Deliverable Report

Deliverable No:	D19.5	**Delivery Month:**	56
Deliverable Title	Evaluate an improved grey mullet larval rearing protocol in a commercial hatchery		
WP No:	19	**WP Lead beneficiary:**	P4. IOLR
WP Title:	Larval Husbandry – grey mullet		
Task No:	19.5	**Task Lead beneficiary:**	P4. IOLR
Task Title:	Testing the improved grey mullet larval rearing protocol in a commercial hatchery		
Other beneficiaries:	P25. DOR		
Status:	Completed	**Expected month:**	55
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Lead Scientist preparing the Deliverable: B. Koven (IOLR)

Other Scientists participating:

Objective: Testing the improved grey mullet larval rearing protocol in a commercial hatchery.

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1.0 Introduction

Most of the flathead grey mullet fry used in commercial aquaculture are collected from the wild, especially in the Eastern and Southern Mediterranean, Saudi Arabia and Gulf States and South East Asia. Grey mullet larvae produced in commercial hatcheries has been reported on a limited scale in Italy, Egypt and Israel (FAO, 2018). However, problems such as egg disinfection and larval survival during the first 10 days of rearing remain challenges to the successful production of juveniles.

A protocol for the larval rearing of grey mullet has been developed at P4. IOLR and is based on the experience garnered by the IOLR group over the last 4 years of project "DIVERSIFY". The protocol has also been tested and refined in both 400 and 1500 l conical V-tanks and has frequently delivered survival at the juvenile stage of *ca.* 20%. In the original DOW, the commercial hatchery P25. DOR DGEY YAM was designated as the test site to test the commercial protocol, but this partner has stopped all activity in the project for a number of years and its responsibilities have been transferred to P4. IOLR. Consequently, the grey mullet larval rearing protocol was tested in 6000 l V-tanks, which are considered of commercial size. The results presented here are the performance of five 6000 l V-tanks which were used to carry out commercial rearing from egg to *ca.* 60-70 dph during 2017. It should be emphasized that this protocol requires control over salinity and temperature in the rearing tanks.

2.0 Materials and methods

2.1 Five 6000 l V-tanks in a flow through system where filtered (10 µm), UV-treated, 40 ‰ seawater (25 ºC) entered from the tank bottom and exited through filters near the top of the tank were used in these commercial trials. Rotifer (*Brachionus rotundiformis*) and *Artemia* nauplii were enriched with the commercial product "Red Pepper" (Bernaqua, Belgium) and the weaning diet used was a 1:1 combination of dried *Ulva lactuca* produced by P4. IOLR and the commercial product "Caviar" (Bernaqua, Belgium).

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

3.0 Results and Discussion

The summary of spawning, egg stocking, juvenile and survival and production details in 2017 are presented in **Table 1**. The production of 78704 juveniles does not include the juveniles harvested from experimental tasks and research studies that were carried out within the framework of DIVERSIFY. This meant that the entire juvenile production at P4. IOLR for 2017 was *ca.* 200,000 fish. Survival was *ca.* 20% from egg to 60 dph during the planned studies. This was somewhat higher than the 10-16% in the commercial trials and may have been a result of variable egg quality. The desired egg stocking level is 50-70 eggs/l at the ambient salinity of 40‰ and temperature of 25-26^{o}C. This was not strictly adhered to among the tanks of the commercial trials as an attempt was made to use all available eggs. It is advisable to stock eggs in darkness until hatching, where there is a 400 % tank exchange/day to wash out organic material from the hatching process. After hatching and during the pre-larval stage, the salinity is then gradually reduced to 25‰ and the exchange rate lowered to a 100 % tank exchange/day. The hypothesis was that lowered salinity would save energy used for osmoregulation and channel it towards growth, which has been shown in gilthead sea bream (Tandler et al., 1995). First feeding occurs 1-2 days after hatching, as the yolk sac is utilized quickly, likely because of the relatively high seawater temperature (Yufera and Darius, 2007) but eye pigmentation and mouth opening should be confirmed. Swim bladder inflation begins at 3 dph and there is ca. 100% inflation by 5 dph. Siphoning the tank bottom should be carried out each morning. Rotifer (*Brachionus rotundiformis*) enrichment (1000 rots/ml) takes place in 28^{o}C seawater with a salinity of 20-25‰ and Red Pepper is added in two rations over 8 h according to manufacturer's instructions (0.36 ml/1 x 106 rotifers) together with 400 mg taurine/l. *Artemia* enrichment takes place in 28^{o}C seawater (40‰) where 0.6 nauplii/ml are fed Red Pepper in two equal rations over 16 h according to manufacturer's instructions (1.5 ml/1 x 106 nauplii). The greening of the tanks using *Nannochloropsis galbana* (Tamaru et al., 1994) and rotifer feeding, unenriched and enriched *Artemia* feeding as well as weaning from *Artemia* onto a dry food is presented in **Tables 2, 3 and 4.**

In summary, the larvae of grey mullet are not difficult to grow after 10 dph and the present protocol has consistently given good juvenile production. However, because of the relatively large yolk sac, the eggs are very buoyant and can pile up on each other in the incubators, which makes disinfection less effective, which may contribute to the first 10 days of mortality. This also may be a result of too high lipid in the diet (*ca.* 15 % total lipid), which is vastly higher than the 5% recommended for rabbit fish (*Siganus rivulatus*), another marine omnivore. Moreover, high visceral fat, which was observed in rabbitfish and grey mullet, may interfere with nutrient transfer to the developing oocyte, which may also influence the first 10 days of mortality.

Table 1. Summary of spawning, egg stocking, juvenile survival and production details during the commercial runs of 2017.

Date of spawning	**4.10.17**			**17.10.17**	**31.10.17**
Tank no.	**C1**	**C2**	**C3**	**C4**	**C5**
Spawning Volume (ml)	90	50	167	220	90
Stocking (eggs/ml)	54	30	100	132	54
Hatching %	64.3	64.3	-	33.5	64.3
Survival (0 DPH)	51.7	51.7	-	68.5	51.7
No. fish produced	17908	13541	16936	17783	12536
Survival (%)	16.6	12.58	10.1	10	11.6
Age of transfer from tank	70	70	61	61	70
Total juveniles produced 78704					

(a) (b) (c)

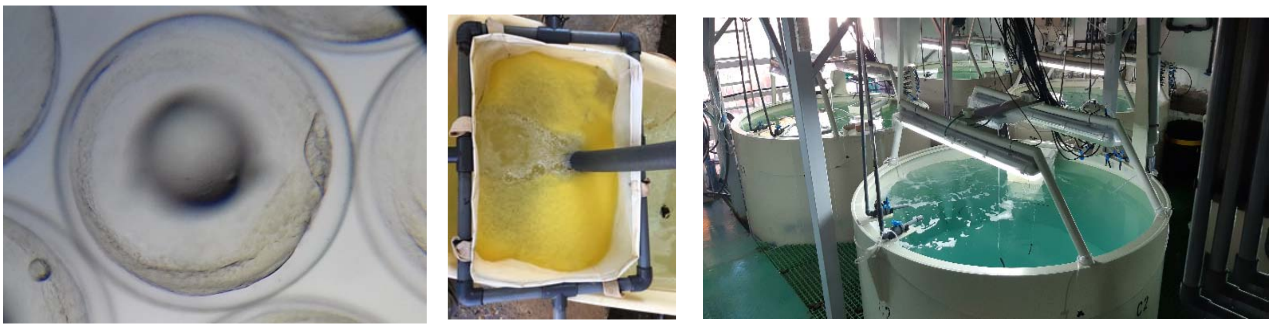

Figure 1. Grey mullet eggs with (a) developing embryo, in (b) the egg collector and (c) the 6 m^3 tanks for larval rearing.

Table 2. “Greening” of 6000 l tanks and provision of enriched rotifers.

dph	**Rotifers (x 1000)/l/day**	***Nannochloropsis*/day**
2	20	1 x 10^6 cells/ml
3	20	1 x 10^6 cells/ml
4	20	1 x 10^6 cells/ml
5	20	1 x 10^6 cells/ml
6	20	1 x 10^6 cells/ml

7	20	1 x 10^6 cells/ml
8	20	1 x 10^6 cells/ml
9	20	1 x 10^6 cells/ml
10	20	1 x 10^6 cells/ml
11	20	1 x 10^6 cells/ml
12	20	1 x 10^6 cells/ml
13	20	1 x 10^6 cells/ml
14	20	1 x 10^6 cells/ml
15	20	1 x 10^6 cells/ml
16	20	1 x 10^6 cells/ml
17	20	1 x 10^6 cells/ml
18	20	1 x 10^6 cells/ml
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21	20	1 x 10^6 cells/ml
22	20	1 x 10^6 cells/ml
23	20	1 x 10^6 cells/ml
24	20	1 x 10^6 cells/ml
25	20	1 x 10^6 cells/ml
26	20	1 x 10^6 cells/ml
27	20	1 x 10^6 cells/ml
28	20	1 x 10^6 cells/ml
29	20	1 x 10^6 cells/ml
30	20	1 x 10^6 cells/ml

Table 3. Providing unenriched *Artemia* nauplii on 14 dph

dph	Nauplii/ml
14	0.1
15	0.1
16	0.1
17	0.2

Table 4. Weaning from *Artemia* nauplii to dry food and juveniles at 59 dph.

dph	Nauplii/ml/day	Dry food	Meal number
18	3		
19	3		
20	4		
21	4		
22	4		
23	4		
24	4		
25	4	50-100	2
26	3.5	50-100	2
27	3.5	100-200	3
28	3	100-200	2
29	3	200-300	2
30	3	200-300	2
31	3	100-200	4

59 dph

32	3	200-300	4
33	2	200-300	4
34	2	100-200	5
35	2	200-300	5
36	2	200-300	5

4.0 References

Tandler, A., Anav, F.A., Choshniak, I., 1995. The effect of salinity on growth rate, survival and swimbladder inflation in gilthead seabream, *Sparus aurata*, larvae. Aquaculture 135, 343–353.

Yúfera, M., Darias, M.J., 2007. The onset of exogenous feeding in marine fish larvae. Aquaculture 268, 53-63

Tamaru, C.S., Murashige, R., Lee, C.S., 1994. The paradox of using background phytoplankton during the larval culture of striped mullet, *Mugil cephalus* L. Aquaculture 119, 167–174.

Deviations from the DOW: This deliverable was completed on time in month 55 of the project. In the DOW, it was erroneously (typographical error probably) written that the Lead Beneficiary for this deliverable is P26. GEI, while the correct is P25. DOR. Furthermore, in the DOW, this partner was designated as the test site to test the commercial protocol, but this partner has stopped all activity in the project its responsibilities were transferred to P4. IOLR.

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