DIVERSIFY

New species for EU aquaculture

Deliverable Report

Deliverable No:	D23.3	**Delivery Month:**		61
Deliverable Title	Comparison of the project's improved grey mullet grow-out feed under the different environmental and water conditions in Israel, Greece and Spain			
WP No:	23	**WP Lead beneficiary:**		P4. IOLR
WP Title:	Grow out husbandry-grey mullet			
Task No:	23.3	**Task Lead beneficiary:**		P4. IOLR
Task Title:	Compare the effect of feeding an improved grey mullet diet on the grow-out in monoculture in earthen ponds in Israel, Greece and Spain			
Other beneficiaries:	P1. HCMR	P18. CTAQUA	P26. GEI	P31. IRIDA
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Objective: Compare the effect of feeding an improved grey mullet diet on the grow-out in monoculture in earthen ponds in Israel, Greece and Spain

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1.0 Introduction

After metamorphosing from larvae to juveniles in seawater, grey mullet move to the less saline waters of estuaries and river mouths. Many countries, such as Israel, Greece and Spain, stock mullet fry and fingerlings in inland freshwater lakes, ponds and reservoirs, as they grow faster in low salinity, for commercial rearing and as a form of fisheries enhancement (culture-based fisheries). In aquaculture, grey mullet are generally grown in polyculture in semi-intensive ponds and netted enclosures in shallow coastal waters with common carp, grass carp, silver carp and Nile tilapia in brackish or freshwater. In monoculture, fish will consume the natural productivity, detritus as well as extruded feeds. However, the order of preference for these nutrient sources is unclear. It is becoming increasingly apparent that if the intensive farming of grey mullet is to succeed, then extruded diets, that are nutritionally dense and satisfy the nutrient requirements of this species, must be selectively consumed over naturally available food. In tropical areas, the growing season will last 7-8 months where the mullet will reach 0.75-1 kg. However, if kept for two on-growing seasons, the fish will reach 1.5-1.75 kg (The fish site; https://thefishsite.com/articles/cultured-aquaculture-species-flathead-grey-mullet).

The aim of the present study was to compare the grow-out of grey mullet at different densities in commercial ponds in Greece, Israel and Spain, which generally represent the range of different environmental and growing conditions existing in the Mediterranean basin. Based on the **P4:IOLR** formula, an extruded mullet grow-out feed was produced by **P31:IRIDA** for these trials. The IRIDA differed from the IOLR formula in that it was extruded instead of pelleted in a California pelleting Mill and that fish meal replaced poultry meal (ca 13% DW diet) in the diet due to European regulations that prohibit the use of poultry byproducts in animal feeds.

2.0 Materials and methods

Greece

The feeding trial was carried out by **P1.HCMR** at the farm of **P.26 GEI** (Vas. Geitonas & Co Ltd EE). Six hundred wild-caught mullets (*Mugil cephalus)* weighing 21 ± 1.4 g were distributed into six 14 m^3 grow-out rectangular cement ponds (4 and 6 individuals per m^2, 80 and 120 fish per pond, respectively) using three replications per density treatment. The cement ponds were continuously supplied with artesian bore water with a dissolved oxygen level around 8 ppm and water temperature at 18-22°C. The photoperiod followed the natural cycle of the season. The feeding ration was 2% of tank biomass and fed to the fish over 2 daily feedings (09:00 and 15:00 h), six days a week and the daily feed intake was recorded. Feeding was not performed when the farm staff observed feed wastes at the bottom of pond from the previous feeding. Monitoring of fish health and feed consumption as well as a recording of water parameters were performed daily. The feeding trial duration was 14 months and at the end of the trial, all fish from each pond were anesthetized and individually weighed (**Fig. 1**). Diet and fish samples for proximate and fatty acid analyses were taken at stocking and at the end of the experiment.

Spain

Wild grey mullet (*Mugil cephalus*) fingerlings (*ca.* 1g) were received at the facilities of **P18.Ctaqua** and acclimatized from 10 ‰ (water salinity from origin) to the 35 ‰ of the recirculation aquaculture system (RAS) water where they were grown until reaching ca. 3.5 g. At this point, they were acclimatized to 12 ‰ and to the **P31.IRIDA** 1.5 mm (3 mm length) feed they would be fed during the trial at the farm in Trebujena in southern Spain. The fish were stocked in two earthen ponds (L3 and L4), which were 1100 m^2 and 800 m^2, respectively, and at densities of 0.5 individuals/m^2 (total of 544 fish) and 1 individual/m^2 (total of 800 fish), respectively. Four samplings were performed during the trial to monitor fish performance as a function of time. These were at stocking, two intermediate samplings after 7 and 11 months and the final sampling at 18 months, where all harvested fish were individually live weighed and measured. During the 18 month trial, the fish were fed manually once in the morning to check fish feeding behavior and with automatic belt feeders for the rest of the day. It should be noted that this type of field sampling of mullet in earthen ponds is problematic since it was not possible to collect all the fish in the net due to the considerable stress it caused (**Figure 2)**, consequently the ponds were not completely emptied on the final sampling (**Fig. 3)**.

Israel

F2 grey mullet (*Mugil cephalus*) juveniles were stocked at two different densities (1 and 2 fish/m^2) in 2 plastic lined earthen ponds (each 144 m^2) at **P4.IOLR** facilities in Eilat, Israel. All fish were weighed individually at the beginning of the study and the average weight for the 1 and 2 fish/m^2 treatments were 20.72 g and 20.51 g, respectively. The ponds were fed with Red sea ambient seawater (40 ‰) at an exchange rate of 2 pond volumes/day where the temperature ranged from 18 ^oC in winter to 30 ^oC in summer. The fish were fed the 1.5 mm **P31.IRIDA** extruded pellets at ration level of 3% of pond fish biomass distributed over two daily feedings and the duration of the trial was 18 months (**Fig. 4**). The weight distribution as a percent of the population at the beginning of the study is shown in **Fig. 14** which demonstrates that the majority of the mullet population in both ponds (ca. 60%) were around 10 g.

3.0 Results and Discussion

In Greece, there were no significant differences in survival and growth performance of wild fry between the two density treatments at the end of the experiment. The survival was 72.1 ± 11.3% and 75 ± 5.3% for the 4 and 6 fish/m^2 treatments, respectively. Fish that were stocked at 4 individuals/m^2 had an average weight of 51.6 ±11.2 g, which was very similar to 51.2 ± 8.5 g of fish from the 6 individuals/ m^2 treatment and represented a growth increase of 143% in both treatments (**Fig. 5**). The frequency analysis showed that the weight range of 20-80 g represented the dominant weights of the fish population in both density treatments (**Fig. 6**). On the other hand, larger fish (80-180 g) from the 6 fish/m^2 treatment represented a somewhat larger part of the population (14 %) than the lower density of 4 individuals/m^2, which was 12 % of the population. To summarize, there wasn't any clear effect of stocking density on size distribution. At both density levels, a very poor growth of fish was observed (only ~30 g weight gain) over the 14-month feeding period. Moreover, the FCR was very high, which means inefficient feed conversion, for the 4 fish/m^2 (4.93) and the 6 fish/m^2 (4.61) treatments. The results from the proximate and fatty acid analyses of the diet as well as fish at stocking and at the end of the feeding trial showed no marked biochemical changes between fish sampled from the 4 and 6 fish/m^2 treatments (apart from some differences in liver n-3 PUFA and monoenes) and are not presented here.

Several factors may have negatively affected the fish growth such as the unsuitability of cement ponds for mullet culture, which could have led to improper feeding by the fish, the lack of natural food, palatability and potential nutrient deficiencies of feed, or husbandry practices as well.

Figure 1 Final sampling of mullet fish by HCMR's staff in GEI fish farm.

Figure 2 Final sampling in the farm (left). It is a labor intensive task and it is very difficult to empty the ponds completely (right) to harvest all the fish.

Figure 3 Set-up for the field sampling including extra oxygen supply.

Figure 4 Harvesting and live weighing of the mullet from the plastic lined 144 m^2 grow-out pond.

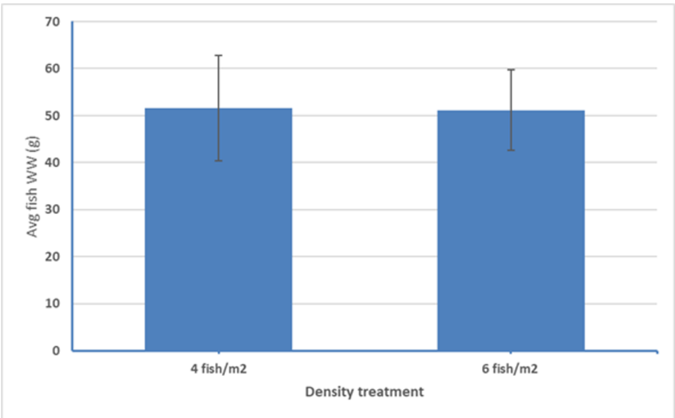

Figure 5 The effect of two stocking treatments (4 and 6 fish/m^2) on the average fish weight in Greece. N=80, 120 of the 4 and 6 fish/m^2 treatments, respectively. ANOVA of values was not significant (P>0.05).

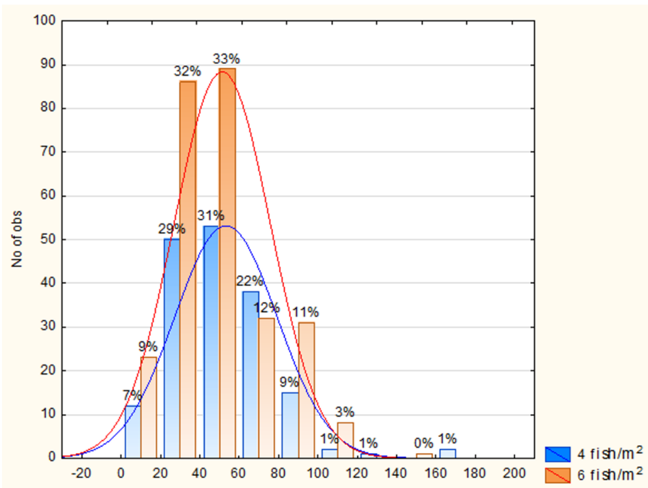

Figure 6 The effect of the two stocking treatments (4 and 6 fish/m^2) on the weight distribution in the grey mullet population of the Greek feeding trial.

Fig. 7 summarizes the Spanish results of the stocking, interim and final sample weighings of the trial. Linear regression analysis shows that the curve for growth (average fish weight with time) of the 0.5 fish/m^2 treatment is significantly ($P<0.05$) different from 0, while the linear curve for 1.0 fish/m^2 was not. Moreover, the slope or rate of growth increase of the lower density treatment is close to significantly higher ($P<0.056$) than the higher density treatment suggesting a density effect on growth which supports the IOLR's results reported in deliverable **D23.2**. **Fig. 8** shows a non-significant ($P>0.05$) growth advantage in the lower density treatment while the condition index was similar for both culture densities (**Fig. 9**), which is an indication of no differences in feeding conditions between the two ponds.

Final specific growth rate (SGR) values from the two culture conditions during the 533 days of the trial were 0.83 and 0.73%/day for the 0.5 and 1.0 fish/m^2 treatments, respectively. The FCR result was not considered reliable as the farm staff did not always load the automatic feeders every day based on their observations during the morning hand feeding. This was not in agreement with the recommendations of the scientific staff and may have modulated fish growth. Taking this into account and considering an estimate of 30% mortality (survival cannot be better estimated since the ponds were not fully harvested), the available data for calculation provides an overall FCR of 0.46 which suggests that the fish were feeding on the natural productivity of the ponds as well.

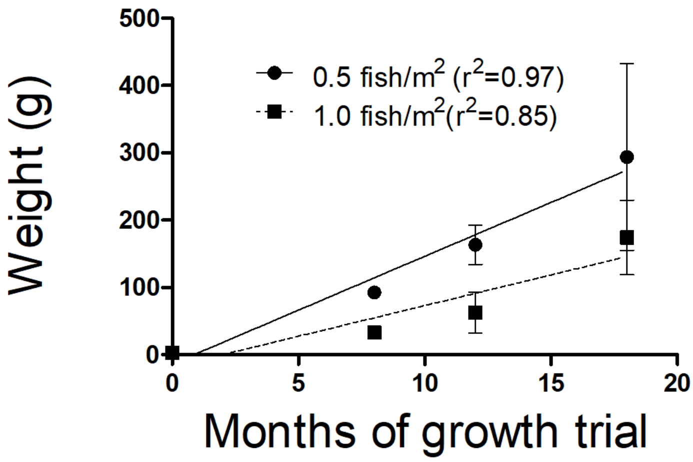

Figure 7 Linear regression of the effect of pond density (fish/m^2) on weight gain with time (months of growth trial)

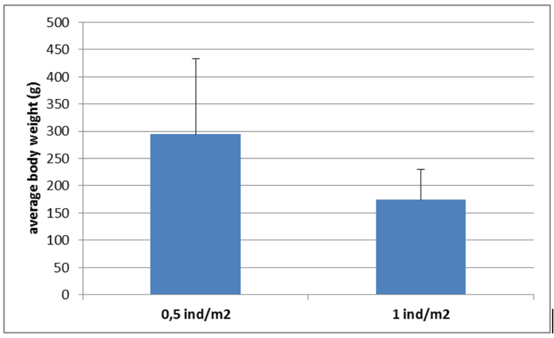

Figure 8 Final average body weight of the grey mullet reared at the two densities in the Spanish trial.

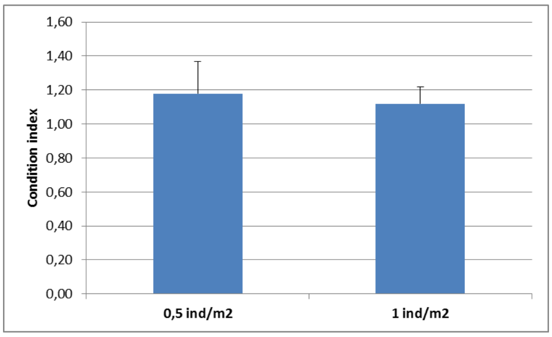

Figure 9 Condition index of the grey mullet reared at the two densities in the Spanish trial.

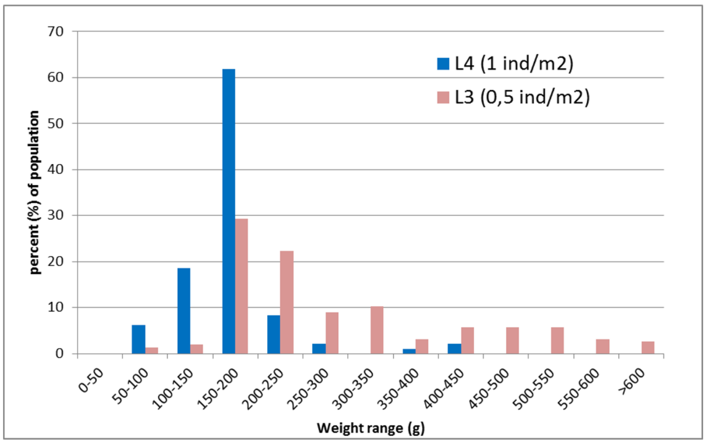

Figure 10 Weight range distribution of the two culture densities in the earthen ponds.

The weight range distribution of the grey mullet at the final sampling (December 2016) shows a density effect on the weight range of the grey mullet (**Fig. 10**). In the 1.0 fish/m^2 treatment, 61.9% of the population is in the range of relatively small fish of 150-200 g while this weight range represented only 29.3% of the population stocked at 0.5 fish/m^2. Moreover, in the high density treatment only 2.06% of the population was above 400 g average body weight, which is considerably lower than the 22.93% of the population in fish stocked at 0.5/m^2. Additionally, among the larger fish representing 22.93%, there was 11.46% of the fish population above 500 g average body weight (**Fig. 10**)

With regard to the histological analyses of the intestines, the samples from the high density pond were not well preserved during the transport from the farm and could not be sent for histological evaluation. Only the intestines from the lower density treatment were processed and histologically evaluated.

All the intestines samples presented a marked and generalized lymphocyte infiltrate (**Fig. 11)** in mucosa and lamina propria; 50% of the samples showed mild to moderate number of intraepithelial degenerated forms with occasional impact of hydropic degeneration and vacuolation of the mucosal epithelium and few infiltrated eosinophilic granular cells (EGC). Intestinal sub-mucosa displayed mild to moderate mixed cellular infiltrate including mononuclear and EGC infiltrate with focal marked EGC infiltrate and moderate congestion of blood vessels in 50% of the samples (**Fig. 11)**. One of the samples presented in the muscle intestinal layer a mild impact of Myxosporean aggregates (Sphaerospora-like), which is a normal condition in fish cultured in earthen ponds (**Fig. 12)**.

Examined intestines presented marked cellular infiltrate in intestinal mucosa and submucosa. These signs are compatible with chronic enteritis with multifocal degenerative signs. No widespread bacteria, parasite or fungal forms were detected in the examined sections.

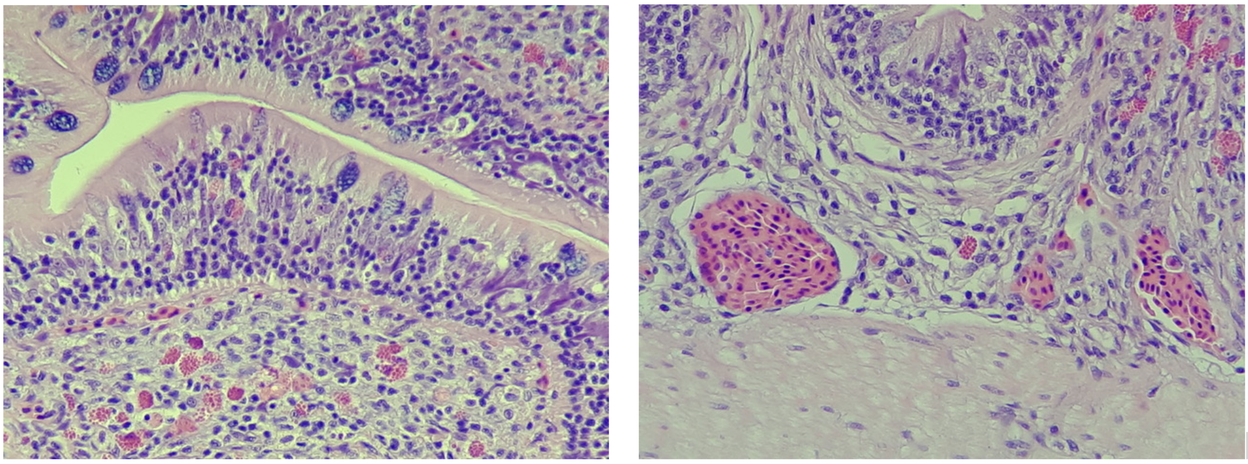

Figure 11. Left**:** Generalized lymphocyte infiltrate in mucosa and lamina propria of the intestine of grey mullet reared at low density. Right: Image of moderate congestion of blood vessels found in 50% of the samples.

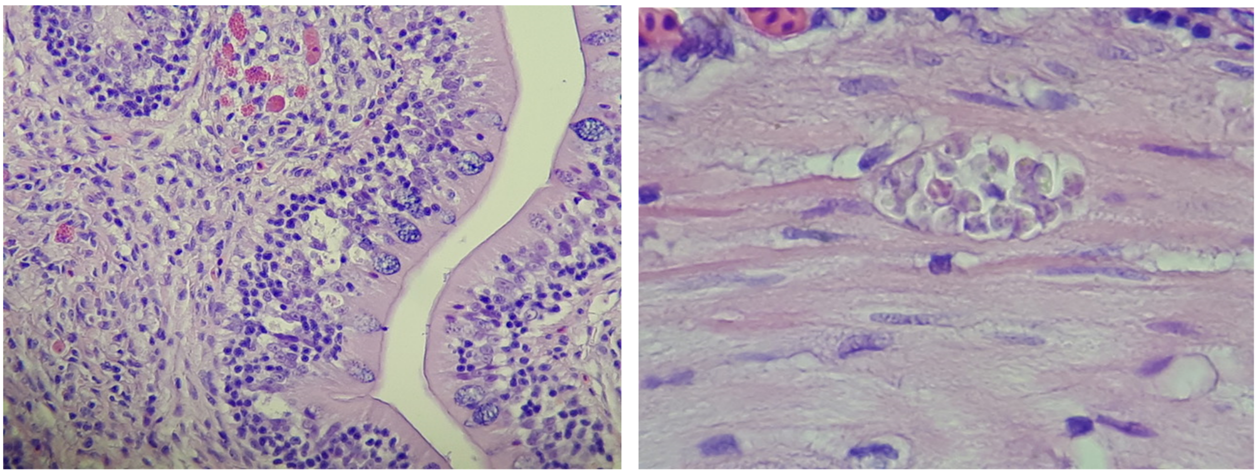

Figure 12. Left: hydropic degeneration and vacuolation of the mucosal epithelium. Right: Myxosporean aggregates (compatible with Sphaerospora-like parasite) detected in one fish (magnification x100).

In conclusion, although the trial was performed with one single replicate of each density condition, the presented results clearly indicated a growth advantage in the lower stocking density (0.5 fish/m^2) and it is consistent with the results from the **P4.IOLR** in Israel, which also showed a density effect (**Task 23.2**).

However, in the plastic-lined earthen pond trial in Israel, the density effect was more muted and there was only a slight growth advantage in 1 fish/m^2 (153.9 g) treatment over the 2 fish/m^2 (145.3 g) treatment (**Fig. 13**). On the other hand, survival in the lower density fish (80.4%) was considerably higher than the 61.1% found in the higher density treatment (**Fig. 13**). Having said that, there was a major problem with herons which managed to enter the ponds under the anti-bird netting and consume large amounts of fish, possibly more in the 2 fish/m^2 pond. In fact, at the end of the feeding trial the density ratio of 1:2 was changed to 0.88:1.34 due to fish predated mortality. This also likely modulated the weight distribution after 18 months. Although at stocking the weight distribution between ponds was very similar (**Fig. 14a**), there was only a slight tendency for the 1 fish/m^2 treatment to have larger fish (90.7% of population over 50 g) than the 2 fish/m^2 treatment (70.9% of population over 50 g) while the higher density treatment fish were comprised of smaller individuals (19.7% of population less than 50 g) compared to the lower density fish (9.5% of population less than 50 g) (**Fig. 14b**).

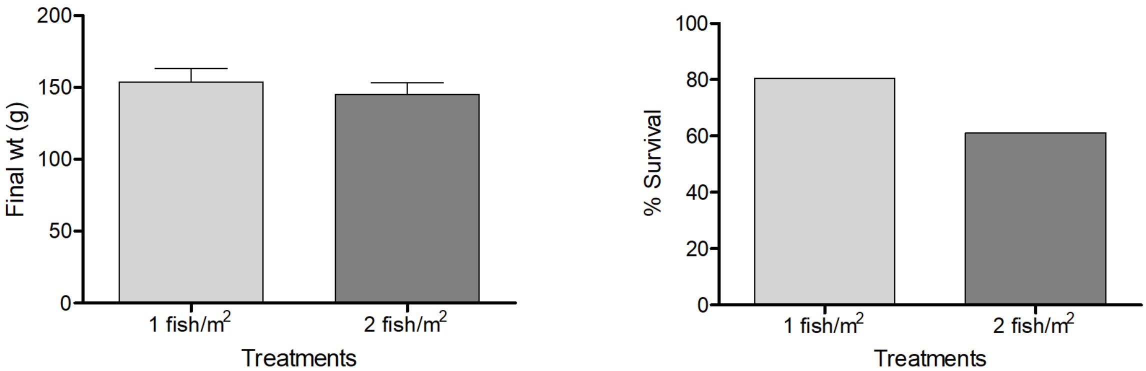

Figure 13 Final weight (wt) and survival in each of the two treatment ponds at the end of growth trial in Israel.

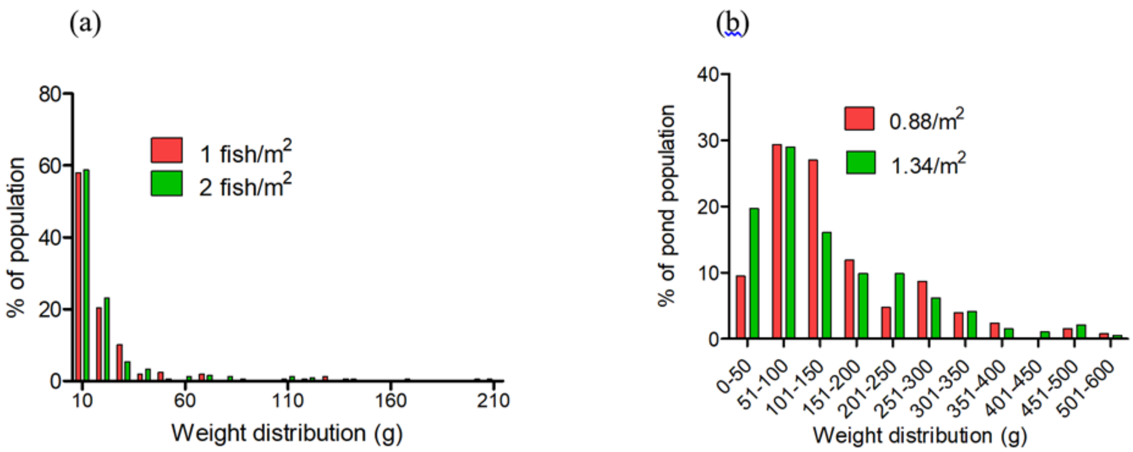

Figure 14 The weight distribution as a percent (%) of the population at (a) stocking and at (b) end of the experiment in Israel.

The generally poor growth performance of the grey mullet in the Greek, Spanish and Israeli trials can be due to a number of factors. Certainly attempting to grow mullet in full strength seawater (40 ‰), which was the case in the Israeli trial, is not going to deliver the best growth, as much energy will be channeled into osmoregulation instead of building tissue. However, a major impediment is likely the extruded diet, which remains not sufficiently attractive to the fish as they appear to prefer the detritus and primary productivity of the pond over the more nutrient dense feed. Moreover, in earthen ponds the mullet are likely using sediment to aid mashing of the plant material in the gizzard for better digestion and absorption . In order to improve the feasibility of intensive monoculture of this species, the dietary formula of the current grey mullet feed must be improved. For instance, the total dietary lipid may be too high, which currently is at about 14% DW diet. It is conceivable that this is causing excessive visceral fat accumulation in the fish, while the lipid levels of food items in their natural environment would suggest a reduced requirement for total dietary lipid. Ghion (1986) concluded that dietary fat in grey mullet should range between 5-10%. Technically, this would also mean producing only extruded diets as low levels of dietary lipid interfere with milled pellet integrity On the other hand, the ca. 35% protein level in the IOLR mullet diet would agree with Nour et al. (1993) who determined the effect of dietary protein levels on growth performance and feed utilization in grey mullet.

Although mullet are omnivorous, micro- and macroalgae would still represent a significant portion of their diet in lower salinity estuarine waters. This means the ingestion of plant protein, carbohydrate and carotenoids. The present level of dried *Ulva lactuca* is ca. 5% in the diet but this may not be sufficient. *U. lactuca* contain chlorophyll a and b (12.2-14.9%) and carotenoids (6.2-29.7%) such α and β carotenes (Abdel-Baky et al.,2009). In fact, Wassef et al. (2001) reported the best weight gain in grey mullet was when fish were fed 20% Ulva meal.

Taken altogether and despite the fact that the growth performance in the participating countries were disappointing, valuable lessons were learned in order to move forward in the development of a suitable diet for the grow-out of the grey mullet.

5.0 References

The fish site: https://thefishsite.com/articles/cultured-aquaculture-species-flathead-grey-mullet

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Deviation from DOW

The Israeli growth trial was originally planned to be carried out in the large brackish ponds at **P25.DOR** DGAY YAM near Haifa. However, this company closed down after the first year in the project. Consequently, smaller and seawater fed ponds at **P4.IOLR** in Eilat, Israel had to be prepared (netting, plastic covering etc.) in order to attempt to carry out this task. These preparations, the delayed arrival of the IRIDA diet due to custom's regulations, collecting sufficient numbers of juvenile mullet and the lack of suitable personnel to carry out this growth trial were the main reasons for the delay of this deliverable.

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