, J., Vivas, J., Real, F., 2010. First report of *Streptococcus iniae* in red porgy (*Pagrus pagrus,* L.). *Journal of Fish Diseases* 33, 901-905.

Padilla, D., Real, F., Gómez, V., Sierra, E., Acosta, B., Déniz, S., Acosta, F., 2005. Virulence factors and pathogenicity of *Hafnia alvei* for gilthead seabream (*Sparus aurata*, L). *Journal of Fish Diseases* 28, 411-417.

Reed, J.L., Muench, M., 1938. A simple method of estimating fifty percent end points. *American Journal of Hygiene* 27, 493-497.

Rodríguez, L.A., Gallardo, C.S., Acosta, F., Nieto, T.P., Acosta, B., Real, F., 1998. *Hafnia alvei* as an opportunistic pathogen causing mortality in brown trout (*Salmo trutta*). *Journal of Fish Diseases* 21, 365-370.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

For this trait, is necessary to challenge different groups of animals (generally settled in different tanks), most often by inoculating each group with different doses of bacteria / bacterial toxin. Such experiments must be conducted in accordance to standards validated by Animal Ethics Committees.

For the strict commitment to the Koch's postulates, the bacterial strain that has been used in the challenge has to be recovered from dead fish. Thus, bacterial analyses have to be performed from dead animals.

See comments in Viral sheet.

RESEARCHER CONTRIBUTION (and date of the last modification):

Fernando Real (ULPGC) Léa Joret (INRA) Ariadna Sitjà-Bobadilla (CSIC) Christian Michel (INRA) (20/06/13)

Link Table 2

Identifiant	ATOL:0000408	AW14
Name of Trait:	Susceptibility to viral infection	
Definition:	Any detectable or measurable disorder or clinical effect reflecting the degree of pathogenicity or lethality induced in animals or animal populations by a viral invasion and multiplication	

SIMILAR TO:

ATOL:0001570

MEASUREMENT METHOD:

The susceptibility to virus is measured in fish exposed to a viral challenge. For such purpose a control group (no exposed) and different challenged groups have to be established. Challenged groups have to include different doses of virus. The size, strain and rearing conditions of the fish, as well as the virus strain or serotype and the infection route have to be defined. Four methods of measuring the effect of viral infection can be used:

Method 1:
Quantification of the number of animals showing clinical signs and/or of the number/importance of lesions typically related to particular viral infections, during a given period after the challenge and a given infectious dose.

Method 2:
Assessment of the median infective dose (LD_{50}), or survival dose fifty (SD_{50}), that is calculated as the minimum quantity of virus that it is necessary to produce the death to the 50% of exposed animals. The lower the LD_{50}, the more toxic or pathogenic is the virus.

Effect of the tested virus is measured in animal groups exposed to increasing doses. Mathematical analysis is conducted according to interpolating methods, as described in Reed and Muench (1938).

Method 3:
If only one challenged group is established, the relative percentage of survival (RPS) can be calculated as:

RPS=1- [(Mortality (%) in challenged group) / (Mortality (%) in control group)] ×100

The higher the value, the higher the survival

Method 4:
Assessment of tissue culture infective dose fifty ($TCID_{50}$), when cell lines derived from the fish species or fish strains to be tested are available. The principle is the same as in LD_{50}, the effect of the virus on confluent cells monolayers being visualized as enumerable cytopathic or lysis plaque forming areas.

MATERIAL (biological, reagents & instrumental):

Pathology unit with tanks with containment measures for virus and their water effluents, and virus-free income water. Each dose has to be set up in different replicated tanks.

Production and handling of viral suspensions requires a laboratory equipped for and used to cell culture work.

Adequate containers for biohazard disposal of material in contact with virus and dead fish.

Virus extracts or solutions obtained from cell cultures and cell culture medium (for Control groups).

UNIT AND RANGE OF VALUE:

Method 1: Number of animals and/or number of lesions (range: 0-100%)

Methods 2 and 4: LD_{50} and $TCID_{50}$ (range:0-100%) infective doses expressed as plaque forming units (pfu /ml).

Method 3: RPS (%) (range: 0-100%)

PARAMETERS TO MEASURE:

Presence, number, intensity of lesions

Lethal doses fifty (LD_{50}): Number of dead animals

$TCID_{50}$: Number of lysis plaques or cytopathic effect areas

BIBLIOGRAPHIC REFERENCES:

Castric, J., de Kinkelin, P., 1984. Experimental study of the susceptibility of two marine fish species, sea bass (*Dicentrarchus labrax*) and turbot (*Scophthalmus maximus*), to viral haemorrhagic septicaemia. *Aquaculture* 41, 203–212.

Dorson, M., Touchy, C., 1981. The influence of fish age and water temperature on mortalities of rainbow trout, *Salmo gairdneri* Richardson, caused by a European strain of infectious pancreatic necrosis virus. *Journal of Fish Diseases* 4, 213–221.

Ramstad, A., Romstad, A.B., Knappskog, D.H., Midtlyng, P.J., 2007. Field validation of experimental challenge models for IPN vaccines. *Journal of Fish Diseases* 30, 723–731.

Reed, J.L., Muench, M., 1938. A simple method of estimating fifty percent end points. *American Journal of Hygiene* 27, 493-497.

Sommerset, I., Skern, R., Biering, E., Bleie, H., Fiksdal, I.U., Grove, S., Nerland, A.H., 2005. Protection against Atlantic halibut nodavirus in turbot is induced by recombinant capsid protein vaccination but not following DNA vaccination. *Fish & Shellfish Immunology* 18, 13-29.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

In experiments performed on animals, it is necessary to challenge different groups of fish (generally settled in different tanks), most often by inoculating each group with viral suspensions at different concentrations. Such experiments must be conducted in accordance to standards validated by Animal Ethics Committees.

For the strict commitment to the Koch's postulates, virus from the whole-body (if larvae are used) or target tissues (if juvenile fish are used) that die during the experiment has to be reisolated in cell cultures.

RESEARCHER CONTRIBUTION (and date of the last modification):

Fernando Real (ULPGC)
Léa Joret (INRA)
Ariadna Sitjà-Bobadilla (CSIC)
Christian Michel (INRA)
Heidi Johansen (NOFIMA)

(20/06/13)

Link Table 2

Identifiant	ATOL:0000804	AW15
Name of Trait:	Swimming behavior	
Definition:	Any measurable or observable characteristic related to movement through the water.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Habituation period to the experimental design before test: Single fish must be moved from its maintenance tank to the experimental tank 2 h before the experiment starts. Video recording must begin 30 min before starting the test and fish homogeneous swimming in the entire tank is required before stimulation.

Behavioral test : swimming behavior to electromagnet stimulation

- *Step 1: stimulation*

The stimulus is dropped by releasing the electromagnet when the fish reached the stimulation zone. Fish behavior is recorded during 1 h after the stimulation.

- *Step 2 : video recording of the swimming response*

Fish which never swim in the tank and which thus cannot be stimulated were characterized as ''shy'' fish. Fish which present a homogeneous swimming in the tank and which could be stimulated are characterized as more ''bold''.

- *Step 3: end of the test*

After the end of the test, fish are removed from the experimental tank and placed in a separate tank to avoid alarm pheromone release within the experimental design where other fish remain to be tested.

Video analyses: The video recordings are analyzed using a software which allows to separate the tank in four virtual zones of the same surface (Z1, Z2, Z3 and Z4), and to track the fish swimming behavior. Each video recording are analyzed in three sequences of 20 min: sequence 1 (S1): before the stimulation, sequence 2 (S2): just after the stimulation, and sequence 3 (S3): 40 min after the stimulation.

Different variables of interest are chosen to analyze the fish behavior: Latency time before the start of the stimulation: The time taken from the start of video recordings to the stimulation application. This variable allowed to measure individual latency before of time spent by tested fish in the experimental tank stimulation.

Time spent in virtual zone: the proportion of time spent by a fish in each zone (residence; in %). This variable allows identifying the fish spatial distribution for each sequence.

Traveled distance: distance travelled by each fish in the tank (in cm). This variable quantified the fish swimming activity level in the tank for each sequence.

Velocity: The fish angular velocity weighted by the time spent by the fish in each zone (in 8 s_1). This variable is calculated for each fish as followed: [(TZ1AVZ1) + (TZ2AVZ2) + (TZ3AVZ3) + (TZ4AVZ4)]/(TZ1 + TZ2 + TZ3 + TZ4) where TZ1, TZ2, TZ3 and TZ4 are the time spent by the fish in each zone (s).

AVZ1, AVZ2, AVZ3 and AVZ4 are the individual angular velocity in each zone (8 s_1). This variable is an indicator of the speed of changing direction and quantifies the swimming path complexity in relation to time spent by fish in each zone.

MATERIAL (biological, reagents & instrumental):

Two tanks. One of them with video camera device. Software to analyze video recordings
UNIT AND RANGE OF VALUE: According to each variable.
PARAMETERS TO MEASURE: Swimming behavior to electromagnet stimulation and locomotor activity as indicator of welfare, through same variables.
BIBLIOGRAPHIC REFERENCES: Millot, S., Begout, M.L., Chatain, B., 2009. Exploration behavior and flight response toward a stimulus in three sea bass strains (*Dicentrarchus labrax* L.). *Appl. Anim. Behav. Sci.* 119, 108-114.
SYNONYMS EXACT:
OTHER ASPECTS TO INCLUDE: Behavioral test to determine swimming behavior may differ depending on the species, fish size or production interest.
RESEARCHER CONTRIBUTION (and date of the last modification): Daniel Montero (ULPGC) Léa Joret (INRA) Marc Vandeputte (INRA) (28/02/13)

Link Table 2

Identifiant	ATOL:0001542	GM1
Name of Trait:	Age at slaughter	
Definition:	Any measurable characteristic related to the age of an animal at the time of slaughter.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*
MEASUREMENT METHOD: Calculate the duration in days between harvest date and hatching date. If hatching lasts for several days, the date of 50% hatching should be considered as hatching date.
MATERIAL (biological, reagents & instrumental):
UNIT AND RANGE OF VALUE: Days
PARAMETERS TO MEASURE: Time elapsed from hatching date to harvest date

BIBLIOGRAPHIC REFERENCES:

Navarro, A., Zamorano, M.J., Hildebrandt, S., Ginés, R., Aguilera, C. and Afonso, J.M., 2009. Estimates of heritabilities and genetic correlations for growth and carcass traits in gilthead seabream (*Sparus auratus* L.), under industrial conditions. *Aquaculture* 289, 225–230

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

It can be affected by several factors like water temperature, feeding level and other rearing conditions.

There are other classical measurements for age in fish, like days post-fertilization and days post first feeding (this latter one is mostly used in salmonids which have a long yolk resorption period)

Days post-fertilization or days post first feeding may be preferred in case hatching lasts for several days (e.g. in salmonids).

RESEARCHER CONTRIBUTION (and date of the last modification):

Rafael Ginés (ULPGC)
Marc Vandepute (INRA)
Jaume Pérez-Sánchez (CSIC)

(20/06/13)

Link Table 2

Identifiant	ATOL:0001650	GM2
Name of Trait:	Body compartment yield	
Definition:	Any measurable characteristic related to the weight of one or more body compartment (members, organs, etc) normalized by the body weight	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Dry and weigh the whole-body fish (ATOL:0000351).

Separate the body compartment from the body fish. Specify which part has been removed (for instance, in fillet yield you have to specify if the fillet include the belly flap or the skin, in head percentage if the head include the gills).

Weigh the body compartment.

MATERIAL (biological, reagents & instrumental):

Dissection material; A scale (accuracy of 0.01 g for kg, and 0.01 mg for g)

UNIT AND RANGE OF VALUE:

%

Depend on each body compartment.

PARAMETERS TO MEASURE:

Body compartment yield (For example, fillet yield, head percentage, visceral percentage, etc..) .

BIBLIOGRAPHIC REFERENCES:

Cozzolino D., Murray I., Scaife J.R., 2002. Near infrared reflectance spectroscopy in the prediction of chemical characteristics of minced raw fish. *Aquaculture Nutrition* 8, 1-6.

Navarro, A., Zamorano, M.J., Hildebrandt, S., Ginés, R., Aguilera, C. and Afonso, J.M., 2009. Estimates of heritabilities and genetic correlations for body composition traits and G×E interactions, in gilthead seabream (*Sparus auratus* L.). *Aquaculture* 295, 183–187

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

RESEARCHER CONTRIBUTION (and date of the last modification):

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(20/06/13)

Link Table 2

Identifiant	ATOL:0000351	GM3
Name of Trait:	Body weight	
Definition:	Any measurable characteristic related to the body weight of an organism.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Fish are caught in their rearing tank with a dip net and placed in anesthetic bath.

Fish can be weighed in two ways:

- *Weighing a sample (mean weight)*: A container with rearing water is placed on an appropriate scale and tare. The number of fish in the sample is counted, and the total weight of the sample is recorded. The sample can be weighed in several times if necessary. The body weight will be obtained in the following way:

$$\frac{\text{Total weight}}{\text{Nº of fish}}$$

- *Weighing individual fish*: A container without water is placed on an appropriate scale and tare. Fish is taken in anesthetic bath and carefully placed on the container. It should be placed before on a wet cloth to allow excess water to drain. The value of the measurement is recorded, and when possible, automatically recorded with a data transfer system.

MATERIAL (biological, reagents & instrumental):

A scale with adequate precision (usually around 1/100 to 1/1000 of the average weight to be measured).

Fish.

A representative sample from a fish population.

UNIT AND RANGE OF VALUE:

mg, g, kg

PARAMETERS TO MEASURE:

The body weight of a fish or the mean body weight of a group of fish.

BIBLIOGRAPHIC REFERENCES:

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

The scales must be checked before each measurement series, and regularly calibrated by an external service provider.

When mean weight of a fish group is estimated, the sample shall be representative of the entire population of the tank. Usually, a minimum of 30 fish, or 10% of the total number of fish in the tank will be sufficient. However, in cases where is suspected that differences between individual fishes are high, the in-tank variance should preferably be checked prior to sampling. The number of fish required can be calculated using standard statistical equation, based on the level confidence required,e.g. 95% confident that the estimated individual weight is within ± 1 SD of the true mean.

In most cases, fish should be fasted before body weight is measured, for a time that allows emptying of the gut (generally 24-48h). If this is not feasible, e.g. when physiological samples are to be taken at the same time, of fed fish (i.e. not of fasted fish), the gastrointestinal contents of a representative number of fish should be dissected out, weighed, and a gut content index calculated on a tank basis, to correct the individual weight.

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Link Table 2

Identifiant	ATOL:0001057	GM4
Name of Trait:	Carcass weight	
Definition:	Any measurable characteristic related to the weight of an animal following slaughter and removal of digestive tract and internal organs.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Open the visceral cavity of slaughtered fish. Remove all viscera, including the gonads, the heart and the kidney but neither the head nor the gills.. Rinse the visceral cavity to remove traces of

blood. Dry and weigh the gutted fish.

MATERIAL (biological, reagents & instrumental):

Dissection material; A scale (minimum accuracy of 1/1000 of the minimum carcass weight)

UNIT AND RANGE OF VALUE:

Gram (g)

PARAMETERS TO MEASURE:

Carcass weight

BIBLIOGRAPHIC REFERENCES:

Eroldogana, O.T., Kumlua, M., Aktas, M., 2004. Optimum feeding rates for European sea bass *Dicentrarchus labrax* L. reared in seawater and freshwater. *Aquaculture* 231, 501–515.

Haffray, P., Bugeon, J., Pincent, C., Chapuis, H., Mazeiraud, E., Rossignol, M.N., Chatain, B., Vandeputte, M., Dupont-Nivet, M., 2012. Negative genetic correlations between production traits and head or bony tissues in large all-female rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 24, 145-152.

SYNONYMS EXACT:

Gutted body weight

OTHER ASPECTS TO INCLUDE:

Sometimes, it is measured by difference between body weight and viscera weight. However, this is not appropriate as loss of body fluids not counted in viscera can account to up to 5% of the body weight.

In some cases, kidney which is adherent to the carcass is not removed. In this case, it should be mentioned. Sometimes also, fins are removed from the carcass. It should also be mentioned.

If measurement of fillet weight/yield is done by further processing of the carcass, it is important that the opening of the visceral cavity is done in the sagittal plane, to ensure the absence of weight bias between the two fillets (although this has no impact on carcass yield)

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Link Table 2

Identifiant	ATOL:0000548	GM5
Name of Trait:	Carcass yield	
Definition:	Any measurable or observable characteristic related to the yield	

obtained after processing of a dead body to remove specific parts.

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Dry and weigh the whole-body fish to obtain body weight (ATOL:0000351).

Open the visceral cavity. Remove all viscera of slaughtered fish, including the gonads, the heart and the kidney, but neither the head nor the gills. Rinse the visceral cavity to remove traces of blood. Dry and weigh the gutted fish to obtain the carcass weight (ATOL:0001057).

Carcass yield will be obtained as the percentage of the body weight (ATOL:0000351): 100 x (carcass weight / whole-body weight).

MATERIAL (biological, reagents & instrumental):

Dissection material; A scale (minimum accuracy of 1/1000 of the minimum carcass weight)

UNIT AND RANGE OF VALUE:

%

60-95 %

PARAMETERS TO MEASURE:

Carcass yield

BIBLIOGRAPHIC REFERENCES:

Eroldogana, O.T., Kumlua, M., Aktas, M., 2004. Optimum feeding rates for European sea bass *Dicentrarchus labrax* L. reared in seawater and freshwater. *Aquaculture* 231, 501–515.

Haffray, P., Bugeon, J., Pincent, C., Chapuis, H., Mazeiraud, E., Rossignol, M.N., Chatain, B., Vandeputte, M., Dupont-Nivet, M., 2012. Negative genetic correlations between production traits and head or bony tissues in large all-female rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 24, 145-152.

SYNONYMS EXACT:

Gutted body weight percentage; dressing percentage; carcass to whole, slaughter yield.

OTHER ASPECTS TO INCLUDE:

In some cases, kidney which is adherent to the carcass is not removed. In this case, it should be mentioned. Sometimes, also fins are removed from the carcass, and it should also be mentioned.

If measurement of fillet weight/yield is done by further processing of the carcass, it is important that the opening of the visceral cavity is done in the sagittal plane, to ensure the absence of weight bias between the two fillets (although this has no impact on carcass yield).

RESEARCHER CONTRIBUTION (and date of the last modification):

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(20/06/13)

Link Table 2

Identifiant	ATOL:0001653	GM6
Name of Trait:	Condition factor	
Definition:	Indicator of the stoutness of an animal, obtain using the formula K = (body weight[g])/(body length^3[cm])x100.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Condition factor = K = (body weight[g])/(body length^3[cm])x100

Where body length corresponds to the fork length (ATOL:0001658) and body weight (ATOL:0000351) to the total weight of the fish after draining.

MATERIAL (biological, reagents & instrumental):

A scale (accuracy of 0.1 g)

Measuring board (accuracy of 0.1 cm)

UNIT AND RANGE OF VALUE:

Length in cm; weight in gram, the condition factor is around 1 (0.5-3) (no unit)

PARAMETERS TO MEASURE:

Fork length of the fish

Body weight of the fish

BIBLIOGRAPHIC REFERENCES:

Nash, R.D.M., Valencia, A.H., Geffen, A.J., 2006. The Origin of Fulton's Condition Factor. Setting the Record Straight. *Fisheries* 31, 236-238. (http://folk.uib.no/nfiag/nfiag/reprints/NashETAL2006Fisheries.pdf).

SYNONYMS EXACT:

Fulton index, condition factor, K

OTHER ASPECTS TO INCLUDE:

Depending on the fin integrity the standard length (ATOL:0001659) could be used instead of the fork length.

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(20/06/13)

Link Table 2

Identifiant	ATOL:0000087	GM7
Name of Trait:	Exterior defects	
Definition:	Any observable external defects of the animal.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

The exterior defects are evaluated by visual examination in combination with palpation and are usually classified according to a score.

In fish, exterior defects or deformities can be grouped into five categories: shape, pigmentation, scales, swim bladder and skeletal (Divanach *et al*., 1996).

The skeletal deformities are related to changes in spine and head. The head deformities are associated with complex opercular and jaw (Cobcroft *et al*., 2001; Kocour *et al*., 2006; Morel *et al*., 2010). The most common deformities related to the spine are lordosis (dorsal deformities, V shape), scoliosis (lateral deformity, zig-zag), kyphosis (deformity ventral way Λ) and fusion of vertebrae (Afonso *et al*., 2000).

See manual of *Visual evaluation of skeletal deformities in farmed salmon* (**ANNEX-2**

).

MATERIAL (biological, reagents & instrumental):

Fish

Photos of normal and deformed fish, if adequate

UNIT AND RANGE OF VALUE:

Score or absence / presence.

PARAMETERS TO MEASURE:

Deformities.

BIBLIOGRAPHIC REFERENCES:

Afonso, J.M., Montero, D., Robaina, L., Astorga, N., Izquierdo, M.S., Ginés, R., 2000. Association of a lordosis-scoliosis-kyphosis deformity in gilthead seabream (*Sparus aurata*) with family structure. *Fish Physiology and Biochemistry* 22, 159-163.

Cobcroft, J. M., Pankhurst, P. M., Sadler, J., Hart, P., 2001. Jaw development and malformation in cultured striped trumpeter *Latris lineata. Aquaculture* 199, 267-282.

Divanach, P., Boglione, C., Menu, B., Koumoundouros, G., Kentouri, M., Cataudella, S., 1996. Abnormalities in finfish mariculture: and overview of the problem, causes and solutions. In: Chatain, B., Saroglia, M., Sweetan, J., Lavens, P. (Eds.), Sea Bass and Sea Bream Culture: Problems and Prospects. Oostende, Belgium. *Eur. Aqu. Soc.* 45, p. 6.

Kocour, M., Linhart, O., Vandeputte, M., 2006. Mouth and fin deformities in common carp: is there a genetic basis?. *Aquaculture Research* 37, 419–422.

Morel, C., Adriaens, D., Boone, M., De Wolf, T., Van Hoorebeke, L., Sorgeloos, P., 2010. Visualizing mineralization in deformed opercular bones of larval gilthead sea bream (*Sparus aurata*). *Journal of Applied Ichthyology* 26, 278–279.

SYNONYMS EXACT:

Body malformations; Morphological malformations

OTHER ASPECTS TO INCLUDE:

The morphological malformations are modifications of fish morphology respect a standard of quality, with important economic consequences for the industry. Thus, deformities can be evaluated at different ages during the fish culturing process (fingerling, juvenile and adult)

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(20/06/13)

Link Table 2

Identifiant	ATOL:0001658	GM8
Name of Trait:	Fork length	
Definition:	Length of a fish from the tip of the snout to the end of the middle caudal fin rays	

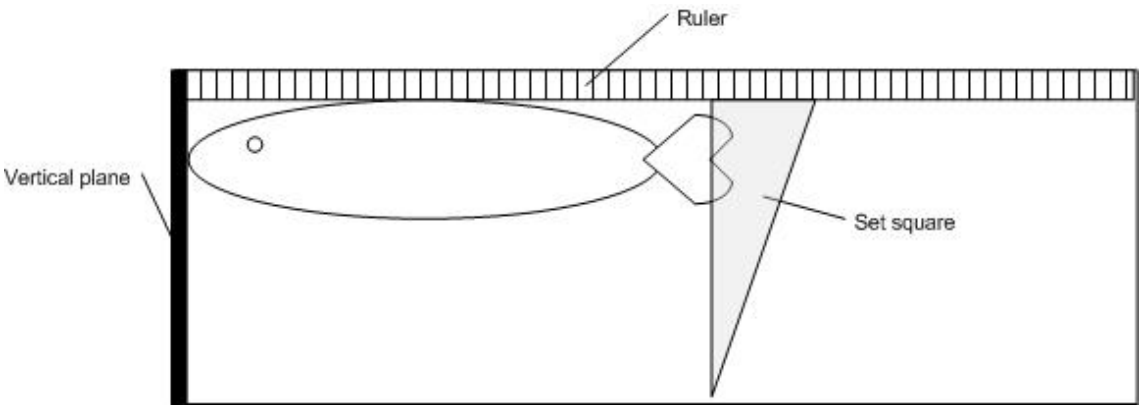
SIMILAR TO:

ATOL:0001660

MEASUREMENT METHOD:

The fork length is measured by laying the fish on a ruler which has a vertical plane at the zero mark. To minimized the parallax error on the reading of the measure, the ruler have to be equip with a set square as shown in the diagram below. The fish is place on the device in taking care of keeping it parallel to the ruler. The fish is softly compressed to the zero mark with the tip of the snout. The length is measure in putting the set square on the end of the middle caudal fin rays

On sea bass it's better to place the belly of the fish in contact to the ruler to have a better parallel position of the fish.



Measures on pictures can be used, with a distance reference and specific softwares, avoiding any

ambiguity.

MATERIAL (biological, reagents & instrumental):

Measuring board (accuracy of 0.1 mm)

Calliper for juvenile

Software for images analysis

UNIT AND RANGE OF VALUE:

mm, cm

PARAMETERS TO MEASURE:

Body length until middle caudal fin rays.

BIBLIOGRAPHIC REFERENCES:

Gaygusuz, Ö., Gürsoy, Ç., Özulu, M., Tarkan, A.S., Acıpınar, H., Bilge, G., Filiz, H., 2006. Conversions of Total, Fork and Standard Length Measurements Based on 42 Marine and Freshwater Fish Species (from Turkish Waters). *Turkish Journal of Fisheries and Aquatic Sciences* 6, 79-84.

Howe, J.C., 2002. Standard length: not quite so standard. *Fisheries Research* 56, 1-7.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

RESEARCHER CONTRIBUTION (and date of the last modification):

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Link Table 2

Identifiant	ATOL:0001017	GM9
Name of Trait:	Meat colour	
Definition:	Any measurable or observable characteristic related to the colour of meat.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Before any measurement, calibrate the Chroma-meter with a white plate reference standard.

Flesh colour can be measured on the internal part of a fillet (corresponding to white muscle) or on a cutlet.

After cleaning the flesh with absorbent paper, put the measuring head sensor on the flesh. All the surfaces of the measuring head sensor should be in contact with the flesh. Due to colour and tissue heterogeneity it is recommended to realize several measurements on different fillet localization. For example, for fillet from a pan-size rainbow trout flesh colour is measured at two locations of the fillet (above the lateral line: behind the head and below the dorsal fin), whereas 3 measurements are done for bigger trout (> 1kg) (above the lateral line: behind the head; below the dorsal fin; and near the tail, below the adipose fin).

After flashing, L*, a* and b* reflected light values are recorded. From a* and b* values the C* (saturation) and H* (Hue) values can be calculated see below for formulae.

MATERIAL (biological, reagents & instrumental):

A fillet or a cutlet.

Chroma-meter for example Minolta CR-series (200, 300, 400).

The illuminant should be specified generally C or D65, as well as the angle of observation (2 or 10 deg).

UNIT AND RANGE OF VALUE:

CIE L*a*b*and L*C*H* colour spaces

L* : luminosity from 0 black to 100 white

a*: negative values indicate green while positive values indicate red

b*: negative values indicate blue and positive values indicate yellow

$C^* = (a^{*2}+b^{*2})^{1/2}$ correspond to saturation

$H^* = \tan^{-1}(b^*/a^*)$ in deg correspond to Hue

PARAMETERS TO MEASURE:

L* a* b* individual and mean value if several measurements were done on the same fillet.

BIBLIOGRAPHIC REFERENCES:

Choubert, G., Blanc, J. M., Vallee, F., 1997. Colour measurement, using the CIELCH colour space, of muscle of rainbow trout, *Oncorhynchus mykiss* (Walbaum), fed astaxanthin: effects of family, ploidy, sex, and location of reading. *Aquac. Research* 28, 15-22.

CIE, Commission Internationale de l'Eclairage, 1976. *Colorimetry.* Publication Nº 1 5. Bureau central de la CIE, Vienna, Austria. http://eilv.cie.co.at/term/157.

SYNONYMS EXACT:

Flesh colour, muscle colour, fillet colour, flesh colour, muscle colour, fillet colour.

OTHER ASPECTS TO INCLUDE:

The time after slaughter and storing condition should be mentioned.

Several devices can be used to measure colour in the L*a*b* colour space like a camera or a flatbed scanner. Nevertheless, due to a different illumination of the flesh, such devices give different absolute colour values and cannot be compared to a Chroma-meter value.

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Link Table 2

Identifiant	ATOL:0001664	GM10
Name of Trait:	Meat cooking loss	
Definition:	Any measurable or observable characteristic related to the decrease in the weight of a cut of meat during cooking, mostly due to moisture loss	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

The fillet is weighed before cooking, and then the fillet is cooked. The fillet is weighed after cooking and elimination of the liquid loss.

MATERIAL (biological, reagents & instrumental):

A fillet or a part of a fillet.

A scale (precision should be adapted to the weight of the sample, 0.1g is generally appropriate).

A cooking apparatus (micro wave oven, steam oven, warm-water bath).

UNIT AND RANGE OF VALUE:

Cooking loss can be expressed in g, but is generally expressed in percent of the raw weight or in a percentage corresponding to the cooking yield (weight of the fillet after cooking/weight of the fillet before cooking) x 100.

PARAMETERS TO MEASURE:

Weight of the flesh/fillet before cooking.

Weight of the flesh/fillet after cooking.

BIBLIOGRAPHIC REFERENCES:

Min, B., Green, B. W., 2008. Use of Microbial Transglutaminase and Nonmeat Proteins to Improve Functional Properties of Low NaCl, Phosphate-Free Patties Made from Channel Catfish (*Ictalurus punctatus*) Belly Flap Meat. *J.Food Sci.* 73, E218-E226.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

The cooking procedure, time and temperature, storing of the fillet have to be detailed, as well as the type and model of cooking material.

RESEARCHER CONTRIBUTION (and date of the last modification):

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Link Table 2

Identifiant	ATOL:0001002	GM11
Name of Trait:	Meat flavour	

Definition:	Any measurable or observable characteristic related to the meat flavour.

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Sensory analysis*:*

The fish taste testing methods is modelled after the Flavour Profile Analysis procedure. A well trained panel can detect odorous compounds at very low levels and make a distinction between types of off-flavours described by flavour wheels (Van der Ploeg *et al*., 1995), as well as flavour intensity. Fish must be tested in constants conditions (temperature, light, etc.), in an environment free of odours (cigarette smoke, the smell of food, perfumes, chemicals, and other strong odours), that can interfere with sensory evaluation. Flavour intensity may be quantified on different scales, depend on species, countries etc. (see Table 1). Fish should be cooked either by microwaving or by steaming above boiling water. Cooking time depends on the method used or size and number of fish analysed simultaneously (Van der Ploeg *et al*., 1995). Testers have to have a time between two samples to rinse their mouth with water and eat a piece of bread or something else with neutral flavour to limit the contamination of a too strong off-flavour sample with the following one (Robin *et al*., 2006). However several experimental protocols for determining off-flavour thresholds have been described in the scientific and technical literature on sensory assays *there is no generally applied standard procedure for the determination.*

Table 1. Assessment of flavour intensity

Sensory class channel catfish (MIB) by Ploeg (1991)	**Intensity scale**	**Sensory class rainbow trout (GSM) by Robin *et al*., (2006)**	**Sensory score**
No off-flavours	0	On-flavour/non tainted	<3
Threshold	T		
Very slight	0.5	Very slight tainted	≥ 3 and <4
Slight	1		
Slight to distinct	1.5	Slightly tainted	≥ 4 and <5
Distinct	2		
Distinct to strong	2.5	Tainted	≥ 5 and <6
Strong	3	Strongly tainted	6

MATERIAL (biological, reagents & instrumental):

Microwave

Water

Trained sensory panel

Sensory lab with inverse pressure and constants conditions (temperature, light, etc.)

UNIT AND RANGE OF VALUE:

0 – 3 for Intensity scale

0 – 6 for Sensory score

PARAMETERS TO MEASURE:

Intensity scale

Sensory score

BIBLIOGRAPHIC REFERENCES:

Baigrie, B. Taints and off-flavors in food. WEB: http://www.woodheadpublishing.com/en/book.aspx?bookID=405'

Olafsdottir, G., Nesvabda, P., Di Natale, C., Careche, M., Oehlenschläger, J., Tryggvadóttir, V., Schubring, R., Kroeger, M., Esaiassen, M., Macagnano, A., Jørgensen, B.M., 2004. Multisensor for fish quality determination. *Trends in Food Sci. Techn.* 15, 86-93.

Papp, Z.G., 2008. Off-flavour problems in farmed fish. In: Øyvind Lie (Ed.) Improving farmed fish quality and safety *Woodhead Publ.* Cambrige, England, pp. 471-489.

Robin, J., Cravedi, J.P., Hillenweck, A., Deshayes, C., Vallod, D., 2006. Off-flavor characterization and origin in French trout farming. *Aquaculture* 260, 128-138.

Taylor, A.J., Mottram, D.S., Flavour science: Recent developments. University of Nottingham and University of Reading, UK. WEB: http://www.woodheadpublishing.com/en/book.aspx?bookID=686

Van der Ploeg, M., Tucker, C., Steeby, J., Weirich, C., 1995. Management plane for blue-green off-flavors in Mississippi pond-raised catfish. *Cooperative Extension Service Missisipi State Univ. Publication 2001,* 500-8-95.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

Flavour is a property of food and it is perceptible both in the mouth (taste) and in the nose (smell).

Quantitative chemical assays are time and cost consuming methods for screening of fish for flavour quality before harvesting avoid marketing fish with environment-related off-flavours. While only a limited number of odorous compounds can be detected by instrumental methods, sensory analysis (taste and odour testing) which treats trained “testers” (panel) as analytical instruments is much effective for routine evaluation of fish flavour quality.

Different gas sensors including electrochemical, metal oxide and organic polymers are available to measure the flavour of fish flesh. Responses of electrochemical and conducting polymer sensors have been shown to correlate with other objective measures of quality in fresh seafood as well. However, the stability of correlation between sensory data and electronic nose response still represents a problem for practical application of gas sensors in seafood shelf life evaluation (Olafsdottir *et al.,* 2004).

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Link Table 2

Identifiant	ATOL:0001663	GM12
Name of Trait:	Meat lipid content	
Definition:	Any measurable or observable characteristic related to the amount in meat of fat-soluble substances (molecules composed of carbon and hydrogen characteristically insoluble in water).	

SIMILAR TO: *If it’s appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Lipids from muscle will be extracted with a chloroform:methanol (2:1 v:v) mixture according to Folch *et al.* (1957).

The meat liquid content can be also evaluated on lived fish by using a Fish fat meter (Distell). This device allows to make an indirect measure of the lipid content. The measure consists in putting the sensor of the device on a wipe part of the skin. The fat meter measure the quantity of water in the fillet which is inversely proportional to the lipid content. The measures have to be done always on the same places on the fish because of the existence of two gradients of lipid deposition in the muscle, anterior-posterior and dorso-ventral. The evaluation of the mean value of one group of fishes is more accurate than individual measurements but the device also allows the identification of the leanest or the most fatty within a group.

MATERIAL (biological, reagents & instrumental):

Flesh without skin

Fat meter Distell

UNIT AND RANGE OF VALUE:

% lipids per wet muscle or % lipids per dry muscle.

http://www.fao.org/docrep/003/T0219E/T0219E01.htm (*)

http://nutraqua.com/index.php?lang=en (**)

(*)This web page gives the values of the yield of edible tissue and the protein and fat contents of the world's commercially more important fish and shellfish species, derived from a survey of published, and some unpublished, material.

(**)This online data basis gives the nutritional contents chart with scientifically proven results on 47 aquatic products whose levels in 20 nutrients (vitamins, minerals, macronutrients) as well as fatty acids have been established.

PARAMETERS TO MEASURE:

Meat lipid content

BIBLIOGRAPHIC REFERENCES:

Douirin, C; Haffray, P; Vallet, JL; et al., 1998 Determination of the lipid content of rainbow trout (*Oncorhynchus mykiss*) filets with the Torry Fish Fat Meter (R). *Sci. Aliment*. 18, 527-535.

Folch, J., Lees, M., Sloane-Stanley G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem*. 226, 497– 509.

http://www.fao.org/docrep/003/T0219E/T0219E01.htm

http://www.distell.com/products/prd-fish-fatmeter/introduction/ffm-principle-of-operation

SYNONYMS EXACT:

Flesh lipid content

OTHER ASPECTS TO INCLUDE:

Indirect method can be used like NMR, NIR after calibration with chemical method

RESEARCHER CONTRIBUTION (and date of the last modification):

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(20/06/13)

Link Table 2

Identifiant	ATOL:0000100	GM13
Name of Trait:	Meat omega-6 to omega-3 fatty acid ratio	
Definition:	Any measurable characteristic of ratio between fatty acids of omega-6 (CHEBI:36009) family to fatty acids of omega-3 (CHEBI:25681) family as an index of healthy value of meat.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

After cold extraction by the Folch method (Folch *et al*., 1957), total lipids are transmethylated (Christie, 1982) and the obtained fatty acid methyl esters separated by GLC and quantified by FID (Izquierdo *et al*., 1992).

Omega-6 to omega-3 fatty acid ratio is calculated as follows:

Sum of ω-6 fatty acids / sum all of ω-3 fatty acids (ω-6/ ω-3)

ω-6 or n-6 fatty acids = polyunsaturated fatty acids with 18 or more carbon atoms and 2 or more double bonds belonging to the linoleic acid family
ω-3 or n-3 fatty acids = polyunsaturated fatty acids with 18 or more carbon atoms and 3 or more double bonds belonging to the alpha-linolenic acid family

MATERIAL (biological, reagents & instrumental):

Flesh, Gas-Chromatograph

UNIT AND RANGE OF VALUE:

No unit.

Because of the low content of n-6 fatty acids in marine fish, the ratio of total n-3 to n-6 fatty acids (essential fatty acid ratio) is high, varying between about 5 and more than 10 (Steffens, 1997).

PARAMETERS TO MEASURE:

ω-6 fatty acids; ω-3 fatty acids (usually expressed as % of total fatty acids)

BIBLIOGRAPHIC REFERENCES:

Christie, W.W., 1982. *Lipid analysis*. Pergamon, Oxford.

Folch, J., Lees, M., Sloane-Stanley G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem*. 226, 497– 509.

Harris, W.S., Lemke, S.L., Hansen, S.N., Goldstein, D.A., DiRienzo, M.A., Su, H., Nemeth, M.A., Taylor, M.L., Ahmed, G., George, C., 2008. Stearidonic acid-enriched soybean oil increased the omega-3 index, an emerging cardiovascular risk marker. *Lipids* 43, 805-811.

Izquierdo, M.S., Arakawa, T., Takeuchi, T., Haroun, R., Watanabe, T. 1992. Effect of n-3 HUFA levels in Artemia on growth of larval Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* 105, 73-82.

Pigott, G.M., Tucker, B.W., 2002. Formulation of Special Feeds. In Halver J.E., Hardy R.W. (Eds.), *Fish Nutrition*, San Diego, Academic Press, pp. 652-661.

Steffens W., 1997. Effects of variation feeds on nutritive in essential fatty acids in fish value of freshwater fish for humans. *Aquaculture* 151, 97-119.

SYNONYMS EXACT:

n-6 to n-3 fatty acid ratio.

OTHER ASPECTS TO INCLUDE:

Meat omega-3 to omega-6 fatty acid ratio (n-3/n-6) is also widely used.

In the case of humans use of the dietary n-6 to n-3 ratio itself has been questioned with the suggestion that it is be replaced by the omega-3 Index. This is a measure of the levels of EPA and DHA in red blood cell lipids and should attain a level of 8% (Harris *et al.,* 2008).

RESEARCHER CONTRIBUTION (and date of the last modification):

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(20/06/13)

Link Table 2

Identifiant	ATOL:0001684	GM14
Name of Trait:	Meat pH	
Definition:	Any measurable or observable characteristic related to the relative acidity or alkalinity of meat, as measured by the concentration of hydrogen ion.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

a) pH method in fish (Einen *et al.,* 1999; Ginés *et al.,* 2002):

Make a lateral incision at the height of the dorsal fin to the whole fish.

Introduce the electrode 1 cm

Measure the pH (as established by supplier).

b) pH method in muscle (Periago *et al.,* 2005; Fuentes *et al.,* 2010):

Mince the skinned fillet to obtain a homogeneous sample.

Mix 10 g of sample with 50 ml of distilled water (previously checked to neutral pH value). Let the solution stabilize for one hour before measurement.

Measure the pH in the mixture (as established by supplier).

MATERIAL (biological, reagents & instrumental):

Penetration electrode connected to a pH-meter (accuracy of 0.01 pH unit) (*method a*)

pH-meter (accuracy of 0.01 pH unit) (*method b*)

UNIT AND RANGE OF VALUE:

pH unit, between 6.0 and 7.4 depending of species, nutritional states and post mortem time.

PARAMETERS TO MEASURE:

Meat pH

BIBLIOGRAPHIC REFERENCES:

Einen, O., Mørkøre, T., Bencze Røra, A.M., Thomassen, M.S., 1999. Feed ration prior to slaughter--a potential tool for managing product quality of Atlantic salmon (*Salmo salar*). *Aquaculture* 178, 149–169.

Fuentes, A., Fernández-Segovia, I., Serra, J.A., Barat, J.M., 2010. Comparison of wild and cultured sea bass (*Dicentrarchus labrax*) quality. *Food Chemistry* 119, 1514–1518.

Ginés, R., Palacio, M., Zamorano, M.J., Argüello, A., López, J.L., Afonso, J.M., 2002. Starvation before slaughtering as a tool to keep freshness attributes in gilthead sea bream (*Sparus aurata*). *Aquaculture International*, 10, 379–389.

Periago, M.J., Ayala, M.D., López-Albors, O., Abdel, I., Martínez, C., García-Alcázar, A., Ros, G., Gil, G., 2005. Muscle cellularity and flesh quality of wild and farmed sea bass, *Dicentrarchus labrax* L. *Aquaculture* 249, 175–188.

SYNONYMS EXACT:

Flesh pH; muscle pH; fillet pH; intramuscular pH

OTHER ASPECTS TO INCLUDE:

Both methods are equivalent and equally valid. Due to a rapid evolution of muscle pH after slaughtering, the post-mortem time of measurement should be mentioned.

RESEARCHER CONTRIBUTION (and date of the last modification):

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(20/06/13)

Link Table 2

Identifiant	ATOL:0001547	GM15
Name of Trait:	Meat protein content	
Definition:	Any measurable characteristic related to the amount in meat of macromolecules consisting of long chains of amino acids in peptide linkage.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Determination of Kjeldahl-nitrogen x 6.25

Or according to Dumas

MATERIAL (biological, reagents & instrumental):

Flesh without skin

UNIT AND RANGE OF VALUE:

% protein per wet muscle or % protein per dry muscle.

http://www.fao.org/docrep/003/T0219E/T0219E01.htm (*)

http://nutraqua.com/index.php?lang=en (**)

(*) This web page gives the values of the yield of edible tissue and the protein and fat contents of the world's commercially more important fish and shellfish species, derived from a survey of published, and some unpublished, material.

(**)This online data basis gives the nutritional contents chart with scientifically proven results on 47 aquatic products whose levels in 20 nutrients (vitamins, minerals, macronutrients) as well as fatty acids have been established.

PARAMETERS TO MEASURE:

Meat protein content.

BIBLIOGRAPHIC REFERENCES:

Dumas: NEN-EN-ISO 16634

http://www.fao.org/docrep/003/T0219E/T0219E01.htm

Kjeldahl-N: NEN-EN-ISO 5983-2

SYNONYMS EXACT:

Flesh protein content.

OTHER ASPECTS TO INCLUDE:

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(20/06/13)

Link Table 2

Identifiant	ATOL:0000130	GM16
Name of Trait:	Muscle to body weight ratio	
Definition:	Weight of all muscles or a specific muscle normalized by the live body weight of the animal.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Dry and weigh the whole-body fish (ATOL:0000351).

Fillet the fish. Specifies which parts of the fish are included in the fillet (for instance, if the belly flap is included).

Weigh both skinless fillets.

Muscle yield (muscle to body weight ratio) will be obtained as percentage of the whole-body fish: 100 x (fillets weight / whole-body fish).

MATERIAL (biological, reagents & instrumental):

Weight (accuracy of 1 or 0.1 g)

UNIT AND RANGE OF VALUE:

% live body weight that often varies between 35% and 60% for most cultured fish species.

FAO web page gives the values of the yield of edible tissue and the protein and fat contents of the world's commercially more important fish and shellfish species, derived from a survey of published, and some unpublished, material.

PARAMETERS TO MEASURE:

Animal body weight and fillets weight.

BIBLIOGRAPHIC REFERENCES:

Haffray, P. Bugeon, J. Pincent, C. Chapuis, H. Mazeiraud, E. Rossignol, M.N. Chatain, B. Vandeputte, M. Dupont-Nivet, M., 2012. Negative genetic correlations between production traits and head or bony tissues in large all-female rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 368-369; 145-152.

http://www.fao.org/docrep/003/T0219E/T0219E01.htm

http://www.youtube.com/watch?v=baYglEECAbU (see 3:28 min)

SYNONYMS EXACT:

Fillet yield

Fillet percentage

Muscle yield

OTHER ASPECTS TO INCLUDE:

In some cases only one fillet can be filleted and weigh, so the formula is 2 x 100 x (fillet weight / whole-body fish). None of the current body composition methods estimates muscle mass directly and non-invasive techniques are estimations with varying degrees of accuracy, based on previous dissection measurements of muscle mass.

RESEARCHER CONTRIBUTION (and date of the last modification):

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(20/06/13)

Link Table 2

Identifiant	ATOL:0000486	GM17
Name of Trait:	Skin colour trait	
Definition:	Any measurable characteristic related to the degree of paleness or	

darkness of the skin.

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Skin colour is measured in dorsal areas of randomly selected fish from at stock tank immediately after capturing them, using a portable spectrophotometer and employing the CIE L* (lightness; dark=0, white=100), a* (red=+values, green=-values), and b* (yellow=+values, blue=-value) colour scale. The three-dimensional characteristics of colour appearance, i.e. the lightness attribute (L*) are measured and the two chromatic attributes hue (H*ab = arctan (b*/a*) and chroma C*ab=(a*2 + b*2) 0.5) are calculated.

MATERIAL (biological, reagents & instrumental):

MiniscanTM XE (HunterLab, Reston,VA, USA)

Minolta Color Meter type CR-400/410 (Minolta, Japan)

UNIT AND RANGE OF VALUE:

CIE L* (lightness; dark = 0, white = 100)

Colour component a* (red = +values, green = -values) (from -127 [green] to +127 [red])

Colour component b* (yellow = +values, blue = -value) (from -127 [blue] to +127 [yellow])

PARAMETERS TO MEASURE:

L*, a*, b*

BIBLIOGRAPHIC REFERENCES:

Pavlidis, M., Papandroulakis, N., Divanach, P., 2006. A method for the comparison of chromaticity parameters in fish skin: Preliminary results for coloration pattern of red skin Sparidae. *Aquaculture* 258, 211-219.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

RESEARCHER CONTRIBUTION (and date of the last modification):

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Léa Joret (INRA)
Wout Abbink (IMARES)

(20/06/13)

Link Table 2

Identifiant	ATOL:0001662	GM18
Name of Trait:	Specific growth rate	
Definition:	Growth rate between two sampling date SGR=100 x[(ln (w_2)-ln(w_1))/(t_2-t_1)]	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

The growth of fish can be expressed in terms of absolute growth rate (i.e. dW/dt of grams per day), or as specific growth rate. Since growth rates depend on the size of the fish, the specific growth rates (SGR) are mostly used, expressing the growth rate divided by the size of the fish:

SGR (%) = $[(\ln W_t - \ln W_0)/t]x100$

Where W_0 is the initial fish dry weight and W_t is the individual dry weight at time t.

If the Food Conversion Rate is known, the SGR can also be calculated by dividing the percentage body weight fed per day by the food conversion rate.

MATERIAL (biological, reagents & instrumental):

Weight (accuracy 0.1 g for juveniles, 0.0001 g or higher accuracy for larvae, depending on the species, and on the need for individual weight measurements of wet and/or dry mass)

UNIT AND RANGE OF VALUE:

Time (days)

Weight (g)

SGR (%)

PARAMETERS TO MEASURE:

Time

Dry weight or wet weight

SGR

BIBLIOGRAPHIC REFERENCES:

Ricker W.E., 1958. Handbook of computations for biological statistics of fish populations, *Fisheries Research Board of Canada,* Ottawa.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

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(20/06/13)

Link Table 2

Identifiant	ATOL:0001661	GM19
Name of Trait:	Thermal growth coefficient	
Definition:	Growth rate between two sampling date taking into account the water temperature variation TGC = [(W2 exp0.333 - W1 exp0.333) x (degree x days)$^{-1}$ × 1000].	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

The thermal growth coefficient (TGC) model has the following basic form:

$$\sqrt[3]{W_t} = \sqrt[3]{W_0} + [(T/1000) \times t]$$

Where, T (a constant) is temperature in ºC; t is time in days; W_0 is initial weight and W_t is final weight (after t days).

Under constant water temperature, TGC is calculated as:

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

Under variable daily water temperature, the concept of degree-days is introduced and TGC is calculated as

$$\mathrm{TGC} = \left[\left(\sqrt[3]{W_t} - \sqrt[3]{W_0}\right)/\left(\sum_{i=1}^{t} T_i\right)\right] \times 1000$$

Where, W_0 is initial weight; W_t is final weight (after t days); T_i is mean daily water temperature and $\sum_{i=1}^{t} T_i$ is the sum of mean daily water temperature over the study duration period of t days or degree-days.

MATERIAL (biological, reagents & instrumental):

Weight (accuracy of 0.1 unit)

UNIT AND RANGE OF VALUE:

Temperature in ºC, time in number of days, weight in g. TGC is a coefficient hence has no unit.

Range of TGC values (examples) 1.53 to 1.74 (rainbow trout); 1.39 (lake trout); 0.99 (brown trout); 0.98 (chinook salmon); 0.60 to 2.1 for Atlantic salmon.

PARAMETERS TO MEASURE:

Growth rate taking into account the water temperature variation.

BIBLIOGRAPHIC REFERENCES:

Dumas, A., France, J., Bureau, D.P., 2007. Evidence of three growth stanzas in rainbow trout (*Oncorhynchus mykiss*) across life stages and adaptation of the thermal-unit growth coefficient. *Aquaculture* 267, 139-146.

Iwama, G.K., Tautz, A., 1981. A simple growth model for salmonids in hatcheries. *Canadian Journal of Fisheries and Aquatic Sciences* 38, 649-656.

Jauralde, I., Martínez-Llorens, S., Tomás, A., Ballestrazzi, R., Jover, M., 2011. A proposal for modelling the thermal-unit growth coefficient and feed conversion ratio as functions of feeding rate for gilthead sea bream (*Sparus aurata*, L.) in summer conditions. *Aquaculture Research*. doi:10.1111/j.1365-2109.2011.03027.x.

Jobling, M., 2003. The thermal growth coefficient (TGC) model of fish growth: a cautionary note. *Aquaculture Research* 34, 581–584.

Johnston *et al.,* 2007 see http://www.st-andrews.ac.uk/fmrg/documents/283.pdf

SYNONYMS EXACT:

VF3/GF3

OTHER ASPECTS TO INCLUDE:

TGC is expected to be constant over a certain size range of the fish, however, its exponent may vary (Dumas *et al.,* 2007)

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(20/06/13)

Link Table 2

Identifiant	ATOL:0001660	GM20
Name of Trait:	Total length	
Definition:	Length from the tip of the snout to the tip of the longer lobe of the caudal fin, usually measured with the lobes compressed along the midline. It is a straight-line measure, not measured over the curve of the body.	

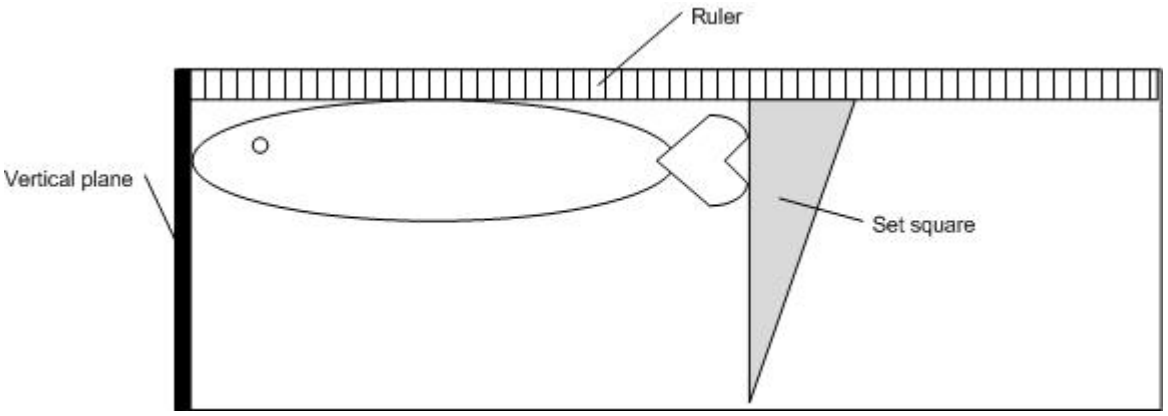
SIMILAR TO:

ATOL:0001658

MEASUREMENT METHOD:

The total length is measured by laying the fish on a ruler which has a vertical plane at the zero mark. To minimized the parallax error on the reading of the measure, the ruler have to be equip with a set square as shown in the diagram below. The fish is place on the device in taking care of keeping it parallel to the ruler. The fish is softly compressed to the zero mark with the tip of the snout. The longer lobe of the caudal fin is compressed along the rule and the length is measured.

On sea bass it's better to place the belly of the fish in contact to the ruler to have a better parallel position of the fish.



MATERIAL (biological, reagents & instrumental):

Measuring board (accuracy of 0.1 mm)

Calliper for small fish
UNIT AND RANGE OF VALUE: mm
PARAMETERS TO MEASURE: Body length
BIBLIOGRAPHIC REFERENCES: Gaygusuz Ö., Gürsoy Ç., Özulu M., Tarkan A.S., Acıpınar H., Bilge G., Filiz H., 2006. Conversions of Total, Fork and Standard Length Measurements Based on 42 Marine and Freshwater Fish Species (from Turkish Waters). *Turkish Journal of Fisheries and Aquatic Sciences* 6, 79-84. Howe J.C., 2002. Standard length: not quite so standard. *Fisheries Research* 56, 1-7.
SYNONYMS EXACT:
OTHER ASPECTS TO INCLUDE:
RESEARCHER CONTRIBUTION (and date of the last modification): Andries Kamstra (IMARE) Léa Joret (INRA) Jaume Pérez-Sánchez (CSIC) Alain Vergnet (IFREMER) Mathilde Dupont-Nivet (INRA) (20/06/13)

Link Table 2

Identifiant	ATOL:0000552	GM21
Name of Trait:	Visceral adipose tissue weight trait	
Definition:	Any measurable characteristic related to the weight of the fat-storing tissue surrounding the internal organs.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*
MEASUREMENT METHOD: Weigh the fish (ATOL:0000351). Open the visceral cavity and remove all viscera of slaughtered fish. Separate manually the fatty tissue (visceral fat) from the internal organs. Weigh the visceral fat.
MATERIAL (biological, reagents & instrumental): Dissection material; A scale (accuracy, usually around 1/100 to 1/1000 of the average weight to be measured)
UNIT AND RANGE OF VALUE:

Gram (g)

PARAMETERS TO MEASURE:

Visceral adipose tissue weight.

BIBLIOGRAPHIC REFERENCES:

Navarro, A., Zamorano, M.J., Hildebrandt, S., Ginés, R., Aguilera, C., Afonso J.M., 2009. Estimates of heritabilities and genetic correlations for body composition traits and G×E interactions, in gilthead seabream (*Sparus auratus* L.). *Aquaculture* 295, 183-187.

SYNONYMS EXACT:

Visceral fat weight

OTHER ASPECTS TO INCLUDE:

Refrigerating the fish may help for fat dissection. Difficult in species with many pyloric caeca.

RESEARCHER CONTRIBUTION (and date of the last modification):

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Link Table 2

Identifiant	ATOL:0000773	NT1
Name of Trait:	Average daily feed intake	
Definition:	Any measurable characteristic related to the amount of feed ingested in a day.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

The amount of feed eaten by an individual or group of fish during a defined period divided by total number of days of the period. Can be expressed per individual or per unit Body Mass (BM).

Feed intake can be determined by self-demand feeders or by feeding until apparent satiation and taking note of the amount of feed dispensed, collecting and weighting uneaten feed

MATERIAL (biological, reagents & instrumental):

A scale (accuracy of 0.1 unit)

UNIT AND RANGE OF VALUE:

$g.day^{-1}$; $g.kg\ BM^{-1}day^{-1}$; $g.kg\ metabolic\ BM^{-1}\ day^{-1}$

PARAMETERS TO MEASURE:

Amount of feed distributed; amount of uneaten feed.

BIBLIOGRAPHIC REFERENCES:

Jobling, M., Arnesen, A.M., Baardvik, B.M., Christiansen, J.S., Jorgensen, E.H., 1995. Monitoring voluntary feed intake under practical conditions, methods and applications. *J. Appl. Ichthyol*. 11, 248-262.

Jobling, M., Coves, D., Damsgard, B., Kristiansen, H.R., Koskela, J., Petursdottir, T.E., Kadri, S., Gudmundsson, O., 2001. Techniques for measuring feed intake. In: Houlihan, D. Boujard, T. Jobling, M. (Eds), *Food Intake in Fish*, Blackwell Science, UK, pp. 49-87.

Helland, S.J., Grisdale-Helland, B., Nerland, S., 1996. A simple method for the measurement of daily feed intake of groups of fish in tanks. *Aquaculture*, 139, 157-163.

SYNONYMS EXACT:

Voluntary feed intake; Daily feed intake.

OTHER ASPECTS TO INCLUDE:

Water temperature, Body mass

RESEARCHER CONTRIBUTION (and date of the last modification):

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Link Table 2

Identifiant	ATOL:0001528	NT2
Name of Trait:	Eating rate	
Definition:	Any measurable or observable characteristics related to the quantity of feed (or diet) eaten by an animal per unit of time; it is used for the measurement of eating motivation.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Eating rate or feeding rate (FR) is the amount of feed given over a period of time

"Feeding rate (or level) is a term used in commercial aquaculture to describe the amount of feed provided over a given time interval (usually 1 day)" (Houlihan *et al.,* 2001. Glossary)

MATERIAL (biological, reagents & instrumental):

Scale and stopwatch

UNIT AND RANGE OF VALUE:

Unit mass per day (g or kg day^{-1})
Units are frequently given per fish weight and sometimes per fish unit

PARAMETERS TO MEASURE: Amount of feed eaten or distributed, duration, body mass
BIBLIOGRAPHIC REFERENCES: Houlihan, D., Boujard, T., Jobling, M., 2001. *Food Intake in Fish*, Blackwell Science, UK, pp418. Houlihan, D., Boujard, T., Jobling, M., 2001. *Food Intake in Fish*, Blackwell Science, UK, Glossary of terms, pp391.
SYNONYMS EXACT: Ration size, Daily allowance
OTHER ASPECTS TO INCLUDE: Description of the husbandry conditions; temperature, body mass.
RESEARCHER CONTRIBUTION (and date of the last modification): Ep Eding (WU) Léa Joret (INRA) Jan Mraz (VURH) Marisol Izquierdo (ULPGC) Sadasivam Kaushik (INRA) (20/06/13)

Link Table 2

Identifiant	ATOL:0001599	NT3
Name of Trait:	Energy requirement	
Definition:	The amount of energy required for a specific purpose: maintenance, weight gain, milk yield, etc.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Animals have a requirement for fuels which can be expressed as their energy equivalents and thus as an "energy requirement". All animals need energy for maintenance, activity, growth and reproduction. The energy requirements of fish are traditionally estimated by constructing a complete energy budget, balancing the energy intake against energy expenditures. Estimated expenditures include energy lost in the faeces, energy lost to excretion (urinary, gill, body surface energy loss), energy used for metabolism and activity, and energy deposited as growth (Cho and Kaushik, 1990; Kaushik and Medale, 1994; Cho and Bureau, 1995; Bureau *et al.,* 2002).

Energy requirement for maintenance in fish reflects the amount of energy required for maintaining vital functions and is often measured based on oxygen uptake (indirect calorimetry) or even through direct calorimetry (Smith *et al.* 1978) and is most commonly expressed as calories or joules per unit body weight or unit metabolic body weight per day. Energy requirement for growth is expressed in terms of digestible energy (DE). DE is measured as the difference between the gross energy (GE) content of the diet, determined by bomb calorimeter or by multiplying the protein, fat and carbohydrate contents of the diets by their respective average combustion values) and the energy content of the faeces (fecal energy FE). DE=GE-FE.

The DE requirements may be defined as digestible energy need (DEN), defined as the apparent

digestible energy required to grow 1 kg of wet body weight (Bailey and Alanärä, 2006).

MATERIAL (biological, reagents & instrumental):

Adiabatic or parr bomb calorimeter, scale, oxygen electrodes (indirect calorimetry).

UNIT AND RANGE OF VALUE:

Unit: joule (J) or calorie

1 calorie = 4,184 J

Example;

Maintenance energy requirements calculated using comparative carcass analysis in salmonids 85 - 110 kJ ME kg−1 day−1 (Kaushik and Medale, 1994), affected by wtaer temperature and body weight.

Range of values on DE required to grow 1 kg of wet body weight for different species of fish (Bailey and Alanärä, 2006): 11 – 24 MJ DE kg^{-1}.

PARAMETERS TO MEASURE:

Feed intake (kg), Dietary Gross energy content (MJ kg^{-1})

Digestible energy (DE) content of the feed (MJ kg^{-1})

The increment of weight gain (kg), whole body energy content ((MJ kg^{-1})

BIBLIOGRAPHIC REFERENCES:

Bailey, J., Alanärä, A., 2006. Digestible energy need (DEN) of selected farmed fish species. *Aquaculture* 251, 438–455.

Bureau, D.P., Kaushik, S.J., Cho, C.Y., 2002. Bioenergetics. In Halver, J.E., Hardy, R.W. (editors), *Fish Nutrition*, San Diego, Academic Press, pp. 2-62

Cho, C.Y., Kaushik, S.J., 1990. Nutritional energetics in fish: energy and protein utilization in rainbow trout (*Salmo gairdneri*). *World Rev. Nutr. Diet.* 61, 132-172.

Cho, C.Y., Bureau, D.P., 1995. Determination of the energy requirements of fish with particular reference to salmonids. *Journal of Applied Ichthyology*. 11(3/4),141-163.

Kaushik, S., Médale, F, 1994. Energy requirements, utilization and supply to salmonids. *Aquaculture* 124, 81-97.

Smith, R.R., Rumsey, G.L., Scott, M.L., 1978. Net energy maintenance requirements of salmonids as measured by direct calorimetry: effect of body size and environmental temperature. *J. Nutr.* 108, 1017-1024.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

Energy requirement can be affected by several effects such as water temperature, salinity, fish size, water current and feeding level, etc.

OTHER ASPECTS TO INCLUDE:

RESEARCHER CONTRIBUTION (and date of the last modification):

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(20/06/13)

Link Table 2

Identifiant	ATOL:0001490	NT4
Name of Trait:	Feces gross energy content	
Definition:	Amount of heat produced during the complete combustion of feces	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Some feed components resist digestion and are voided as faeces. The energy that would have been liberated by the combustion of the faecal material is lost to the animal and is referred as faecal energy loss (FEL). The measurement involves collection of faeces, desiccation or freeze-drying the feces and determining the energy content.

The energy content of feces is determined using an adiabatic bomb calorimeter, where dry samples are completely combusted in an oxygen filled container, and the heat released is measured by the elevation of temperature and energy content calculated according to each manufacturer´s manual.

MATERIAL (biological, reagents & instrumental):

Adiabatic bomb calorimeter, scale

UNIT AND RANGE OF VALUE:

PARAMETERS TO MEASURE:

Elevation of temperature due to the combustion of one gram of faeces.

BIBLIOGRAPHIC REFERENCES:

Cho, C.Y., Slinger, S.J., 1979. Apparent digestibility measurement in feedstuff for rainbow trout. In: Halver, J.E. and Tiews, K. (Eds.), *Finfish Nutrition and Fishfood Technology*, Vol. 2, Heenemann GmbH, Berlin, pp. 239–247.

Choubert, G., Noue, J. de la, Luquet, P., 1979. Continuous quantitative automatic collector for fish faeces. *Prog. Fish-Cult*. 41, 64-67.

Kaushik, S.J., Coves, D., Dutto, G., Blanc, D., 2004. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. *Aquaculture* 230, 391–404.

Robaina L., Izquierdo M.S., Moyano F.J., Socorro J., Vergara J.M., Montero D., Fernández-Palacios H., 1995. Soybean and lupin seed meals as protein sources in diets for gilthead seabream *(Sparus aurata)*: nutritional and histological implications. *Aquaculture* 130, 219-233.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

Care should be taken to separate fish faeces from water and to avoid contamination of the faeces with uneaten feed. The most frequently used methods to collect faeces to determine FEL were developed by Choubert *et al.* (1979) and Cho and Slinger (1979). Choubert *et al.* (1979) used a mechanically rotating screen to filter out faecal material. To avoid stress to fish and to simplify faeces collection, a system was developed by Cho and Slinger (1979) to allow the separation of faeces by sedimentation. It is used for routine measurement of digestibility of feed ingredients. This method allows fish to feed normally, determinations can be repeated and fish handling is

avoided. Although soluble material may be lost from the faeces due to leaching. This method has been also adapted for marine fish studies (Robaina *et al.,* 1994; Kaushik *et al*., 2004).

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(20/06/13)

Link Table 2

Identifiant	ATOL:0001449	NT5
Name of Trait:	Feces total lipid content	
Definition:	Any measurable or observable characteristic related to the concentration of lipid (CHEBI:18059) in feces.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Ether extraction (ISO, 1999) in collected feces. The main methods used for feces collection are stripping (Austreng, 1978), collection (Choubert *et al.,* 1979) and use of decantation columns (Cho and Slinger, 1979). Feces are then freeze-dried before analysis.

MATERIAL (biological, reagents & instrumental):

See bibliographic reference ISO (1999).

UNIT AND RANGE OF VALUE:

% crude fat or g fat per kg sample (often in g fat per kg dry matter).

PARAMETERS TO MEASURE:

See bibliographic reference ISO (1999).

BIBLIOGRAPHIC REFERENCES:

Austreng, E., 1978. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. *Aquaculture* 13, 265-272.

Austreng, E., Skjrede, A., Eldegard A., 1980. Digestibility of fat and fatty acids in rainbow trout and mink. *Aquaculture* 19, 93-95.

Cho, C.Y., Slinger, S.J., 1979. Apparent digestibility measurement in feedstuff for rainbow trout. In: Halver, J.E. and Tiews, K. (Eds.), *Finfish Nutrition and Fishfood Tech*nology, Vol. 2, Heenemann GmbH, Berlin, pp. 239–247.

Choubert, G., Noue, J. de la, Luquet P., 1979. Continuous quantitative automatic collector for fish faeces. *Prog. Fish-Cult*. 41, 64-67.

ISO 1999. ISO, Animal Feeding Stuffs-Determination of Fat Content. ISO 6492. *International Organization for Standardization.*

SYNONYMS EXACT:

Feces crude fat content. Fecal fat content.

OTHER ASPECTS TO INCLUDE:

It should be reported when hydrolysis is applied in the analysis**.** Care should be taken to separate fish faeces from water and to avoid contamination of the faeces with uneaten feed. The most frequently used methods to collect faeces are stripping (Austreng 1978; Austreng *et al.,* 1980), sieving water using a continuous feces collector (Choubert *et al.,* 1979) or through sedimentation (Cho and Slinger,1979).

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(20/06/13)

Link Table 2

Identifiant	ATOL:0001397	NT6
Name of Trait:	Feces major mineral content	
Definition:	Any measurable or observable characteristic related to the concentration of major inorganic components in feces.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Fish must be well adapted to the diets prior to faeces collection.The main methods used for feces collection are stripping (Austreng, 1978), collection (Choubert *et al.,* 1979) and use of decantation columns (Cho and Slinger, 1979). Feces are then freeze-dried before analysis. All the mineral elements put together are determined as total ash. Measurement of ash consists of oxidizing all organic matter in a weighed sample by combustion and determining the weight of the remaining ash. For individual macro and micro minerals, different methods are used.

MATERIAL (biological, reagents & instrumental):

Total mineral (ash) content after combustion in a muffle furnace; ashed samples subject to analysis of individual minerals (colorimetry, atomic absorption, inductivity-coupled plasma)

UNIT AND RANGE OF VALUE:

g or mg mineral/g dry matter faeces

PARAMETERS TO MEASURE:

Content of each mineral in collected faeces.

BIBLIOGRAPHIC REFERENCES:

Austreng, E., 1978. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. *Aquaculture*, 13, 265-272.

Cho, C.Y., Slinger, S.J., 1979. Apparent digestibility measurement in feedstuff for rainbow trout. In: Halver, J.E. and Tiews, K. (Eds.), *Finfish Nutrition and Fishfood Tech*nology, Vol. 2, Heenemann GmbH, Berlin, pp. 239–247.

Choubert, G., de la Noue, J., Luquet, P., 1979. Continuous quantitative automatic collector for fish faeces. *Prog. Fish-Cult*. 41, 64-67.

Sugiura, S.H., Dong, F.M., Rathbone, C.K., Hardy, R.W., 1998. Apparent protein digestibility and

mineral availabilities in various feed ingredients for salmonid feeds. *Aquaculture* 159, 177-202.

Vandenberg, G.W., de la Noue, J., 2001. Apparent digestibility comparison in rainbow trout (*Oncorhynchus mykiss*) assessed using three methods of faeces collection and three digestibility markers. *Aquaculture Nutrition* 7, 237-245.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

The main methods used for faeces collection to study mineral content are stripping (Austreng, 1978), collection (Choubert *et al*., 1979) or use of decantation columns (Cho and Slinger, 1979, Robaina *et al*., 1994). Much variability can occur depending on the method of feces collection (Sugiura *et al*., 1998; Vandenberg and de la Noueet, 2001)

The environment in which sample is obtained and prepared for analysis, especially ultra-trace elements, may affect accurate measurements.

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(20/06/13)

Link Table 2

Identifiant	ATOL:0001259	NT7
Name of Trait:	Feed apparent digestible nutrient	
Definition:	Any measurable characteristic related to the quantity of feed apparent digestible nutrient: feed (or diet) nutrient content x nutrient apparent digestibility.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Feed apparent digestible nutrient is determined by multiplying the apparent digestibility coefficient (ADC) for a nutrient by the feed nutrient content.

The apparent digestibility coefficient (ADC) for a nutrient in a diet is determined by collection of feces using an indigestible marker in the feed (Austreng, 1978; de la Noue and Choubert, 1986; Glencross, 2007). The difference in concentrations of this indigestible marker in the feed and in the feces allows the determination of the ADC.

MATERIAL (biological, reagents & instrumental):

Feeds containing a known amount of an indigestible marker. Feeding fish over a given period with such feeds and collection of feces using one of different methods; analyses of feeds and feces for the marker and the nutrients.

UNIT AND RANGE OF VALUE:

%, below 100%

PARAMETERS TO MEASURE:

The apparent digestibility coefficient (ADC diet) of each specific nutritional variable is based on

Equation:

ADC of a diet (%) = 100 (1 – (% marker in diet / % marker in faeces))

ADC of a nutrient in a diet (%)

ADC (%) = 100 (1- (% marker in diet x % nutrient in faeces) / (% marker in faeces x % nutrient in diet))

Where, Marker in diet and Marker in feces represent the marker content of the diet and faeces, respectively, and Nutrient in diet and Nutrient in faeces represent the content in the nutritient concern (e.g. protein or energy) in the diet and feces, respectively.

Feed apparent digestible nutrient level = Feed nutrient content x ADC of that nutrient in a diet (%)

BIBLIOGRAPHIC REFERENCES:

Austreng, E., 1978. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. *Aquaculture*, 13, 265-272.

de la Noue, J., Choubert, G., 1986. Digestibility in rainbow trout: Comparison of the direct and indirect methods of measurement. *Progressive Fish-Culturist*. 48, 190-195.

Glencross, B.D., Booth, M., Allan, G., 2007. A feed is only as good as its ingredients – a review of ingredient evaluation strategies for aquaculture feeds. *Aquaculture Nutrition* 13, 17-34.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

RESEARCHER CONTRIBUTION (and date of the last modification):

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(20/06/13)

Link Table 2

Identifiant	ATOL:0001232	NT8
Name of Trait:	Feed component apparent digestibility (include: nitrogen, amino acid, protein, structural carbohydrate and phosphorus)	
Definition:	Difference between input food and output food reported to input food ((input-output)/input) in the whole digestive tract.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Apparent digestibility (AD) is measured by finding the difference between the feed component (whole diet, ingredient or nutrient) intake (I) and the feed component content in faeces (F) divided by the feed component intake:

AD=(I-F)/I x100

Either a Direct Method or an Indirect Method can measure the Apparent Digestibility.

The Direct Method requires the complete quantitative control of feed intake and collection of all faecal losses corresponding to the amount eaten, which is tedious and complex.

The Indirect Method requires the addition of an inert non-digested non-absorbed tracer to the diet, feed to satiation and follow its concentration in the faeces. Only representative samples of faeces are collected, but it is not required to collect all of them. Then the apparent digestibility coefficient (ADC) may be calculated for any feed component including the whole diet, an ingredient or a nutrient by the formula:

ADC of a diet (%) = 100 (1 – (% marker in diet /% marker in faeces))

ADC of a nutrient in a diet (%) : ADC (%) = 100 (1- ((% marker in diet x % nutrient in faeces)/(% marker in faeces x % nutrient in diet)))

MATERIAL (biological, reagents & instrumental):

Feeds containing a known amount of an indigestible marker. Feeding fish over a given period with such feeds and collection of feces using one of different methods; analyses of feeds and feces for the marker.

UNIT AND RANGE OF VALUE:

%

PARAMETERS TO MEASURE:

Direct Method:

I= total ingested food (diet, ingredient or nutrient ingested)

F=the nutrient content in faeces

Indirect Method:

MD=inert marker concentration in the diet,

MF=inert marker concentration in the faeces,

ND=nutrient (diet or ingredient) concentration in the diet,

NF=nutrient (diet or ingredient) concentration in the faeces,

Then the apparent digestibility coefficient is determined by the formula:

ADC (%)=100-100x(MDxNF/MFxND)

BIBLIOGRAPHIC REFERENCES:

Cho, C.Y., Slinger, S.J., 1979. Apparent digestibility measurement in feedstuff for rainbow trout. In: Halver, J.E. and Tiews, K. (Eds.), *Finfish Nutrition and Fishfood Technology*, Vol. 2, Heenemann GmbH, Berlin, pp. 239–247.

Cho, C.Y., Kaushik, S.J., 1990. Nutritional energetics in fish: Energy and utilization in rainbow trout. *World Rev. Nutr. Diet.* 61, 132–172.

De la Noue, J., Choubert, G., 1986. Digestibility in rainbow trout: Comparison of the direct and indirect methods of measurement. *Progressive Fish-Culturist*. 48, 190-195.

Refstie, S., Helland, S.J., Storebakken, T., 1997. Adaptation to soybean meal diets for rainbow trout, *Oncorynchus mykiss*. *Aquaculture* 153, 263– 272.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

Since apparent digestibility values approach true digestibility values as the feed intake increases, it is important to feed the animals as close to satiation as possible to get reliable apparent digestibility values. Moreover, fish must be adapted to the diet for several days previous to feed intake measurements and faeces collection. Faeces are freeze-dried immediately after collection and kept at -20ºC until analysis. Internal markers should have no taste, flavour or colour for the fish and should be inert, non-toxic, easy to quantify, homogeneously distributed in the diet, whose physical and chemical properties will not be affected. Some frequently used markers are

chromium oxide (1-2% in diet), yttrium or titanium dioxide and, particularly for lipids, cholestan.

RESEARCHER CONTRIBUTION (and date of the last modification):

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(20/06/13)

Link Table 2

Identifiant	ATOL:0001258	NT9
Name of Trait:	Feed component true digestibility	
Definition:	Any measurable or observable characteristic related to the true digestibility ((input-output+endogenous+microbial)/input) of feed (or diet) component in the digestive tract.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Faeces include undigested food components (F) together with faecal residues of metabolic origin (Fm) including unabsorbed residues from body origin, such as mucosal cells, digestive enzymes and other secretions, or intestinal tract microflora.

True digestibility also called "corrected" digestibility (CD) is determined by calculating the difference between feed (or diet) nutrient intake and the faecal nutrient (F) minus the metabolic origin nutrient in faeces (Fm)

CD=(I-(F-Fm))/I x100

MATERIAL (biological, reagents & instrumental):

UNIT AND RANGE OF VALUE:

%

PARAMETERS TO MEASURE:

BIBLIOGRAPHIC REFERENCES:

Anderson, J.S., Lall, S.P., Anderson, D.M, Chandrasoma, J., 1992. Apparent and true availability of amino acids from common feed ingredients for Atlantic salmon (*Salmo salar*) reared in sea water. *Aquaculture* 108, 111– 124.

Cho, C.Y., Slinger, S.J., Bayley, H.S., 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. *Comp. Biochem. Physiol*. 73, 25-41.

Vergara, J.M., López-Calero, G., Robaina, L., Caballero, M.J., Montero, D., Izquierdo, M.S., Aksnes A., 1999. Growth, feed utilization and body lipid content of gilthead seabream (*Sparus aurata*) fed increasing lipid levels and fish meals of different quality. *Aquaculture* 179, 35–44.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

The food characteristics and the level of feed intake affect Fm. Fm should be determined in fasting animals, where the amount of faeces is so small that in practice Fm is of little significance for

animals fed to satiation. Therefore, in fish the apparent digestibility is frequently preferred over corrected digestibility. Nevertheless, true or corrected digestibility of ingredients, diets or nutrients obtained in terrestrial animal models such as mink, has been also used as a reference for fish.
RESEARCHER CONTRIBUTION (and date of the last modification): Marisol Izquierdo (ULPGC) Léa Joret (INRA) Sadasivam Kaushik (INRA) (20/06/13)

Link Table 2

Identifiant	ATOL:0001580	NT10
Name of Trait:	Feed efficiency	
Definition:	Ratio between the amount of product on the amount of feed intake.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Feed efficiency: $(BW_1-BW_0)/FI$, where BW_0 and BW_1 are initial and final live mean body weight, respectively, and FI is total dry matter intake during the same l interval.

MATERIAL (biological, reagents & instrumental):

A scale

UNIT AND RANGE OF VALUE:

Without unit

PARAMETERS TO MEASURE:

Total amount of feed intake (feed distributed – uneaten feed) ; dry matter content of feed.

Body weights of fish at the beginning and at the end of a given period of time.

BIBLIOGRAPHIC REFERENCES:

Gropp, J.M., Tacon, A.G.J., 1994. Report of the EIFAC workshop on methodology for determination of nutrient requirements in fish. *EIFAC occasional paper*, n° 29. FAO, Rome, Eichenhau, Germany, 92p.

Houlihan, D., Boujard, T., Jobling, M., 2001. *Food Intake in Fish*, Blackwell Science, UK, Glossary of terms, pp 391.

SYNONYMS EXACT:

Feed efficiency ratio

OTHER ASPECTS TO INCLUDE:

Feed: Gain ratio (Dry feed intake / Wet weight gain) is the inverse of Feed Efficiency

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Bendik Fyhn Terjesen (NOFIMA)

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Link Table 2

Identifiant	ATOL:0001121	NT11
Name of Trait:	Hepato-somatic-index	
Definition:	Ratio of weight of liver with the empty biliary vesicle to fasted body weight.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Hepatosomatic index is the ratio of liver weight to body weight Generally expressed as a percentage of whole body weight

MATERIAL (biological, reagents & instrumental):

Weight balance

UNIT AND RANGE OF VALUE:

Per cent of body weight. It varies depending to the energy reserves of fish. Indicative values are 1.5- 3.0%.

But can be sometimes very high in some species such as the Atlantic cod (>15-20%; Rosenlund *et al,.* 2004)

PARAMETERS TO MEASURE:

Body weight (g)

Liver weight (g)

BIBLIOGRAPHIC REFERENCES:

Rosenlund, G., Karlsen, O., Tveit, K., Mangor-Jensen, A., Hemre, G.I., 2004. Effect of feed composition and feeding frequency on growth, feed utilization and nutrient retention in juvenile Atlantic cod, *Gadus morhua* L. *Aquaculture Nutrition*. 10, 371-378.

SYNONYMS EXACT:

Liver index

OTHER ASPECTS TO INCLUDE:

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(20/06/13)

Link Table 2

Identifiant	ATOL:0000374	NT12
Name of Trait:	Nutrient absorption	
Definition:	Any measurable or observable characteristics related to the transport of nutrients from gastrointestinal tract to blood or lymphatic system.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Digested molecules of macro and micronutrients from the diet are absorbed from the intestine and cross the mucosa into the bloodstream and transported to other organs for storage or further chemical changes. Absorption is generally assessed by following the concentration of nutrients (amino acids, glucose, macro and micro-minerals, triglycerides, lipoproteins…) in the blood or plasma after a meal (post-prandial) of known composition and quantity. The levels of nutrient concentrations and the patterns of changes over time after a meal can vary with the quantity and quality of nutrients supplied through the diet, the size of fish and water temperature. The pattern of absorption is linked to the rate of passage (intestinal transit) of foodstuffs through the digestive tract.

In vitro techniques using portions of digestive tract and measuring the passage of nutrients from the mucosal side to the serosal side can also be used to quantify absorption. In larvae histological observations (morphometric, microscopic or cytochemical) are useful to determine the capacity of nutrient absorption across the intestinal epithelium. For instance, as a measure of lipid absorption, lipoprotein size and number of lipoprotein particles per unit area are calculated by counting and measuring these particles in a given area of the intestinal section.

MATERIAL (biological, reagents & instrumental):

UNIT AND RANGE OF VALUE:

Plasma concentrations of nutrients (µmoles / ltr)

PARAMETERS TO MEASURE:

Biochemical analyses of nutrients in the whole blood, serum or plasma using methods for each specific nutrient under consideration.

The percentage of intestinal epithelium occupied by a given nutrient by image analysis.

Size and number of nutrient particles per unit area.

BIBLIOGRAPHIC REFERENCES:

Bergot, F., 1979. Effects of dietary carbohydrates and their mode of distribution on glycaemia in rainbow trout (*Salmo gairdneri* Richardson). *Comp. Biochem. Physiol*. 64A, 543-547.

Caballero, M.J., Izquierdo, M.S., Kjørsvik, E., Montero, D., Socorro, J., Fernández A.J., Rosenlund, G., 2003. Morphological aspects of intestinal cells from gilthead seabream (*Sparus aurata*) fed diets containing different lipid sources. *Aquaculture* 225, 325-340.

Geurden, I., Kaushik, S., Corraze, G., 2008. Dietary phosphatidylcholine affects postprandial plasma levels and digestibility of lipid in common carp (*Cyprinus carpio*). *Br J Nutr.* 100, 512-517.

Krogdahl, A., Nordrum, S., Sorensen, M., Brudeseth, L., Rosjo, C., 1999. Effects of diet composition on apparent nutrient absorption along the intestinal tract and of subsequent fasting on mucosal disaccharidase activities and plasma nutrient concentration in Atlantic salmon *Salmo salar* L. *Aquacult. Nutr.* 5, 121-133.

Liu, J., Caballero, M.J., Izquierdo, M.S., El Sayed Ali, T., Hernández-Cruz, C.M., Valencia, A., Fernández-Palacios, H., 2002. Necessity of dietary lecithin and eicosapentaenoic acid for

growth, survival, stress resistance and lipoprotein formation in gilthead sea bream *Sparus aurata. Fisheries Science* 68, 1165-1172.

Santigosa, E., García-Meilán, I., Valentin, J.M., Pérez-Sánchez, J., Médale, F., Kaushik, S., Gallardo, M.A., 2011. Modifications of intestinal nutrient absorption in response to dietary fish meal replacement by plant protein sources in sea bream (Sparus aurata) and rainbow trout (*Onchorynchus mykiss*). *Aquaculture* 317, 146–154.

Walton, M.J., Wilson, R.P., 1986. Postprandial changes in plasma and liver free amino acids of rainbow trout fed complete diets containing casein. *Aquaculture* 51, 105-115.

SYNONYMS EXACT:

Postprandial pattern

OTHER ASPECTS TO INCLUDE:

Fish size, temperature

RESEARCHER CONTRIBUTION (and date of the last modification):

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(20/06/13)

Link Table 2

Identifiant	ATOL:0001597	NT13
Name of Trait:	Nutrient balance	
Definition:	Any measurable characteristic related to the balance between nutrient input and nutrient output.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Standardized chemical analysis methods (AOAC) for quantitative measurements of a given nutrient in feeds and body samples to calculate the balance between the supply and its loss from the body.

MATERIAL (biological, reagents & instrumental):

Frozen and lyophilized samples of feeds and whole body.

Laboratory equipment for quantitative analysis of nutrients (e.g Kjeldahl for N determinations, Shoxlet apparatus for gravimetric determinations of lipids and gas chromatography for fatty analysis).

UNIT AND RANGE OF VALUE:

% intake, range of values is highly variable depending on nutrient, species and physiological condition.

For fast growing fish, N retention and lipid retention are higher than 30% and 50%, respectively.

PARAMETERS TO MEASURE:

Total level of proteins and lipid and any other nutrient considered of interest.

BIBLIOGRAPHIC REFERENCES:

Diez, A., Menoyo, D., Pérez-Benavente, S., Calduch-Giner, J., Vega-Rubín de Celis, S., Obach, O., Favre-Krey, L., Boukouvala, E., Leaver, M.J., Tocker, D.R., Pérez-Sánchez, J., Krey, G., Bautista, J.M., 2007. Conjugated linoleic acid affects lipid composition, metabolism and gene expression in gilthead sea bream. *J. Nutr.* 137, 1363-1369.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

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Jaume Pérez-Sánchez (CSIC)
Marc Vandeputte (INRA)
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Link Table 2

Identifiant	ATOL:0001620	NT14
Name of Trait:	Trace mineral element requirement	
Definition:	The amount of trace mineral element required for a specific purpose: a weight gain, a milk yield, etc...	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Defining the methodology applied to the determination of requirements for each trace mineral should consider some general recommendations:

a) A practical point of view to establish nutritional requirements is preferred.

b) If optimal culture conditions for the tested species have been established, the requirements should be assayed in such conditions. If not, it should be reviewed what it is known from this species in its natural conditions.

c) As far as possible, experimental conditions similar to those used at commercial scale should be used (feed preparation technique, water quality, photoperiod or fish stocking density, among others).

d) Only one hypothesis tested per experiment is preferred.

e) At least triplicate tanks of fish should be used per dietary treatment, as one tank of fish represents a single block observation.

f) To determine quantitative requirements it is important to considerer different factors related to the species (such as age and size), related to the medium (such as temperature, salinity, culture density, to the type of culture: extensive/intensive), related to the feeding strategy and feeding regime and related to the feed (dietary energy content, nutrient availability in the ingredient source and interactions with other dietary nutrients or ingredients).

g) Complete feed ingredient descriptions should be provided, including International Feed Number (IFN), chemical composition and particle size, when reporting dietary formulations and the results of nutritional feeding trials. If a commercially prepared diet is used, the trade name and manufacturer should be indicated.

h) A standard diet should be used as a control in addition to any local diet also designed as a

control. In most cases the use of different control diets makes among different authors complicates comparison of results among them.

i) A minimum of six dietary nutrient levels or treatments is recommended for nutrient requirement studies.

j) Carcass analysis should be carried out at the beginning and at the end of the experiment.

k) An appropriate statistical analysis is always necessary.

l) Fish should be fed until “apparent satiation” instead of restricted feeding rates.

Specific parameters related to each mineral deficiency should be determined. Mineral concentration in tissues is determined following ash digestion in 10% nitric acid solution using Ion chromatography.

MATERIAL (biological, reagents & instrumental):

UNIT AND RANGE OF VALUE:

PARAMETERS TO MEASURE:

The most important parameters to be determined are:

- Calcium (Growth, carcass concentration, Nutrient and mineral ADC).
- Copper (Concentration in liver, Concentration in whole body, Cu/zn SOD gene expression, Toxicity).
- Iodine (Bacterial kidney disease infections).
- Iron (Haematological values, hepatic and whole body iron concentration, Concentration in whole body and liver, Deficiency, Toxicity).
- Magnesium (Weight gain, serum Mg and muscle Mg, Weight gain, whole body Mg, Concentration in whole body, serum and bone, Deficiency).
- Manganese (Concentration in whole body, vertebrae and heart, Concentration in whole body, Cu-Zn superoxide dismutase activity, Mn superoxide dismutase activity, Deficiency, Concentration whole body, SOD activity, Mn SOD gene expression) .
- Selenium (Concentration in liver, Concentration in muscle, Weight gain, GSH-Px activity, Catalase activity, SOD activity, Liver malondialdehyde concentration, Gluthathione peroxidase activity, Deficiency, Toxicity).
- Zinc (Concentration in whole body, vertebrae and plasma, Concentration in whole body and serum, Cu/zn SOD gene expression, Deficiency).

BIBLIOGRAPHIC REFERENCES:

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

RESEARCHER CONTRIBUTION (and date of the last modification):

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Link Table 2

Identifiant	ATOL:0001527	NT15
Name of Trait:	Voluntary feed intake	
Definition:	Any measurable or observable characteristics related to the amount of feed consumed by an animal having a continuous unrestricted access to feed and diet (ad libitum) over a given period (generally one day).	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

There are several ways for measuring feed intake on a group of fish reared in the same tank. They are all based on the same principle which is measuring the difference between the amount of feed delivered and the amount of waste feed collected. It exist many methods to do this properly, depending of the type of feed you are using. Some methods are described below:

- Voluntary feed intake (g of dry matter/day) will be determined by dividing total feed provided to the tank (in g of dry matter), corrected for uneaten pellets, by the number of fish per tank (Saravanan *et al*., 2012).

To measure feed intake of individual fish, X ray methods can be used as describe by Jobling (2001).

MATERIAL (biological, reagents & instrumental):

UNIT AND RANGE OF VALUE:

g/day

PARAMETERS TO MEASURE:

Feed delivered

Waste feed

Body weight

BIBLIOGRAPHIC REFERENCES:

Helland, S. J., Grisdale Helland, B., Nerland, S., 1996. A simple method for the measurement of daily feed intake of groups of fish in tanks. *Aquaculture* 139,157-163.

Jobling, M., 2001. Techniques for measuring feed intake. Pg 49 in Food Intake in Fish. D. Coves, B. Damsgard, H. R. Kristiansen, J. Koskela, T. E. Petursdottir, S. Kadri, and 0. Gudmundsson (Eds). Blackwell Sciences, Oxford.

Saravanan, S., Geurden, I., Figueiredo-Silva, A.C., Kaushik, S.J., Haidar, M.N., Verreth, J.A.J., Schrama, J.W., 2012. Control of voluntary feed intake in fish: a role for dietary oxygen demand in Nile tilapia (*Oreochromis niloticus*) fed diets with different macronutrient profiles. *British Journal of Nutrition* 108, 1519-1529.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

- Helland *et al*. (1996). These two systems are using electric feeders controlled by the fish themselves (self-feeder) or manually controlled, deep cylindro-conic or cylindro-spheric tanks, fecal trap and sinking pellets. The electric feeders have to by adjust to minimize the amount of waste pellet without imposing any restriction on the food access. Deep tank maximized the time when the pellet can be eaten by the fish before reaching the bottom of the tank. The tank shape and the tangential inlet water flow allow a good concentration of the uneaten pellet on the middle

of the tank where they are carried in the water flow along an outlet pipe. Pellets are trapped using fecal trap (sediment trap) link to the bottom of the tank or link to the outlet pipe. These devices allow a good capture of the pellet in doing a highest suction than in the outlet pipe. The uneaten pellets have to be count precisely. For this the fecal trap are empty and the uneaten pellets are separated using a bucket where an open window close by a mesh have been done on a side of it. By this way the pellet are concentrated in the bucket and can be easily counted.

The two systems only varied on the type of feeder use (self or manually control). The choice is determined by the water stability of the pellet when is withstand the mechanical stress caused by the flow water over them and the procedure used for separation of the waste feed and feces.

With extruded feeds with high stability in water, self-feeder can be used. The fish will eat following their own rhythm. The uneaten pellet will be harvest and count twice a day.

With pressed pellet or experimental pellet with bad water stability, the meal is given using electric feeders manually operated. The operator activates an electric switch placed outside of the tank. The operator and the switch must be out of the fish view. Any impulses on the switch initiate a release of feed. All the tanks are fed slowly one impulse by one. The meal stops when the first uneaten pellet is caught by the fecal trap. If the rhythm of feeding is too high meal will stop too early so the fish will not have a full meal. The fecal trap is empty and the waste pellet count, when the entire uneaten pellets on the bottom of the tank have been caught by the fecal trap.

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Link Table 2

Identifiant	ATOL:0001775	RP1
Name of Trait:	Fertilization rate	
Definition:	Any measurable or observable characteristic related to the ability of oocyte to be fertilized after natural or artificial insemination: number of fertilized eggs / number of total oocytes.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Depending on the species and the applied water temperature after fertilization process, when the embryo reached minimally the stage with clearly visible notochord, up to eye stage can be calculated fertilization rate. There is a common formula in the hatchery practice which can be used for the calculation described by Muir and Robert (1985):

Fertilization rate (%) = nº. of fertilized eggs / total nº. of eggs x 100.

However, the assessment of fertilization rate at first development stages, when species allow it (for example 4cell stage in seabass or turbot), can offer the possibility to sharply analyse precocious embryo mortality in egg quality studies.

It is recommended to sample triplicates to assess at least 100 eggs per replicate in order to validate the reproducibility and precision of the counting method. This is of course different from

fertilisation variability which will be assessed using fertilization replicates.

MATERIAL (biological, reagents & instrumental):

About 200-300 eggs from each fertilized egg batches are needed for the calculations. For the counting of eggs a stereomicroscope (binocular with 50x magnification) is required for many species with culture dishes and needles.

UNIT AND RANGE OF VALUE:

Proportion (%); Range: 0 – 100.

PARAMETERS TO MEASURE:

Total number of eggs and number of developing embryos (fertilized eggs) within the sample.

BIBLIOGRAPHIC REFERENCES:

Muir, F.J., Robert, J.R., 1985. *Recent advances in aquaculture*. Croon Helm, London & Sydney; Westview Press Boulder, Colorado. p. 2.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

In most of marine fish, fertilized eggs are floating while unfertilized or dead eggs sink. Attention must be paid to avoid selective collection of samples.

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Link Table 2

Identifiant	ATOL:0001531	RP2
Name of Trait:	Live offspring number	
Definition:	Any measurable characteristic related to the number of live offspring at the time of parturition or hatched.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

For species that spontaneously produce fertilized eggs in captivity:

- Eggs produced naturally by female broodstock are collected every day during the experimental period.
- Eggs are collected from each tank and stocked in 5-litre beakers. From this pool of eggs, 5 randomized 5-ml samples are removed.

For species that require egg collection (stripping) after spontaneous or hormone-induced ovulation followed by in vitro fertilization:

- unfertilized eggs are collected by stripping at the appropriate time depending on the species to avoid excessive post-ovulatory ageing (overipening)

- sperm is collected from individual spermiating males
- in vitro fertilization is performed under standard conditions to avoid any negative effect of the procedure

The eggs are counted and observed under binocular microscope to calculate:

-Total amount of eggs produced per kg of female per stripping.

- Percentage of fertilized eggs determined as the percentage of developing embryos (i.e. reaching 8-cell stage and beyond) after direct observation in the case of transparent embryos (e.g. cod. Kjkørsvik *et al.,* 1990) or after appropriate fixation and staining (e.g. rainbow trout).

- Early embryo development rate determined as the percentage of morphologically normal eggs at the morula stage and described as transparent, perfectly spherical with clear, symmetrical early cleavages (Kjkørsvik *et al*., 1990).

- Percentage of abnormal eggs including eggs with more than one oil globule, when relevant (i.e. depending on the species).

- Percentage of non-fertilized eggs, determined by the morphological characteristics of the eggs (Kjkørsvik *et al*., 1990) or after appropriate fixation and staining.

-Egg and oil droplet diameter of 150 randomized samples from each tank are measured by a profile projector, when relevant (i.e. depending on the species).

After that, 3-4 samples of 100-500 eggs from each tank or individual female are carefully monitored throughout development as follows (Aegerter *et al,.* 2005; Bonnet *et al.,* 2007):

- Eyeing rate (% of embryos reaching eyed stage)
- Hatching rate
- % of embryos reaching yolk sac resorbtion (YSR) or autonomous feeding
- % of malformed embryos at YSR or % of malformed larvae
- Types of larval malformation observed at YSR or larval stage based on image analysis of at least 50-100 embryos/larvae (Bonnet *et al.,* 2007).
- Total length of 45 larvae from each tank are measured by a profile projector (V-12A, Nikon Co., Tokyo, Japan).

MATERIAL (biological, reagents & instrumental):

- Eggs and larvae

- Egg collector

- Egg incubation device for batches of 100-500 eggs

- Camera

- Binocular microscope

- Profile projector

- Water container

UNIT AND RANGE OF VALUE:

According to each parameter

PARAMETERS TO MEASURE: (and UNIT)

- Total amount of eggs produced per kg of female (Nº eggs/kg of female)

- Egg fertilization rate (%)

- Percentage of abnormal eggs (%)

- Percentage of non-fertilized eggs (%)

- Egg and oil droplet diameter of 150 randomized samples (mm)

- Eyeing rate (%)

- Hatching rate (%)
- Larval survival (%) at larval stage or yolk sac resorption (YSR)
- Percentage of abnormal larvae (%), at larval stage or YSR.
- Larva total length (mm).

BIBLIOGRAPHIC REFERENCES:

Aegerter, S., Jalabert, B., Bobe, J., 2005. Large scale real-time PCR analysis of mRNA abundance in rainbow trout eggs in relationship with egg quality and post-ovulatory ageing. *Molecular Reproduction and Development* 72, pp.377–385. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16075464.

Bonnet, E., Fostier, A., Bobe, J., 2007. Characterization of rainbow trout egg quality: a case study using four different breeding protocols, with emphasis on the incidence of embryonic malformations. *Theriogenology* 67, pp.786–794. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17118435.

Fernández-Palacios, H. 2005. Efecto de determinados nutrientes en la composición de dietas para reproductores de dorada (*Sparus aurata*) sobre la calidad de sus puestas. *Tesis Doctoral*. Universidad de Las Palmas de Gran Canaria. 315 pp.

Fernández-Palacios, H., Izquierdo, M. S., Robaina, L., Valencia, A., Salhi, M., J. Vergara, 1995. Effect of n-3 HUFA levels in broodstock diet on egg quality of gilthead seabream (*Sparus aurata*). *Aquaculture* 132, 325-337.

Kjkørsvik, E., Mangor-Jensen, A., Holmefjord, L., 1990. Egg quality in fishes. *Advances in Marine Biology* 26, 71-113.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

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Link Table 2

Identifiant	ATOL:0001723	RP3
Name of Trait:	Oocyte yield	
Definition:	Number of oocytes produced in relation to body weight over a laying period.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

For species whose oocytes are released naturally in the fish tanks, oocytes are usually collected in a spawning container and have to be removed with a net. When oocytes are collected by hand-stripping on anaesthetized females, oocytes are collected in a container and, after draining, weight can be determined directly. After determination of the total weight (TW) or total volume (TV) of the spawn, a subsample on volume (SV) or weight-basis (SW) is taken and the number of oocytes

(SNO) in the sample counted. The total number of oocytes (TNO) corresponds to: SNO * (TW (or TV)/SW (or SV). The oocytes yield corresponds to: TNO/BW (BW is the body weight of the female).

MATERIAL (biological, reagents & instrumental):

Tank with spawning container.

Dish for collection of eggs.

A scale (a simple 5% accuracy is acceptable on volume or weight).

UNIT AND RANGE OF VALUE:

Number/kg body weight of the female

Total number/female (it is important information for farmers).

PARAMETERS TO MEASURE:

Number of oocytes

BIBLIOGRAPHIC REFERENCES:

SYNONYMS EXACT:

Eggs yield

OTHER ASPECTS TO INCLUDE:

In aquaculture, oocyte yield can represent the capacity production of a broodstock among a whole reproductive season; non spawning females of the stock may decrease the mean individual oocyte yield.

Individual oocyte yield along the season can be obtained by the isolation of females or couples and obtention of oocytes by stripping or spontaneous spawning. In that case, the assessment of yield may be biased by the long term retention in spawning facility.

A lot of species are batch spawners i.e. they release oocytes as consecutive discrete clutches spawned at both specific and individual rhythms. In fisheries, the ovulated oocytes found in the ovaries are assessed as batch fecundity.

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Link Table 2

Identifiant	ATOL:0000393	RP4
Name of Trait:	Oogenesis	
Definition:	Characteristic related to the formation of female germ cells.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

a) Total number of oocytes per female during spawning season

b) Plasma hormonelevels determination: 17bestradiol (E2), Fsh, Lh, Vitellogenin (Vtg).

c) Number of oocytes at different stages of follicular development by routine histological techniques.

d) Gonadosomatic index.

MATERIAL (biological, reagents & instrumental):

Oocytes, plasma, and tissue.

UNIT AND RANGE OF VALUE:

a) Number of oocytes per kg female and spawning.

b) ng/ml of plasma.

c) Number of oocytes at different stages of follicular development.

d) Gonad size expressed as percentage of the body weight.

All parameters with many interspecific range of value.

PARAMETERS TO MEASURE: (and UNIT)

Number of oocytes per female, ng/ml of E2 and Fsh, oocytes at different stages.

BIBLIOGRAPHIC REFERENCES:

García-López, A., Sánchez-Amaya, M.I., Tyler, Ch., Francisco Prat, F., 2011. Mechanisms of oocyte development in European sea bass (*Dicentrarchus labrax* L.): investigations via application of unilateral ovariectomy. *Reproduction* 142, 243–253.

Gómez, J.M., Weil,, C., Ollitrault M., Le Bail, P.Y., Breton, B., Le Gac, F., 1999. Growth hormone (GH) and gonadotropin subunit gene expression and pituitary and plasma changes during spermatogenesis and oogenesis in rainbow trout (*Oncorhynchus mykiss*). *General and Comparative Endocrinology* 113, 413-428.

Lubzens, E., Young, G., Bobe, J., Cerdà, J., 2010. Oogenesis in teleosts: How fish eggs are formed. *General and Comparative Endocrinology* 165, 367-389.

Martins, Y.S., Pereira Arantes, F., Sato, Y., Enemir dos Santos, J., Rizzo, E., Bazzoli, N., 2012. Comparative analysis of gonadal morphology in six fish species of the Incertae Sedis genera in Characidae of occurrence in the Sao Francisco River Basin, Brazil. *Acta Zoologica* 93, 48–56.

Miura, C.H., Higashino, T., Miura, T., 2007. A Progestin and an Estrogen Regulate Early Stages of Oogenesis in Fish. *Biology of Reproduction* 77, 822–828.Patiño, R., Thomas, P., Yoshizaki, G., 2003. Ovarian follicle maturation and ovulation: An integrated perspective. *Fish Physiology and Biochemistry* 28, 305-308.

Taranger, G.L., Carrillo, M., Schulz, R.W., Fontaine, P., Zanuy, S., Felip, A. Weltzien, F.A., Dufour, S., Karlsen, O., Norberg, B., Andersson, E., Hansen, T., 2010. Control of puberty in farmed fish. *General and Comparative Endocrinology* 165, 483–515.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

The method of cannulation (ovarian biopsy) is also a routine non-invasive method to estimate the stage of gonad maturation of the females, by quantifying the amount of oocytes at each stage (diameter and morphology) on a biopsy sample. Also, for some species, it is also very useful the

estimation of the stage of maturation by evaluating external signs, such as the degree of abdominal swelling in some flatfishes.
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Link Table 2

Identifiant	ATOL:0000327	RP5
Name of Trait:	Ovarian follicle morphology	
Definition:	Any measurable or observable characteristic related to the shape, structure, or color of the ovarian structure containing an oocyte.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*
MEASUREMENT METHOD: Score ovarian follicle development stage on histology section
MATERIAL (biological, reagents & instrumental): Ovarian biopsy or part of the ovary after dissection Histological processing up to staining Microscope
UNIT AND RANGE OF VALUE: % of follicles of each size class / stage based on species specific or general (Wallace and Selman, 1990) histological tables of follicle development.
PARAMETERS TO MEASURE: % of follicles of each stage Size of the oocyte Presence of follicular layers Intracellular characteristics of the oocyte
BIBLIOGRAPHIC REFERENCES: Tyler, C.R., Sumpter, J.P., 1996. Oocyte growth and development in teleosts. *Reviews in Fish Biology and Fisheries* 6, 287–318. Wallace, R.A., Selman, K., 1990. Ultrastructural aspects of oogenesis and oocyte growth in fish and amphibians. *J Electron Microsc Tech.* 16, 175-201.
SYNONYMS EXACT:
OTHER ASPECTS TO INCLUDE:

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Link Table 2

Identifiant	ATOL:0001676	RP6
Name of Trait:	Ovary weight	
Definition:	Any measurable characteristic related to the weight of the female reproductive gland containing the germ cells.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Fish are caught in their rearing tank with a dip net and placed in anesthetic bath with an overdose. Fish must be killed before being handled. The fish is placed on a container and its abdomen is open with a knife or scissors from the anus to the head. Ovaries are carefully removed from the abdomen, taking care to remove the whole ovary by cutting from the top around the esophagus to the anus.

To weigh ovary: A dry container is placed on an appropriate scale and tared. The ovary, after wiping, is placed on the container. The value of the measurement is recorded, and when is possible, automatically recorded with a data transfer system.

Weighing should if possible be performed in a closed room to avoid influence of air movements.

MATERIAL (biological, reagents & instrumental):

A scale with adequate precision (usually around 1/100 to 1/1000 of the average weight to be measured).

Scissors and forceps are required for dissection.

UNIT AND RANGE OF VALUE:

Gram (g)

PARAMETERS TO MEASURE:

Fish ovary weight.

BIBLIOGRAPHIC REFERENCES:

SYNONYMS EXACT:

Female gonad weight, female reproductive gland.

OTHER ASPECTS TO INCLUDE:

The scale must be checked before each measurement series, and regularly calibrated by an external service provider.

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Link Table 2

Identifiant	ATOL:0000431	RP7
Name of Trait:	Puberty	
Definition:	Any measurable characteristic related to the age at which animal develops the ability to produce live offspring.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

1) Letal method during the reproductive season:
   - Dry and weigh the whole-body fish.
   - Remove and weigh the gonads.
   - Gonadosomatic index (GSI, weight of gonads dissected related to the total fish weight) will be obtained as percentage of the whole-body fish:
   - GSI=100 x (gonad weight/body weight)

2) Alternative method (invasive but not letal): availability of sperm under abdomen pressure in males and ovarian biopsy followed by oocyte observation in females.

MATERIAL (biological, reagents & instrumental):

Dissection material

Weight (accuracy of 0.001 unit)

UNIT AND RANGE OF VALUE:

Weight unit (g)

GSI unit (%) For salmon range between 0.001 and 20%.

For salmon a GSI above 0.05 (male) and 0.2 (female)

In the case of ovarian biopsy, oocyte diameter, cytoplasm aspect (transparency of non vitellogenic vs opacity of vitellogenic oocytes) can both indicate the start of genital activity even before the reproductive season (variable among the species).

PARAMETERS TO MEASURE:

Gonad weight

Gonadosomatic index (GSI)

BIBLIOGRAPHIC REFERENCES:

Taranger, G.L., Carrillo, M., Schulz, R.W., Fontaine, P., Zanuy, S., Felip, A., Weltzien, F.A., Dufour, S., Karlsen, O., Norberg, B., Andersson, E., Hansen, T., 2010. Control of puberty in farmed fish. *General and Comparative Endocrinology* 165, 483–515.

Tyler, C.R., Sumpter, J.P., 1996. Oocyte growth and development in teleosts. *Reviews in Fish*

Biology and Fisheries 6, 287–318.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

This measurement (GSI) indicates if the individual has entered puberty.

For salmon a GSI above 0.05 (male) and 0.2 (female), for the first time of its life, will normally indicate that the individual has entered puberty. In fact, puberty occurs sooner when gametogenic process starts i.e. cortical alveoli stage in females and spermatocyte production in males.

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Link Table 2

Identifiant	ATOL:0001005	RP8
Name of Trait:	Sperm mobility	
Definition:	Any characteristic related to the proportion of mobile spermatozoa in a given ejaculate.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

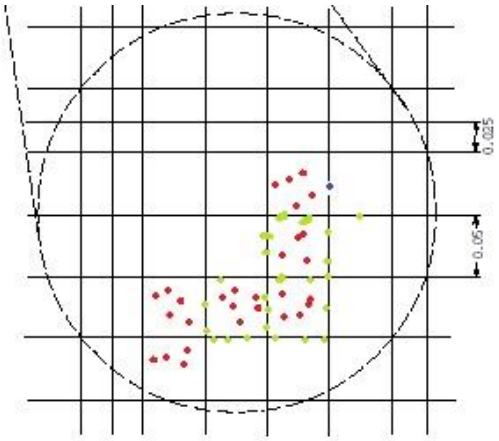
Sperm Mobility:

Once the sperm is collected with a syringe via abdominal massage and saved at 4°C, dilute sperm with an immobilizing diluent. Take 1 µl of sperm with a micropipette of 20 (can be with the cut tip) and place it into 49 µl of activating extender (sea or fresh water previously set, and depending on the species). Mix both with the tip of micropipet for sperm activation. Immediately under a microscope with objective 40x, estimate sperm mobility in percentage (0% to 100%) for movement. The duration of sperm mobility is obtained by measuring how long it takes from sperm motility activation with sea/fresh water until the end of sperm movement. Mobility is a sperm that moves from one place to another, a vibrating sperm is not mobile. In case of a too high density of sperm, initial milt should be pre-diluted in a diluent (see RP9).

Sperm Density:

Take 10 µl of sperm with a micropipette of 20, and dilute it in a tube with 190 µl of formalin solution (1:20). Following this dilution, take another 10 µl from the first dilution, and dilute it again in 190 µl of formalin. From this second dilution fill the Thoma chamber, let sediment for > 5 min and count cells (squares) up and down. It is recommended to count sperm on 16 to 25 small squares (the ones with 0.05 mm) in 4 different areas of the Thoma chamber, in order to take into account artifactual discrepancies over the surface. Then calculate the mean value of the 4 counts. Count at least 25 cells per 16 or 25 small squares for counting accuracy. If the sperm is too concentrated, add another dilution step. If the sperm is too diluted, remove one dilution step or change the

dilution ratio. From this, calculate the number of sperm per ml. Divide the mean cell count by the number of small squares counted (16 or 25). Multiply this number by 4.10^6 to obtain the number of cells per mL. Do not forget to go back to the initial sperm concentration by multiplying the concentration by the dilution ratios (x20x20 in this example).



Thoma chamber, red ones are acceptable for counting. For green ones, always count the ones on the top side and the left side of a given square, in order to avoid counting them twice in the adjacent square because they are on the line. Count reds and greens.

MATERIAL (biological, reagents & instrumental):

Syringe

Micropipette of 20 µl

400x microscope

Formalin

Thoma chamber

UNIT AND RANGE OF VALUE:

Sperm mobility (%)

Sperm density (spermatozoas / mL (or per cm^3)

PARAMETERS TO MEASURE:

Sperm mobility (the movement of the sperm)

Sperm concentration

BIBLIOGRAPHIC REFERENCES:

Mylonas, C.C., Kyriakou, Y., Sigelaki, I., Georgiou, G., Stephanou, D., Divanach, P., 2004. Reproductive biology of the shi drum (*Umbrina cirrosa*) in captivity and induction of spawning using GnRHa. *Bamidgeh* 56, 75-92.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

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Link Table 2

Identifiant	ATOL:0000427	RP9
Name of Trait:	Sperm motility	
Definition:	Quality of the movement and swimming of spermatozoa in a given ejaculate, scored from 0 to 5.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Freshly obtained sperm from a male fish is diluted with specific immobilizing diluents and then sperm is activated with sea water or fresh water (depending on the fish species), for sperm motility measurement. The important feature of such diluents is that they should prevent motility, therefore the osmolarity, has to be established for each species, and has to be as similar as possible to that of the seminal fluid. pH should range between 6.8 and 8.9. Diluted sperm is drawn into a Neubauer chamber and then, motility intensity and percentage of motile spermatozoa is estimated under a dark field light microscope, immediately after sperm dilution, according to a scale shown below.

Alternatively to this subjective method, activated sperm is drawn into a Neubauer chamber, and sperm motility is recorded by a video camera attached to the microscope. The resulting images are analyzed with specific software named CASA (Computer-assisted sperm analysis).

MATERIAL (biological, reagents & instrumental):

Freshly obtained sperm, fresh or sea water, immobilizing diluent with the appropriate osmolarity for each fish species, microscope with x 400 magnification, Neubauer chamber. For CASA: also digital camera, software for computerized image analysis, PC. Any agent such as Pluronic acid to avoid sperm sticking to glass slide and sperm clumping.

UNIT AND RANGE OF VALUE:

0: 100% immobile

I : < 30% motile with slight movement, 70% immobile

II: 20-50% move vigorously, 50-80% immobile

III: 50-70% move vigorously, 30-50% have slight shaking

IV: 70-80% move quickly, 20-30% have slower speed

V: > 80% move quickly, impossible to fix the view on any spermatozoa.

CASA parameters: distance per second and derivate (see Parameters to measure section)

PARAMETERS TO MEASURE:

Percentage of motile sperm and total duration of the motility period.

Additionally sperm volume and concentration are also determined to give a better measurement of sperm quality.

CASA parameters:
- *Percent motility*: Percent of sperm moving in a manner fitting motility determination parameters

- *Velocity curvilinear (VCL)*: Point to point velocity (total distance traveled) per second
- *Velocity average path (VAP)*: Point to point velocity on a path constructed using a roaming average
- *Velocity straight line (VSL)*: Velocity measured using the first point and the average path and the point reached that is furthest from this origin during the measured time period
- *Linearity (LIN): VSL/VAP*, describes path curvature
- *Wobble (WOB): VAP/VCL*, describes side to side movement of the sperm head
- *Progression (PROG)*: The average distance of the sperm from its origin on the average path during all frames analyzed
- *Beat cross frequency (BCF)*: This value is determined in the plugin by detecting the frequency at which VCL crosses VAP

BIBLIOGRAPHIC REFERENCES:

Chambeyron, F., Zohar, J., 1990. A diluent for sperm cryopreservation of gilthead seabream, *Sparus aurata*. *Aquaculture* 90, 345-352.

Billard, R., Cosson, J., Crim, L.W., Suquet, M., 1995. Spermphysiology and quality. In: Bromage, N.R., Roberts, R.J. (Eds). *Brood stockmanagement and egg and larval quality*. Blackwell Science, p. 25-52.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

RESEARCHER CONTRIBUTION (and date of the last modification):

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(20/06/13)

Link Table 2

Identifiant	ATOL:0000342	RP10
Name of Trait:	Spermatogenesis	
Definition:	Any characteristic related to the formation of the male gametes.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Spermatogenesis (formation of male gametes) can be measured/characterized by: the percentage of different germ cell stages.

a) Weighing. Gonad Somatic Index (GSI), the proportion of testis weight in relation to total body weight times 100.

b) - Germ Cell stages (see Schulz *et al.,* 2010):
- type A undifferentiated spermatogonia (stem cell)
- type A undifferentiated spermatogonia
- type A differentiated spermatogonia (A_{diff})
- spermatogonia type B (early–late)

- leptotenic/zygotenic primary spermatocytes (L/Z)
- pachytenic primary spermatocytes (P)
- diplotenic spermatocytes/metaphase I (D/MI)
- secondary spermatocytes/metaphase II (S/MII)
- early spermatids (E1)
- intermediate spermatids (E2)
- final spermatids (E3)
- spermatozoa (SZ)

c) Radio immunoassays, Enzyme-linked immunosorbent assays (ELISA's).
Determination of pituitary hormones, growth factors and steroid hormones (see Schulz *et al.,* 2010).

MATERIAL (biological, reagents & instrumental):

- Histology (Equipment: e.g. Bouin's fixation fluid, tissue dehydration steps, paraffin embedding equipment, microtome, staining procedures, stereo microscope, image analysis tools)
- Electron microscope
- Radio Immune Assay (RIA), ELISA test kit

UNIT AND RANGE OF VALUE:

Gonad Somatic Index (GSI): %

PARAMETERS TO MEASURE:

Fish age, fish weight, fish length, period of the year the male gonad is sampled, external appearance of the male gonad morphological aspects, environmental culture conditions, GSI.

BIBLIOGRAPHIC REFERENCES:

Schulz, R.W., de França, L.R., Lareyre, J.J., LeGac, F., Chiarini-Garcia, H., Nobrega, R.H., Miura, T., 2010. Spermatogenesis in fish. *General and Comparative endocrinology* 165, 390-411.

SYNONYMS EXACT:

OTHER ASPECTS TO INCLUDE:

RESEARCHER CONTRIBUTION (and date of the last modification):

Ep Eding (WU) (18/01/13)
Léa Joret (INRA) (28/02/13)
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(20/06/13)

Link Table 2

Identifiant	ATOL:0001679	RP11
Name of Trait:	Testes weight	
Definition:	Any measurable characteristic related to the weight of the male reproductive glands.	

SIMILAR TO: *If it's appropriate, in connection with other Identifiant number*

MEASUREMENT METHOD:

Fish are caught in their rearing tank with a dip net and placed in anesthetic bath with an overdose. Fish must be killed before being handled. The fish is placed on a container and its abdomen is open with a knife or scissors from the anus to the head. Testes are carefully removed from the abdomen, taking care to remove the whole ovary by cutting from the top around the esophagus to the anus. Testes are weighed on a scale. The accuracy of the scale can vary dependent on what the weight of the testes is used for, but 0.1 % of the minimum weight in the range that are measured will normally be appropriate. The value of the measurement is recorded, and when is possible, automatically recorded with a data transfer system. Weighing should if possible be performed in a closed room to avoid influence of air movements.
MATERIAL (biological, reagents & instrumental): Weight (accuracy of 0.1 % of the minimum weight).
UNIT AND RANGE OF VALUE: Gram (g)
PARAMETERS TO MEASURE: Testes weight.
BIBLIOGRAPHIC REFERENCES:
SYNONYMS EXACT:
OTHER ASPECTS TO INCLUDE:
RESEARCHER CONTRIBUTION (and date of the last modification): Tom Hansen (IMR) (18/01/13) Léa Joret (INRA) (28/02/13) Evaristo Mañanós (CSIC) Alain Vergnet (IFREMER) Robbert Blonk (WU) (20/06/13)

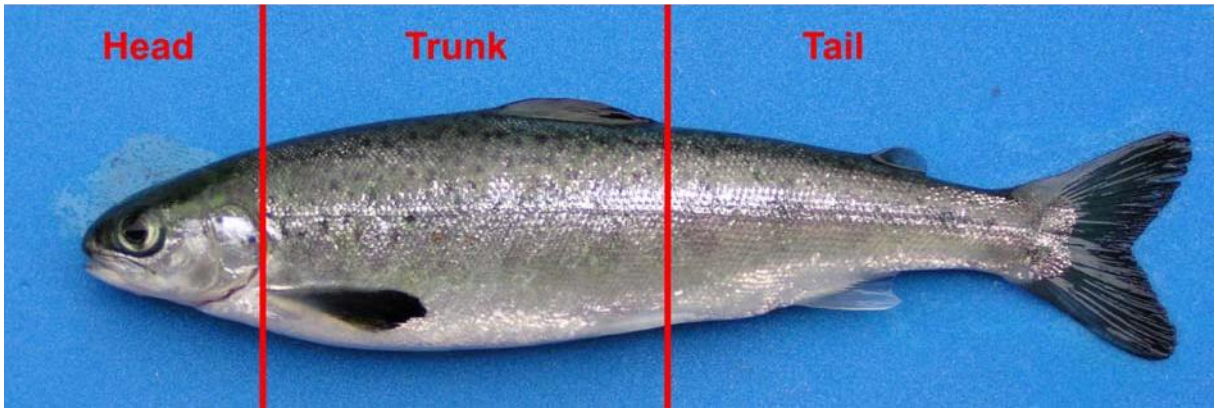
Link Table 2

ANNEX-2

VISUAL EVALUATION OF SKELETAL DEFORMITIES IN FARMED SALMON & SEABASS

Visual evaluation of skeletal deformities in farmed salmon

The body is divided in three main regions; head, trunk, and tail.



Scoliosis (S)

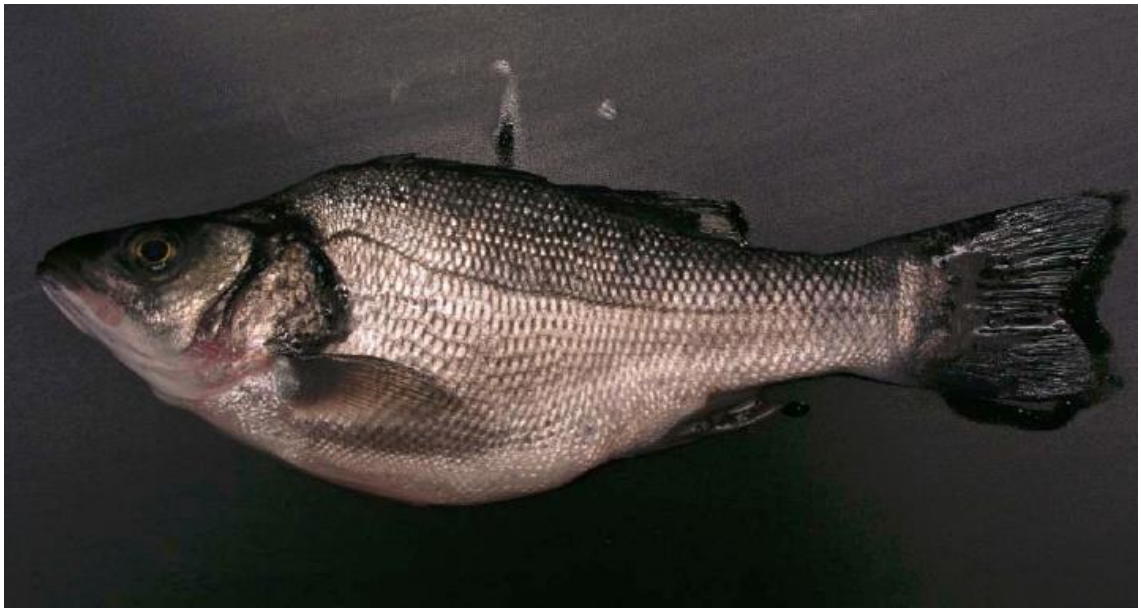
A curvature in the vertebral column, both trunk and tail. Visible in a dorsal – ventral view.

Lordosis (L)

A curvature in the tail region of the vertebral column. Visible in a lateral view.

Kyphosis (K)

A curvature in the trunk region of the vertebral column. Visible in a lateral view.



Short trunk (STR)

A shortening in the trunk region of the vertebral column.

Short tail (STA)

A shortening in the tail region of the vertebral column.

Short operculum (SO)

A shortening of the operculum.

Deformed lower jaw (LJ)

A curvature of the lower jaw.

Deformed upper jaw (UJ)

A shortening of the upper jaw.