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1 Short Communication

2 **A novel method to individually track spawning females in aquaculture tanks**
3 **using the European sea bass (*Dicentrarchus labrax*) as a model**

4

5 Stephane Lallement^a, Allan Bengue^b, Benjamin Geffroy^b

6 ^aLaboratoire Service d'Experimentations Aquacoles, Ifremer, Palavas Les Flots, France

7 ^bMARBEC, Univ Montpellier, Ifremer, IRD, CNRS Palavas, France

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10 Keywords: Natural spawning; Spawn traceability; Identification of breeders

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12

13 **ABSTRACT**

14

15 Mass reproduction is widely used in fish farms because it is relatively easy and inexpensive. It
16 also enables the development of mass selection programmes that provide gains for traits with
17 high heritability. The main weakness of this approach is that it is currently impossible to know
18 which females participated in reproductive events, unless by conducting expensive genomic
19 experiments. We tested oviduct-inserted floating passive integrated transponder tags as a new
20 method to detect (i) the identity of spawners and (ii) the timing of spawning. We first
21 conducted a preliminary experiment using four small tanks (1.5 m³) each containing one male
22 and one female tracked by infrared video cameras to test the experimental device. We then
23 tested it in “real” aquaculture conditions, using a bigger tank (10 m³) containing 89 adult fish.
24 Results showed that this tracking system accurately identified the timing of spawning of
25 individual fish. We confirm that European sea bass preferentially spawn at sunrise or sunset.
26 This proof-of-concept developed for one commercially important fish species could also be
27 used for novel species of interest for aquaculture, for example, to determine the exact timing of
28 spawning after hormonal treatment of novel species.

29 **1. Introduction**

30 Natural spontaneous spawning is extensively used by hatcheries to obtain a relatively large
31 number of eggs with a minimum of constraints (Bromage, 1995). Natural reproduction has also
32 been found to produce better quality spawn (egg quality and larval survival) than selective
33 breeding, which requires fish manipulation and hormonal induction (Mylonas et al., 2010).
34 However, the main drawback of depending on natural spawns is that inbreeding is difficult to

35 control since many parents are kept in batches (Gjerde et al., 1996). Kinship relationship are
36 nonetheless possible to obtain as well as parentage contribution, but this require relatively
37 expensive genomic experiments (Superio et al., 2021). In any case, it remains impossible to
38 predict the timing of spawning, which precludes advances in the homogenization of larval
39 development protocols. Eggs are usually collected the morning after spawning (Barnabé, 1980)
40 so a tracking system able to detect the exact time of spawning would make it easier to collect
41 the eggs. For all these reasons, a device that can detect spawning events would clearly be an
42 asset for aquaculture. Oviduct-inserted radio transmitters have been successfully used to track
43 spawning events and to locate wild fish, the first attempt having been made on northern pike
44 (*Esox lucius*) (Pierce, 2004). Since then, similar experiments have been performed on wild
45 muskellunge (*Esox masquinongy*) (Pierce et al., 2007), European perch (*Perca fluviatilis*)
46 (Skovrind et al., 2013) and lake trout (*Salvelinus namaycush*) (Binder et al., 2014). However,
47 the transmitters are quite large (> 1 g), which may have prevented them from successfully
48 releasing the device on each occasion. The main objective of this article is to describe the use
49 of an oviduct-inserted passive integrated transponder (PIT) tag, associated with a detector
50 device placed on the outflow of the tank to track and monitor spawning events of European sea
51 bass.

52 **2. MATERIALS AND METHODS**

53 We designed encapsulated PIT-tags such that they would float. The PIT-tags (BIOLOG-ID
54 FDX-B transponder enables radio frequency, TINY 955 ISO 11784) used in both experiments
55 were 8 mm long and 1.4 mm wide (Figure 1A) and were placed in a section of 3 mm wide and
56 14 mm long angle pipelle (in fact a piece of Cornier pipelle). The assembly was then closed
57 with silicone at each end to trap an air bubble inside (Figure 1A), thus ensuring the buoyancy of
58 the device (hereafter named “modified PIT-tag”). Once inserted into the ovarian cavity of
59 females, it would be expelled during spawning and detected by a tag reader (Biolog PRD 640)
60 positioned close to the outflow of the tank.

61 **2.1. Preliminary tests**

62 On the 19th of October 2017, a few randomly selected females from our “advanced” fish stock
63 (fish intended to reproduce in autumn that are maintained at a temperature of 13 °C with a
64 corresponding winter photoperiod: sunrise at 8:00 am and sunset at 6:00 pm) were anaesthetised
65 (Benzocaine, 300 ppm), and their eggs analysed to determine their maturation stage according
66 to (Fauvel and Suquet, 1988). Briefly, 4 main stages have been described: stage A (vitellogenic

67 oocytes, central germinal vesicle, no lipid-droplet coalescence), stage B (post-vitellogenic
68 oocytes, hyalinisation of the periphery of the cytoplasm), stage C (central germinal vesicle,
69 early lipid-droplet coalescence) and stage D (migration of the germinal vesicle, lipid-droplet
70 coalescence). A Cornier pipelle was inserted into the ovarian cavity of the females to collect
71 oocytes (Figure 1B). Once collected, the females were kept in the second tank until the egg
72 maturation stage was analysed with a binocular (Figure 1C). Three females presented eggs at
73 stage C and one at stage B (Table 1) and were thus chosen for hormonally stimulation by
74 injection of D-Trp⁶-Luteinizing hormone releasing hormone (LHRH) at a dose of 10 µg/kg.
75 Following the injection, the modified PIT-tag was placed in the oviduct (Figure 1D). The four
76 females were then placed in four individual 1.5 m³ tanks. Each tank was filled with fresh
77 seawater and the temperature was regulated at 13 °C (mean ± sd: 13.3 ± 0.3 °C during this first
78 trial). We also added one male to each tank to stimulate reproduction so we could analyse
79 spawning behaviour.

80 An infrared camera (SZ-CVI003 hd-cvi 720P) was installed above each tank to record fish
81 behaviour night and day (Figure 1E). Another camera was installed close to the outflow of each
82 tank to track eggs collected in the net (Figure 1E, F). The PIT-tag reader was also placed close
83 to the outflow (Figure 1F). The reader was connected to a computer that recorded the exact
84 detection time of the modified PIT-tag, and hence spawning (Figure 1F). In this trial, the aim
85 was to validate the approach before conducting a second trial in a tank containing more fish in
86 real conditions.

87 **2.2. Experimental approach in aquaculture conditions**

88 To conduct the second experiment, fish were sampled in a 10 m³ tank containing 89 adults
89 homozygous albino European sea bass with a balanced sex ratio (1F/1M). We preferentially
90 used albinos' fish to facilitate the monitoring of the behaviour during the night, thanks to the
91 infrared cameras. Those fish are the second generation produced from a first artificial cross
92 made in 2006 using homozygous (based on their phenotypes) males and females, artificially
93 reproduced with heterozygous individuals
94 ([https://wwz.ifremer.fr/mediterranee/aquaculture/Cheptels-experimentaux-de-bar/Selection-](https://wwz.ifremer.fr/mediterranee/aquaculture/Cheptels-experimentaux-de-bar/Selection-albinos)
95 [albinos](https://wwz.ifremer.fr/mediterranee/aquaculture/Cheptels-experimentaux-de-bar/Selection-albinos)). The fish were already individually marked by PIT-tags (12 mm long and 21 mm wide)
96 positioned dorsally. The weight and sex of the fish were therefore known (Table 1), enabling us
97 to easily identify females. Two infrared cameras (SZ-CVI003 hd-cvi 720P) were installed
98 above the tank. A similar protocol to that used in the 2017 experiment was applied on the 2^d

99 and 23th of March 2021. For technical and phenological reasons, the experiment started
100 relatively late in the season (March). In the Mediterranean Sea, the ovulation period of the
101 European sea bass is known to last from the beginning of December until the end of March
102 (Asturiano et al., 2000). But, due to unusually high temperatures in 2019-2020, in 2020, peak
103 reproduction occurred in mid-February, explaining why we targeted the same period in 2021.
104 Females with eggs at a stage B, C, D of maturation or that were starting laying eggs (n=19)
105 were selected for the experiment and the modified PIT-tag was inserted in their oviduct (Figure
106 1D). Note that these fish were not hormonally stimulated to spawn. They were then returned to
107 their original tank containing all the males (n=44) and females at other developmental stages
108 (n=26). A PIT-tag reader was placed at the outflow and connected to a computer. Temperature
109 was monitored every day (mean \pm sd: 14.4 \pm 0.5 °C between the 2^d and 23th of March 2021)
110 and fish were fed *ad libitum* by an automatic food dispenser.

111

112 **2.3. Data analysis**

113 We collected two main variables: first, the exact time of spawning (thanks to the cameras
114 placed above the tank) and second, the time the modified PIT-tag was detected. The difference
115 between the two times corresponds to the latency in time, i.e., the time that elapses between
116 when the modified PIT-tag is released into the tank and the time it is read by the tag reader.

117 **3. Results**

118 In the first experiment, two spawning events took place in the four tanks containing the isolated
119 pairs. The first modified PIT-tag was detected on 20th of October 2017 at 6:53 pm and the
120 second on the 21st of October 2017 at 5:36 am. We then looked at the video recording made a
121 few minutes before the tag was detected, which allowed us -for the first time to our knowledge-
122 to describe the reproductive sequence of the European sea bass (Supplementary video 1). The
123 male (smaller and in this case albino) stimulated the female (bigger and blackish in colour) by
124 first nuzzling the female's abdomen. Then the male and the female entered the centre of the
125 small tank sequentially, first the female, which apparently released the eggs (the water surface
126 first appeared troubled at 6:53 pm) followed by the male, which likely fertilized them. This first
127 experiment validated the use of the device.

128 In the second experiment in March 2021, three spawning events were detected by reading the
129 modified PIT-tag. Based on analysis of the video and detection of the tag at the water surface,

130 the first spawning event took place on the 5th of March 2021 at 6:29 am and the modified PIT-
131 tag was detected at 6:43 am. The time latency was therefore 14 minutes. The second spawning
132 event took place on the 10th of March 2021 at 6:01 pm and the modified PIT-tag was read at
133 6:05 pm, giving a time latency of four minutes. Finally, the third spawning event took place on
134 the 23rd of March 2021 at 5:55 pm. The tag reader detected the modified PIT-tag at 6:44 pm.
135 The time latency was 49 minutes.

136

137 **4. Discussion**

138 The aim of this study was to test the possibility of identifying spawning females in aquaculture
139 tanks. The modified PIT-tag we developed appeared to provide reliable information to identify
140 each individual and the spawning time, two variables that could be extremely useful for
141 aquaculture. One may wonder why only three females out of the 19 females implanted spawned
142 successfully in the 2021 trial. Due to relatively high temperatures in 2021, most females were
143 at the regressing stage, which may explain why many other females at stage C and D did not
144 spawn. In any case, all the females that spawned released the modified PIT-tag, as expected.

145

146 In 2017, the two spawning events obtained after LHRH injection occurred respectively at 5h36
147 am and 6h53 pm. Similar timings were observed for natural spawning in 2021 (6:29 am, 6:01
148 pm and 6:44 pm). This strongly suggests that hormonal stimulation did not affect the
149 reproduction rhythms of the fish. In addition, our results corroborate those of (Villamizar et al.,
150 2012) indicating that reproductive activity of European sea bass is predominantly nocturnal.

151 We detected the same peak of spawning activity, one hour before sunrise, but did not find the
152 one 6 hours after sunset described in (Villamizar et al., 2012). It is known that fish reproductive
153 activity is adaptively regulated to ensure the highest fitness of offspring (Oliveira and Sánchez-
154 Vázquez, 2010). Therefore, the specific reproductive timing observed here is likely explained
155 by the fact that the reproductive strategies of the fish are intended to reduce predation on their
156 eggs. The fact that the highest egg viability was found for eggs laid just before sunset
157 (Villamizar et al., 2012) tend to corroborate this fact.

158 Individual monitoring of spawning activity enabled further insights into the reproductive
159 behaviour of European sea bass. Here we provide evidence that courtship and stimulation is
160 similar to that of other fish species (Calado et al., 2017; Hamamoto et al., 1992; Moyer et al.,
161 1983; Thresher, 1982), starting by the tactile stimulation by the male, nuzzling and touching the

162 female's abdomen. However, this behaviour was only observed in one video, and further
163 recordings are needed to depict this behaviour more thoroughly. Regarding the average time
164 latency between the release of the modified pit-tag and its detection, the overflow system
165 placed on the side of the tank in our study should preferably be positioned in the centre of the
166 tank to ensure a consistent and average latency for all spawners in large tanks. Importantly, the
167 modified PIT-tag not released by the other females of the study (n = 18) had no impact on the
168 subsequent health of these fish, as neither injuries nor specific illness were detected in the
169 months following the experiment. This is important for the wellbeing of the animals used.
170 To conclude, we foresee different applications and further development of this novel method in
171 aquaculture, particularly to master reproduction of novel fish species. For instance, it could
172 help identify the timing of response (i.e. spawning) after hormonal treatment of a novel species,
173 and thus contribute to the diversification of aquaculture. The device could also be combined
174 with an alert system, enabling the eggs resulting from a particular spawning event to be
175 collected before they start being mixed with eggs from other spawns. We expect that both
176 professional fish farmers and researchers in aquaculture will use this novel method in the near
177 future.

178

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180

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182 anonymous reviewers for improving the manuscript.

183

184

185 **Figure Legend**

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187

188 Figure 1: The different steps to insert and detect the modified PIT-tag. A) The modified PIT-

189 tag; B) Collection of females and sampling of the eggs; C) Assessment of oocytes development

190 stages; D) Insertion of the modified PIT-tag into the oviduct using a Cornier pipette; E)

191 Video-recording of fish behaviour using cameras placed above each tank and F) Eggs released

192 and the modified PIT-tag (within the white circle) detected by the PIT-tag reader.

193

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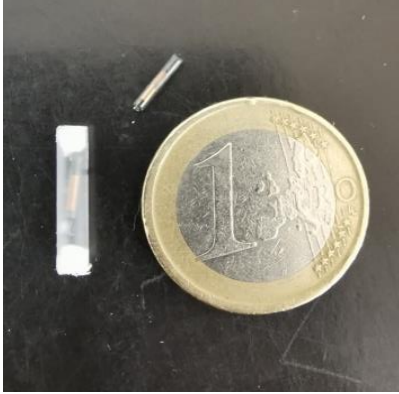
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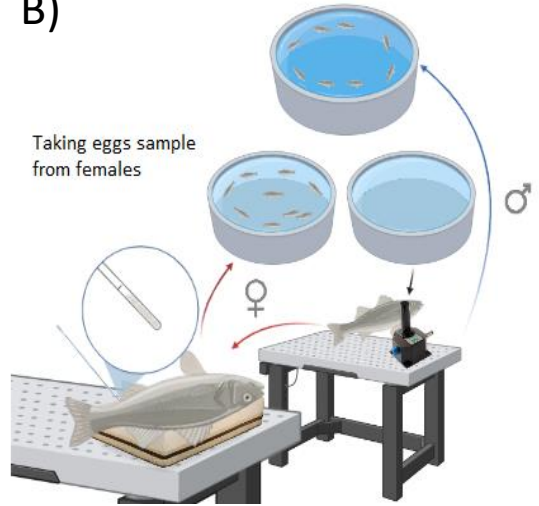
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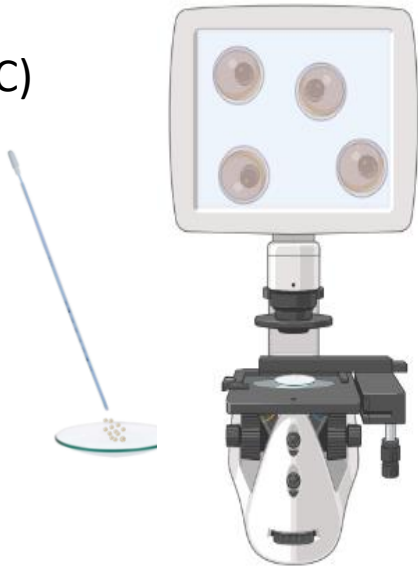
A)



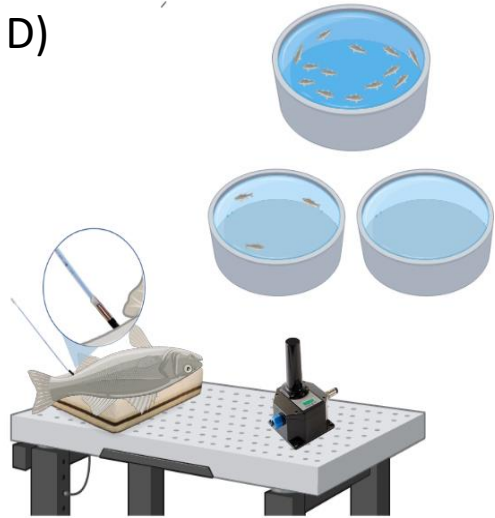
B)



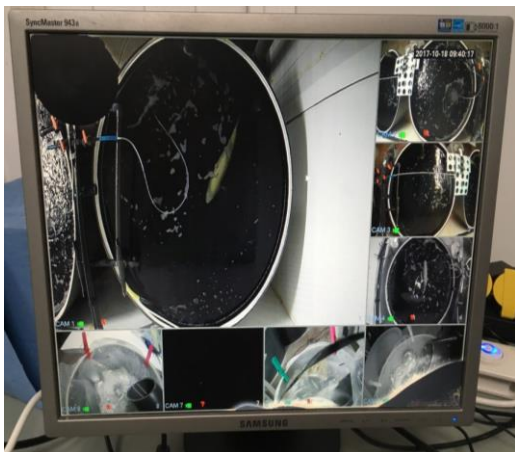
C)



D)



E)



F)



Implant date	Weight (Kg)	LHRH injection	Oocyte stage	Egg-laying	Egg-laying time
17/10/2017	5.3	yes	C	yes	06:53pm
	3.5	yes	C	yes	05:37am
	4.3	yes	C	no	
	3.8	yes	B	no	
02/03/2021	3.4	no	C	no	
	3.2	no	C	no	
	2.6	no	C	no	
	2.4	no	B	yes	06:05pm
	2.2	no	Start of laying	no	
	2.5	no	C	no	
	3.4	no	C	no	
	1.7	no	C	no	
	3.0	no	C	yes	06:43am
	3.3	no	C	no	
	23/03/2021	2.0	no	Start of laying	yes
1.9		no	C	no	
3.1		no	D	no	
2.0		no	B	no	
2.6		no	Start of laying	no	
2.2		no	D	no	
2.2		no	C	no	
2.4		no	Start of laying	no	
2.9		no	C	no	

Table 1: Summary of the characteristics of females implanted with a modified pit-tag in the two trials.