Aquaculture 494 (2018) 10–18

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Short communication

# Evaluation of different tags on survival, growth and stress response in the flatfish Senegalese sole

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ARTICLE INFO

*Keywords:*
Flatfish
PIT-tags
Loss rates
Sex effect
Growth

ABSTRACT

Internal electronic tagging is a major issue in flatfish species due to the small size of abdominal cavity. In this study, three tag types, referred to as Nonatec, nano and mini, were evaluated in three weight classes of Senegalese sole: small (0.3 g), middle (0.8 g) and large (2.0 g). Tags were injected from the blind side and fish were carefully handled to minimize sharp movements. Tag losses were 8% in the small size class, between 5.2 and 15.1% in the middle size class and 2–4% in the large size class. The mortality rates ranged between 2.0 and 15.0% with the lowest values in the large size class. No negative effects of tags on growth (tagged vs non-tagged fish using a middle size class) were found after 57 days of culture. Four additional trials using mini tags in a large size class at industrial scale validated our experimental results. With respect to morphology, no differences in the area, ellipticity and circularity were found except for a slight higher aspect ratio index in mini- and nano-tagged soles when compared with untagged fish. A longitudinal analysis of growth using the tag type, sex and tag position (anterior, medium or posterior) as fixed factors revealed a significant and strong effect of sex, with females appearing significantly heavier (13.6%) than males. In addition, the significant interactions between tag position and tag type with the time indicated a delayed growth of Nonatec-tagged fish and specimens with tags in the posterior section of abdomen. Expression analysis of stress-related genes revealed an activation of HPI axis and cellular stress defenses at 2 days just after tagging (dat) not evident at 11 dat. All these data indicate that soles can be successfully tagged at very small sizes both at experimental and industrial scales if tag type is properly selected and fish correctly handled. Moreover, sex and tag position are significant factors affecting growth that need to be controlled in longitudinal studies and selective breeding programs.

## 1. Introduction

Internal tags have become a valuable tool for longitudinal studies in fish aquaculture and ecology (Jepsen et al., 2005; Bégout et al., 2016; Cousin et al., 2012; Ferrari et al., 2014; Mahapatra et al., 2001). The PIT (Passive Integrated Transponder) tags are the most common devices since they can be used in large populations in an operational way. In the last years, PIT tags ranging between 6 and 12 mm in length and with a low weight (~2–4% tag/body weight ratio) have been successfully used in fish (Jepsen et al., 2005; Baras et al., 2000; Cousin et al., 2012; Ombredane et al., 1998). However, the great diversity in fish morphology requires that both the tag type and the injection procedure have to be specifically validated in a species- and class sizes-specific way and the effects on growth, survival, behavior and stress concurrently assessed. In pelagic fish, several studies reported optimal retention ratios (> 90%) with little or no effects on fish survival and growth although retention ratios were highly influenced by fish size (Acolas et al., 2007; Larsen et al., 2013; Mahapatra et al., 2001; Navarro et al., 2006; Soula et al., 2011). In flatfish, internal tagging is a bit more complicated by some methodological constraints due to the small size of the abdominal cavity and fish handling. Although specific procedures in large-size flatfish species such as plaice and turbot were optimized by accessing from the eyed-side (Moser et al., 2005; Oesau et al., 2013; Sparrevohn et al., 2014), specific methods need to be optimized in smaller and more ellipsoid flatfishes such as soles.

The Senegalese sole (*Solea senegalensis*) is an economically

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https://doi.org/10.1016/j.aquaculture.2018.05.009
Received 29 October 2017; Received in revised form 3 May 2018; Accepted 4 May 2018
Available online 05 May 2018
0044-8486/ 

important flatfish species in Southern Europe. The aquaculture production under recirculation aquaculture systems (RAS) grew exponentially in the last five years. However, some bottlenecks related with reproduction success and size dispersion still persist (Morais et al., 2016) that require advanced studies to identify hatchery and management solutions. Up to now, the visible implant elastomer (VIE) has been the most used individual tagging method in this species to evaluate growth performance and to study social interactions in population (Salas-Leiton et al., 2010a,b). However, the use of distinct patterns based on color and shape applied on the blind side of juveniles was prone to errors due to the complexity to read and interpret the VIE tagging patterns. Hence, new methodologies based on internal electronic tags are required to provide unique identification codes, easy to read, functional for long periods and that can be applied to a large range of fish sizes. Currently, PIT tags are intramuscularly applied to sole breeding stocks (Anguís and Cañavate, 2005) and juveniles in behavioral trials (Ibarra-Zatarain et al., 2016). However, an intraperitoneal tagging method needs to be optimized and validated in small size soles just at the beginning of pre-ongrowing stage (2–5 g) in order to design and implement selective breeding programs. Moreover, the analysis of some expression markers related to the hypothalamus-pituitary-interrenal axis and cellular stress (Benitez-Dorta et al., 2013, 2017; Manchado et al., 2008; Montero et al., 2015; Salas-Leiton et al., 2012) will allow to assess the impact on animal welfare and the suitability to evaluate complex traits in longitudinal studies.

The aim of this study was to evaluate the suitability of three internal PIT tags of different sizes and to monitor effects on tag losses, mortality, growth, morphology and expression of some stress-related genes in sole. This evaluation included different fish weight classes and were performed both at experimental and industrial scales over a long term. The data gathered highlight the importance of individual tagging to evaluate growth performance in young sole juveniles controlling some other variables such as sex and tank effects which both have a profound impact on the statistical models and results accuracy.

## 2. Material and methods

### 2.1. Tagging protocol and fish trials

All soles used in this study belonged to the same spawning batch and they were supplied by CUPIMAR (San Fernando, Cadiz, Spain). All procedures were authorized by Bioethics and Animal Welfare Committee of the IFAPA and registered with number 06-11-15-337 by National authorities for regulation of animal care and experimentation.

Before carrying out the fish trials, the tag injection procedure was optimized and adapted to the morphology and behaviour of sole. All staff was trained to acquire skills in needle, tags and fish handling prior to the trial. Specimens were fasted for 1 day before tagging and they were deeply anesthetized before handling (phenoxyethanol, 150 ppm). Before injection, PIT tags were disinfected by immersion in 70% ethanol followed by a distilled water washing. Animals were flipped blind side up and a small incision in the posterior part of the abdominal cavity was done using a G18 or G19 needle (previously disinfected in 70% ethanol and rinsed in distilled water). The size of the incision was wide enough to allow for the tag injection by exerting a bit pressure with forceps (Supplementary fig. 1). Large incisions were avoided to accelerate the healing of the wounds. When a high number of specimens were tagged, the needles were periodically changed to be sharp enough to easily cut through the skin and muscle wall. The introduction of the tag was always parallel to the ventral fin by gently pressing on the top of the abdominal cavity to control tag injection and avoid any damage to internal organs. After completing this procedure, the tag was softly displaced in the abdominal cavity to move away from the incision. Once completed, the incisions were covered with iodine gel (betadine gel) to prevent infections and to facilitate wound healing. Thereafter fish were transferred back to their home tanks in their natural position (standing on the blind side) to avoid agitation after anaesthesia recovery. To minimize movements in the tank after tagging, animals were fasted for 24 h. Average handling time required to tag one fish ranged between 30 and 60 s.

To evaluate the effects of PIT tags on survival, tag loss rate, growth and stress responses, different tagging sessions were carried out that resulted in three experimental trials to evaluate: i) tag losses and survival (experiment 1), ii) growth performance (experiment 2a) and iii) effects on gene expression (experiment 2b). A last experiment was conducted at an industrial scale (experiment 3) In all cases, the above procedure was followed paying special attention to the following points: a) animals were deeply anesthetized before handling and during all the procedure; b) careful return to the home tank in natural position to avoid agitation; c) further fish handing was reduced as much as possible by using self-cleaning tanks (1.0 m × 0.5 m, volume = 0.05 $m^3$) and just manipulating belt-feeders once day.

Experiment 1: In the first tagging session, a trial to evaluate tag losses and fish survival was carried out. Three PIT tag types were tested (Supplementary fig. 1): a) Nonatec™ (1 × 6 mm, 7.25 mg); b) Nano transponder (ID-100A/1.25, Trovan; 1.25 × 7 mm, 25 mg); c) Mini transponder (ID-100A/1.4 Trovan, 1.4 × 8 mm 30 mg). Moreover, these three tags were injected in three weight classes: small (average 0.3 g), middle (average 0.8 g) and large class (average 2.0 g). All individuals were randomly selected and assigned to each tag experimental group by class size. Mean weights for each tag type and size class are presented in Table 1. In the small weight class, only the Nonatec tags could be evaluated since the small size of the abdominal cavity impeded the injection of larger tags. After tagging, all individuals ($n$ = 25) were placed in one tank. In the middle size class, soles were injected with the three tag types and dispatched in triplicate tanks (the total number of individuals by tag type ranged between 145 and 157, with 45 to 53 soles per tank, 9 tanks in total). In the large size class, 50 individuals for each tag type were injected and distributed into separate tanks (one tank per tag type, 3 tanks in total). For each fish, weight, length and surgeon identity (five persons in total) were recorded. After tagging, soles were dispatched in self-cleaning rectangular tanks in an open flow circuit with one water renewal each 2 h that ensured a high water quality and cleanness of surfaces. Food (2% of fish biomass; Gemma Micro Skretting, Spain) was supplied from the second day after tagging onwards by using 12 h-belt feeders. Tag losses and mortality were recorded daily for 15 days. Water temperature and salinity were 18.1 ± 0.5 °C and 37 ppt, respectively. A treatment with hydrogen peroxide (100 ppm) was also weekly applied to facilitate wound healing and prevent diseases. Weight was automatically registered for Nano and Mini transponders using the FR-200 FishReader W (Zeuss, Trovan, Spain). For Nonatec tagged fish, the hand-held Bluetooth reader was used to read the tag and weight was manually inserted in a spreadsheet.

In a second tagging session, two trials were carried out.

Experiment 2a: Firstly, the effects of the three PIT tag types on growth performance and morphology were evaluated using a middle weight class (1.0 g). A set of 360 soles was tagged using the Nonatec, nano- and mini transponders and distributed separately into nine tanks ($n$ = 40 per tank). Animals were daily inspected for ten days and only 30 individuals with completely healed wounds were randomly selected and maintained in each tank. Then, thirty untagged soles from the same larval batch and of similar size were added to each tank (Table 2). Food (2% fish biomass; Gemma Micro Skretting, Spain) was supplied from the second day after tagging onwards by using 12 h-belt feeders. The tanks were daily inspected for tag losses and mortality. Weight and standard length (from the mouth until the base of caudal fin) were recorded at 20, 36 and 57 days after adding untagged fish. Body width (in the maximal width point excluding the dorsal and ventral fins) was measured at the end of the trial. In the first biometry (at 20 days, i.e. 30 days post tagging), the presence of a scar on the blind side was used to distinguish untagged fish from those that had lost their tag. In the last two samplings, due to the absence of any skin lesion, the expected