New species for EU aquaculture

Deliverable Report

Deliverable No:	D23.2	**Delivery Month:**	60
Deliverable Title	Stocking protocols for pond monoculture grow out of F1 and wild caught grey mullet		
WP No:	23	**WP Lead beneficiary:**	P4
WP Title:	Grow-out husbandry-grey mullet		
Task No:	23.2	**Task Lead beneficiary:**	P4
Task Title:	Compare the effect of feeding an improved grey mullet diet on the grow-out in monoculture of F1juveniles stocked at two different densities in cement and earthen ponds		
Other beneficiaries:	P25. DOR		
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Objective: Stocking protocols for pond monoculture grow out of F1 and wild caught grey mullet.

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SUMMARY

Three separate experiments tested the effect of stocking various sizes of grey mullet fish (8.23, 44.8, 108 g) at different densities (4, 6, 10, 12, 29, 55 and 286 fish/m^2) in a range of cement and polypropylene tanks. The results generally show that increasing the fish stocking density above 6 fish/m^2 can lead to decreased growth in an increasing segment of the population resulting in larger numbers of smaller fish, potentially a result of higher stress among cohorts from increasing competition for the same food source. Another possible factor was that the grey mullet were tested using the high salinity of the Red sea (40 ‰). This contrasts with commercial farms, where the grow out of grey mullet is usually carried out in lower salinity or fresh water ponds, where growth rate has been shown to be higher than in sea water. In future studies, the effect of increased ration size, use of extruded and not pelleted diets as well as the number of meals per day (simulate continuous feeding) will be employed. This should reduce in the population the number of slower growing (high FCR), smaller fish and increase the efficiency of grow-out.

Introduction

The grow out of grey mullet (*Mugil cephalus*) to market weight has been traditionally carried out by extensive polyculture with tilapia and carp in Israel and Egypt as well as other countries around the Mediterranean (FAO, 2006-2018). However, if grey mullet is to be a main stream aquaculture species in Europe, protocols have to be developed to intensively farm this species in monoculture. One of the major problems encountered in the monoculture of grey mullet is that fish growth is closely tied with stocking density. Bakeer (2006) reported that increasing stocking density from 1 to 3 fish/m^3 in earthen ponds reduced growth performance although fish yield increased but with no improved profitability. Size variability during grow-out is also a critical problem as feed, the largest component of the cost of production, is not being converted efficiently by smaller individuals into wet weight gain and is exacerbated with increasing stocking density (Biswas et al., 2012). Studies have shown that a low density of 1.5-2 fish/m^2 gave the best fish performance and the lowest size variability (Bakeer 2006; Biswas et al., 2012), provided that pond management included feeding both a manufactured diet and pond fertilization. As these densities are very low and not suitable for intensive culture, a number of studies over the course of the DIVERSIFY project were performed on various tank types and stocking densities using an improved P4.IOLR mullet feed and no pond fertilization with the aim to increase stocking density and improve production.

Materials and methods

Experiment 1

Grey mullet juveniles (F2) after being anesthetized and weighed singly (8.23 ± 0.23 g) were stocked in two cement (19.0 m^2) and one polypropylene (3.5 m^2) tanks at densities of 55, 29 and 286 fish/m^2, respectively, which showed an approximately normal weight distribution (**Fig. 1a)**. The experimental tanks were in an open system fed by filtered (10 µm) and ambient sea water (40 ‰). Unfortunately, due to unexpected Israeli customs bureaucracy and demands, the arrival of the extruded mullet feed from P31.IRIDA was delayed and did not arrive in time for the growth trial. Nevertheless, the stocked fish were fed with a similar formula pelleted IOLR feed (IRIDA feed formula is based on the IOLR formula but used fish meal instead of poultry meal). Fish were fed a ration at 2% of tank biomass, which was divided into two daily feedings and individuals were weighed after 1 year at the end of the experiment.

Experiment 2
Four 5 m^3 polypropylene tanks, which were in a flow through, filtered (10 µm), ambient sea water (40 ‰) system were stocked with F2 grey mullet juveniles weighing an average of 108 ± 0.35 g. Two of the tanks were stocked with 29 fish each representing a density of 4 fish/m^2 while the other two tanks were stocked with 42 fish each or 6 fish/m^2 . The fish were fed pelleted feed produced at the P4.IOLR for grow-out diets for grey mullet. The fish were fed a ration size according to feed tables developed at P4.IOLR. At the end of about 6 months, all fish were individually live weighed.

Experiment 3
Two 18 m^3 cement tanks were stocked with 200 and 245 fish (44.8 ± 1.44 g), which allowed for the testing of densities of 10 and 12 fish/m^2, respectively. The tanks were in a flow-through filtered (10 µm), ambient sea water (40 ‰) system and the fish were fed a ration size according to feed tables developed at P4.IOLR. The feed used in this study was extruded and produced by P31.IRIDA. The IRIDA diet replaced the poultry meal with fish meal as poultry meal is prohibited in fish feeds in Europe. The study was terminated after almost 8 months where all fish were individually live weighed.

Ethics statement
All animal experimental procedures were conducted in compliance with the Guidelines of the European Union Council (86/609/EU) for the use of laboratory animals.

Results and Discussion

The 1 year growth trial in *Experiment 1* demonstrated a clear indication of stocking density on size distribution. As there was almost no mortality, the reported density effect was almost certainly due to a density driven interaction between individuals. At all density levels over 50% of individuals in the population were below the average weight, which is an indication of poor growth in the majority of fish. Nevertheless, as density decreased there was concomitant increase in average weight (**Fig. 1b**). These results have implications for the monoculture of grey mullet and suggest a significant delay in fish growth and consequently a poor overall FCR in the farmed fish population.

(a) (b)

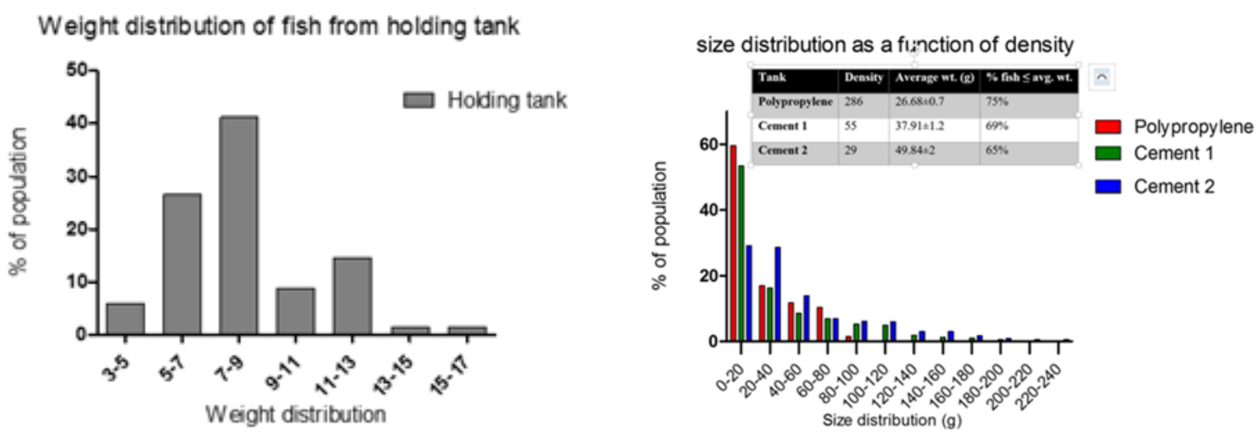

Figure 1 The (a) weight distribution of a representative sample of 68 fish with an average weight of ± 0.23 8.23 g and the (b) size distribution in the population as a function of density in the tanks at the end of the trial.

The fish at the end of *Experiment 2*, that were stocked at 4 and 6 individuals/m^2 (285.34 ±14.0 g, 294.65± 15.9 g, respectively) represented a growth increase of 164.2% and172.8%, respectively. Although the wet weight gain was not significantly (P>0.05) different in both density groups (**Fig. 2**), the fish from the 4 fish/m^2 were more concentrated in the weight range of 200-350 g which represented 77.6% of the population **(Fig. 3)** while this range represented only 48.8% of the population of fish stocked at 6 fish/m^2.

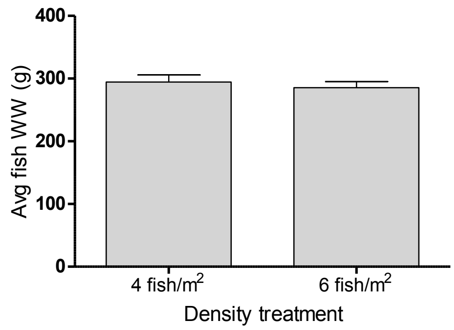

Figure 2 The effect of two stocking treatments (4,6 fish/m2) on the average fish weight. N=86, 58 of the 4 and 6 fish/m^2 treatments, respectively. ANOVA of values was found not significant (P>0.05).

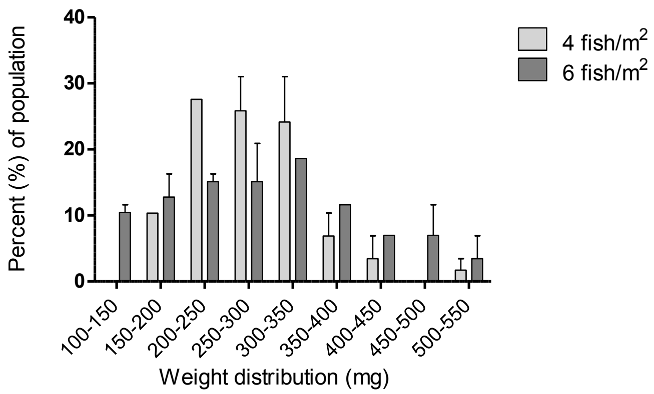

Figure 3 The effect of the two stocking treatments (4 and 6 fish/m^2) on the weight distribution in the population.

On the other hand, larger fish (350-550 g) from the 6 fish/m^2 treatment represented a larger part of the population (29.1 %) than the lower density of 4 individuals/m^2 which was only 12.1 % of the population (**Fig. 3**). Moreover, the 4 fish/m^2 treatment demonstrated an FCR of 3.26 whereas fish in the higher stocking density showed a FCR of 3.78. Overall, the results suggest that average growth rate was not markedly affected by these low stocking densities although the size distribution and FCR may be influenced.

In *Experiment 3*, the effect of higher stocking densities did have an effect on average wet weight (WW) and size distribution in experiment. At the end of the study, the average wet weight of fish in the 12 individuals/m^2 was 219.5 ± 5.0 g (390% increase), which was significantly less ($P<0.0001$) than fish from the 10 individuals/m^2 that weighed 276.4 ± 8.7 g (517% increase) (**Fig. 4**). In the higher density treatment, 71.2 % of the fish population was represented by smaller fish ranging from 100-250 g whereas the percent of these smaller individuals represented less than half (44.7%) of the population from the lower density of 10 fish/m^2 (Figure 23.2.5). In contrast, larger fish (250-550 g) from the lower density treatment represented 52.3% of the population while only 27% of the population from the high density treatment were in this size range (**Fig. 5**). In addition, the FCR was improved with a decrease in stocking density. Fish at higher density (12 fish/m^2) demonstrated an FCR of 3.5 while the FCR for fish in the 10 fish/m^2 treatment was 3.0.

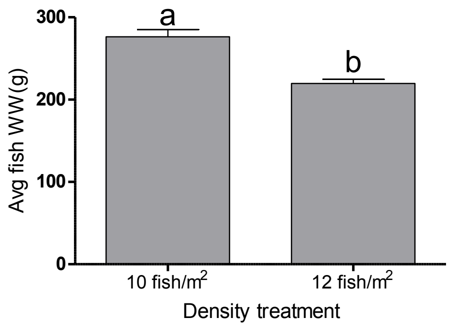

Figure 4 The effect of two stocking treatments (10 and 12 fish/m2) on the average fish weight. N=200 and 177 of the 12 and 10 fish/m^2 treatments, respectively. 2 tailed T test was performed and was found significant ($P<0.0001$).

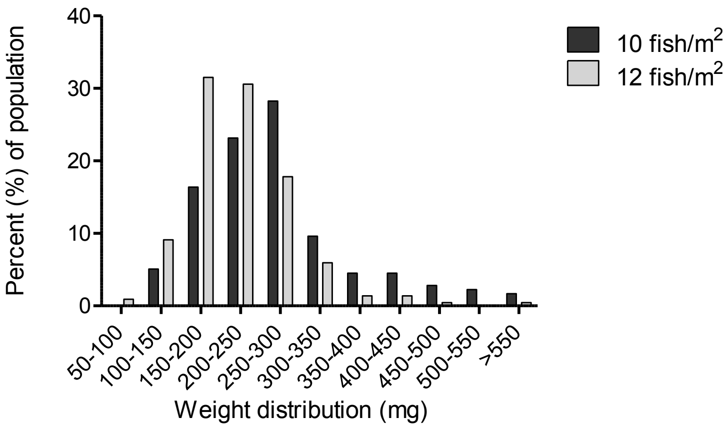

Figure 5 The effect of three stocking treatments (10,11,12 fish/m^2) on the weight distribution in the population.

Although there was not enough tank replicates to come to robust conclusions, the higher FCR of the P4.IOLR diet compared to the FCR of P31.IRIDA diet may be expected. The P31.IRIDA diet was extruded which gave it better water stability, which made it available to the fish over a longer period than the pelleted P4.IOLR diet. Moreover, the P31.IRIDA diet replaced poultry meal with fishmeal, which may have provided other nutrients or a more favorable amino acid composition that promoted growth.

The results consistently show that increasing the fish stocking density can lead to decreased growth in an increasing segment of the population resulting in larger numbers of smaller fish, potentially a result of higher stress among cohorts. Despite the fact that adult grey mullet can grow even in the high salinity of the Red sea (40 ‰), in commercial farms the grow out of grey mullet is usually carried out in lower salinity or fresh water ponds, where growth rate has been shown to be higher than in seawater (Oren, 1981). Moreover, juveniles in nature would be moving to lower salinity estuaries at approximately the ages tested. Studies at P4.IOLR have shown (deliverable D13.1) that a significant amount of enzyme activity (Na+/K+ ATPase) and metabolic pump energy is necessary to maintain osmotic homeostasis (Boef and Payan, 2001). DHA modulates Na^+/K^+ ATPase activity, which would likely be through its contribution to membrane fluidity (Izquierdo and Koven, 2011) leading to reduced activity of this enzyme and energy demand. This may also explain part of the benefit of replacing poultry meal with fish meal (as IRIDA did with the IOLR formula) with its constituent n-3 long chain polyunsaturated fatty acids in its lipid fraction.

Nevertheless, a wide size distribution is still a problem in low salinity water in grow out. This is often attributed to increasing competition for the same food source. However, a recent study carried out on juvenile grey mullet at the P4.IOLR (not listed as a task in the DOW), showed that the effect of increased stocking density on the percent of smaller fish in the population can be significantly reduced if the ration size is doubled and distributed over a higher number of meals. Omnivores and herbivores are constantly grazing resulting in continuous consumption. Carnivores, in contrast, consume prey in discreet meals. Although grey mullet was shown to be an omnivore in this project, the percent biomass used to feed the fish in the present studies ranged from approximately 1.3 to 2% and was provided in discreet meals, according to the P4.IOLR mullet feeding table. In future studies, the effect of increased ration size, use of extruded and not pelleted diets as well as the number of meals per day (simulate continuous feeding) will be employed. This should reduce, in the population, the number of slower growing (high FCR), smaller fish and increase the efficiency of grow-out. As feed represents a significant cost in the production of grey mullet, pond management that would produce a population with a normal size distribution would be more efficient and profitable.

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Deviation from DOW

This deliverable was based on the IOLR task to test different stocking protocols of F1 juveniles in cement ponds but we included as well polypropylene tanks to test different densities. This is primarily due to the fact that we were observing a clear density effect on size distribution and therefore took the opportunity to test this further. A wide size distribution is a critical obstacle to the monoculture of this species. Chemical analyses were not done because of a shortage of analytical staff and difficulties finding suitable replacements to work in Eilat. In this task partner P25. Dor Dgey Yam was, according to the DOW, meant to use juveniles from the wild but this was changed to using juveniles grown at the IOLR since this partner was producing hundreds of thousands of juveniles and it would be more efficient to use these fish. Unfortunately, the partner P25. Dor Dgey Yam closed down and were not able to run the study in two 6000 m^2 ponds testing densities of 0.5 and 1.0 fish per m^2. Moreover, the P25. Dor Dgey Yam study was meant to be compared to similar experiments run in Greece and Spain and would have contributed to D23.3. Instead, the IOLR carried out the study by stocking plastic lined 400 m^3 earthen ponds. However, these ponds had not been used for a number of years and they had to be refitted with anti-bird netting and new plumbing, which postponed further the carrying out of this task.

Also, this deliverable is being submitted much later than planned in the project due to technical difficulties finding staff, available tank space, getting sufficient numbers of fish and the closing of partner P25. Dor Dgey Yam.

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