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2 **Quantity discrimination in angelfish (*Pterophyllum scalare*):**

3 **A novel approach with food as the discriminant**

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21 The ability to distinguish between different quantities of items is fundamental in many  
22 ecological contexts, and it has been shown in different animal species. This ability may also  
23 be context specific. Quantity estimation in fish has mainly been analysed in the context of  
24 social behaviour, whereas a majority of studies conducted with species other than fish tested  
25 it in the context of foraging. Surprisingly, little is known about the capacity of fish to  
26 discriminate between food quantities, possibly because of difficulties in testing individual  
27 fish in a novel, and thus aversive, test environment. Here, we present a novel approach that  
28 allows us to test single angelfish (*Pterophyllum scalare*) while minimizing isolation related  
29 stress. In binary choice tests, sets composed of similarly-sized discrete food items differing in  
30 numerical size, were presented and the spontaneous (untrained) choice of angelfish was  
31 investigated. In all contrasts tested in three experiments, angelfish preferred the numerically  
32 larger to the smaller food set. The performance of the fish was ratio-dependent in the small  
33 but not in the large number range ( $> 4$  food items, contrasts that were investigated for the first  
34 time in fishes), and there was no significant difference in the magnitude of preference in the  
35 small versus the large values. However, overall results indicated that the response was ratio-  
36 dependent, with an increase in accuracy as the numerical ratio between the contrasts  
37 increased. Furthermore, the same numerical ratios that were successfully discriminated with  
38 small quantities were also similarly discriminated with large quantities. Altogether, our  
39 results thus imply that angelfish utilize the Approximate Number System of quantity  
40 representation for the entire numerical range tested, and that their response attempts to  
41 maximize foraging success.

42 **Keywords** Angelfish, Food sets, Numerical cognition, Quantity discrimination

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44           Quantity discrimination is a basic form of numerical competence. This ability allows  
45 individuals to choose between quantities that differ in the number of elements, and it has been  
46 shown in a range of animal species, including humans (e.g. see Lourenco, 2016; Vallortigara,  
47 2015). Such capacity can provide fitness benefits in diverse ecological scenarios including  
48 inter-group conflicts (Bonanni, Natoli, Cafazzo, & Valsecchi, 2011), parental investment  
49 (Lyon, 2003) or predation risk contexts (Hager & Helfman, 1991). Most studies investigating  
50 quantity discrimination abilities have employed foraging situations, because in nature  
51 discrimination of the relative differences between food quantities available can directly affect  
52 survival rates. According to optimal foraging theory (Stephens & Krebs, 1986), when animals  
53 are faced with alternative foraging options, they should choose the one that provides the  
54 greatest net energetic gain. Therefore, the ability to assess different quantities is helpful to  
55 select the food source that provides the best payoff.

56           Most studies on quantity discrimination dealing with foraging decisions have been  
57 carried out in mammals and birds and under controlled laboratory conditions. This approach  
58 has allowed investigators to assess potential cognitive mechanisms underlying the  
59 discrimination. A variety of research methods have been adopted to investigate numerical  
60 abilities of animal species (reviewed in Agrillo & Bisazza, 2014), but a commonly adopted  
61 methodology to measure quantity discrimination involving foraging behaviour is the binary  
62 choice test. Under this paradigm, subjects have to select between two visible, simultaneously  
63 presented, numerically different sets of food items, which generally remain in view at the  
64 time of choice. This spontaneous quantity discrimination has been employed in studies with  
65 mammals (Baker, Morath, Rodzon, & Jordan, 2012; Bánszegi, Urrutia, Szenczi, & Hudson,  
66 2016; Beran, Evans, & Harris, 2008; Cox & Montrose, 2016; Hanus & Call, 2007; Miletto  
67 Petrazzini & Wynne, 2016; Parrish, Evans, & Beran, 2015) and birds (Bogale, Aoyama, &  
68 Sugita, 2014; Garland, Low, & Burns, 2012; Rugani, Vallortigara, & Regolin, 2013), but it

69 has also been utilized in other animal species such as amphibians (Krusche, Uller, & Dicke,  
70 2010; Stancher, Rugani, Regolin, & Vallortigara, 2015; Uller, Jaeger, Guidry, & Martin,  
71 2003) and reptiles (Miletto Petrazzini, Fraccaroli, Gariboldi, Agrillo, Bisazza, Bertolucci, &  
72 Foà, 2017).

73         These studies have shown that animals are sensitive to quantitative differences in food  
74 sets, as most species studied were found to be able to discriminate between the item sets and  
75 showed significant preference for the larger quantity. Often, individuals are subjected to  
76 discrimination tests that involve small ( $\leq 4$ ) and also large ( $> 4$ ) quantities of food items, and  
77 sometimes discrimination ability was found to be not uniform across these two number  
78 ranges. The results have suggested the existence of two distinct representational mechanisms:  
79 one to account for performance when numerically small sets are presented, and another when  
80 discrimination between numerically large sets was required. The latter system, named  
81 Approximate Number System (ANS), was found to be imprecise. It adheres to Weber's law  
82 in that discrimination depends on the ratio, and not the absolute numerical difference,  
83 between the number of elements of the sets compared. In contrast, the mechanism proposed  
84 to operate with small quantities, named Object File System (OFS), is precise. It does not  
85 depend on the ratios between the two quantities, but is limited to discrimination of elements  
86 in the small number range, i.e. maximum 3-4 elements (Feigenson, Dehaene, & Spelke,  
87 2004). Nevertheless, some evidence indicates the existence of only one system (the ANS) for  
88 the whole numerical range, as performance in some studies has been found to be dependent  
89 upon the numerical ratio in both the large and the small number range (Beran, 2004; Cantlon  
90 & Brannon, 2006; Perdue, Talbot, Stone, & Beran, 2012).

91         A growing number of studies have focused on the analysis of numerical cognition and  
92 quantitative abilities in fishes too (see Brown, 2015). Most of these studies have examined  
93 the discrimination between sets constituted by a different number of conspecifics, when the

94 sets (shoals) are placed in each of the opposite sides of a test aquarium (see Agrillo, Miletto  
95 Petrazzini, & Bissazza, 2017). By transferring an individual test fish of a social species into a  
96 novel, and potentially dangerous environment (the test aquarium), it was expected that, if the  
97 subject was able to distinguish between quantities of conspecifics, it should join the larger  
98 shoal as this offers better protection, diluting the potential predation risk for a solitary fish. In  
99 several fish species, a natural ability to assess quantities of conspecifics has been  
100 demonstrated (Agrillo, Dadda, & Serena, 2008a; Buckingham, Wong, & Rosenthal, 2007;  
101 Piffer, Agrillo, & Hyde, 2012; Potrich, Sovrano, Stancher, & Vallortigara, 2015; Seguin &  
102 Gerlai, 2017; Stancher, Sovrano, Potrich, & Vallortigara, 2013; Thünken, Eigster, &  
103 Frommen, 2014). As in other vertebrates, a controversy exists, however, over the  
104 representational mechanism(s) underlying discrimination in fishes. Some of the studies  
105 support the existence of two distinct mechanisms (Agrillo, Miletto Petrazzini, & Bisazza,  
106 2014; Agrillo, Piffer, Bisazza, & Butterworth, 2012; Piffer et al., 2012), whereas other studies  
107 support the idea of a single mechanism operating over the entire numerical range (Mehlis,  
108 Thünken, Bakker, & Frommen, 2015; Miletto Petrazzini & Agrillo, 2016; Potrich et al.,  
109 2015).

110 In contrast with other animal species, however, only a very few studies in fish have  
111 used food as discriminative stimulus, and the focus on foraging behaviour in this type of test  
112 has only begun recently. Difficulties of testing an individual fish in a novel, potentially  
113 frightening environment, together with complications arising from presenting food in water,  
114 including odour cues, may account for the lack of food quantity discrimination studies in fish.  
115 In fact, in the only two studies published to date, each individual fish had to be acclimatized  
116 to the novel environment (the test aquarium) for a week, and smaller conspecifics were also  
117 introduced to reduce the potential effects of individual housing as well as to facilitate  
118 adaptation and response to the food stimulus (Luxon-Xiccato & Dadda, 2017; Lucon-

119 Xiccato, Miletto Petrazzini, Agrillo, & Bisazza, 2015). These studies, conducted with guppies  
120 (*Poecilia reticulata*), tested only a few contrasts of sets of similarly-sized food items. Lucon-  
121 Xiccato et al. (2015) reported that the guppies were able to distinguish between different  
122 number of food items up to a 2:1 ratio (4 versus 1 and 4 versus 2 items), but not between  
123 smaller ratios (number of elements in the larger set divided by the number of elements in the  
124 smaller set). For example, they were unable to discriminate between 6 versus 4 sets of  
125 discrete food items (Lucon-Xiccato & Dadda, 2017). However, no study has examined the  
126 abilities of fish to discriminate between food quantities in the large versus the small number  
127 range using multiple contrasts systematically varied.

128         The angelfish (*Pterophyllum scalare*) have been used in the analysis of quantity  
129 discrimination abilities. These fish have been shown to spontaneously discriminate shoals of  
130 conspecifics differing in numerical size when the contrasted shoals were in the large number  
131 range, when they were in the small number range and also when one of the contrasted shoals  
132 belonged to the large and the other to the small number range (Gómez-Laplaza & Gerlai,  
133 2011a, 2011b, 2015, 2016a, 2016b). Furthermore, in addition to being able to show  
134 significant preference for the larger shoal when both contrasted shoals were simultaneously  
135 visible, angelfish were also found to be able to remember where the larger shoal used to be  
136 shown, a result that demonstrated mental representation (memory) of different quantities of  
137 items in this small teleost (Gómez-Laplaza, Caicoya, & Gerlai, 2017). However, quantity  
138 discrimination abilities of angelfish in contexts other than social has not been investigated,  
139 although two studies have used training procedures with food as reward (Agrillo, Miletto  
140 Petrazzini, Tagliapietra, & Bisazza, 2012; Miletto Petrazzini, Agrillo, Izard, & Bisazza,  
141 2016). It is possible that natural selection shaped discrimination abilities for quantities of  
142 shoals and for quantities of food items differently. If performance is context specific (Miletto  
143 Petrazzini, Agrillo, Piffer, & Bisazza, 2014), a different ecological context employed

144 experimentally may reveal different, previously unknown, numerical cognitive features of  
145 angelfish. The goal of the current study is to explore this possibility, and to investigate  
146 discrimination ability of angelfish when the items to be discriminated are food. In chicks, for  
147 example, the response was not found to be context-specific, i.e. preference for the  
148 numerically large quantity was found both when discriminating between numerically distinct  
149 social partners as well as between food quantities. The discrimination response to social  
150 attractors, however, was found to be better than that to food attractors (Rugani, Cavazzana,  
151 Vallortigara, & Regolin, 2013).

152         In the present study, we investigated the spontaneous ability of angelfish to  
153 discriminate between food quantities using a two-choice discrimination task between sets  
154 composed of discrete homogeneously-sized food items differing only in numerical size.  
155 Initially, we employed the same procedure we previously utilized for the analysis of  
156 discrimination between shoals of conspecifics. This procedure required individual housing  
157 and testing of the subjects (which motivated them to choose conspecifics). The results of our  
158 pilot experiments showed, however, that the priority of the subjects after being individually  
159 transferred to the test aquarium was not foraging, but rather fear, associated with high activity  
160 and/or immobility (active or passive defense reactions). In fact, similar effects of short term-  
161 individual housing in angelfish on feeding behaviour have been reported (Gómez-Laplaza &  
162 Morgan, 1993). Therefore, to avoid the behavioural consequences of individual housing in  
163 this shoaling (group forming) species, we developed a novel methodology by which subjects  
164 were individually tested while in a shoal. Using the novel method, we examined the ability of  
165 angelfish to discriminate between a range of food sets of different numerical size. The task  
166 consisted of fish having to discriminate and freely approach the zone close to the larger of  
167 two food sets simultaneously presented. In Experiment 1, we validated the new approach that  
168 allowed us to manipulate and measure the discriminability between stimulus pairs. Also, this

169 experiment served to verify the engagement of individuals in the task and screen for potential  
170 side bias. In Experiment 2, we investigated the response of angelfish when confronted with  
171 pairs of food sets in the small number range, and explored the potential limit of  
172 discriminability (i.e. 4 versus 3 food items) to compare it to that obtained with our previous  
173 studies using stimulus shoals. Finally, in Experiment 3 we tested discrimination between food  
174 sets in the large number range ( $\geq 4$  items in each set), a numerical range surprisingly not  
175 studied before in fish. We also tested an additional contrast that crossed the boundary  
176 between large and small quantities (5 versus 2) in order to clarify whether one or two number  
177 representation systems may operate in the foraging context in angelfish. The failure to  
178 discriminate sets across the large-small boundary has often been interpreted as reflecting  
179 incompatibility between representational systems, thus supporting the idea of the existence of  
180 two distinct systems (e.g. Cordes & Brannon, 2009; Piffer et al., 2012). The assumption  
181 underlying all these tests is that, if angelfish are able to discriminate between the two food  
182 quantities, they are expected to select the most advantageous option to maximize food intake,  
183 i.e. the larger quantity.

184

## 185 **METHODS**

### 186 *Subjects and housing conditions*

187 The experimental subjects used in the experiments were juvenile angelfish (3.0-3.3  
188 cm standard length) obtained from local commercial suppliers. Only juveniles of this sexually  
189 monomorphic species were studied, so as to avoid possible confounding effects arising from  
190 territoriality or sexual/courship behaviour. The fish were housed in the laboratory in glass  
191 maintenance aquaria (60 x 30 x 40 cm, length x width x depth) in groups of 18-20 individuals



192 per aquarium, and were allowed a minimum of 2-week acclimation period prior to  
193 behavioural testing.

194 The maintenance aquaria were filled with dechlorinated tap water, kept at  $26 \pm 1^\circ\text{C}$   
195 throughout the study using thermostat-controlled heaters. Each aquarium was illuminated by  
196 a 15-W white fluorescent light tube placed above the tank, and a 12:12 h light:dark cycle was  
197 maintained with lights on at 0830 hours. External filters continuously cleaned the aquaria,  
198 which had a 2-cm deep gravel substratum. Except for the front, all exterior walls of the  
199 aquaria were lined with white cardboard. The fish were fed twice daily, at 1000 and at 1800  
200 hours, on commercial food flakes (JBL GALA, JBL GmbH & Co. KG, Neuhofen, Germany)  
201 presented on the water surface. All fish were returned to the supplier at the end of the study.

#### 202 *Experimental apparatus and stimuli*

203 The experimental aquarium (60 x 30 x 33 cm, length x width x depth) was maintained  
204 under the same conditions as the maintenance aquaria. All exterior walls of the experimental  
205 aquarium were lined with white cardboard to prevent the fish being influenced by external  
206 visual stimuli. The aquarium was divided into three compartments along the short axis by  
207 inserting two transparent plastic partitions 25 cm from each lateral short side of the aquarium  
208 (see Fig. 1). In the center of each partition, a small rectangular guillotine window (6.5 width x  
209 16 cm height) was opened to allow the fish to pass through from one compartment to the  
210 other. The guillotine windows were handled by the experimenter, and could be closed or  
211 opened by placing or removing (raising or lowering) a panel, of the same material as the  
212 partitions, that could cover the windows (see below). The central part of the smaller middle  
213 compartment constituted the starting box (10 x 10 x 33 cm high) from where the  
214 experimental fish were released for behavioural testing (Fig. 1).

215 The two lateral compartments of the experimental aquarium were alternated across  
216 subjects and were considered as the ‘home compartment’ and the ‘testing compartment’,

217 respectively, where stimuli were presented during testing. In the middle of the testing  
218 compartment, a transparent plastic divider (10.5 x 33 cm width x height) was placed to divide  
219 that end side into two equally-sized halves: the ‘preference zones’ (10.5 x 15 cm, width x  
220 length; Fig. 1). At least three-quarters of the body length of the fish had to be within the  
221 boundary for the fish to be considered inside such zones.

222         In each of the preference zones the food stimuli were presented. These consisted of  
223 two sets of discrete food items of different numerical size (number of food pieces)  
224 simultaneously presented. The food sets remained visible during the test period. In order to  
225 avoid any potential chemical cue that could guide the subjects in the selection, during tests  
226 food quantities were presented outside the experimental tank (see below). Food items were  
227 pasted on a 5 x 5 cm area at the terminal part of transparent plastic panels (10 x 35 cm  
228 height), 4 cm from the bottom end. The panels were inserted between the external part of the  
229 glass of the corresponding end wall of the aquarium and the white cardboard lining the wall.  
230 Thus, the stimuli were positioned flush against the exterior end wall in the testing  
231 compartment at a distance of 10 cm apart from each other. Food was provided in discrete  
232 items prepared by making a homogeneous mass with the flakes using some water. The mass  
233 was agglutinated, and uniform-sized (0.4 cm Ø) circular pieces were obtained by means of a  
234 methacrylate mold sheet (0.1 cm thick) perforated with homogeneous holes (0.4 cm Ø) into  
235 which portions of the agglutinate were introduced to obtain food items with the shape and  
236 size uniform.

237         To avoid discrimination based on the overall configuration of the stimuli, for each  
238 quantity presented 12 different configuration patterns (spatial arrangement of the food items)  
239 were elaborated (see Appendix Fig. A1), in such a way that for any pair of contrasts fish were  
240 presented with a different stimulus configuration.

241 *Procedure*

242 As angelfish is a shoaling cichlid species that forms groups in the wild and also under  
243 laboratory conditions, as mentioned above, to minimize the consequences of social isolation,  
244 subjects were tested only when they voluntarily swam away from their shoal mates. First, all  
245 experimental angelfish underwent an acclimation phase to the procedure in the experimental  
246 aquarium. For this, the fish were placed to the aquarium in groups, i.e. with their shoal mates.  
247 Afterwards, during the testing phase, subjects continued to stay in their group, and a subject  
248 was tested only when it voluntarily entered the start box on the way to the test compartment  
249 where two sets of food items in panels showing different quantities of food were presented.  
250 Tests were performed in the same experimental aquarium as in the acclimation phase in order  
251 to minimize stress and ensure engagement with the task.

#### 252 *Acclimation phase*

253 Shoals of 10 angelfish, randomly chosen, were transferred from their maintenance  
254 aquaria into the experimental aquarium 7 days before the start of the experiments. During this  
255 period, individuals could swim freely among the three compartments of the apparatus and  
256 familiarize themselves with passing through the small windows of the partitions, which was  
257 facilitated by the interaction with other fish. Likewise, during this period, instead of  
258 providing food flakes on the water surface, food was provided in the form as described above  
259 (i.e. in discrete items). Items were pasted onto the terminal part of transparent panels by  
260 adding a drop of water, which allowed the food pieces to remain pasted when the panels were  
261 lowered into the water long enough to be consumed by the fish. During the acclimation  
262 phase, one food item was pasted in the lower part of each of four transparent panels, which  
263 were introduced into the experimental aquarium, and distributed in such a way that two  
264 panels (i.e. two food items) were placed in one of the long walls of the aquarium (one panel  
265 in the middle of each of the two large lateral compartments) and leaning against the walls,  
266 and the other two panels were placed on the opposite long wall of the aquarium. In this way,

267 monopolization of food by more dominant foragers, i.e. potential competition over food  
268 among conspecifics, was reduced. Likewise, the distribution of the food items throughout the  
269 aquarium and their location in the long walls (instead of in the short wall as during actual  
270 choice tests) prevented angelfish from associating the food with one specific location in the  
271 aquarium.

272         Subjects were fed in two daily sessions (morning and afternoon) following the same  
273 schedule as in the maintenance aquaria. The amount of food available during a session was  
274 similar to that provided in the maintenance aquaria, and subjects were soon habituated to feed  
275 in this way. Notably, although in the wild many species prefer feeding in the water column,  
276 domesticated fish prefer the surface to feed (Reinhardt, Yamamoto, & Nakano, 2001, quoted  
277 in El Balaa & Blouin-Demers, 2011), and based upon the mouth structure and behaviour  
278 (slow swimming species) of angelfish, these fish likely forage in nature by picking up food  
279 items (small crustaceans, worms, decaying organic matter) from solid surfaces. A habituation  
280 period during which experimental fish practiced how to feed near the gravel substratum was,  
281 therefore, successful and properly acclimatized the experimental fish to this new feeding  
282 method before the start of experiments.

### 283 *Testing phase*

284         Before starting each trial, an opaque white partition identical to the transparent  
285 partitions, including the guillotine window, was superimposed over one of the transparent  
286 partitions. The transparent partition to be covered by the opaque partition was  
287 counterbalanced between the two transparent partitions that delimited the lateral  
288 compartments, according to the schedule of each experiment. Thus, the position of the home  
289 compartment (that delimited by the opaque white partition) and the testing compartment  
290 (delimited by the transparent partition) were exchanged to avoid any lateral side bias.  
291 Consequently, the divider delimiting the preference zones was also exchanged. Thereafter, all

292 10 fish were gently guided to, and kept in, the compartment now delimited by the opaque  
293 partition (home compartment). As soon as the fish were in the corresponding home  
294 compartment, the opaque guillotine window was closed by the experimenter, thus blocking  
295 the view of the other side of the aquarium. Also, the transparent guillotine window that  
296 delimited the other compartment (the testing compartment) was closed. While all fish were in  
297 the home compartment, two different quantities of same-sized food items glued on the panels  
298 were simultaneously placed in the external side of each of the preference zones of the testing  
299 compartment. After a 3-min period, the opaque white guillotine window of the home  
300 compartment was raised by the experimenter to allow fish passing through it. We waited until  
301 one subject spontaneously swam through the window into the starting box (typically from a  
302 few seconds to a few minutes), and we immediately closed the opaque white guillotine  
303 window. That is, we limited the entrance of only one subject into the start box. The remaining  
304 subjects could not see what happened on the other side of the partition. After a period of 30 s  
305 in the start box, during which the subject could see the two sets of food items through the  
306 transparent partition, we gently raised the transparent guillotine window and the fish was  
307 released and allowed to freely enter the testing compartment to make the choice. As the  
308 transparent guillotine window was equidistant from the two sets of stimuli no location bias  
309 existed before the choice. Generally, subjects rapidly approached the preference zones to feed  
310 on one of the two food sets. The transparent guillotine window was closed to prevent fish  
311 from returning to the start compartment.

312 Tests took place in the morning at the usual feeding time (1000 - 1015 hours), thus  
313 subjects were not food-deprived, but they were sufficiently motivated to perform the task. A  
314 camera placed above the experimental aquarium recorded the behaviour and position of the  
315 subjects for 5 min. This recording period was chosen because fish, after approaching one of  
316 the food sets trying to eat the items (actually inaccessible to consume), generally swam to the

317 other preference zone to try to feed on the other set. After several unfruitful attempts to feed  
318 in one and the other set, often the fish stopped the attempts, and tried to return to the other  
319 compartments. Preliminary experiments revealed that after 5 min, the subjects rarely made  
320 new attempts to feed in the sets or to approach the preference zones, as they had learned that  
321 no food item was possible to consume.

322 To control for possible side preferences, we counterbalanced the left-right  
323 presentations (from the approaching subject's point of view) of the larger and smaller stimuli  
324 across fish and, as already mentioned, reversed the presentation of the sets between the two  
325 lateral sides of the aquarium by changing the overlapping opaque partition and placing it  
326 covering one or the other of the transparent partitions. We also randomized the order of  
327 presentation of each stimulus combination across subjects.

328 Each fish was tested only once for a single numerical contrast, and after having been  
329 tested, each subject was removed from the experimental aquarium and placed in another tank  
330 where it was fed. Likewise, the remaining fish of the shoal were fed in the usual way in the  
331 experimental aquarium, after raising the guillotine windows. Every second day, when two  
332 fish had been tested and removed, two new fish were transferred to the experimental  
333 aquarium to make sure that the size of the shoal in the experimental aquarium remained  
334 relatively constant.

335 In all experiments, all fish entered both preference zones at least once during the  
336 choice tests and, therefore, no fish was excluded from the statistical analyses.

### 337 *Statistical Analysis*

338 We recorded the first preference zone selected by the experimental angelfish, i.e., the  
339 first choice, measured the time spent (sec) in each preference zone, and calculated an index to  
340 quantify preference for one set over the other as follows: the time spent in the preference  
341 zone near the numerically larger food set was divided by the total time spent in both

342 preference zones. We also recorded the frequency of entries to the preference zones, as well  
343 as the latency to enter the preference zones.

344 In each experiment, the data were tested for normality (using the Kolmogorov-  
345 Smirnov one sample test) and for equality of variance (using Levene's test) before analysis.  
346 Data of latency to enter one or another preference zone were log transformed before the  
347 analyses to meet assumptions of parametric statistics.

348 The time spent in the preference zones was considered a measure of each test fish's  
349 preference for a particular food set, and a one sample *t*-test was employed to investigate  
350 whether the observed preference index was significantly ( $P \leq 0.05$ ) different from chance  
351 (50%). The Holm-Bonferroni sequential correction method was employed to correct for type  
352 I error resulting from multiple comparisons. A one-way ANOVA for independent samples  
353 was used to analyze the effect of the comparisons on preference. In case of a significant  
354 result, it was followed by a Tukey honestly significant difference (HSD) post hoc multiple  
355 comparison test. Binomial tests comparing the number of fish initially choosing the larger or  
356 smaller food set were used for each combination of stimuli, and frequency and latency scores  
357 were analyzed using paired *t* tests. All tests are two-tailed.

### 358 *Ethical note*

359 The experiments described here complied with the current law of the country (Spain)  
360 in which they were performed and were approved by the Ethics Committee of the University  
361 of Oviedo (permit ref.: 13-INV-2010). Essentially, the experiments involved behavioural  
362 observations with as little intervention by the observers as possible, and no invasive  
363 manipulation was performed on fish. The fish exhibited no signs of stress and remained  
364 healthy over the course of the experiments.

## 365 **RESULTS**

### 366 *Experiment 1*

367           The purpose of this Experiment was to determine whether the new approach is  
368 adequate to examine quantity discrimination using food as the discriminant in a binary choice  
369 test with angelfish, a shoaling species. To accomplish this, we examined the ability of  
370 angelfish to locate a food set composed of three food items vs no food set, i.e. 3 versus 0  
371 discrimination. The side of the testing compartment where the stimulus set was presented was  
372 initially randomly chosen, after which the location of the food set and the no food set were  
373 systematically alternated across test fish. A total of 12 fish were tested.

### 374 *Results*

375           In this control treatment, test fish reliably discriminated, and spent significantly more  
376 time in, the preference zone close to the 3-food item set than in the zone close to the no food  
377 set. The mean proportion of time, i.e. the preference index was Mean  $\pm$  SE = 0.9135  $\pm$   
378 0.0256,  $N = 12$ , a significant preference above chance (one-sample t-test,  $t_{11} = 16.159$ ,  $P <$   
379 0.001). Likewise, 12 out of 12 fish chose to enter first the preference zone adjacent to the  
380 food set (binomial test:  $P < 0.001$ ). These results indicated the viability of the novel  
381 methodology employed to test discrimination in angelfish. The strong preference for the side  
382 where the food was presented was also supported by the other behavioural parameters  
383 measured (see Table 1).

### 384 *Experiment 2*

385           In this Experiment we investigated whether angelfish were able to discriminate  
386 between two sets of equally-sized food items differing in the number of the items. The  
387 contrasts consisted of all possible binary combinations within the small number range (i.e.  $\leq$   
388 4 items). Thus, subjects were observed in their spontaneous preference between the following



389 six pairs of contrasts: 4 versus 1, 3 versus 1, 2 versus 1, 4 versus 2, 3 versus 2, and 4 versus 3.  
390 We observed 12 subjects in each contrast, i.e. a total of 72 fish were tested.

### 391 *Results*

392 When test fish were confronted with a choice between two food sets of different  
393 numerical size in the small number range, they exhibited a preference for the set containing  
394 the larger number of food items (Fig. 2). In most of the contrasts tested, angelfish spent  
395 significantly more time than expected by chance in the preference zone close to the larger  
396 quantity (one-sample  $t$  test, with Holm-Bonferroni correction: 4 versus 1,  $t_{11} = 5.669$ ,  $P =$   
397  $0.003$ ; 3 versus 1,  $t_{11} = 3.576$ ,  $P = 0.016$ ; 2 versus 1,  $t_{11} = 2.931$ ,  $P = 0.028$ ; 4 versus 2,  $t_{11} =$   
398  $3.021$ ,  $P = 0.036$ ; and 3 versus 2,  $t_{11} = 5.111$ ,  $P = 0.005$ . However, preference was found not  
399 to be significant in the contrast 4 versus 3, ( $t_{11} = 1.926$ ,  $P = 0.080$ ). Angelfish also showed a  
400 significant preference for first entering the preference zone close to the larger set: at least 10  
401 out of 12 fish exhibited this initial preference in most contrasts (binomial probability test: all  
402  $P < 0.05$ , Table 1). Interestingly, the exception was the contrast 4 versus 3, in which the first  
403 choice of 8 out of 12 fish was the large food set, a number that was found not significantly  
404 different from chance ( $P > 0.05$ ). This contrast represented a ratio of 1.33:1 (number of items  
405 in the larger set divided by number of items in the small set) and was the lowest ratio tested  
406 in this experiment.

407 The latency to approach the larger food set was significantly lower than the latency to  
408 approach the smaller set in most contrasts (all  $P \leq 0.031$ , Table 1). However, in the contrast 4  
409 vs 3 the difference was again not significant ( $P = 0.211$ , Table 1). This result, together with  
410 those obtained when considering the preference index and also the first choice, indicates an  
411 unsuccessful discrimination of the 4 versus 3 contrast.

412 One-way ANOVA showed that the difference in the magnitude of the preference for  
413 the larger set among the six contrasts approached significance ( $F_{5,66} = 2.328$ ,  $P = 0.052$ ), and

414 Tukey HSD test indicated that the magnitude of the response was significantly greater when  
415 the ratio was 4:1 compared to when the ratio was 4:3 ( $P = 0.023$ ). This result supports the  
416 notion that ratio affected the discrimination, and as the ratio decreased, although a differential  
417 response remained, discrimination became more difficult for angelfish.

418 Angelfish visited both preference zones in all contrasts indicating that they actively  
419 explored the stimuli, and repeatedly assessed the most profitable set. Consequently, the  
420 number of entries in the preference zone close to the larger food set was generally  
421 significantly higher than the number of the entries to the smaller food set (all  $P < 0.034$ ,  
422 Table 1), indicating the persistence of trying to catch food from the large quantity (i.e. fish  
423 after having been unable to catch food from the larger set, gave up for a while, and  
424 subsequently returned to the larger set without entering the zone of fewer food items). The  
425 exception was the 2 versus 1 and the 4 versus 2 contrasts, where, although fish showed the  
426 above tendency, no significant differences were found in the frequency of visits to one or the  
427 other food set ( $P > 0.05$ ).

### 428 *Experiment 3*

429 In Experiment 2 angelfish, with the exception of the 4 versus 3 contrast, were found  
430 to be able to discriminate between two food quantities when the item sets to be discriminated  
431 were within the small numerical range (1-4). In Experiment 3, we also examined the  
432 angelfish's choice between sets of food items of different numerical size, but now in the large  
433 number range. Five numerical contrasts were presented, including a contrast that crossed the  
434 boundary between the small and large number range, i.e. 5 versus 4, 8 versus 6, 9 versus 6, 8  
435 versus 4, and 5 versus 2. Some of these contrasts correspond to ratios employed in  
436 Experiment 2 and those employed before in a social context (e.g. Gómez-Laplaza & Gerlai,  
437 2011a, 2011b). Twelve subjects were observed in each contrast, with a total of 60 fish tested  
438 under the same protocol and dependent variables as described above.

439 *Results*

440 In all contrasts, fish showed a preference for the location where the larger food set  
441 was presented (Fig. 3). The preference was significantly above chance for contrasts whose  
442 ratios were 1.5:1 and greater (*t* test with Holm-Bonferroni correction: 9 versus 6,  $t_{11} = 3.084$ ,  
443  $P = 0.05$ ; 8 versus 4,  $t_{11} = 3.007$ ,  $P = 0.048$ ; 5 versus 2,  $t_{11} = 2.891$ ,  $P = 0.045$ ). However, for  
444 ratios smaller than 1.5:1 preference, as in Experiment 2, did not reach the threshold of  
445 significance (8 versus 6,  $t_{11} = 1.831$ ,  $P = 0.094$ ; 5 versus 4,  $t_{11} = 2.183$ ,  $P = 0.052$ ). One-way  
446 ANOVA showed no significant difference in the magnitude of the preference between the  
447 five contrasts tested ( $F_{4,55} = 0.460$ ,  $P = 0.765$ ). Interestingly, a similar pattern of results was  
448 found in Experiment 2 and Experiment 3. Thus, the same ratios between the contrasted sets  
449 that were successfully discriminated in the small number range (i.e. equal or above 1.5:1,  
450 Experiment 2), were now also found to be discriminated within the large number range  
451 (compare Fig. 2 and Fig. 3).

452 Some differences relative to Experiment 2 emerged, however, when we analyzed the  
453 other behavioural parameters. For example, although the first choice for 8-9 out of 12 fish  
454 was the larger set, this number of fish was found not to differ from chance in any of the  
455 contrasts tested (binomial tests, all  $P > 0.05$ , Table 1). Also, with the exception of the 8  
456 versus 4 contrast (see Table 1), the latency to approach the larger food set was not  
457 significantly shorter compared to the latency to approach the smaller set. Nevertheless, the  
458 overall time taken to enter the preference zone near the larger food quantity was significantly  
459 shorter than the time taken to enter the zone with the smaller food quantity ( $t_{59} = 3.656$ ,  $P =$   
460 0.001).

461 Analysis of the frequency of visits to the preference zones also indicated that  
462 angelfish actively assessed the sets and appeared to enter the zone of the larger food quantity  
463 with higher frequency, although this was not significantly different from the number of

464 entries to the zone where the small food sets were located (all  $P > 0.05$ , Table 1). However,  
465 considering all contrasts together, the overall frequency of entries in the larger set was, as in  
466 Experiment 2, significantly greater than the number of visits to the preference zone close to  
467 the smaller set ( $t_{59} = 2.015$ ,  $P = 0.048$ ).

#### 468 *Comparison of results obtained in experiments 2 and 3*

469 Comparison of all contrasts tested in Experiment 2 and 3 demonstrated that the  
470 magnitude of the preference was not significantly different among the 11 treatment groups  
471 (contrasts) ( $F_{10,121} = 1.410$ ,  $P = 0.184$ ). Furthermore, comparison of the overall magnitude of  
472 the preference for the large food set in Experiment 2 versus in Experiment 3, also showed no  
473 significant difference in performance between these two experiments ( $F_{1,130} = 1.490$ ,  $P =$   
474  $0.224$ ). Nevertheless, for Experiment 2 and 3 together, linear regression analysis revealed that  
475 the magnitude of the preference for the large food set increased significantly with increasing  
476 ratio between the food sets contrasted, and as the ratio of the larger to the smaller food set  
477 approached one, discrimination (i.e. preference) became increasingly difficult ( $F_{1,9} = 11.347$ ,  
478  $P = 0.008$ ; Fig. 4). This result is in accordance with Weber's law, and Pearson correlation  
479 coefficient confirmed such result: a significant correlation was found between the numerical  
480 ratio and the preference index (i.e. the magnitude of the preference) ( $r = 0.747$ ,  $P = 0.008$ ).  
481 Therefore, we conclude that overall accuracy was positively correlated with the ratio, and  
482 angelfish preference for the larger food set increased significantly with greater ratios.

483 We also found the frequency of visits to the zone of the larger food set to be  
484 significantly greater in Experiment 2 than in Experiment 3 ( $F_{1,130} = 17.034$ ,  $P < 0.001$ ),  
485 indicating perhaps greater persistence to enter the side where the larger quantity of food was  
486 located when the smaller quantity was composed of very few, i.e. only 1-2 items, instead of  
487 4-6 food items. This greater number of entries may be due to motivational factors. Possibly  
488 both of the large food sets provided enough food to satisfy the fish, consequently they did not

489 preferentially visit more times one zone over the other. On the other hand, differences in  
490 latency to approach the preference zones close to both the larger food set (one-way ANOVA,  
491  $F_{1,130} = 1.366$ ,  $P = 0.245$ ) and the smaller food set ( $F_{1,130} = 2.885$ ,  $P = 0.092$ ) were not  
492 significantly different between Experiment 2 and 3.

493

## 494 **DISCUSSION**

495        Idiosyncratic numerical abilities may be required, or may have evolved for,  
496 distinguishing quantities of items under distinct contexts. Previously, we investigated  
497 numerical abilities of angelfish when they were required to choose between different numbers  
498 of conspecifics, a social context. Most studies analyzing numerical or quantity estimation  
499 abilities of fish worked within this context. However, similar studies employed with other  
500 species, e.g. mammals and birds, often utilized foraging, a context in which numerical  
501 abilities of fish are virtually unknown.

502        In the present study we investigated the capacity of angelfish to discriminate  
503 numerically larger food quantity from the smaller one when the two sets of food items were  
504 simultaneously presented. This task was difficult because the choice had to be quantified  
505 using a single, isolated test subject, a condition under which fish of most species would  
506 experience stress or fear, and thus would not perform in a food choice test. The angelfish, like  
507 several other fish species, is a shoaling fish, at least before reaching sexual maturity, that  
508 would suffer from being isolated. To circumvent this issue, we developed a new test  
509 aquarium and procedure. Although we tested the subjects individually, i.e. in isolation, the  
510 experimental fish was allowed to remain within its shoal of conspecifics, and would enter the  
511 test session only once it voluntarily left its shoal.

512        A series of important features characterized the new approach: (1) although subjects  
513 were individually tested, the procedure did not require the artificial (experimenter forced)

514 separation of the subject from the rest of its companions in the shoal; (2) habituating the test  
515 fish to, and keeping them in, the test aquarium for a period of time before the actual choice  
516 task reduced handling stress and the stress of being introduced into a novel tank (Gómez-  
517 Laplaza & Morgan, 1993); (3) by presenting the stimuli outside the aquarium,  
518 chemical/olfactory cues were excluded, and (4) the procedure allowed assessment of  
519 spontaneous preference (as opposed to trained preference) for food quantity within a short  
520 period of time, allowing us to collect data for each fish using a 5 min long recording session.  
521 Other researchers have also tried to reduce the stressors that may confound the results in this  
522 type of tests, however, their procedures required periods of individual training and  
523 habituation of each subject, as well as several trials for each discrimination contrast (Lucon-  
524 Xiccatto & Dadda, 2017; Lucon-Xiccatto et al., 2015).

525         Using the novel methodological approach, our results provide the first evidence about  
526 the abilities of angelfish to discriminate between food quantities and, to our knowledge, the  
527 first results with testing food quantities in the large number range. Experiment 1  
528 demonstrated that the procedure is appropriate to study discriminability in a shoaling species  
529 of fish. Subjects were able to distinguish food from non-food, with all parameters measured  
530 indicating a clear preference for the zone where the food was presented when the alternative  
531 was the absence of food. The performance of experimental fish demonstrated that the subjects  
532 were sufficiently motivated for the choice, which, in turn, evidenced another advantage of our  
533 experimental protocol: there is no need of depriving the subjects of food. Since motivation  
534 may play an important role in the response, food deprivation is sometimes employed (e.g.  
535 Bánszegi et al., 2016; Lucon-Xiccatto et al., 2015). It may be argued that experimental  
536 angelfish could not have perceived the panel without food, and consequently may not have  
537 behaved as if the task offered a binary numerical choice (i.e. angelfish just approached the  
538 only visible stimulus in the compartment). This possibility, is unlikely since all fish could see

539 both preference zones while in the starting box, and although they spent most of the time in  
540 the preference zone with food, occasionally they did move around the compartment, and had a  
541 chance to see the zone without food even if they did not enter in it.

542         Although angelfish have shown good abilities to discriminate shoals of conspecifics  
543 differing in numerical size in a social, shoaling context, also thought of driven by predator  
544 avoidance (Gómez-Laplaza & Gerlai, 2011a, 2011b, 2015, 2016a, 2016b, Gómez-Laplaza et  
545 al., 2017), to date no study has tested these abilities in a foraging context. The current results  
546 demonstrate that angelfish can spontaneously select the larger quantity of food items, and are  
547 able to process quantitative information related to obtaining food. The successful  
548 discrimination, i.e. the preference for the larger food set both in the small and in the large  
549 number range is in accordance with optimal foraging (Stephens & Krebs, 1986). Since the  
550 distance from the start box to the food patches was identical in all contrasts, the costs in terms  
551 of time and effort to reach one or the other set were identical, and angelfish spontaneously  
552 selected the larger set of food items, i.e. the most advantageous option. Given the importance  
553 of this task for gaining fitness benefits in nature, it is likely that natural selection favoured  
554 fish with an ability to detect and show preference towards sets containing more food items.

555         Especially, in Experiment 2 angelfish were able to discriminate all pairings of  
556 combinations in the small number range, except the lowest ratio tested (4 versus 3). As in the  
557 current study, in none of the previous studies with angelfish, using shoals as stimuli and  
558 under different conditions, was the contrast 4 versus 3 elements found to be successfully  
559 discriminated, while the rest of the contrasts were discriminated in some of them (Gómez-  
560 Laplaza & Gerlai, 2011b, 2015, 2016b; Gómez-Laplaza et al., 2017). These previous studies  
561 showed that angelfish could not, or did not, preferentially distinguish between 4 versus 3  
562 stimulus shoals and the limit of discrimination within the small number range was found to  
563 be 1.5:1, i.e. 3 versus 2 (Gómez-Laplaza & Gerlai, 2011b). This ratio was also found to be

564 discriminated here in a foraging context. Thus, it appears that under spontaneous choice test  
565 conditions, angelfish do not exhibit the ability to distinguish ratios below 1.5:1, such as  
566 1.33:1 (4 versus 3) irrespective of the ecological context. Similarly, results showing context  
567 independency have been reported in chicks (Rugani, et al., 2013). When chicks were tested in  
568 a social (objects were used as social attractors) and a foraging context (food was used as  
569 attractor) they distinguished between quantities with similar accuracy. Although our findings  
570 also suggest that there is no context dependency in the discrimination of small quantities in  
571 angelfish, further experiments are required to systematically explore under what contexts and  
572 how angelfish and other species may perform with regard to their numerical estimation  
573 abilities.

574         Our present results are in line with those found in some other animal species. In the  
575 only other fish species that quantity discrimination of food has been investigated, guppies  
576 successfully discriminated the larger food set in 4 versus 2 and 4 versus 1 items but failed  
577 with comparisons of 4 versus 3 and 3 versus 2 items (Lucon-Xiccato et al., 2015). Difficulties  
578 in discriminating 4 versus 3 food items have also been found in amphibians, such as  
579 salamanders (Uller et al., 2003) and frogs (Stancher et al., 2015), that were able to  
580 discriminate the larger food set in 2 versus 1 and 3 versus 2 contrasts but not in 4 versus 3.  
581 Likewise, dogs (*Canis lupus familiaris*) select the larger set of food items up to 3 versus 2 but  
582 not 4 versus 3 (Miletto Petrazzini & Wynne, 2016), and similarly 10-12-month-old infants  
583 are able to select the larger quantity of crackers when the contrasts consist of 2 versus 1 and 3  
584 versus 2, but they fail with 4 versus 3 (Feigenson, Carey, & Hauser, 2002). However,  
585 contrasting evidence has also been reported. Rhesus monkeys were found to successfully  
586 discriminate comparisons of 2 versus 1, 3 versus 2, and 4 versus 3 apple slices, although  
587 failed in other comparisons such as 5 versus 4 and 6 versus 4 (Hauser, Carey, & Hauser,  
588 2000). Similar results with individuals being able to choose the greater food quantity in the 4



589 versus 3 comparisons have been shown in birds (e.g. New Zealand robins: Hunt, Low, &  
590 Burns, 2008, jungle crows: Bogale et al., 2014). All these studies suggest that there is a set  
591 size limit of 3-4 items on discrimination performance, with individuals having more difficulty  
592 (or failing) in comparisons between larger numbers (e.g. 5 versus 4, 6 versus 5, 6 versus 4,  
593 etc.). The latter findings have led to the suggestion that for representing small quantities,  
594 individuals use the Object File System, which is distinct from the system used to represent  
595 large quantities (Feigenson et al., 2004; see Introduction). In fact, in previous studies with  
596 angelfish, we also suggested the existence of two distinct numerical representation systems,  
597 with the Object File System being employed for contrasts with an upper limit of three  
598 elements (shoals of three conspecifics: Gómez-Laplaza & Gerlai, 2011b, 2015). Nevertheless,  
599 the failure in contrasts of 4 versus 3 shoals of conspecifics, and now 4 versus 3 sets of food  
600 items could also reflect a limit ratio that angelfish were not able to discriminate using the  
601 Approximate Number System, and the results in the large number range also appear to  
602 support the existence of that ratio limit in the current foraging context.

603         The results of Experiment 2 also indicated that the performance of angelfish was  
604 affected by the numerical ratio, i.e. the accuracy of discrimination performance decreased  
605 with decreasing the ratio of the different contrasts. Differences between contrasts were more  
606 evident with respect to 4 versus 3, the lowest ratio tested. This is a feature that characterizes  
607 the ANS. However, no extended accuracy was found here in the discrimination between food  
608 sets of different numerical size in the small number range compared to the ability in the  
609 discrimination in a shoal-preference task found previously, suggesting again that in angelfish  
610 different contexts do not activate different performance in quantity estimation. The consistent  
611 nature of our findings is notable given the different experimental paradigms employed in  
612 these studies. For example, in the current study motivational aspects of the test, e.g.  
613 exploratory drive or level of hunger, differed from those in previous studies, in which the

614 main motivating force was shoaling and/or anxiety. Given that we allowed experimental fish  
615 to make a spontaneous choice, i.e. voluntarily leave their shoal for making a food set choice,  
616 a possibility exists that our results represent a bias in sampling for bolder (more exploratory)  
617 or hungrier experimental fish, a potential problem that was absent in our prior studies using  
618 shoals as stimuli, and one which we intend to explore in the future. Another difference  
619 between the current study and the previous ones conducted in the context of shoaling to  
620 measure quantity discrimination abilities is the manner in which the stimuli may be perceived  
621 and quantified by angelfish. In other words, perceptible features of moving, living  
622 conspecifics may greatly differ from those of inanimate objects (e.g., see Agrillo, Dadda,  
623 Serena, & Bisazza 2008b). Although making direct comparisons between studies conducted  
624 in the contexts of shoaling versus foraging is complex, similarity in the acuity of the response  
625 despite differences in procedures and context indicates the robustness of the discrimination  
626 abilities of angelfish. Similarity in the response under different testing conditions and  
627 contexts has also been found, for example, in chicks (Rugani, Fontanari, Simoni, Regolin, &  
628 Vallortigara, 2009; Rugani, et al., 2013). In contrast, numerical acuity of some other animal  
629 species has been shown to be context-dependent, which may include the sensory modality  
630 involved in the task (e.g., visual, auditory: see Agrillo et al., 2017). Context-specific  
631 differences in performance in different quantitative tasks have been reported in other fish  
632 species too (Lucon-Xiccato & Dadda, 2017; Miletto Petrazzini et al., 2014).

633         With large numerical quantities (Experiment 3) experimental angelfish were  
634 successful in discriminating the different binary comparisons of food quantities presented,  
635 when the ratio between contrasts was again 1.5:1 or greater. In most of the parameters  
636 measured (Table 1) the response of fish was found to be similar and we found no significant  
637 difference in general performance among all contrasts of the large number range. For  
638 example, although a slight ratio dependency is apparent on Fig. 3, discrimination was not

639 significantly affected by the numerical ratio between the sets compared (e.g. 8 versus 6 was  
640 discriminated with similar accuracy as 8 versus 4, and 5 versus 4). These results contrast with  
641 those we have previously found using shoals of conspecifics as stimuli. In the latter context,  
642 discrimination accuracy of large numbers positively correlated with ratio of the contrasts, and  
643 became indistinguishable from chance level below a numerical ratio of 2:1 (see also Gómez-  
644 Laplaza & Gerlai, 2016b; Gómez-Laplaza et al., 2017).

645         Likewise, we did not find statistically appreciable difference in overall preference for  
646 the larger food set between the large number (Experiment 3) and the small number  
647 (Experiment 2) range task. This suggests, unlike in the context of shoaling behaviour (e.g.  
648 Gómez-Laplaza & Gerlai, 2011a; Gómez-Laplaza et al., 2017), similar discrimination  
649 abilities within the small and large number ranges in the context of foraging in angelfish.  
650 However, we did find differences in some parameters of the response between small and  
651 large numerical quantities (e.g. in the frequency of visits to the zone of the larger food set),  
652 and the capacity to discriminate small quantities seemed to be initially more precise (the first  
653 choice of a significantly large number of experimental fish was the larger food set, Table 1).  
654 These findings may be due to motivational factors. For example, any one of the two  
655 contrasted food sets in the large number range presented a number of food items that may  
656 provide enough food to satisfy the subjects, which may have led to similar frequency of visits  
657 to the sets and to a nonsignificant initial election of one food set over the other.

658         Although apparently none of the two key features to experimentally differentiate the  
659 Object File System from the Approximate Number System (i.e. greater accuracy when  
660 comparing small quantities as compared to large quantities, and sensitivity to the numerical  
661 ratio when discriminating between large quantities: Weber's law; see Feigenson et al., 2004)  
662 were accomplished in the current study, we found an overall significant increase in accuracy  
663 as the numerical ratio between the contrasts increased (results of linear regression analysis).

664 Such ratio-dependent discrimination is a sign of the ANS activation. Also, a similarity in the  
665 discrimination between quantities having the same ratio in the small and large numerical  
666 values, evidencing the numerical distance and size effect, are features that characterize the  
667 existence of the ANS. The finding of a similar discrimination sensitivity in the two numerical  
668 ranges has been reported in other animal species (DeLong, Barbato, O'Leary, & Wilcox,  
669 2017; Irie-Sugimoto, Kobayashi, Sato, & Hasegawa, 2009; see also Beran & Parrish, 2016;  
670 Jones & Brannon, 2012), and a ratio effect found both in the small (as in the current study)  
671 and in the large number range (unlike the current study), supported the idea of one system  
672 (the ANS). Therefore, even though the pattern of discrimination exhibited by angelfish is, in  
673 some aspects, not fully consistent with the ANS, overall our results suggest that angelfish  
674 employed this system in the current study. In support of this conclusion are our results  
675 showing the successful discrimination between quantities that cross the large-small boundary:  
676 the comparison 5 versus 2 food items. Notably, however, unlike in the context of foraging  
677 shown in our current study, previous findings with angelfish in the shoaling context generally  
678 indicated the functioning of two systems, and demonstrated ratio dependent discrimination  
679 ability only for numerically large shoals of conspecifics (ANS), while absolute number  
680 difference-based discrimination ability for numerically small shoals (OFS). However,  
681 variations in testing and procedural conditions occasionally did indicate the existence of only  
682 one mechanism, the approximate number system (ANS).

683 Another possibility to account for the lack of ratio sensitivity in the large number  
684 range found in the current study is that the ratio comparisons were not large enough to allow  
685 ratio effects to emerge, a hypothesis to be tested in the future. Indeed, we tested ratios up to  
686 2:1 (8 versus 4) and up to 2.5:1 (5 versus 2, in this case crossing the large-small number  
687 range divide). A ratio of 2:1 or greater has been successfully discriminated by angelfish in the  
688 context of shoal size discrimination, but greater ratios such as 3:1 and 4:1 were also included

689 in the large number range in the past, but not in the current study (Gómez-Laplaza & Gerlai,  
690 2011a, 2016a, 2016b, Gómez-Laplaza et al., 2017). An alternative explanation that might  
691 account for the ratio insensitivity shown by subjects in Experiment 3, is a putative ceiling  
692 effect in performance. Such ceiling effect is unlikely, however, since in Experiment 1 the  
693 preference index was larger than in the rest of the experiments, i.e. a ceiling effect was not  
694 reached.

695         The question remains whether angelfish relied upon the number of food items in the  
696 sets or upon non-numerical variables when making their choices. Since we did not control for  
697 continuous variables (e.g. cumulative surface area, density, or the overall space occupied by  
698 the sets), the performance of our experimental angelfish could have been affected by multiple  
699 cues that differentiated the stimulus sets. Likewise, similarities in performance in the small  
700 and large number range contrasts could have been due to the use of the same perceptual  
701 variables that covary with item number. Indeed, non-numerical attributes of the stimuli have  
702 been shown to influence quantity discrimination in fish in other contexts (e.g. Agrillo, Piffer,  
703 & Bisazza, 2011; Gómez-Laplaza & Gerlai, 2012, 2013a, 2013b). Further investigation  
704 controlling for non-numerical cues of the stimuli could clarify the role they may play in  
705 foraging contexts.

706         In sum, our new procedure opens the way for developing adequate methods to test  
707 quantity discrimination in fish. The overall evidence points to a cognitive system underlying  
708 discrimination that is ratio-dependent and likely be driven by the ANS.

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714

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877 Tabla 1. Performance of angelfish when faced with the different contrasts in experiments 1, 2 and 3 ( $N = 12$  in each contrast)

Contrasts	First choice (out of 12 fish) <sup>a</sup>			Frequency of entries <sup>b</sup>				Latency <sup>c</sup>			
	Larger food set	Smaller food set	Binomial test	Larger food set	Smaller food set	t test		Larger food set	Smaller food set	t test	
						$t_{11}$ value	Probability			$t_{11}$ value	Probability
Experiment 1											
3 vs. 0	12	0	$P < 0.001$	$8.00 \pm 0.78$	$1.08 \pm 0.29$	7.287	$P < 0.001$	$3.75 \pm 1.74$	$173.33 \pm 28.32$	12.943	$P < 0.001$
Experiment 2											
4 vs. 1	12	0	$P < 0.001$	$5.17 \pm 0.47$	$2.75 \pm 0.74$	3.446	$P = 0.005$	$1.00 \pm 0.00$	$138.58 \pm 32.37$	16.728	$P < 0.001$
3 vs. 1	10	2	$P = 0.039$	$5.83 \pm 1.13$	$3.92 \pm 0.72$	2.418	$P = 0.034$	$9.75 \pm 5.27$	$43.17 \pm 10.92$	3.134	$P = 0.010$
2 vs. 1	10	2	$P = 0.039$	$5.25 \pm 0.79$	$3.67 \pm 0.83$	1.247	$P = 0.238$	$6.16 \pm 3.44$	$39.08 \pm 8.87$	3.055	$P = 0.011$
4 vs. 2	10	2	$P = 0.039$	$8.00 \pm 0.70$	$6.25 \pm 0.80$	1.481	$P = 0.167$	$14.00 \pm 5.86$	$80.58 \pm 26.83$	2.481	$P = 0.031$
3 vs. 2	10	2	$P = 0.039$	$9.17 \pm 1.02$	$4.92 \pm 0.65$	3.787	$P = 0.003$	$14.58 \pm 9.69$	$63.75 \pm 18.59$	2.958	$P = 0.013$
4 vs. 3	8	4	$P > 0.050$	$9.08 \pm 1.41$	$7.00 \pm 1.07$	2.803	$P = 0.017$	$15.50 \pm 6.43$	$43.58 \pm 11.61$	1.329	$P = 0.211$
Experiment 3											
5 vs. 4	8	4	$P > 0.050$	$6.08 \pm 1.02$	$5.83 \pm 1.04$	0.201	$P = 0.844$	$24.33 \pm 14.06$	$41.83 \pm 16.81$	1.350	$P = 0.204$
8 vs. 6	8	4	$P > 0.050$	$4.00 \pm 0.71$	$2.50 \pm 0.34$	1.964	$P = 0.075$	$22.92 \pm 12.88$	$43.75 \pm 16.57$	1.097	$P = 0.296$
9 vs. 6	8	4	$P > 0.050$	$3.50 \pm 0.36$	$3.42 \pm 0.47$	0.162	$P = 0.874$	$20.17 \pm 10.35$	$42.17 \pm 15.82$	1.320	$P = 0.214$
8 vs. 4	9	3	$P > 0.050$	$6.67 \pm 1.21$	$4.67 \pm 1.14$	1.214	$P = 0.250$	$15.75 \pm 9.56$	$48.25 \pm 11.13$	2.269	$P = 0.044$
5 vs. 2	9	3	$P > 0.050$	$2.83 \pm 0.34$	$2.08 \pm 0.34$	1.682	$P = 0.121$	$24.00 \pm 11.85$	$109.50 \pm 31.95$	1.948	$P = 0.077$

878 *Note.* Subjects were tested individually. Descriptive statistics includes means  $\pm$  SE. The tests used to compare the scores are also included.879 <sup>a</sup>Number of fish whose first choice was one or the other stimulus set. <sup>b</sup>Frequency, number of times that subjects entered to the preference zones.880 <sup>c</sup>Latency to enter the preference zone near one or the other stimulus set.

881 **Figure captions**

882 **Figure 1.** The experimental apparatus. Schematic representation of the experimental  
883 aquarium including partitions and panels. Left: Side view of the experimental aquarium  
884 showing the preference zones and the partitions (transparent and white opaque), with the  
885 guillotine windows, that delimited the compartments. Middle: Transparent panels with an  
886 example of the food sets presented. During testing, the panels were placed outside the  
887 experimental aquarium leaned against the glass wall to avoid olfactory cues (see texts for  
888 details). Right: Top view of the experimental aquarium showing the large compartments  
889 (home and testing) separated by a smaller middle compartment that was subdivided by  
890 additional transparent partitions into three parts: in one compartment, closest to the rear wall,  
891 the tube of the water filter and a thermostat-controlled heater were placed to ensure constant  
892 water conditions, whereas the central part constituted the starting box. The preference zones  
893 are also indicated (dashed line), separated by a transparent plastic divider. The time the test  
894 fish spent within the preference zones was recorded together with the latency to enter, the  
895 frequency of entries and the first choice made by the subjects.

896 **Figure 2.** Discrimination of small quantities. Mean  $\pm$  SE proportion of time (*preference*  
897 *index*) spent by test fish in the preference zone close to the larger quantity of food. Numbers  
898 in parentheses indicate the ratio of the larger to the smaller food set, and the contrasts are  
899 shown in decreasing ratios. Values above 0.5 indicate a preference for the larger food set.  
900 Significant departure from the null hypothesis of no preference is indicated by asterisks: \*\*\*  
901  $P \leq 0.005$ , \*  $P < 0.05$ .

902 **Figure 3.** Discrimination of large quantities. Mean  $\pm$  SEM proportion of time (*preference*  
903 *index*) spent by test fish in the preference zone close to the larger quantity of food. Numbers  
904 in parentheses indicate the ratio of the larger to the smaller food set, and the contrasts are

905 shown in increasing ratios. Values above 0.5 indicate a preference for the larger food set.  
906 Significant departure from the null hypothesis of no preference is indicated by asterisks:  $*P <$   
907 0.05.

908 **Figure 4.** Regression line. Relationship between the proportion of time (preference index)  
909 test fish spent in close proximity of the food sets and the numerical ratio of the comparisons  
910 (number of elements in the larger set divided by the number of elements in the smaller set).

911 **Figure 1A.** Examples of the comparisons tested and the corresponding ratios.