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

Lallement Stephane [1], Bengue Allan [3], Geffroy Benjamin [2, *]

[1] Laboratoire Service d'Experimentations Aquacoles, Ifremer, Palavas Lees Flots, France
[2] MARBEC, Univ Montpellier, Ifremer, IRD, CNRS Palavas, France
[3] MARBEC, Univ Montpellier, Ifremer, IRD, CNRS Palavas, France

* Corresponding author : Benjamin Geffroy, email address : bgeffroy@ifremer.fr

Abstract :

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

Highlights

► There is a need to track individual spawning events to improve aquaculture practices. ► The oviduct-inserted floating Pit-tag accurately indicates the timing of spawning and identity of spawner. ► Associated to video-recording, the device allows to provide cues on reproductive behaviour of fishes

Keywords : Natural spawning, Spawn traceability, Identification of breeders

1. Introduction

Natural spontaneous spawning is extensively used by hatcheries to obtain a relatively large number of eggs with a minimum of constraints (Bromage, 1995). Natural reproduction has also been found to produce better quality spawn (egg quality and larval survival) than selective breeding, which requires fish manipulation and hormonal induction (Mylonas et al., 2010). However, the main drawback of depending on natural spawns is that inbreeding is difficult to control since many parents are kept in batches (Gjerde et al., 1996). Kinship relationship are nonetheless possible to obtain as well as parentage contribution, but this require relatively expensive genomic experiments (Superio et al., 2021). In any case, it remains impossible to predict the timing of spawning, which precludes advances in the homogenization of larval development protocols. Eggs are usually collected the morning after spawning (Barnabé, 1980) so a tracking system able to detect the exact time of spawning would make it easier to collect the eggs. For all these reasons, a device that can detect spawning events would clearly be an asset for aquaculture. Oviduct-inserted radio transmitters have been successfully used to track spawning events and to locate wild fish, the first attempt having been made on northern pike (*Esox lucius*) (Pierce, 2004). Since then, similar experiments have been performed on wild muskellunge (*Esox masquinongy*) (Pierce et al., 2007), European perch (*Perca fluviatilis*) (Skovrind et al., 2013) and lake trout (*Salvelinus namaycush*) (Binder et al., 2014). However, the transmitters are quite large (> 1g), which may have prevented them from successfully releasing the device on each occasion. The main objective of this article is to describe the use of an oviduct-inserted passive integrated transponder (PIT) tag associated with a detector device placed on the outflow of the tank to track and monitor spawning events of European sea bass.

2. MATERIALS AND METHODS

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

2.1. Preliminary tests

On the 19th of October 2017, a few randomly selected females from our "advanced" fish stock (fish intended to reproduce in autumn that are maintained at a temperature of 13 °C with a corresponding winter photoperiod: sunrise at 8:00 am and sunset at 6:00 pm) were anesthetised (Benzocaine, 300 ppm), and their eggs analysed to determine their maturation stage according to (Fauvel and Suquet, 1988). Briefly, 4 main stages have been described: stage A (vitellogenic oocytes, central germinal vesicle, no lipid-droplet coalescence), stage B (post-vitellogenic oocytes, hyalinisation of the periphery of the cytoplasm), stage C (central germinal vesicle, early lipid-droplet coalescence) and stage D (migration of the germinal vesicle, lipid-droplet coalescence). A Cornier pipelle was inserted into the ovarian cavity of the females to collect oocytes (Figure 1B). Once collected, the females were kept in the second tank until the egg maturation stage was analysed with a binocular (Figure 1C). Three females presented eggs at stage C and one at stage B (Table 1) and were thus chosen for hormonally stimulation by injection of D-Trp^6-Luteinizing hormone releasing hormone (LHRH) at a dose of 10 µg/kg. Following the injection, the modified PIT-tag was placed in the oviduct (Figure 1D). The four females were then placed in four individual 1.5 m^3 tanks. Each tank was filled with fresh seawater and the temperature was regulated at 13 °C (mean ± sd: 13.3 ± 0.3 °C during this first trial). We also added one male to each tank to stimulate reproduction so we could analyse spawning behaviour.

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

2.2. Experimental approach in aquaculture conditions

To conduct the second experiment, fish were sampled in a 10 m^3 tank containing 89 adults homozygous albino European sea bass with a balanced sex ratio (1F/1M). We preferentially used albinos' fish to facilitate the monitoring of the behaviour during the night, thanks to the infrared cameras. Those fish are the second generation produced from a first artificial cross made in 2006 using homozygous (based on their phenotypes) males and females, artificially reproduced with heterozygous individuals (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) positioned dorsally. The weight and sex of the fish were therefore known (Table 1), enabling us to easily identify females. Two infrared cameras (SZ-CVI003 hd-cvi 720P) were installed above the tank. A similar protocol to that used in the 2017 experiment was applied on the 2^{d} and 23th of March 2021. For technical and phenological reasons, the experiment started relatively late in the season (March). In the Mediterranean Sea, the ovulation period of the European sea bass is known to last from the beginning of December until the end of March

(Asturiano et al., 2000). But, due to unusually high temperatures in 2019-2020, in 2020, peak reproduction occurred in mid-February, explaining why we targeted the same period in 2021. Females with eggs at a stage B, C, D of maturation or that were starting laying eggs (n=19) were selected for the experiment and the modified PIT-tag was inserted in their oviduct (Figure 1D). Note that these fish were not hormonally stimulated to spawn. They were then returned to their original tank containing all the males (n=44) and females at other developmental stages (n=26). A PIT-tag reader was placed at the outflow and connected to a computer. Temperature was monitored every day (mean ± sd: 14.4 ± 0.5 °C between the 2[d] and 23[th] of March 2021) and fish were fed *ad libitum* by an automatic food dispenser.

2.3. Data analysis

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

3. Results

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

In the second experiment in March 2021, three spawning events were detected by reading the modified PIT-tag. Based on analysis of the video and detection of the tag at the water surface, the first spawning event took place on the 5[th] of March 2021 at 6:29 am and the modified PIT-tag was detected at 6:43 am. The time latency was therefore 14 minutes. The second spawning event took place on the 10[th] of March 2021 at 6:01 pm and the modified PIT-tag was read at

6:05 pm, giving a time latency of four minutes. Finally, the third spawning event took place on the 23rd of March 2021 at 5:55 pm. The tag reader detected the modified PIT-tag at 6:44 pm. The time latency was 49 minutes.

4. Discussion

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

In 2017, the two spawning events obtained after LHRH injection occurred respectively at 5h36 am and 6h53 pm. Similar timings were observed for natural spawning in 2021 (6:29 am, 6:01 pm and 6:44 pm). This strongly suggests that hormonal stimulation did not affect the reproduction rhythms of the fish. In addition, our results corroborate those of (Villamizar et al., 2012) indicating that reproductive activity of European sea bass is predominantly nocturnal. We detected the same peak of spawning activity, one hour before sunrise, but did not found the one 6 hours after sunset described in (Villamizar et al., 2012). It is known that fish reproductive activity is adaptively regulated to ensure the highest fitness of offspring (Oliveira and Sánchez-Vázquez, 2010). Therefore, the specific reproductive timing observed here is likely explained by the fact that the reproductive strategies of the fish are intended to reduce predation on their eggs. The fact that the highest egg viability was found for eggs laid just before sunset (Villamizar et al., 2012) tend to corroborate this fact.

Individual monitoring of spawning activity enabled further insights into the reproductive behaviour of European sea bass. Here we provide evidence that courtship and stimulation is similar to that of other fish species (Calado et al., 2017; Hamamoto et al., 1992; Moyer et al., 1983; Thresher, 1982), starting by the tactile stimulation by the male, nuzzling and touching the female's abdomen. However, this behaviour was only observed in one video, and further recordings are needed to depict this behaviour more thoroughly. Regarding the average time latency between the release of the modified pit-tag and its detection, the overflow system placed on the side of the tank in our study should preferably be positioned in the centre of the tank to ensure a consistent and average latency for all spawners in large tanks. Importantly, the modified PIT-tag not released by the other females of the study (n = 18) had no impact on the subsequent health of these fish, as neither injuries nor specific illness were detected in the months following the experiment. This is important for the wellbeing of the animals used.

To conclude, we foresee different applications and further development of this novel method in aquaculture, particularly to master reproduction of novel fish species. For instance, it could help identify the timing of response (i.e. spawning) after hormonal treatment of a novel species, and thus contribute to the diversification of aquaculture. The device could also be combined with an alert system, enabling the eggs resulting from a particular spawning event to be collected before they start being mixed with eggs from other spawns. We expect that both professional fish farmers and researchers in aquaculture will use this novel method in the near future.

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Figure Legend

Figure 1: The different steps to insert and detect the modified PIT-tag. A) The modified PIT-tag; B) Collection of females and sampling of the eggs; C) Assessment of oocytes development stages; D) Insertion of the modified PIT-tag into the oviduct using a Cornier pipelle; E) Videao-recording of fish behaviour using cameras placed above each tank and F) Eggs released and the modified PIT-tag (within the white circle) detected by the PIT-tag reader.

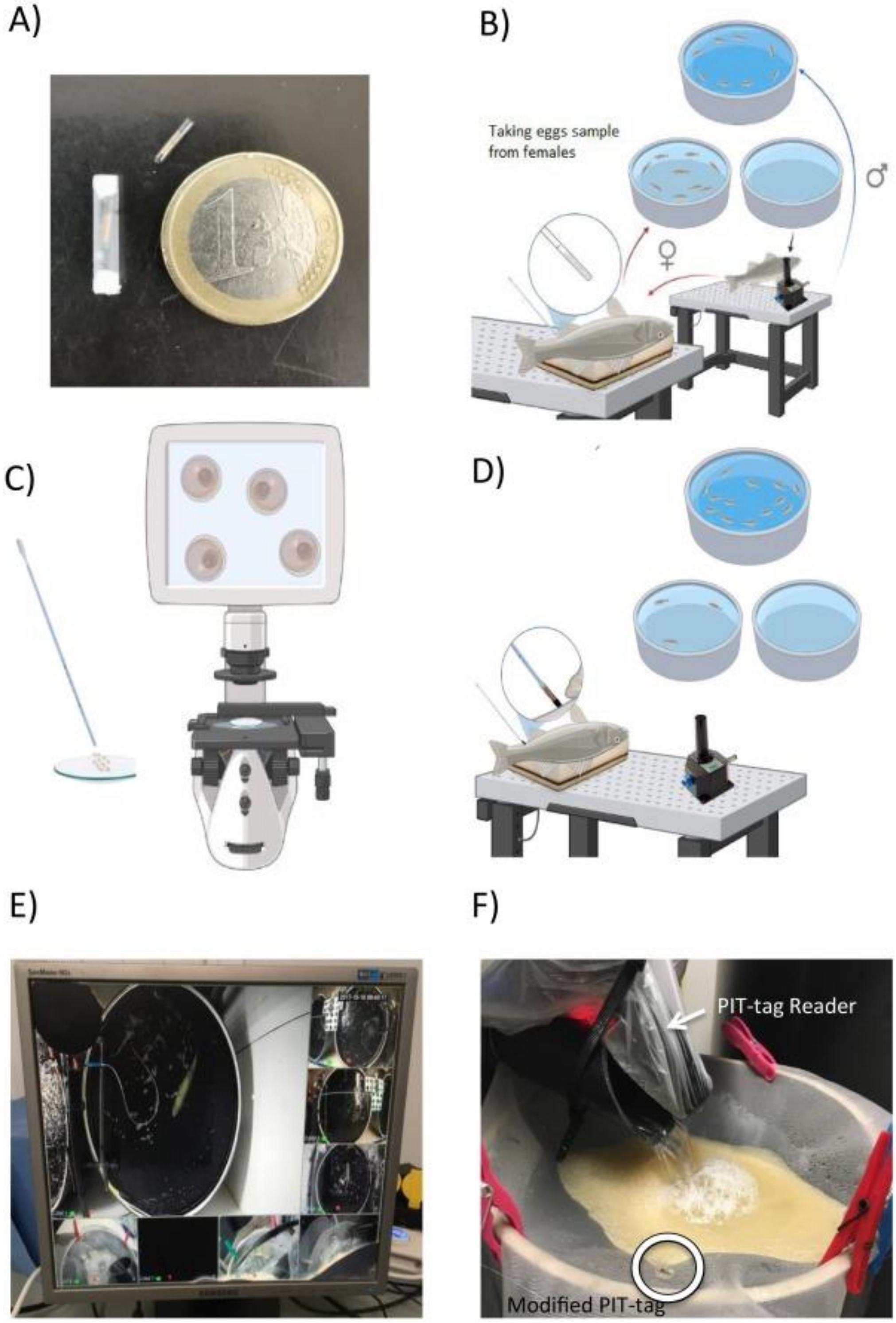
Credit Author Statement

Stephane Lallement: Writing- Original draft, Methodology, Data curation. **Allan Bengue :** Investigation. **Benjamin Geffroy:** Conceptualization, Methodology, Writing- Reviewing and Editing, Supervision.

Declaration of interests

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

Figure 1


A)
B)
Taking eggs sample
from females
♀
♂
C)
D)
E)
F)
PIT-tag Reader
Modified PIT-tag

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Implant dat	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	05:55pm
	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.

Highlights

- There is a need to track individual spawning events to improve aquaculture practices
- We provide evidence that an oviduct-inserted floating Pit-tag accurately indicates the timing of spawning and identity of spawner.
- Associated to video-recording, the device allow to provide cues on reproductive behavior for species of interest for aquaculture.