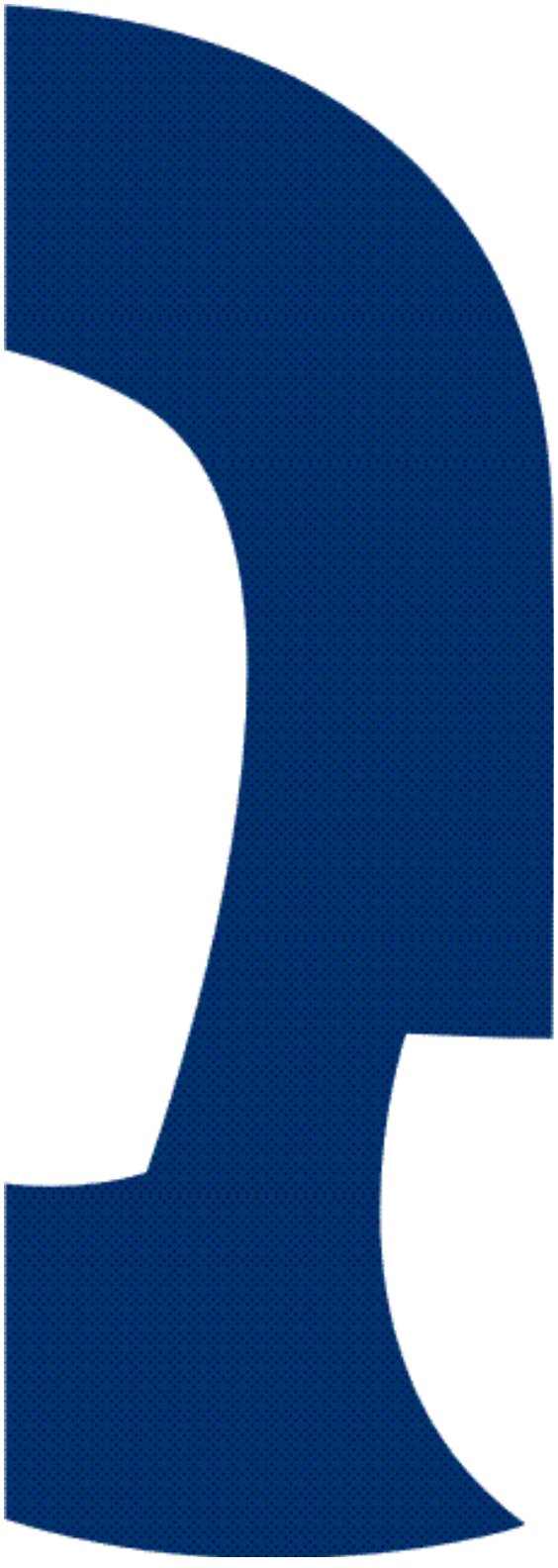

FACULTAD DE CIENCIAS DEL MAR

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

Opportunity of integrated production of *Ceratophyllum demersum* in a nutrition trial with *Carassius auratus*

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Curso 2015/2016

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Abstract

The integrated culture of fish and plant in a recirculated system is called aquaponics, where a mutual benefit have been demonstrated for the cocultured species. Today this practice is still not well known for the most studied consumed freshwater species, and almost nothing has been done for aquarium species. The objective of present work was to study the opportunity of growing one of the most selled aquarium plant coontail (*Ceratophyllum demersum*) in a coculture with the goldfish (*Carassius auratus*) from which a nutritional trial was planned. For the trial 6 aquariums of plants were connected with 6 aquariums of fish where 2 diets were tested: a well known commercial one (Tropifish) (Diet Com), and an experimental one based on fish meal and fish oil enriched with 2% of locally produced grape by-product meal (Diet Exp). Besides growth fish paramenters and plant gowth, the evolution of some of the main physicochemical parameters involving aquaponic systems, such as nitrates, nitrites and ammonium were tested and the opportunity for their coculture evaluated.

Experimental and comercial diets responded with growth rates very similar still within the expected range for this species. Generally speaking, the fish growth was slow as expected with a survival rate of 100% and with no many differences between the used commercial diet and the experimental one (a previous one already tested and enriqued with local grapes by-products). On the side of the plants 273% growth was observed respect to the initial weight, with good appearance and no problems observed along the assay. It was also noted that nitrites, nitrates and ammonium decreased and even disappeared from the waterborne under coculture.

As a resume, results showed the viability of this first experimental diet for goldfish compared with the best knowledge commercial one, although best results could be obtained in the first case by improving feed stability. It was also demonstrated the opportunity for the production of *C. demersum* in coculture with goldfish, which represent a goodness of the water quality for the fish, while generate an extra valuable product. The present study showed promising results to continue working in this field, as well as in the aquarium production sector.

Keywords: Goldfish, *Ceratophyllum demersum*, aquarium diets, aquaponics.

1. Introduction

1.1. World trade in fishkeeping. Interest and perspective

The fishkeeping is defined as breeding fish and other aquatic organisms in aquariums under controlled conditions (Lucas, 2012). It has become a popular and great expansion worldwide pastime facilited for enormus advances in new technologies (Lango-Reynoso *et al*., 2012). Between 1.5 and 2 million people worldwide have marine aquarium, being a hobby that moves large amounts of money worldwide, between 150 and 270 million euros per year according to Wabnitz, (2003).

Among the major exporters in the world it can be find the Maldives, Vietnam, Sri Lanka, Singapore and Hawaii. In addition, other exporters are Colombia, Peru and Brazil in South America; Nigeria, Congo and Malawi in Africa and Thailand and Indonesia in Southeast Asia, with the peculiarity that they are all tropical and subtropical countries (Tlusty, 2002; Lango-Reynoso *et al*., 2012). European Union represent, all countries together, the largest market for ornamental fish in the world; however, the United States is the largest importer globally for ornamental fish (Livengood and Chapman, 2007). Among European countries it can be highlighted the Czech Republic, Spain, Belgium and Holland as some of the more active ones in aquarium bussiness (Tlusty, 2002).

The market of ornamental species has increased due to the benefits they entail, as a kilogram of coral reef fish aquarium would have an approximate value from 450 to 1600 euros; although there are exceptions where prices may increase much more (Fig. 1). Prices for a kilogram of fish for human consumption will range between 5 and 15 euros (Fig. 2) (Livengood and Chapman, 2007).

Figure 1. *Peppemint angelfish*. Market price 27.000 euros. Source: Internet www.uncaprichoperfecto.blogspot.com

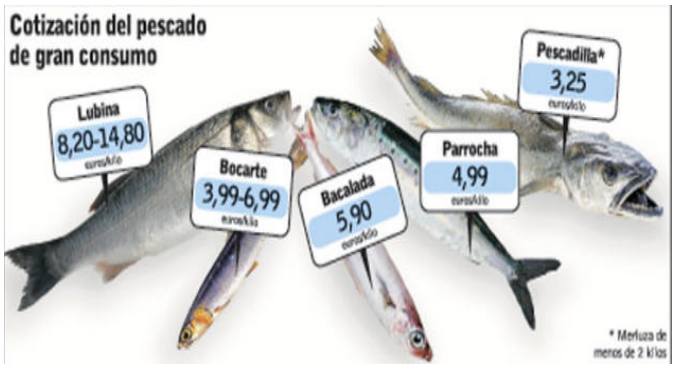

Figure 2. Quote of fish for human consumption. Prices around 10 euros. Source: Internet (www.lne.es)

Most of the ornamental business focussed on freshwater species however marine aquarium trade has encreased steadily on the last three decades except for 2010 due to the global financial crisi. The international market for marine aquarium is dominated by fish,

with an average annual production volume of approximately 20 million of tropical fish; also are extracted and sold about 9-10 million invertebrates, 12 million hard corals and 390,000 pieces of soft corals (Lango-Reynoso *et al*., 2012). Among them it can be found 1539 species of fish, 102 species of coral and 293 species of invertebrates (Table 1) (Livengood and Chapman, 2007).

Table1. Approximate number of fishes, corals, and other invertebrates in the ornamental (aquarium) trade. The majority of these species are collected from the wild being only freshwater fish species extensively farm-raised (Livengood and Chapman, 2007)

Ornamental species	Approximate number of species	Principal geographic regions
Fresh-, Salt-, and Brackish Water Fishes	1539	Southeast Asia, Americas, Africa, Indonesia
Corals (hard and soft)	102	Indo-Pacific, Caribbean, the Red Sea
Invertebrates, other (i.e. shrimps, crabs, snails, starfish)	293	Indo-Pacific, Caribbean, the Red Sea

Fish belonging to the family *Pomacentridae* constitute almost half of the fish trade, including *Chromis viridis, Amphiprion ocellaris,*etc. Below are those from the *Pomacanthidae*, *Acanthuridae*, *Labridae*, *Gobiidae* and *Chaetodontidae* families constituting 25-30% of the trade (Wabnitz, 2003).

Aquatic plants are those that have adapted to living in aquatic environments, both saltwater and freshwater. These have never been popular in botany, as growing in habitats that are uncomfortable or difficult to examine (Preston *et al*., 2014). Ecological factors and climate in particular are known for limiting the distribution of plant species. In the case of tropical zone, the most abundant species are for example Podostemaceae, Hydrocharitaceae, Limnichariataceae, Mayacaceae, Xyridaceae, Eriocaulaceae, Pondeteriaceae and Aponogetonaceae families (Crow, 1993); in temperate zones the most abundant families of aquatic plants are Potamogetonaceae, Juncaginaceae, Sparganiaceae, Halogaceae, Elatinaceae, Callitrichaceae and Hippuridaceae (Crow, 1993); aquatic plants also found in cold areas, such as Potamogeton and Coleogeto*n* families, which have suffered glaciations (Santamaria, 2002).

For aquatic plants there are also a large variety for aquarium trade, from which the most popular are *Ceratophyllum demersum*, *Limnophila sessififlora*, fern java families, Anubia, Hygrophilas, Vallisneria, Echinodorus and Cryptocorynes families (Hiscock, 2003).

1.2. Data production and cultivation of goldfish (*Carassius auratus*).

Kingdom: *Animalia*

Phylum: *Chordata*

Class: *Actinopterygii*

Order: *Cypriniformes*

Family: *Cyprinidae*

Genus: *Carassius*

Specie: *Carassius auratus (Fig.3)*

***Figure 3. Carassius auratus* (goldfish)**
Source*: Internet (www.aquaterraria.com)*

1.2.1. Production

This species in its different varieties is one of the fish most common and worldover commercialized for aquaria mainly from China (Choi *et al*., 2014), also from countries, such as Russia, Korea, Japan, Taiwan (Schofield, 2005), Laos, Macau and Myanmar. This species was introduced in different countries but the place where it had the greatest impact was in the US (Fig. 4). Also it can be found in UK (Tarkan *et al*., 2010) as well as in South America, such as in Colombia (Martinez *et al*., 2011), among many others like Afghanistan, Argentina, Australia, Bolivia, Brazil, Chile, etc.

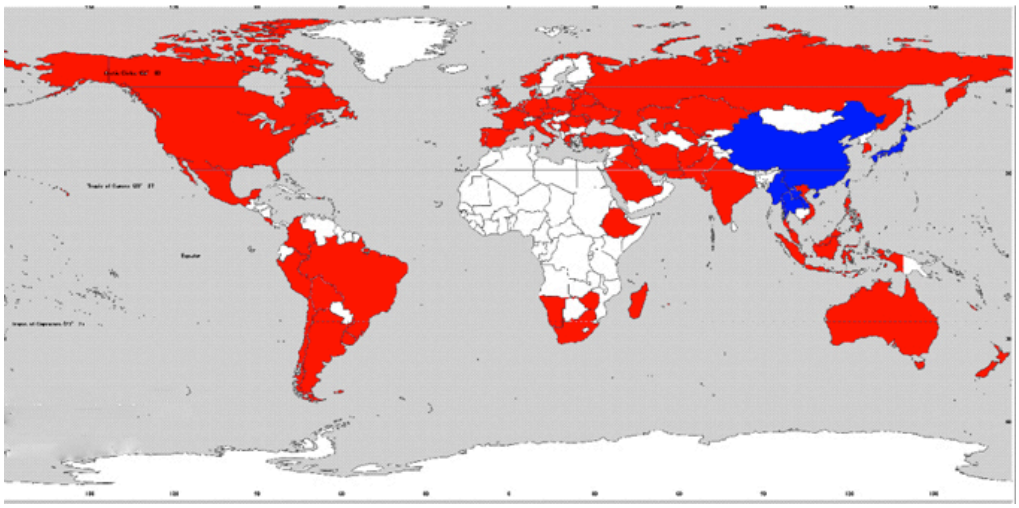

Fig. 4. The blue zone are the countries of origin of the goldfish and red zone are countries where this species has spread. Source: www.advancedaquariumconcepts.com

This organism is very tolerant to different environmental conditions, however, there are some ranges where shown to better growth: pH from 6.8 to 7.8 and temperature between 20 and 30 degrees centrigrates (Ramirez *et al*., 2009), although some authors say that it can withstand temperatures from 0 to 41 degree (Panne *et al*., 2008).

1.2.2. Culture

The goldfish is a colorful fish which is easily obtained from natural spawning and it can be grown at a different temperature of water, from that of the Sabana de Bogotá (14°C), without rise it; can also survive in waters where the conditions are not optimal, why working with them is economically profitable (Ramirez *et al.*, 2009).

In nature, sexual maturity is reached at 1 to 2 year age and reproduction occurs annually up to 6-7 years after. The females put their eggs on vegetation or other fixed objects in the environment. This species has the breeding season in spring and summer; however, in the aquarium, under controlled conditions, goldfish can maintain constant reproductive cycles. To induce spawning, temperature must be around 20-23ºC; during this time the male will chase the female for several days, then the female will release eggs and the male will fertilize them. After spawning, parents should be removed from the tank because they try to eat their own eggs. The eggs hatch in 4-7 days depends on the temperature.

1.2.3. Feeding

The goldfish are omnivorous fish, so they can eat all kinds of food both plant and animal. In captivity it can be fed on frozen food, flakes and pellets. In the market it can found many types of feed for tropical fish, although there are not many reported studies about their utilization.

1.3. Data production and cultivation of *coontail* (*C. demersum*)

Domain: *Eukaryota*

Kingdom: *Plantae*

Phylum: *Spermatophyta*

Subphylum: *Angiospermae*

Class: *Dicotyledonae*

Order: *Nymphaeales*

Family: *Ceratophyllaceae*

Genus: *Ceratophyllum*

Specie: *Ceratophyllum demersum* (Fig 5)

Figure 5. *Ceratophyllum demersum* (*coontail*)
Source: www.plantsrescue.com

It is a cosmopolitan free-floating aquatic plant, well distributed in many countries around the world, but is believed to be native from the United States and most part of Canada (Fig.6). It can be found in fresh and slightly brackish water, and as a peculiarity mostly in waters with a rich nutrients status, thereby providing a benefit to water quality because it acts as a filter. It is a popular aquatic plant for aquaria, and ornamental ponds (Goulder *et al*., 1971).

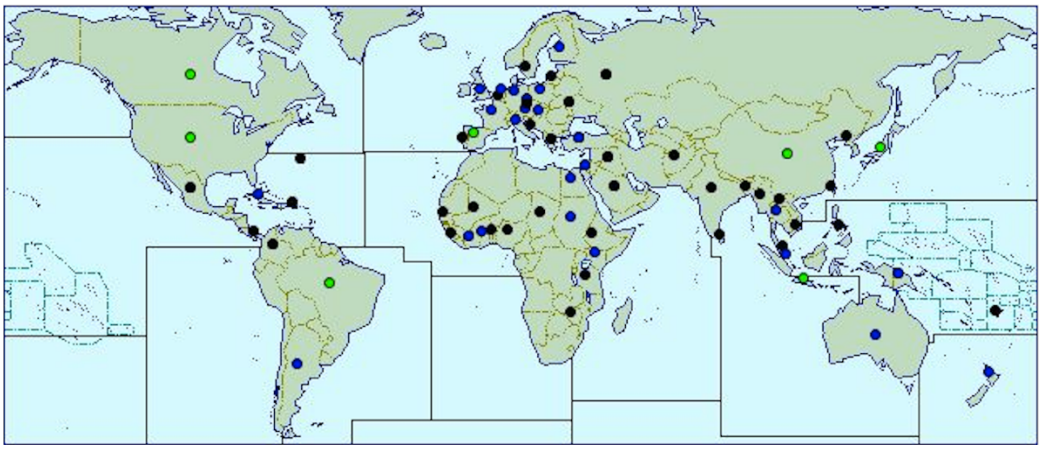

Figure 6. Global distribution of *C. demersum.* The black dots show where they are present, the green dots show regional map for distribution with the country and the blue dots show where it has spread. Source: www.cabi.org

C. demersum is a perennial aquatic angiosperm that is always submerged, is branched but with one branch per node. The leaves are dark green, sessile, rigid, also lack roots, so that capture nutrients through the leaves. It has a wide ecological tolerance and grows relatively fast.

1.4. Integrated culture in aquaponic systems

1.4.1. Aquaponic definition

Aquaponics is the combination of aquaculture and hydroponics production systems (Hart *et al*., 2013), representing the interaction of growing fish and plants in the same growing water bed, where is generated a mutual benefit (Fig. 7). These systems arise with an enormes future expectatives as a food production strategy to solve various global problems like soil deterioration or the use of large amounts of water, while reducing the food miles.

Excretions of fish are rich in nutrients for plants but toxic to fish themselves. In an aquaponic culture the plants act as a filter to absorb these substances previously treated for some beneficial bacteria; the role of bacteria is to turn the fish excreta into compounds more profitable for plants and less toxic to fish. Also it is a system that is booming as it has some advantages, such as saving water in crops due to recirculation (up to 70% less

in some cases), generates a higher quality of food because it does not require the use of agrochemicals and represents a saving in the productive process (Colagrosso, 2014).

Figure 7. Example of aquaponic system where tilapia and lettuce are produced. Source: Article (Hart, 2013)

The basic elements to have an aquaponic system are: a tank for fish (or other feeding aquatic organisms), solids filter, biofilter, water pumps and aeration systems (Fig. 8). These elements are all connected so that the nutrient rich water passes from the fish tank to solids filter, where most of undissolved particles are removed, then it passes through the biofilter where the ammonium is converted to nitrite and nitrite to nitrate due to bacteria metabolism, and finally reaches the plants where they absorb nitrates and subsequently, the water returns cleaned back to the tank (Ramírez *et al*., 2009).

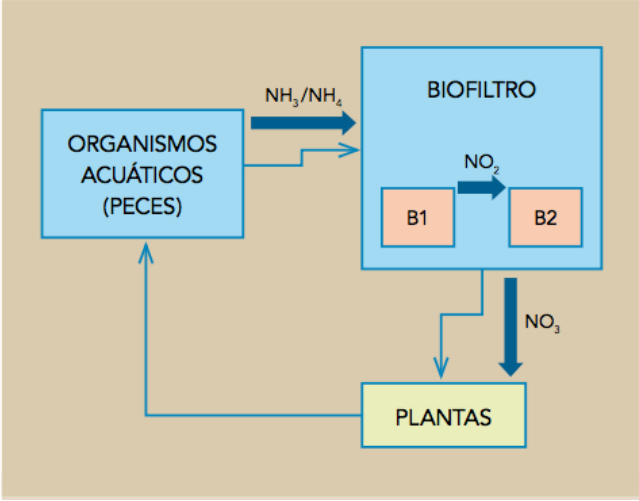

Figure 8. Scheme of a basic aquaponic system. The thin arrow indicates the direction of water flow through the system and the thick arrow indicates the fow nitrogen compounds through the system components. B1 = nitrifying bacteria belonging to the genus Nitrosomonas. B2 = nitrifying bacteria belonging to the genus Nitrobacter. Source: Modified from Ramirez *et a*l., 2009

1.4.2. Biofiltration with aquatic plants

The biological filtration (or biofiltration) is a technology that has already been experienced for the treatment of municipal effluents, which exhibit large load variations throughout the year and thus at low temperatures; in fact, more than 500 plants water treatment using biofiltration in Europe (Sekoulov *et al*., 2008). Treatments of water systems with aquatic plants are an efficient and economical treatment for wastewater, removal of microorganisms and chemical physical contaminants.

The use of *C. demersum* in this experiment of biofilter, since it will consume nitrite, ammonium and nitrate in the medium, thereby achieving the water reaches clean to the tanks.

1.5. Objetives

In the present work, the main objectives were:

1.5.1. To compare an alternative locally produced versus a well known commercial feed for goldfish focusing on the fish feed intake and fish performance.

1.5.2. To determine the opportunity and potential benefits for the combined production of two commercial aquarium species, the goldfish (*C. auratus*) and the coontail (*C. demersum*).

2. Material and methods

2.1. Facilities and species assayed

The Marine Scientific Technological Park of the University of Las Pamas de Gran Canaria, located in Telde, was the center where the present experiment was carried out, belonging to the Aquaculture Research Group (GIA). GIA is a team of voluntary association of professors and researchers from the university, to achieve common goals such as teaching, research, technology transfer and development cooperation in aquaculture, which functions as such since 1990. In 1999 he was recognized as a research group within the organizational structure of the ULPGC research, and in 2016 started to work under the Universitary Institute ECOAQUA.

For this experiment, a module with 12 aquariums was used, 6 of them on the top for the plants and 6 on the bootom for the fish (triplicates per the 2 tested diets). From the system, bootom located aquaria had a capacity of 34.96 liters (23x40x38 cm) and the aquaria on the top had a capacity of 17.48 liters (23x40x19 cm) (Fig. 9). All tanks were provided with adjustable oxygenators keys and addition to a water recirculation system for the oxygen control. A cleanning protocol of once a week was stablished, early morning before supplying the feed with the help of a sponge and a siphon.

Figure 9. General installation and detail of the used aquariums

Aquariums were well supplied with fresh water and with physical and biological filtration Systems (550 Aquamedic Riff, Germany) (Fig. 10), and sterilization by ultraviolet (UV) (Teco, TR 10 series, Italy) (Fig. 11). Photoperiod of 10 hours of light and 14 hours of darkness was stablished (AquaMedic of North America T5, 24w and 10000K).

Figure 10. Physical and biological filtration systems (Aquamedic Riff 550, Alemania)

Figure 11. Sterilization by ultraviolet (UV) (Teco, TR 10 series, Italy).

2.1.1. Fish (*C. auratus*)

The experiment was conducted with twentyfour goldfish acquired from specialized suppliers, and selected considering that fish fins did not present disrepair, erratic swimming, not lossed scales or external injuries. The test animals were handled in accordance with the ULPGC ethics protocol for experimental animals. The specimens had an initial average weight of 12.71 ± 6.84g and a size of 6.9 ± 1.2 cm; after being individually measured and weighed they were introduced in groups of 4 fish in any of the 6 fish aquariums (Fig. 9), thus avoiding differences between tanks at the begginig of the trial (Fig. 12 and Fig. 13).

Fish were hand feeding three times daily (9:00, 11:30 and 14:00) to apparent satiation. In order to keep track of such feeding, each aquarium had their own feed cups, which were weigthed 3 times per day, before and after supplying the 3 doses to know about goldfish time feeding preferences under our conditions.

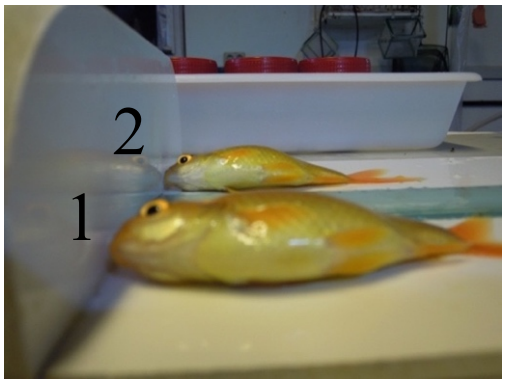

Figure 12. Measuring goldfish.

Figure 13. Goldfish weighed on a balance.

2.1.2. *Coontail (C. demersum)*

The water plants (158 g in total) were also obtained by specialized local supplier. After one week of running the trial with only the fish, the determined average amount of water nitrites, nitrates and ammonia were considered adequated to introduce the plants into the system. An amount of 26.34 ± 0.28g was used as initial batch for each of the 6 upper aquarium, attempting to obtain similar grams of plants in each tank (Fig. 14).

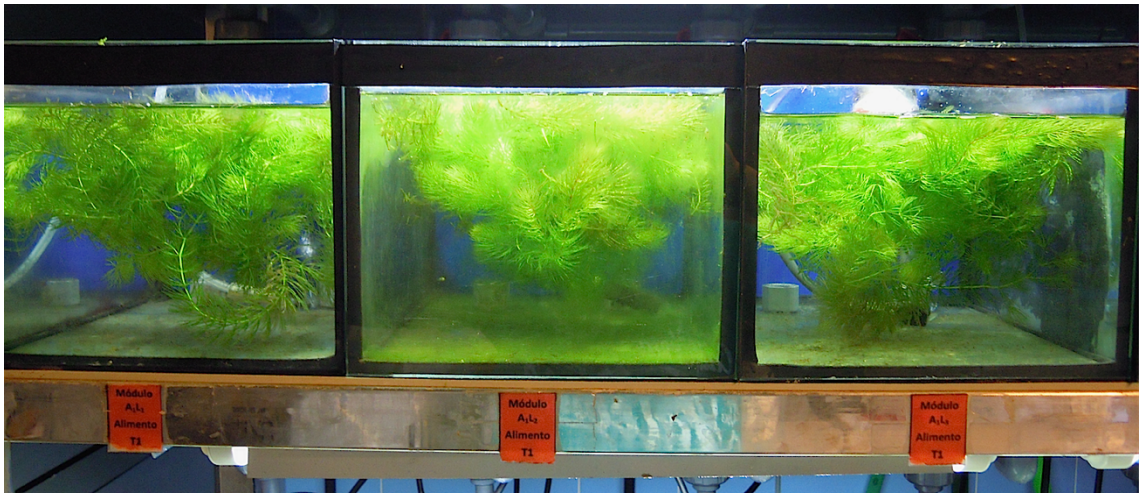

Figure 14. Upper aquariums of *C. demersum*.

2.2. Diets

Two different diets were used in the present experiment: a common commercial diet (Tropifish) (Diet Com) corresponding with the fish tanks 1, 2 and 3, and an experimental one laboratory produced at GIA facilities (Diet Exp) used for tanks 4, 5 and 6. Diet Exp was previously tested, the feed formula demonstrated good results in tilapia juveniles (Graterol, 2015), and it was enriched with 2% of grape by-products meal produced from the local wine processing.

Figure 15. Comercial diet (Tropifish 1.88mm). Source: www.comipez.net

Tropifish 1.8mm is a granulated diet witha diameter of 1.8 mm, with a composition of 56.1% crude protein, oil and fat 18.6%, 1.3% crude fiber, crude ash 9.1%, Ca 1.6%, and P 1.38%, Cumming from fish meal, fish oil, cereals and derivatives, vegetable proteins, yeast and lecithin. Moisture of 6.4% and Na 0.6%, plus additives such as minerals and vitamins (vitamin A E672 (IU/Kg) 7250, vitamin D3 E671 (IU/Kg) 1115 and antioxidants: E324 (mg/ kg) 75 (Fig.15).

The analysis of Diet Exp composition showed 36% protein (Kjeldahl technique (AOAC, 2010) and 9% lipid (Folch *et al.*, 1957) and the formula was based on 22g fishmeal, 68.5g of vegetable meals (soybean, wheat and corn), 5.0g vegetable oil, 3.5 grams of vitamins and minerals and 1.0 grams of binder per 100g of diet (Fig. 16). This diet was enriched with 2% grape by-product as a best ratio observed from previous experiences carried out in this laboratory. This product was obtained from pressing the grapes before fermentation for the production of wine in a canary wine company. Processed in the laboratory and dried at 40°C for one day and then grinding 0.25µ and leave at 10°C in vacuum bags with an antioxidant. The product obtained was mixed with the other dry ingredients and oils are subsequently mixed with a small amount of water (about 10% by weight) to form a dough, which was pelleted using a pelletizing machine through a die diameter of 1.6 mm; the obtained wet granules were dried at 38°C for twelve hours and stored under 10°C.

Figure 16. Experimental diet enriched with local grapes.

2.3. Sampling scheme and tested parameters

During the experiment three sampling were carried out: the first one at the beginning of the trial (D0), the second at 15 days (D15) and the last one after 30 days feeding the fish (D30), which it considered the final of the experiment based on the growth results of the aquatic plant and the little fish growth of this fish species. Therefore, the duration of the experiment was 30 days. In order to calculate goldfish growth, length and weight individually measured using a measuring board and a balance (Fig. 12, Fig. 13 and Fig. 17), whereas for aquatic plants weight was determined using a balance (Fig. 14 and Fig. 18). Nevertheless, fish were feeding for 60 days more, to assure not any inconveniente in fish feeding and performance was observerd in fish fed on the experimental diet versus the fish fed on the commercial one. Thus, at D90 fish were weighted and liver extracted and also weighted to determine hepatosomatic indexes (HSI). Liver is the most estudied organ under culture conditions, as it is affected by any paramenter change like feed and

feeding and culture management. Livers were externaly obserbed and embebed in diluted formol for their later histological evaluation (not in the present work).

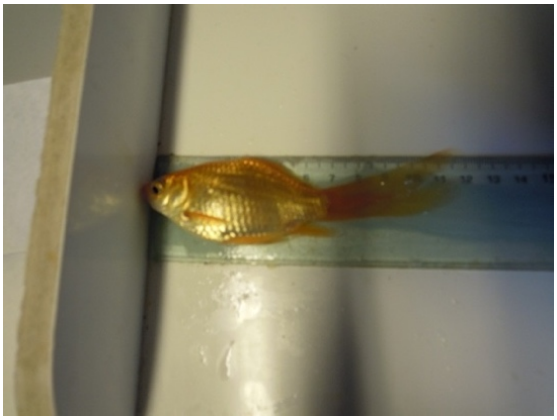

Figure 17. Sampling of a goldfish with a measuring board.

Figure 18. Water removal before weighting of *C. demersum*

Figure 19. Image of fishes from one tank in a container, while the tank is cleaning.

Different parameter was calculated to compare in experimental fish the effectivity of the different diets: Feed intake (% per fish per day), survival (% respect to the initial fish), condition factor (fish weight/length ratio), weight gain (% growth from initial weight), and feed conversion ratio (g feed intake per g gain fish weight), according to the subsequent formula:

- Intake per day (%/day) = (100*feed (g)/days of feeding)/ (final weight-initial weight) x (nº fish)
- Survival (%) = (final number of fish-initial number of fish) x100/initial number of fish
- K (condition factor (%)) = (fish weight) x100/ (fish length)^3
- Weight gain (%body wt /day) = (Final weight-initial weight) x100
- FCR (feed conversion ratio) = Feed intake (g)/Weight (g)
- HSI (hepatosomatic index) = (Liver weight (g) / Fish weight (g)) × 100
- VSI (viscerosomatic index) = (Visceral weight (g)/ Fish weight (g)) x 100

For the plants, only weight increment was measured.

To control the water parameters and valorate the interaction between fish and plants in the aquaponic system, lectures of nitrites and nitrates (Easy-life 5 in 1 test strips) (Fig. 12) was done twice a week; to do this, a test strip was introduced in the water for 30 seconds and the obtained data, expresed in ppm, compared with the colors of the used guide. In addition, to properly control of ammonia (Red Sea, Ammonia test kit) (Fig. 13), $NH3^{+}/NH4^{+}$ ratio was monitored by collecting a water sample and following the instructions on the guide box (data were also expresed in ppm).

Figure 20. Kit for nitrite and nitrate analysis, (Easy-life 5® in 1 test strips)

Figure 21. Ammonia control, (Red Sea, Ammonia test kit)

2.4. Statistic analysis

First analysis was performed to test the normality and homogeneity of variance using the Kolmogorov-Smirnov and Levene's test ($P < 0.05$), respectively (Zar, 2009). When the data were transformed, it was necessary to obtain a homogeneous variance. Differences between groups were determined by one-way ANOVA using Tukey's test for multiple comparisons, a significance level of 95% was used. Help of statistical analysis SPSS 15.0 package was used for all statistical analyses and Excel to create graphs and tables.

3. Results

3.1. Fish

After 90 days of experiment, there were no significant differences in fish weight and length were observed between the two diets, (Fig. 22 and Fig. 23). It is further argued that there was no episode of mortality throughout the experiment with then a 100% of survival for both diets.

At the beggining of the experiment fish showed a weight of 12.71± 6.84 g and at the end (30 days) a weight of 16.00 ± 8.68, therefore an increased body weight by 25.89% in total; after 90 days fish grew a total of 37.97% respect to the initial weight. In the case of the length, fish began with a size of 6.9 ± 1.2 cm and finished with 7.58 ± 1.31 cm at 30 days, increasing 9.85% in length.

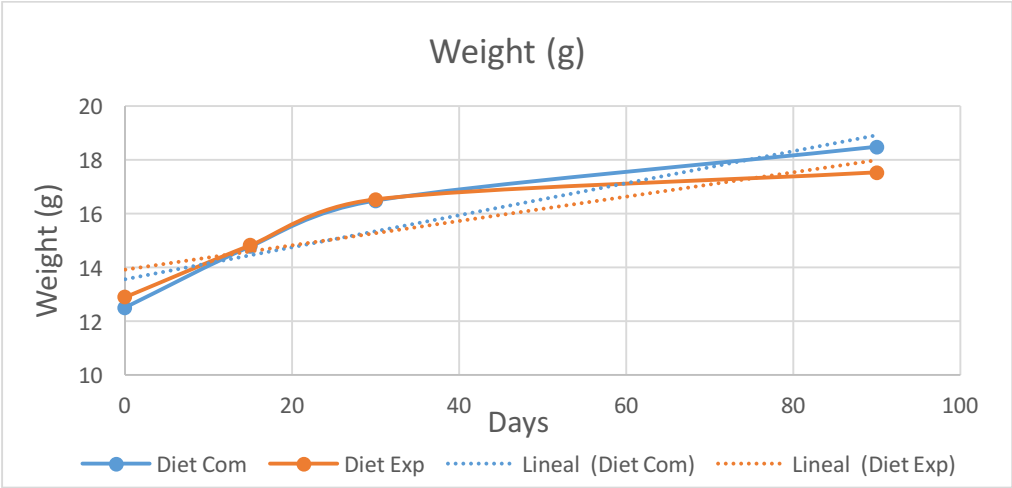

Figure 22. Weight of goldfish along the trial.

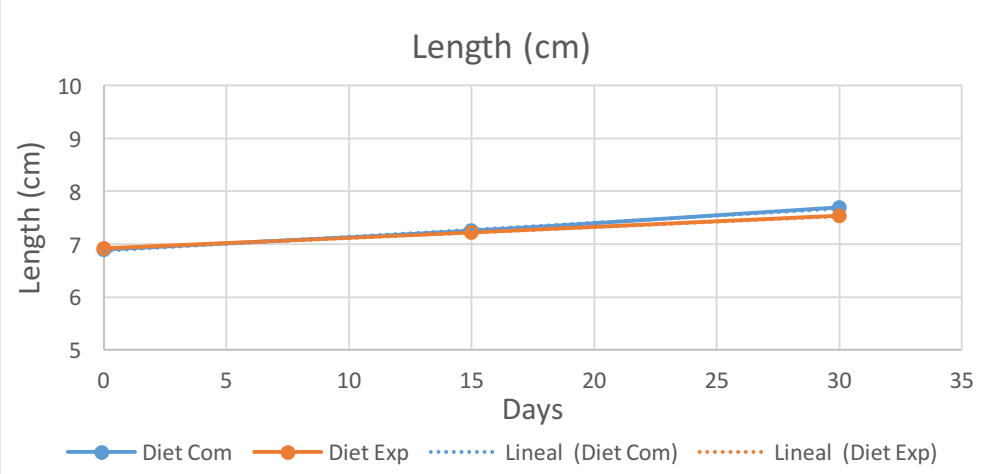

Figure 23. Length of goldfish measured along the trial.

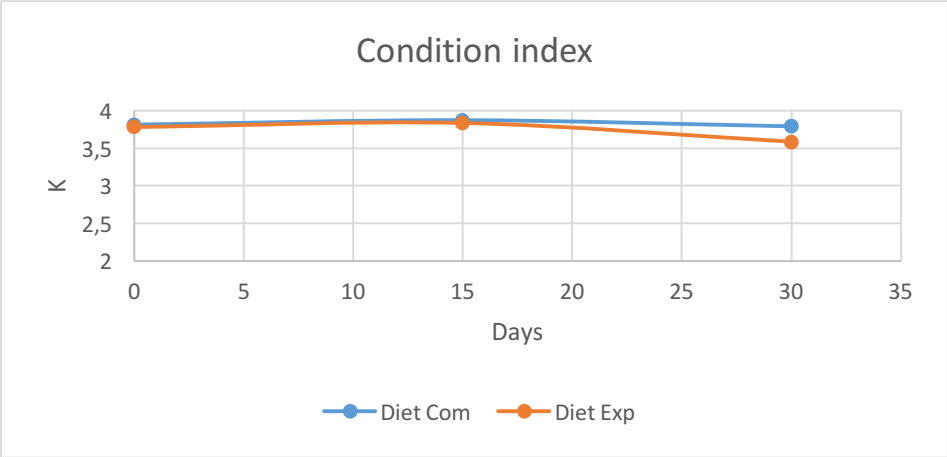

Figure 24. Condition index for goldfish according to their different diets (blue for commercial diet (Diet Com) and orange for experimental diet (Diet Exp)).

Table 2. Fish growth and productive and fish biometric parameters in the experiment (mean values ± standard deviation).

	Initial	Final Diet Com	Final Diet Exp
Weight	12.71 ± 0.28	16.48 ± 0.75	16.53 ± 2.04
Lenght	6.90 ± 0.02	7.69 ± 0.27	7.53 ± 0.45
Weight increment (%)		29.66 ± 0.53	30.05 ± 1.57
Length increment (%)		11.45 ± 0.14	9.13 ± 0.22
Condition index	3.80 ± 0.02	3.58 ± 0.32	3.79 ± 0.52
HSI		1.68 ± 0.86	2.41 ± 0.62
VSI		11.53 ± 4.11	14.20 ± 3.87
Feed intake (g/tank/day)		2.07 ± 0.40^{a}	3.32 ± 0.55^{b}
Feed Intake (100g fish/day)		2.86 ± 0.62^{a}	4.18 ± 1.09^{b}
FCR		3.18 ± 0.33^{a}	4.69 ± 0.99^{b}

Rows with same letters for final data show non statistical differences (P<0.05)

Subsequently as seen in Table 2, growth for the comercial diet was 11.45% in centimeters and 29.66% in grams, while the experimental diet growth 9.13% in centimeters and 30.05% in grams, noting that the condition index remained stable for both diets (Fig. 25). The hepatosomatic indexes for fish fed Diet Exp presented values of 2.41 ± 0.62 and 1.68 ± 0.86 for diet Com, and those for VSH 14.20 ± 3.87 and 11.53 ± 4.11 for the Diet Com and Diet Exp, respectively, with no differences between diets for both indexes. Respect to feed intake (g/tank/day) values of 2.07 ± 0.40 for the commercial diet and 3.32 ± 0.55 for the experimental diet were obtained with no changes along the trial as shown in Fig. 26, which represented percentage of intakes (100g fish/day) of about

2.86 ± 0.62 and 4.18 ± 1.09 for the Diet Com and Diet Exp, respectively, significantly higher in the latter. The peaks are due to the sampling days, where only were fed at the last time because the sampling must be done fasting and Saturday and Sunday when the goldfish did not feed. Besides, the feed conversion ratio had values of 4.69 ± 0.99 for the experimental diet and 3.18 ± 0.33 for the Diet Com.

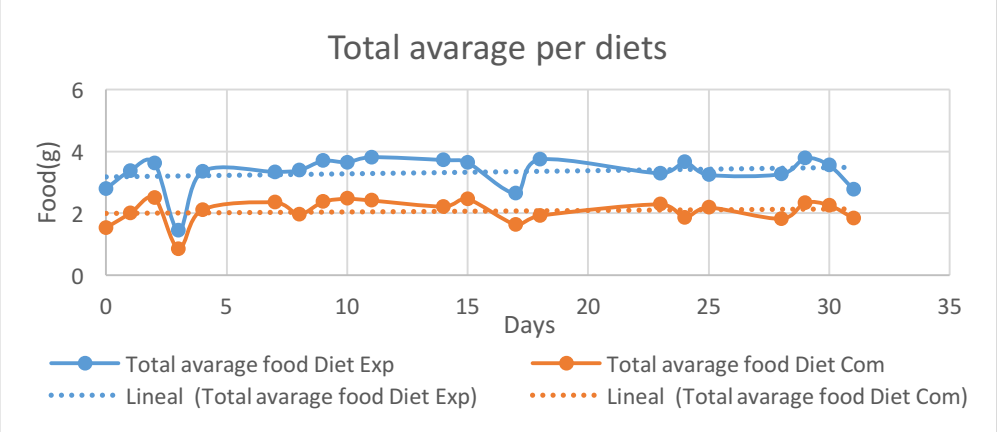

Figure 25. Feeding rate (g/tank/day) for the goldfish and the different diets (blue for commercial diet (Diet 1) and orange for experimental diet (Diet Exp)).

The daily fish supplied at the different doses may be seen in Figure 26, in which is represented the tanks with their trend lines. Clearly the first feed (9:00), is where goldfish ate more food than the others, also, in the next two feedings (11:30 and 14:00), eat about the same amount of feed.

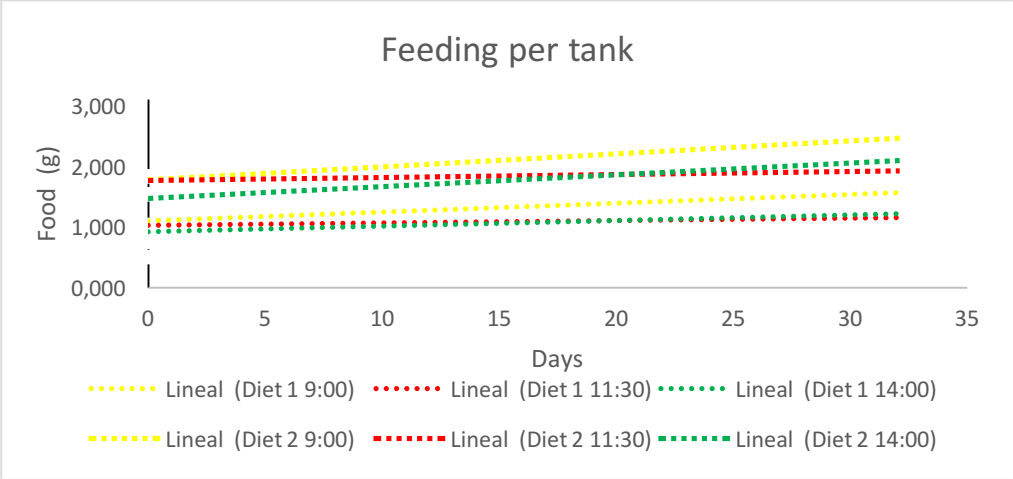

Figure 26. Daily fish feeding rate trend for the goldfish; yellow hues correspond with the morning feeding (9:00), blue with the second feeding (11:30) and green with the last fedding (14:00).

3.2. Plant *C. demersum*

3.2.1. Water plant growth and water parameters

The growth of *C. demersum* during the experiment, from day 7 when nutrition in the system were suitable for the plants to Day 30 of the experiment is shown in Figure 27. Plants showed at the beginning of the experiment a weight of 132g in total, reaching up to 360.5 ± 9.72 grams after 30 days, with a significant 273.3% growth. One of the tanks was eliminated from the final statistic as it plants growth was shown affected from different light insight.

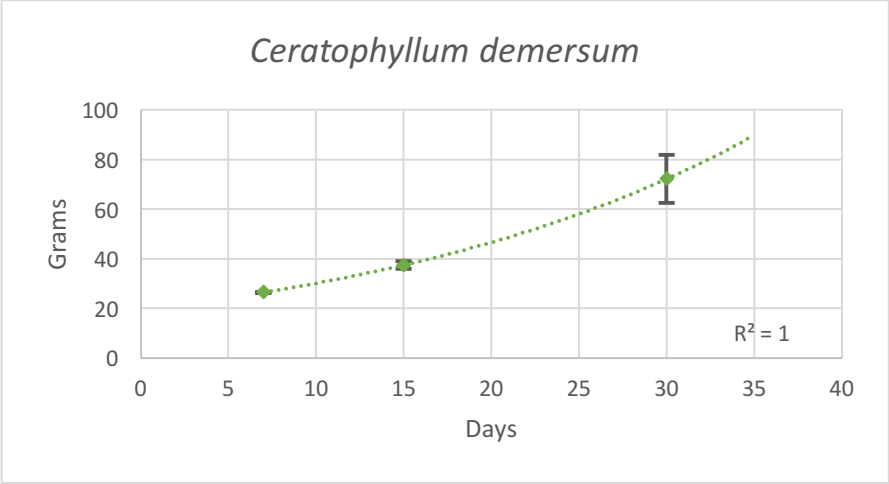

Figure 27. Growth of *C. demersum* during the experiment (mean values ± error bars).

As it is shown in Figures 26 and 27 once plants were introduced in the system levels of nitrites, nitrates and ammonia went down to levels close to 0, 20 and 0.1, respectively, with no changes along the trial.

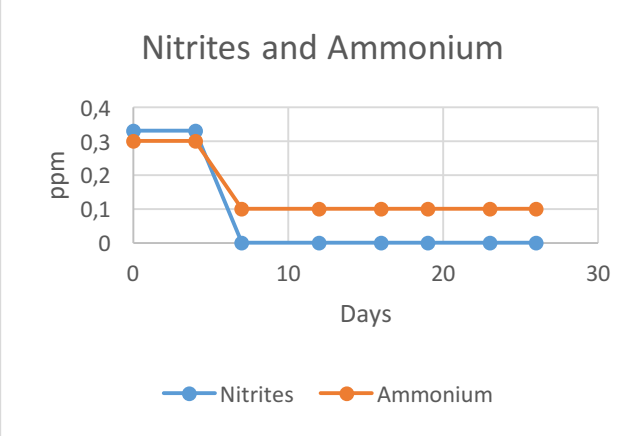

Figure 28. Meassured values for water nitrite and ammonium content throughout the experiment in the coculture of *C. dermersum* and goldfish.

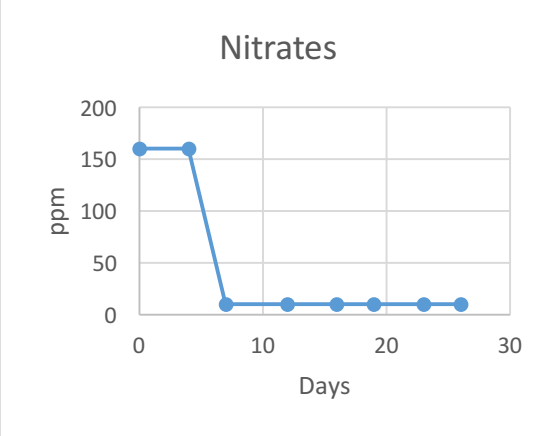

Figure 29. Measured values of water nitrates content throughout the experiment in the coculture of *goldfish* and *C. demersum*

4. Discussion

In present experiment, the observed growth rates for goldfish was very low, about 0.83% per day in weigth and 0.32% in length after 30 days feeding. Apart from the present one, there is only a previous published work done with same species under aquaponic conditions by Ramírez *et al.* (2009), who also found similar slow growth in a co-culture between goldfish and lettuce; these authors ascribing the poorer results to the low pH levels 5.27 ± 1.37 having in their experiment. This is not the case of present trial where pH values were higher. In the study by Moreira *et al*. (2011) where different feeds were also tested for goldfish growing both, a closed recirculated system and open flow system, slow growth of this species was also confirmed with 0.8 to 2.0 g weight increment in 60 days. Thus, although there is no data about goldfish growth at higher size to compare, fish used in present trial were bigger than in the previous ones and thus the expected growth would be less than the previous reported ones as it was shown.

No differences were observed for the experimental diet compared to the commercial one showing both a similar growth trend along the trial. However, it was observed however a higher feed intake for the experimental diet compared with the commercial one, which should be in some manner related with the lower water stability observed for the latter one but mostly to the differences in the diet biochemical composition and quality, as fish were fed to apparent satiation thus able to controlling the amount of feed ingested. In this case, commercial diet showed a higher percentage of proteins and lipids (56.1/18.6) respect to the experimental one (36/9 for protein and lipid) as it was described.

Regarding the daily feeding pattern, the obtained results for both diets showed the highest feed intake for the early morning (9:00) feeding respect to the other two doses (11:30 and 14:00); this is because it is the moment when the fishes starved for a longer period of more time (18 hou) and it is therefore when fish seems hungrier and eat more amount of feed.

The variations of K values for a given species can vary widely because it influence on such factors as temperature, quantity and quality of food and reproductive stage. Obtained K indexes for fish feed on both diets in present trial were statistically similar and remained stable along the experiment as shown in Fig. 25. However, it was observed a prominent lateral inflation in those fish fed the commercial diet after 30 days feeding respect to that from the experimental one with showed a normal shape. It has been reported than excess dietary lipid would produce fat deposition in fish visceral cavity and muscles, reducing the percentage of useful meat and increasing the possibility of rancidity during storage. The experimental diet had half of the lipid content than the commercial.

Therefore, from present results it can be sayid that although more feed was needed to obtain similar fish growth, which should be reduced by improvement in feed processing, the commercial diet seems to content excess lipid content or low lipid quality for this species. This observation continues under analysis in GIA labs. Lower growth rate fish may need high quality feed with lower energy contents which seem to be better adjusted in the experimental one.

In addition, although was not under the control in present trial, it is well known that coloration of aquarium fish can be influenced by the feed employed (Kalinowski *et al.*, 2005; Harpaz and Padowicz, 2007; Kalinowski *et al*., 2007; Garcia *et al*, 2010; Segade, 2012), and may represent a beneficial effect from a specific formula feed. At the end of the trial no differences or improvement of fish skin coloration from fish fed on the commercial feed (specific one), respect to the tested one were visually observed.

Regarding water analysis, a reduction of ammonia, nitrites and nitrates was observed, this decline coincides with the aquatic plant inclusion in the system. Similar quick lowering results were described by Ramirez *et al*. (2009), in a co-culture trial between goldfish and lettuce with decreasing values for ammonium, nitrite and nitrate. Therefore, the production of ammonia confirms the description from Keiser (2000), in which according to the process of bacterial transformation lowering values from high initial concentrations at 0.2-0.4 ppm to values of 0-0.2 ppm were able to diminish over time. In the present experiment, the values of nitrites were around 0 ppm, confirming the conversion of nitrite to nitrate (Timmons *et al*., 2007). In the case of nitrates, these values were of 160 ppm at beginning and during the experiment decreased to have much lower values, as 0 - 20 ppm.

According to the study of Walstad (2003), *C. demersum* specie prefers ammonium than nitrates in the environment, this is due to the absorption of ammonium is less difficult for this species. In the present experiment it was observed that both nutrients decreased and this could be due to the better plant growth in the presence of ammonium or a mixture of ammonium and nitrates, than in an environment with only nitrates.

Finally, we note that the environment was ideal for the aquatic plants used because in a short time nearly tripled its weight, starting with values of 26 grams per tank and ending with values of 72 grams per tank. Therefore, it could be said that the co-culture has great advantages in comparison with conventional growing systems with only goldfish where a higher maintenance for the system is needed; in present case for example, although a cleaning protocol was initialy defined for once a week, only once

every 2 weeks was necessary. An aquaponic integrated system has maintained the water quality suitable for the experimental fish and both species fish and plant have commercial interest and could be selled with the subsequent economic profit.

5. Conclusions

1. The slow growth of this species at higher size was confirmed, since both diets, commercial and experimental, responded with growth rates very similar still within the expected range for this species.

2. Although the condition index was similar and remained stable along the trial, there was observed a poorer appearance from fish fed the commercial diet respect to the experimental one. Further studies are needed to better adapt the quality of the diet for higher goldfish size, which should need lower lipid content while high quality fatty acid profile as suspected from the experimental formula feed diet based on fish oil as sole lipid source.

3. It is claimed that at the first done the fishes ingested more food.

4. The observed declines of nitrites, nitrates and ammonium in the system with the co-culture of goldfish and *C. demersum*, made a suitable environment for the culture of both species, thus reducing effort and energy imput into the system. Reduction of cleaning effort and lowering water removal from once a week to one time every two weeks was required.

5. The observed decrease in the amounts of nitrites, nitrates and ammonium, from the beggining of the trial may represent that the number of fish in the system were not high enough for the weight of plants used. Thus, although the co-culture has increased the water quality for the fish while gain a 273.3% growth for the plants, further studies are needed to better adjust the proper biomass of goldfish and the plants at the initial of the trials for the next experiences.

6. This experiment represented the first one carried out in GIA facilities with the goldfish as a fish model for aquarium feed research, being demonstrated the viability of the tested diet respect to a commercial one. Moreover, the opportunity for the co-culture of the two tested species tested was confirmed under experimental scale and it could be implemented in future experiences.

6. Acknowledgments

To the Universidad of Las Palmas de Gran Canaria for give me the opportunity to perform this work. Also to the Aquaculture Research Group (GIA) for allows me to make this study.

Concerning the development of the work, I wish to express my gratitude to my tutors Lidia Robaina Robaina y Lucía Molina Domíuez because they are helping when I needed. I would also like to pay special tribute to Ángel Segade for the help he provided me, throughout all the work Also to my partners at university and my family, because they are who have been supporting me all this time at the university.

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Memoria final del Trabajo Fin de Grado (TFG)

GRADO EN CIENCIAS DEL MAR. ASIGNATURA: 40630 - Trabajo Fin de Grado

Año Académico: 2015/2016 Alumno: Gustavo J. Santana García

1. Descripción detallada de las actividades desarrolladas durante la realización del TFG

La realización del TFT fue en el Parque Científico Marino de la Universidad de Las Palmas de Gran Canaria, localizada en el municipio de Telde (Taliarte), junto el Grupo de Investigación de Acuicultura (GIA), a cargo de las dos tutoras Lidia Robaina Robaina y Lucía Molina Domínguez, donde a su vez también tuve una gran ayuda por parte el recién doctorado Ángel Segade Botella. El comienzo del experimento fue el día 17 de noviembre de 2015, tras conseguir las dos especies objetivo del mismo, 24 golfish (*Carassius auratus*) y 72 g de coontail (*Ceratophyllum demersum*).
Las actividades desarrolladas durante la estancia de prácticas fueron las siguientes:

- Explicación detallada de los sistemas pertenecientes a los acuarios donde se iba a realizar el experimento. Sistema de filtración biológico y físico, sistema de esterilización ultravioleta, sistemas de aireación, etc.

- Limpieza de todo el tren de acuarios, donde se hizo un saneamiento exhaustivo de los mismos con el fin de lograr un hábitat impecable para las especies. Se utilizaron diferentes técnica de limpieza.

- Realización de las diferentes tablas de datos a ordenador con el fin de lograr una organización de trabajo y no perder ningún dato obtenido.

- Los golfishs se obtuvieron del mismo centro, donde fueron aclimatados y alimentados un mes antes, para su introducción en los acuarios se hizo una selección aleatoria, donde posteriormente fueron pesados y medidos todos los especímenes y apuntados los datos en la tabla pertinente.

- En el caso de la planta acuática, las cuales se obtuvieron por un proveedor local especializado, se introdujeron tras ser pesadas en los acuarios superiores

intentando lograr una equidad de gramos en los mismos y posteriormente introduciendo los datos en la tabla habilitada para ella, las mismas se introdujeron una semana más tarde logrando de este modo un medio idóneo para ellas.

- Tras la puesta en funcionamiento del experimento, se hicieron análisis de agua dos veces por semana con la finalidad de obtener diferentes parámetros del agua, apuntando dichos datos.

- Estudio de diferentes dietas, obteniendo la mejor dieta comercial para la especie y comparándola con una experimental que ya había tenido buenos resultados con tilapia (*Oreochromis niloticus*). Dicha dieta experimental fue introducida en diferentes recipientes según cada acuario logrando de este modo una organización y un seguimiento de cada acuario según su ingesta.

- Suministro del alimento a los peces durante todo el experimento de tres dosis diarias (9:00, 11:30 y 14:00), 5 días a la semana (lunes a viernes). Y haciendo pesadas de cada recipiente antes y después de cada dosis para saber lo que habían consumido los peces de cada tanque, anotándolo en su lugar correspondiente.

- Limpieza de acuarios diariamente mediante sifonado antes y después de la alimentación con la finalidad de eliminar todos los desechos posibles que hubieran en el acuario.

- Limpieza una vez por semana de todos los acuarios exhaustivamente eliminando posibles organismos que hubiesen en el mismo.

- Seguimiento de los peces, la planta acuática y todos los sistemas durante todos los días observando la calidad de los mismos y evitando que hubiera ningún desperfecto e inconvenientes con los mismos.

- Búsqueda y lectura de artículos para la realización del trabajo.

2. Formación recibida

La formación recibida durante todo el periodo de prácticas fue a cargo de las tutoras y sobre todo Ángel Segade, el cual estaba doctorando en ese momento. Donde se me fue suministrada toda la información pertinente para poder lograr realizar un trabajo adecuado a lo que se esperaba.

Su información se basó en diferentes aspectos, como fueron por ejemplo la explicación exhaustiva de todos los sistemas para que pudiera comprender el por qué de su introducción y su funcionamiento dentro del sistema. Además, fueron proporcionadas técnicas de trabajo eficientes con las cuales era más fácil el manejo de todo el material usado, como por ejemplo la colocación de diferentes aireadores, colocación de rejillas en los desagües de los acuarios, la limpieza de los mismos, etc.

Por otro lado, durante todo el período de realización del trabajo se me fue suministrada mucha información relevante con mi trabajo con el fin de abrirte la mente e introducirte a su vez en el entorno de la acuicultura.

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3. Nivel de integración e implicación dentro del departamento y relaciones con el personal.

Desde el principio el trato de todo el personal fue agradable por lo que me fue muy fácil integrarme en el lugar. Al principio el trato era quizás más cordial, pero a lo largo del tiempo fue cada vez más amistoso incluso llegando a poder decir que me llevo grandes amigos del centro.

Siempre contaron conmigo para todos los proyectos que pudiesen surgir sintiéndome como uno más del grupo, dando opinión, dando soluciones, etc. El buen ambiente que había en el lugar consiguió que no hubiese ningún tipo de problema a nivel de conducta ni de trato personal.

4. Aspectos positivos y negativos más significativos relacionados con el desarrollo del TFG

En este apartado solo tengo aspectos positivos, ya que no me surgió ningún problema a la hora de realizar mi trabajo fin de grado, pero si una propuesta de mejora.

La propuesta de mejora va hacia el grado de Ciencias del Mar, donde creo que hay demasiada teoría y poca práctica. Creo que saldríamos mejor preparados se realizaran más prácticas en los diferentes campos, ya que a la hora de empezar mi trabajo fin de grado me vi un poco perdido en el tema. A pesar de esto, solo tengo buenas palabras a la hora de hablar sobre el desarrollo de mi TFG.

El aspecto positivo que más puedo resaltar es el clima de trabajo en el lugar, la disponibilidad tanto de los tutores como del personal de lugar a la hora de resolverme alguna duda o solventar cualquier problema que pudiera surgir, ofreciéndome toda la ayuda que fuera posible. Además de la ayuda mutua que reciben entre ellos con la finalidad de alcanzar una meta.

Otro aspecto positivo, es la disponibilidad de recursos y herramientas para poder trabajar, facilitando de este modo el trabajo a realizar.

También es de mención el aprendizaje que me fue suministrado durante todo el periodo de realización del trabajo, donde además de afianzar conocimientos adquiridos a lo largo de la carrera, he aprendido grandes aspectos de la acuicultura tanto a nivel nacional como mundial. Donde a su vez he logrado aprender la forma de trabajo y organización de un grupo de investigación.

5. Valoración personal del aprendizaje conseguido a lo largo del TFG.

La valoración del aprendizaje ha sido realmente positiva, ya que además de encontrarme en un lugar de trabajo con un clima muy agradable he podido aprender como es el "mundillo" de la acuicultura, y observar como trabaja un grupo de investigación.

También tener la satisfacción de hacer un buen trabajo donde se aprende ha ser organizado y cuidadoso a la hora de hacer todas las cosas, tanto cuando estás siendo ayudado a cuando estas solo.

Además de cómo dije antes, el hecho de poder llevarme amigos en mi experiencia en este lugar.