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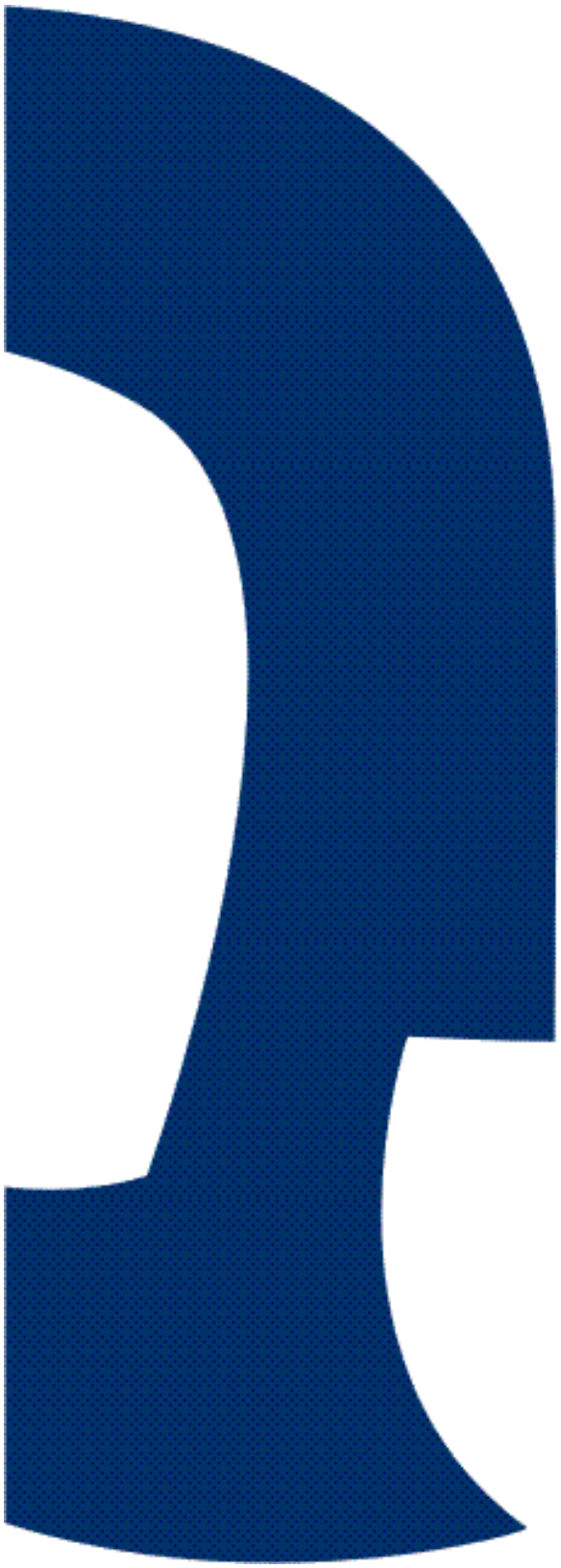

Effects of squid meal as dietary fish meal protein substitution for the aquarium fish *Amphilophus citrinellus*. Opportunities for the co-culture with *Ceratophyllum demersum*

Demetrio Plasencia Plasencia

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Lidia Esther Robaina Robaina

Lucía Molina Domínguez

Trabajo Fin de Título para la obtención del título de graduado en Ciencias del Mar

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Trabajo presentado por Demetrio Plasencia Plasencia para la obtención del título de graduado en Ciencias del Mar en la Universidad de Las Palmas de Gran Canaria y dirigido por la Doctora Doña Lidia Esther Robaina Robaina, del Grupo de Investigación en Acuicultura, como tutora y la Doctora Doña Lucía Molina Domínguez, del Instituto Universitrario Ecoaqua, como cotutora.

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Abstract

There exist no information regarded specific diets for the Midas species, although is an important species in acuariophilia. The main objective of the present trial was to test four diets for the *Amphilophus citrinellus* (Midas cichlid) , formulated with increased percentages of squid meal instead of the fish meal protein (Control 100/0; D1 75/25; D2 50/50; D3 25/75).

The aquaponics consists of a sustainable recirculated system for the co-production of plants and fish. It is an emerging food production system in Europe, and also becoming the interest in aquarium fish production in the world. In the present experiment the co-culture of the Midas cichlid and the aquatic plant *Ceratophyllum demersum* (coontail) was studied. The coontail co-culture was stablished to moreover determine the plant benefits on the culture system and the growth and opportunities for produce both species at same time.

After 60 days of the experiment, only statistical differences between the different diets were observed in fish length, being the diets with the highest percentage of squid meal those which presented the highest values. Besides, the biochemical results revealed that in the diets with higher percentage of squid meal, the fish presented a higher percentage of lipids, whereas in those that predominated the fish meal was higher the percentage of proteins. Regarding the plants, it showed a rapid growth rate, reaching a 310.4% increase in their weight after 60 days while reducing the nitrates of the system by 50% in a single week. Results showed that for Midas cichlid, Diet 3 (75% squid meal/ 25% fish meal) presented higher fish length even compared to those fish fed with 100% fish meal protein, in turn, the plants effectively reduced nitrate water resulting in a thriving environment for fish growth.

Keywords: Midas cichlid, Coontail, Co-culture, Aquaponics.

1. Introduction

The generic terms for ornamental species describes all aquatic organisms used in aquariums, including fish and invertebrates such as molluscs, crustaceans, echinoderms, corals and live rocks (rocks encrusted with a variety of organisms) (Pomeroy *et al*., 2006; Panné-Huidobro and Luchini, 2008).

The ornamental aquatic organisms sector remains one of the most popular hobbies in developed countries, and also, in recent years, in developing countries, representing a great opportunity for economic growth in rural communities (Panné-Huidobro, 2010). The largest markets for ornamental fish are North America, Europe and Japan, while the main suppliers of this product are the developing countries of the tropics (Wabnitz *et al*., 2003; Veron *et al*., 2011). In the last decade, global exports in value have had an annual growth rate of 6.2% being in the year 2000 of the order of 176 million dollars, reaching 342 in 2010 (Tissera, 2012).

Most of the ornamental business focussed on freshwater species however marine aquarium trade has encreased steadily on the last three decades. The international market for marine aquarium is dominated by fish, with an average annual production volume of approximately 20 million of tropical fish (Lango *et al*., 2012).

Among the main exporters in the world are the Maldives, Vietnam, Sri Lanka, Singapore and Hawaii, being Singapore the largest exporter of tropical ornamental fish worldwide. Other suppliers that determine a large share of wild freshwater fish from the Amazon and other Central and South American countries are Brazil, Colombia and Peru, while in Africa they are Congo, Nigeria and Malawi and in Southeast Asia, Thailand and Indonesia (Livengood and Chapman, 2007; Monticini, 2010). The following figure (Figure 1) shows an example of a supply / trade chain of live ornamental organisms, among all sectors and systems concerned.

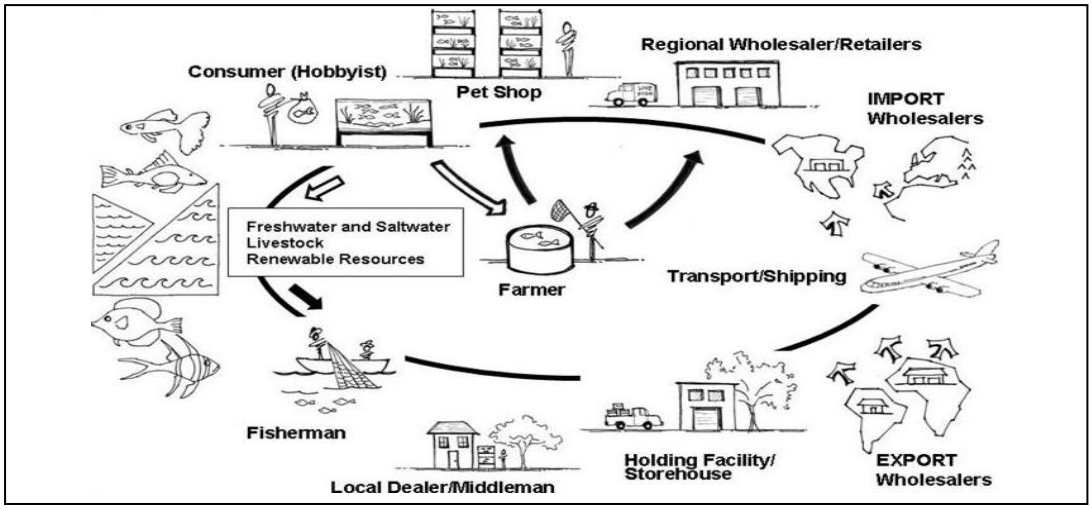

Figure 1. Scheme of a supply / trade chain of ornamental organisms (Livengood and Chapman, 2007).

In the case of aquatic plants, there is also a great variety for the aquarium business, the most popular species are *Ceratophyllum demersum*, *Limnophila sessififlora* and *Microsorum pteropus*, and the genera Anubia, Hygrophilas, Vallisneria, Echinodorus and Cryptocorynes (Hiscock, 2003).

Aquatic plants are those that have adapted to live in aquatic environments, both salt water and fresh water. In the tropical zone, the most abundant species belong to the families Podostemaceae, Hydrocharitaceae, Limnocharitaceae, Mayacaceae, Xyridaceae, Eriocaulaceae, Pontederiaceae and Aponogetonaceae; in temperate zones the most abundant families are Potamogetonaceae, Juncaginaceae, Sparganiaceae, Halogaceae, Elatinaceae, Callitrichaceae and Hippuridaceae (Crow, 1993); aquatic plants also found in cold areas, such as Potamogetonaceae family (Santamaria, 2002).

1.1. Midas Cichlid (*Amphilophus citrinellus*)

Cichlids are economically important fish, either as food (Nile tilapia) or as ornamentation (Midas), and all exhibit territorialism and aggressive food behaviors depending on the availability of food and space (Rodríguez and Dabrowski, 2014).

The species *Amphilophus citrinellus* (Midas cichlid) (Figure 2), family Cichlidae (Order Perciformes), is one of the most popular aquarium species worldwide. Males of this species can reach sizes close to 30 cm, it presents a very characteristic protuberance on their head, while females, in case of having it, is smaller.

Figure 2. Midas Cichlid (*A. citrinellus*),
from www.aquaterraria.com

The Midas cichlid is original from Central American Atlantic coast, more specifically found in Managua, Jiloá, Masaya and Apoyo lakes (Nicaragua) and the San Juan river basin (Costa Rica) (Martínez-Sánchez *et al*., 2001).

1.1.1. Coloration

Most Midas cichlids present a grayish color with black markings, being this color known as “normal”. The more colorful morphs start life as normals, but lose their typical markings at various ages and become white, yellow, orange, or even reddish. Some individuals have dark brown or black blotches, and some are mixtures of the various colors. The yellow through orange morphs are called "gold". Golds cannot change their markings after they have metamorphosed, although their colors intensify when they breed. The advantages and disadvantages of being of a particular color are said to depend on the relative and absolute frequencies of the various morphs, as well as on the colors of the environment in which they occur. In some species, polychromatism is thought to occur as a form of mimicry; in others, it may be related to climatic factors (Barlow, 1976a).

1.1.2. Aggregations

A noteworthy difference in behavior between aggregations of Midas cichlids in nature and those in aquariums is their aggressiveness. When kept in small groups, for example from two to seven fish, in aquariums with a capacity of 100 to 400 liters, there are almost continuous battles. This situation results in injury and, ultimately, the death of weaker individuals. In contrast, the Midas cichlid is found in groups of peaceful forms in nature. Thus, under conditions of large spaces the aggressions are minimal (Barlow, 1976b).

1.1.3. Food habits

Underwater observations of Midas cichlid revealed that this species feeds in various ways. Individuals can be seen sifting gravel or sand when feeding in the open areas, or sifting the detritus taken from crannies among the rocks. In addition to these feeding movements are excavation episodes with the mouth, through which the fish perform a small hole where they deposit food as small snails and insect larvae. An examination of the intestinal contents of 29 fish collected at Masaya Lake revealed that algae are the most commonly found food, followed by insects, snails and some fish remains (Barlow, 1976b).

The very young fish that are still protected by their parents feed on the plankton that is transported to them by the soft currents of the lake. They also feed on copepods and organisms attached to the rocks (Noakes and Barlow, 1973). Consequently, the pattern obtained is that of a highly omnivorous and opportunistic animal. It begins its life like predator, but soon changes to a more omnivorous way of life, consuming appreciable amounts of vegetal matter. But, as he gets closer to his maximum size, he seems to change again and become more piscivorous (Barlow, 1976b).

1.2. Coontail (*Ceratophyllum demersum*)

Ceratophyllum demersum (coontail) (Figure 3), family Ceratophyllaceae (Order Nymphaeales), is a fast-growing submerged plant native of North America but, nowadays, has a cosmopolitan distribution in temperate and tropical regions. It is commonly seen in ponds, lakes, ditches and quiet streams with moderate to high nutrient levels. It does not produce roots, instead it absorbs all the nutrients it requires from the surrounding water. It can absorb high concentrations of elements like phosphorus and nitrogen for its growth, acting like a filter. This plant is also very effective against algae, as it feeds on the same nutrients as these and in turn produces harmful substances that inhibit its development (Foroughi *et al*., 2013).

Figure 3. Coontail (*C. demersum*) in the present trial.

1.3. Aquaponic culture systems

Aquaculture is a form of agriculture encompassing the propagation, cultivation and marketing of aquatic animals and plants, mainly for food and ornamentation.

Hydroponics by definition means “water working”. In practical use, it refers to growing plants in a water and nutrient solution, without soil. A hydroponic culture allows a farmer to grow plants in a more efficient and productive manner with less labour, less water and less fertilizer because the plants are provided with the ideal water and nutrient ratios and optimum conditions for growth.

Aquaponics is a combination of aquaculture and hydroponics. It doesn’t require soil and very less or no any chemicals to produce a large amount of fish and plants in a small space. In aquaponics, the nutrient-rich water that results from raising fish provides a source of nutrients (urea) for the nitrogen-consuming bacteria, which helps to clean the water where the fish live in by breaking down these compounds into nitrates, which then feed the plants and keeps them healthy. As such the combination of aquaculture and hydroponics help to sustain an environment in which they both can thrive (Kopsa,

2015). In short, aquaponic systems can function because fish waste is similar (but not identical) to the nutritional requirements of plants to grow and develop, helping to clean the water where the fish live (Rakocy, 2006).

In general terms, an aquaponic system consists of the following elements (Selock, 2003; Nelson, 2007): fish tank (or other aquatic organisms), clarifier (or solid filter), biofilter, tanks for the plants, water pumping and aeration systems. These elements are connected in such a way that nutrient-rich water passes from the fish tank to the clarifier, where most large and small dissolved particles are removed (Lennard, 2004). After passing through the clarifier, the flow continues to the biofilter, which has a large surface that allows it to house a large number of bacteria that convert ammonium to nitrite, and others that convert nitrite to nitrate (Walsh 1998, Rakocy, 2006). Then the flow passes to the tank of the plants where the nitrates are filtered. The water can be sent directly back to the fish tank, or first pass through a siphon that collects the water from all the tanks, then be taken back to the fish tank and restart the cycle (Ramírez *et al*., 2009) (Figure 4).

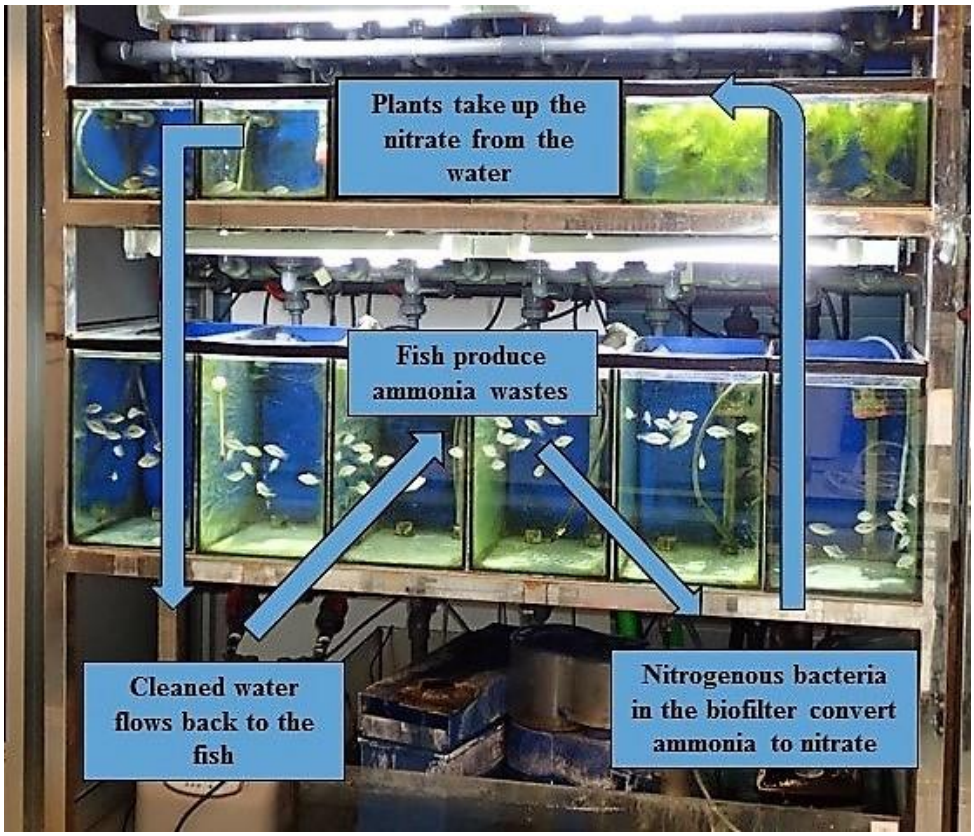

Figure 4. Scheme of a basic aquaponic system. The arrows indicate the direction of water flow through the system.

1.4. Objetives

In present experiment, cichlids of the species *A. citrinellus* (Midas cichlid) were used to measure their growth in an aquaponic system with the aquatic plant *C. demersum* (coontail), administering a series of diets with different percentages of proteins from fish meal and squid meal. Thus, this study was developed with two main objectives:

1) To better understand about diets and requirements for the growth of *A. citrinellus*, by measuring fish length, weight rates and fish biochemical analyzes at the end of the experiment.

2) To test the opportunity for co-producing *C. demersum* by measuring the growth of this plant of commercial interest at the same time that they filter the nitrates from the water, generating a benefit for both organisms and the culture system.

2. Material and methods

For the development of this experiment, the aquaculture facilities of the Taliarte Marine Science Park of the University of Las Palmas de Gran Canaria were used, specifically those belonging to the Aquaculture Research Group (GIA, in their Spanish acronym) and the Aquaculture and Biotechnology Highly Specialized Service (SABE, in their Spanish acronym), where the co-culture experiment and the biochemical analyzes of the fish were carried out, respectively.

2.1. Facilities and maintenance

For the development of the first part of the experiment the following materials have been used: 18 tanks (Figure 5); 12 tanks of 34.96 liters (23x40x38 cm) for the fish and 4 tanks of 17.48 liters (23x40x19 cm) for the aquatic plants. The tanks are located in a module supplied with fresh water and with physical and biological filtration systems (Aqua Medic Reef 500, Germany) and sterilization by ultraviolet radiation (UV) (Teco®, TR 10, Italy). The input flow to the aquariums was 30 l/h and the water was maintained, throughout the experiment, at 23.52 ± 1.31 ºC and 8.05 ± 1.13 mg/l of dissolved oxygen, with a photoperiod of 10 hours of light / 14 hours of darkness (AquaMedic© of North America) T5, 24w y 10000K.

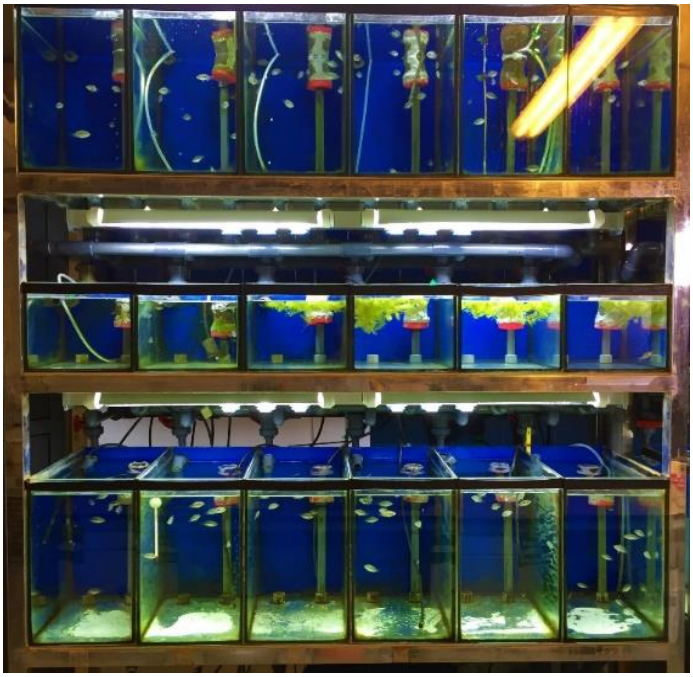

Figure 5. Freshwater aquaria module where the experiment was developed.

For the cleaning and maintenance of the tanks a siphon and brush were used daily, and for the control of water quality, the amount of nitrate in the system was determined by the Kit for the analysis of nitrates (Easy-life 5®) weekly.

2.2. Diets

The diets were formulated based on those previously used in an experiment to optimize the diet in different production stages of another aquarium fish species, the false clown fish (*Amphiprion ocellaris*) (Espinosa, 2014). For both experiments, 4 isoprotein and isolipidic inert diets were used, containing fish meal and defatted squid meal as dietary protein sources and fish oil (Peruvian anchoveta) as the only added lipid source. These diets differ only in the proportions of fish meal and squid meal protein.

The four experimental diets used in the study and their respective replicated tanks were: Control Diet (Tanks 1, 2 and 3), Diet 1 (Tanks 4, 5 and 6), Diet 2 (Tanks 7, 8 and 9) and Diet 3 (Tanks 10, 11 and 12). The produced pellet size was 1.4 mm diameter with the following composition (Table 1):

Ingredients (%)	Diets			
	Control	D1 (75/25)	D2 (50/50)	D3 (25/75)
Fish meal (FM)	50	38	25	13.5
Squid meal (SM)*	0	11	20.5	31.2
Corn	5	5	5	5
Soy 47	12	12	12	12
Wheat	12	11.4	9	11
Wheat gluten	6	5	6.4	5.1
Corn storch	4.5	6	9.3	8.4
Fish oil	4	5.1	6.3	7.3
Mix of vitamins	2	2	2	2
Mix of minerals	2	2	2	2
Carboxymethylcellulose	0.5	0.5	0.5	0.5
Alpha-cellulose	2	2	2	2

Table 1. Percentage of the ingredients of each diet * (SM) defatted squid meal.

2.2.1. Preparation of experimental diets.

The four inert diets were elaborated in the the Aquafeed and Processing Pilto Plant at the Scientific Marine Science Park, Las Palmas de Gran Canaria, Spain.

The main steps for pellet production were the following:

1) Prepare the mix of water-soluble vitamins and minerals; 2) Prepare the mix of fat-soluble vitamins; 3) Mix all dry ingredients; 4) Slowlly mix of the fat-soluble vitamins in the oil until a homogeneous mass was obtained; 5) The resulting mixture was passed through a granulator (CPM Mod. CL3, USA) and the grains obtained were air/oven dried at 30°C for 12 hours; 6) The resulting dry pellets were passed through a mechanical sieve (AS 200 Retsch®, Germany) to obtain the desired grain size; 7) Store the resulting feeds in a well closed bag at 4°C until use.

2.3. Experimental species

A total of 144 fish of the species *A. citrinellus* (Midas cichlid) produced in Taliarte were used to perform this experiment, being their growth measured in an aquaponic system with the aquatic plant *C. demersum* (coontail).

The fish were distributed in 12 tanks (12 individuals per tank), selecting those specimens with a weight between 0.30 and 0.60 g with an obtained average length of

3.17 ± 0.26 cm. The coontail plants, obtained from a local supplier, were distributed in 4 tanks (20 grams per tank) after one month, when the determined amount of nitrates in the system was adequate (around 160 ppm).

2.4. Fish feeding and samplings

During the first week of the experiment the fish were fed 3 times per day (at 9:00, 11:30 and 14:00) to apparent satiation with their respective diets. From second week to the end of the trial feeding was reduced to 2 times per day (at 9:00 and 14:30), to better adjust the total feed intake. Fish weight were recorded every 15 days after fish fastening during 24h, and the respective fish length once a month; an ictiometer and a digital balance (Mettler PE 400) were used (Figure 6), making a total of 5 sampling (including the initial sampling), 2 to measure only the weight and 3 to measure weight and length. The plants were also weighed every 15 days using the same method from the time they were introduced into the tanks (one month later than the fish).

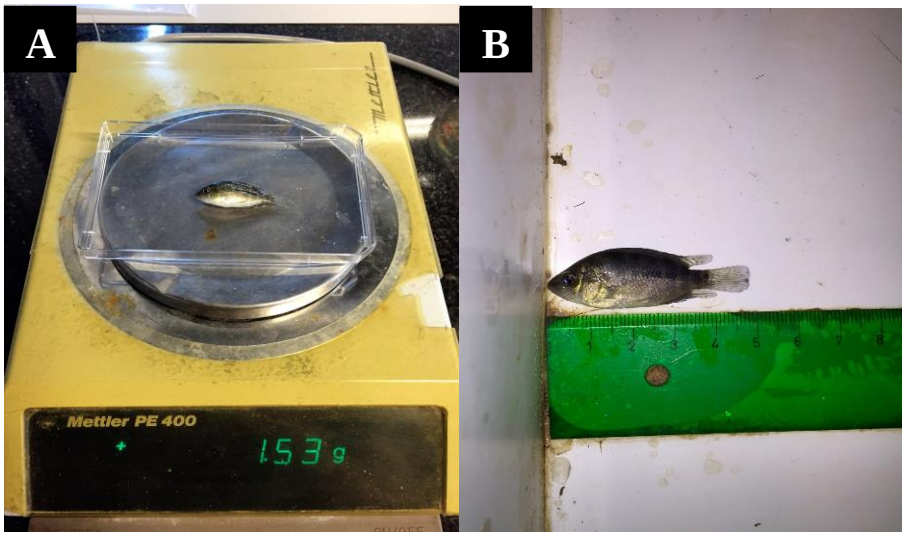

Figure 6. Midas cichlid weighed on digital balance (A), and lemgth measured with ictiometer (B).

2.5. Growth and feed utilization parameters

To compare the effectiveness of the different diets, the following parameters were calculated:

- Specific Growth Rate (**SGR**) (%/day) = 100 x (ln final mean weight – ln initial mean weight) / days. A term used in aquaculture to estimate the daily growth of the fish after a certain period.

- Feed Conversion Rate (**FCR**) = Feed intake (g) / Biomass gained (g). The FCR represent the necessary amount of feed to grow a kilogram of fish.
- Protein Efficiency Ratio (**PER**) = Weight gain (g) / Protein intake (g) x Number of fish. PER is the ratio of grams of body weight gain (in specified time) to the grams of protein consumed.
- **Survival** (%) = 100 x (final number of fish / initial number of fish).
- **Feed intake** = Amount of feed consumed over a period of time.
- **Daily intake** (%body weight/day) = 100 x Feed intake (g) / Weight gain (g) x days.

2.6. Biochemical analysis

The analysis was run at the SABE labs to determine the diets and fish biochemical contents. The samples to be analyzed were obtained by crushing 5 initial fish and 5 of the 12 fish of each tank (3 tank samples per diet). The following biochemical analysis were carried out:

2.6.1. Total lipids

The method used for the extraction of total lipids was that described by Folch *et al.* (1957), using a mixture of chloroform-methanol (2:1 v/v) containing 0.01% BHT (Butylated hydroxytoluene). Once the solvent is evaporated with a stream of nitrogen, the lipids were weighed and stored under nitrogen and dissolved in chloroform to prevent oxidation. The data were obtained from the following formula: % Lipids = g Lipids / g Sample x 100

2.6.2. Protein

Proteins were calculated from the total nitrogen composition of the samples as determined by the Kjeldahl technique (AOAC, 1995). This method involves the digestion of samples with concentrated sulfuric acid at 400°C in the presence of a copper catalyst, followed by distillation. The data were obtained from the following formula: % Proteins = ((ml HCl sample - ml HCl white) x 0.1 x 14.007 x 6.25 x 100) / Sample weight in mg. Where 0.1 = Normality of hydrochloric acid; 14.007 = Atomic weight of N_2; 6.25 = Protein factor.

2.6.3. Ash

The ash content was determined by incineration of the sample in a muffle furnace at a temperature of 600°C to constant weight (AOAC, 1995). The data were

obtained from the following formula: % Ash = 100 x (C-A) / (B-A). Where A = Crucible weight; B = Crucible weight + simple; C = Crucible weight + Ash.

2.6.4. Moisture

The moisture of the samples was determined by oven drying at 110°C to constant weight (AOAC, 1995). The data were obtained from the following formula: % Moisture = 100 x (B-C) / (B-A). Where A = Weight of weighing bottle; B = Weight of the weighing bottle + Wet simple; C = Weight of the weighing bottle + Dry sample.

2.7. Statistical analysis

Throughout the experiment the data were saved in Excel from which the necessary graphs and means of weight and length were obtained to later obtain the statistics parameters from the program SPSS using the HSD of Tukey Test.

3. Results

3.1. Midas cichlid (*A. citrinellus*) growth

After 60 days of experiment, statistical differences in length between different diets could be observed, with the highest values for fish fed on Diet 3. As for weight, differences were observed up to day 45 only between the Control Diet and Diet 3, but these disappeared at the last sampling (day 60) (Table 2).

Measures	Control	D1 (75/25)	D2 (50/50)	D3 (25/75)
W0	0.45 ± 0.09 ^{a}	0.43 ± 0.09 ^{a}	0.43 ± 0.09 ^{a}	0.45 ± 0.09 ^{a}
W15	0.68 ± 0.17 ^{b}	0.68 ± 0.13 ^{b}	0.71 ± 0.15 ^{ab}	0.73 ± 0.15 ^{a}
W30	0.87 ± 0.20 ^{b}	0.94 ± 0.22 ^{ab}	0.96 ± 0.22 ^{a}	1.00 ± 0.23 ^{a}
L30	3.69 ± 0.28 ^{b}	3.73 ± 0.31 ^{b}	3.76 ± 0.35 ^{b}	3.88 ± 0.31 ^{a}
W45	1.25 ± 0.40 ^{b}	1.3 ± 0.37 ^{ab}	1.32 ± 0.35 ^{ab}	1.42 ± 0.38 ^{a}
W60	1.58 ± 0.59 ^{a}	1.59 ± 0.50 ^{a}	1.61 ± 0.49 ^{a}	1.77 ± 0.47 ^{a}
L60	4.50 ± 0.58 ^{ab}	4.48 ± 0.51 ^{b}	4.54 ± 0.45 ^{ab}	4.70 ± 0.47 ^{a}

Table 2. Weight (g) and length (cm) of Midas cichlids fed on the experimental diets along the trial and statistical results (mean ± standard deviation). Different letters in same row means significant differences ($P<0.05$).

3.1.1. Midas cichlid weight

At the beginning of the experiment, fish showed a weight of 0.44 ± 0.01 g, being increased after 60 days as follows (Figure 7): a) The fish of the Control Diet weighed 1.58 ± 0.59 g, having increased their body weight by 251.11%; b) the fish of the Diet 1 weighed 1.59 ± 0.50 g, having increased its body weight by 269.77%; c) the fish of the Diet 2 weighed 1.61 ± 0.49 g, having increased their body weight by 274.42%; d) the fish of the Diet 3 weighed 1.84 ± 0.47 g, having increased its body weight by 308.89%.

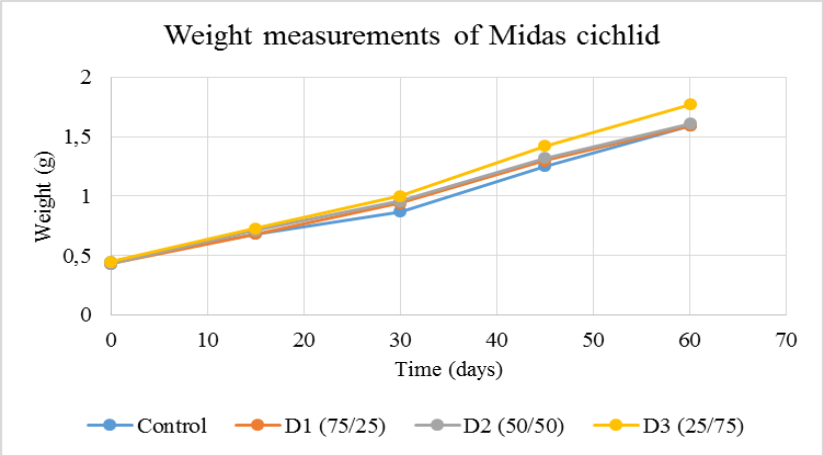

Figure 7. Weight measurements of Midas cichlids along the experiment.

3.1.2. Midas cichlid length

In terms of length, the initil fish measured 3.17 ± 0.26 cm. After 60 days, this length increased, as follows (Figure 8): a) The fish of the Control Diet measured 4.50 ± 0.58 cm. having increased its length by 41.96%; b) the fish of the Diet 1 measured 4.48 ± 0.51 cm. having increased its length by 41.32%; c) the fish of the Diet 2 measured 4.54 ± 0.45 cm. having increased its length by 43.22%; d) the fish of the Diet 3 measured 4.70 ± 0.47 cm. having increased its length 48.26%.

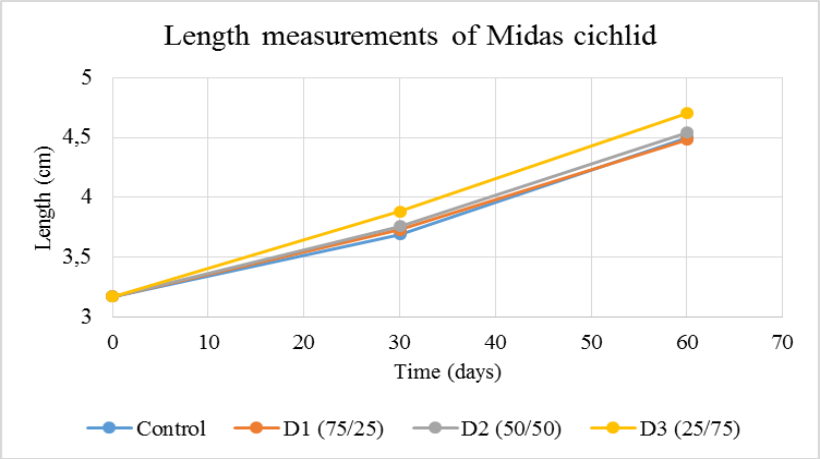

Figure 8. Length variation of Midas cichlids fed on the experimental diets along the trial.

3.1.3. Growth parameters and survival

In all diets, general lowering of the feed intake (Table 3) was observed from day 0 to day 30 of the experiment, thereafter some general increase for all diets till day 60; total feed intake per tank ranged between 2.47 to 4.30 for the different periods. The fish from Diet 3 consumed some higher amount of feed throughout the study in same way as for the daily intake (Table 4), being the range of the daily feed intake between 5.24% and 9.13%.

Time (days)	Feed intake (gr feed/tank)			
	Control	D1 (75/25)	D2 (50/50)	D3 (25/75)
0-15	3.89 ± 0.11	3.70 ± 0.03	4.20 ± 0.32	4.11 ± 0.29
15-30	2.48 ± 0.16	2.47 ± 0.22	2.65 ± 0.28	2.83 ± 0.24
30-45	3.55 ± .028	3.50 ± 0.39	3.49 ± 0.13	3.96 ± 0.37
45-60	3.94 ± 0.72	3.31 ± 0.16	3.84 ± 0.49	4.30 ± 0.18

Table 3. Feed intake by Midas cichlids fed on the experimental diets along the trial (mean ± standard deviation).

Time (days)	Daily intake (%body weight/day)			
	Control	D1 (75/25)	D2 (50/50)	D3 (25/75)
0-15	9.13 ± 0.23	8.23 ± 0.33	8.36 ± 0.60	8.93 ± 0.64
15-30	7.65 ± 1.89	5.43 ± 0.32	5.93 ± 0.66	6.39 ± 0.60
30-45	5.24 ± 0.61	5.37 ± 0.33	5.50 ± 0.35	5.66 ± 0.52
45-60	6.74 ± 1.45	6.36 ± 0.37	7.37 ± 0.46	8.41 ± 0.86

Table 4. Daily intake by Midas cichlids fed on the experimental diets along the trial (mean ± standard deviation).

The Specific Growth Rate (SGR) (Table 5) was calculated with the weights obtained from the different sampling, where it can be observed that throughout the experiment the fish increased their weight significantly during the first 15 days and then continued to grow more moderately.

Time (days)	SGR (%/day)			
	Control	D1 (75/25)	D2 (50/50)	D3 (25/75)
0-15	2.84 ± 0.11	3.04 ± 0.13	3.32 ± 0.25	3.24 ± 0.19
15-30	1.61 ± 0.32	2.10 ± 0.11	2.00 ± 0.17	2.10 ± 0.12
30-45	2.41 ± 0.24	2.18 ± 0.10	2.08 ± 0.06	2.37 ± 0.10
45-60	1.58 ± 0.26	1.34 ± 0.09	1.33 ± 0.05	1.44 ± 0.09

Table 5. Specific Growth Rate (SGR) of Midas cichlids fed on the experimental diets along the trial (mean ± standard deviation).

Using the weight gained and the food consumed, the Feed Conversion Rate (FCR) (Table 6) was calculated. The highest values were observed in the first 15 days

of the experiment in all diets. At day 60, Diet 3 stood out with higher values than the rest.

Time (days)	FCR			
	Control	D1 (75/25)	D2 (50/50)	D3 (25/75)
0-15	4.11 ± 0.10	3.70 ± 0.15	3.76 ± 0.27	4.02 ± 0.29
15-30	3.44 ± 0.85	2.44 ± 0.14	2.67 ± 0.30	2.88 ± 0.27
30-45	2.36 ± 0.28	2.41 ± 0.15	2.48 ± 0.16	2.55 ± 0.24
45-60	3.03 ± 0.65	2.86 ± 0.17	3.32 ± 0.21	3.78 ± 0.38

Table 6. Feed conversion rate (FCR) of Midas cichlid for every diet along the trial (mean ± standard deviation).

Then, the Protein Efficiency Ratio (PER) was obtained with the weight gained and the protein consumed (Table 7). Similar values between diets were obtained during the entire experiment, being the lowest those for the first 15 days, according with the observed higher feed intake for this period.

Time (days)	PER			
	Control	D1 (75/25)	D2 (50/50)	D3 (25/75)
0-15	0.55 ± 0.01	0.61 ± 0.02	0.61 ± 0.04	0.57 ± 0.04
15-30	0.83 ± 0.18	1.13 ± 0.07	1.03 ± 0.11	0.94 ± 0.09
30-45	1.05 ± 0.13	1.02 ± 0.06	0.99 ± 0.06	0.97 ± 0.09
45-60	0.81 ± 0.20	0.83 ± 0.05	0.72 ± 0.04	0.63 ± 0.06

Table 7. Protein efficiency ratio (PER) of Midas cichlids fed on the experimental diets along the trial (mean ± standard deviation).

Survival (Table 8) was 100% in 3 of the 4 diets used.

SURVIVAL %	Control	D1 (75/25)	D2 (50/50)	D3 (25/75)
	100	100	100	83.33

Table 8. Survival of Midas cichlids fed on the experimental diets along the trial.

3.1.4. Biochemistry of diets

Biochemical analysis of the different diets are shown in (Table 9):

	Diets			
Biochemical composition (%)	Control	D1 (75/25)	D2 (50/50)	D3 (25/75)
Proteins	40.79 ± 0.32	41.54 ± 0.11	40.87 ± 0.20	42.05 ± 0.11
Lipids	10.59 ± 0.08	10.34 ± 0.08	10.25 ± 0.21	9.58 ± 0.19
Ash	8.64 ± 0.06	7.57 ± 0.07	5.86 ± 0.04	4.92 ± 0.03
Moisture	10.87 ± 0.02	11.34 ± 0.13	12.34 ± 0.10	12.59 ± 0.03

Table 9. Biochemical results for the different diets (mean ± standard deviation).

3.1.5. Biochemistry of the Midas cichlid

As for the biochemical results obtained from the initial fish (day 0) (Table 10), a high percentage of proteins and a low percentage of lipids could be observed in comparison with the fish at the end of the study (day 60). At the end of the experiment the statistical results obtained from the biochemical analyzes performed on the fish (Table 11) revealed the existence of significant differences in lipids among all diets, with the lowest lipids percentage on those fish fed on Diet 1 and the highest for Diet 3. In proteins, a statistical difference between the Control Diet and the others occurred, being the Control Diet the one that presented the highest percentage. In moisture and ash there weren't significant differences between the diets.

Initial fish	
Biochemical composition (%)	**Day 0**
Proteins	21.44 ± 0.67
Lipids	3.88 ± 0.28
Ash	4.69 ± 0.26
Moisture	75.02 ± 1.39

Table 10. Biochemical results of the whole fish at the beginning of the experiment (Day 0) (mean ± standard deviation).

Final fish				
Biochemical composition (%)	**Control**	**D1 (75/25)**	**D2 (50/50)**	**D3 (25/75)**
Proteins	21.20 ± 0.29 ^{a}	18.35 ± 0.58 ^{b}	17.89 ± 0.34 ^{b}	17.19 ± 0.70 ^{b}
Lipids	5.71 ± 0.24 ^{c}	4.67 ± 0.29 ^{d}	6.64 ± 0.58 ^{b}	8.20 ± 0.06 ^{a}
Ash	4.20 ± 0.28 ^{a}	4.52 ± 0.58 ^{a}	4.68 ± 0.33 ^{a}	3.95 ± 0.25 ^{a}
Moisture	72.21 ± 0.90 ^{a}	72.98 ± 1.81 ^{a}	72.23 ± 0.67 ^{a}	70.93 ± 0.49 ^{a}

Table 11. Biochemical results of the whole fish analysis at the end of the experiment (Day 60) (mean ± standard deviation). Different letters in same row means significant differences ($P<0.05$).

3.2. Coontail (*C. demersum*) growth

In the case of aquatic plant coontail, starting at about 20 g per tank, there was observed a significantly higher growth in tank 3, reaching a weight of 62.08 g that means an increase of 310.4%; conversely, in the rest of the tanks, this growth occurred in a much more moderate way, reaching a weight of 33.52 g at best, that means an increase of 167.6% (Figure 9).

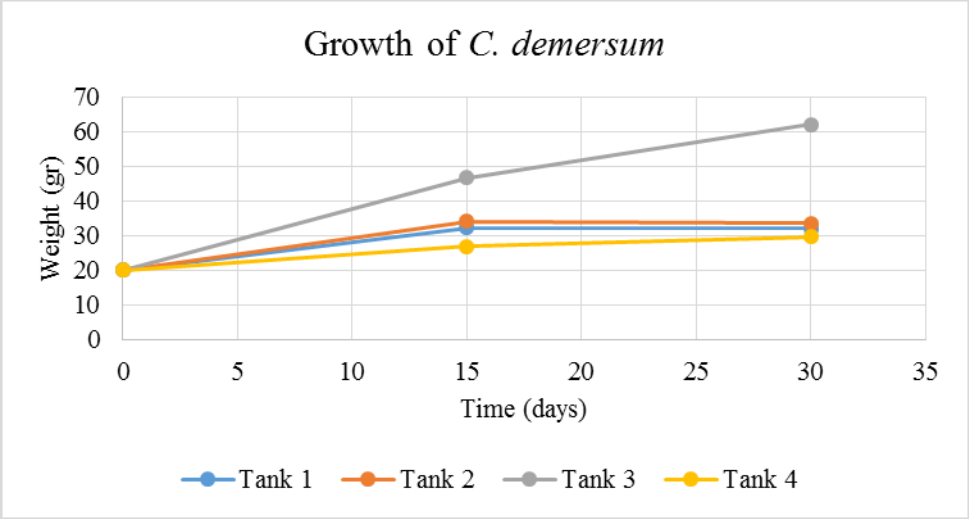

Figure 9. Growth of *C. demersum* in the four tanks along the experiment.

When plants were introduced into the aquaria the nitrates concentration in the water had values of 160 ppm (mg/l). This concentration was reduced to 30 ppm in 14 days and to 20 ppm in 28 days. (Figure 10).

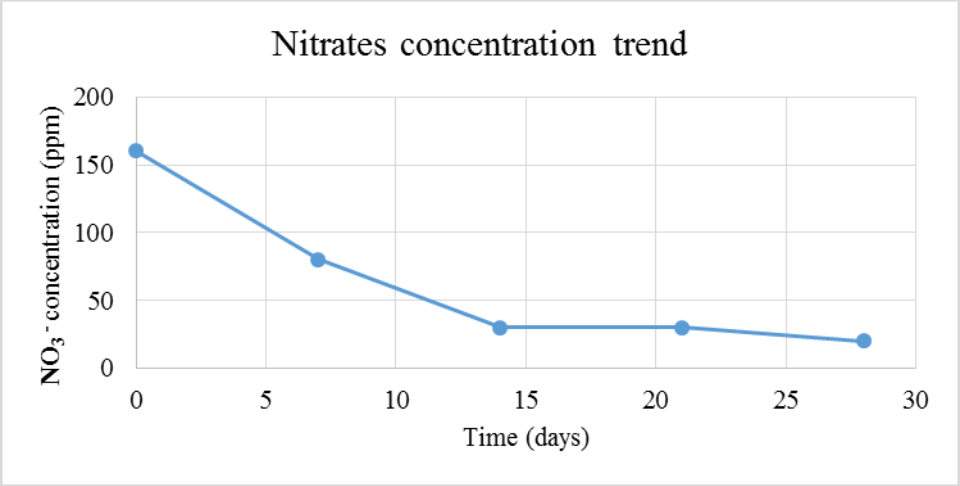

Figure 10. Culture nitrates content during the experiment in the co-culture of Midas cichlid and *C. demersum*.

4. Discussion

To our knowledge no references have been found for the target species studied (*A. citrinellus*) under controlled culture conditions, although it is one of the most popular species for aquarium purpouses. All tested diets, both the Control based on fish meal, and those containing squid meal, were well accepted by the animals from the beginning of the trial, no difference or adaptation period were needed. Under the experimental conditions, it was convenient to reduce the number of daily intakes from 3

to 2 times per day, in order to reduce the loss of feed and to better adjust the animal daily feed intake. The observed growth rates for the Midas cichlid were high throughout the experiment, around 2.19% per day, compared to the results reported by Espinosa (2014) for the false clownfish (*Amphiprion ocellaris*), under the same conditions of culture and the same formula feed. There was also obtained a high survival rate with values around 100%, the lowest values (83.33%) for Diet 3 were not a consequence of diet, but a failure in the aquarium filter.

It was noticed no differences in fish colour as a consecuence of different formulation; the colors that characterize this species was not observed in the trial so fish may need longer size to demonstrate this colour changes.

Throughout the experiment it was evident that the increasing inclusion of squid meal promoted a greater growth both in weight and length of the organisms, being in the diets with greater percentage the highest values. It should be noted, however, comparing the results obtained from the different diets once the experiment was completed, that there were no statistical differences in relation to weight on the last day of the experiment. This is mainly due to the dominance of some fish observed for Diet 3, which became more noticeable during last 15 days, thus increasing the weight standard deviations for this diet and causing that statistical differences observed in the previous sampling disappear. In terms of length, we can observe statistical differences between Diets 1 and 3, being also in this case Diet 3 which presented the highest values, both day 30 and day 60.

In the experiment performed by Espinosa (2014), the results did not reveal significant differences between the diets, except for the parameters gain weight and SGR in males (between 90-120 days of experiment), in which higher values were observed using Diet 1 (25% SM) and lower with Diet 3 (75% SM). In the results obtained for individuals in pre-reproductive stage the highest values of length were observed in Diets 1 and 3. As for the weight, in Diets 1 and 3 obtained higher results in the females while in the males they gained more weight with the Control Diet the first 60 days, and with the Diets 1 and 3 the rest of the experiment.

In general, the fish throughout the experiment had a positive growth pattern, with a weight gain between (0.21 and 0.43) g every 15 days, a gain in length about 0.70 cm each month, an SGR between (1.33 and 3.32) %/day, a FCR between (2.36 and 4.11) and a PER between (0.55 and 1.13). These results are generally better than those reported by Espinosa in 2014, being the only reported data for a species close to the one tested in present trial.

Regarding the biochemical results of the whole fish, three of the highest percentages coincided with Espinosa's experiment (2014). These were; proteins in Control Diet, lipids in Diet 3 and ash in Diet 2. In contrast, the highest percentage of moisture in the Midas cichlid was given in Diet 1, whereas in clownfish it was given in the Diet 2. It was observed an increase of the lipid content in final fish respect to the initial ones as is normaly observed in all culture fish species. Moreover, an increment of the fish lipid content while lowering in the proteins was obtained as the amount of squid meal increased in the diets, which could be related to the better quality of the squid meal respect ot the fish meal with more disponible energy for the fish in the first case, thus fovouring a higher lipid deposition.

As a summary of the obtained results it could be said that squid meal is a beneficial ingredient for the Midas cichlid species in same way as it was found for the false clownfish, being even better in the case of Midas cichlid. This benefit has been observed in other species of culture because the squid meal has an amino acid profile closer to the nutritional requirements of fish than those of the fishmeal (ARRAINA, 2016). In fact, some specific feed for commercial aquaculture includes nowadays small amounts of squid meal for animals during the weaning and reproduction stages (Fernández-Palacios *et al*., 1997), even though the squid meal is more expensive than the fish meal.

Among the few experiments carried out with aquarium species, Gonzáles (2011) conducted an experiment to determine the influence of four inert diets on the growth of the dwarf cichlid *Apistogramma eunotus*. A total of 120 specimens of *A. eunotus* were distributed in groups of 10 individuals and placed within 12 aquariums. The fish were fed a feeding rate equivalent to 6% of the biomass presented in each aquarium daily for 77 days. The growth of *A. eunotus* in terms of final weight was significantly influenced ($P < 0.05$) by the flake NutraFin® diet, which presented a significantly high percentage of carbohydrates respect to that in present experiment.

In relation to the water analysis, a reduction of nitrates was observed from the moment than the plant *C. demersum* was introduced into the system, reducing nitrates by 50% in the first week. Similar reduction results were described by Foroughi (2013), in a study focusing on nitrogen removal by *C. demersum*; treatments were performed on raw municipal wastewater (RMW), treated municipal wastewater (TMW), and diluted fresh latex (DFL). The results showed that the plant *C. demersum* reduced nitrate by more than 41.66% in the first six days.

Under the same concentrations of nitrates Santana (2016) achieved with *Carassius auratus* (goldfish) and initial 132 grams of *C. demersum* reduce nitrates to 20 ppm in 30 days. These same results were obtained with *A. citrinellus* and initial 80

grams of *C. demersum* in a same period of time. Which could mean that, in order for the plant to continue to grow properly after the experiment, a greater biomass of fish would have been required.

As for the growth of the plants, in a period of 30 days a 310.4% growth was obtained. In the experiment carried out by Santana (2016) with the same plant, growth of 273.3% was achieved in the same period of time. In both experiments there were growth problems related to the incidence of light in the different tanks, arriving in the case of Santana (2016) to have to discard one of the tanks of the final statistic. The growth of *C. demersum* was significantly faster in those tanks in which the plant received direct light; in contrast, the plants located in the aquaria where the light arrived with less intensity had a growth significantly less.

5. Conclusions

At the end of the experiment, it can be concluded that for the Midas cichlid, Diet 3 (25% fish meal/ 75% squid meal) presented better growth results (mainly in length) than those diets with higher fish meal content. In addition, an increase in the lipid content of the fish was observed when the squid meal was increased in the diets.

As for the quality of the water in the co-culture, the *C. demersum* plant reduced nitrates effectively from the moment it was added to the system, creating a suitable environment for the cultivation of Midas cichlid. Moreover plant increased its weight by up to 310.4% from the initial stocking.

6. Acknowledgments

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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: 2016/2017 Alumno: Demetrio Plasencia Plasencia

1. Actividades desarrolladas

A lo largo del Trabajo de Fin de Título (TFT), se han desarrollado una serie de actividades que pueden resumirse en los siguientes apartados:

1.1. Cultivo y mantenimiento de peces y plantas en circuito cerrado.

Alimentación del cíclido Midas (*Amphilophus citrinellus*) en circuito cerrado junto a la planta Cola de zorro (*Ceratophyllum demersum*), a la vez que se medía el crecimiento de ambos organismos cada 15 días y se realizaba un seguimiento de los nitratos presentes en el agua.

2. Mantenimiento de acuarios.

Limpieza de acuarios que implica la limpieza exterior e interior de los cristales y el sifonado del fondo.

3. Experimentación de distintas dietas en cíclidos.

Creación de distintas dietas para peces de acuario, en concreto para el cíclido Midas (*A. citrinellus*), variando el porcentaje de harina de calamar y harina de pescado presente en cada una ellas. Una vez elaboradas las dietas se pesó diariamente la cantidad de pienso ingerido por lo animales y se muestreó el peso y la talla de estos cada 15 y 30 días respectivamente.

4. Análisis bioquímico.

Determinación de cenizas, humedad, lípidos totales y proteínas, en los peces de acuario con los que se ha experimentado, mediante los siguientes procedimientos:

El contenido de ceniza se determinó por incineración de la muestra en un horno de mufla a una temperatura de 600º C hasta peso constante.

La humedad de las muestras se determinó por desecación en estufa a 110ºC hasta peso constante (AOAC, 1995).

El método utilizado para la extracción de lípidos totales fue el descrito por Folch *et al*., (1957), haciendo uso de una mezcla de cloroformo-metanol (2:1 v-v) conteniendo 0.01% de BHT. Una vez evaporado el solvente con una corriente de nitrógeno, los lípidos se pesaron y se almacenaron en atmósfera de nitrógeno y se disolvieron en cloroformo para evitar su oxidación.

Las proteínas se calcularon a partir de la composición en nitrógeno total de las muestras determinadas mediante la técnica de Kjeldahl (AOAC, 1995). El método consiste en la digestión de las muestras con ácido sulfúrico concentrado a 400º C en presencia de un catalizador de cobre, seguido de una destilación.

2. Formación recibida

La formación recibida fue llevada a cabo por los técnicos del GIA, que me explicaron su forma de operar en sus labores diarias para así yo poder realizar correctamente las actividades necesarias para la elaboración del TFT.

Cabe destacar también el estudio de los temas de acuariología que se impartían en la asignatura de fisiología de los organismos marinos del Grado de Ciencias de Mar, con los que empecé a interesarme por esta rama de la biología.

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

En el GIA se realizan múltiples tareas y todas en diferentes sectores. La variedad de experimentos que se llevan a cabo hace necesaria, en muchas ocasiones, la ayuda y colaboración de los empleados de las diferentes salas. Afortunadamente, el ambiente que se respira en el GIA es de compañerismo, con un trato cercano y abierto a la integración.

Durante mi estancia colaboré e interactué con diferentes estudiantes de ciclo medio y superior, compañeros de carrera, estudiantes de máster y empleados del GIA.

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

El hecho de tener que desempeñar actividades que, a priori, nada tienen que ver con la elaboración de mi TFT, supone una ampliación de los conocimientos generales impartidos y por lo tanto una ventaja, pues adquieres versatilidad a la hora de trabajar.

Sin embargo, centrándonos exclusivamente en el experimento personal necesario para este trabajo, cabe destacar el aprendizaje adquirido a la hora de elaborar un trabajo de investigación mediante el cual pude ampliar mis conocimientos en acuariología, que era en este caso el campo de interés. Como inconveniente a la hora de desarrollar el TFT cabe destacar el problema que me ha supuesto el tener que elaborarlo en un idioma que no domino y que no he podido practicar a lo largo de la carrera, el depender de este idioma ha provocado que se alargue en el tiempo la posibilidad de presentar el trabajo en la fecha pensada inicialmente.

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

A lo largo de la realización del TFT adquieres conocimientos y experiencias que no se obtienen en un aula. El hecho de haber pasado tantas horas diarias realizando este trabajo ha supuesto un aumento en el interés de profundizar en este campo, además de los conocimientos obtenidos durante el experimento como pueden ser; el mantenimiento y cuidado de acuarios y peces en circuitos cerrados, la elaboración de piensos experimentales y el análisis bioquímico para la cuantificación de lípidos, proteínas, humedad y cenizas en peces.

Termino el TFT habiendo mejorado mi aptitud para trabajar en grupo, teniendo la capacidad de solucionar problemas inesperados y siendo capaz de buscar, sintetizar y redactar ideas para llegar a un objetivo concreto.