Feeding habits and the influence of pellet diameter on the feeding responses of the flathead grey mullet (*Mugil cephalus*) in captivity

Sandra Ramos-Júdez¹ and Neil Duncan^{1*}

¹ IRTA, Sant Carles de la Ràpita Ctra. de Poble Nou km. 5.5, 43540 Sant Carles de la Ràpita, Tarragona, Spain.

Abstract

The feeding habits and effect of the diameter of pelleted feeds on the feeding responses of wild juvenile and adult flathead grey mullet (*Mugil cephalus*) in captivity were examined. Optimal pellet size for feeding was defined according to the behavioural responses and ingestion of pellets with different diameters (2, 4, 6, 8 mm) that were dropped into the tank in a random sequence. Larger pellets (6 and 8 mm) were more attractive (lower reaction time, high percentage of capture), but the small to medium-sized pellets (2 and 4 mm) were consumed the most. The optimal size was the 2- and 4-mm pellet diameter for juvenile individuals (365.50 ± 36.90 g; 28.8 ± 0.84 cm) and the 4-mm diameter pellet for adults (937.49 ± 146.54 g; 40 ± 1.12 cm). The preferred feeding area of adult mullet was also studied to estimate preference in relation to pellet characteristics such as floating or sink. Two pellet types, floating or sinking, were offered simultaneously in the water column: at the surface, mid-water column and bottom of the tank. The flathead grey mullet had a preference to feed in the mid-water column and the bottom of the tanks indicating that sinking or slow-sinking pellets would be the optimal feed type in relation to mullet feeding behaviour.

Keywords: *Mugil cephalus*, food, pellet diameter, feeding habits

* Corresponding author: Neil Duncan, Email: neil.duncan@irta.cat, Tel.: +34 977 745427 extension: 1815

1. Introduction

Mullet species are of great ecological and economic importance for both fisheries and aquaculture around the world, which is mainly concentrated in the Mediterranean, Black Sea region and South East Asia (Crosetti, 2016). There is increasing interest in the intensive culture of mullets as it is necessary to diversity marine aquaculture products away from carnivorous fish, to supply demand in local market where mullet is appreciated and to reduce fishing pressure on mullet species. Mullet species present good biological characteristics and potential for aquaculture. Positive aquaculture characteristics include, the euryhaline capabilities of many mullets, the fast growth (~ 1 kg per year) (FAO, 2019), the high efficiency in converting food to body mass (EUMOFA, 2020) and the omnivorous diet (Cardona, 2016). The euryhaline capabilities enable that flathead grey mullets (*Mugil cephalus*) are cultured in freshwater, brackish water, and marine water aquaculture facilities and the omnivorous diet indicates the species' high potential for culture with diets that do not contain fishmeal and fish-oil. In the Mediterranean region, in addition to flathead grey mullet, other species being cultured are: thicklip grey mullet (*Chelon labrosus*), golden grey

mullet (*Liza aurata*), thinlip mullet (*Liza ramada*), and leaping mullet (*Liza saliens*) (Crosetti, 2016).

The expansion of flathead grey mullet aquaculture depends on the development of intensive-farming techniques for which, current limitations include diet formulation, characteristics such as pellet size and feeding practices, i.e., how, when and where to deliver feed to optimize feeding efficiency. It is important to develop feeds that are readily accepted to meet fish nutritional requirements for maintenance, growth and guarantee the developmental competence of breeders and offspring. The flathead grey mullet and many species from the Mugilidae family have been described as omnivorous (see review by Cardona, 2016; Jamabo and Maduako, 2015). This omnivorous classification has focused research on the growth performance of fry and juveniles using feeds formulated with vegetable protein sources (Gisbert et al., 2016; Koven et al., 2020; Torfi et al., 2015), which will allow aquaculture to be a sustainable activity reducing and/or eliminating fishmeal and fish oil from the diets. Despite of the recent interest in Mugilidae diets, the feeding responses of mullets concerning pellet characteristics have received no attention in comparison to other cultured species. As a consequence, there is a lack of commercial pelleted feed for flathead grey mullet juveniles or breeders which, additionally, seem to reject commercially produced pellets during acclimatisation once captured from the wild. Efforts to maximise feed utilisation in mullet species fed artificial feeds in intensive aquaculture should take into account the species feeding behaviour and the characteristics of the pellet.

The physical characteristics of pellets, such as size (shape, diameter and length), colour (contrast), texture (hardness) and density (sinking rate; floating, slow sinking, or fast sinking) that offers a different distribution of the feed into the water, determine the ability of fish to detect the pellet, capture the pellet, and once captured, the acceptance to ingest the feed (Stradmeyer, 1989). Fish species could be reluctant to eat pellets with characteristics that make them not perceived as a desirable food item (Stradmeyer, 1989) and do not match with the type of feeding habits the species occupies; surface, surface/column, column feeder or benthic/bottom feeder (Jobling et al., 2001; Rahman et al., 1992). For instance, catfish, salmon, and shrimp require floating, slow-sinking, or fast-sinking feeds, respectively (Lall and Tibbetts, 2009). In addition, pellet characteristics such as size can directly influence growth, as described for Atlantic salmon (*Salmo salar*) (Wańkowski and Thorpe, 1979) and Nile tilapia (*Oreochromis niloticus*) (Azaza et al., 2010). Therefore, providing an adequate pellet particle presented according to the species feeding habits will improve the feed acceptance, growth and development of flathead grey mullet in culture.

The present study aimed to identify pellet characteristics to improve delivery of diets to flathead grey mullet by determining the appropriate pellet size accepted by juveniles and adults that meet feeding habits in intensive captive conditions.

2. Methods

2.1 Experimental animals

Wild flathead grey mullets were captured from Ebro River and brought to IRTA facilities (Sant Carles de la Ràpita, Spain) and held for 16-17 months before the experiments. Fish were stocked in 10 m³ tanks. During the first year, the fish did not accept pellets and were fed at 1.5% of the body weight a soft mixture of 20% sardines,

20% hake, 15% mussels, 10% squid, 10% shrimp and 25% of a ground commercial diet (Skretting, Spain) with 0.1% spirulina. After one year and before the present experiment, fish were presented different sizes of pellet from a commercial on-growing diet for sea bream (*Sparus aurata*) (Skretting, Spain) at 1.5% body weight. Twelve juveniles and twelve breeders were selected to examine pellet size preference and returned back to the main tank. A total of 21 fish were then selected to evaluate the feeding habits of this species. In both tests, fish was allowed to acclimatize to the tanks for 7 days prior to the start of the experiment. Throughout the acclimatization period, fish were fed with polychaetes (TOPSY Bait, Netherlands). Forty-eight hours prior to the behavioural tests the fish were not fed to increase their potential activity and appetite.

The study was performed in accordance with the European Union, Spanish and Catalan legislation for experimental animal protection (European Directive 2010/63/EU of 22nd September on the protection of animals used for scientific purposes; Spanish Royal Decree 53/2013 of February 1st on the protection of animals used for experimentation or other scientific purposes; Boletín Oficial del Estado (BOE), 2013; Catalan Law 5/1995 of June 21th, for protection of animals used for experimentation or other scientific purposes and Catalan Decree 214/1997 of July 30th for the regulation of the use of animals for the experimentation or other scientific purposes).

2.2 Preferred pellet diameter

In order to record the response of the flathead grey mullet to pellets of different diameters, three fish were placed per tank in a total of four circular tanks of 2000 L (1.7 m diameter x 1 m depth). The test was performed with twelve juveniles (mean weight: 365.50 ± 36.90 g; mean standard length: 28.80 ± 0.84 cm) and twelve adults (937.49 ± 146.54 g; 40 ± 1.12 cm) separately. In the case of the juveniles, three different diameters (2, 4 and 6 mm) were tested and in the case of the adults, four (2, 4, 6 and 8 mm). The pellets were all the same commercial on-growing diet for sea bream (*Sparus aurata*) (Skretting, Spain).

In each tank, a feeding tube was positioned just below the water surface to introduce the pellets and guide them into the water. The part of the tank that had the feeding tube was curtained with black plastic to screen the fish from any movement or disturbance caused by introducing the pellets. The responses to pellets were recorded with a video system. A video camera (Camera KPC-SN505U, Korea Technology and Communications, Seoul, Korea) encased in a waterproof housing (Integraqua Technology, A Coruña, Spain) was placed inside the water to show a direct view of the entrance of the pellets into the tank and allow observations of the full water column in the tank from surface to bottom (Fig. 1A). The camera was connected to a video recorder (Presentco, Xmotion HD Enterprise 08 Video Recorder, Rister, Barcelona, Spain) and responses were recorded. Pellets were randomly dispensed individually through the tube (143 ± 19 pellets / diameter) at time intervals of 50 s. The test was repeated on three different days at 9:00 h, as the morning was found to be the peak of flathead grey mullet feeding activity, which was diurnal (Collins, 1981).

Different behavioural responses and measures were made from the video recordings. The responses evaluated are based on studies by Stradmeyer (1989) and Smith et al. (1995) and are related to the parameters that influence the final success of feeding; the detectability or attractiveness and acceptability of the food item. Linked to the detectability and attractiveness two parameters were registered, (i) the percentage

134 reaction (rapid movement of the head towards the pellet) or no-reaction of the fish and
 136 (ii) the time elapsed since the pellet entered the water and the first catch. Linked to
 138 acceptability the following parameters were registered, (i) the percentage of pellets
 140 ingested or rejected - those spat out and not recaptured for ingestion- and (ii) the
 142 handling time (time elapsed between the first capture and its final ingestion). A
 144 preference index (scale from 0 to 1) was calculated dividing the total number of pellets
 ingested by the number of times the pellet was captured; some pellets were captured
 more than once as the pellet was spat out and captured a second time (recaptured) or
 more. The preference index was applied only in those pellets that were ingested. Those
 pellets with an index close to 0 would be less acceptable than those with an index close
 to 1. An index of 1 would indicate that all the pellets were ingested when first captured.

146 **Figure 1. Schematic representation of the tank systems set-up for A) the pellet diameter preference**
 148 **test and B) evaluation of feeding habits.** In the first trial, four fiberglass tanks (2000 L each) were
 150 prepared with three individuals per tank for adults and juveniles. In the second trial, two 10 m³-tanks
 152 were used with 15-16 fish each. Each feeding area (water inlet, middle, water outlet of the tank)
 154 was coupled with three tubes with different depths to allow the presentation of food in three levels; surface,
 mid-water column and bottom. Dotted line indicates water limit. Dark areas represent the part of the tank
 that was curtained with black plastic to avoid the disturbance of fish caused by the introduction of pellets.
 Dark square with white circle indicates the camera position.

2.3 Feeding habits

156 Thirty-one individuals (mean weight: 1044.82 ± 388.92 g; mean standard length: 41.27 ± 3.89 cm)
 158 were split in two groups ($n = 15$, $n = 16$) and placed in two rectangular 10 m³ tanks of 6.1 x 2.1 x 1.2 m.
 160 Three feeding areas were designated in the tanks; water inlet, the middle and water outlet of the tank and a video camera was placed in each
 162 feeding area to record the activity as described in the previous trial. In each feeding area, three polyethylene tubes of different lengths were placed to guide pellets into the
 164 water column to different depths; surface, in which the tube was placed above the water surface; mid-water column, in which the tube was positioned approximately 25 cm
 166 below the water surface to have the pellets fall and appear in the water column; and the bottom, the tube reaching the bottom of the tank (see schematic representation in Fig.
 168 1B). The part of the tank that had the opening of the feeding tubes was curtained with black plastic to avoid disturbance of fish by the introduction of pellets. The tank was
 170 illuminated with a strip of LEDs that were positioned in the middle of the tank from the inlet to the outlet and programmed to switch on and off at sunrise and sunset with a
 172 gradual increase in intensity. The LEDs were set to emit blue light and the intensity at the water surface increased from 10 lux at the inlet and outlet to 30 lux in the central
 area of the tank. The lux at the feeding points was approximately, inlet 25 lux, middle 30 lux and outlet 25 lux.

174 Pellets with the same nutritional formula and the same commercial dietary presentation
 176 (Optiline, Skretting, Spain) fabricated for rainbow trout (*Oncorhynchus mykiss*), but
 178 with different characteristics were used depending on the depth of the tube in the water
 column. Floating 4-mm pellets (Optiline AE, Skretting, Spain) were administered
 180 through the tube at the surface, and non-floating 4-mm pellets (Optiline AE Ouro,
 Skretting, Spain) were administered through the tubes positioned mid-water column and
 the bottom. Five minutes before the feeding activity, a vibrating alarm was presented in
 both tanks. Subsequently, pellets were administered in the three feeding areas (inlet,
 182 middle and outlet of the tank) in one different tube depth (surface, mid-water column or
 bottom) per feeding area. In this way, the presentation of pellets at three depths were
 184 made at the same time with each depth in a different feeding area. Pellets were dropped
 into the tubes in the following sequence: bottom – mid-water column – surface, in order
 186 that pellets were presented to the fish at the same moment. The same pattern of
 administration (depth and area) was followed for seven days and on the eighth day, it
 188 was randomly changed. This sequence was repeated four times over 28 days. This set
 up ensured that each feeding area in both tanks had periods when feed was administered
 190 at each water column depth.

The distribution of feeding fish in the tank was determined by quantifying the number
 192 of fish feeding at each feeding point where feed was introduced. The feeding points
 were a combination of area (inlet, middle and outlet of the tank) and depth at which the
 194 pellets were presented (surface, mid-water column and bottom of the tank). The feeding
 activity was considered to last a total of 25 s, as this was the time that the pellets in the
 196 water column had not yet reached the bottom and during which most of the pellets were
 eaten. Each day, the number of individuals feeding at each feeding point were counted
 198 each 5 s during the 25 s feeding period. The proportion of fish feeding at each feeding
 point was calculated by dividing the number of fish feeding at a feeding point by the
 200 total number of fish feeding at the three feeding points on the specific day.

2.4 Statistical analysis

202 The differences between responses to different pellet diameters were evaluated by
 means of a two-way repeated measures ANOVA with normal distributed data (Shapiro-
 204 Wilk test) and equal variance (Levene test), considering the tanks as subjects and the
 diameter and the days on which the test was performed as factors. Pairwise statistical
 206 analysis was performed with the Holm-Sidak post hoc test. The Friedman non-
 parametric test followed by Wilcoxon test with the Bonferroni correction was applied
 208 when the data did not pass the normality test. Feeding habits data did not met normality
 assumptions. Therefore, the non-parametric Scheirer-Ray-Hare test was applied in each
 210 tank. The test is an extension of the Kruskal-Wallis test and represents the alternative to
 two-way ANOVA (Sokal and Rohlf, 1995). Proportion of feeding fish was the
 212 dependent variable and the independent variables were feeding area (inlet, middle and
 outlet) and depth (surface, mid-column and outlet). The Scheirer-Ray-Hare test was
 214 done by applying a two-way ANOVA on ranked data. The H statistic was computed by
 dividing the Sum of Squares (SS) for each factor and interaction by the total Mean
 216 Square (MS). The significance of H was tested as a chi-square variable with the degrees
 of freedom of the SS being tested. The Dunn's post hoc test of pairwise multiple
 218 comparisons based on rank sums was performed.

A P value of < 0.05 was set to indicate significant differences. Data is presented as
 220 mean $\pm$ standard deviation (SD) if not indicated otherwise. Statistical analyses were

performed using SigmaPlot version 12.0 (Systat Software Inc., Richmond, CA, USA) and MS Excel was used to calculate the H statistic and the significance of H.

3.0 Results

3.1 Pellet diameter preference

There was no significant effect of the day in which the test was performed on the reaction and ingestion percentages of different pellet diameter in adults or juvenile fish. However, the day in which the test was performed had a significant effect on the manipulation time of pellets in the adults ($P = 0.001$), as a quicker ingestion was showed in the first day, and on the response time in the case of juveniles ($P = 0.005$) with a slower response on the first and second days.

The diameter of the pellets had an influence on the reaction of the adult individuals ($P < 0.001$). The adult flathead grey mullet detected the larger pellets more easily ($P = 0.002$) (Fig. 2A), although the response time did not significantly differ between the diameters presented (9.7 ± 2.1 s on average) (Table 1). Nevertheless, the initially most attractive sizes were not those consumed once captured, since the diameter was critical in their ability to consume the pellets ($P < 0.001$). The 4 mm pellets were consumed in a higher proportion followed by the 2- and 6-mm pellets (Fig 2A). The decrease in the preference index from the 2, 4, 6 to the 8-mm pellets (Table 1) indicated that smaller pellets tended to be rejected least often. The preference index's value for the 8 mm pellets was 0.07 as just one pellet was ingested and this pellet was captured several times before being swallowed. The handling time of the pellets before ingestion was significantly different depending on the pellet diameter ($P < 0.001$). The smaller the pellet, the shorter the observed manipulation time. Pellets of 2 mm required 2.5 ± 2.4 s to be ingested, 4-mm pellets required 18.7 ± 4.8 s, 6-mm were eaten in 36.5 ± 9.9 and the only 8-mm pellet consumed was consumed after 100 s of manipulation (Table 1).

Figure 2. Percentage reaction to pellets and percentage ingestion of pellets (mean $\pm$ SD) of different diameters by (A) adult and (B) juvenile flathead grey mullet (*Mugil cephalus*). Three flathead grey mullets were held in each of four tanks that were tested on three days and a percentage reaction / ingestion to 143 $\pm$ 19 pellets / diameter was calculated for each tank on each day ($n = 12$). Different lowercase letters indicate significant differences ($P < 0.05$) in reaction and capital letters in ingestion. Analysis was performed by the Holm-Sidak post-hoc test after two-way RM ANOVA or the Wilcoxon test with the Bonferroni correction after the Friedman test in no normally-distributed data.

Table 1. Reaction and handling time (s) of different pellets diameter and Index of preference for adult and juvenile flathead grey mullet (*Mugil cephalus*). Reaction time refers to the time that fish detects the pellet; handling time refers to the total manipulation time by the fish from the first capture of the pellet until ingestion. The preference index was calculated dividing the total number of pellets ingested by the number of times the pellet was captured (scale from 0 to 1, from less acceptable to direct ingestion). Different letters indicate significant differences ($P < 0.05$). N (in brackets) represents the number of tanks and days in which data was available following the examination of the responses to 143 ± 19 pellets introduced per each diameter in adults and juveniles.

Pellet diameter (mm)	Mean reaction time (s) per tank per day		Mean handling time (s) per tank per day		Mean Preference Index per tank per day	
	Adults	Juveniles	Adults	Juveniles	Adults	Juveniles
2	12.5 ± 6.7^a (12)	7.0 ± 2.9^a (12)	2.5 ± 2.4^a (12)	6.2 ± 2.8^a (12)	1.00 ± 0^a (12)	0.98 ± 0.04^a (12)
4	10.1 ± 4.7^a (12)	5.1 ± 1.6^{ab} (12)	18.7 ± 4.8^b (12)	19.1 ± 8.4^b (12)	0.80 ± 0.12^b (12)	0.79 ± 0.16^a (12)
6	7.9 ± 5.0^a (12)	3.9 ± 1.2^b (12)	36.5 ± 9.9^c (12)	39.6 ± 9.4^c (7)	0.58 ± 0.17^c (12)	0.41 ± 0.30^b (7)
8	8.5 ± 4.8^a (12)	-	100 (1)	-	0.07 (1)	-

In juvenile individuals, significant differences were found amongst the percentage reaction to 2-, 4- and 6-mm pellet diameters ($P = 0.01$). Although the juvenile mullet exhibited a high percentage of detection of the three pellet sizes, the reaction to 2 mm pellets was lower than 6 mm pellets (Fig. 2B). Flathead grey mullet also took a significantly longer time to respond to the smaller pellet diameters (Table 1). The time of response was significantly different ($P = 0.01$) between the biggest (6 mm, 3.9 ± 1.2 s of response) and smallest pellets (2 mm, 7.0 ± 2.9 s), while there was no significant difference between intermediate pellets and biggest. On the other hand, smallest and intermediate pellets (2 mm and 4 mm) were consumed at a significantly higher proportion (Fig. 2B) and their preference index values were closer to 1 (Table 1). The largest diameter (6 mm) was more likely to be rejected. The mean time for pellets to be eaten varied significantly with the diameter ($P < 0.01$). Ingestion of the pellets took significantly longer with increasing pellet diameter, from 6.2 ± 2.8 s, 19.1 ± 8.4 s to 39.6 ± 9.4 for 2-, 4- and 6-mm pellet diameter, respectively.

3.2 Feeding habits

The distribution of fish during the feeding activity significantly depended on the feeding area (inlet, middle and outlet of the tank) ($P < 0.001$) and the depth in the water column where the food was presented (surface, mid-water column and bottom) ($P = 0.009$) in both tanks. There was no significant interaction between both factors indicating that the presence of mullet feeding at the surface, in mid-water column or at the bottom did not depend on the feeding area; the inlet, middle or outlet. A significantly higher proportion of fish fed in the middle of the tanks ($64 \pm 4\%$ and $63 \pm 6\%$ in tanks 1 and 2, respectively) (mean $\pm$ SEM) in comparison with the inlet ($15 \pm 2\%$ and $29 \pm 6\%$) and outlet ($21 \pm 3\%$ and $4 \pm 2\%$) ($P < 0.001$) (Fig 3A). Regarding the distribution of fish in the water column, tank 1 showed a significantly higher proportion of fish exhibiting feeding activity in the mid-water column ($52 \pm 5\%$) ($P < 0.001$), and tank 2, both in the mid-water column ($36 \pm 6\%$) and in the bottom ($47 \pm 8\%$) ($P < 0.001$). Significant lower proportion of fish was found feeding in the water surface ($20 \pm 3\%$ and $13 \pm 4\%$ in tanks 1 and 2, respectively) in comparison with the depth where the fish fed the most in each tank (Fig 3B).

296

Figure 3. Proportion of fish (mean $\pm$ SEM) observed to be feeding in (A) each feeding area (water inlet, middle or water outlet) and in (B) each depth where the food was presented (surface, mid-water column or bottom). Analysis was performed with the non-parametric Scheirer-Ray-Hare with feeding area and depth as factors followed by a Dunn's post-hoc test. Different small letters represent significant differences ($P < 0.05$) between feeding areas and depths in tank 1, and different capital letters represent significant differences in tank 2. N per each feeding area and depth is 28 days.

304

4.0 Discussion

306 The feeding responses of flathead grey mullet recorded in this study reveals the effects
 308 of the pellet diameter on the feeding activity and manifests a pattern of feeding habit
 310 preferences for this species. The tests were performed in groups to favor responsiveness
 312 of the fish for the feed, as flathead grey mullet appear to have a quicker response to
 314 feeding in the presence of a feeding group than when isolated (Olla and Samet, 2011).
 316 Isolated individuals could become stressed (Pagnussat et al., 2013) and reduce feed
 318 intake (Pankhurst et al., 2008). Pellets with different diameters have a different
 320 detectability / attractiveness and acceptability that influence the final success of feeding.
 322 The percentage of pellets detected and the response time to the pellet represent
 324 measures of the pellet detectability and attractiveness. The percentage of pellets
 326 consumed and the number of captures followed by rejection (index preference) are
 328 measures of the pellet acceptability. Both juveniles (~360 g) and adults (~930 g) of
 330 flathead grey mullet responded with higher percentages of responses and a shorter or
 332 quicker response time towards the largest and intermediate pellets compared to the
 334 smallest pellets. However, the pellet diameters that appeared to be at first the more
 336 attractive, were more frequently rejected and were not ingested immediately once
 338 captured. The larger pellets were less often captured and the time from capture to
 340 ingestion was longer. It is possible that those larger pellets that were finally ingested
 342 had softened because of longer contact with water and some manipulation in the mouth
 344 of the mullet. Therefore, the present study suggests that the optimum diameter,
 346 indicated by those diameters least often missed or rejected and therefore, optimal for
 348 feeding, would be 2 and 4 mm for juveniles of ~360 g and 4 mm for adult breeders
 350 weighing ~930 g. These results manifest that flathead grey mullet would require pellets
 of smaller diameter compared to what is expected in other fish species of the same size.
 If we compare with the manufacturer recommendations for gilthead seabream (*Sparus
 aurata*), larger diameters are recommended for individuals of similar sizes; 4 to 6-mm
 pellet diameter for seabream juveniles from 71 to 500 g of weight and 8 mm pellet
 diameter for seabream > 500 g (Ballester-Moltó et al., 2016). The ability of the sea
 bream to ingest pellets of larger diameter could relate to this species well-developed
 chewing apparatus enabling bream to break the pellet into smaller pieces (Ballester-
 Moltó et al., 2016). In contrast, the flathead grey mullet has been observed to capture
 the food and keep it in the oral cavity or spit it out and recapture before the pellet was
 consumed. Mullet did not chew the food item; therefore, the pellets were not observed
 to be broken into smaller particles. Besides, the teeth of flathead grey mullets are
 described to be weak and their mandibles are thought to favor the ingestion of sand/mud
 and organic matter and to select the particles for their final ingestion (Cardona, 2016).
 Although flathead grey mullets are mainly detritivores and usually feed on plant
 materials, algae, dinoflagellates, or diatoms, bigger items such as crustaceans, annelids,
 fish parts, insect parts have also been identified in their stomach contents (Jamabo and
 Maduako, 2015). It may be possible that flathead grey mullet could eat bigger diameter
 pellets, but softer than the ones tested in the present study. Soft pellets of a given
 diameter have been more acceptable in several species (Knights, 1985) including
 salmon juveniles (Stradmeyer, 1989). Therefore, it would be interesting to test pellets of
 different hardness and evaluate the relationship between the hardness of the pellet, the
 diameter, and acceptability.

In regard to flathead grey mullet feeding habits, the present study identifies that feeding mullet exhibited a distribution throughout the water column, including the water surface and the bottom. Nevertheless, it was detected that mullet have a preference for feeding in the water column and the bottom rather than in the surface. This result matches with the natural feeding habit of the species, which feeds on suspended plant materials and forages the bottom (Cardona, 2016). The same feeding habits were identified in juveniles held in tanks by Jimenez-Rivera et al. (2021), in contrast with Ghion (1986) which identified that although eating in all parts of the tank, the feeding increased when juveniles were fed at the surface. According to the present results, slow-sinking or fast-sinking feeds would meet the preferred feeding habits in the flathead grey mullet. The present study, also detected a preference for feeding location in the tank, the middle of the tank. There are several possible explanations for this preference as the tank environment was not completely uniform. For example, the inlet area had more disturbance from the incoming water and the outlet area may have had slightly lower water quality. In addition, the blue light intensity emitted by the LEDs was slightly higher in the middle of the tank than at the ends (inlet and outlet) and might have affected motivation for feeding in these areas.

5.0 Conclusion

The present study has identified the preferred characteristics of pellets in terms of size; diameter (2 and 4 mm for juveniles and 4 mm for adults), and density (sinking or slow-sinking pellets) according to the feeding responses and feeding habits of juveniles and adults of flathead grey mullet (feeding in the water column and the bottom). The present study provides a basis for the future development of an optimal pelleted diet for this species in intensive culture conditions.

6.0 Acknowledgements

Thanks to IRTA staff for their technical help, to Alex Rullo Reverté and Mario Villalta Vega for help performing the tests and to Anna Belén Castilló Pérez who helped in video evaluation. This study was funded by the National Institute for Agricultural Research and Technology and Food [grant number FEDER-INIA RTA2014-0048]; the Spanish Ministry of Science and Innovation [grant number RTI2018-094710-R-I00]; and the Seventh Framework Program of the European Community for research, technological development and demonstration actions [grant number KBBE-2013-07 single stage, GA 603121, DIVERSIFY]. The participation of S. Ramos-Júdez was financed by a FI-AGAUR (Generalitat de Catalunya) predoctoral scholarship co-financed by the European Social Fund.

7.0 References

- Azaza, M.S., Dhraief, M.N., Kraiem, M.M., Baras, E., 2010. Influences of food particle size on growth, size heterogeneity, food intake and gastric evacuation in juvenile Nile tilapia, *Oreochromis niloticus*, L., 1758. *Aquaculture* 309, 193–202. <https://doi.org/10.1016/j.aquaculture.2010.09.026>
- Ballester-Moltó, M., Sanchez-Jerez, P., García-García, J., Cerezo Valverde, J., Aguado-Giménez, F., 2016. Controlling feed losses by chewing in gilthead sea bream (*Sparus aurata*) on-growing may improve the fish farming environmental

394 sustainability. Aquaculture 464, 111–116.
<https://doi.org/10.1016/j.aquaculture.2016.06.018>

396 Cardona, L., 2016. Food and feeding of Mugilidae, in: Crosetti D, B.S. (Ed.), Biology,
 Ecology and Culture of Grey Mullet. Boca Raton, FL: CRC Press, pp. 165–195.

398 Collins, M.R., 1981. The feeding periodicity of striped mullet, *Mugil cephalus* L., in
 two Florida habitats. J. Fish Biol. 19, 307–315. [https://doi.org/10.1111/j.1095-](https://doi.org/10.1111/j.1095-8649.1981.tb05834.x)
 400 8649.1981.tb05834.x

Crosetti, D., 2016. Current State of Grey Mullet Fisheries and Culture, in: Crosetti, D.,
 402 Blaber, S. (Eds.), Biology, Ecology and Culture of Grey Mullet. Boca Raton,
 FL: CRC Press, pp. 398–450.

404 EUMOFA, 2020. Blue Economy Report, Maritime Affairs and Fisheries. Luxembourg.
https://doi.org/10.2771/33246_KL-02-20-897-EN-N

406 FAO., 2019. The State of World Fisheries and Aquaculture. (SOFIA). Rome: Food and
 Agriculture Organization of the United Nations, 1-230. [WWW Document].

408 Ghion, F., 1986. Elevage intensif du mullet, in: Techniques d'élevage Intensif et
 d'alimentation de Poissons et de Crustacés. FAO Project Reports, AF014/F. p.
 410 253.

Gisbert, E., Mozanzadeh, M.T., Kotzamanis, Y., Estévez, A., 2016. Weaning wild
 412 flathead grey mullet (*Mugil cephalus*) fry with diets with different levels of fish
 meal substitution. Aquaculture 462, 92–100.
<https://doi.org/10.1016/j.aquaculture.2016.04.035>

414 Jamabo, N.A., Maduako, N.C., 2015. Food and feeding habits of *Mugil cephalus*
 416 (Linnaeus, 1758) in Elechi Creek, Niger Delta, Nigeria. Int. J. Fish. Aquac. 7,
 25–29.

418 Jimenez-Rivera, J., Boglino, A., Linares Córdova, J., Duncan, N., Ruiz-Gómez, L.,
 Rey-Planellas, S., Ibarra, Z., 2021. Characterization of the different behaviours
 420 exhibited by juvenile grey mullet (*Mugil cephalus*) under rearing conditions.
<https://doi.org/10.1101/2021.02.23.432396>

422 Jobling, M., Gomes, E., Dias, J., 2001. Feed types, Manufacture and Ingredients, in:
 Houlihan, D., Boujard, T., Jobling, M. (Eds.), Food Intake in Fish. Blackwell
 424 Science Ltd, USA, pp. 25–48.

Knights, B., 1985. Feeding behaviour and fish culture, in: Cowey, C.B., Mackie, A.M.,
 426 Bell, J.G. (Eds.), Nutrition and Feeding in Fish. Academic Press, London, pp.
 223–241.

428 Koven, W., Gisbert, E., Meiri-Ashkenazi, I., Nixon, O., Israeli, D., Tandler, A., Nolasco
 Soria, H., Solovyev, M.M., Rosenfeld, H., 2020. The effect of weaning diet type
 430 on grey mullet (*Mugil cephalus*) juvenile performance during the trophic shift
 from carnivory to omnivory. Aquaculture 518, 734848.
<https://doi.org/10.1016/j.aquaculture.2019.734848>

432 Lall, S.P., Tibbetts, S.M., 2009. Nutrition, Feeding, and Behavior of Fish. Vet. Clin.
 434 North Am. Exot. Anim. Pract. 12, 361–372.
<https://doi.org/10.1016/j.cvex.2009.01.005>

436 Olla, B., Samet, C., 2011. Fish-to-Fish Attraction and the Facilitation of Feeding
 Behavior as Mediated by Visual Stimuli in Striped Mullet, *Mugil cephalus*. J.
 438 Fish. Res. Board Canada 31, 1621–1630. <https://doi.org/10.1139/f74-204>

Pagnussat, N., Piato, A.L., Schaefer, I.C., Blank, M., Tamborski, A.R., Guerim, L.D.,
 440 Bonan, C.D., Vianna, M.R.M., Lara, D.R., 2013. One for All and All for One:
 The Importance of Shoaling on Behavioral and Stress Responses in Zebrafish.
 442 Zebrafish 10, 338–342. <https://doi.org/10.1089/zeb.2013.0867>

- 444 Pankhurst, N.W., Ludke, S.L., King, H.R., Peter, R.E., 2008. The relationship between
acute stress, food intake, endocrine status and life history stage in juvenile
446 farmed Atlantic salmon, *Salmo salar*. *Aquaculture* 275, 311–318.
<https://doi.org/10.1016/j.aquaculture.2008.01.001>
- 448 Rahman, M.M., Varga, I., Chowdhury, S.N., 1992. Manual on polyculture and
integrated fish farming in Bangladesh. Dep. Fish. Food Agric. Organ. United
Nations.
- 450 Smith, I.P., Metcalfe, N.B., Huntingford, F.A., 1995. The effects of food pellet
dimensions on feeding responses by Atlantic salmon (*Salmo salar* L.) in a
452 marine net pen. *Aquaculture* 130, 167–175.
[https://doi.org/https://doi.org/10.1016/0044-8486\(94\)00207-5](https://doi.org/https://doi.org/10.1016/0044-8486(94)00207-5)
- 454 Sokal, R., Rohlf, F., 1995. Biometry: the principles and practice of statistics in
biological research., W.H. Freeman and Co. New York.
456 <https://doi.org/10.2307/2343822>
- Stradmeyer, L., 1989. A behavioural method to test feeding responses of fish to pelleted
458 diets. *Aquaculture* 79, 303–310. [https://doi.org/10.1016/0044-8486\(89\)90471-7](https://doi.org/10.1016/0044-8486(89)90471-7)
- Torfi, M., Estevez, A., Gisbert, E., 2015. Fish meal substitution in diets for flathead
460 grey mullet *Mugil cephalus* fry. Book of Abstracts, Aquaculture Europa 2015,
Rotterdam, The Netherlands, 20-23 October 2015
- 462 Wańkowski, J.W.J., Thorpe, J.E., 1979. The role of food particle size in the growth of
juvenile Atlantic salmon (*Salmo salar* L.). *J. Fish Biol.* 14, 351–370.
464 <https://doi.org/10.1111/j.1095-8649.1979.tb03530.x>