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1 **Learning and anticipatory behaviour in a “sit-and-wait” predator: the**
2 **Atlantic halibut**

3

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14

15 **Abstract**

16 We studied the learning capacities and anticipatory behaviour in a “sit-and-wait” predatory
17 fish, the Atlantic halibut, *Hippoglossus hippoglossus*. In Experiment 1 two groups of halibut
18 received series of light flashes (conditioned stimulus, CS) that started before delivery of food
19 (unconditioned stimulus, US) and persisted until after food delivery, i.e. delay conditioning.
20 Control groups received unpaired CS and US presentations. The anticipatory behaviour of
21 delay conditioned halibut consisted mainly of take-offs towards the surface shortly after onset
22 of the CS. In Experiment 2 six groups of halibut were trained in three trace conditioning
23 procedures: Two groups with 20 s, two groups with 60 s and two groups with 120 s trace
24 interval. Learning was evident in the 20 and 60 s trace groups and in one of the 120 s trace
25 groups. In contrast to delay conditioning the anticipatory behaviour of trace conditioned
26 halibut was characterised by subtle movements near the tank floor with orientation towards
27 the CS. The cautious responses of halibut after trace conditioning differed markedly from
28 what is observed in other fish species and are suggested to reflect a “sit-and-wait” foraging
29 strategy that requires the predator to remain undetected until the prey is within lunging range.

30

31 Key words: Flatfish; Foraging; Pavlovian conditioning; Response systems

32

33 **1. Introduction**

34 The past decade has seen a wealth of studies on fish learning (Brown et al., 2006).
35 However, studies on the mechanisms involved in learning are scanty. For studies of fish
36 ecology it is crucial that we gain more insight into the learning capacity and constraints in
37 different species. One context in which learning plays an important role is foraging (Croy and
38 Hughes, 1991; Warburton, 2006). A type of learning that has a significant impact on the
39 ability to recognize and respond to prey is Pavlovian (classical) conditioning (Lieberman,
40 2000), i.e. associations between initially neutral cues (conditioned stimulus, CS) and
41 biologically relevant stimuli such as food (unconditioned stimulus, US). For fish, stimuli that
42 announce a greater probability of encountering food could involve visual features of the prey
43 itself or cues that occur together with prey, e.g. sounds or odours. Such cues may increase
44 attention and preparedness to attack, and thus increase capture success. The anticipatory
45 behaviour, i.e. the form and timing of the response, may be expected to reflect the
46 informational value of the cue (e.g. type of prey, temporal and spatial relationship between
47 cue and prey encounter), but also the foraging strategy of the predator.

48 Cues and prey encounter often overlap in time, i.e. the cue persists until the prey is
49 detected and caught (delay conditioning). In other occasions the cue may disappear before the
50 prey is detected (trace conditioning), for instance prey-induced movements of vegetation or
51 sediment. Most of the experimental works on Pavlovian learning in fish have used delay
52 conditioning, and it has long been known that fish rapidly learn to associate different cues
53 with rewards (Bull, 1928). Little, however, is known about the abilities of trace conditioning
54 in fishes. In a recent study we found that groups of Atlantic cod, *Gadus morhua*, could be
55 conditioned to associate a light signal in the feeding area with a food reward at trace intervals
56 as long as 120 s (Nilsson et al., 2008a), demonstrating that some fish have excellent capacities
57 for trace conditioning.

58 Once an association between a CS and a rewarding US has been formed, the CS alone
59 elicits a response. The response often reflects stimulus substitution, where subjects direct their
60 behaviour to the CS, e.g. by approaching it or even trying to catch or ingest it (Brown and
61 Jenkins, 1968; Purdy et al., 1999), a behaviour referred to as sign-tracking (Hearst and
62 Jenkins, 1974). For instance, archer fish, *Toxotes chatareus*, respond to a CS light above the
63 surface by squirting water at it when it is paired with fruit flies delivered on the surface
64 (Waxman and McCleave, 1978). Cod always approached the CS light, which was located on
65 the opposite side of the feeding site in a 3 m tank, before they gathered in the feeding area
66 prior to food arrival (Nilsson et al., 2008b). A hunting cod generally orients to and approaches
67 prey upon detection (Brawn, 1969; Steingrund and Fernö, 1997). Sign-tracking may thus lead
68 to efficient responses to cue signals for cod and other species with similar foraging strategies.
69 Cue-induced anticipatory responses in fishes have been studied little, and not much is known
70 about learning capacities and anticipatory responses in fish with other foraging strategies,
71 such as “sit-and-wait”.

72 In “sit-and-wait” ambush strategies, an immediate approach response (sign-tracking) to
73 food-announcing cues may not be adaptive, for at least two reasons. First, rapid approaches
74 could frighten away prey that is out of lunge range. Secondly, the place where a cue is
75 detected may not be the same as where the prey is caught. As a successful “sit-and-wait”
76 ambush requires attack at a suitable distance and angle, cues should be expected to induce
77 increased attention and adjustments of body posture and position rather than release
78 immediate attack-like responses. The spatial and temporal relationship between the cue and
79 the associated prey should then determine the form and timing of the response, i.e. which
80 behavioural components that are involved in the response and at which stimuli these are
81 directed, as well as when to respond. In fact, the “sit-and-wait” predator domestic cat, *Felis*
82 *silvestris catus*, becomes hypoactive while the rat, *Rattus norvegicus*, that applies a “search

83 behaviour”, becomes hyperactive in the same appetitive conditioning procedure with the
84 different anticipatory responses reflecting their foraging strategies (van den Bos et al., 2003).

85 Flatfish are “sit-and-wait” ambush predators (Gibson, 2005), famous for their unusual
86 morphology and excellent camouflage, but little is known about their learning abilities. We
87 studied the capacity for associative learning and anticipatory behaviour in the Atlantic halibut,
88 *Hippoglossus hippoglossus*. Halibut is the largest flatfish species and may reach a weight of
89 >300 kg and ages of over 50 years (Haug, 1990). Small individuals live quite localized on
90 sandy bottoms at moderate depths (20-60 m) and feed mainly on benthic crustaceans, while
91 fish become more important prey as the halibut grow (Haug, 1990). Prey detection relies both
92 on vision and olfaction (de Groot, 1969; Yacoob and Browman, 2007). When the distance to
93 the prey is short enough prey are captured in a rapid lunge with simultaneous opening of the
94 mouth and protrusion of the jaws, with the prey being sucked into the mouth (Gibson, 2005).

95 With a feeding strategy that relies on camouflage, ambush and surprise, we hypothesised
96 that halibut should not react to reward-associated cues by immediate approach responses or
97 sign-tracking, but rather prepare an attack by more subtle responses. In Experiment 1 we used
98 delay conditioning to study learning and anticipatory response with overlapping CS-US
99 presentations and a relatively short CS-US interval. In Experiment 2 we used trace
100 conditioning with three different trace intervals to examine whether halibut have the capacity
101 of trace conditioning, and if so, whether the anticipatory behaviour is expressed differently
102 than during delay conditioning.

103 **2. Experiment 1 – delay conditioning**

104 *2.1. Methods*

105 *2.1.1. Experimental setup*

106 Four circular 1 m diameter tanks of black plastic were used for the experiment. To facilitate
107 a smooth water exchange and removal of uneaten food and faeces by the exchanged water, the
108 tank floor was kept plain without substrates or other structural features. The tanks were filled
109 with 60 cm seawater (≈ 450 L) at 12° C and 90% O_2 saturation, and continuously illuminated
110 by underwater fluorescence tubes (33 lux). The water was exchanged at a rate of 10 L min^{-1} .
111 A video camera hung above each tank. A cover with a 60 cm diameter hole was placed over
112 each tank to prevent escape of fish from the tanks and to avoid direct light from the
113 fluorescence tubes on the recordings. The field of view of the camera thus did not cover the
114 upper sides of the tanks (Fig. 1). A 3 W light-bulb was placed on the floor of the tank about
115 10 cm from the wall opposite to the illumination. Food was delivered at the surface from a
116 feeder. Uneaten food was removed through the drain within a few minutes.

117 *2.1.2. Fish and food*

118 Four groups of 20 one year old halibut, hatched in captivity from eggs from wild-caught
119 parents, were measured for length (15 ± 1.0 cm, mean \pm S.D.) and allowed to recover in the
120 experimental tanks for 9 days in advance of the experiment. When lying on the black tank
121 floor halibut attained a skin pattern of white spots and a darker brown colour than when
122 swimming off the floor, reflecting an attempt to camouflage. Halibut have low motivation to
123 eat common formulated food (Kristiansen et al., 2004), and a pilot conditioning experiment
124 revealed that formulated food often fails to induce immediate feeding response and is thus not
125 appropriate as reward (own unpublished observations). Shrimp, *Pandalus borealis*, induces
126 strong feeding behaviour in juvenile halibut (Yacoob and Browman, 2007), and boiled and

127 chopped-up shrimp was used as reward in the experiment. On the first introduction of shrimp
128 halibut did not respond immediately at the sight of shrimp, but a strong response similar to
129 that reported by Yacoob and Browman (2007) occurred after around 10 s and first feed intake
130 after around 15 s. This indicates that they mainly responded to the olfactory cue. In order to
131 facilitate familiarity with this food and ensure that halibut also responded to the sight of
132 shrimp, they were fed shrimp instead of formulated food the last three days before the start of
133 the experiment. On the last feeding sessions before the start of the experiment the time to first
134 feed intake was reduced to around 7 s. At delivery, the shrimp spread out on the surface, and
135 sank at a rate of about 6 cm s^{-1} following the slow clockwise flow in the tank.

136 *2.1.3. Procedures*

137 For two groups each feeding was announced by a series of light flashes (conditioned
138 stimulus, CS; 1 s on: 1 s off) from the light-bulb. The CS had a duration of 20 s and started 10
139 s before delivery of shrimp (unconditioned stimulus, US), i.e. 10 s overlapping with the US
140 (delay conditioning). In two unpaired control groups shrimp was delivered one hour after CS
141 presentation. The scheduled procedure was 6 trials per day with 2-hours intertrial intervals.
142 Due to a few deviations from the planned schedule, with for instance the CS bulb not
143 working, the average number of trials per day was 5.6 (range 4 – 6). The fish received these
144 schedules for 5 days. On days 6 – 8, when a response had been acquired in the delay groups,
145 the duration of the CS was increased to 40 s (starting 30 s before food delivery in the delay
146 procedure) in order to provide longer observation times of the anticipatory behaviour. The
147 trials were recorded on DVD for subsequent analysis.

148 *2.1.4. Behavioural analysis*

149 Halibut usually spend most of the time lying motionless on the bottom. Our prediction was
150 that anticipatory behaviour induced by the CS would make the halibut change position more

151 often. For all trials on the first 5 days of the experiment, the position of the snout of each fish
152 was registered on the video image immediately before the CS, and the number of fish that
153 remained in position 10 s later (i.e. immediately before food delivery in the delay groups) was
154 recorded. To estimate the baseline level of motion the same procedure was made for a 10-s
155 interval immediately before the CS (pre-CS). While this analysis did not give any details of
156 how and when the halibut responded, the form and temporal distribution of responses were
157 studied in more detail in all trials on the last day of the experiment (day 8), when the CS
158 duration had been extended. A time period starting 20 s before and ending 30 s after the onset
159 of the light flashes (i.e. at food delivery in the delay groups) was divided into 5-s intervals,
160 and the number of fish lying motionless at the end of each 5-s interval was recorded. We also
161 registered how many times the following three behavioural patterns were initiated in each 5-s
162 interval: *Take-off*: Lift from the bottom and swim in the water column for more than 10 s;
163 *Bottom-swim*: Moving more than three body lengths along the bottom; *Reposition*: A small
164 move, less than three body lengths, usually straight forward or a turn.

165 Whether fish approached the CS (sign-tracked) was determined on the basis of analyses of
166 days 6 – 8. The image of the tank on the screen was divided into four equal 90° sectors, with
167 the CS light bulb in the centre of one sector (CS sector), and the number of fish on or
168 immediately above the floor in the CS sector was recorded 5 s before and 10 s after the onset
169 of the CS. In order to determine whether fish directed their attention towards the CS without
170 approaching it, the number of fish on or immediately above the floor with their heads directed
171 $\pm 45^\circ$ towards the CS light bulb was recorded on the same images.

172 2.1.5. Statistics

173 The data resulting from the video analysis were categorical variables that indicated the
174 frequency of different behaviours. As it was difficult to confirm or transform these variables

175 into a normal distribution, nonparametric statistical methods were used. An anticipatory
176 response to the CS should lead to fewer fish maintaining their positions on the tank floor
177 during the CS-US interval as compared to before the CS, i.e. a change from the pre-CS level.
178 Kendall tau rank correlation was used to test if there was a correlation between the magnitude
179 of the change and trial number, indicating learning. Wilcoxon signed rank test was used to
180 compare the number of fish in the CS sector and the number of fish oriented towards the CS
181 bulb before and during the CS. For the observations on the last day of number of motionless
182 fish, take-offs, bottom-swims and repositions in 5-s intervals, an aligned ranks test for
183 randomized complete blocks (Stokes et al., 2000) was used. In short, each procedure was
184 divided into four periods, pre-CS, first third, second third and last third of the CS-US interval,
185 and the test identified if there were significant effects from these periods for each behavioural
186 pattern. The test was first performed using the entire dataset, and if an effect was found the
187 test was performed on each of the three CS-US periods at a time to detect differences from
188 pre-CS levels. All tests were performed separately for each replicate group. All tests were
189 two-tailed and the level of significance was set at 0.05.

190 **2.2. Results**

191 *2.2.1. Response acquisition*

192 There was little response in the delay groups to the CS on the first day (Fig. 2a). On the
193 subsequent days the number of fish remaining motionless on the floor decreased during the
194 CS compared to pre-CS level, and the magnitude of the decrease was correlated with trial
195 number (Group 1: $\tau = -0.37$, $p < 0.01$; Group 2: $\tau = -0.57$, $p < 0.001$). In the control groups
196 there was no significant correlations (Group 1: $\tau = -0.06$, $p > 0.05$; Group 2: $\tau = 0.23$, $p >$
197 0.05), and the change from pre-CS level to CS was generally small (Fig. 2b).

198 2.2.2. *Form of the response*

199 In the delay groups the effect of period (pre-CS, first third, second third and last third of
200 the CS-US interval) on the number of motionless fish was significant, with the number of
201 motionless fish lower than pre-CS level throughout the CS in both groups (Table 1, Fig. 3a).
202 *Take-off* was the main response, and most take-offs occurred during the first seconds after the
203 onset of the CS (Table 1, Fig. 3a). The take-off response was especially dominant in Group 2,
204 in which most of the fish swam near the surface after take-off. Group 1 also responded with
205 *bottom-swim*, which occurred more often throughout the CS than pre-CS (Table 1, Fig. 3a).
206 The rate of *repositions* during the CS did not differ from pre-CS level (Table 1, Fig. 3a).

207 In contrast to in the delay groups, the number of motionless fish in the control groups
208 increased slightly throughout the CS (Table 1, Fig. 3b). None of the three behavioural patterns
209 occurred at a higher rate during the CS than pre-CS (Table 1, Fig. 3b).

210 2.2.3. *Sign-tracking*

211 The delay groups did not sign-track by moving to the CS. The number of fish in the CS
212 sector was low during the CS in both groups and did not differ from pre-CS level in Group 1
213 ($T_+ = 34.5$, $p > 0.05$), while the number decreased in Group 2 ($T_+ = 113$, $p < 0.01$, Fig. 4a).
214 However, the number of fish directed towards the CS light bulb slightly increased during the
215 CS in Group 1 ($T_+ = 9$, $p < 0.01$, Fig. 4c), while there was no difference in Group 2 ($T_+ = 48$,
216 $p > 0.05$).

217 In the control groups the number of fish in the CS sector was slightly higher during the CS
218 than pre-CS in Group 2 ($T_+ = 0$, $p < 0.05$), but not significantly so in Group 1 ($T_+ = 0$, $p >$
219 0.05 , Fig. 4b). The average increase in the CS sector (25% of the tank floor) was 0.39 and
220 0.44 fish for Group 1 and 2, respectively, and is thus in accordance with the total increase on
221 the tank floor of 1.33 and 1.83 (see Fig. 3b). The number of fish directed towards the CS light

222 bulb during the CS did not change from pre-CS level (Group 1: $T_+ = 2$, $p > 0.05$; Group 2: T_+
223 $= 10$, $p > 0.05$, Fig. 4d).

224 **3. Experiment 2 – trace conditioning**

225 Experiment 1 demonstrated that halibut can rapidly become conditioned when trained with
226 overlapping CS-US presentations. Contrary to our hypothesis that anticipatory behaviour
227 would be characterized by subtle movements, the main response was to lift from the bottom
228 and swim actively in the water column, more similar to an ongoing attack of prey than an
229 attempt to prepare for a future attack. An explanation for this could be that the halibut were
230 trained with overlapping CS-US presentations and a relatively short CS-US interval, a CS-US
231 relationship resembling situations where the reward is or will soon be within range. Then
232 there would be little time and little reason to avoid being detected by the prey.

233 In a situation in which a “sit-and-wait” predator gets a cue about the presence of a prey
234 before the prey is available for attack, e.g. out of a halibut’s vision or lunge range, a more
235 cautious behaviour may be crucial to come within range without alarming the prey. Trace
236 conditioning, in which the CS is terminated before the presentation of the US, would resemble
237 such a situation. In Experiment 2 we therefore trained groups of halibut at three different trace
238 interval durations, 20, 60, and 120 s, in order to study if a) the anticipatory behaviour of
239 halibut is expressed differently during trace conditioning than delay conditioning, b) halibut
240 has the capacity to be trace conditioned at long trace intervals and if the anticipatory
241 behaviour is affected by the duration of the interval.

242 *3.1. Methods*

243 The CS duration was 10 s, and the offset of the CS was separated from the US with a trace
244 interval of 20 s, 60 s, or 120 s with two replicate groups of 20 halibut for each trace duration.
245 The CS-US interval (the CS and the trace interval) was thus 30 s or more, giving sufficient

246 time to analyse anticipatory responses. The length of the fish was 18.7 ± 1.1 cm (mean \pm
247 S.D.) in the 20 s and 60 s trace procedure and 21.3 ± 1.4 cm in the 120 s trace procedure,
248 which were carried out three weeks later than the 20 s and 60 s procedures. As learning was
249 assumed to be slower with trace conditioning, which is more demanding than delay
250 conditioning (Lieberman, 2000), the fish were trained for 12 days instead of 5 in Experiment
251 1, with on average 5.8 trials per day. The setup, procedure and analyses were otherwise the
252 same as in Experiment 1.

253 **3.2. Results**

254 *3.2.1. Response acquisition*

255 20 s trace groups: With the exception of the first day the number of fish remaining
256 motionless on the floor decreased during the CS-US interval compared to the pre-CS level,
257 and on the last days very few fish remained motionless throughout the CS-US interval (Fig.
258 5a). The magnitude of the change from pre-CS to the CS-US interval was correlated with trial
259 number (Group 1: $\tau = -0.44$, $p < 0.001$; Group 2: $\tau = -0.26$, $p < 0.01$).

260 60 s trace groups: The difference between the CS-US interval and pre-CS in number of fish
261 remaining motionless was less pronounced and the response acquisition slower (Fig. 5b) than
262 in the 20 s trace groups. Still, on the last days few fish remained motionless throughout the
263 CS-US interval, and the magnitude of the change from pre-CS to the CS-US interval was
264 correlated with trial number (Group 1: $\tau = -0.46$, $p < 0.001$; Group 2: $\tau = -0.42$, $p < 0.001$).

265 120 s trace groups: The difference between the CS-US interval and pre-CS in number of
266 fish remaining motionless was small in both groups throughout the experiment (Fig. 5c). In
267 Group 1 the number of motionless fish was higher during the CS-US interval than pre-CS on
268 the first days and lower on the last days (Fig. 5c), with a correlation between the magnitude of
269 the change and trial number ($\tau = -0.35$, $p < 0.001$). In Group 2 the number of motionless fish

270 was slightly higher during the CS-US interval throughout the experiment (Fig. 5c), and the
271 magnitude of the change was not correlated with trial number ($\tau = 0.03$, $p > 0.05$).

272 3.2.2. Form of the response

273 20 s trace groups: The number of motionless fish fell after the onset of the CS and
274 remained lower throughout the CS-US interval (Table 2, Fig. 6a). The main response to the
275 CS was *repositions*. The rate of repositions showed a peak in the first seconds following the
276 onset of the CS, though it was higher than pre-CS level also later in the CS-US interval (Table
277 2, Fig. 6a). *Bottom-swims* were less frequent than reposition, but more frequent than pre-CS
278 level throughout the CS-US interval in Group 1, while the rate during the CS-US interval did
279 not differ from pre-CS level in Group 2 (Table 2, Fig. 6a). *Take-offs* were relatively rare
280 during the CS-US interval in both groups (Fig. 6a), but more frequent than pre-CS level in
281 Group 1. In Group 2 there was no difference (Table 2).

282 60 s trace groups: As in the 20 s trace groups, the number of motionless fish fell during the
283 CS and was lower throughout the CS-US interval (Table 2, Fig. 6b). Also here the main
284 response was *repositions*. In contrast to the 20 s trace groups, the occurrence of repositions
285 had no clear peak at the onset of the CS but was more evenly distributed during the CS-US
286 interval (Fig. 6b). The rate was higher than pre-CS level throughout the CS-US interval
287 (Table 2). *Bottom-swim* occurred at a much lower rate than reposition but more often during
288 the CS-US interval than pre-CS (Table 2, Fig. 6b). *Take-offs* were very rare and the rate did
289 not differ from pre-CS level (Table 2, Fig. 6b).

290 120 s trace groups: The number of motionless fish decreased slightly after the onset of the
291 CS in Group 1 and was lower than pre-CS level in the first two thirds of the CS-US interval,
292 while there was no difference in Group 2 (Table 2, Fig. 6b). The rate of repositions was
293 somewhat elevated early in the CS-US interval in Group 1 (Fig. 6c), but the difference did not

294 reach significance (Table 2). *Bottom-swim* and *take-off* were rare in both groups (Fig. 6c) and
295 their rates did not differ from the pre-CS levels (Table 2).

296 3.2.3. *Sign-tracking*

297 20 s trace groups: Sign-tracking by moving to the CS was not a major response. The
298 number of fish in the CS sector during the CS was low in both groups and did not differ from
299 pre-CS level in Group 1 ($T_+ = 31.5$, $p > 0.05$), while it was slightly increased in Group 2 ($T_+ =$
300 5 , $p < 0.01$, Fig. 7a). More common than CS approach was orienting towards the CS. The
301 number of fish directed $\pm 45^\circ$ towards the CS light bulb during the CS was higher than pre-CS
302 level (Group 1: $T_+ = 1$, $p < 0.001$; Group 2: $T_+ = 0$, $p < 0.001$, Fig. 7d), although the majority
303 of the fish was not directed towards the CS.

304 60 s trace groups: The number of fish in the CS sector during the CS did not differ from
305 pre-CS level (Group 1: $T_+ = 40.5$, $p > 0.05$; Group 2: $T_+ = 23$, $p > 0.05$, Fig. 7b). Similar to
306 the 20 s trace groups, more fish were directed towards the CS bulb during the CS than pre-CS
307 (Group 1: $T_+ = 0$, $p < 0.01$; Group 2: $T_+ = 19.5$, $p < 0.05$, Fig. 7e).

308 120 s trace groups: The number of fish in the CS sector during the CS did not differ from
309 pre-CS level (Group 1: $T_+ = 2.5$, $p > 0.05$; Group 2: $T_+ = 7.5$, $p = p > 0.05$, Fig. 7c). Slightly
310 more fish were directed towards the CS bulb during the CS than pre-CS in Group 1 ($T_+ = 2.5$,
311 $p < 0.05$, Fig. 7f). There was no difference in Group 2 ($T_+ = 5$, $p > 0.05$, Fig. 7f).

312 4. Discussion

313 This is the first study of anticipatory behaviour in a fish with a “sit-and-wait” foraging
314 strategy. Atlantic halibut are clearly able to associate events separated by at least 60 s.
315 Learning was most rapid during delay conditioning and slowest at the longest trace intervals.
316 The form and temporal distribution of the anticipatory behaviour differed strikingly between

317 delay- and trace conditioning, with the fish in the delay procedure swimming towards the
318 surface at the onset of the CS and very few fish remaining on the bottom during the CS-US
319 interval, while in the trace procedure almost no fish left the bottom and at any time in the CS-
320 US interval many fish were lying motionless.

321 The repositions of halibut in the long-trace procedures were so slight that when we first
322 glanced at the videos we doubted that there were any conditioned responses in the 60 s and
323 120 s trace groups. A preliminary analysis (not presented here), in which the numbers of fish
324 lying motionless 5 s before onset of the CS and 1 s before US release were compared,
325 supported this impression; no clear change from pre-CS to pre-US was detected in the 60 s
326 and 120 s trace procedures. However, the more sensitive analyses of all movements
327 throughout the CS-US interval revealed that this was not the case. For both 60 s trace groups
328 the number of fish holding their position was lower during the CS-US interval than during an
329 equally long pre-CS interval, and the magnitude of the difference increased with trial number.
330 A similar pattern was seen in one 120 s trace group. This demonstrates the importance of in-
331 depth analysis of anticipatory behaviour when subtle responses can be expected.

332 We trained halibut in groups, which might have influenced their behaviour. Since
333 individuals could not be recognized, we do not know how many individuals actually
334 responded to the CS, but the finding that few fish maintained their position throughout the
335 CS-US interval suggests that most fish responded in the delay, 20 s and 60 s trace groups. In
336 the 120 s trace group 1, more fish maintained their position, and here only some individuals
337 may have learned. Some individuals may have responded to the behaviour of other
338 individuals rather than to the CS, and social interactions (Brown and Laland, 2006) may have
339 speeded up the learning process. However, halibut is a non-social species (Haug, 1990), and
340 social behaviour presumably had a limited influence on their learning. In any case, social
341 behaviour cannot explain that anticipatory responses were differently expressed in delay and

342 trace procedures, and that learning was slower during conditioning with long trace intervals.
343 Individual halibut may, however, differ in their ability to learn. Kristiansen and Fernö (2007)
344 suggested that feeding motivation of halibut depended on the coping style of the individuals
345 and on how demanding the feeding method was. Longer trace intervals present the halibut
346 with more difficult cognitive situations, and individuals with low coping ability may have
347 changed to a “wait and see” coping strategy (reactive strategy), with fewer of them motivated
348 to learn.

349 In learning experiments the response rate often increases towards the time of the arrival of
350 rewards (Gallistel and Gibbon, 2000), but this was not found in the present study. Responses
351 were most often initiated early in the CS-US interval (delay and 20 s trace procedures) or had
352 a relatively flat temporal distribution (60 s trace). Also in cod the response peak occurs well
353 before the time of food release (Nilsson et al., 2008a, b). Cod approached the CS (sign-
354 tracked) immediately at its onset, whether the CS was located in the feeding area or on the
355 opposite side of the tank, and regardless of whether they were trained in a delay or trace
356 procedure. Moreover, cod gathered below the feeder waiting for food to arrive throughout a
357 60 s trace interval (Nilsson et al., 2008a).

358 Cod are cruising predators that search actively for food and usually pursue or attack prey
359 immediately upon detection (Brawn, 1969; Steingrund and Fernö, 1997). The immediate sign-
360 directed response thus reflects cod feeding strategy. The response of the “sit-and-wait”
361 predator halibut was very different. With the exception of one 20 s trace group, the number of
362 fish near the CS did not increase after the onset of the CS. In contrast to cruising predators,
363 “sit-and-wait” ambush predators often attack prey later and at another location than where it
364 was detected, i.e. when it has entered the lunge range of the predator. This may explain the
365 absence of sign-tracking in halibut. In one delay group, all 20 s and 60 s trace groups and one
366 120 s trace group, the number of fish oriented towards the light-bulb rose after the onset of the

367 CS. Cues can thus draw the attention of halibut, in spite of not evoking approach responses.
368 Interestingly, in spite of the significant amount of attention paid to the CS, in all procedures
369 most individuals did not orient themselves directly towards the stimulus. Flatfish eyes are
370 independently mobile, giving a 360° angle of vision (Gibson, 2005), and halibut at the floor
371 may have had visual contact with the CS even at an angle of more than 45°.

372 While approach behaviour towards the CS was generally absent, take-off, i.e. an approach
373 towards the surface, was the main response in the delay procedure. The surface was where
374 food was delivered, and take-off may be seen as a goal-directed behaviour (Boakes, 1977).
375 When the cue temporally overlapped with the reward, goal-directed responses were thus
376 evoked immediately. In contrast, when there was a trace interval between the cue and the
377 reward, few goal-directed approaches were observed, with the response almost exclusively
378 consisting of subtle movements near the floor (repositions and bottom-swims). Furthermore,
379 when the trace interval was long (60 s) these responses had a relatively flat temporal
380 distribution during the CS-US interval. The anticipated time between cue and reward thus has
381 an influence on the form and temporal distribution of the response. The immediate, goal-
382 directed response of the delay conditioned halibut could be seen as attack behaviour. The CS
383 announced that food would be delivered within a few seconds (the CS-US delay was 10 s
384 through most of the experiment), giving the fish little time and little reason to prepare
385 themselves by reorientations on the floor. In contrast, in the trace procedures the CS
386 announced food availability in a more distant future, with enough time to prepare and little
387 reason for immediate approach. In a natural situation with live prey, conspicuous responses
388 might frighten prey not yet within range and thus decrease the chances of successful capture.
389 The differences in response pattern between halibut and cod, with cautious responses in trace
390 conditioned halibut and immediate sign-tracking in cod, appears to be similar to the
391 differences in anticipatory behaviour between the rat and the cat, with increased activity

392 during the CS-US interval in the former and decreased activity in the latter, in accordance
393 with their respective feeding strategies (van den Bos et al., 2003).

394 Under natural conditions, the time between cue and encounter will not always be the same,
395 but rather differ from one occasion to another and between different prey types (e.g. free-
396 swimming fish versus bottom-dwelling invertebrates). Where specialized diets are involved,
397 similar responses on all cues may be advantageous. For more diverse diets the time between
398 cues and prey encounters is presumably highly variable, with appropriate form and timing of
399 response difficult to achieve. Predators may either learn to respond differently to different
400 cues, or choose an intermediate response. It would be interesting to train halibut in a
401 procedure with a highly variable CS-US interval or to switch from a period with short-interval
402 trials to long-interval trials and vice versa, in order to see how this species deals with more
403 realistic temporal relationships.

404

405 **Acknowledgments**

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409

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- 467

468 **Tables**

469

470 **Table 1.** P Probability levels given by the aligned ranks test for randomized complete blocks
 471 on the effect of period (pre-CS, 1st third, 2nd third and last third of the CS-US interval) on the
 472 number of motionless fish, take-offs, bottom-swims and repositions in the delay procedure
 473 and the control procedure. The column “Main effect” gives the probability level when the
 474 entire dataset (all four periods) was included. The columns “1st third”, “2nd third” and “Last
 475 third” gives the probability level when each of these periods was compared with pre-CS. *p <
 476 0.05; **p < 0.01; ***p < 0.001; n.s., not significant. Note that the effects on “Motionless” are
 477 due to a decrease in number of motionless fish during the CS-US interval in the delay
 478 procedure, while it is due to an increase in the control procedure.

Behaviour	Procedure	Replicate	Main effect	1st third	2nd third	Last third
Motionless	Delay	1	***	***	***	***
		2	***	***	***	***
	Control	1	***	**	***	***
		2	***	*	***	***
Take-off	Delay	1	*	**	n.s.	n.s.
		2	***	**	n.s.	n.s.
	Control	1	n.s.			
		2	n.s.			
Bottom-swim	Delay	1	**	***	**	**
		2	n.s.			
	Control	1	n.s.			
		2	n.s.			
Reposition	Delay	1	n.s.			
		2	n.s.			
	Control	1	n.s.			
		2	n.s.			

479

480

481 **Table 2.** Probability levels given by the aligned ranks test for randomized complete blocks on
482 the effect of period (pre-CS, 1st third, 2nd third and last third of the CS-US interval) on the
483 number of motionless fish, take-offs, bottom-swims and repositions in the 20 s trace, 60 s
484 trace and 120 s trace procedures. The column “Main effect” gives the probability level when
485 the entire dataset (all four periods) was included. The columns “1st third”, “2nd third” and
486 “Last third” gives the probability level when each of these periods was compared with pre-
487 CS. *p < 0.05; **p < 0.01; ***p < 0.001; n.s., not significant.

488

Behaviour	Procedure	Replicate	Main effect	1st third	2nd third	Last third	
Motionless	20 s	1	***	***	***	***	
		2	***	***	***	*	
	60 s	1	***	***	***	***	
		2	***	***	***	***	
	120 s	1	**	*	*	n.s.	
		2	n.s.				
	Take-off	20 s	1	*	*	n.s.	*
			2	n.s.			
60 s		1	n.s.				
		2	n.s.				
120 s		1	n.s.				
		2	n.s.				
Bottom-swim	20 s	1	**	*	***	**	
		2	n.s.				
	60 s	1	**	*	**	n.s.	
		2	*	n.s.	*	**	
	120 s	1	n.s.				
		2	n.s.				
	Reposition	20 s	1	**	***	*	n.s.
			2	**	**	n.s.	*
60 s		1	***	***	***	***	
		2	**	*	***	**	
120 s		1	n.s.				
		2	n.s.				

489 **Figure legends**

490

491 **Fig. 1.** The experimental setup (side view).

492 **Fig. 2.** Mean \pm S.E. number of fish maintaining their positions on the floor of the tank during
493 a 10-s period immediately before the onset of the CS (open circles) and throughout the first 10
494 seconds of the CS (the CS-US interval in the delay procedure, filled circles) in a) the delay
495 procedure, b) the control procedure. Left and right figures represent replicate groups.

496 **Fig. 3.** Behaviour of groups of halibut in relation to time from onset of the CS. Triangles:
497 number of fish lying motionless on the floor at the end of each 5-s interval (x-value is the first
498 second of the interval, e.g. the interval “0” is 0-5 s from onset of the CS); filled circles:
499 repositions; open circles: bottom-swims; squares: take-offs. Mean \pm S.E. values based on the
500 final six conditioning trials day 8. a) delay procedure, b) control procedure. Left and right
501 figures represent replicate groups.

502 **Fig. 4.** Sign-directed behaviour of halibut. Open bars: 5 s pre-CS; filled bars: 10 s after the
503 onset of the CS. Number of fish in the CS sector in a) the delay procedure and b) the control
504 procedure, and number of fish directed $\pm 45^\circ$ towards the CS bulb in c) the delay procedure
505 and d) the control procedure. Mean \pm S.E. based on all (17 for the delay groups, 18 for the
506 control groups) trials on days 6 – 8.

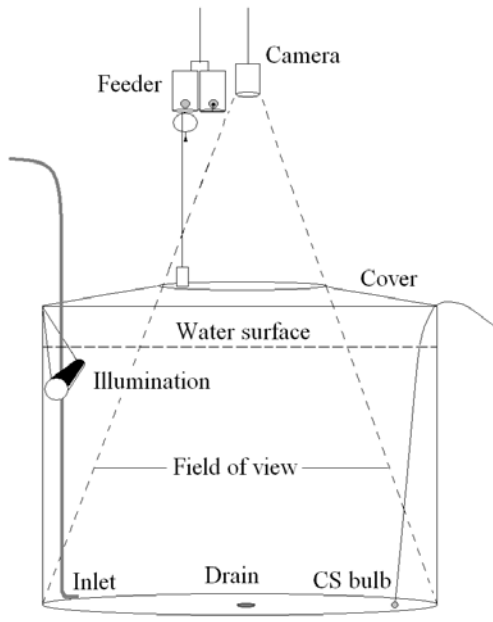
507 **Fig. 5.** Mean \pm S.E. number of fish maintaining their positions on the floor of the tank
508 throughout the CS-US interval (filled circles) and through an equal-length period immediately
509 before the onset of the CS (open circles) in a) the 20 s trace procedure, b) the 60 s trace
510 procedure, and c) the 120 s trace procedure. Left and right figures represent replicate groups.

511 **Fig. 6.** Behaviour of groups of halibut in relation to time from onset of the CS. Triangles:
512 number of fish lying motionless on the floor at the end of each 5-s interval; filled circles:

513 repositions; open circles: bottom-swims; squares: take-offs. Mean \pm S.E. values based on the
514 final six conditioning trials. a) the 20 s trace procedure, b) the 60 s trace procedure, and c) the
515 120 s trace procedure.

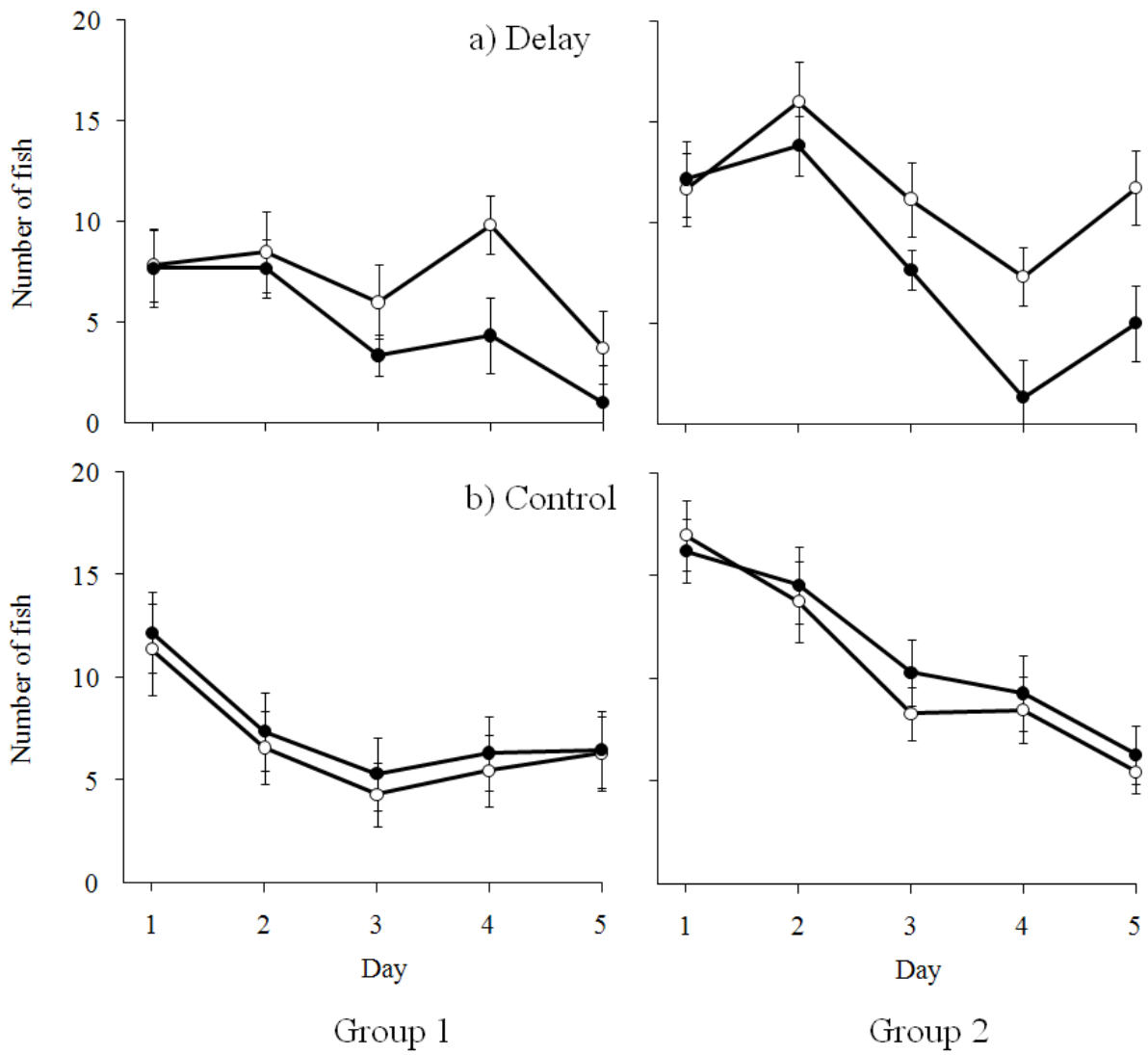
516 **Fig. 7.** Sign-directed behaviour of trace conditioned halibut. Open bars: 5 s pre-CS; filled
517 bars: 10 s after the onset of the CS. Number of fish in the CS sector in a) the 20 s trace
518 procedure, b) the 60 s trace procedure and c) the 120 s trace procedure, and number of fish
519 directed $\pm 45^\circ$ towards the CS bulb in d) the 20 s trace procedure, e) the 60 s trace procedure
520 and f) the 120 s trace procedure. Mean \pm S.E. based on all trials on days 10 – 12 (16 trials for
521 20 s trace group 1, 17 trials for all other groups).

522



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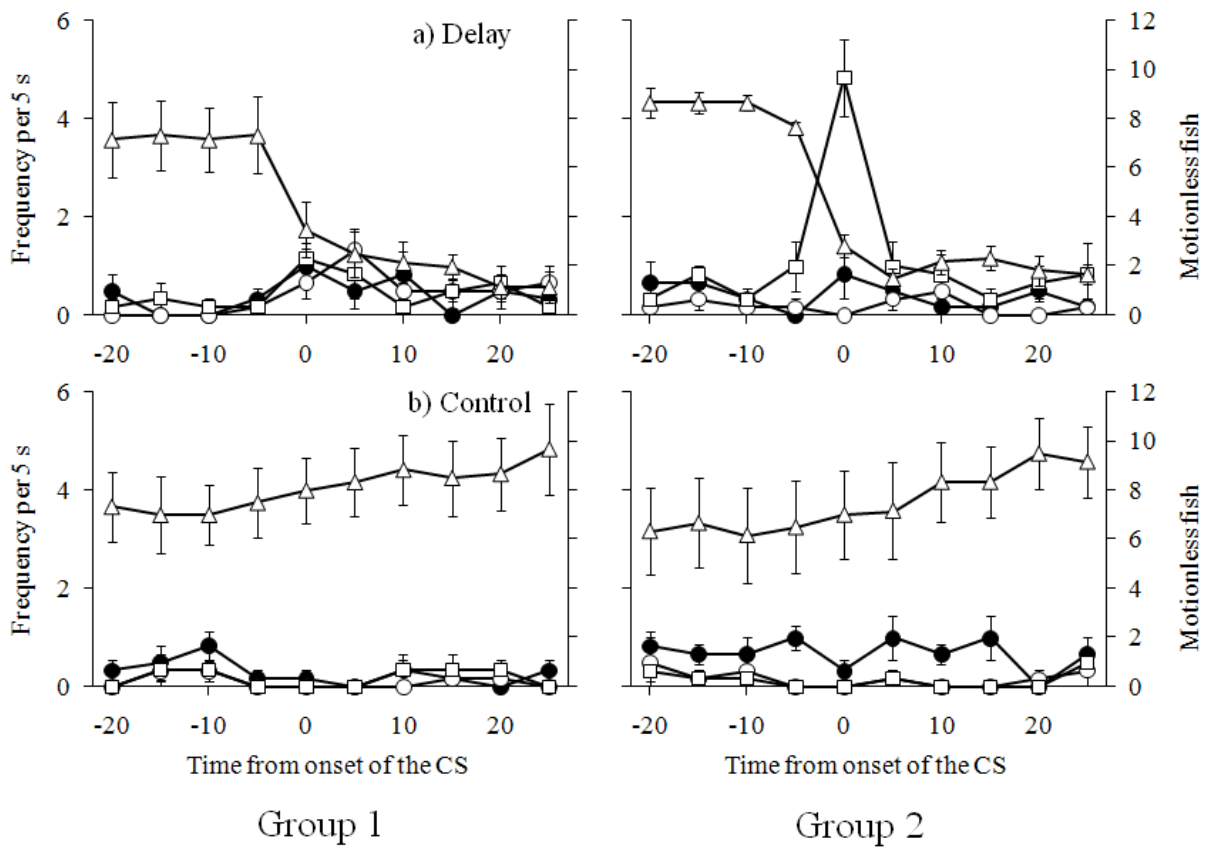
524 **Fig. 1.**



525

526 **Fig. 2.**

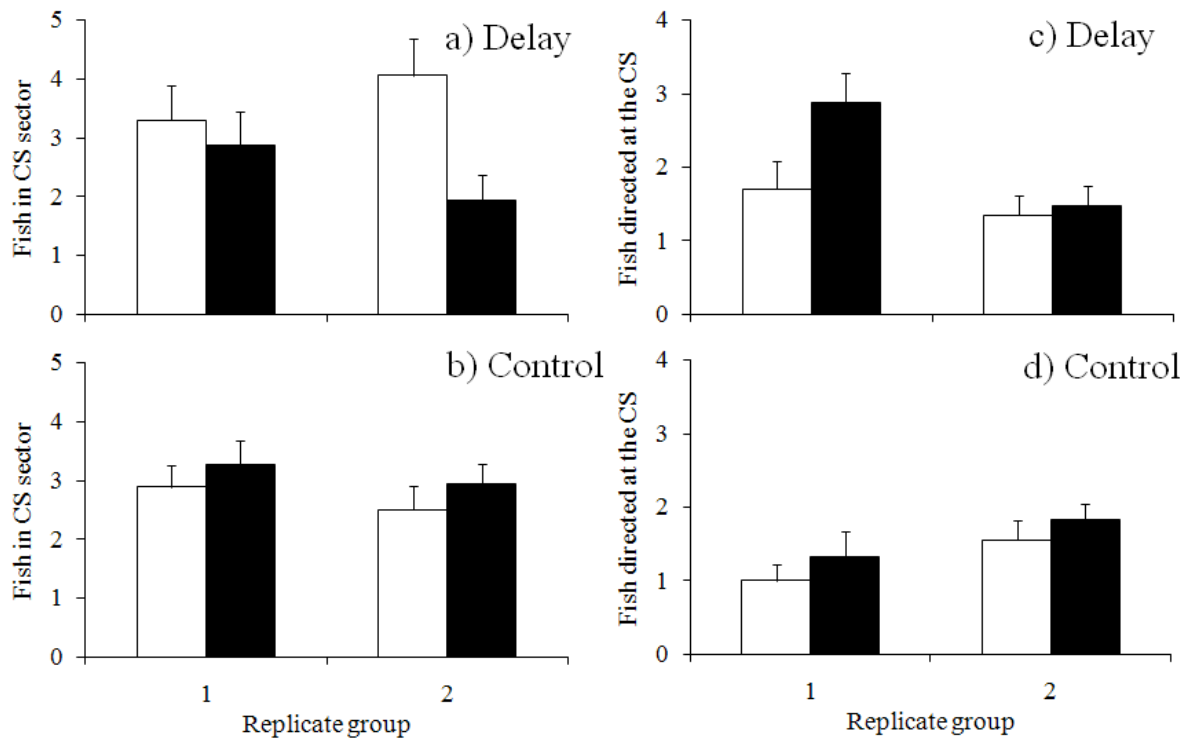
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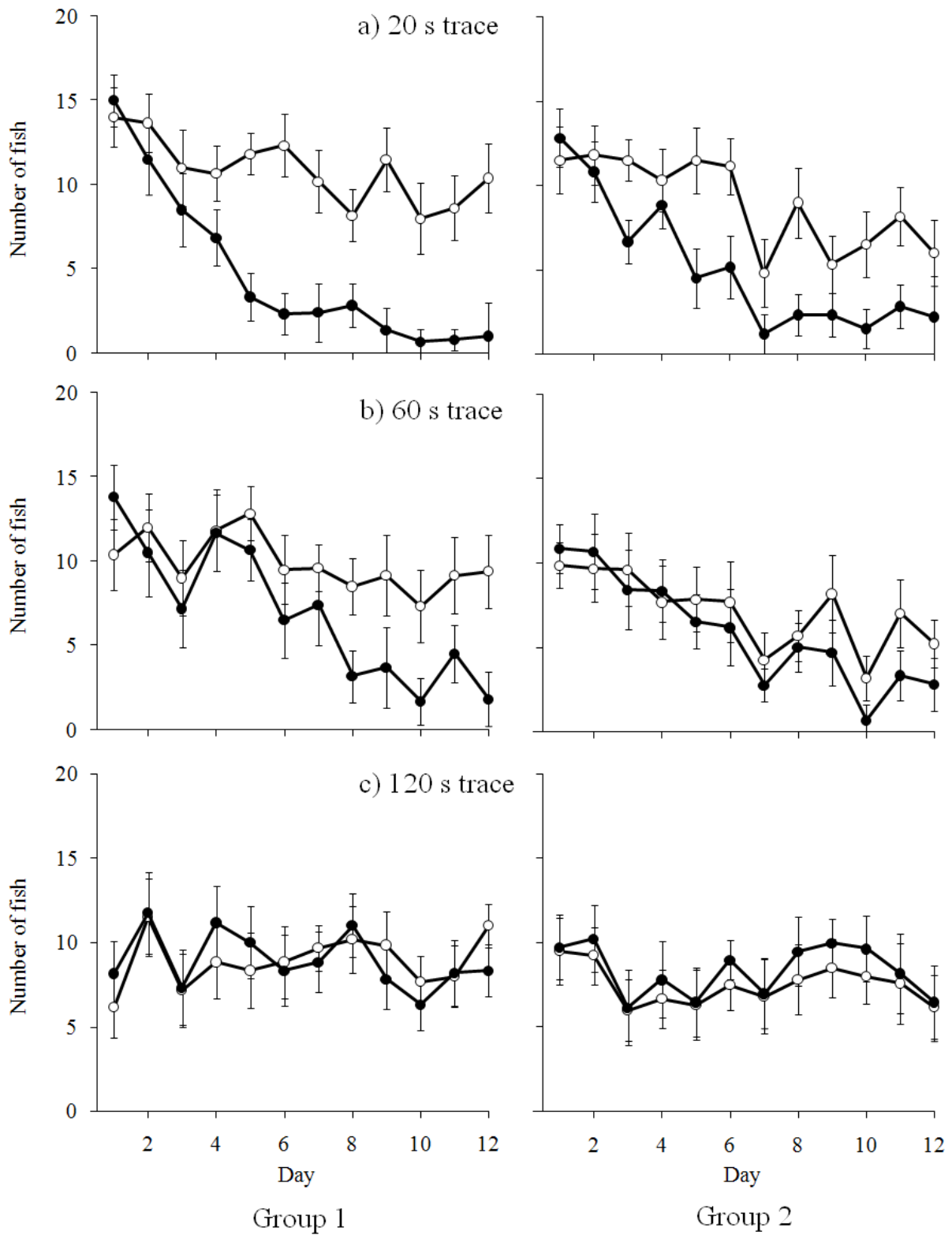
530 **Fig. 3.**

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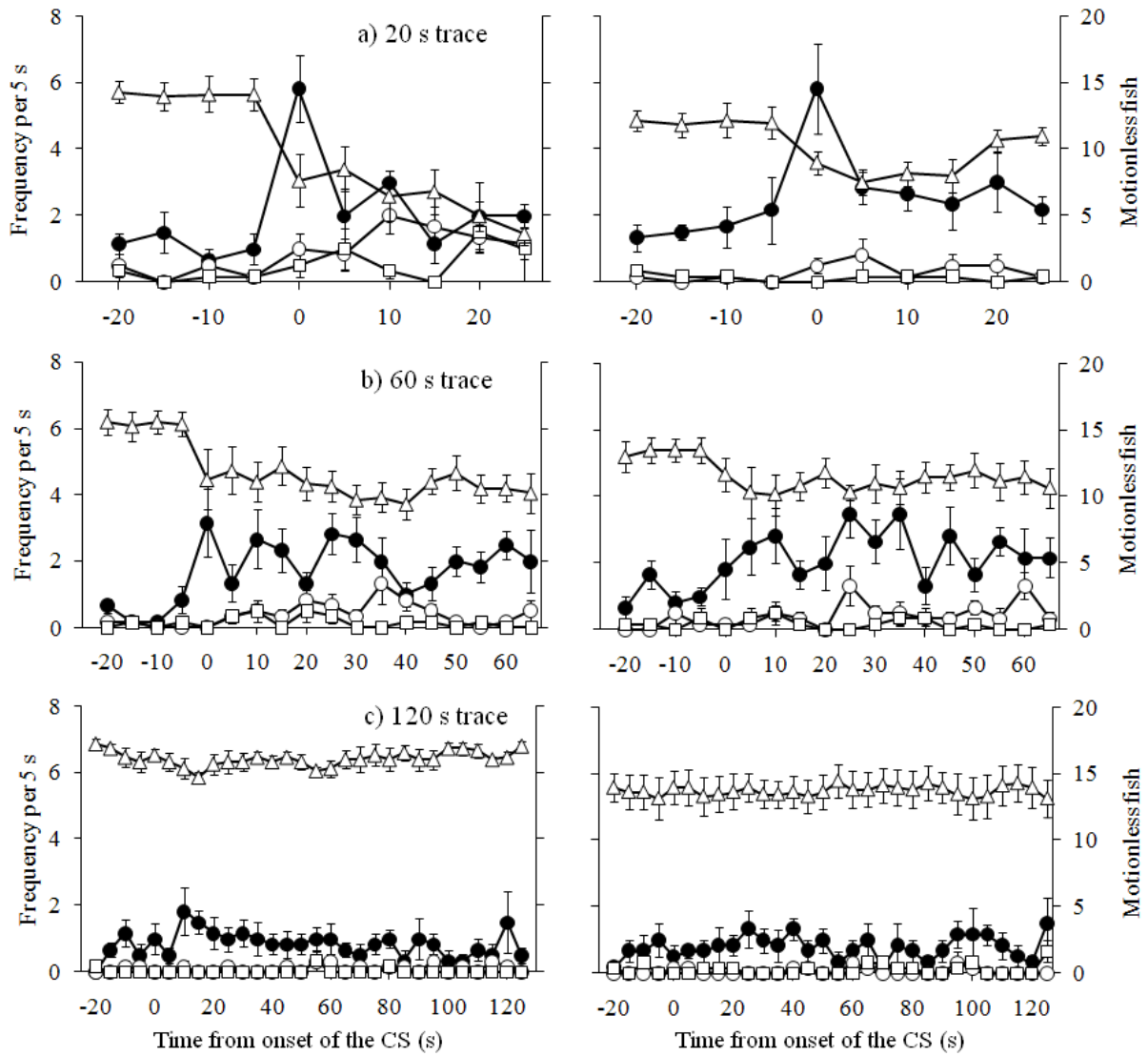
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533 **Fig. 4.**



534

535 **Fig. 5.**

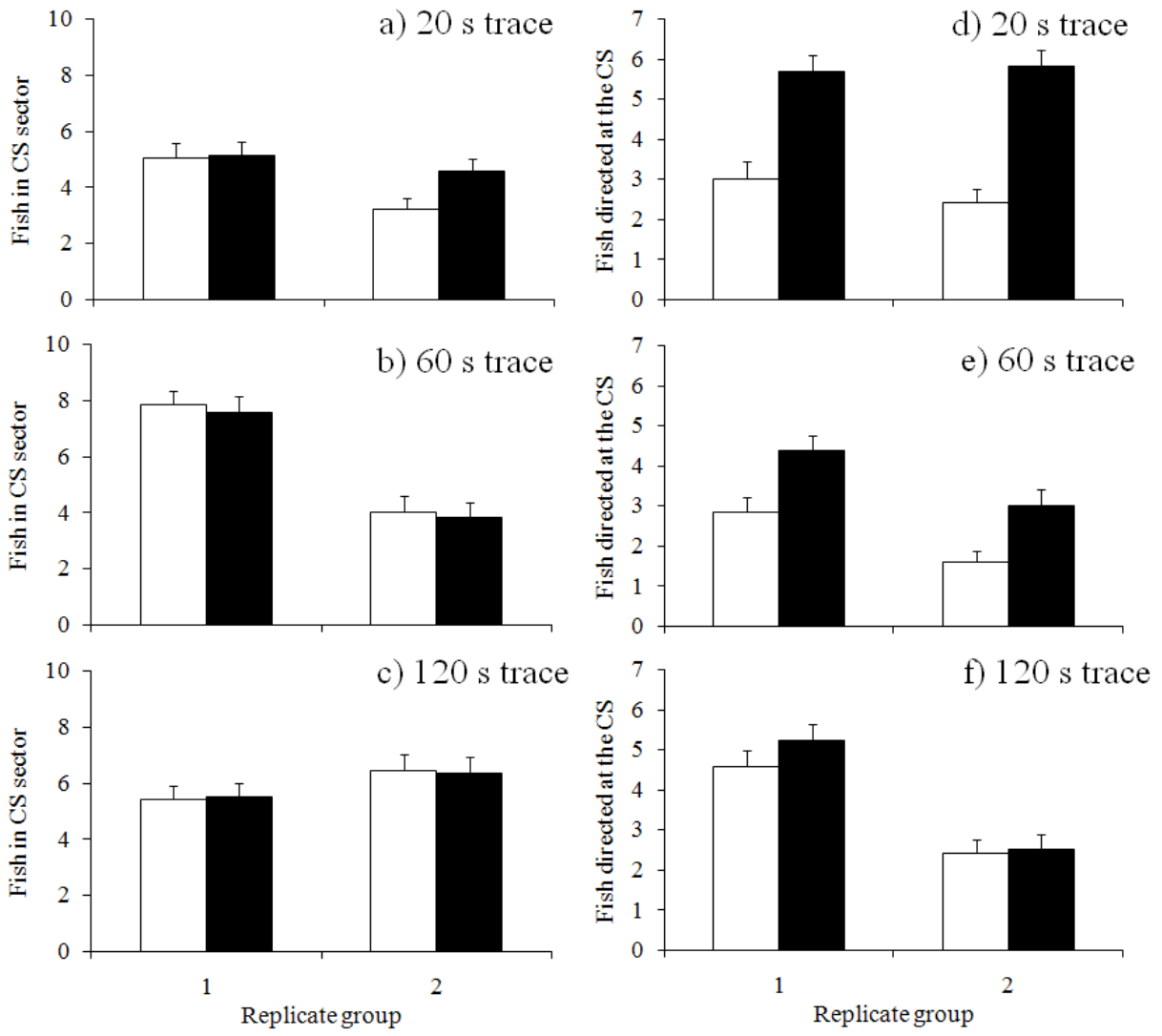


536

Group 1

Group 2

537 Fig. 6.



538

539 **Fig. 7.**