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RESEARCH ARTICLE

Effect of visible implant elastomers (VIE) and coded wire tags (CWT) on the growth, survival, and tag retention of juvenile European whitefish (*Coregonus lavaretus*)

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Abstract. This study examined the effect of visual implant elastomers (VIE) and coded wire tags (CWT) on the growth, survival, and tag retention of juvenile European whitefish, *Coregonus lavaretus* (L.), (mean body weight 18.7 ± 0.4 g, mean body length 9.7 ± 0.4 cm). The VIE tags were implanted subcutaneously in the anterior head, and the CWTs were implanted intramuscularly under the left gill operculum. The experiment consisted of two stages. The first stage (70 days) examined the effects of tagging on growth and survival, while the second stage (days 71-140) focused on assessing tag retention. Daily and specific growth rates, feed conversion factor, condition factor, coefficient of body weight variation, and survival did not differ significantly between the control fish and those tagged with the VIE tags and CWTs. Tag retention was high in the fish tagged with both VIE tags (100%) and CWTs (93%).

Keywords: tagging, rearing, recirculating aquaculture system

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Introduction

Whitefish, *Coregonus lavaretus* (L.), belongs to the family *Coregonidae* that occurs in the cold, clean waters of northern Europe, Asia, and North America (Elliott 2008, Gomułka et al. 2014). They are valuable to commercial and recreational fisheries for their meat and roe (Aronsuu and Huhmarniemi 2004, Mills et al. 2004, Tournay 2006). However, studies using commercial yield data have shown a declining trend in catch abundance (Mickiewicz and Trella 2015, Wołos et al. 2016). This decline has been hypothesized to be a function of lake degradation in spawning grounds and hybridization (Łuczyński et al. 1992, Winfield and Durie 2004, Falkowski and Wołos 2007, Thomas and Eckmann 2007). Subsequently, whitefish has become a threatened species, and it has been included on the IUCN Red List (Freyhof and Kottelat 2008). To improve this situation, stocking is conducted with material reared in recirculating aquaculture systems (RAS) (Szczepkowski et al. 2010, Wunderlich et al. 2011). Knowledge of its effectiveness, however, is relatively scarce, which precludes using the most appropriate forms of stocking material. Assessing the effects of stocking requires tagging the material that is released.

Among many tagging methods, visible implant elastomers (VIE) and coded wire tags (CWT) are very important (Brennan et al. 2007, Simon and Dörner 2011, Szczepkowski et al. 2015, Zhu et al. 2016). Short-term evaluations (< 180 d) of VIE tags as a batch mark for juvenile salmonids have revealed high retention, high visibility, and minimal effects on growth and survival in rainbow trout, *Oncorhynchus mykiss* (Walbaum), (Hale and Gray 1998, Walsh and Winkelman 2004), brown trout, *Salmo trutta* L., (Hale and Gray 1998, Olsen and Vøllestad 2001), bull trout, *Salvelinus confluentus* (Suckley), and cutthroat trout *Oncorhynchus clarkii* (Richardson), (Bonneau et al. 1995). They are easy to use, and they can be used for external identification in different locations on the body and in different implant colors (Willis and Babcock 1998, Curtis 2006, Brennan et al. 2007, Imbert et al. 2007, Kozłowski et al. 2017). A CWT is a stainless steel wire 0.25 mm in diameter that is permanently magnetized and has a special code for a given series of tags. Decreased growth rates and increased mortality were noted with CWT fish tagging. This is only observed for a few weeks after tagging (Vander Haegen et al. 2005, Zakęś et al. 2015). CWTs are simple to administer and have been applied for mass tagging several species of salmonid fish (Blankenship 1990, Weitkamp and Neely 2002), including whitefish.

Before using any tagging method on a wider scale, it is appropriate to examine its impact on the growth, survival, and tag retention in a given species. The objective of the study was to determine the effect of the VIE and CWT tagging methods in juvenile whitefish reared in RAS for stocking material. The visibility of VIE tags was also assessed in natural light and in the dark using a dedicated UV flashlight.

Materials and methods

The research material was whitefish fry obtained through artificial spawning and initial rearing at the Department of Sturgeon Fish Breeding in Pieczarki, Inland Fisheries Institute in Olsztyn. The fish were trained to consume feed during the initial rearing stage of three months. The experiment was conducted in a RAS with a volume of 7 m^3 equipped with a SDK CN 3.2 biofilter (SDK, Poland) filled with synthetic Light Bioelementer with a total volume of 1.5 m^3 (RK Plast A/S, Denmark). The fish were kept in plastic tanks with a volume of 1 m^3. After initial rearing, the fish were moved to another RAS where the experiment was conducted. The RAS was equipped with an identical biofilter, and the tanks had a volume of 2.0 m^3 and a total volume of 32 m^3.

The sample of 900 fish with an average body weight of 18.7 ± 0.4 g and a body length of 9.7 ± 0.4 cm (mean ± standard deviation) was selected for the experiment. The fish were marked with visible implant elastomers (VIE) and coded wire tags (CWT) (Northwest Marine Technology, USA). The control group comprised untagged fish. One hundred specimens from each group (V, C, Control) were placed in separate tanks (n = 3, 100 fish per tank). Three replicates were performed for each of the variants analyzed (3 groups × 3 repetitions = 9 tanks). Visible implant elastomers made of a liquid polymer with a curing agent were injected subcutaneously with 0.3 ml syringes. Coded wire tags were applied with a manual applicator (Handheld Multishot Tag Injector, NMT, USA). VIE tags were implanted in the anterior head, and CWTs in the muscle of the left gill operculum. The experiment was conducted in two stages. The first stage (70 days) examined the effects of tagging on fish growth and survival. After its completion, fish from the different replications of each variant were combined (3 groups: VIE, CWT, Control) and reared for the following 70 days (stage II) to assess tag retention (over the course of 140 days).

During rearing, water temperature was monitored daily. Oxygen content and water pH were measured at the tank water outflows with a Cyber Scan 5500 (Eutech Instruments, USA). Total ammonia nitrogen was determined by the Nesslerization method and nitrites were determined with the sulphanilic method using a spectrophotometr at the tank water outflows. The mean water temperature in stage I was 17.0 ± 1.5°C and in stage II it was 15.2 ± 0.4°C.

Oxygen concentration at the tank outflow did not decrease below 5.8 mg O_2 l^{-1}, and water pH ranged from 6.6 to 6.9. The maximum ammonia nitrogen content did not exceed 0.1 mg CAA l^{-1}, and nitrites did not exceed 0.04 mg NO_2- l^{-1}. A constant water flow rate of 12 l min^{-1} was maintained throughout the rearing period.

An automatic band feeder (FIAP, Fischtechnik Gmbh, Germany) was used to feed the fish Aller Performa Ex, 4 gr. (Aller-Aqua, Denmark) that contained 54% protein, 15% fat, and 13.6% carbohydrates (NFE). The granule size ranged from 1.6 to 2.4 mm. The digestible energy of the feed was 19.5 MJ kg^{-1}. The daily feed ration in experiment I was 2% of the fish biomass, and in experiment II it was reduced to 1% of fish biomass. Every morning, the tanks were cleaned of feces and unconsumed feed. The fish were measured every 14 days by removing 15 specimens at random from each tank to determine weight (0.1 g) and body length (1 mm). After the measurements, all the fish from each tank were counted to determine stock survival and tag retention. The fish were measured after being anesthetized in a Propiscin solution (Kazuń and Siwicki 2001) at a dose of 0.7 ml l^{-1}. VIE tags were read with a dedicated UV flashlight, and CWTs were read with a manual scanner (Handheld Wand Detector, NMT, USA). VIE visibility was also assessed during the measurements according to the visibility scale by Simon and Dörner (2011).

The data collected were used to calculate the values of the following indicators:

daily growth rate – DGR = $(BW_f - BW_i)\ t^{-1}$; specific growth rate – SGR = $100\ (\ln BW_f - \ln BW_i)\ t^{-1}$; feed conversion ratio – FCR = $TFC\ (FB - IB)^{-1}$; condition factor F = $100\ BW_m\ TL^{-3}$; body weight variation coefficient V = $100\ (SD\ BW^{-1})$; survival – S = $100\ (FN\ IN^{-1})$; tag retention R = $100\ (NFT\ FN^{-1})$, where: BW_i and BW_f – initial and final body weight (g); BW – body weight (g); BW_m – mean body weight (g); t – experiment duration (days); TFC – total feed consumption (g); SD – body weight standard deviation; IB and FB – initial and final fish biomass (g); IN and FN – initial and final number (ind.); NFT – number of fish with tags on the final day of the experiment (ind.).

The results are presented as means ± standard deviation (SD). Statistical differences in stages I and II of the experiment were analysed with single variant analysis (ANOVA). Homogeneity of variance was verified with Levene's test. The post-hoc Tukey's test was applied ($P \leq 0.05$) to determine whether differences among groups were statistically significant. Statistical calculations were performed with STATISTICA 12 PL (StatSoft Polska).

Results

During the experiment, VIE and CWT tagging had no significant effect on the results of rearing juvenile whitefish (Table 1). The daily and specific growth rates, feed conversion factor, condition factor,

Table 1

Results of rearing whitefish in the first stage of the experiment (K – control group, group V – fish tagged with VIE tags, group C – fish tagged with CWTs, mean values ± SD, N=3)

Parameter	Group K	Group V	Group C
Body weight (BW, g)	78.4 ± 2.6[a]	78.7 ± 2.1[a]	79.2 ± 2.6[a]
Total length (TL, cm)	19.6 ± 0.2[a]	19.5 ± 0.2[a]	19.7 ± 0.2[a]
Standard length (SL, cm)	17.2 ± 0.2[a]	17.1 ± 0.2[a]	17.2 ± 0.3[a]
Daily growth rate (DGR, g d^{-1})	0.86 ± 0.04[a]	0.85 ± 0.03[a]	0.86 ± 0.04[a]
Specific growth rate (SGR, % d^{-1})	2.07 ± 0.09[a]	2.05 ± 0.05[a]	2.04 ± 0.04[a]
Feed conversion ratio (FCR)	0.75 ± 0.03[a]	0.76 ± 0.03[a]	0.76 ± 0.03[a]
Condition factor (CF)	1.54 ± 0.02[a]	1.58 ± 0.02[a]	1.54 ± 0.04[a]
Body weight variation coefficient (V, %)	11.9 ± 0.9[a]	13.4 ± 3.3[a]	14.9 ± 4.3[a]
Survival (S, %)	97.3 ± 1.2[a]	96.3 ± 0.6[a]	95.7 ± 1.2[a]
Tag retention (R, %)	-	100.0 ± 0.0[a]	94.4 ± 4.3[a]

Groups marked with the same letter index in the same row do not differ statistically significantly (P > 0,05)

Table 2
Tag retention of visible implant elastomers and coded wire tags of juvenile whitefish (from day 70 the fish from individual replications in each variant were combined – for details see the Materials and methods section)

	Marking VIE			Marking CWT	
Time (days)	0*	1*	2*	Present	Absent
Stage I of the experiment					
14	0.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0
28	0.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0
42	0.0 ± 0.0	95.2 ± 2.1	4.8 ± 2.1	97.6 ± 2.2	2.4 ± 2.2
56	0.0 ± 0.0	90.6 ± 3.8	9.4 ± 3.8	95.5 ± 3.2	4.5 ± 3.2
70	0.0 ± 0.0	85.5 ± 0.1	14.5 ± 0.7	94.4 ± 4.3	5.6 ± 4.3
Stage II of the experiment					
84	0.0	85.4	14.6	93.3	6.7
98	0.0	85.3	14.7	93.2	6.8
112	0.0	83.7	16.3	93.2	6.8
126	0.0	83.0	17.0	93.1	6.9
140	0.0	81.2	18.8	93.1	6.9

0 – undetected by naked eye; 1 – visible to naked eye; 2* – visible using a UV flashlight

coefficient of body weight variation, and survival did not differ significantly between the VIE and CWT tagged and untagged fish ($P \leq 0.05$). Tag retention in the fish was high for both the VIE tags (100%) and CWTs (93%).

Until day 28 of rearing, VIE tags were visible to the naked eye, and CWT retention was 100% (Table 2). After this period, VIE tag visibility decreased, and some CWTs were lost. The gradual loss of CWTs occurred until day 84 of rearing, after which the remaining CWTs were retained until the end of the experiment.

Discussion

The experiment results indicate that tagging with VIE tags and CWTs had no effect on juvenile whitefish growth rates. The rearing indicator values were similar to those of Wunderlich et al. (2011) during intensive rearing of the same species in RAS. Favorable FCR values (0.76) indicated that tagging with VIE tags and CWTs did not adversely affect whitefish growth or condition. Differences in growth rates between untagged and tagged fish were also not noted in other species such as *Lutjanus campechanus* (Poey) (Brennan et al. 2007) or *Schizopygopsis younghusbandi* Regan (Zhu et al. 2016).

The tagging methods tested were also not found to have had an affect on whitefish survival, which was in the range of 95-97%. VIE tags are usually considered safe (Astorga et al. 2005, Simon 2007, Simon and Dörner 2011), but in some cases increased mortality is reported in smaller fish (body weight < 1 g) (Soula et al. 2012). No increased mortality of fish tagged with CWTs is noted (Brennan et al. 2005, Dőrner et al. 2006, Simon and Dörner 2011).

VIE tag and CWT retention in whitefish during the study period of 140 days was high. This species is considered to be delicate and difficult to manipulate, and this includes performing tagging procedures (Zakęś et al. 2017). High tagging efficiency using the methods analyzed is also confirmed by observations of other fish species. VIE tag retention in largemouth bass, *Micropterus salmoides* (Lacepde) (Catalano et al. 2001), European eel, *Anguilla anguilla* (L.) (Imbert et al. 2007), and rainbow trout, *O. mykiss* (Leblance and Noakes 2012), exceeded 80%, and CWT retention in common snook, *Centropomus undecimalis* (Bloch) (Brennan et. al. 2005), perch, *Perca fluviatilis* L. (Dörner et al. 2006), and European eel (Simon and Dörner 2011) was over 90%.

In the case of VIE tags, short-term (< 180 days) retention is usually very high (Bonneau et al. 1995, Hale and Gray 1998, Olsen et al. 2004), but after this period retention and visibility can decrease considerably (Close and Jones 2002, FitzGerald et al. 2004, Brennan et al. 2005). Tag retention depends on the technique and experience of the person tagging (Elrod and Schneider 1986, Champigneulle et al. 1987), the tag insertion site (Hale and Gray 1998), and the conditions in which the fish are kept (Guy et al. 1996). CWTs generally have a high retention rate (Brennan et al. 2007, Zhu et al. 2016), and they do not affect fish growth, survival, or condition (Blankenship 1990, Barnes 1994).

The results of the present experiment suggest that VIE tag retention in whitefish is largely determined by how the tags are read. When they were read with a UV flashlight, retention was 100%, but when UV light was not used, it decreased to 81.2% on the final (140th) day of the experiment. Similar results were obtained by FitzGerald et al. (2004) when rearing Atlantic salmon, *Salmo salar* L.; 17 months after tagging, VIE tag retention was 92%, and after 28 months retention decreased to 52.2% in daylight, but it increased to 87.8% under UV light. According to Josephson and Robinson (2008), the visibility of VIE tags is largely determined by ambient light conditions rather than other potential factors such as growth rate, feeding, or rearing environment. The authors suggest that ambient sources of light (fluorescence and solar light) reduce the visibility of VIE tags. Close and Jones (2002) provided evidence that VIE tags in rainbow trout, *O. mykiss*, were more visible when they were analysed in the dark (under a blanket or a shroud). These findings concur with the VIE tag research of other authors (Willis and Babcock 1998, Olsen et al. 2004).

CWT loss during the experiment occured only 28 days after tagging and lasted until day 84 of rearing. Tag loss in this period is difficult to explain since CWTs are usually lost during the first month after tagging (Blankenship 1990, Guy et al. 1996, Hale and Gray 1998, Thomassen et al. 2000, Simon and Dörner 2011). Tag loss is observed after a longer period only in very small salmonid fish (< 1 g) (Blankenship 1990). Kolari and Hirvonen (2006) published similar findings on Arctic charr, *Salvelinus alpinus* (L.), where CWT loss was noted as long as 173 days after tagging. These authors also failed to determine why tag loss occurred over such a long period of time.

VIE tag and CWT retention is highly dependent on tagging location (Hale and Gray 1998, Brennan et al. 2005). In the present experiment, CWTs were implanted in the muscle of the left gill operculum, because this muscle is well developed in whitefish. Moreover, this tagging location is also used successfully in other fish species (Brennan et al. 2007, Szczepkowski et al. 2015, Zakęś et al. 2015). VIE tags implanted in this location were not visible to the naked eye immediately after the implantation procedure. This was probably related to the strong pigmentation of the cheek muscle, which is why we had to change the implantation location. The better tagging location that we finally applied in the experiment was the anterior head, which caused no problems with either tag implantation or visibility immediately after the procedure. The results of the experiment suggest that the two tagging locations (anterior head for VIE tags and the cheek muscle of the gill operculum for CWTs) are suitable for tagging whitefish above a body weight of 18.7 g. Damage to internal organs during implantation is highly improbable in these locations.

The effectiveness of this tagging method depends on many factors such as the duration of the procedure, the experience of the person tagging, the tagging location, the size of the fish, and the use of an anaesthetic. The procedure in the current experiment was performed by a team of two people (one tagging and one manipulating the fish and applying anesthesia) who were experienced in tagging with VIE tags and CWTs in pike, *Esox lucius* L., (Szczepkowski et al. 2015), pikeperch, *Sander lucioperca* (L.), (Zakęś et al. 2015), and Atlantic sturgeon, *Acipenser oxyrinchus* Mitchill (Kozłowski et al. 2017). In this experiment, the time required to implant VIE tags was 350 fish h^{-1} and for CWTs it was 450 fish h^{-1}. Our tagging rate was similar to that described by other researchers (Dewey and Zigler

1996, Bailey et al. 1998, Astorga et al. 2005, Brennan et al. 2005, 2007, Zakęś et al. 2015).

When tagging with elastomers, the color of the tag is important. In our experiment we used orange, because it is one of the most visible VIE tag colors (Szczepkowski et al. 2012, Kozłowski et al. 2017). Other researchers drew similar conclusions concerning VIE colors in their papers concerning various fish species (Willis and Babcock 1998, Brennan et al. 2007, Imbert et al. 2007).

Our study provides evidenced that tagging whitefish with body weights of more than 18 g with VIE tags and CWTs has no negative effect on growth or survival. The tag implantation location proved suitable for tag retention at a high level (> 90%) over a period of 140 days of rearing, and it can be recommended for tagging this species. During the experiment, it was also noted that the whitefish cheek muscle is highly pigmented and VIE tags are not visible in this location. It would also be advisable to conduct research on smaller sized material, which would permit determining the minimum whitefish size that can be tagged effectively and safely.

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Author contributions. M.K. designed the experiments; M.K., M.S., I.P., B.S. performed the experiments; M.K., M.S. analyzed the data; M.K wrote the paper.

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