

Impact of intraperitoneal and intramuscular PIT tags on survival, growth, and tag retention in juvenile pikeperch, *Sander lucioperca* (L.)

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Marek Hopko, Zdzisław Zakęś, Agata Kowalska, Konrad Partyka

Abstract. The aim of the study was to compare the effects of intraperitoneal (group P) and intramuscular (group M) methods of implanting PIT tags on the growth, condition, and survival of juvenile pikeperch, *Sander lucioperca* (L.) (body weight about 82 g) and tag retention. After tagging, the fish were held in recirculating aquaculture systems (RAS) and reared on commercial feed for 56 days. No significant differences were noted in fish growth rate or condition between the experimental groups (groups P and M) and the control group that was not tagged (group C; $P > 0.05$). The feed conversion ratios were also of similar values (FCR – 1.2-1.3; $P > 0.05$). Tagging did not impact fish survival, which was 98.9% in group P and 100% in groups M and C on the final day of the experiment ($P > 0.05$). Tag retention in both groups was also 100%. The results of this study confirm that both tagging methods are suitable for juvenile pikeperch. The PIT tags were noted to migrate in the body cavity in group P (47.2% of tagged fish). Accordingly, the intramuscular method for implanting PIT tags is recommended for juvenile pikeperch (body weight > 60 g).

Keywords: passive integrated transponders, tagging evaluation, tag effects, percids

Introduction

Tagging is an important research tool that permits tracking fish migration, spawning behavior, growth, and survival. Fish farms also use tagging for selection and rearing, as well as for determining the effectiveness of stocking open waters (Nielsen 1992). Group tagging, such as the application of dyes, branding or freeze-branding, are usually used to analyze stocking effectiveness (growth and survival). Recently, individual tagging methods, including Passive Integrated Transponder (PIT) tags, are being applied more frequently. The indisputable advantages of this method include its practically unlimited period of operation, the vast number of individual code combinations, and its small size (Dębowski et al. 1998, Baras et al. 2000). Unfortunately, PIT tags also have disadvantages. They are still fairly costly, and require a special scanner to identify fish. PIT tags have been and continue to be used for tagging salmonid fish (Dębowski et al. 1998, Dare 2003). Recently, the spectrum of species with which this system is used has been broadened to include other predatory fish (Baras et al. 2000, Cucherousset et al. 2007, Wagner et al. 2007, Hopko et al. 2010). An analysis of the available literature indicates that using this type of tag ensures high retention rates while having little impact on fish mortality, behavior, or growth rates (Baras et al. 2000, Wagner et al. 2007). Negative side effects

M. Hopko, Z. Zakęś [✉], A. Kowalska, K. Partyka
Department of Aquaculture
The Stanisław Sakowicz Inland Fisheries Institute in Olsztyn
Oczapowskiego str. 10, 10-719 Olsztyn-Kortowo, Poland
Tel. +48 89 5241046, e-mail: hopko@infish.com.pl

have been noted, too, such as increased mortality among tagged fish. This occurs primarily when the tagged fish are of a small body size (Dębowski et al. 1998, Baras et al. 2000, Gries and Letcher 2002).

When designing a method for tagging a given species with PIT tags, it is important to determine the minimum fish size that permits effective tag implantation (Baras et al. 2003, Hopko et al. 2010). With sea trout, *Salmo trutta* L., higher mortality and a decidedly negative impact was noted in fish that were under 8 cm in length (Dębowski et al. 1998). Similar results were noted among Atlantic salmon, *Salmo salar* L., that were under 6 cm long (Gries and Letcher 2002). In fish, the PIT tags are usually implanted in the body cavity (intraperitoneal tagging) or in the muscles (intramuscular tagging). Tags are implanted under the first or second dorsal plate in sturgeon spawners, while intramuscular implantation just posterior to the dorsal fin is indicated in European wels, *Silurus glanis* L. (Wunderlich et al. 2007). With juvenile pikeperch, *Sander lucioperca* (L.), and spawners of this species, the intraperitoneal method was determined to be effective (Zakęś 2009, Hopko et al. 2010). Tags implanted into the body cavity can, however, shift position (Baras et al. 2000), and in extreme cases, for example during artificial spawning manipulations, the tags can be expelled along with the spawn (Parker and Rankin 2003, Z. Zakęś, unpublished materials). Thus, it is justifiable to compare PIT tagging methods (intraperitoneal and intramuscular) in pikeperch and to determine the impact the implantation methods (or the location of the tag) have on chosen biological characters of the species.

The aim of the study was to determine the impact the PIT tags implantation method (intraperitoneal or intramuscular) has on the growth rate, condition, survival, feeding effectiveness, and tag retention in juvenile pikeperch.

Materials and methods

The study material was obtained through out-of-season pikeperch spawning conducted at the Department of Sturgeon Fish Breeding, Inland Fisheries Institute in

Olsztyn (IFI Olsztyn) (Zakęś 2007). After the initial rearing period in recirculating aquaculture systems (RAS), during which the material attained a mean body weight of 4–5 g (June 2009), the fish were transported to the Department of Aquaculture IFI Olsztyn in polyethylene bags under the following conditions: the bags were supplied with oxygen ($20 \text{ dm}^3 \text{ water} + 20 \text{ dm}^3 \text{ oxygen}$); transport time – 2 h; water temperature – 20.5°C . The fish were stocked into tanks with a volume of 600 dm^3 , where they were reared under optimal environmental conditions (Zakęś 2009).

The experiment proper began in December 2009. The reared material attained a mean body weight (BW) of 81.9 g and a mean body length (SL) of 18.8 cm. The juvenile pikeperch were stocked into a RAS with nine rearing tanks with cubic volumes of 0.2 m^3 . Thirty fish were stocked into each tank (the mean initial stocking density was 12.29 kg m^{-3}). There were three groups of fish (each one in three replicates): group P (fish tagged intraperitoneally); group M (fish tagged intramuscularly); group C (control group of untagged fish).

Before tagging, the fish were anesthetized in an aqueous solution of etomidate (Propiscin, IFI Olsztyn) at a concentration of $2 \text{ mm}^3 \text{ dm}^{-3}$ (Kazuń and Siwicki 2001). The fish were tagged with PIT tags (Fish Eagle, Lechlade, Great Britain) (material: bio-glass; length: $12.0 \pm 0.4 \text{ mm}$; diameter: $2.12 \pm 0.07 \text{ mm}$; tag weight: 93 mg). The tags were implanted into the fish with a syringe and needle with an internal diameter of 2.86 mm, at an angle of 30° , and a puncture depth of 8–10 mm. The fish from group P were tagged intraperitoneally on the lateral abdominal wall the end of the left abdominal fin (Hopko et al. 2010). However, the fish from group M were tagged intramuscularly. The tags were implanted at the first dorsal fin (2–3 fin ray) halfway between the dorsal fin and the lateral line (Fig. 1). The tags and the applicators were disinfected with 96% ethyl alcohol before each individual was tagged. The fish from group C were only anesthetized.

The fish were returned to the rearing tanks in which they had been held prior to tagging. The water flow rate was maintained at $4.0 \text{ dm}^3 \text{ min}^{-1}$ (1.2 water exchange h^{-1}). Water temperature and oxygen concentration were

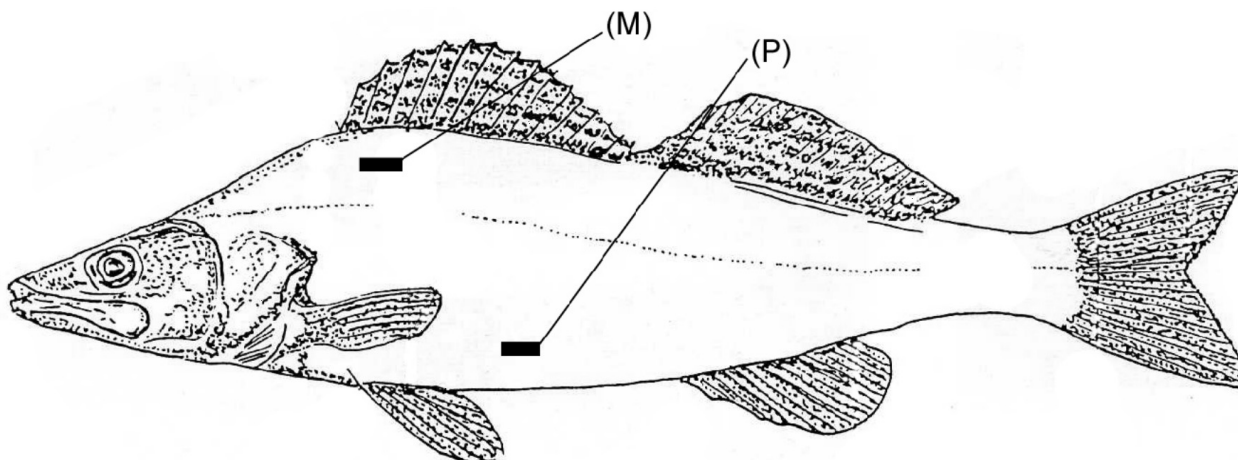


Figure 1. Location of PIT tag implantation in juvenile pikeperch: M – intramuscular implantation, P – intraperitoneal implantation.

monitored daily. Additionally, the contents of total ammonia nitrogen ($\text{TAN} = \text{NH}_4^+ - \text{N} + \text{NH}_3 - \text{N}$), nitrite nitrogen ($\text{NO}_2 - \text{N}$), and pH were monitored weekly. The mean water temperature was 21.9°C (± 0.1). The oxygen concentration at the inlets and outlets did not fall below 7.67 and 5.12 $\text{mg O}_2 \text{ dm}^{-3}$, respectively. The content of ammonia nitrogen and nitrite nitrogen, measured at the tank inlets and outlets ranged from 0.108–0.118 mg TAN dm^{-3} ; 0.114–0.167 mg TAN dm^{-3} ; 0.002–0.016 $\text{mg NO}_2 - \text{N dm}^{-3}$; 0.002–0.019 $\text{mg NO}_2 - \text{N dm}^{-3}$, respectively. However, the water pH ranged from 7.99 to 8.30. A 24 h light photo-period was applied, and the light intensity measured at the water surface of the rearing tanks was 1–2 lx.

The fish were fed E-1P Stella feed (Skretting, Norway). According to the manufacturer, the feed composition was as follows: protein – 47.0%; fat – 14.0%; carbohydrates – 21.0%; granule diameter – 2.5 mm. The digestible energy of the feed was 18.5 MJ kg^{-1} . The feed was delivered continually for 19 h d^{-1} using 4305 FIAP automatic band feeders (Fishtechnik GmbH, Germany). The daily feed ration was determined based on the mean stock biomass. The ration was adjusted weekly throughout the rearing period and ranged from 1.0% of the stock biomass (beginning of rearing) to 0.8% of the stock biomass (final period of rearing).

Individual measurements of fish body length (SL; ± 1.0 mm) and body weight (BW; ± 0.1 g) were taken at the beginning of the experiment (d0), after 28 days of rearing (d28), and at the end of the experiment on day 56 (d56). On days 28 and 56 of the experiment, the presence of the tags was checked with a hand-held scanner (Fish Eagle, Lechlade, Great Britain). The tanks were monitored daily for feed consumption, expelled tags, fish behavior, and mortality. The biomass of the fish and their wound condition following PIT tag implantation were also determined weekly. Additionally, the weight of the viscera (liver, gastrointestinal tract, visceral fat; W_i ; ± 0.01 g) was determined on the final day of the experiment using the viscera obtained from 15 fish from each tank. The location of the tags in the fish was determined, and any pathological changes resulting from the tag implantation procedure were identified. The fish were anesthetized with an overdose of anesthetic (3–4 mm^3 Propiscin dm^{-3}), and then they were headed. The following were determined based on the results obtained:

- Fulton's condition coefficient; $F = 100 \times (\text{BW} \times \text{SL}^{-3})$
- daily growth rate; $\text{DGR} (\text{g d}^{-1}) = (\text{BW}_2 - \text{BW}_1) \times \Delta t^{-1}$
- specific growth rate; $\text{SGR} (\% \text{ d}^{-1}) = 100 \times (\ln \text{BW}_2 - \ln \text{BW}_1) \times \Delta t^{-1}$

- viscerosomatic index; $VSI (\%) = 100 \times (W_t \times BW^{-1})$
- feed conversion ratio; $FCR = TFS \times (FB - IB)^{-1}$
- survival; $S (\%) = 100 \times \text{final number of fish (indiv.)} \times \text{initial number of fish}^{-1} (\text{indiv.})$
- tag retention, $R (\%) = 100 \times \text{number of tags confirmed in the fish on the final day of the experiment (no.)} \times \text{the number of tags implanted on the first day of the experiment}^{-1} (\text{no.})$

where:

BW_1 – initial fish body weight (g), BW_2 – final fish body weight (g), W_t – viscera weight without gonads (g), Δt – rearing period (days), SL – fish body length (cm), FB – final stock biomass (g), IB – initial stock biomass (g), TFS – total feed supply (g).

Single factor analysis of variance (ANOVA) with repetitions was used to analyze the results of the experiment. When statistically significant differences were noted among groups ($P \leq 0.05$), Tukey's test (Statistica-StatSoft Poland, Kraków). The *arc sin* function was used to transform the percentage data.

Results

No significant differences in fish weight increases were noted among groups. This was true of both the absolute and relative measures of this parameter (DGR and SGR) noted on day 28 of rearing (d28) and on the final day of the experiment (d56) ($P > 0.05$; Table 1). The condition coefficient and the viscerosomatic index (VSI) of the fish with PIT tags (groups P and M) did not differ significantly from the values of these indexes calculated for the control group of fish. No significant differences among groups were noted with regard to FCR, and during the 56-day experiment the values of this coefficient ranged from 1.2 to 1.3 ($P > 0.05$; Table 1).

Fish survival was high and ranged from 98.9 to 100% ($P > 0.05$; Table 1), and PIT tag retention in both of the tagged groups was 100%. In group P the tags remained at the implant location in 52.8% of the fish (in the visceral fat). In 47.2% of the fish, the tags had shifted to the posterior part of the body cavity.

Neither of the tagged fish groups exhibited signs of body cavity (group P) or muscle infection (group M) that could have arisen from the tagging operation.

Discussion

PIT tag retention and survival in juvenile pikeperch was very high. A review of the literature focusing on the effectiveness of this type of tagging indicates that these tags are tolerated well by various species. Tag retention usually ranges from 85 to 100%, while mortality among tagged fish does not exceed a few percent (Baras et al. 2000, Dare 2003, Parker and Rankin 2003, Navarro et al. 2006). Studies of the impact PIT tags have on fish usually focus on the dependence of tag retention on fish size and the survival of tagged individuals. One of the principle aims of these types of studies was to determine the minimal size limit of the fish that guarantees the results of the procedure will be acceptable (see Baras et al. 2000, Navarro et al. 2006). The effect of the location of the implanted PIT tag and its impact on the parameters mentioned previously was studied slightly less frequently (Parker and Rankin 2003, Wagner et al. 2007). The tag implantation site did not have a significant impact on either tag retention or fish survival in juvenile pikeperch. Wagner et al. (2007) came to similar conclusions in a study of muskellunge, *Esox masquinongy* Mitchill. It should also be emphasized that in both of these studies the pikeperch and muskellunge tagged were older juveniles (weighing between several tens of grams to several hundred grams). In a tagging study of juvenile gilthead seabream, *Sparus auratus* L., with a mean body weight of 3.5 g, Navarro et al. (2007) confirmed that the percentage of fish that expelled tags was significantly higher in individuals which had been tagged intramuscularly than those which had been tagged intraperitoneally at 40 and 14%, respectively. It appears, thus, that in fish this size it is justifiable to apply intraperitoneal PIT tag implantation. The muscle mass of fish in this stage of development (body weight < 5 g) is probably too low to ensure the retention of tags of this size.

Table 1

Growth, condition, survival, and PIT tag retention in different groups of juvenile pikeperch (C – control group of untagged fish, P – fish tagged intraperitoneally, M – fish tagged intramuscularly) at different stages of rearing (d0 – beginning of rearing, d28 – day 28 of rearing, d56- day 56 of rearing) (mean values \pm SD; N = 3). No significant differences were noted among groups ($P > 0.05$)

Parameter/day of rearing	Group C	Group P	Group M
Body weight – BW (g)			
d0	81.75 \pm 0.44	81.89 \pm 0.54	81.98 \pm 1.95
d28	102.03 \pm 3.52	102.63 \pm 2.24	103.19 \pm 2.67
d56	119.79 \pm 7.00	120.20 \pm 3.38	121.58 \pm 2.74
Body length – SL (cm)			
d0	18.67 \pm 0.04	18.93 \pm 0.03	18.93 \pm 0.21
d28	20.14 \pm 0.13	20.32 \pm 0.11	20.38 \pm 0.32
d56	21.45 \pm 0.27	21.72 \pm 0.22	21.73 \pm 0.18
Daily growth rate – DGR (g d ⁻¹)			
d0-d28	0.72 \pm 0.11	0.74 \pm 0.09	0.76 \pm 0.05
d29-d56	0.63 \pm 0.12	0.63 \pm 0.07	0.66 \pm 0.02
d0-d56	0.68 \pm 0.12	0.68 \pm 0.07	0.71 \pm 0.03
Specific growth rate – SGR (% d ⁻¹)			
d0-d28	0.79 \pm 0.11	0.81 \pm 0.09	0.82 \pm 0.04
d29-d56	0.57 \pm 0.08	0.56 \pm 0.05	0.59 \pm 0.02
d0-d56	0.68 \pm 0.09	0.68 \pm 0.06	0.70 \pm 0.03
Condition coefficient – F			
d0	1.24 \pm 0.02	1.20 \pm 0.01	1.20 \pm 0.01
d28	1.22 \pm 0.05	1.20 \pm 0.02	1.19 \pm 0.03
d56	1.15 \pm 0.04	1.12 \pm 0.00	1.13 \pm 0.02
Viscerosomatic index – VSI (%)	6.24 \pm 0.22	6.13 \pm 0.22	6.02 \pm 0.36
Biomass increases (g)			
d0-d28	608.5 \pm 94.43	588.7 \pm 115.08	636.3 \pm 39.41
d29-d56	532.6 \pm 104.65	520.7 \pm 51.80	551.8 \pm 19.87
d0-d56	1141.1 \pm 199.03	1109.4 \pm 138.40	1188.1 \pm 54.99
Feed conversion ratio – FCR			
d0-d28	1.1 \pm 0.15	1.2 \pm 0.20	1.1 \pm 0.10
d29-d56	1.4 \pm 0.29	1.4 \pm 0.17	1.4 \pm 0.06
d0-d56	1.3 \pm 0.23	1.3 \pm 0.17	1.2 \pm 0.06
Survival – S (%)			
d0-d28	100	98.9	100
d29-d56	100	100	100
d0-d56	100	98.9	100
Tag retention – R (%)	-	100	100

When intraperitoneal tagging is applied, the tags can migrate within the body cavity. In the current study, PIT tag migration was noted in 47.2% of the pikeperch individuals from group P. Symptomatically, this was not observed in previous studies

of juvenile pikeperch, and the PIT tags were found in the visceral fat at the original implantation site (Hopko et al. 2010). The phenomenon of tag migration might have a significant impact on their retention. In some instances, there is also a risk of

abdominal wall perforation. It is also possible that the tag perforates the intestine and is expelled along with the feces (Baras and Westerloppe 1999). Examinations of the body cavities of pikeperch from group P conducted 56 days after tagging did not confirm any symptoms of the perforation of internal organs by the PIT tags. When PIT tags are implanted intramuscularly, the sudden contraction of muscle fibers during flight responses or attacks, can force the tag out of its place of implantation. According to Navarro et al. (2006), the tag expulsion phenomenon in fish tagged intramuscularly is linked to the size of the fish. It is mainly observed in cases when early juvenile stages are tagged (at body weights of several grams), while no tag loss is noted in larger fish (body weight of about 200 g). In the current study, PIT tag retention of those implanted intramuscularly was also 100%.

In the current study, a syringe with a needle was used to implant the tags. Studies by Navarro et al. (2006) indicated that in gilthead seabream this method of implantation can be used with fish weighing > 5 g. Tagging smaller individuals lowered tag retention and fish survival. The negative side effects of this method were also confirmed in perch, *Perca fluviatilis* L., with body weights of about 5 g. Mortality in the group in which implantation was performed with a syringe and needle was 40%, while in other groups in which the tag was implanted into the body cavity through a 3 mm incision in the abdominal wall made with a surgical scalpel was significantly lower and was close to the control group that was not tagged. The PIT implantation method with a needle is fairly invasive. Even with larger specimens, this must be performed with great precision so as not to damage any internal organs and lead to increased mortality. Mortality among fish that were tagged intraperitoneally was observed primarily in the first week, and sometimes into the second week, following tagging (Baras et al. 2000, Dare 2003, Navarro et al. 2006). In the current study, fish deaths were related to the mechanical damage of the specimen and was not the result of the tagging procedure.

The PIT tag implantation method had an impact on the rate the wounds healed. After 14 days, the wounds from intramuscular implantation were completely healed. In turn, the wounds left after intraperitoneal implantation healed more slowly, and about 20% of the individuals were not fully healed after three weeks. The current results correspond to those obtained for juvenile gilthead seabream by Navarro et al. (2006), who reported that the mean healing period after the implantation of PIT tags was 20 days. The first specimens with fully healed wounds were noted 15 days following tag implantation, and the last were noted 26 days following implantation.

Usually, no adverse effects on growth rate are noted following PIT implantation (Baras et al. 2000, Navarro et al. 2006, Wagner et al. 2007). In the current study, the implantation site was not noted to have an impact on either fish growth rate or condition indexes, which were similar to the values noted in the control group. A certain slowing of the growth rate of the fish might be noted in the first week following tagging; however, in the following weeks this is compensated for and growth does not differ between the tagged and untagged fish (Baras et al. 2000, Navarro et al. 2006). In the current study, indirect, individual measurements of fish were taken 28 days after they had been tagged. After this period, the negative effects of tagging on the growth of the fish might no longer be apparent (Baras et al. 2000). It should be emphasized that tagging was not noted to affect the intensity or effectiveness of pikeperch feeding. The FCR coefficient in the groups of tagged fish had similar values to those noted in the control group.

The labor intensity of the implantation method also has an impact on its effectiveness. The time required to implant the tags either intramuscularly or intraperitoneally during the current study was similar at about twenty seconds (not including anesthetizing the fish). Tagging fish using a syringe and needle is decidedly less labor intensive than making a surgical incision and implanting the PIT tag into the fish's body (Baras et al. 2000). It should also be taken into consideration that using anesthetic can sometimes be difficult, especially in the field, and it can lengthen the time

required to tag a significant number of fish. In this situation, intramuscular implantation might be safer for the fish. When PIT tags are implanted into the body cavities of non-anesthetized fish, there is a greater risk of damaging internal organs. It is also worth noting that using anesthetic or not during tagging did not have a significant impact on the PIT tag retention index in the study by Parker and Rankin (2003).

In summation, either the intraperitoneal or intramuscular PIT tag implantation method can be used with juvenile pikeperch. These methods guarantee high tag retention, and neither has a negative impact on the growth or survival of the fish. However, because the PIT tags that were implanted intraperitoneally were more difficult to identify (they migrate within the body cavity), and the wounds from this type of implantation took longer to heal, it is recommended to use the intramuscular method for implanting PIT tags. This method of implantation should find wider applications in selection and breeding activities, such as tagging pikeperch spawners. This method appears to be safe to use in the field if the fish are tagged without anesthetic. However, before drawing definitive conclusions, wider studies should be performed, especially on smaller fish.

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Streszczenie

Wpływ dootrzewnowej i domięśniowej implantacji znaczków PIT na przeżywalność, wzrost i trwałość oznakowania młodocianego sandacza, *Sander lucioperca* (L.)

W ostatnich latach coraz większą popularność zdobywa metoda indywidualnego znakowania ryb pasywnymi transponderami, tzw. znaczkami PIT (Passive Integrated Transponder). Dobór właściwego systemu znakowania ryb jest zależny od gatunku, stadium rozwoju osobniczego, sposobu i miejsca implantacji w ciele oraz specyficznych celów danego programu badawczego. Celem prezentowanych badań było porównanie efektów stosowania dootrzewnowej (grupa P) i domięśniowej (grupa M) metody implantacji znaczków PIT (rys. 1) na tempo wzrostu, kondycję, przeżywalność młodocianego sandacza, *Sander lucioperca* (L.) (masa ciała ok. 82 g) i retencję znaczków. Po poznakowaniu ryby przetrzymywano w obiegach recyrkulacyjnych i podchowivano na paszy sztucznej przez 56 dni. Nie odnotowano istotnych różnic w tempie wzrostu i kondycji ryb z grup doświadczalnych (grupy P i M) względem,

niepoznakowanej grupy kontrolnej (grupa C; $P > 0,05$; tab.1). Współczynniki pokarmowe pasz przyjęły również zbliżone wartości (FCR – 1,2-1,3; $P > 0,05$). Znakowanie nie wpłynęło na przeżywalność ryb, która w dniu zakończenia podchowu wynosiła 98,9% (grupa P) i 100% (grupa M i C) ($P > 0,05$; tab. 1). Retencja znaczków w obydwu grupach była równa 100%. Uzyskane wyniki potwierdziły, iż w przypadku młodocianego sandacza dobrze sprawdzają się obie analizowane metody znakowania. W grupie P zaobserwowano zjawisko przemieszczenia się PIT w jamie ciała (47,2% znakowanych ryb). Potencjalnie może to utrudniać identyfikację/odczyt znaczków. W związku z tym wydaje się, że w przypadku juwenalnego sandacza (masa ciała > 60 g) należy raczej rekomendować metodę domięśniowej implantacji znaczków PIT.