



Characterization of the different behaviours exhibited by juvenile flathead grey mullet (*Mugil cephalus* Linnaeus, 1758) under rearing conditions

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Abstract

Aim of study: To describe the common behaviour of flathead grey mullet (*Mugil cephalus*) under rearing conditions.
Area of study: Tepic, Mexico.

Material and methods: Behaviours exhibited by mullets were videorecorded with submersible cameras installed inside of three tanks. A total of 690 min per day (07:30 - 18:30 h) were recorded per tank during a week. Afterwards, the different behaviours exhibited by juvenile *M. cephalus* were described, identified and characterized in an ethogram and grouped into two categories: a) locomotion, including three different observed behaviours (resting, swimming and fast swimming) and b) feeding, including three behaviours (surface feeding, bottom feeding and rubbing). Each of the behavioural variables were quantified.

Main results: *M. cephalus* is a species with a constant locomotion associated to feeding, since fish showed continuous movement during most of day light period. On the contrary, fish exhibited reduced movement during dark periods. Mulletts were observed to be a non-aggressive fish species under conditions of the present study, since the absence of dominance and aggression towards conspecifics was observed, which suggested a high predisposition for adaptation to captivity. Finally, behavioural frequencies of grey mullet juveniles were similar among the three tanks for most of the behavioural variables analysed ($p > 0.05$) except for the variable bottom feeding ($p = 0.02$).

Research highlights: Results from this study could be of interest for the aquaculture industry to optimize rearing techniques and welfare for the production of grey mullet.

Additional key words: welfare; ethogram; captivity; aquaculture.

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Introduction

Mugil cephalus, commonly known as flathead grey mullet, is a cosmopolitan fish species. It is found in all oceans and in a large variety of aquatic environments. It has a high tolerance to different environmental conditions that includes a wide range of temperatures and salinities (Saleh, 2008). These eurytopic attributes of grey mullet, in combination with the foraging feeding habits and fast-growing rate (~0.70 kg per year), enable this species to be considered for both freshwater and marine aquaculture (Whitfield *et al.*, 2012; FAO, 2020). Overall, grey mullet has been used as a model in different research areas, such as ecotoxicology, population dynamics, parasitism and gametes cryo-preservation (Chao & Liao, 2001; Mahanty *et al.*, 2011; Crosetti & Blaber, 2015; Colín *et al.*, 2020). There is an interest in this fish species for human consumption, due to its flesh nutritional quality. In 2019, worldwide production reached 118,056 tons, of which aquaculture production represented 6,124 tons, Egypt being the main producer, followed by the Republic of Korea, Italy, the Chinese Province of Taiwan and Israel (FAO, 2020). Likewise, in some countries the polyculture practices of *M. cephalus* (Soto, 2009) with other species such as *Penaeus vannamei* (Hosseini Aghuzbeni *et al.*, 2017) and *Penaeus monodon* (Mondal *et al.*, 2020), have been promoted as a sustainable alternative.

The economic importance of grey mullet is based on its consumption and its cost varies from one region to another, relying on several factors; for example, female's grey mullet gonads, also known as caviar or bottarga, could reach 65 € kg⁻¹ in the European markets (Aldana, 2015; Rodríguez, 2018). Therefore, significant advances have been made on nutrition, growth, larval culture, and reproduction of individuals reared in captivity (Martínez *et al.*, 2019; Besbes *et al.*, 2020; Talukdar *et al.*, 2020; Ramos-Júdez & Duncan, 2022). Furthermore, several studies have demonstrated that grey mullet can also be an adequate candidate species for mariculture which can contribute to food production and reduction of fishing impact (Saleh, 2008; Robles & Mylonas, 2017).

For the purpose of domestication and rearing of a fish species, welfare is important, since confinement conditions trigger physiological and behavioural responses which impact growth performance, disease outbreaks and reproduction, among other factors (Ashley, 2007). It has also been recognized that most of the typical behaviours exhibited by organisms in captivity, such as feeding, swimming, sociability, dominance, reproduction, etc., are related to environmental stimuli and aquaculture management practices (Rowland, 1999; Lall & Tibbetts, 2009; Baran & Streelman, 2020). Additionally, the behavioural responses of individuals under rearing conditions are often used as operational welfare indicators, since they might indicate potential stressful situations of individuals in their environment (Huntingford *et al.*,

2006). Hence, understanding the behaviour of cultured species can be an early warning indicator of alterations in animal stress status and health, and a useful tool to provide adequate environmental conditions, enrichment and facilities promoting welfare of the reared organisms (Kristiansen *et al.*, 2004; Saraiva *et al.*, 2019).

In behavioural studies, ethograms provide reliable information about the behavioural responses of animals in their environment. According to McDonnell & Poulin (2002), an ethogram could be defined as a formal description of a species behavioural repertoire or a major segment of it. It may be a complete list of all behaviours or it may focus on a particular functional group or category of behaviours. A species ethogram shows the actions, interactions and overall activity typically performed by animals in the wild and such activities are expected to be replicated in captivity (Marsh & Hanlon, 2004) if animals are under the right conditions. Currently, ethological studies of fish in captivity include the analysis of behavioural functional categories, such as reproductive (Ibarra-Zatarain & Duncan, 2015) or feeding behaviours (Huntingford, 2004; Carvalho *et al.*, 2007). However, studies that analyse in detail the behaviour exhibited by organisms in captivity are scarce or inexistent for many species that are currently produced in aquaculture (Lahitte *et al.*, 2002; Bolgan *et al.*, 2016). In the case of *M. cephalus*, there are no studies describing the behaviour of this fish species in the wild or under rearing conditions. Hence, the aim of this study was to describe and measure the normal behaviour frequencies of locomotion and feeding of grey mullet (*Mugil cephalus*) in rearing conditions, integrating them into an ethogram. The description of the behavioural patterns of this species in captivity, may represent a forecasting tool to evaluate the preferences and requirements of the animals, and to provide adequate management protocols and facilities in order to improve welfare (Castanheira *et al.*, 2017; Saraiva *et al.*, 2019).

Material and methods

The number, handling and manipulation of the organisms used in this study were established following the criteria of the National Centre for the Replacement, Refinement and Reduction in Animals in Research (NC3Rs, UK). Locally, the protocol for handling and use of animals was authorized by the Bioethics Commission of the State of Nayarit, Mexico (permit number CEBN/05/2017).

Collection and maintenance of organisms

Fish were captured from the wild on the pacific coast, Mazatlán, Mexico, in September 2019. A total of 300 fish were caught (average weight and length 30.2 ± 6.9 g and 15.1 ± 1.2 cm, respectively). Fish were transported to the

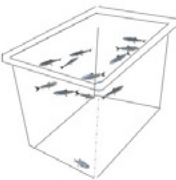
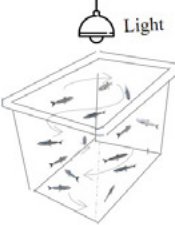
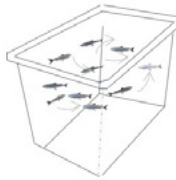

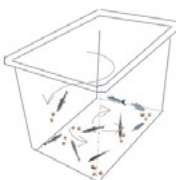
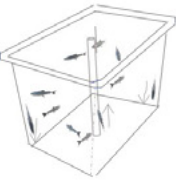
LOCOMOTION			FEEDING		
A. Resting	B. Swimming	C. Fast swimming	D. Surface	E. Bottom	F. Rubbing
					
Before 08:30 and after 18:40 hours	Between 08:45 and 18:30 hours	30 minutes before a feeding event	Three feeding events 9:30 – 12:30 and 15:30	60 min after feeding surface "event"	Along the day at unspecific times
Maintained a stationary position or slow swimming	Fluid and constant swimming in group or individually	Locomotion with quickly and prolonged in all over the tank	Capture of pellets in the water surface with multidirectional movements	Capture of pellets from the bottom of tanks until the food was finished	Fish rubbing the mouth against tank surface with a zigzag movement

Figure 1. Ethogram of the behaviours identified in *Mugil cephalus* in captivity.

Nayarit Centre for Innovation and Technological Transference (CENITT-UAN) in Tepic, Nayarit. Individuals were acclimated in two 500-L rectangular tanks (100 × 140 × 55 cm) connected to a recirculation system (RAS). Once acclimation was completed (45 days), a total of 36 fish were randomly selected and transferred to three 220-L rectangular tanks (80 × 68 × 48 cm) to reach a final density of 12 fish per tank (1.6 kg m⁻³) during the experiment. Water parameters were maintained as follows: temperature, 25-27°C; salinity, 27-29 mg L⁻¹; pH, 6-7; and oxygen, 5-7 mg L⁻¹. All water parameters were monitored daily in the morning. Photoperiod was adjusted to follow the natural seasonal cycle (light:dark, 11:13) by using an automated external dimmer (MyTouchSmart, General Electric®) turning on-off white lamps (OSRAM 85 W) from 08:00 to 19:00 h during the experiment. Mulletts were fed to satiety with a commonly used commercial diet for marine fish (floating pellets Skretting®, The Netherlands, with specifications: 55% crude protein and 16% lipids) because there is no specific diet for the species. Tanks were siphoned daily 30 min after the last feeding, to remove the food remains and faeces to maintain adequate water quality conditions.

Data collection

A high-definition camera system (Swann/2K Series-1080p) was installed underwater in three tanks. Each camera was positioned on the lateral wall of the tank, 10 cm below the water surface to capture more than 90% of the total area of the tank. Video recording started at 07:30 and finalized at 19:00 h every day for one week. All different behaviours exhibited by the fish were noted by three observers until no more different behaviours were observed. The identification and selection of behaviours were based on focal observations of the video recordings performed by a single observer. The behaviours observed

included feeding responses, swimming and overall activity at the beginning and the end of seven consecutive days, following the recommendations of Bolgan *et al.* (2016). A total of 690 min per day were recorded from each tank, from which the timing of the different behaviours was associated to changes during the schedule used to maintain the fish, changes in light and feeding were established for each behaviour associated with environmental stimuli such as light and feeding analysing a total 90 min per tank (Mas-Muñoz *et al.*, 2011; Ibarra-Zatarain & Duncan, 2015; Thomsen *et al.*, 2020).

Behavioural variables analysed

Two behavioural categories were analysed: 1) locomotion and 2) feeding. For the first category, the time that fish spent resting and swimming (normal and fast) was measured, and for the second category, the time of feeding preference (surface and bottom) and rubbing were quantified. The selected behaviours are some of the most commonly used and reliable in the field of descriptive studies performed with marine and freshwater fish species (Myrberg, 1972; Mas-Muñoz *et al.*, 2011; Pink & Fulton, 2014; Ibarra-Zatarain & Duncan, 2015; Bolgan *et al.*, 2015). Regarding the behavioural frequencies, the behaviours of each fish in each tank were registered during each 1-min time period to quantify the behaviours observed during the time course, following recommendations by Altman (1974) and Carr & Aldrich (1982) for observational studies.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 25 software. Data were checked for normality and homoscedasticity with a Kolmogorov-Smirnov test

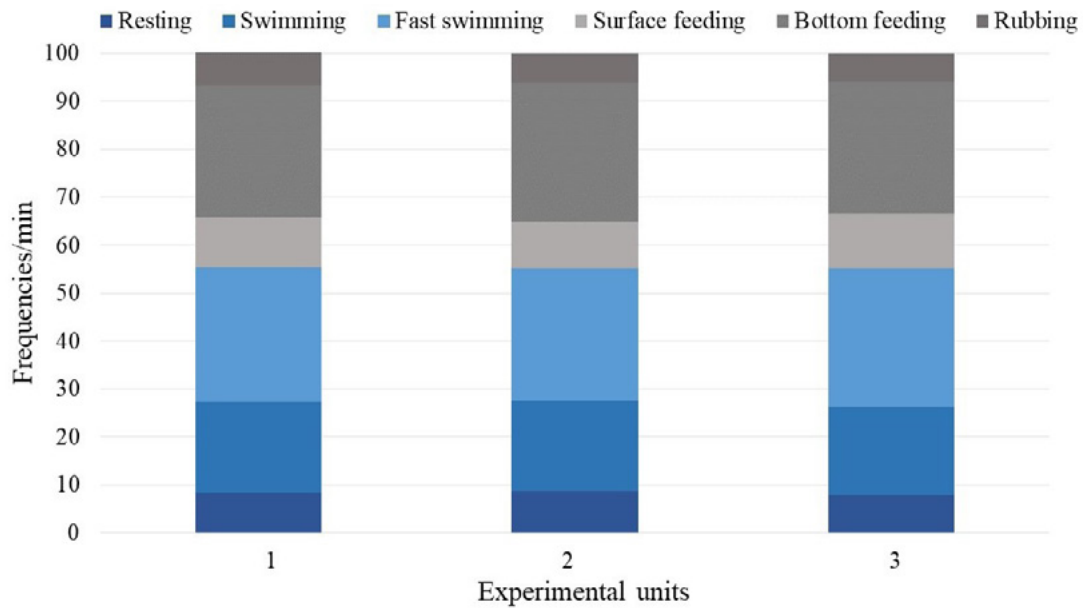


Figure 2. Average frequencies per minute observed in each experimental unit (experimental unit = 220-L tank) over a week of monitoring of the different behaviours exhibited in juvenile *Mugil cephalus* in captivity in the experimental units (the blue tones represent the locomotion category and the grey tones the feeding category).

and a Levene's test, respectively. A multivariate analysis of variance (MANOVA) was performed to assess the time course of the behaviours amongst the different tanks in the behaviours analysed among the three tanks where the cameras were installed. Additionally, a Tukey's post-hoc test was performed on data when significant differences were detected among tanks. A 95% confidence interval ($p=0.05$) was set for all analyses.

Results

Mugil cephalus exhibited resting behaviour during the dark periods from 07:30 to 08:00 and from 18:30 to 19:00 h. Lights were switched on from 8:00 to 18:30 h and locomotor activity of grey mullet juveniles increased gradually during the day and this behaviour was similar in all tanks throughout the experiment. Additionally, mullets were observed to be a social, non-aggressive and highly active fish species.

Locomotion

Resting. This behaviour was mainly characterized by the fish remaining stationary in the water column, where fish's pectoral and tail fins remained close to their body with slow undulations to give a kind of stationary swimming behaviour. More than 90% of the fish maintained a stationary position in the water column; however, in few occasions, they performed slow movements. Moreover, fish did not exhibit social interactions, since they occupied

different positions in the water column (Fig. 1A). This inactivity always occurred in the absence of light, and could be described as sleeping (Keene & Appelbaum, 2019).

Swimming. This behaviour was characterized by active swimming, in which fish swam at a constant speed throughout the tank. Additionally, mullets formed small groups or fish shoals with some individuals swimming independently. While fish were swimming, they took different directions and interacted with other individuals in repeated occasions. Moreover, no aggressive behaviours such as chases, bites, fin erections, etc. were detected. This behaviour was exhibited by more than 90% of all fish and was observed during light hours (Fig. 1B).

Fast swimming. This was defined as high activity or fast swimming, in which fish presented a subcarangiform locomotion consisting in constant undulations of the posterior part of the body, accompanied by movements of the tail fin and with or without extensions of the pectoral fins. Fish showed a schooling formation with prolonged movements from one point to another in the water column, with constant interactions between fish, but with no aggression. This behaviour was observed in all individuals from all tanks analysed and occurred approximately 30 min before a feeding event (Fig. 1C).

Feeding

Surface feeding. When feed was offered, fish quickly approached and ingested all possible pellets. However, it was also noted that after capturing the pellets, fish frequently spat the pellets out. During the feeding frenzy, mullets

showed multidirectional movements around the food. In addition, a cooperative event between fish was observed in which animals that first consumed pellets, moved immediately to another point in the water column, allowing other individuals to rise at the surface to feed. Besides, no signs of aggression or dominance over food were detected (Fig. 1D). This behaviour was displayed by 100% of the fish.

Bottom feeding. After the feed sunk, fish began a second feeding event in which they consumed the food from the bottom of the tank in a vertical position. In this context, all fish, from all tanks, were observed to exploring the bottom of the tank searching for uneaten pellets, and constantly interacting with other fish, but no aggressive behaviours were observed (Fig. 1E). Unlike surface feeding, all food consumed at the bottom of the tank was swallowed and not spat out.

Rubbing. This behaviour was distinctive of fish by rubbing their mouths against the walls or bottom of the tank doing zigzag movements. While performing this behaviour, fish maintained a vertical position with the pectoral fins close to the body. Rubbing was observed individually or in group (less than 60% of the fish) in different occasions along the day (Fig. 1F).

Comparison of behavioural frequencies

No statistical differences ($p > 0.05$) were detected in the frequencies of the six behavioural variables analysed among tanks during the light and dark times (Fig. 2). Specifically, no significant differences were detected for resting ($F_2 = 2.19, p = 0.124$), swimming ($F_2 = 2.93, p = 0.064$), fast swimming ($F_2 = 0.667, p = 0.519$), surface feeding ($F_2 = 2.20, p = 0.122$) and rubbing behaviours ($F_2 = 3.16, p = 0.053$). Fish from tank 2 presented significant higher frequencies in bottom feeding than fish from tank 3 ($F_2 = 3.96, p = 0.026$).

Discussion

This study described for the first time the behaviour of *M. cephalus* juveniles in captivity. Overall, mullets are individuals with high activity during daytime due their high rates of locomotion, swimming, social interactions, which decreased immediately after lights were turned off, as a transition to resting behaviour. In this context, Helfman (1986) suggested that the behaviour in animals such as feeding, breeding, aggregations and resting, are influenced by artificial light alternation (light and dark) as was the case for this fish species. Moreover, the biological rhythm exhibited by the grey mullet juveniles, of high activity during the daytime and resting during time without light, demonstrated that constant light/dark periods and environmental conditions might favour feeding synchronization and adaptation to environment. In this context, Oliveira *et al.* (2017) and Sánchez-Vázquez *et al.* (2019) suggested

that consistent photoperiod and environment are beneficial and promote welfare, since fish could easily cope with the given conditions to entrain into a circadian rhythm, which ultimately, facilitates their adaptation to given conditions. Therefore, it is possible to hypothesize that mullets entered in a circadian rhythm.

Locomotion

The locomotor activity of grey mullets started with a period of resting behaviour or slow swimming, registered during the first minutes of the day (before lights were turned on (from 07:30 to 08:00 h) and when lights were turned off (from 18:30 to 19:00 h). Bolgan *et al.* (2015) described a similar resting behaviour in the arctic charr (*Salvelinus alpinus*) held in captivity. Authors classified resting behaviour as a state of inactivity, in which fish held a stationary position most of the time with no forward locomotion. Similarly, Ibarra-Zatarain & Duncan (2015) reported that gilthead seabream tended to swim slowly either alone or in small groups around the tank during early morning (08:30 h). Likewise, Park *et al.* (2018) reported the same resting behaviour in the Korean endemic cobitid (*Iksookimia hugowolfeldi*) in the wild. Killen *et al.* (2016) have suggested that resting behaviour is associated to energy conservation. Also, Zimmerman *et al.* (2008) and Elbaz *et al.* (2013) have described that prolonged period of behavioural quiescence could be defined as sleep in fish. Those authors pointed out that fish decrease locomotor activity and metabolic rate in order to save energy. Therefore, energy is conserved to be allocated for functional activities such as feeding, growing or reproduction, which are important biological parameters for aquaculture.

The second locomotor pattern analysed, swimming behaviour, was the most common in grey mullet juveniles and it was characterized by a constant movement of the fish during the daylight hours. This has been typically described for other fish species and is linked to their physiology; for example, Farwell & McLaughlin (2009) and Brownscombe *et al.* (2017) suggested that constant swimming behaviour is environmentally adaptive and may be linked to biological functions such as: metabolism, respiration and digestion. A similar behaviour to that described in the present study was reported by Ibarra-Zatarain & Duncan (2015), who observed that gilthead seabream (*Sparus aurata*) had a swimming activity characterized by a constant swimming speed in all fish. Furthermore, swimming behaviour of white mullet (*Mugil curema*) in their natural environment coincided with the behaviour reported in the present study (Carvalho *et al.*, 2007). Relative to *Mugil cephalus*, a study performed by Carr & Aldrich (1982) evaluated the effect of densities on swimming behaviour and concluded that this fish species exhibited constant swimming during light periods, which is in agreement to reported results. Downie *et al.* (2020) mentioned that con-

stant swimming behaviour denotes optimal welfare and a good physiological condition.

Regarding fast swimming behaviour, it has been documented that locomotor activity tends to increase when it is associated to behaviours such as: feeding, foraging and mating in captivity or predator avoidance and migration in the wild (Brownscombe *et al.*, 2017). Therefore, the fast swimming behaviour exhibited by *M. cephalus* in captivity, may be related to an anticipatory activity to food, which is similar to what happens in other aquaculture species adapted to captivity. For example, gilthead seabream (Montoya *et al.*, 2010) and tambaqui (*Colossoma macropomum*) (Fortes-Silva *et al.*, 2018) showed an increased or anticipatory swimming activity before feeding. In a different context, other studies have reported that fast swimming, could be associated with fast growing fish (Palstra *et al.*, 2010), as they present optimal muscular-skeletal development and osmoregulation (Huntingford & Kadri, 2013) and exhibits stress resistance (Martins *et al.*, 2012).

Feeding

Mulletts showed two distinctive feeding behaviours. First, individuals showed a preference to swallow pellets in the surface of water, the same reaction was identified by Ghion (1986), when observing that feeding of the same fish species increased when juveniles were fed at the surface. This initial feeding reaction could represent an intuitive strategy securing food (Montoya *et al.*, 2010), as reported in European seabass (*Dicentrarchus labrax*) (Azzaydi *et al.*, 1998) and *Parachaeturichthys ocellatus* (Panicker, 2020). Moreover, it is known that feeding fish regularly, like in aquaculture rearing conditions, induces an internal mechanism of synchronization with food. Additionally, Lall & Tibbetts (2009) proposed that the feeding behaviour in fish is associated to cognition, similarly to birds and mammals. Therefore, it is possible that grey mullet (*Mugil cephalus*) adapted its behaviour to fixed schedules of feeding in captivity conditions, which reinforce the probable assumption of adaptation to rearing conditions and development of circadian rhythms.

After grey mullets consumed the pellets in the water surface, it was observed that some individuals frequently spat out the pellets that they had just swallowed resulting in approximately 60% of the offered food sinking to the bottom of the tank when second feeding started. This behaviour lasted until fish consumed all the pellets and it was accompanied by exploration and social interactions and coincided with the report by Ramos-Júdez & Duncan (2022), who found the same preference to feed in the middle water and the bottom of the tanks in adult mullet. Islam *et al.* (2009) suggested that grey mullet is a bottom feeder, that exhibits herbivore preferences, based on gut content, which may explain the presence of bottom feeding behaviour in captivity. Similar results have been found

by Anders *et al.* (2017) and Park *et al.* (2018) in cod (*Gadus morhua*) and the Korean endemic cobitid, suggesting that this preference to feed is associated with their natural feeding habits. When analysing the frequencies of this behavioural variable, significant differences were detected among tanks. This variation could be related to feeding practices, since fish were fed *ad-libitum* and, thus, it is possible that fish did not receive the same amount of feed. To confirm this previous assumption, it will be important to evaluate different food rations for this fish species as suggested by Wassef *et al.* (2001), who reported that 4% of total biomass in food might result in similar behavioural frequencies. Moreover, it is recommended to perform more studies on diets adapted for this omnivorous fish species and determine their possible effects on behaviour, since no related studies have been performed to our knowledge.

On the other hand, rubbing behaviour has been related to the lifestyle and feeding strategy of this fish species (Almada *et al.*, 1999), and a similar observation was reported in the Korean endemic cobitid. This fish species showed a zig zag swimming behaviour which was associated with their habitat and feeding behaviour (Park *et al.*, 2018). Additionally, Islam *et al.* (2009) described that mullet fed in the wild mainly with algae and detritus; thus, the adaptation of feeding behaviour in the Mugilidae family is instinctively based on searching for food at the bottom or edges by rubbing its body to graze and ingest phytoplankton and other detritus (Bowen, 1984). Therefore, by showing this kind of behaviour, grey mullet may be adapting to captivity, hence, this behavioural response can be used as a measurement of optimal performance of the species.

Lastly, grey mullet seemed to be a social species without any trait of aggression or the establishment of social hierarchies. Additionally, grey mullet juveniles exhibited a cooperative behaviour when consuming food, swimming in a synchronized way, allowing all individuals to eat. This non-aggressive and cooperative behaviour exhibited by the grey mullet juveniles in the present study agreed with the observations performed by Carr & Aldrich (1982) and Sampaio *et al.* (2001) for the same fish species and for *Mugil platanus*, maintained in captivity or in conditions similar to the present study. Wey *et al.* (2008) described that animals with social behaviour form complex social interactions and structures that might present advantages to obtain resources for all the shoal (*i.e.* food, feeding areas, others). On the contrary, in other reared species, it was demonstrated that some individuals compete among their congeners for resources and are conferred an advantageous access to food over others, such as in rainbow trout (Øverli *et al.*, 2004) and Senegalese sole (Fatsini *et al.*, 2017). Therefore, the absence of hostile behaviours in the grey mullet could be considered as an important factor for the aquaculture of this fish species.

Conclusion

Behaviours are responses exhibited by fish in their environment to access to resources, which allow them to meet their basic requirements and survive. Considering that welfare of reared animals generally leads to a production of quality, it is relevant to know the expression of natural behaviours as a forecasting model of welfare status and physiological performance (Huntingford, 2004). Thus, this research described the normal behaviour of mullet juveniles (*Mugil cephalus*), where all the functional categories observed in rearing conditions were described, that is, the behaviour observed in chronological order in a production farm. Moreover, the social behaviour may establish a preliminary basis for mullet as an attractive aquaculture species in Mexico. Thus, this study contributes to the knowledge of the species for a correct management of the biological processes and to implement rearing protocols that will improve aquaculture production of a species of economic interest. Moreover, this study represents a starting point for any behavioural study in mullet and a reference for comparison with other studies in the wild and under confined and stressful conditions for this species.

Authors' contributions

Conceptualization: Z. Ibarra-Zatarain, A. Boglino, N. Duncan, S. Rey-Planellas, M.L. Ruiz-Gómez.

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Writing – review & editing: Z. Ibarra-Zatarain, A. Boglino, N. Duncan, S. Rey-Planellas, M.L. Ruiz-Gómez.

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