



## Dietary discrimination using a dual-choice self-feeding system in seabream (*Sparus aurata*)

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### ABSTRACT

Self-feeding systems have been extensively used to study animal behaviour and food preferences in vertebrates. In this study, we used a dual-choice self-feeding system to explore feeding behaviour and dietary discrimination based on organoleptic cues. Experimental tanks were provided with 2 self-feeders activated by string sensors and connected to a computer system. In the first experiment (E1) [6 tanks, 10 animals/tank, body weight (BW) 266 g ± 19.4 g], both feeders delivered the same control standard diet to evaluate the potential preference for one feeder. E1 had 3 phases: 1st, animals were allowed to demand from both feeders; 2nd, from only one feeder and 3rd from both feeders again. The second experiment (E2) (11 tanks, 15 animals/tank, BW range 25–50 g) examined whether seabream can discriminate between 3 isoenergetic diets: the control diet; a positive/palatable diet containing high levels of fish, squid and krill meal; and a negative diet in which 1.5% quinine hydrochloride was added to the control diet. E2 had 3 phases: 1st acclimation with control diet; 2nd dual-choice; 3rd dual-choice with reversal of feeders. The third experiment (E3) (8 tanks, 10 animals/tank, BW range 25–50 g) was performed using a single self-feeder *per* tank, with each tank being fed either control or quinine diet, to corroborate the aversive response to quinine in the absence of dietary choice. E1 demonstrated that animals demanded significantly less when only one feeder/sensor was available. E2 shows that seabream can easily discriminate a bitter compound (quinine) in the diet, showing negative preference ( $P < 0.05$ ) in both phases, 2nd and 3rd. When no choice was available (E3), seabream equally demanded control or quinine diet, presumably driven by its energy requirements. However, the presence of the bitter substance increased feed refusals, 14.3% rejection of demanded feed in quinine diet, *versus* 2.6% in control diet, indicating a role of sensory systems in assessing food quality. A positive conditioning with a palatable diet was not effective and no positive choices were measured over a basal diet, although results were possibly influenced by the previous familiarization with the basal diet and by the experimental conditions (fast growing juveniles at high temperature). This poor dietary selection during active feeding periods could partly explain the easy adaptation of this species to substitution diets. Finally, results suggest that food intake is conditioned by agonistic behaviour in an age-dependent manner. The information will be useful for diet and feeding strategy design in seabream.

### 1. Introduction

Several studies have confirmed that fish are suitable for vertebrate behavioural research as they have the ability to perform non-associative and associative tasks including habituation (Best et al., 2008; Wong et al., 2010), conditioned place preference (Mathur et al., 2011),

avoidance learning (Xu et al., 2007), quantity (Potrich and Sovrano, 2015; Messina et al., 2020), visual (Dagget et al., 2019) and spatial (Arthur and Levin, 2001) discrimination, working memory (Bloch et al., 2019) and appetitive choice discrimination (Bilotta et al., 2005). The application of operant conditioning, in which animals carry out simple tasks in order to gain a reward, has demonstrated that fish can self-

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regulate feeding to meet their nutritional and energetic requirements (reviewed by Fortes-Silva et al., 2016). By operating self-feeding devices, fish learn how to activate mechanical sensors (conditioned stimulus) that trigger feed delivery (unconditioned stimulus) from automatic feeders. Self-feeding systems have also been extensively used to study different behavioural aspects in fish such as feeding activity rhythms (Sánchez-Vázquez et al., 1995), operant learning ability (Nilsson and Torgersen, 2010), feeding dynamics in fish groups (Millot and Begout, 2009), fish welfare (Attia et al., 2012) and food preferences (Aranda et al., 2000). Although such feeding devices have also been tested on seabream (*Sparus aurata*), studies on their feeding behaviour are scarce. Montoya and co-workers demonstrated that seabream can select diets with different levels of oil oxidation (Montoya et al., 2011) and compose a balanced diet by selecting different incomplete diets from three alternative feeders (Montoya et al., 2012). The present work contributes to ongoing efforts aimed towards a better understanding of seabream feeding behaviour. The ability of seabream to discriminate food sensory properties using dual-choice self-feeding systems was studied. It was observed that seabream can easily operate self-feeding systems and select a diet using sensory (olfactory and/or gustative) traits.

## 2. Material and methods

Two-year-old (experiment 1) and one-year-old (experiment 2 and experiment 3) immature seabream (*S. aurata*) were kept in 2000 L tanks supplied with aerated sea water in a flow through system and equipped with an automatic feeder (Arvotec) which was activated by a homemade string sensor placed 3 cm below the water surface. The feeders were connected to a computer system which recorded the date, time and tank from which each food demand originated. Animals were maintained under natural temperature and photoperiod conditions for at least 3 months and self-fed on a commercial diet (Biomar, EFICO 3073)

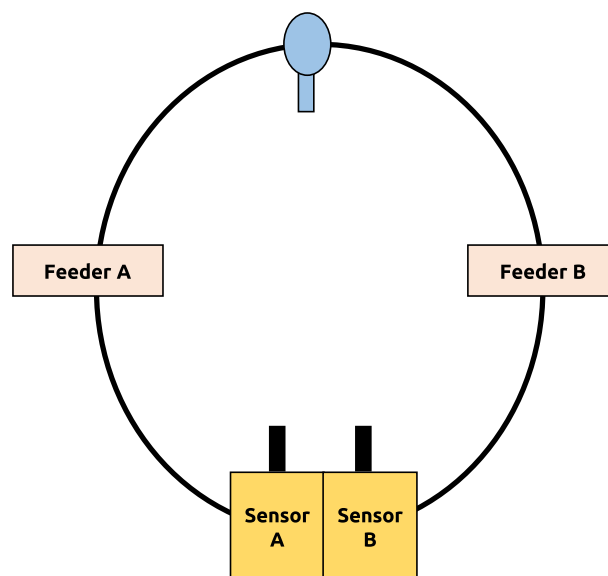
**Table 1**  
Diet composition.

Components	Commercial EFICO 3073 Biomar	Basal	Negative	Positive
Soy protein concentrate	–	17	17	–
Fish meal SuperPrime	–	12.5	12.5	30
Fish meal 60	–	–	–	10
Fish protein hydrolysate	–	–	–	6
Krill meal	–	–	–	6
Squid meal	–	–	–	6
Wheat gluten	–	6	6	–
Corn gluten	X	10	10	–
Soymeal	–	20	20	–
Canola meal	X	5	5	–
Sunflower meal	–	–	–	–
Wheat meal	–	10	8.5	28.5
Blood meal	X	–	–	–
Pea starch	X	–	–	–
Peanut meal	X	–	–	–
Fish oil	X	4.8	4.8	12.5
Soy oil	X	–	–	–
Canola oil	–	11.2	11.2	–
Palm oil	–	–	–	–
Vitamin premix	X	1	1	1
MCP	–	2	2	2
L-Lysine	–	0.3	0.3	–
DL-Methionine	–	0.2	0.2	–
Quinine (%)	–	–	1.5	–
Protein	41–45	44	43.9	44
Fat	16–20	18.2	18.2	18.2
Carbohydrate	18–31	–	–	–
Fibre	2–4	–	–	–
Ash	5–8	–	–	–
Energy (MJ/kg)	21.7–22.3	21.5	21.4	21.5

(Table 1). Prior to the experiments, fish were placed in 500 L experimental tanks and acclimation was carried out for at least 2 weeks before the experimental period. Tanks were constantly supplied with flow through sea water and equipped with two identical self-feeding systems activated by independent string sensors that were placed together to ensure the distance from the sensor to the feeder was the same. The water supply was placed on the opposite side of the string sensors (Fig. 1). The full water volume of the tank was replaced every 60 min. The number of demands was integrated every 5 min and feed reward per sensor activation was set at approximately 1 g/demand. The experimental tanks were visually isolated from the remaining tanks in the aquaculture facilities in order to avoid disturbances by daily routine activities. Access to the experimental area was limited to sampling and cleaning procedures. Sampling for measurement of fish weight and length was performed by pre-anaesthetizing the animals in their tanks with a solution of 2-phenoxy-ethanol (0.02%) for 3–5 min prior to netting. Subsequently, the animals were transferred to a sampling tank containing 0.1% of the same anaesthetic, where they remained for 2 min. Twenty-four hours prior to the start of sampling, sensors were removed from the water (at 10.00 a.m.). All experiments were carried out in accordance with the principles published in the European animal directive (86/609/EEC) for the protection of experimental animals.

### 2.1. Experiment 1

This experiment involved three consecutive phases. Sixty animals were distributed randomly into six 500 L tanks (10 fish per tank) equipped with two self-feeding systems. The animals were sampled at the start of the experiment to confirm the absence of statistical differences in size [body weight (BW) = 266.87 ± 19.4 g and length (L) = 23.21 ± 1.52 cm]. Phase 1 was aimed at exploring the potential preference of the animals for one feeder, thus both feeders were provided with the same basal diet (Table 1) for 15 days. After recording the total feeding demands in each feeder, the sensor with a higher number of demands was removed during phase 2. The animals were then solely allowed to self-feed from the least preferred feeder for 7 days. During phase 3, both sensors were reinstalled and feeding demands were



**Fig. 1.** Schematic vision of the 500 L tanks used in the dual choice experiments. Both feeders were oppositely placed in the sides of the tank yet string sensors were positioned together in order to ensure the same distance to the feeders. Water supply (blue) was placed opposite the sensors. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

recorded for 14 days. The average temperature throughout the three phases remained at  $23.5 \pm 0.5$  °C.

### 2.2. Experiment 2

In this experiment, 165 juvenile seabream (BW range = 25–50 g) were split up into 11 experimental tanks ( $n = 15$  animals / tank) equipped with two self-feeding systems. The experiment comprised 3 consecutive phases. During *phase 1* all feeders were provided with the same basal diet (Table 1) and animals were allowed to self-feed for 18 days. During *phase 2*, three different diets were used: a basal, a negative and a positive diet (Table 1), which were randomly assigned to demand feeders as exhibited in Fig. 2. The negative diet consisted of 1.5% quinine hydrochloride dihydrate (Sigma) which was added to the basal diet (Table 1). The positive diet was a high palatability diet containing high levels of fish, squid and krill meal. All three diets were isoenergetic thus exhibiting similar protein and fat levels (Table 1). Preliminary experiments using different experimental conditions demonstrated that positive diet is better accepted than both basal and negative diets (unpublished data). The animals were allowed to self-feed for 7 days. During *phase 3*, the diets in each tank were switched between feeders and demands were recorded for a period of 18 days. The average temperature throughout the three phases remained at  $25.2 \pm 0.5$  °C.

### 2.3. Experiment 3

This experiment was set up to corroborate the negative effect of quinine on feed demands by comparing fish groups in the absence of choice, that is, with access to a single type of diet (basal or negative). Therefore, 80 seabreams with BW ranging between 25 and 50 g were distributed in 8 tanks equipped with only one feeding system. Four tanks were provided with a basal diet and the remaining 4 experimental groups were fed with a negative diet for a period of 14 days. Initially, feeders were loaded with a recorded quantity of feed thus the total amount of food distributed at the end of the experiment was calculated by weighing the food remaining in the food dispensers. Such quantity was used to calculate the delivery rate for each electronic feeder using the number of food demands registered during the experimental period. Subsequently, the daily delivery of food was calculated using the feeder delivery rate and the number of daily demands. The non-consumed pellets were recovered daily from the bottom of the tank and subsequently weighed and dried overnight at 100 °C in order to obtain dry weight. The average temperature during the experimental period remained at  $13.6 \pm 0.5$  °C.

### 2.4. Data analysis

The preference index (PI) represents the feeding-demand balance

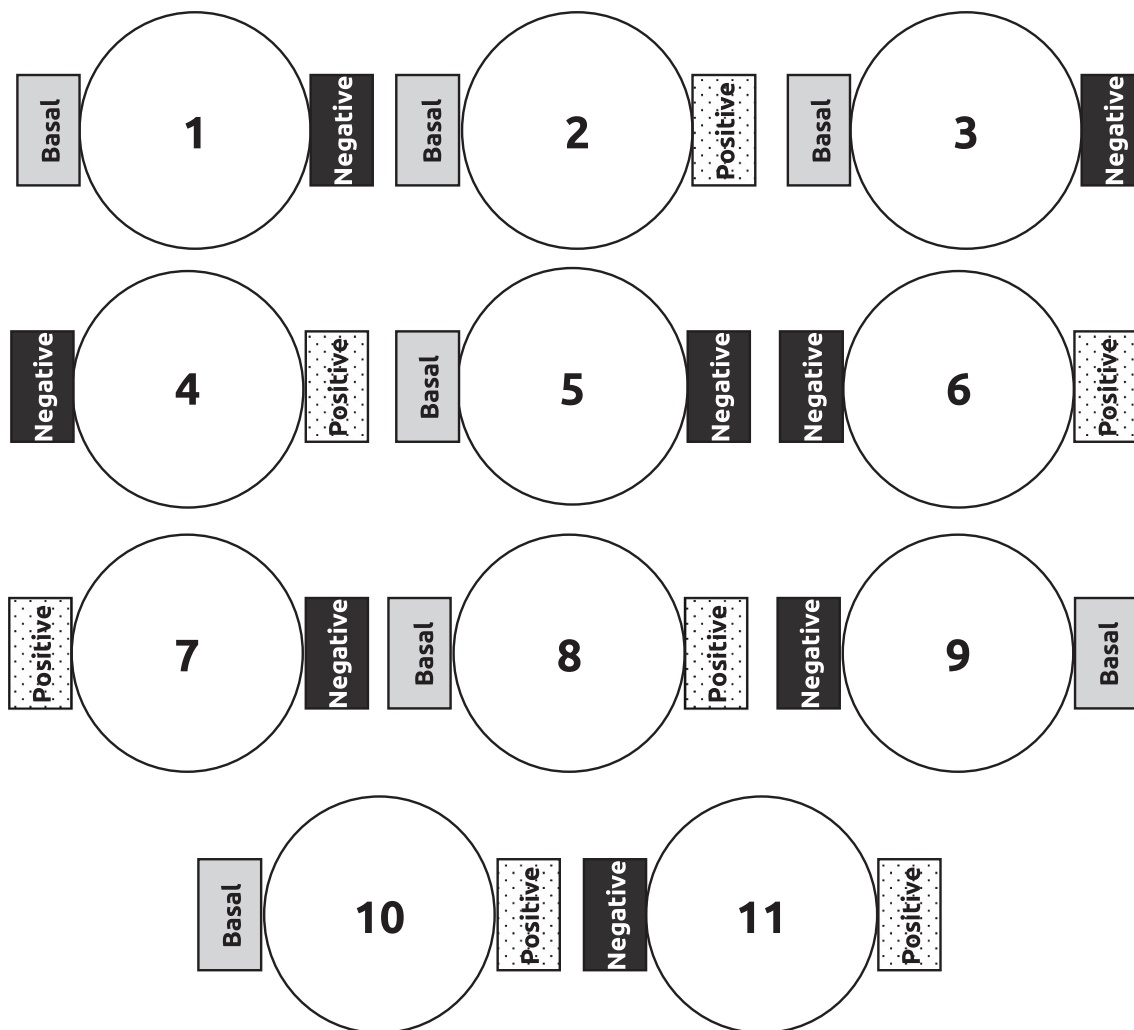


Fig. 2. Distribution of the isoenergetic diets: basal, negative (basal + quinine 1.5%) and positive (enriched with marine meals) diets in the different feeders of the tanks according to phase 2 of the experimental design 2. Subsequently, the diets were switched between feeders of the same tank during the experimental phase 3. During the initial experimental phase, all feeders contained the basal diet.

between both self-feeders of the same tank and is calculated as the quotient between the total demands of both feeders (A/B) throughout the experimental phase. A PI close to 1 indicates demand parity between both feeders, on the contrary,  $PI > 1$  or  $PI < 1$  represents a higher number of demands on feeder A or B, respectively. For the experiment 2, we also calculated the PI based on the feed diet, irrespectively of which feeder delivered the feed. Therefore, the PI for basal/negative, basal/positive and positive/negative diets was calculated as the quotient between the total demands for each diet during the phase 2 and 3 of the experiment 2. Data are expressed as means  $\pm$  standard error of the mean (SEM). Differences were analysed by one-way analysis of the variance (ANOVA) followed by Tukey's multiple range test (Experiment 1 and 2) or t-student test (Experiment 3) ( $P < 0.05$ ).

### 3. Results

#### 3.1. Experiment 1

No significant differences in weight and length were detected at the start of the experiment (data not shown). The number of total demands during phase 1 is shown in Table 2. Statistical comparison of daily average demands between the feeders of the same tank during phase 1 demonstrated that only one tank (16%) exhibited a statistically similar number of demands between both feeders (Fig. 3A). The remaining five tanks exhibited a PI far from the reference neutral index value (Table 2), displaying a clear preference for one of the feeders. During the phase 2, fish were driven to demand from the unpreferred feeder by removing the sensor with a higher number of demands during phase 1. Although no previous differences were observed in tank 1, the sensor showing the highest number of demands in phase 1 was also removed. Once the second sensor was reinstalled in the tank during the third phase, preference for the feeder available during phase 2 (Fig. 3A and B) was observed in most tanks, yet no differences were found in the number of daily demands between both feeders in two of the tanks (5 and 6) (Fig. 3B). Such changes were obtained owing to an increased drive of the fish towards the least preferred feeder in phase 1 (Table 2).

No previous differences were observed in the fish in tank 1, despite this, the sensor showing the lower number of demands in phase 1 was also removed. One must assume that the preference of fish during phase 3 switched to the available sensor in phase 2, now displaying significant differences. This was observed in tanks 2 and 3 where preferences notably shifted thus resulting in significant differences contrary to those recorded in phase 1. Preferences remained the same for fish in tank 4 yet the PI was severely reduced (Table 2) denoting an increased motivation for the least preferred feeder during phase 1. The number of demands per day during phase 1 and 3 was significantly higher than that recorded for phase 2, when a single feeder was used and no choice was available. No differences were observed between phase 1 and 3 (Fig. 4).

#### 3.2. Experiment 2

Experiment 2 was carried out on smaller fish showing a BW ranging

**Table 2**  
Feeding demands during the different phases of the experiment 1.

Tank	Phase 1 (15 days)			Phase 2 (7 days)			Phase 3 (14 days)		
	Feeder A	Feeder B	PI	Feeder A	Feeder B	PI	Feeder A	Feeder B	PI
1	156	386	0,40	137	–	–	455	121	3,76
2	58	664	0,09	233	–	–	565	75	7,53
3	590	31	19,03	–	437	–	58	748	0,08
4	78	48	16,42	–	150	–	608	185	3,29
5	733	268	2,74	–	228	–	501	351	1,43
6	173	538	0,32	305	–	–	479	239	2,00

Total self-feeding demands in feeders A and B of the same tank in the different phases of experiment 1. See material and methods for experimental design. Preference index (PI) is calculated as the quotient between the total demands of feeder A vs B.

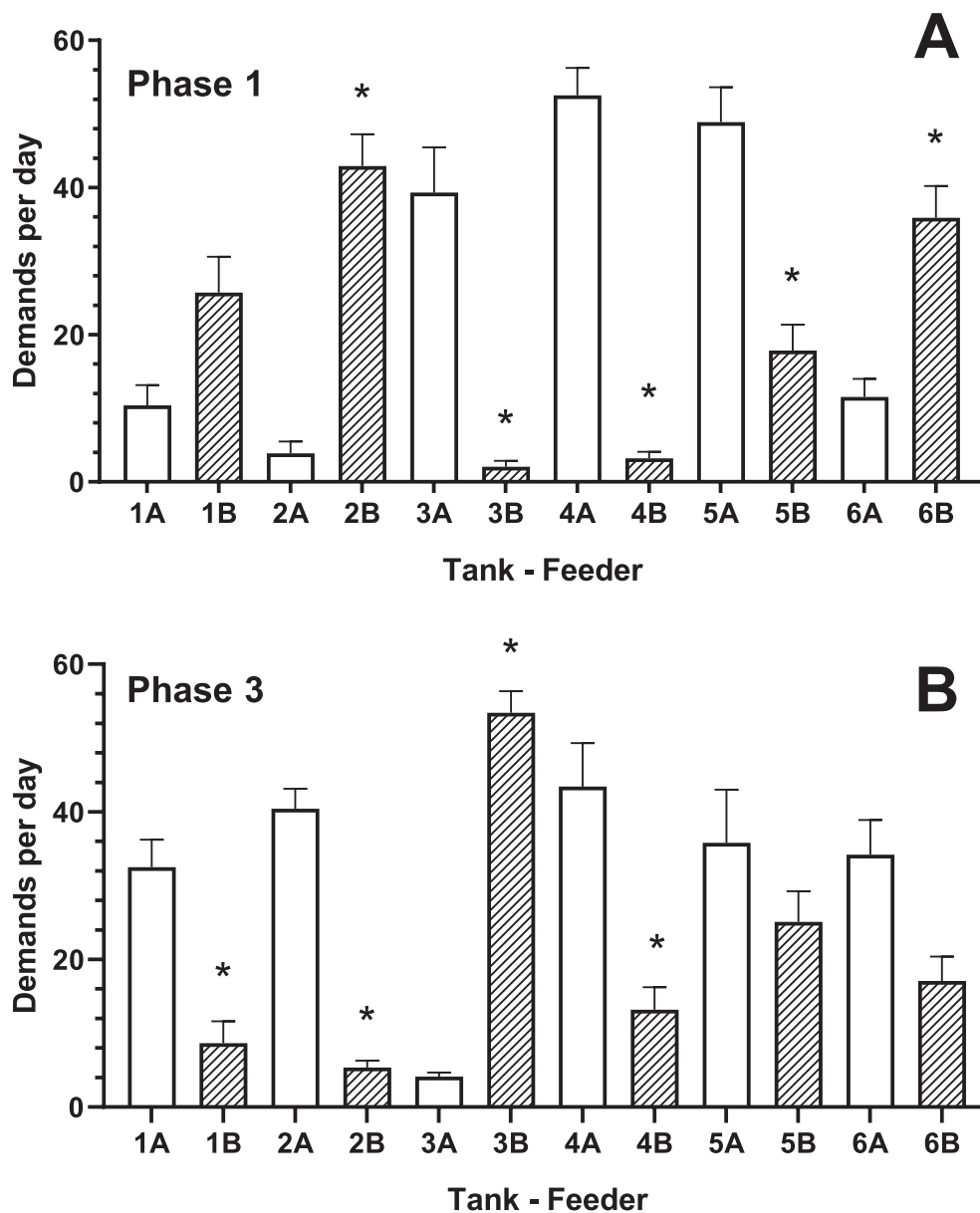
from 25 to 50 g. Fish were initially weighed and distributed randomly among the experimental tanks. Individual sizes and weights were not recorded after distribution in order to avoid stressful conditions which could influence feeding behaviour. During phase 1, all feeders were loaded with a basal diet and food demands were recorded for 18 days. Contrary to experiment 1, no significant differences were observed in most tanks (72%, 8 out of 11) in the number of demands between both feeders of the same tank (Fig. 5A, Table 3). When positive (palatable) and negative (quinine) diets were added in the experimental phase 2, the animals significantly demanded less of the negative diet when compared to the basal diet (Fig. 6A), and the same occurred when diets were switched between feeders in the same tank during the experimental phase 3 (Fig. 6B). However, when food demands between feeders of the same tank during phase 2 are compared, statistically notable differences are only observed in two tanks (8 and 9, Fig. 5B, Table 3). The same occurred during phase 3 when diets were switched, in which significant differences were only found in tank 7 (Fig. 5C, Table 3). However, when considering the integrated demands of all feeders providing different diets during both phases, quinine pellets were demanded in significantly lower quantities than the basal and positive (only phase 3) diets, despite switching the feeders between the two phases (Fig. 6A and B). Similarly, when all demands of each diet were integrated irrespectively of the feeder from they were delivered and the PI calculated for basal/negative, basal/positive and positive/negative for each experimental phase, the value for basal/positive was always close to 1 indicating no preference for positive diet. However, when negative diet was administered (basal/negative and positive/negative) PI values were always over 1 showing a preference for the alternative diet (basal or positive) (Table 4).

#### 3.3. Experiment 3

This experiment was carried out in order to evaluate the response to a negative diet in the absence of a dietary alternative. Results indicate that fish equally demanded basal and negative feeds, and feed delivery was also found to be similar (Fig. 7). However, the quantity of non-consumed pellets containing quinine throughout the whole experimental period was approximately 5 times higher compared to that of non-consumed pellets in the basal treatment (128.85 vs 27.16 g). Therefore, animals fed with negative pellets rejected 14.34% of demanded feed while those fed on a basal diet only rejected 2.57%, which results in 5.6 times more feed rejections.

### 4. Discussion

Feeding strategies have been widely used to study animal behaviour, as food is often used as an experimental reward, but can also involve a limited resource in time and space which animals defend from their congeners by exhibiting agonistic behaviour. This response is based on the evaluation of cost/benefits of the contest and can be associated to the access to good quality resources (Dominguez-Castanedo, 2021). In our studies evaluating the preference for two alternative feeders



**Fig. 3.** Daily average demands in feeders A and B (see Fig. 1) of the different tanks in phase 1 (A) and 3 (B) of experiment 1. During phase 1 animals were allowed to self-feed from both feeders delivering the basal diet (Table 1) for 15 days. During phase 2, the preferred feeder was removed for 7 days and subsequently during phase 3 both sensors delivering basal diets were reinstalled for 14 days. Data are expressed as means  $\pm$  standard error of the mean (SEM). Asterisks indicate significant differences ( $p < 0.05$ ) after one-way ANOVA.

(experiment 1), it was observed that animals commonly develop preferences for one feeder despite both systems delivering the same diet. However, when fish are forced to feed from the least preferred feeder for a required amount of time and are subsequently provided with the initial alternative, they are often unable to overcome this biased behaviour and learn to develop a preference for the opposite feeder system. Animals provided with only one feeder demanded less food than when provided with two but presumably their nutritional and energetic requirements remained unaltered. This suggests that social interactions could compromise feeding behaviour and food intake in seabream. It has been demonstrated in several fish species including rainbow trout (Brännäs and Alanärä, 1994; Alanärä and Brännäs, 1996), seabass (Millot and Begout, 2009), and cod (Millot et al., 2012), that the majority of sensor activations are performed by a reduced number of fish (1–2) in the tank population. Such dominant fish in the social hierarchy get a higher share of available food than the submissive fish, hence having better growth ratios (Brännäs and Alanärä, 1994; Alanärä and Brännäs, 1996). Undeniably, body size also defines the social ranking of the animals as in some species a size difference of only 5% is sufficient to ensure dominance of larger animals (Abbott et al., 1985).

Seabream exhibit aggressive behaviour for food (Goldan and Popper, 2003) which is intensified when resources are limited and defensible. In some species, such agonistic behaviour seems to be more severe when food is delivered in a predictable area compared with a wider and unexpected distribution (Oikonomidou et al., 2019). Food competition regulates growth in seabream as medium-sized juveniles exhibit specific growth rates three times as high when surrounded by smaller rather than larger fish because of the monopolization of food by larger animals (Karplus et al., 2000). In addition, animals fed under high feeding rates display a lower number of aggressive acts during feeding time than animals fed at lower ratios (Oikonomidou et al., 2019). It can be suggested that seabream can also develop agonistic behaviour associated to the conditioned stimulus while dominant animals will defend the sensor from subordinates, thus leading the feeding demands of the population. In tanks equipped with only one sensor, dominant fish will be more efficient in its guard but in tanks with two sensors the defence will be less effective and subordinate fish will gain some opportunities to demand by themselves. Therefore, the presence of an additional sensor can weaken agonistic behaviours, thus explaining the decrease in the number of feeding demands in the tanks equipped with only one sensor. In

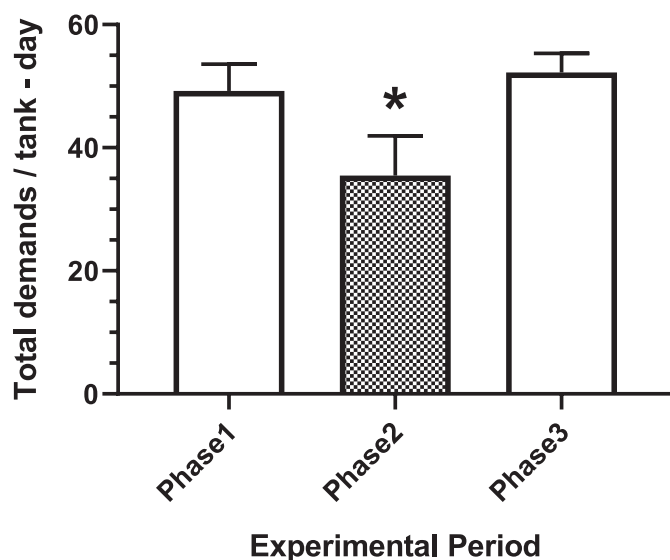


Fig. 4. Average demands throughout all three experimental phases (see Material and Methods for description of the experimental design) of experiment 1. Data are expressed as means  $\pm$  standard error of the mean (SEM). Asterisk indicates significant differences ( $p < 0.05$ ) after one-way ANOVA.

In addition, data suggest that the potential agonistic behaviour when using single self-feeding systems can mask energetic and nutritional requirements as well as organoleptic preferences of the population. This is to say, self-feeding systems allowing agonistic behaviour will register the preferences and requirements of the dominant animal(s). Such agonistic behaviour could also explain the imbalance of demands between sensors releasing identical diets from the feeders. The dominant animal(s) will defend and demand the preferred sensor whereas the demands on the opposite sensor, defended less efficiently by the leader, will be made sporadically by subordinate animals. The preference of dominant(s) fish can be altered by removing the preferred sensor for a period of time; however, the agonistic behaviour now falls on the opposite sensor.

The demand imbalance between both sensor/feeder systems was severely reduced during the second experiment carried out on smaller animals. It is very likely that the agonistic behaviour is less established in smaller juvenile animals as was demonstrated in killifish (*Milnerichthys robustus*) (Dominguez-Castanedo, 2021). Similarly, studies in zebrafish have demonstrated a positive correlation between aggressive behaviour and developmental maturity suggesting that social ranking initially emerges between weeks 4 and 6 of development (Ricci et al., 2013). The onset of exogenous feeding during development could promote concomitantly the aggressive behaviour due to competition for resources, yet it seems to be species dependent. In fact, some species do display cannibalism in the larval stages (Höglund et al., 2005). Data suggest that agonistic behaviour in seabream could be positively correlated with developmental maturity as demonstrated in zebrafish (Ricci et al., 2013). Alternatively, the enhanced nutritional requirements of smaller fish (Oliva Teles et al., 2020), and consequently an increased voracity, could be the reason of the reduced agonistic behaviour in younger fish since subordinate animals could show augmented feeding motivation thus making the defence of dominant fish less efficient.

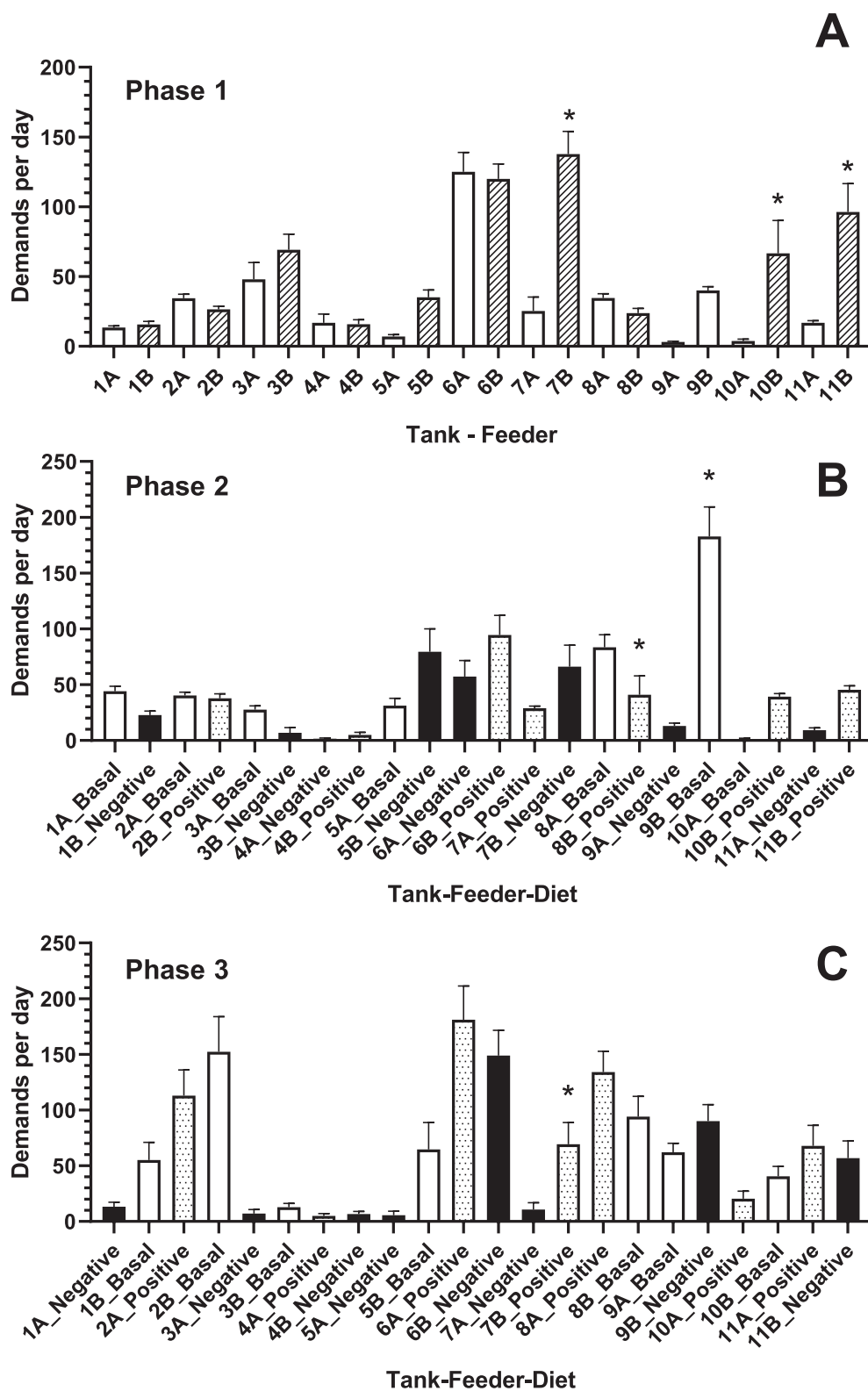
Previous studies reported that seabream can select diets with reduced lipid oxidation rates using self-feeding systems (Montoya et al., 2011), and also compose a balanced diet by selecting incomplete diets delivered by self-feeding systems (Montoya et al., 2012). According to our study, and with the aim to corroborate that seabream can also select diets from an organoleptic point of view, food demands among three different isoenergetic diets were compared, including a basal, positive and negative (basal with added quinine) diet in two different phases in

which diets were switched between feeders of the same tank. In both phases, animals demanded quinine diets in lesser quantity, soundly supporting the negative discrimination of this diet. However, in the absence of an alternative diet (experiment 3), seabream demanded similarly both basal and negative diets but, importantly, the quantity of unfed pellets increased with the presence of the bitter substance. Consequently, we can conclude that seabream can discriminate the presence of bitter flavours in diets especially when an alternative diet without quinine is provided, such as a basal or positive diet, otherwise animals will demand both diets indistinctively but reject pellets containing these bitter substances. Quinine is a highly deterrent substance for most fish species (Kasumyan and Doving, 2003), including seabream (Rigos et al., 2013). These results can be understood as an interplay between the energetic/homeostatic and sensory/hedonic systems controlling feeding behaviour in fish. Homeostatic systems could be regulating food demands to meet fish energetic requirements. This would explain the similar number of demands when fish have solely one dietary alternative: a basal or negative (deterrent) diet (experiment 3). Once in the oral cavity, intraoral gustatory systems screen sensory qualities of the captured food to promote ingestion or rejection (Morais, 2017). Bitter flavours often indicate toxic or harmful molecules and promote aversive responses such as pellet rejection (Kasumyan and Doving, 2003). However, when fish had a potential dietary choice (experiment 2), the alternative diet was demanded at a higher rate than the bitter feed.

Surprisingly, seabream did not show a preference for a diet enriched with marine ingredients, as no significant differences between positive and basal diets were found during both experimental phases. During the second phase of experiment 2, the number of demands between positive and negative diets were both lower than the basal diet, which could be due to the novelty of the diets and the short experimental period (7 days). Fish were accommodated to the basal diet during the first experimental phase, which lasted 18 days, followed by exposure to new diets (positive and negative). This could explain the lack of preference for the positive, putatively more palatable diet, with familiarization to the basal diet possibly having a higher influence in food choice. However, during the third experimental phase the differences between positive and negative diets were patent suggesting that fish could overcome the novelty effect. Such results, to a certain extent, support the strong effect of aversive substances, as animals were unable to overcome the lower preference for the negative (quinine) diet throughout the different experimental phases. On the other hand, this experiment was performed with rapidly growing juveniles at high water temperature, a situation in which it is not unlikely that the homeostatic system, associated with high energetic demands, overrides the hedonic regulation of feeding behaviour. Therefore, during the active feeding period of the annual cycle when temperatures reach maximal values, it seems plausible that seabream will not discriminate diets efficiently unless they contain deterrents or anti-nutritional molecules. Our preliminary results suggest that positive conditioning by palatable diets can exhibit different efficiency depending on the physiological state of the seabream (Morais et al., 2019) This poor dietary choice during active feeding periods could explain, to a certain extent, the easy adaptation of this species to diets in which alternative protein/oil sources are included in partial replacement of fish meal/oil (Pulido-Rodríguez et al., 2021).

## 5. Conclusion

Our studies demonstrate that seabream can discriminate different diets according to their organoleptic characteristics. The response associated to aversive diets was clearly more consistent. The negative conditioning of the quinine diet was revealed only when the animal was provided with an alternative choice, which resulted in a lower demand of the less palatable or aversive diet (experiment 2), but when no choice was available seabream equally demanded both diets (experiment 3), likely driven by the animal's homeostatic system and responding to



**Fig. 5.** Daily average demands in feeders A and B (see Fig. 1) of the different tanks in phase 1 (A) 2 (B) and 3 (C) of experiment 2. During phase 1 animals were allowed to self-feed from both feeders delivering the basal diet (Table 1) for 18 days. During phase 2, feeders were loaded with the experimental diets (basal, positive and negative) according to Fig. 2. For phase 3, diets were switched between feeders of the same tank. Data are expressed as means  $\pm$  standard error of the mean (SEM). Asterisks indicate significant differences ( $p < 0.05$ ) after one-way ANOVA.

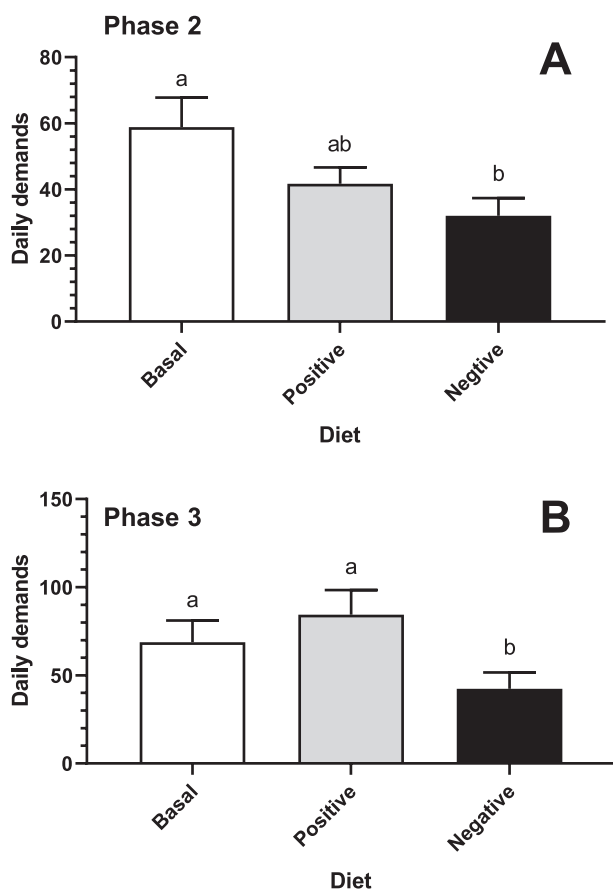
energetic requirements. However, in spite of equally demanding feed, the presence of the bitter substance resulted in higher feed refusals (experiment 3), indicating an important role of sensory systems in assessing food quality and ultimately determining food intake. In our experimental conditions involving highly active feeding periods, seabream was not able to discriminate positive diets (experiment 2) suggesting that energetic requirements may overcome hedonic regulation

of food intake. Finally, results also suggest that particular attention should be paid to social interactions that drive animals towards agonistic behaviour and can influence experimental results. Such potential agonistic behaviour seems to be less prominent in younger animals.

**Table 3**  
Feeding demands during the different phases of the experiment 2.

Tank	Phase 1 (18 days)			Phase 2 (7 days)				Phase 3 (18 days)			
	Feeder A	Feeder B	PI	Basal	Positive	Negative	PI	Basal	Positive	Negative	PI
1	243	283	0.86	309 A	–	159 B	1.94	991 B	–	240 A	0.24
2	619	476	1.30	282 A	265 B	–	1.06	2740 B	2031 A	–	0.74
3	864	1244	0.69	194 A	–	48B	4.04	229 B	–	129 A	0.56
4	303	286	1.06	–	35 B	11 A	0.31	–	90 A	121 B	0.74
5	127	631	0.20	219 A	–	554 B	0.40	1164 B	–	101 A	0.08
6	2250	2161	1.04	–	662 B	401 A	0.61	–	3258 A	2680 B	1.21
7	457	2480	0.18	–	202 A	463 B	0.44	–	1247 B	191 A	0.15
8	622	429	1.45	585 A	287 B	–	2.04	1694 B	2414 A	–	1.42
9	55	720	0.08	1280 B	–	91 A	0.07	1120 A	–	1632 B	0.69
10	70	1201	0.06	10 A	275 B	–	0.04	729 B	366 A	–	0.50
11	302	1732	0.17	–	318 B	64 A	0.20	–	1122 A	1023 B	1.19

Total self-feeding demands in feeders A and B of the same tank (phase 1) or different diets (phase 2 and 3) in the different phases of experiment 2. See material and methods for experimental design. Letters in phase 2 and 3 indicate feeders in which the different diets were loaded. Preference index (PI) is calculated as the quotient between the total demands of feeder A vs B.



**Fig. 6.** Average demands during experimental phase 2 (A) and 3 (B) of experiment 2 (see Material and Methods for description of the experimental design). Data are expressed as means ± standard error of the mean (SEM). Asterisk indicates significant differences ( $p < 0.05$ ) after one-way ANOVA.

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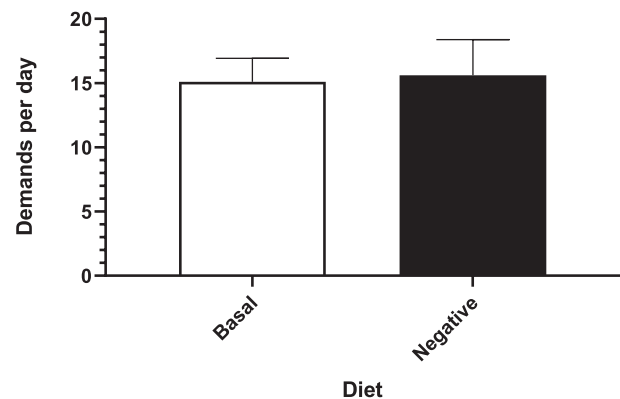
**CRedit authorship contribution statement**

**Sara Puchol:** Data curation, Formal analysis, Investigation,

**Table 4**  
Preference index (PI) for experimental diets during the different phases of the experiment 2.

	Basal/Negative	Basal/Positive	Positive/Negative
Phase 2 (7 days)	2.34	1.06	1.29
Phase 3 (18 days)	1.66	1.07	1.42

Preference index (PI) is calculated as the quotient between the total demands of experimental diets indistinctly of the feeder they were delivered during the phase 2 and 3 of the experiment 2.



**Fig. 7.** Daily average demands of basal and negative diets in tanks provided with one string sensor (experiment 3). Data are expressed as means ± standard error of the mean (SEM). Asterisk indicates significant differences after t-student test ( $p < 0.05$ ).

Methodology, Validation, Visualization, Writing – review & editing. **Esther Leal:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **Rita Angotzi:** Investigation, Methodology, Validation, Writing – review & editing. **Jesús Rosel:** Formal analysis, Writing – review & editing. **Sofia Morais:** Conceptualization, Resources, Writing – review & editing. **J.M. José Miguel Cerdá-Reverter:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



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