

# Multiple alizarin immersion marking of juvenile sea trout *Salmo trutta m. trutta* L. otoliths as a practice in the stocking program



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## Background

Fish stocking is one of the most commonly applied methods to compensate damages resulting from human impact on the environment and aquatic living resources (Cowx 1994). It is also a tool in sustainable fisheries management. As a complementary action, otolith marking is used to compare efficiency of stocking with the use of different fish life stages. Mass marking of larvae by immersion in fluorochrome solutions was developed in the mid-1980s (Hettler 1984) and it is still widely applicable (Baer & Rösch 2008, Martyniak et al. 2013).

## Materials & Methods

A large scale marking experiment was conducted in 2014–2016.

Ten-day-old sea trout larvae dedicated to Łeba River stocking (northern Poland; Fig. 1) were divided into two groups: I) fish intended for alevins stocking; and II) fish intended for fry 0+ stocking.

### The marking procedure:

Group I – fish single marked by 3 h immersion in a solution of alizarin red S (ARS) using a concentration of 200 mg dm<sup>-3</sup>.

Group II – fish double marked by 3 h immersion in a solution of alizarin red S (ARS) using a concentration of 200 mg dm<sup>-3</sup> with 14 days (± 90°D) interval between first and second marking session.

Mean values of water temperature between first and second immersion in fish from group II varied between years and was 4.4°C, 7.5 °C, 6.7 °C, respectively.

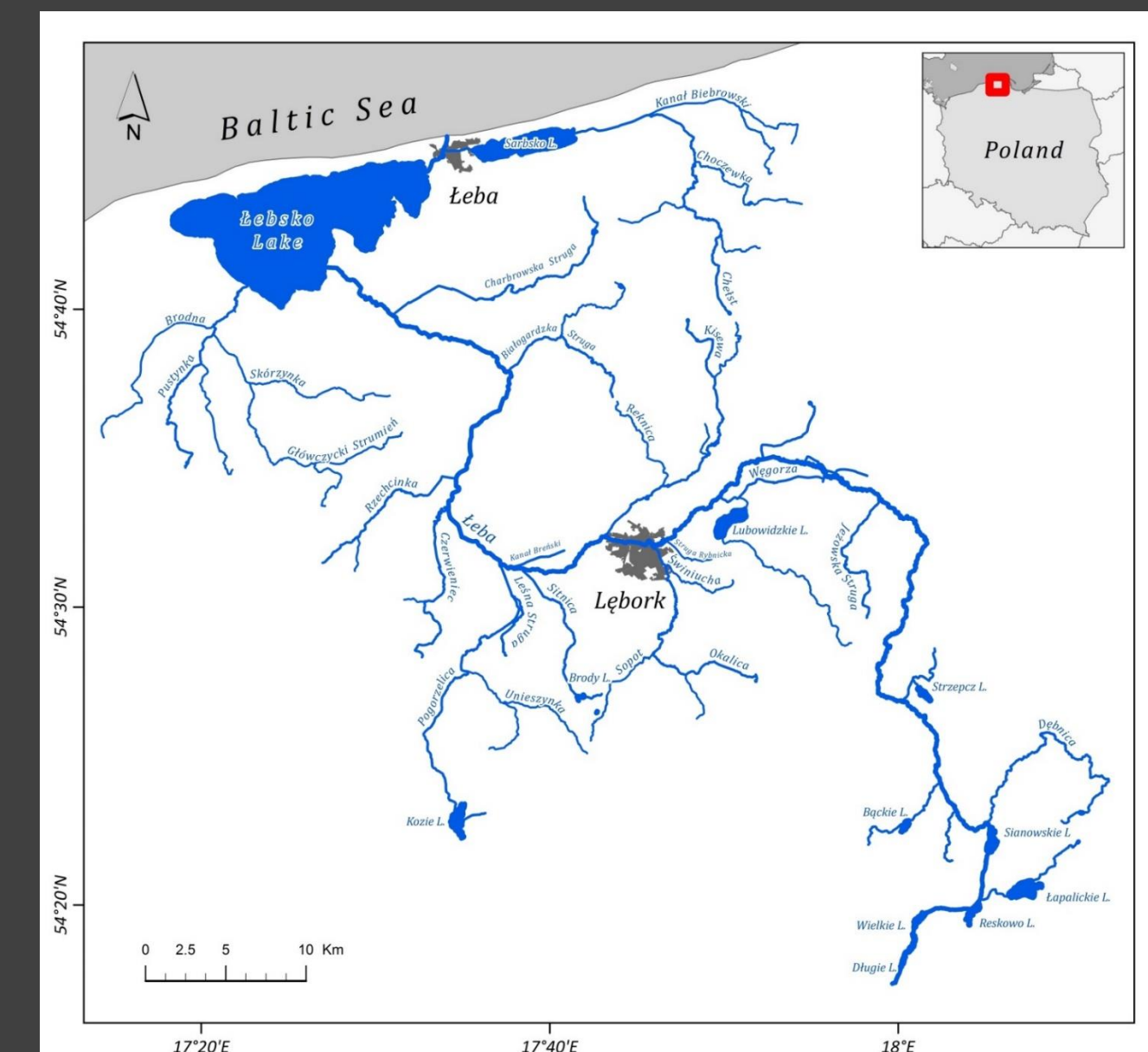


Fig. 1 . Geographic location of the Łeba River system.



## Results

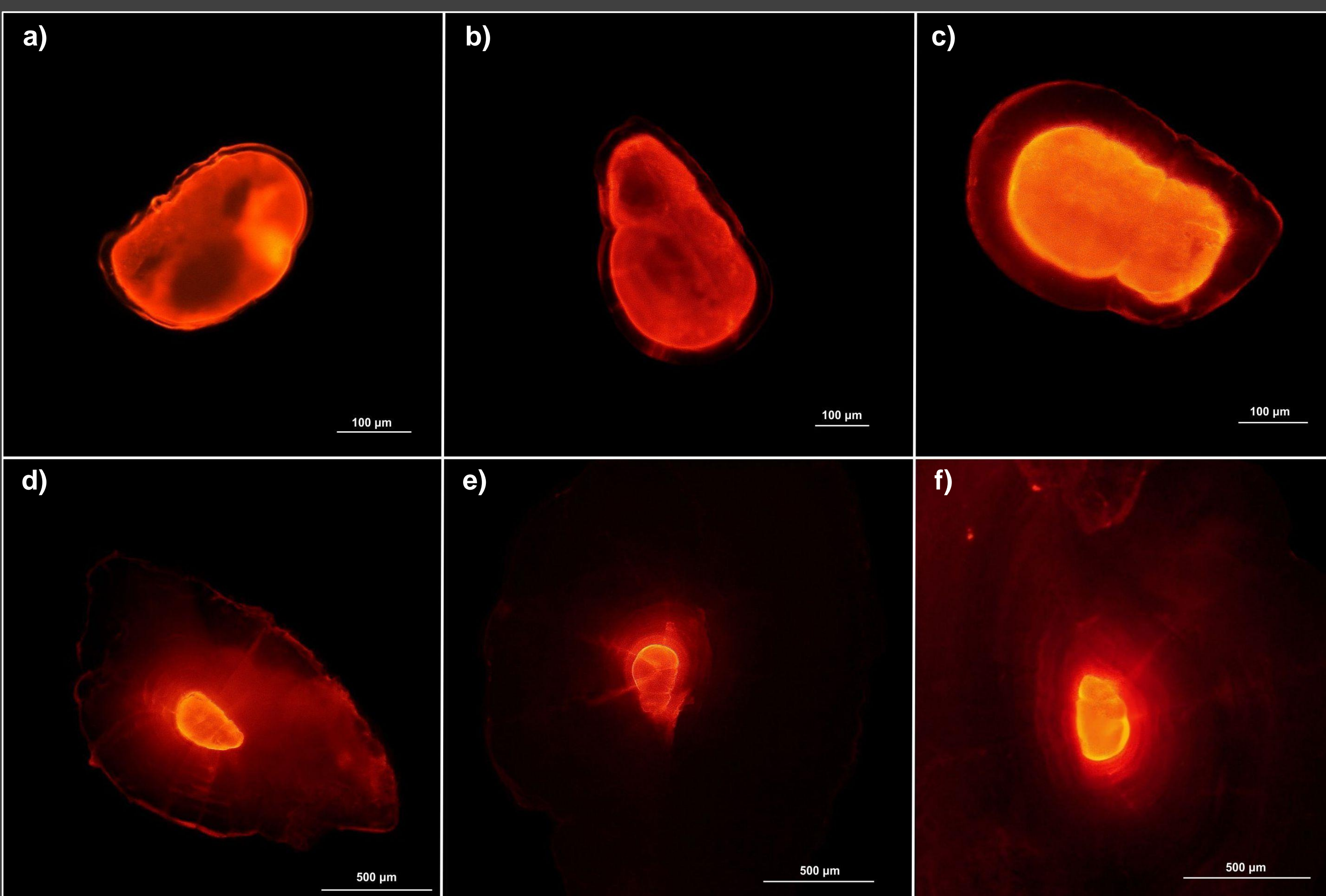


Fig. 2. Single alizarin marks (ARS) in the sea trout otoliths: a) 10 days, b) 30 days, c) 60 days, d) 8 months, e) 20 months, f) 44 months after batch marking; magnification: a-c ) 100x and d-e) 40x respectively.

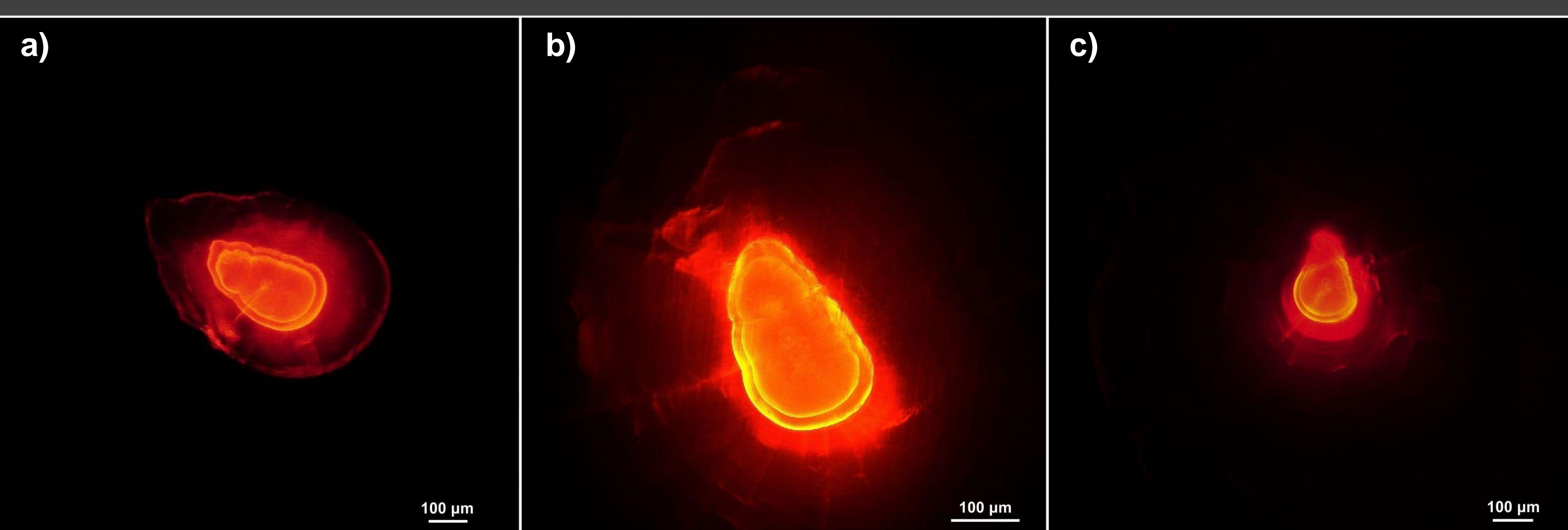


Fig. 3. Double alizarin marks (ARS) in the otoliths of sea trout originating from fry stocking : a) 60 days, b) 8 months and c) 20 months after batch marking; magnification: a,c) 40x and b) 100x respectively.

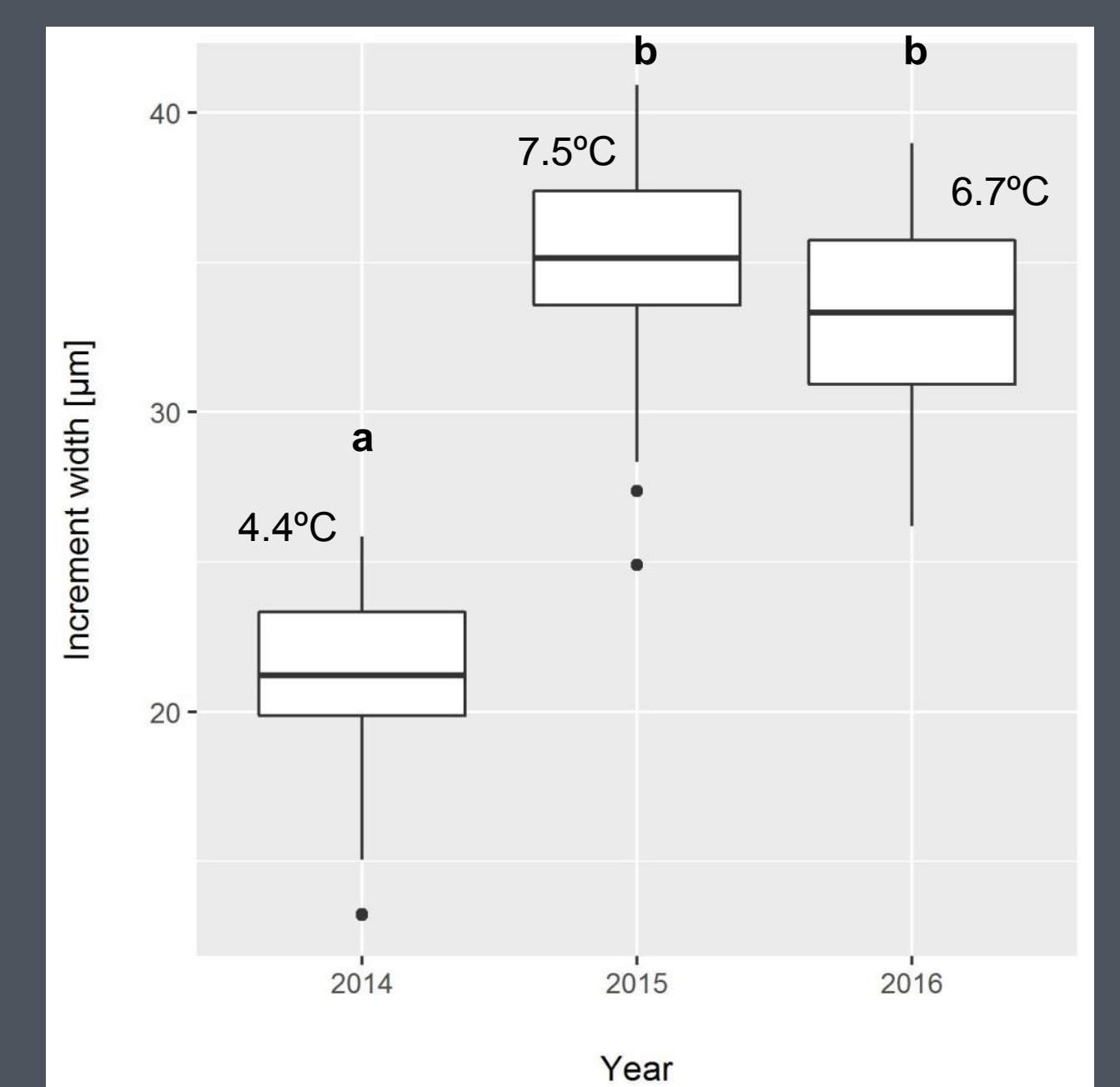
The effectiveness of the applied marking method was verified after 60 days and was 100% in both groups - clear and well-visible ARS marks were present (Fig. 2, Fig.3).

Post-marking mortality was not observed.

The time interval between each batch marking and temperature regime has been found to provide the proper distance between the ARS marks (Fig. 3, Fig. 4) in the otoliths (sagittae), observed as two well-separated rings.

In an experiment, a total of 204,830 fish from group I and 452,804 fish from group II were marked and released into the Łeba River system in 2014–2016.

Fig. 4. Increment width (µm) in the sea trout otoliths between first and second ARS marks; lines, boxes, and whiskers are means, interquartile range (IQR), and 1.5x IQR of the distance between the marks, respectively. Data marked with different superscripts were statistically different (Kruskal-Wallis H test, P < 0.05)



## Conclusions

Presented method can be advised as a routine procedure that provides clear and well-visible single including double alizarin marks in otoliths and helps to distinguish fish originating from different groups.



## Acknowledgements

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