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RESEARCH PAPER

Marking of Burbot (*Lota Lota* L.) with Fluorescent Dyes Using the *Per Os* Method

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Abstract

The aim of this study was to investigate the effectiveness of the *per os* marking method of otoliths in burbot at different early life stages. In experiment E1 burbot larvae 12 (DPH) and in E2 - 22 (DPH) were fed with live *Artemia salina* nauplii immersed in alizarin red S (ARS) and tetracycline hydrochloride (TC), for 1, 3, or 6 subsequent days (in E1) and for 1, 4, or 8 subsequent days (E2). In experiment E3 the marking procedure involved feeding burbot juveniles (92 DPH) using a commercial feed supplemented with ARS at a doses of 0 to 100 g per kg of feed. In E1, the highest percentage of marked otoliths was recorded in the group fed for 6 days with *A. salina* immersed in TC. During E2, 100% of marked otoliths were recorded in the groups fed for 4 or 8 days, regardless of the type of dye used. The highest percentage of marked otoliths in E3 was noted in the group fed with 40 g ARS/kg feed. In conclusion, for mass marking of burbot using the *per os* method, feeding larvae at 22 DPH with ARS-immersed *A. salina* nauplii continuously for 4 days is recommended for fishery practice.

Keywords: alizarin red S, *Artemia salina*, commercial feed, otoliths, tetracycline hydrochloride.

Introduction

The constant decline of the burbot populations in the natural environment due to anthropogenic pressure has caused increasing interest in intensive aquaculture of this species (Paragamian and Wakkinen, 2008). It has led to significant scientific activities aiming at the development of effective production methods of burbot fry under controlled conditions (Harzevili *et al*., 2003; Trabelsi *et al*., 2011; Palińska-Żarska *et al*., 2014).

Marking and/or tagging the fish being restocked to the waterbodies is one of the most effective tools in ichthyologic studies, enabling determination and providing evidence for the life span, growth rate, mortality, and migration of fish in the natural environment (Everhart *et al*., 1975; Hilborn *et al*., 1990). It is also applied to evaluate the effectiveness of large-scale restocking operations of endangered fish species (Cowx, 1998; Champigneulle and Cachera, 2003). Most of the standard methods of fish tagging (fin clipping, tattoos, PITs, magnetic or conventional tags) cannot be applied at the earliest stages of life (larvae or juveniles), yet these stages are the ones mainly restocked. Therefore, fluorochromes (fluorescent dyes) are often used for the mass marking of larvae (Brothers, 1990), as they are capable of forming complexes with calcium ions that are embedded in the skeletal structures of fishes (i.e. vertebras, bones, scales, and otoliths). However, the fluorochrome remains for the entire life of fish only in otoliths. The presence of fluorescent marks may then be easily detected under UV light (Weber and Ridgway, 1962). In fishery practice, a few fluorochrome markers have been used for this procedure, such as tetracycline, oxytetracycline, tetracycline hydrochloride, calcein, alizarin red S, and alizarin complexone (Nagięć *et al*., 1983; Brooks *et al*., 1994; Liu *et al*., 2009).

Different methods of fluorochrome administration have been used for the mass marking of early life stages of fish. This includes immersion, injection, body spray, and feeding (Weber and Ridgway, 1962; Tsukamoto, 1985; Leskelä *et al*., 2004; Babaluk and Craig, 1990). Until now, most of the mass markings of fish larvae with fluorochromes have been done by immersion. However, the effectiveness of fish marking by immersion is largely determined by the physicochemical parameters of water (hardness, pH, oxygenation). The immersion method during the marking of older fish stages, possessing the gill apparatus and scales, is usually ineffective. In this case, fluorochromes are introduced into a fish body via water; therefore, the effects of the

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procedure are determined by its physicochemical parameters. Most of the applied markers significantly decrease the pH of water, thereby having a negative effect on fish survival (Hettler, 1984; Tsukamoto, 1985). Therefore, attempts to use feed as a vector for fluorochromes have been undertaken in order to eliminate the above-mentioned difficulties resulting from varying water parameters and to mark the fish in an easier, cheaper, and safer way for both the fish and the natural environment (Stańczak *et al*., 2015). Initial attempts at fish marking based on this method consisted in a few days of feeding larvae with the supplemented feed (Nagięć *et al*., 1983; Thomas *et al*., 1995) or zooplankton that had earlier been immersed in a fluorescent dye (Nagięć and Nagięć, 1983).

It has been assumed that, as in other species, the marking of a comparable amount of burbot larvae/fry with the *per os* method (administration of *A. salina* nauplii or feed supplemented with fluorochromes) requires a lower amount of dye compared to the immersion technique (Hettler, 1984; Tsukamoto, 1985). It is important for the quality of the natural environment to minimise the effects of water contamination and the volume of generated post-production water. The conservation of fish populations requires successive actions aimed at improving environmental conditions and carrying about the quality of their structure. In this context, optimisation of the mass-marking method of stocking material with the *per os* method may contribute to the evaluation of the ichthyofauna structure of a selected freshwater ecosystem and minimise the risk of negative anthropogenic effects on the natural environment.

Considering the limitations and possibilities, the aim of the present study was to determine the optimal conditions for marking larvae and juveniles of burbot using the food source as a vector for two fluorochromes: tetracycline hydrochloride (TC) and alizarin red S (ARS).

Materials and Methods

Origin of Fish and Rearing Conditions

Experiments were conducted in three successive seasons (2013–2015). The material (larvae/juveniles) for all experiments originated from controlled reproduction of fish cultured in the fish farm Czarci Jar near Olsztyn (NE Poland). The artificial reproduction was conducted using only thermal stimulation of spawners following the methodology described by Żarski *et al*. (2010). Two days after hatching, the larvae were transported to the laboratories of the Centre of Aquaculture and Ecological Engineering, University of Warmia and Mazury in Olsztyn. In each experiment, the administration of *Artemia salina* nauplii (Ocean Nutrition Ltd., USA) started on the 3rd day post hatch (DPH) and continued until the beginning of the appropriate experiments. In experiment 3 (E3), juveniles of burbot were fed *A. salina* nauplii until 50 DPH, followed by the weaning of the fish onto commercial feed according to the methodology described by Palińska-Żarska *et al*. (2014).

Preparation of the Live Food as a Vector of Fluorescent Dyes

In experiments 1 and 2 (E1; E2) *Artemia salina*, the vector for the fluorescent dyes, was incubated according to the producer's instruction and then subjected to immersion in either ARS or TC (Sigma-Aldrich Ltd). Time (12 h) and concentration of ARS (200 mg L^{-1}) or TC (600 mg L^{-1}) as optimal for immersion of *Artemia salina* nauplii were adopted according to Stańczak *et al*. (2015).

Experiment 1

The experimental material was comprised of 12-day-old burbot larvae with a mean total body length of 4.9 (±0.2) mm and a mean body weight of 0.67 (±0.13) mg. The experimental rearing lasted 12 days. The fish were divided into seven experimental groups (triplicated) and kept in separate aquaria (200 fish in each aquarium representing a separate replicate among respective treatments) with a total volume of ca. 2 L that were set into a recirculating system. The larvae were fed *ad libitum* three times daily with the dye-immersed *A. salina* nauplii with ARS (ARS.1, ARS.3 and ARS.6) or TC (TC.1, TC.3 and TC.6) for 1, 3, and 6 subsequent days, respectively. Burbot larvae in the control group (C.1) were fed throughout the study with dye-free *A. salina* nauplii. After feeding the larvae with dye-immersed *A. salina* sp., the feeding was continued in all groups with the use of dye-free *A. salina* nauplii. The experiment was carried out at a temperature of 10 (±1)°C, with water oxygenation at 80% and ammonia and nitrate concentrations below 0.01 mg L^{-1}.

Experiment 2

In E2, 22-day-old larvae of burbot with a mean total body length of 10.6 (±0.1) mm and a mean body weight of 7.8 (±4.3) mg were reared for 12 days. The larvae were fed *ad libitum* three times daily with dye-immersed *A. salina* nauplii with ARS (ARS2.1, ARS2.4 and ARS2.8) or TC (TC2.1, TC2.4 and TC2.8) for 1, 4, and 8 subsequent days, respectively. Similarly to E1, fish in the control group (C.2) were fed throughout the study with dye-free *A. salina* nauplii. The fish from each replication among respective treatments were kept in separate tanks (200 fish each) with a total volume of ca. 2 L set into a recirculating system. Also as in E1, after the end of the fluorochrome administration period, the feeding was continued in all groups with the use of dye-free

A. salina nauplii. The rearing was carried out at a water temperature of 15 (±1)°C, with water oxygenation at 80% and ammonia and nitrate concentrations below 0.01 mg L^{-1}.

Experiment 3

A 25-day experiment was conducted with 92-day-old burbot juveniles with a mean total length of 6.0 (±0.6) cm and a mean body weight of 1.8 (±0.2) g. In E3, the fish were divided into six experimental groups (each in two replicates) and fed with commercial feed (Perla Eel Proactive 5.0, SKRETTING Ltd.) without ARS (C.3-control group) or with different doses of ARS (i.e. 20, 40, 60, 80, and 100 g of fluorochrome per 1 kg of feed for the ARS.20, ARS.40, ARS.60, ARS.80 and ARS.100 groups, respectively). The fish were kept in separate tanks (20 fish each) with a total volume of ca. 3 L that were set in a recirculating system with monitored physicochemical parameters of water. The feed was administered manually four times a day for 10 days in a dose equal to 5% of the biomass of the fish in each tank. Afterwards, the fish were fed exclusively ARS-free (pure) feed for another 15 days.

Sampling and Mark Analyses

In all experiments, the tanks were cleaned of uneaten food and fish waste once daily in the morning before the first feeding. At the same time, dead fish were collected and counted. At the end of each experiment, all remaining fish were counted, and 30 larvae or juveniles per aquarium were euthanised by overexposure in an anesthetic (2-phenoxyethanol) in dose 2 ml/ L^{-1}) and then individually weighed (±0.1 mg) and measured (±0.01 cm). After measuring, whole fish were preserved in 70% ethyl alcohol. Next, otoliths were dissected from each specimen, placed on microscopic slides, embedded in Entellan (Sigma-Aldrich Ltd.), and analysed under a Nikon Eclipse 90i fluorescent microscope equipped in a Lumen 200 UV lamp (Prior Scientific) (at wavelengths of 450–490 and 510–560) in order to detect the mark.

Statistical Analysis

Growth parameters and survival of fish were compared using one-way ANOVA. Normality of distribution was *arcsin* transformed prior to the statistical analysis. The significance of differences between groups was estimated using a post-hoc LSD Fisher test ($P<0.05$). Analyses were performed using Statistica software (StatSoft).

Results

Experiment 1

No marked otoliths were detected in the control group (C.1). Marked otoliths were found in all treatments in which 12-day-old larvae of burbot were administered *A. salina* nauplii immersed in a dye solution. However, the percentage of marked otoliths was positively correlated with the number of days of feeding the fish with dye-immersed food (Table 1). The lowest percentage of marked otoliths (61.1%) was found in the group TC.1, whereas the highest was found in group TC.6 (Table 1) (Figure 1). The survival of burbot larvae throughout the experiment was high and ranged between 87.8% (TC.6) and 92.7% (ARS.1) (Table 1). The final values of body weight and total length of burbot larvae between the groups were similar (Table 1).

Experiment 2

As in E1, no marked otoliths were detected in the control group (C.2). Marked otoliths were noted in all variants wherein 22-day-old larvae of burbot were fed live *A. salina* nauplii supplemented with both fluorescent dyes (Table 2). The lowest percentage of marked otoliths was found in groups ARS2.1 and TC2.1 (93.3% and 66.7%, respectively) fed with the dye-immersed *A. salina* nauplii for only 1 day (Table 2) (Figure 2a, d). In the other groups fed the dye-immersed nauplii with ARS or TC for 4 and/or 8

Table 1. Mean values (± SD; n = 3 for each group) of total length (TL), wet body weight (BW) as well as survival rate and percentage of marked otoliths obtained after 12-day long controlled rearing of burbot larvae (initial age – 12 days post hatch) fed for 1, 3 or 6 days with *Artemia* sp. immersed in alizarin red S (groups ARS.1, ARS.3 and ARS.6, respectively) or tetracycline hydrochloride (groups TC.1, TC.3 and TC.6, respectively).

Group	TL (mm)	BW (mg)	Survival (%)	Marked otoliths (%) n = 30
ARS.1	8.6 ± 0.2	4.9 ± 0.2	92.7 ± 3.2	61.1
ARS.3	8.7 ± 0.2	4.4 ± 0.3	89.0 ± 0.5	72.2
ARS.6	8.6 ± 0.1	4.7 ± 0.1	91.3 ± 2.5	87.8
TC.1	8.7 ± 0.1	4.9 ± 0.8	91.3 ± 2.5	73.3
TC.3	8.6 ± 0.1	4.8 ± 0.2	90.7 ± 1.3	76.7
TC.6	8.5 ± 0.2	4.7± 0.2	87.8 ± 5.7	100
C.1	8.5 ± 0.2	5.0 ± 0.3	90.3 ± 1.8	0

The results were compared to the control group fed exclusively with dye-free *Artemia* sp. nauplii (group C.1, respectively). No significance differences among treatments were found ($P>0.05$).

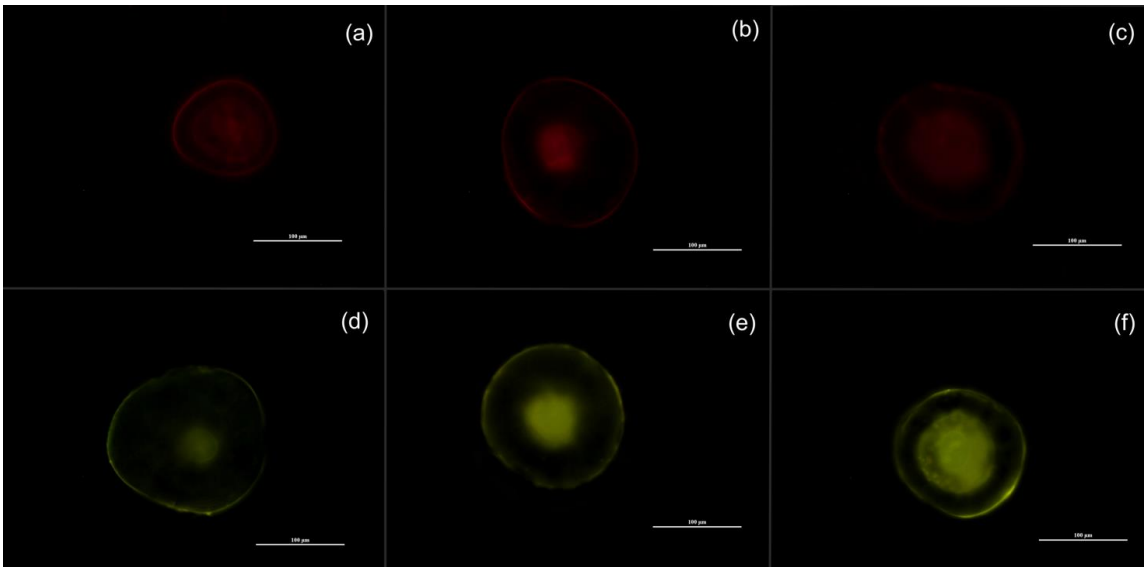

Figure 1. Otoliths of 24-days old burbot larvae marked by feeding the fish with *Artemia* sp. nauplii immersed in either alizarin red S (ARS) or tetracycline hydrochloride (TC). Fish were fed (starting from 12 day post hatch) with ARS-immersed *Artemia* for 1, 3 or 6 days in groups a) ARS.1, b) ARS.3 and c) ARS.6, respectively. Fish were fed with TC-immersed *Artemia* for 1, 3 or 6 days in groups d) TC.1, e) TC.3 and f) TC.6, respectively. Photos taken under filtered UV light. Bar represents 100 µm.

Table 2. Mean values (± SD; n = 3 for each group) of total length (TL), wet body weight (BW) as well as survival rate and percentage of marked otoliths obtained after 12-day long controlled rearing of burbot larvae (initial age – 22 days post hatch) fed for 1, 4 or 8 days with *Artemia* sp. immersed in alizarin red S (groups ARS2.1, ARS2.4 and ARS2.8, respectively) or tetracycline hydrochloride (groups TC2.1, TC2.4 and TC2.8, respectively)

Group	TL (mm)	BW (mg)	Survival (%)	Marked otoliths (%) n = 30
ARS2.1	$15.4^{ab} \pm 0.1$	$38.1^{abc} \pm 0.5$	82.7 ± 3.8	93.3
ARS2.4	$15.3^{b} \pm 0.4$	$35.7^{c} \pm 3.4$	87.7 ± 3.5	100
ARS2.8	$15.8^{ab} \pm 0.4$	$39.3^{abc} \pm 1.8$	87.0 ± 2.6	100
TC2.1	$15.9^{ab} \pm 0.5$	$39.6^{bc} \pm 2.9$	83.3 ± 1.1	66.7
TC2.4	$16.1^{a} \pm 0.6$	$41.4^{b} \pm 3.0$	86.3 ± 5.5	100
TC2.8	$15.3^{b} \pm 0.5$	$35.3^{a} \pm 2.4$	87.0 ± 5.3	100
C.2	$16.0^{ab} \pm 0.4$	$40.4^{b} \pm 1.9$	86.0 ± 4.6	0

The results were compared to the control group fed exclusively with dye-free *Artemia* sp. nauplii (group C.2, respectively). The values in the same column marked with different letters differ at P<0,05.

days, the effectiveness of marking reached 100% (Table 2) (Figure 2b, c, e, f). At the end of E2, the survival of burbot larvae did not differ significantly between the groups and ranged from 82.7 to 87.7%. Significant differences were found only in the final body weight of fish from two experimental groups (ARS2.4 and TC2.8) versus the control group (Table 2).

Experiment 3

No marked otoliths were found in the control group (Figure 3f). Marked fish were identified in all experimental groups in which 92-day-old juveniles were fed with feed supplemented with ARS. The highest (100%) percentage of marked otoliths (Figure 3 b) was found in the ARS.40 group (Table 3), in contrast the lowest percentage of marked otoliths (76.6%) was determined in the ARS.60 group (Figure 3c). At the end of E3, the survival in all experimental groups was 100%, and the values of final body weight and length of burbot juveniles did not differ significantly between the experimental groups (Table 3).

Discussion

The optimal marking method should provide 100% effectiveness in the procedure, and in the case of techniques using the fluorochrome dye, it should also be characterized by a high quality of obtained ‘marks’(Brown and Harris, 1995). The results of the present study for the first time provide data on the possibility of marking burbot otoliths with two different fluorescent dyes administered *per os.* It confirmed that feeding the fish with live *A. salina*

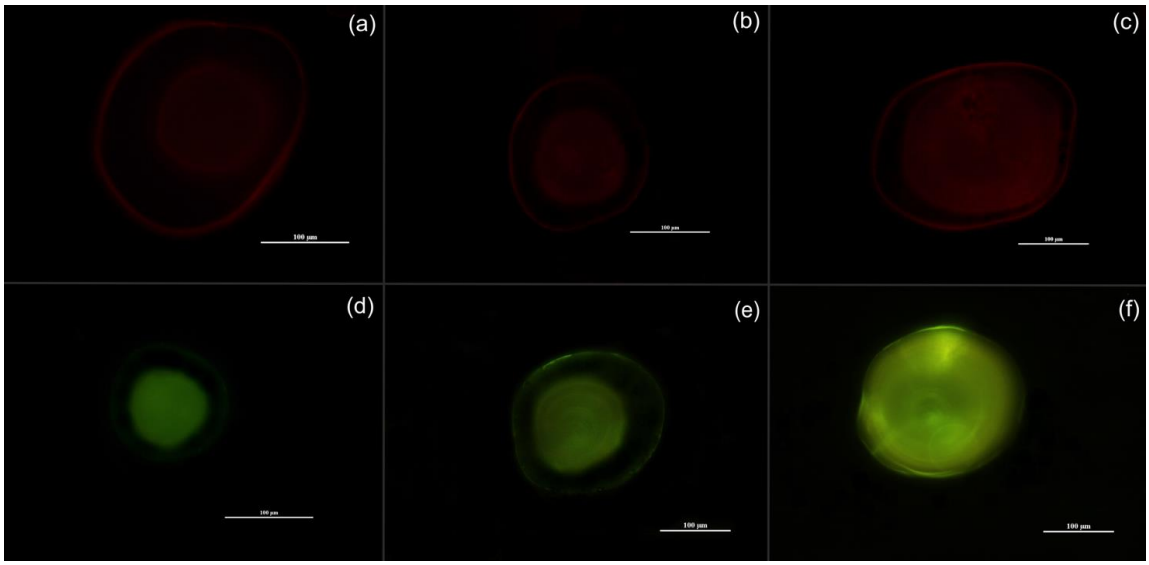

Figure 2. Otoliths of 34-days old burbot larvae marked by feeding the fish with *Artemia* sp. nauplii immersed in either alizarin red S (ARS) or tetracycline hydrochloride (TC). Fish were fed (starting from 22 day post hatch) with ARS-immersed *Artemia* for 1, 4 or 8 days in groups a) ARS2.1, b) ARS2.4 and c) ARS2.8, respectively. Fish were fed with TC-immersed *Artemia* for 1, 4 or 8 days in groups d) TC2.1, e) TC2.4 and f) TC2.8, respectively. Photos taken under filtered UV light. Bar represents 100 µm.

Table 3. Mean values (± SD; n = 2 for each group) of total length (TL), wet body weight (BW) as well as survival rate and percentage of marked otoliths obtained after 25-day long controlled rearing of burbot larvae (initial age – 92 days post hatch) fed with commercial feed or with different doses of ARS, i.e. 20, 40, 60, 80 and 100 g of fluorochrome per 1 kg of feed.

Group	TL (cm)	BW (g)	Survival (%)	Marked otoliths (%) n = 30
C.3	7.4 ± 1.0	2.7 ± 0.9	100	0
ARS.20	7.6 ± 0.7	3.0 ± 0.8	100	90
ARS.40	7.5 ± 0.9	3.0 ± 1.0	100	100
ARS.60	7.6 ± 0.8	2.9 ± 1.0	100	76.6
ARS.80	7.3 ± 0.9	2.5 ± 1.0	100	93.3
ARS.100	7.3 ± 0.8	2.6 ± 0.8	100	86.6

The results were compared to the control group fed exclusively with dye-free commercial feed (group C.3, respectively). No differences between the groups regarding to any of recorded parameter were found (P>0.05).

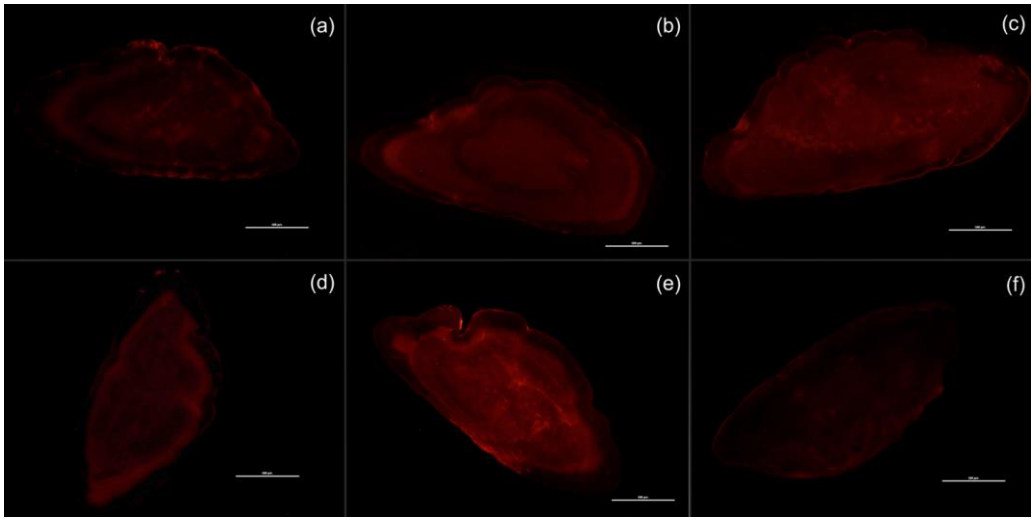

Figure 3. Otoliths of 117-days old burbot larvae marked by feeding the fish with commercial feed with different doses of ARS. Fish were fed (starting from 92 day post hatch) for 10 days in groups a) ARS.20, b) ARS.40, c) ARS.60, d) ARS.80, e) ARS.100 and the control group fed exclusively with dye-free commercial feed f) C.3, respectively. Photos taken under filtered UV light. Bar represents 500 µm.

nauplii already immersed in fluorochromes can be successfully applied in 22-day-old burbot. The procedure for the earlier life stage of burbot (12 DPH) resulted in full marking efficiency in only one case (where 100% of otoliths were marked). Moreover, our results confirmed that in burbot larvae the ease of fluorochrome mark identification and the higher percentage of marked fish are linked with the number of days of supplemented feed administration. Hence, a 4-day period of administration of fluorochrome-supplemented live feed is recommended for burbot larvae. The same method of mass marking was applied in 4 DPH ide larvae (*Leuciscus idus* L.) administered *Artemia* sp. nauplii stained with one of the three fluorescent dyes: ARS, TC, or calcein. Fish fed for 2 and 4 days with supplemented *A. salina*, showed high efficiency of marking, regardless of marker type (Stańczak *et al*., 2015). Satisfying results (70 to 100% marking otoliths) were also obtained during the marking of 14 DPH larvae of tench (*Tinca tinca* L.) fed with ARS-supplemented *A. salina* nauplii (Stańczak *et al*., 2014) .

Nagięć and Nagięć (1983) used live food (lake zooplankton) as a vector of marker transfer to a fish body for the first time ever. They successfully applied a 10-minute bath of zooplankton in a TC and chlortetracycline (CTC) solution later supplied to whitefish (*Coregonus lavaretus* L.) larvae. An average of 30% of zooplankton had antibiotics either in gut content or built into the body. TC-treated fish did not avoid the antibiotic bearers. Daily doses of orally administered TC antibiotics for treated whitefish in the study were at 500–700 mg L^{-1}. Contrary to Nagięć and Nagięć's data (1983), in our E1 and E2 live *A. salina* nauplii were used as sterile material that is easy to purchase and use. A high survival rate of larvae was always considered to be an important indicator of the effectiveness of rearing procedures. In burbot larviculture, one of the biggest problem is a very high mortality rate observed in the first stages of post-embryonic development (Żarski *et al*., 2010). However in the present study, the survival rate of the burbot during experiments E1–E3 did not fall below 80%, like in other tests (Harzevili *et al*., 2004; Wocher *et al*., 2012). In both described experiments concerning the administration of supplemented *A. salina* nauplii to burbot larvae, there was no negative effect of any of the analyzed fluorochrome dyes on the final survival of the stocks. Likewise, high survival was reported in the case of ide and tench larvae during marking procedures (Stańczak *et al*., 2014, 2015).

In burbot juveniles, the administration of feed supplemented with ARS in a dose of 40 g/kg feed for ca. 10 days proved to be an effective method of *per os* marking and may be recommended for use in the fishery practice. The administration of supplemented feed for 10 days allows for easier identification marks in otoliths of adult fish. It is noteworthy that the marking procedure with the *per os* method did not affect stock survival, which was also confirmed in whitefish (Nagięć *et al*., 1983), red drum (*Sciaenops ocellatus L.*) (Thomas *et al*., 1995) and cod (*Gadus morhua* L.) (Nordeide *et al*., 1992). Moreover, the feed containing fluorochromes as well as non-supplemented feed were consumed by burbot juveniles with equal willingness.

In conclusion, for mass marking of burbot using the *per os* method, feeding larvae at 22 DPH with ARS-immersed *Artemia salina* nauplii continuously for 4 days as well as for fry feed supplemented ARS (40 g/kg) for 10 days is recommended. Comparing the commonly applied immersion technique versus the presented *per os* method for marking burbot larvae, we stated that the latter is less time-consuming, easier to perform, cheaper, and safer for fish. In the context of environmental safety, the total amount of ARS used for the proposed *per os* method is 75% less than in common immersion methods used for other fish species.

References

Babaluk, J.A. and Craig, J.F. 1990. Tetracycline marking studies with pike, *Esox lucius* L. Aquaculture and Fishery Management, 21: 307–315. doi: 10.1111/j.1365-2109.1990.tb00469.x ;

Brooks, R.C., Heidinger, R.C. and Kohler, C.C. 1994. Mass-marking otoliths of larval and juvenile walleyes by immersion in oxytetracycline, calcein, or calcein blue. North American Journal of Fisheries Management, 14 (1): 143–150.doi: 10.1577/1548-8675(1994)014<0143:MMOOLA>2.3.CO;2 ;

Brothers, E.B. 1990. Otolith marking. American Fisheries Society Symposium 7: 183–202.

Brown, P. and Harris, J.H. 1995. Strontium batch-marking in golden perch (*Macquaris ambigua*, Richardson) and trout cod (*Maccullochella macquariensis*, Cuvier). In: D.H. Secor, J.M Dean, S.E. Campana (Ed.), Recent developments in fish otolith research. University of South Carolina Press, Columbia: 303–318.

Champigneulle, A. and Cacher, S. 2003. Evaluation of large-scale stocking of early stages of brown trout, *Salmo trutta*, to angler catches in the French-Swiss part of the River Doubs. Fisheries Management and Ecology, 10: 79–85. doi: 10.1046/j.1365-2400.2003.00325.x ;

Cowx, I.G. 1998. Stocking strategies: issues and options for future enhancement programmes?. In: I.G. Cowx (Ed.), Stocking and Introduction of Fish. Fishing News Books. Blackwell Scientific Publications, Oxford: 3–13.

Everhart, W. H., Eipper, A.E. and Youngs, W.D. 1975. Fish marking. In: W.H. Everhart, W.D. Youngs (Ed.), Principles of Fishery Science. Cornell University Press, New York: 141–216.

Harzevili, A.S., Charleroy, D.De., Auwerx, J., Vught, I., Slycken, J.Van., Dhert, P. and Sorgeloos, P. 2003. Larvae rearing of burbot (Lota lota) using Brachionus calyciflorus rotifer as starter food. Journal of Applied Ichthyology, 19: 84–87. doi: 10.1046/j.1439-0426.2003.00442.x ;

Harzevili, A.S., Dooremont, I., Vught, I., Auwerx, J.,

Quataert, P. and Charleroy, D.De. 2004. First feeding of Burbot, *Lota lota*(Gadidae, Teleostei) larvae under different temperature and light conditions. Aquaculture Research, 35: 49-55. doi: 10.1111/j.1365-2109.2004.00982.x ;

Hettler, W. F. 1984. Marking otoliths by immersion of marine fish larvae in tetracycline. Transactions of the American Fisheries Society, 113: 370–373. doi: 10.1577/1548-8659(1984)113<370:MOBIOM>2.0.CO;2 ;

Hilborn, R., Walters, C.J. and Jester, D.B. 1990. Value of fish marking in fisheries management. In: N.C. Parker, A.E. Giorgi, R. Heidinger, B.J. Douglas, E. Prince, G.A. Winans (Ed.), Fish-Marking Techniques. American Fisheries Society Symposium, Maryland: 5–8.

Leskelä, A., Jokikokko, E., Huhmarniemi, A., Siira, A. and Savolainen, H. 2004. Stocking results of spray-marked one-summer old anadromous European whitefish in the Gulf of Bothnia. Annales Zoologici Fennici, 41: 171–179.

Liu, Q., Zhang, X.M., Zhang, P.D. and Nwafili, S.A. 2009. The use of alizaryn red S and alizarin complexone for immersion marking Japanese flounder *Paralichthys olivaceus* (T.). Fisheries Research, 98: 67–74. doi: 10.1016/j.fishres.2009.03.014 ;

Nagięć, M. and Nagięć, C. 1983. Marking of juvenile whitefish (*Coregonus lavaretus* L) by tetracycline antibiotics. Roczniki Nauk Rolniczych, 100(3): 107–114.

Nagięć, M., Nagięć, C., Dąbrowski, K. and Morawska, E. 1983. Marking of juvenile whitefish *Coregonus lavaretus* L. with tetracycline antibiotics. Acta Ichthyologica et Piscatoria, XIII (2): 47–56.

Nordeide, J.T, Holm, J.C., Ottera, H., Blom, G. and Borge, A. 1992. The use of oxytetracycline as a marker for juvenile cod (*Gadus morhua* L.). Journal of Fish Biology, 44: 21–30. doi: 10.1111/j.1095-8649.1992.tb03167.x ;

Palińska-Żarska, K., Żarski, D., Krejszeff, S., Nowosad, J., Biłas, M., Trejchel, K., Brylewski, A., Targońska, K. and Kucharczyk D. 2014. The effect of age, size and digestive tract development on burbot, *Lota lota* (L.), larvae weaning effectiveness. Aquaculture Nutrition, 20: 281–290.doi: 10.1111/anu.12076 ;

Paragamian, V.L. and Wakkinen, V.D. 2008. Seasonal movement of burbot in relation to temperature and discharge in the Kootenai River, Idaho, USA and British Columbia, Canada. In: V.L. Paragamian, D.H. Bennett (Ed.), Burbot: ecology, management and culture. The American Fisheries Society, Maryland: 55–77.

Stańczak, K., Krejszeff, S., Dębowska, M., Mierzejewska, K., Woźniak, M. and Hliwa, P. 2015. Mass-marking of *Leuciscus idus* (Linnaeus 1758) larvae using *Artemia salina* (Linnaeus 1758) as a vector of fluorescent dyes. Journal of Fish Biology, 87: 799–804. doi: 10.1111/jfb.12753 ;

Stańczak, K., Krejszeff, S., Mierzejewska, K., Wiszniewska, K., Kucharczyk, D., Woźniak, M. and Hliwa, P. 2014. Marking tench (*Tinca tinca*) larvae alizarin Red S via live food supplementation. In: Z., Zakęś, K. Demska-Zakęś, A. Kowalska (Ed.). Wylęgarnictwo organizmów wodnych a bioróżnorodność. Wydawnictwo Instytutu Rybactwa Śródlądowego w Olsztynie: 149-156. [in Polish]

Thomas, L.M., Holt, S.A. and Arnold, C.R. 1995. Chemical marking techniques for larval and juvenile red drum (*Sciaenops ocellatus*) otoliths using different fluorescent markers. In: D.H. Secor, J.M. Dean, S.E. Campana (Ed.). Recent developments in fish otolith. Univ. South Carolina Press: 703–717.

Trabelsi, A., Gardeur, J.N., Teletchea, F. and Fontaine P. 2011. Effects of 12 factors on burbot *Lota lota* (L., 1758) weaning performances using fractional factorial design experiment. Aquaculture, 316: 104–110. doi: 10.1016/j.aquaculture.2011.03.027 ;

Tsukamoto, K. 1985. Mass-marking of ayu eggs and larvae by tetracycline tagging of otoliths. Bulletin of the Japanese Society for the Science of Fish, 51 (6): 903–911. doi: org/10.2331/suisan.51.903;

Weber, D.D. and Ridgway, G.J. 1962. The deposition of tetracycline drugs in bones and scales of fish and its possible use for marking. The Progressive Fish-Culturist, 4 (24): 150–155.

Wocher, H., Harsanyi, A. and Schwarz, F.J. 2012. Larviculture of Burbot (*Lota lota* L.): larval rearing using *Artemia* and weaning onto dry feed. Aquaculture Research, 44: 106–113. doi: 10.1111/j.1365-2109.2011.03015.x ;

Żarski, D., Kucharczyk, D., Sasinowski, W., Targońska, K. and Mamcarz, A. 2010. The influence of temperature on successful reproductions of burbot, *Lota lota* (L.) under hatchery conditions. Polish Journal of Natural Sciences, 25: 93–105. doi: 10.2478/v10020-010-0007-9 ;