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Original study

Application of PIT tags for individual identification of turbot (*Scophthalmus maximus*)

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Abstract

Passive integrated transponder (PIT) tags only work in very close proximity of the receiver (cm distance). Therefore, active transponders are mainly used in behavioural monitoring. In the present study, the effects of intraabdominal implantation of PIT tags on survival, well-being and growth performance of juvenile turbot (*Scophthalmus maximus*) were investigated. Furthermore, the tag retention rate and read out error rate of all tags were examined. Passive integrated transponder tags were implanted in the abdominal cavity of nearly 6 000 turbots. All tags were readout and checked for correct function over a period of 122 days every five and a half weeks. No significant effects of tagging on fish survival (mortality rate <0.2 %), health or growth were detected during the trial period. Tag retention rate was 100 % and no malfunctions were observed. Results suggest that turbots can be marked with PIT tags in the abdominal cavity without obvious negative influences on performance traits and tag retention rate.

Keywords: passive integrated transponder (PIT), radio frequency identification system (RFID), individual identification, turbot, *Scophthalmus maximus*

Abbreviations: PIT: passive integrated transponder

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Introduction

In the early nineteen-seventies, first attempts were made to produce turbot (*Scophthalmus maximus*) in marine aquaculture systems in Scotland. In the mid-eighties, France and Spain began to produce noteworthy amounts of farmed turbot. Subsequently, the European production has steadily increased to more than 9 200 t in 2009 (Bjørndal & Øiestad 2011) and considerable progress has been made in terms of stock management and keeping systems during these decades. However, only limited genetic gain has been achieved regarding important traits like growth performance and uniformity, feed efficiency or robustness. This can primarily be attributed to a lack of advanced breeding schemes including performance testing and breeding value estimation. Such programmes are rarely applied in most aquaculture species compared to simple mass selection schemes (Herbinger *et al.* 1995). Disadvantages of mass selection are increased inbreeding levels and limited genetic gain, especially for traits with low heritability. Advanced breeding programmes are based on individual selection and it is necessary to establish a reliable method for individual identification of animals in the breeding stock. Performance testing is crucial and offspring from different matings should be raised together, as the environmental effects of different tanks are else confounded with genetic effects (Estoup *et al.* 1998, Herbinger *et al.* 1999, Borrell *et al.* 2004). This is, however, complicated by the fact that the larvae are initially too small to be physically marked. It is necessary to rear full-sib groups in separate tanks until the size of the fish allows individual marking (Doyle & Herbinger 1994). Alternatively, genetic markers can be used for parental assignment nowadays.

Pedigree information further allows the construction of relationship matrices for the estimation of genetic parameters and breeding values (Herbinger *et al.* 1999, Castro *et al.* 2007).

In general, marks are classified according to their application. External marks include anchor and jawbone marks, cold brandings, pigment and colour codings, visible implant tags or fin clips. Low costs and simple attachment are in favour of these common methods in aquaculture (Moffet *et al.* 1997, Navarro *et al.* 2006). However, a high risk of loss, inaccurate recognition, the possibility of secondary infections and a limited number of different labels are clear disadvantages (Das Mahapatra *et al.* 2001). Internal marking procedures require the implantation of passive integrated transponder (PIT) tags, which can be detected via an external reading device. The tags are usually appliqued intraperitoneal, intramuscularly or subcutaneously (Gheorghiu *et al.* 2010, Hopko *et al.* 2010). Passive integrated transponder tags have been used in different aquatic species, like prawns (Caceci *et al.* 1999), gilthead sea bream (Navarro *et al.* 2006), brown trout (Acolas *et al.* 2007), Nile tilapia (Baras *et al.* 1999), Eurasian perch (Baras *et al.* 2000) and Atlantic salmon (Gries & Letcher 2002). Small tag size and the almost unlimited number of different labels are the main advantages of PIT tags (Hopko *et al.* 2010). Furthermore, many studies have reported that PIT tags have no or only very little influence on growth and on the survival rate of fish (Prentice *et al.* 1989, Quartararo & Bell 1992, Baras *et al.* 1999, Baras *et al.* 2000, Gries & Letcher 2002). However, PIT tags are expensive and implantation requires special equipment and species-specific procedures to assure that the implant does not cause injuries upon migration (Baras *et al.* 2000, Das Mahapatra *et al.* 2001, Gheorghiu *et al.* 2010, Hopko *et al.* 2010). Especially if fish are marked at a young age, the subcutaneous or intramuscular application may be problematic (Baras *et al.* 2000, Gries & Letcher 2002).

Therefore, the aim of the current study was to establish a protocol for the application of PIT tags in turbot. Key parameters such as the rate of tag losses, the mortality of marked fish and the behaviour of the implants in terms of possible migration or encapsulation processes were analysed to evaluate feasibility under practical conditions.

Material and methods

Experimental conditions and animals

The entire experiment comprised an initial number of 5 986 turbot obtained from a Norwegian (n=3 423) and an Icelandic (n=2 563) hatchery. The initial fish weight varied between 9.6 g and 168.4 g (Table 1). The animals were kept under commercial conditions in a recirculating seawater system with 20 cylindrical basins and a total tank volume of 50 m^3 at the »Gesellschaft für Marine Aquakultur mbH (GMA)« (Büsum, Germany). During the experiment, water conditions were as follows: O_2-content ≈ 8.2 mg/l, NH_4^+-content ≈ 0.3 mg/l, NO_2^--content ≈ 2.5 mg/l, salinity ≈ 29 ‰, temperature ≈ 18.5 °C. Fish were fed a commercial turbot feed, »Aller 505« (Aller Aqua, Christiansfeld, Denmark) at a rate of 1 % of the actual stock biomass per day.

Table 1
Size range and mean values of the two turbot origins in g at the time of tag implantation

	Min	Max	Mean values
Iceland	9.6	119.0	47.7 (14.1)
Norway	17.8	168.4	82.0 (21.1)
Total	9.6	168.4	67.28 (25.1)

Tagging procedure

Glass encapsulated PIT tags (Hallprint, PTY Ltd., Hindmarsh Valley, Australia) with a length of 7 mm and a diameter of 2 mm were used to mark the fish. The transponder codes were read out using a hand-held reader (PetScan RT100 V5, Real Trace, Villebon-sur-Yvette, France) connected to a laptop computer. Transponders were implanted in all 5 986 turbots within a period of three weeks.

The fish were starved for two days before implantation in order to prevent problems caused by partially digested food in the digestive system (Gheorghiu *et al.* 2010). Prior to tag implantation, the turbots were anesthetized using tricaine methane sulphonate (MS222, Sigma-Aldrich, Steinheim, Germany) in a concentration of 100 mg/l water. The tags were disinfected with alcohol and then implanted into the abdominal cavity via a 3 mm stitch incision approximately 1.5 cm abdominal of the occipital region, i.e. where head and backbone meet (Figure 1). The incision itself was not closed artificially nor treated in any other way. After implantation, the animals were kept in clear water and observed until they had completely recovered from anaesthesia and then were transferred to the basins. The fish were starved for the following two days to avoid possible tag losses which might occur via the incision due to extensive movements during feeding.

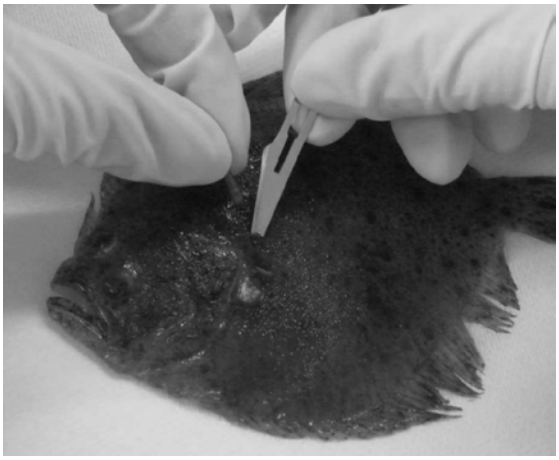

Figure 1
Implantation position of the PIT (passive integrated transponder) tag, approximately 1.5 cm abdominal of the occipital region

Monitoring of the transponder status

The tag position was determined by radiography in 24 randomly chosen living turbots at day 14 post-implantation (Figure 2). Eighteen of these animals were still alive at day 122 post-implantation and available for a second radiography. Furthermore, the tag position and encapsulation status were determined in 800 turbots during routine slaughtering.

Individual measurement of body weight started at the day the animals were tagged. The individual weight of each turbot was recorded over a period of 122 days every five and a half weeks (Figure 3). During these routine controls the presence and function of transponders were checked by scanning the fish with a reader (Acolas *et al.* 2007, Cooke *et al.* 2011). The obtained alphanumeric codes were compared with the initial set of codes at the time of implantation to detect possible reading errors.

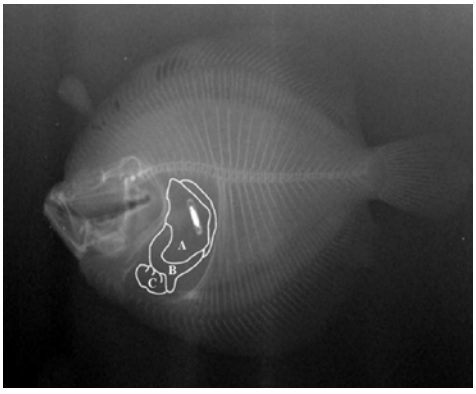

Figure 2
Radiograph of a turbot plus visceral illustration: (A) liver, (B) midgut and (C) rectum

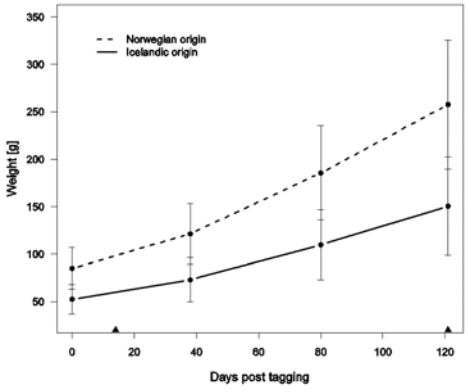

Figure 3
Average weight of the both turbot origins after tag implantation at subsequent dates (●). Dates of the radiographs (▲).

Results and discussion

Individual identification is a key prerequisite in performance testing schemes. This is because the estimation of genetic parameters and breeding values depends on the availability of pedigree and individual performance data of the offspring (Rodríguez-Ramilo *et al.* 2007). Therefore, a simple and reliable individual identification method is required that does not affect animal health, is easy to handle and provides a large number of different identifications. In many species, radio frequency identification systems are an adequate alternative to meet these requirements (Baras *et al.* 2000, Das Mahapatra *et al.* 2001, Navarro *et al.* 2006, Hopko *et al.* 2010). In the present study, we applied PIT tags to almost 6 000 turbots. All animals survived the intraabdominal application process and recovered well from anaesthesia. The mortality within the following four weeks was 0.2 %. This has to be considered as very low, even in fish that have not undergone the procedure. Furthermore, no signs of secondary infections or intraperitoneal inflammation like enlarged abdomen were observed.

The radiographic control of the tag position in 24 fishes on days 14 and 122 post-implantation revealed no substantial variation in tag localization. No changes of the localization were detected radiographically between days 14 and 122 and it can be assumed that the tag takes a constant position in the abdominal cavity within the first two weeks (Figure 4). During the final section, it was observed that all transponders were enclosed in a fibrinoid capsule and thus attached to an intraperitoneal surface. Fibrinoid encapsulation of tags has also been reported in other studies (Baras & Westerloppe 1999, Baras *et al.* 2000, Gheorghiu *et al.* 2010). However, the time required for a complete encapsulation is unknown. Regarding the invariant position between the two radiographic controls (Figure 4), it can be assumed that the transponders become fixed not later than day 14.

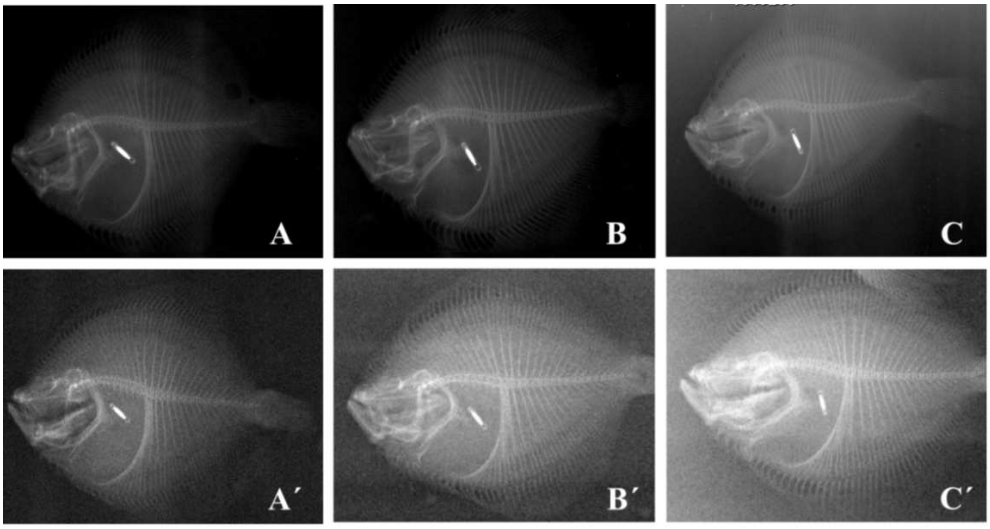

Figure 4
Radiographs of three turbots with passive integrated transponder (PIT) tags. (A), (B) and (C) are the first radiographs 15 days after tag implantation. (A´), (B´) and (C´) are the second radiographs of the same turbot 122 days after tag implantation.

The intraabdominal position of the transponder and the encapsulation status were determined in a total of 800 animals during the final slaughtering process. Four different localizations can be distinguished. The most frequent position found in 672 fish (84.0 %) was between the liver and the intestine close to the spleen (Figure 5A). Eighty-one transponders (10.1 %) were attached to the abdominal cavity wall (Figure 5B), in 39 observations (4.8 %) the tag was found on the liver surface (Figure 5C) and only eight transponders (1.0 %) were positioned lateral to the liver on top of the intestine (Figure 5D).

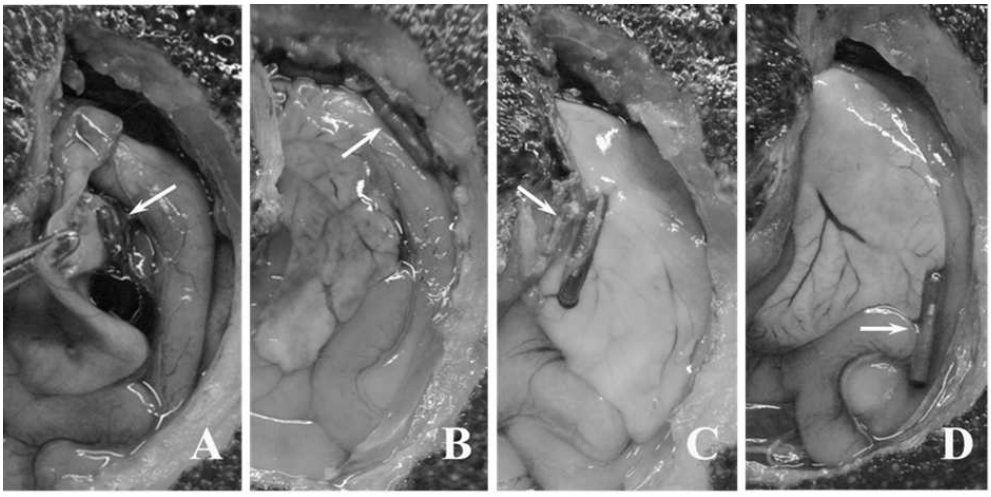

Figure 5
Encapsulated passive integrated transponder (PIT) tags following interperitoneal implantation in turbot: (A) Tag encapsulated between liver and viscera, (B) tag encapsulated on the liver, (C) tag encapsulated on the abdominal cavity wall and (D) tag encapsulated by lateral to the liver on top of the intestine.

No transponder was found outside the abdominal cavity. Regarding the mode of application, the most frequently observed position is also the most plausible one. Those 16.0 % of cases with a different position might result from secondary tag migration, probably due to intestinal peristaltic activity. It can be hypothesized that the tag position is more variable in larger fishes. We did not observe a significant weight difference between groups of animals with different transponder positions.

It could be argued that the intraabdominal tag application might affect the well-being and performance of the fish. The assessment of an animal's well-being is, however, difficult and afflicted by a large subjective error, especially in aquatic species. On the other hand, performance parameters can be objectively used as indicator traits (Cooke *et al.* 2011). Within the current study, the animals were starved for two days following the tagging procedure. The desired feed intake of 1 % of the stock biomass per day was then re-accomplished immediately. The fish grew with 1.2 % per day on average during the time following tag implantation (Figure 3). Considering the feeding regime, this growth performance has to be regarded as normal in turbot (Schram *et al.* 2009). Additionally, the fish were obviously

vital, no signs of diseases were observed and neither ulcers and bleedings nor other signs of intraabdominal inflammation were found within the abdominal cavity during evisceration. These findings are in concordance with several previous studies in other species (Baras *et al.* 2000, Das Mahapatra *et al.* 2001, Acolas *et al.* 2007). Based on these results, it can be concluded that the intraabdominal implantation of PIT tags has no negative effects on animal health and performance in turbot.

During the regular readout of all transponders, no tag losses or failures occurred and no readout errors were observed. Similar results with tag retention rates of 100 % have previously been reported (Baras *et al.* 2000, Navarro *et al.* 2006, Hopko *et al.* 2010). Additionally, the readout procedure in flatfish turned out to be uncomplicated because the area of the abdominal cavity is very small as compared to torpedo-shaped fish where it almost extends along the entire body. Thus, the mobility of the transponder is strongly restricted, what in turn might enhance the effective encapsulation. Furthermore, the borders of the abdominal cavity are defined by bony structures to a larger extent in flatfish than in torpedo-shaped fish. This reduces the probability of tag losses and has also a positive aspect for marketing because the migration of tags into the edible muscle tissue might be a problem when tagging fish that are intended for human consumption (Gheorghiu *et al.* 2010).

In conclusion, the present study is, to our knowledge, the first one investigating intraabdominal implantation of PIT tags in turbot. It was demonstrated that this method is feasible without affecting survival, health and growth performance. However, high costs for PIT tags (≈ 2.5 $ per tag) have to be considered. Passive integrated transponder tags represent an effective tool for individual identification of fish as well as for animals in selective breeding programmes. In view of the results, intraabdominal implantation of PIT tag is a safe and efficient marking method in turbot.

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