## FIRST SUMMARY OF STUDIES CARRIED OUT ON THE GREY MULLET (*Mugil cephalus*) IN THE EU PROJECT: DIVERSIFY

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## Introduction

The grey mullet (*Mugil cephalus*) is a euryhaline omnivore that is grown in pond/extensive culture in a number of countries around the Mediterranean basin. Recently, this species has attracted a great deal of interest as a good candidate for intensive aquaculture providing a relatively inexpensive source of protein while its roe (bottarga) is a highly valued product. A number of studies on grey mullet were carried out or are currently underway in the EU project: Diversify that have provided promising results in "Reproduction and genetics (WP7)", "Nutrition (WP13)", "Larval Husbandry (WP19)" and "Growout husbandry (WP23)".

## Summary of studies

The deliverable (D7.1) of WP7 "Establishment of a Computer Assisted Sperm Analysis (CASA) for the evaluation of grey mullet sperm" has been submitted. Preliminary analyses show that mullet sperm motility pattern is not similar to that of sea bass (*Dicentrarchus labrax*) or rainbow trout (*Oncorhynchus mykiss*) in terms of velocity as well as motility duration (ca 1 min; Fig. 1a). Our preliminary results, indicate that the major bio-technical settings, dilution of sperm, quality of activation and mastering of video recordings have been determined and can now be applied to experimental protocols. Since fish sperm quality is usually assessed through subjective observation, in terms of motility classes and duration, statistical analysis becomes less precise. The current results will help improve assessment of the effect of different treatments on reproductive performances of mullet males.

The aim of the taurine studies (IOLR) in WP13 was to compare the effect of dietary taurine on the performance of grey mullet larvae and juveniles in order to determine if the requirement, synthesis and benefits of this nutrient varies with developmental stage and the shift from carnivory to herbivory. In the first experiment three taurine levels (0, 400, 600mg  $I^{-1}$  medium) were used to enrich rotifers and/or *Artemia* nauplii, which were fed to 2-12 and 13-19dph grey mullet larvae, respectively. All fish were weaned onto a starter diet from 20-44dph. The trial was carried out in eighteen 400 l V-tanks in an open, filtered (10µ) and UV-treated seawater system. This allowed the testing of 6 live food taurine treatments (Trotifer-*Artemia*); T0-0, T400-0, T600-0, T0-400, T400-400, T600-600 in replicates of 3 tanks treatment<sup>-1</sup>. The DW of 12dph larvae fed the highest taurine enriched rotifers (600 mg  $I^{-1}$ ) grew significantly (P<0.05) better than larvae consuming the low and medium taurine enriched live food (0, and 400 mg  $I^{-1}$ ). The high taurine larvae were still markedly (P<0.05) larger than the control at 19dph despite the feeding of all fish non-taurine enriched *Artemia* nauplii for 5 days. In fact, at 44dph the taurine treatment fish (32 days after the rotifer treatments) continued to be significantly (P<0.05) larger than the non-taurine enrichment of *Artemia* alone or together with the rotifers did not demonstrate a clear growth advantage over the only rotifer taurine enrichments. Nevertheless, better (P<0.05) larval survival was achieved when both rotifer and *Artemia* were enriched with the highest level of taurine (600 mg taurine  $I^{-1}$ ).

In another study, 40 juvenile grey mullet (ca 5.5g) were stocked in each of twenty 400 l V-tanks in the same experimental system as the larval trial. The fish were fed five 1mm extruded pelleted treatments which were identical in lipid, protein and micronutrient composition but differed in their taurine levels (0, 0.5, 1, 2% DW diet) for a period of 60 days. This allowed the testing of the 5 taurine treatments in replicates of 4 tanks treatment<sup>-1</sup>. Dietary taurine supplementation in juvenile mullet continued to give a growth advantage that can be expressed as 2% > 1% > 0.5% > 0% suggesting that the fish cannot synthesize taurine or at insufficient levels after the mode of feeding shift and that juveniles are likely omnivorous in order to ingest adequate taurine levels in nature.

As part of WP19, the effect of "greening" larval rearing tanks (from 2-30 dph) with one of two different algal species (*Nannochloropsis oculata* or *Isochrysis galbana*) at two different turbidities (A: 0.56 and B: 0.89 NTU) and its consequent effect on prey capture was investigated (IOLR). The fifth treatment was the control, which contained no algae (0.26 NTU). The experimental system consisted of fifteen 1500 l tanks (100 eggs l<sup>-1</sup>) allowing three replicate tanks for each of the 5 treatments. As *Nannochloropsis oculata* and *Isochrysis galbana* cells differ in size, each turbidity level (measured twice daily) meant different algal concentrations were used (turbidity A; *Nannochloropsis*-0.2x10<sup>6</sup> cells ml<sup>-1</sup>, *Isochrysis*-0.0144x10<sup>6</sup> cells ml<sup>-1</sup>, turbidity B; *Nannochloropsis*-0.4x10<sup>6</sup> cells ml<sup>-1</sup>, *Isochrysis*-0.0228x10<sup>6</sup> cells ml<sup>-1</sup>). Although no algal species or turbidity level significantly (P>0.05) affected rotifer consumption, linear regressions between turbidity and tank biomass was significant (P=0.043) with *Isochrysis* and *Isochrysis* were highly significant (P=0.0020 and P=0.0010, respectively). Moreover, larval survival was markedly improved (P=0.0016) at turbidity B compared to the no algae control and turbidity A (Fig. 1b). However, no difference in survival and tank biomass was found between algal species at the high turbidity level. This study concluded that algal turbidity and not algal type was the dominating factor affecting mullet larval performance from 2-30 dph.

A large multi-partner (Israel, Greece, Spain) 1 year study is currently underway evaluating the performance of an improved mullet grow-out diet (IOLR) in monoculture as a function of stocking density and pond type. The feed company IRIDA is producing all the pelleted feed necessary to carry out these growth trials over at least 1 year. The IOLR and the SME; DOR are feeding the grow-out diet to F1 juveniles stocked at different densities in cement (30 m<sup>2</sup>; 4 and 6 juveniles m<sup>2-1</sup>) and earthen ponds (6000 m<sup>2</sup>, 0.5 and 1 juvenile m<sup>2-1</sup>), respectively. The HCMR will similarly test this diet on wild caught juveniles at the same densities in 6 cement ponds (20 m<sup>2</sup>) while CTAQUA will evaluate the diet for wild caught juveniles at the earthen pond densities (2 ponds at 1100 m<sup>2</sup> each). The diet will be evaluated in terms of FCR, PER, SGR, overall weight gain and survival.