



### Deliverable Report

<b>Deliverable No:</b>	D9.3	<b>Delivery Month:</b>	60
<b>Deliverable Title</b>	Performance of grow-out diets for greater amberjack developed in order to maximize growth potential		
<b>WP No:</b>	9	<b>WP Lead beneficiary:</b>	P2. FCPCT
<b>WP Title:</b>	Nutrition-greater amberjack		
<b>Task No:</b>	9.3	<b>Task Lead beneficiary:</b>	P28. CANEXMAR
<b>Task Title:</b>	Development of diets for grow-out of amberjack to maximize growth		
<b>Other beneficiaries:</b>	P2. FCPCT		
<b>Status:</b>	Delivered	<b>Expected month:</b>	58

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**Other Scientists participating:** Robaina, Lidia (P2. FCPCT)

**Objective:** Performance of grow-out diets for greater amberjack developed in order to maximize growth potential:

**Description:** A report with the effect of the improved diet on the growth-out of greater amberjack will be provided. Description of the effects of the improved diet including a) detailed information about the on-growing rearing conditions, b) growth performance, c) survival d) feed efficiency and e) significance for the industry.



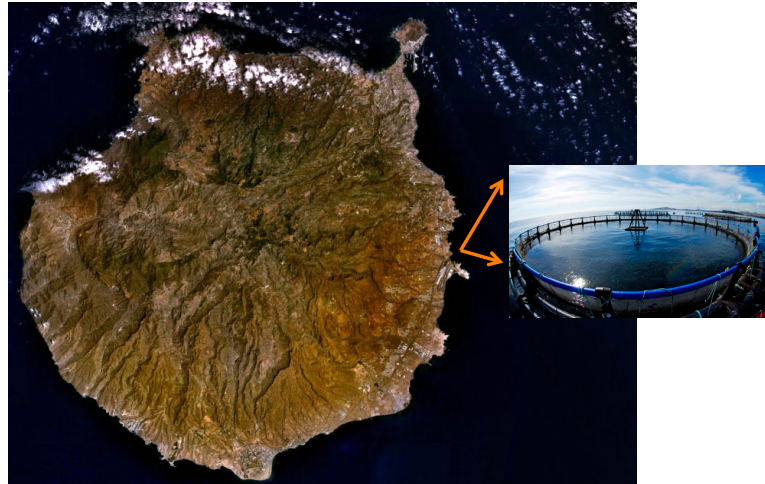
### Introduction

The results from previous studies included in deliverable D9.2 by HCMR indicated that the dietary lysine requirements based on the Broken-line model, which can support maximum weight gain of greater amberjack juveniles fed on a diet based mainly on plant ingredients, containing 45% protein, 18% lipid and 25% fish meal inclusion was 2.11% of diet.



### Material and methods

Based on the lysine content in the described formulae for greater amberjack in D9.2, a study was conducted in sea cages belonging to CANEXMAR and sited in the east coast of Gran Canaria Island (Canary Islands, Spain) (Fig. 9.3.1).



**Figure 9.3.1.** Site of CANEXMAR farm, at east coast of Gran Canaria Island (Canary Islands, Spain)

Greater amberjack juveniles were produced by P2. FCPCT in their aquaculture facilities. Two different amberjack juvenile populations (around 5000 individuals each) were placed in CANEXMAR facilities (Fig. 9.3.2). A total of 5,000 juvenile amberjack of 100 g body weight were produced with the standardized methodology for greater amberjack in 2015 and were placed in an experimental cage of 5x5 m in a harbour area, in preparation for movement to the CANEXMAR cages. The same was done in 2016.



**Figure 9.3.2.** Transport of the amberjack juveniles from PCTM facilities to the experimental cage in CANEXMAR



These two populations (2015 & 2016) even when fish were very well acclimated (**Fig. 9.3.3**), failed to reach the CANEXMAR farm site for different reasons. The first batch of fish did not survive the transport to the offshore cages due to operational reasons and most of the fish died. The second batch of fish was lost due to cage breakdown during a big storm and hard weather. After those incidences, CANEXMAR needed to make a new design and improvement in the fish transport system and cage construction for the sea. Then, a new batch of greater amberjack juveniles was produced at FCPCT at the end of 2016 and CANEXMAR focused in improvements of transport and viability in sea cages with this batch.



**Figure 9.3.3.** Different batches of greater amberjack juveniles acclimated in 5x5 cages waiting to be carried into CANEXMAR farm.

### **Fish transport and acclimation**

Following the fish transport protocol for similar species to the greater amberjack, the next points were followed carefully:

- ✓ The acclimatization of the fishes to face the transport (**Fig. 9.3.4**)
- ✓ Equalize the water conditions between the exit tanks and the transport tanks.
- ✓ Controlling the pH level and ammonium in the transport tanks during its loading.
- ✓ Controlling the oxygen in the tanks before and during the fish loading to avoid important oscillations in its level.
- ✓ To double the transport capacity with more containers and minimize the fish density as possible as can.
- ✓ The fish were deposited in containers covered with a cap to avoid the stress involved in the transport (**Fig. 9.3.5**).
- ✓ The unloading was from a tank to another tank. In this process we renewed the water inside two hours before the unloading, controlling the seawater temperature and equalizing the oxygen level.



**Figure 9.3.4.** Acclimation procedures and transport to off-shore sea cages



### Cage construction and farm nets

After the problems with the materials used, CANEXMAR decided to use tougher and higher quality materials, which are not usually used in a standard fish farm. The fish of the third batch were relocated from the cages that CANEXMAR has in the farm to a more secure zone, protecting the cages and nets of confront brushes, abrasion, vandalism, and any other contingency that the cage could suffer in the zone.

### Daily feeding and fish studying of the feed and the environment conditions

The starting months of feeding were very hard due to a strong winter (more stormy than usual in the Canaries), being very difficult to see the fishes in the cage. The sea conditions generated additional stress and the feeding was very difficult. A feeding regime of three times per day to solve the situation was adopted. After the adaptation months, the fishes feed better and the feeding task is easier. This stressful situation could have had negative effects in the growth or the health of the fishes.

While the fish grew, they improved their adaptation to the offshore conditions, in the sixth month, CANEXMAR decided to maintain feeding twice per day (one in the morning and the other one in the afternoon). There were no differences between the feedings, with the fish behavior being equal during all the experimental time, with fish showing a nervous behavior when boats reached the cage.

### Daily feeding control

A routine monitoring was done during the different months, filling in different sheets with different parameters to be observed by operators, including feeding, behavior, temperature, and different sea conditions (an example is shown in **Table 9.3.1**). **Table 9.3.2** shows the different diets used during the whole on-growing cycle. A diet with the levels of Lysine proposed by HCMR and included in Deliverable 9.2 was used.

**Table 9.3.1.** Feeding control sheet from January 2017

DAY	N° CAGE	Suggested feed/Kg	Real feed/Kg	Type feed	N° codic feed	Behavior	mortality	Net bird	Cage	T.°C	Sea conditions	Ocean currents
11/01/2017	9x	12	22	1,9		3		ok	ok	19	3	2
12/01/2017		12	22	1,9		3		ok	ok	19	3	2
13/01/2017		12	22	1,9		3	2	ok	ok	19	3	2
14/01/2017		12	22	1,9		3		ok	ok	18	3	2
15/01/2017		12	22	1,9		3		ok	ok	18	3	2
16/01/2017		12	22	1,9		3		ok	ok	18	3	2
17/01/2017		12	22	1,9		3		ok	ok	18	3	2
18/01/2017		12	22	1,9		4		ok	ok	18	3	2
19/01/2017		12	22	1,9		4		ok	ok	18	3	2
20/01/2017		13	23	1,9		3	3	ok	ok	18	3	2
21/01/2017		13	23	1,9		3		ok	ok	18	1	1
22/01/2017		13	23	1,9		3		ok	ok	18	1	1
23/01/2017		13	23	1,9		3		ok	ok	18	1	1
24/01/2017		13	23	1,9		3		ok	ok	18	1	1
25/01/2017		13	23	1,9		3	7	ok	ok	18	1	1
26/01/2017		13	23	1,9		3		ok	ok	18	1	1
27/01/2017		13	23	1,9		3		ok	ok	18	1	1
28/01/2017		13	23	1,9		3		ok	ok	18	1	1
29/01/2017		13	23	1,9		3		ok	ok	18	1	1
30/01/2017		13	23	1,9		3	2	ok	ok	18	1	2
31/01/2017		13	23	1,9		3		ok	ok	18	1	1



**Table 9.3.2.** Commercial diets used during the on-growing at sea cages. Levels of Lysine are according to the previously determined levels in D9.2.

		3 mm	4,5 mm	6,5/9 mm	12/15 mm
<b>CRUDE PROTEIN</b>	%	54	54	54	52
<b>CRUDE LIPIDS</b>	%	18	18	20	20
<b>CARBOHYDRATES</b>	%	12.3	12.3	10.4	13.1
<b>CELLULOSE</b>	%	0.6	0.6	0.5	0.6
<b>ASH</b>	%	9.2	9.2	9.5	8.8
<b>PHOSPHORUS</b>	%	1.4	1.4	1.4	1.3
<b>CRUDE ENERGY</b>	MJ/KG	21,7	21.7	22.2	22.1
<b>DIGESTIBLE ENERGY</b>	MJ/KG	19.1	19.1	19.7	19.4
<b>DIGESTIBLE PROTEIN</b>	G/MJ	25.1	25.1	24.3	23.7
<b>VITAMIN A</b>	I.U/Kg	10000	10000	10000	10000
<b>VITAMIN D3</b>	I.U/Kg	500	500	500	500
<b>VITANIM E</b>	MG/Kg	200	200	200	200
<b>VITAMIN C</b>	MG/Kg	100	100	100	100
<b>LYSINE</b>	gr/Kg	36.5	35.3	36.7	

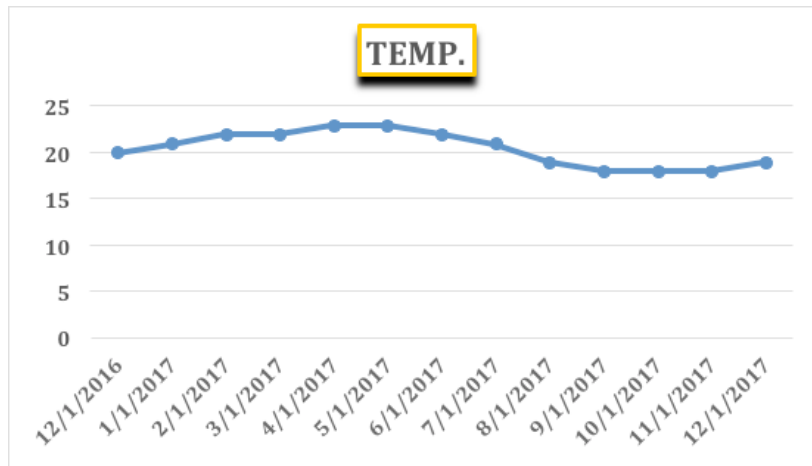
### Sampling protocols

Four different sampling points were determined during the on-growing cycle. The sea conditions in the zone prevented more samplings with the security guarantees, because the fish suffer more stress when we use the standard methods to pick them up and get the sampling with the fish tools (**Fig. 9.3.5**). The method used to calculate the weight was a dynamometer. The anesthetic used to guarantee the welfare during the process was clove oil in a concentration of 25 ml/1000 l.



**Figure 9.3.5.** Sampling procedures at sea cages

The average temperature of the year was recorded around the sea cage located in CANEXMAR farm, being temperature according to the average temperature for the geographical region (**Fig. 9.3.6**).



**Figure 9.3.6.** Temperature variations during the experimental period

In each sampling point, 107 fish were sampled (**Table 9.3.3**). Average body weigh was 121.9 g in March 2017, increasing to 230 g in May, to 303 g in June and to 538.4 g at the end of September. High variation in fish size was observed in each sampling point.

**Table 9.3.3.** Average body weight of experimental fish during the on-growing cycle.

DATE SAMPLING: 06/03/2017		DATE SAMPLING: 02/05/2017	
CAGE	J09X	CAGE	J09X
FISH NUMBER SAMPLING	107	FISH NUMBER SAMPLING	107
AVERAGE WEIGHT SAMPLING	121,9	AVERAGE WEIGHT SAMPLING	230
MAX. WEIGHT	320	MAX. WEIGHT	409
MIN. WEIGHT	100	MIN. WEIGHT	110

DATE SAMPLING: 26/06/2017		DATE SAMPLING: 22/09/2017	
CAGE	J09X	CAGE	J09X
FISH NUMBER SAMPLING	107	FISH NUMBER SAMPLING	107
AVERAGE WEIGHT SAMPLING	303,4	AVERAGE WEIGHT SAMPLING	538.4
MAX. WEIGHT	501,5	MAX. WEIGHT	736.5
MIN. WEIGHT	196,5	MIN. WEIGHT	431.5

The growth predicted and measured is shown in **Fig. 9.3.7**. Mean Weight (MW) L1 shows the growth data obtained from the samplings whereas MW L2 data from the theoretical calculations with the objective weight of one kg, that is very easily obtained in P2. FCPCT indoor facilities. The FCRs measured were higher than the predicted ones (**Fig. 9.3.8**) for the normal feed utilization obtained at FCPCT indoor facilities. FCR L1 shows growth data obtained from the samplings whereas FCR L2 shows data from the theoretical calculations with the objective weight of one kg.

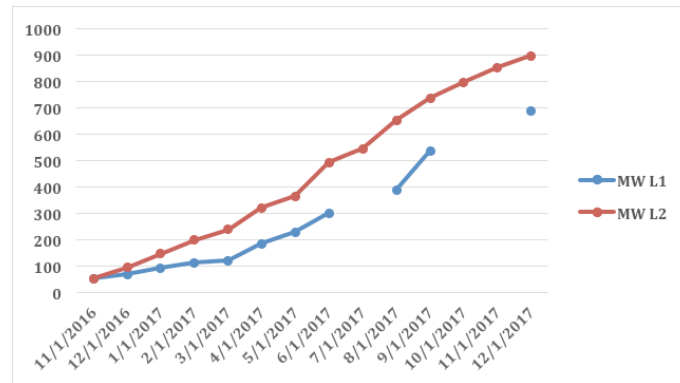


Figure 9.3.7. Comparison between growth projections vs growth from samplings.

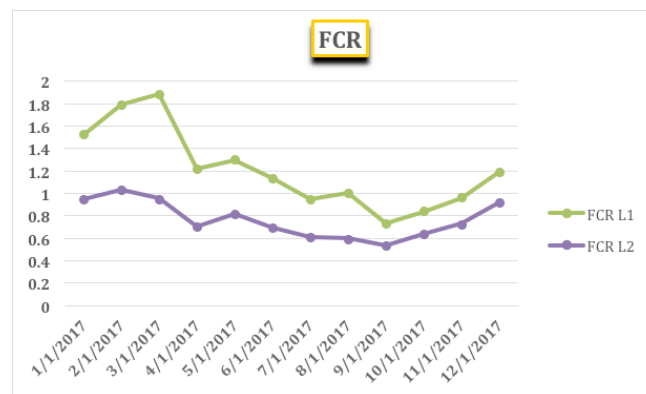


Figure 9.3.7. Comparison between FCR projections vs FCR from samplings.

Sampled animals presented no parasitic infection during the three first samples, but at the fourth sampling point, monogenean parasite infection occurred, with a incidence at level 2 according to the scores defined by Fernandez-Montero et al. (2018), previously reported in a D25.5 of amberjack health (Fig. 9.3.8)

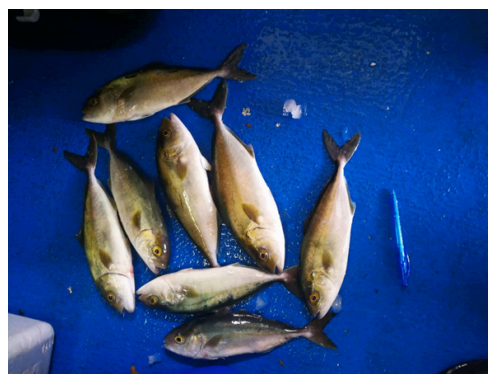


Figure 9.3.8. Juvenile amberjack after sampling. Some ulcerative processes can be observed in the skin due to parasite infection.



## Deviations

The fish mortality in the first three days in this cage was caused by the un-adaptability to the new environment. While the other fishes only have to adapt themselves to a new feeding method, the fishes of the submerged cage had to confront the sea depth changes of the cage. We believed that this adaptability problem causes additional stress, which increased the stress of the downloading from the transport. This is the most important difference with the other fish lot. Probably, the best option if we have to try this experiment again, would be to introduce all the fishes in the same conventional cage and when all the fishes show a successful adaptation to the new environment, we could pick up the fishes to introduce them in a submerged cage.

The feeding in the starting months was very hard and we have had to do many different feedings per day to guarantee that all the fishes get all the feed they need. The scuba diver supervisions always had to be very fast and efficient to spend no more time than necessary near to the nets to prevent stress to the fishes. It should be pointed out that in one of our routine pathology controls to our commercial animals in the enterprise's cages, specially in the species *Sparus aurata*, we found typical parasitic infection of monogenea of the greater amberjack, this is a very important fact for us currently and for future proofs with this species.

One of the most important reasons to decide the finalization of the project in our enterprise structures was found this kind of parasitic in the *Sparus aurata* species, this parasitic existence increases with the times and is a serious threat to the enterprise production. We have to determine yet the danger and incidence of this parasitic between both species, however the most logical consequence is avoiding the risk for all the company production for a project in the final stage.

## Project Coordinator's Comments:

The description in the DOW of D9.3 includes "Performance of grow-out diets for greater amberjack developed in order to maximize growth potential: A report with the effects of the improved diet on the grow-out of greater amberjack will be provided in this deliverable. The deliverable will describe the effects of the improved diet and will include: a) detailed information about the on-growing rearing conditions, b) growth performance, c) survival, d) feed efficiency and e) significance for the industry. The efficiency of the developed grow-out diet on fillet quality will be also determined. [month 58].

There was no suggestion of the use of a submerged cage and there is no description in the above Deliverable report of a submerged cage being used. Therefore, it is not clear what the authors of the Deliverable refer to in the Deviations above. Also, there was supposed to be an evaluation on fillet quality at the end of the rearing, which was not done, without any explanation by the Deliverable responsible.

Although not explicitly stated in the report, the company seems to suggest in the Deviations that they discontinued the experiment because they noticed a parasitic infection in greater amberjack, which was also found in their gilthead seabream (*Sparus aurata*) stocks and it appeared to increase in time. There is no mention or description of the identity of the parasite, apart from belonging to the Monogenea group. It is important to note that, as far as I know, parasites are extremely species-specific and they do not usually infect fish other than their natural host. So, it seems extremely doubtful that the same parasite species infected both the amberjack and the gilthead seabream. However, without any proper description and documentation of the parasite, it is impossible to know what was the situation. Unfortunately, as the Project Coordinator, this is the first time I heard this issue.

