



Comparison of Hard Structures for Age Estimation and Chemical Composition Analysis of Otoliths of Perch from Lake Trzesiecko under Reclamation

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1. Introduction

The study was carried out on perch harvested from Lake Trzesiecko in 2009. The lake has been subject to reclamation measures since 2005, involving: aeration of the near-bottom zone by means of an aerator, application of chemical compounds, e.g. iron sulphate, aimed at the release of phosphates from water depths and their accumulation in the near-bottom layer in the form of insoluble salts, as well as biomanipulation measures involving controlling of the abundance of planktivorous fish by predatory fish (Heese et al. 2013). One of such predatory fish species is perch. It is a typical predator of European waters, and is commonly used in biomanipulation (Eklöv & Van Kooten 2001). All of the measures, both chemical and technical, are aimed to contribute to the improvement of the ecological state of the lake discussed.

Measures involving biomanipulation are always difficult to perform. It is important to continuously control the participating groups of specimens/organisms/populations. The accurate determination of their health condition or population features, including the age structure, is of key importance in the execution of such a task (Newman & Dunk 2002). This results in the necessity to seek a simple but reliable element permitting obtaining possibly accurate results. The objective of this paper was to find such an element among: the scale, rays of the dorsal and anal fins, and the otolith, for the purpose of precise age estimation, determination

of the readability of the aforementioned structures, and calculation of the growth rate for perch from Lake Trzeciecko. The chemical composition analysis of the otolith was also performed for the purpose of determining the ability of the applied iron sulphate to penetrate biological membranes and accumulate in its structure. Its presence suggests a certain threat resulting from human interference into the ecosystem of Lake Trzeciecko through the introduction of chemical agents to the waters.

2. Material and methods

The present study covered 35 individuals of perch harvested from Lake Trzeciecko on 8-9 October 2009. Each of the individuals was weighed by means of an Axix scale with an accuracy to 0.01g, and measured by means of a ruler. Both total length and body length were determined with an accuracy to 1 mm. A scale was then sampled from the left side, from the area of the body over the lateral line under the first dorsal fin. Mucus and other contaminants were removed from the place before sampling the scale. The sampled material was placed in previously labelled paper bags. Before the age analysis, each of the scales was rinsed in 5% ammonia solution. Several clean scales of the same fish were mounted on a microscope slide and covered with a cover slip. Otoliths and dorsal (D) and anal (A) fin rays were sampled during the anatomical study, also involving the identification of the sex of the individuals analysed. Fin rays were cut off together with their base immersed in muscles. The obtained fin rays were then labelled and dried at room temperature until residues of skin and muscle could be easily removed from the rays. The resulting material was submerged in binary synthetic resin. The obtained blocks were cut by means of a slow-speed circular saw Izomet by Buehler into slices with a thickness of 0.75 mm. This way, microscope slides were prepared. The otoliths were sampled from the head by performing a cut from the top of the head to the inner ear. Both of the sagitta were removed and placed in labelled paper bags. Before further analyses, the otoliths were submerged in water, and residues of membranes were removed. They were then rinsed in deionised water. They were weighed with an accuracy to 0.001 g. Further works were conducted under a stereoscopic microscope Olympus SZX16. For all of the four structures, microscopic photographs were taken by means of a camera Olympus CX50. With the application of the cellSens

Standard software, measurements and labelling were performed, permitting age estimation and calculation of growth rate. The works were conducted following the methodology proposed by Secor et al. (1991) and Gabriel et al. (2000). Chemical composition analysis of the otoliths was also performed by means of a scanning electron microscope by JEOL with an EDS analyser. Each of the otoliths was rinsed with deionised water prior to the analysis. The analysis was conducted in one plane from the right side of the otolith to the left one. Three places were selected for detailed chemical composition analysis (Pattanaik 2004).

3. Calculation

The age of the studied fish was estimated based on the scale, D and A fin rays, and the otolith (Fig. 4). When the age could not be estimated based on a given structure, an empty space was left in the table. When the estimated age varied between one year and another, years were recorded using a slash (Table 1). When different age was determined for the same fish based on the analysed structures, the result obtained for at least two of the structures was adopted.

The growth rate of the fish was calculated based on the scale, by means of the back-reading method, in accordance with the Fraser-Lee equation: $L_n = C + S_n/S_c \cdot (L_c - C)$, where L_n – length of fish at age n , C – length of fish at which a scale is formed in the case of perch 2.5 cm, S_n – length of scale radius corresponding to particular annual rings, S_c – total length of scale radius at the moment of harvesting of a given fish Heese (1992).

Basic statistic data, i.e. the mean and range, were also determined for total length, body length, body weight, gutted weight, length and weight of the otoliths, and for the chemical composition of the otoliths.

The significance and strength of correlation between the weight and length of otoliths was also verified with the application of the Statistica 10.0 PL software. The correlation scales were adopted following Stanisz (1998). The results of the chemical composition analysis were averaged for all of the three places analysed.

Table 1. Morphological characteristics of the perch individuals studied and comparison of age on the basis of hard structures and scales**Tabela 1.** Charakterystyka morfologiczna okonia oraz porównanie struktur twardego do szacowania wieku

Lp.	Total length [cm]	Length body [cm]	Body mass [g]	Gutted weight [g]	Longest axis otolith [mm]	Otolith mass [g]
1	25.1	22.2	192.98	167.65	7.526	0.03
2	16.4	13.5	44.06	39.93	6.430	0.018
3	25.2	21.5	213.41	186.17	7.581	0.027
4	29.0	25.6	352.15	303.62	9.334	0.036
5	24.3	21.3	192.92	165.56	8.037	0.029
6	28.0	24.5	278.46	244.54	8.548	0.031
7	21.2	18.8	106.5	98.11	6.716	0.017
8	20.5	17.8	90.24	82.83	6.112	0.013
9	30.0	27.0	350.1	305.33	9.619	0.044
10	25.6	22.5	217.67	189.64	8.659	0.032
11	25.6	22.8	206.02	183.27	8.192	0.025
12	12.2	10.9	20.22	16.84	4.557	0.007
13	25.3	21.8	210.03	189.07	7.577	0.024
14	25.0	21.9	191.33	164.68	8.455	0.027
15	23.1	20.4	159.58	140	6.860	0.02
16	26.5	23.7	267.9	234.6	8.659	0.028
17	27.5	23.8	270.77	230.88	8.867	0.033
18	20.9	18.3	103.92	88.15	7.263	0.024
19	28.9	25.2	327.48	258.67	8.356	0.028
20	30.7	27.1	365.44	316.52	9.099	0.04
21	30.0	28.0	353.31	307.07	9.801	0.041
22	28.0	27.5	306.82	269.7	9.188	0.033
23	38.4	32.6	811.7	691.4	11.164	0.073
24	27.9	24.7	286.87	257.77	9.026	0.034
25	37.9	32.7	793	680.2	11.375	0.057
26	36.8	31.5	699.7	604.2	11.596	0.062
27	30.3	25.4	412.9	365.4	9.225	0.041
28	30.6	25.8	354.7	310.9	8.810	0.03
29	37.5	31.7	784.8	674.5	11.473	0.065
30	31.8	26.8	479.4	416.2	9.924	0.047
31	31.4	26.8	451.6	395	10.748	0.046
32	31.7	26.4	454.8	390.9	9.650	0.04
33	31.5	26.7	454.1	392.6	9.519	0.037
34	27.3	23.1	254.1	220.6	7.987	0.026
35	31.6	27.0	441.3	383.4	9.486	0.036
min	12.2	10.9	20.22	16.84	4.56	0.01
max	38.4	32.7	811.7	691.40	11.60	0.07
average	27.82	24.21	328.58	284.74	8.73	0.03

Table 1. cont.

Tabela 1. cd.

Lp.	Males or females	Age scale	Age dorsal spine	Age anual spine	Age otolith
1	F	5+	5+	5+	5+
2	M	2+	2+	-	-
3	F	4+/5+	5+	5+	5+
4	F	6+	6+	-	-
5	F	4+	4+	4+	4+
6	F	6+	6+	6+	6+
7	F	4+	4+	-	-
8	M	3+	3+	3+	3+
9	F	5+/6+	6+	-	5/6+
10	F	5+	5+/6+	5+/6+	5+
11	F	5+	5+	5+	5+
12	M	2+	2+	2+	2+
13	F	4+/5+	5+	-	5+
14	M	5+	5+	4+/5+	-
15	F	4+	4+	4+	-
16	F	5+	5+	5+	5+
17	F	6+	6+	4+/5+	5+
18	M	4+	5+	4+	-
19	F	6+	4+	5+	6+
20	F	6+	6+	5+	-
21	F	5+	5+	4+	5+/6+
22	F	6+	6+	6+	6+
23	F	7+/8+	8+	8+	8+
24	F	6+	6+	-	6+
25	F	8+	7+/8+	-	7+/8+
26	F	8+	8+	8+/7+	7+/8+
27	F	6+	6+	5+/6+	6+
28	F	6+	6+	6+	6+
29	F	8+	8+	8+	8+
30	F	7+	7+	6+	6+
31	F	6+/7+	7+	6+	7+
32	F	6+	6+	6+	6+
33	F	6+	6+	6+	6+
34	F	6+	6+	6+	6+
35	F	6+	6+	6+	6+
min	M=5				
max	F=40				

4. Results

The studied individuals had a total length from 12.2 to 38.4 cm. Their body length varied from 10.9 to 32.7 cm (Table 1). The smallest fishes turned out to be the youngest, with the estimated age of 2+. They also had the lowest weight (Table 1). The number of such young individuals in the study amounted to 2 individuals. The age of the remaining individuals varied from 3+ to 8+. The most numerous were fishes aged 6+ (15 individuals) and 5+ (8 individuals). The least numerous were individuals aged 3+ and 7+, represented by one and two fishes, respectively (Fig. 1). The obtained data concerning the total scale radius and partial radiuses in particular years of life of the fish were used to calculate the growth rate of the analysed perch (Fig. 2). It was determined to grow the fastest in the first year of life. Perch in the second and third year of life also had a quite fast growth rate. Somewhat slower growth was observed in older fishes (Fig. 3).

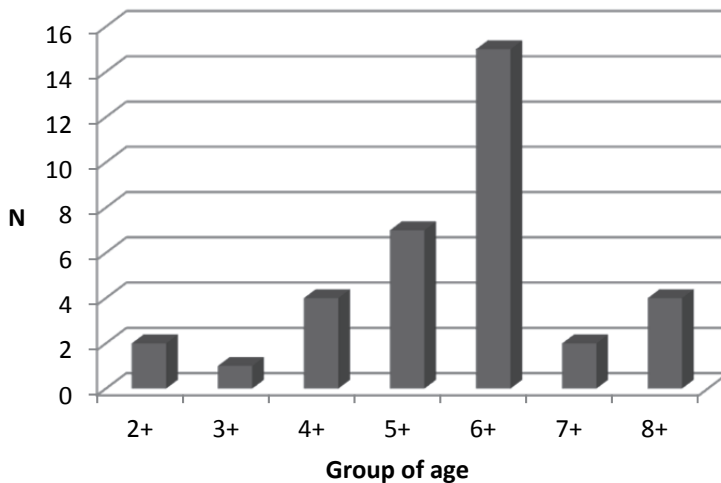


Fig. 1. Age of perch *Perca fluviatilis*

Fig. 1. Liczebność w grupach wiekowych okonia *Perca fluviatilis*

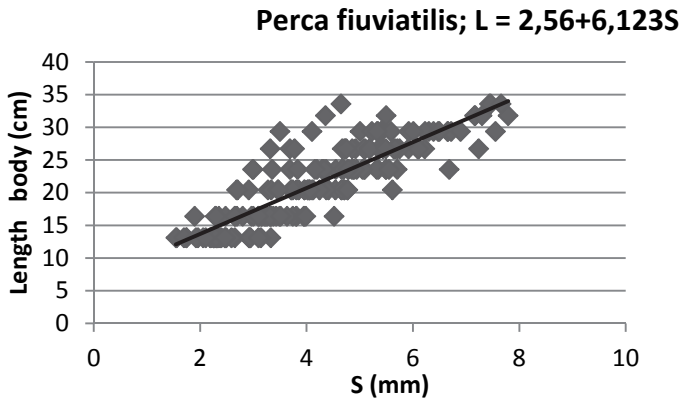


Fig. 2. Correlation between the scale radius (S) and body length of the perch analysed

Fig. 2. Zależność pomiędzy promieniem łuski (S) a długością ciała badanego okonia

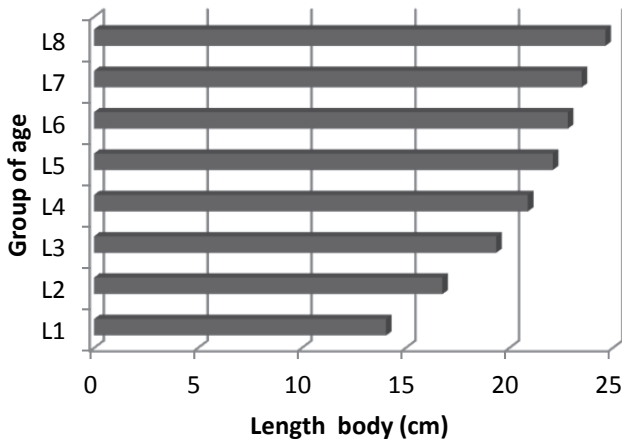


Fig. 3. Growth rate for particular age groups of the perch studied

Fig. 3. Tempo wzrostu dla poszczególnych grup wiekowych u badanego okonia

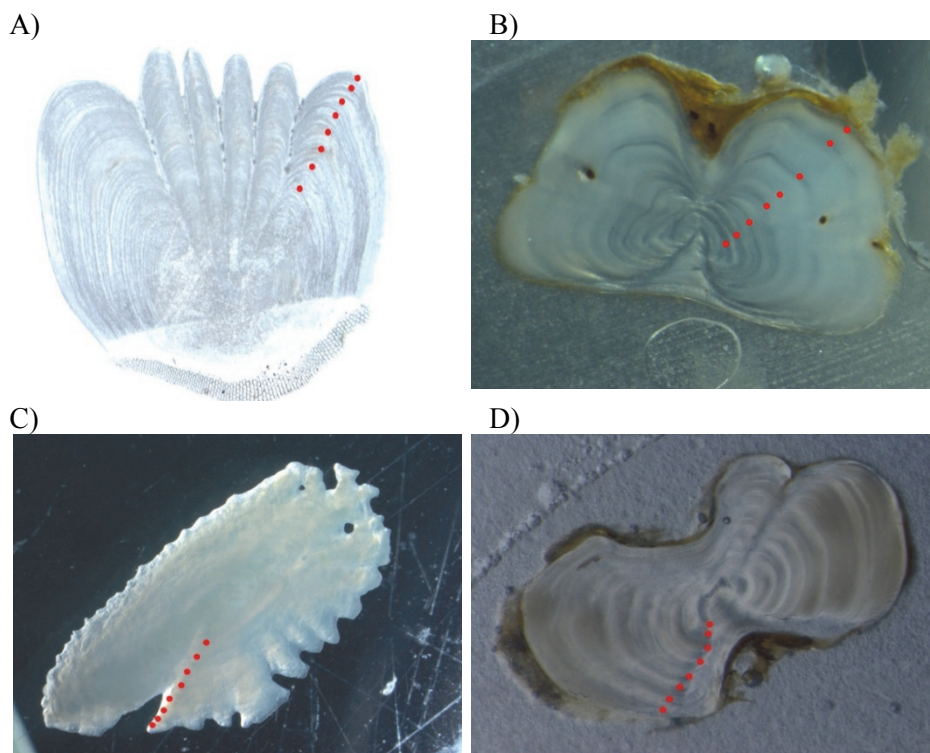


Fig. 4. Age of perch 8+ based on the analysed structures: a) scale, b) anal fin ray, c) otolith and d) dorsal fin ray

Fig. 4. Wiek okonia 8+ na podstawie analizowanych struktur: a) łuska, b) promień płetwy A, c) otolith i d) promień płetwy D

Table 2. Chemical composition of the perch specimens studied

Tabela 2. Skład chemiczny otolitów badanego okonia

Chemical element	Range weight %	Range atomic %
C	19.99-32.13	23.59-39.32
O	46.25-63.85	45.14-62.44
N	5.43-13.95	6.40-19.94
Ca	0.18-21.62	0.02-9.75
Al	0.02-10.27	0.01-5.26
Ba	0.0-1.43	0.00-1.88
Mg	0.02-4.27	0.01-1.74

Table 2. cont.**Tabela 2.** cd.

Chemical element	Range weight %	Range atomic %
Na	0.01-0.34	0.02-0.24
P	0.01-0.29	0.00-0.17
Mn	0.01-0.46	0.00-0.16
Cr	0.01-0.38	0.00-0.12
Fe	0.01-0.26	0.03-0.07
Mo	0.02-0.21	0.01-0.03
Si	0.01-0.16	0.00-0.09
S	0.02-0.06	0.01-0.03

Chemical analyses provided data on the chemical composition of the otoliths. They showed very high percent weight and percent mass contribution of the following elements: carbon, calcium, oxygen, and nitrogen. Varied amounts of other elements were also recorded, including metals such as: aluminium, manganese, chromium, molybdenum, or iron (Table 2).

5. Discussion

accurate estimation of the age of particular individuals is a very important issue in studies on population structure. It requires the selection of a reliable structure based on which the reading can be performed. The reliability of the element is strictly related to its readability, i.e. facility of identification of annual marks (Lampart-Kałużniacka et al. 2013). Since the moment of discovery of annual growth marks on the scale, the assessment of those structures has been continuously discussed, and the most readable structure has been searched for. These may be opercula, fin rays, rings, or otoliths (Zymonas & McMahon 2009). Otoliths have gained a lot of recognition over the latest years. Their popularity is not only limited to the estimation of the age and growth rate, but also to the determination of the effect of the environment on the organism through their chemical composition analysis, the estimation of the duration of the larval stage of a given fish, or in the case of diadromous fish, the estimation of time spent in particular ecosystems (Campana 2005). It should be mentioned that each otolith located in the inner ear of a fish, in the number of 6, i.e. three pairs (Payan et al. 2004), is suitable for age estimation.

Sagitta is used for this purpose the most frequently, although for fish from family Cyprinidae, it is the lapillus (Bao-Shan et al. 2012). The size and readability of the otolith seem to be features determining its selection to the largest degree, but they are also mutually excluding features, because smaller fish have a smaller otolith which is more difficult to extract, but usually more readable. A larger otolith is less readable, but easier to identify. Readability is related to the biomineralisation process, i.e. the development of consecutive layers of calcium carbonate during the growth, i.e. aging of a fish (Melancon et al. 2005). It is assumed that otoliths “grow” in such a manner that the oldest part is located on the inside, around the nucleus, and the youngest on the edges (Radtke 1984). Annual growth marks are two zones, light and dark, around the nucleus (Panfili et al. 2002). Unfortunately, not every otolith is distinguished by such readability. Very often, it is difficult to determine particular annual marks, i.e. zones. It has been evidenced that their development is affected both by physiological and environmental factors (Clark & Friedland 2004; Checkley et al. 2004; Fukuda et al. 2009). Therefore, it is difficult to unequivocally determine the cause of low readability of otoliths. Similarly, in the present study, the readability of sagittas was dissatisfactory. In 11 out of 35 cases, no or an inaccurate reading was obtained (the age varied between one year and another). According to Panfili et al. (2002), much more readable otoliths could be obtained by means of cutting them into a microscope slide which could then be polished. Extracted otoliths of perch from Lake Trzeciecko, however, were distinguished by high brittleness. Attempts to cut slides on a slow-speed circular saw caused crumbling of the material. Such analyses were therefore given up. The causes of the “brittleness” of the studied otoliths might be numerous, but they are all related to the crystal structure of calcium carbonate (Gebauer et al. 2010). It can take the form of aragonite, vaterite, and calcite. It was determined that otoliths composed of aragonite are less brittle and more readable. They can be used for more accurate identification of annual marks, which cannot be done in vaterite or calcite structures (Melancon et al. 2005). Whether calcium carbonate in otoliths takes the form of aragonite, vaterite, or calcite determines not only their readability, but also other properties, e.g. brittleness, because a change in the crystal structure results in obtaining different, new, and not necessarily favoured properties (Pattanaik 2004). In the case of problems with the identifica-

tion of annual marks on the otolith as a structure recognised as very reliable, the analysis of its crystal structure should be conducted. A high contribution of vaterite and calcite crystals suggests that an otolith will not be very useful for age estimation, and the risk of error of over- or underestimation of age is much higher. Both cases will result in inaccurate conclusions affecting the calculation of the growth rate, and determination of the population's age structure, the protected length of fish, and sexual maturity of a given species.

The occurring difficulties in age estimation based on otoliths resulted in the lack of possibility to determine correlations between body length and otolith radius. This made the application of the back-reading method for the estimation of fish length in a given year of life impossible. Therefore, it is important that the estimation of age of a given fish is not only be based on one structure. In the present study, such estimation was also based on rays of the dorsal fin D and anal fin A, and the scale. Both the rays and the scale in the perch studied were distinguished by high readability. Particularly the dorsal fin ray can be recognised here as a readable structure distinguished by high facility of identification of annual marks. High variability of shapes of dorsal fin rays in the microscope section resulted in no possibility to retain the constant direction of the structure's ray, which made it impossible to apply the back-reading method for the determination of fish length. Therefore, in the case of perch from Lake Trzesiecko, only the scale turned out to be the structure for which the Fraser-Lee equation could be applied, permitting the determination of the growth rate for the perch studied. The obtained results suggest that in the first years of life, the perch grows the most intensively, although reaching 12 cm body length in the first year of life is improbable. Such body length probably covers two years of life, because following Heese (1992), perch from environments rich in food in the first year of life reaches a body length of approximately 7 cm. This is caused by the difficulty to identify the first mark on the scale in older fishes aged 5+ and older which are dominant in the study. Scales of such fish are often so non-transparent in the middle that the first years are identified only in their further sections. This may result from a certain allometry occurring during the scale's growth (Fig. 4A). Such an interpretation is supported by the fact that the same age for the same specimen was often obtained for different structures, e.g. for ray D. The obtained results for

scales, however, contributed to the overestimation of the body length of perch for group L1 in the present study.

The estimated age of the fish studied varied from 2+ to 8+, and was largely coherent for the structures analysed. Several cases were recorded, where the age varied for all of the structures, and could not be unequivocally determined. This can result from among others the period of harvesting of fish for analysis. Differences in the term of development of the annual mark for a given structure were determined. It is assumed that in autumn, the ring for the scale and otolith is formed (Heese 1992), and in summer, for rays (Zymonas & McMahon 2009). Considering this information, it can be observed that in the case of fins, particularly D, rings were already developed, and any doubts only existed in two cases. In the case of the remaining structures, problematic readings were more numerous, particularly for the scale which could be sampled before the full development of the annual ring.

Another objective of the present study was the analysis of the chemical composition of otoliths. Maximum percent mass and weight contributions were recorded for elements both composing the mineral part of the otolith (CaCO_3), and constituting its organic part (N) (Asenath-Smith 2012). Particular attention was paid to the presence of metals, and particularly iron, which due to the temporary application of $\text{Fe}_2(\text{SO}_4)_3$ can occur in excess in water, or be released from bottom sediments in anaerobic conditions (Kajak 1996). Therefore, it should be thoroughly assessed whether the application of chemical compounds has any negative effect on the biotope of Lake Trzesiecko. This requires more detailed analytical-toxicological studies showing not only the metabolical paths of the elements introduced to the ecosystem by man, but also showing potential threats for the organisms inhabiting the environment. It is also disturbing to record the presence of other elements in the otoliths, including heavy metals, suggesting other threats.

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Przydatności struktur do szacowania wieku i analiza składu chemicznego otolitów okonia *Perca fluviatilis* (L.) z Jeziora Trzesiecko, poddanego zabiegom w celu obniżenia trofii wód

Streszczenie

Prezentowany Porównanie przydatności struktur twardych do szacowania wieku i analiza składu chemicznego otolitów okonia *Perca fluviatilis* (L.) pochodzącego z rekultywowanego Jeziora Trzesiecko.

Prowadzone badania wskazały, że najbardziej czytelną strukturą do szacowania wieku u okonia pochodzącego z Jeziora Trzeskiecko był promień płetwy grzbietowej D. Otolity okazały się strukturami bardziej problematycznymi. Trudno było na ich podstawie szacować wiek, jednocześnie były bardzo „kruche”, co uniemożliwiło wykonanie na ich podstawie preparatów mikroskopowych. Prawdopodobnie było to związane z ich budową krystaliczną. Tempo wzrostu można było obliczyć tylko na podstawie łuski. Stąd rozsądne wydaje się działanie w którym, szacowanie wieku i wyznaczenie tempa wzrostu prowadzi się w oparciu o kilka elementów. Opieranie badań tylko na jednej wybranym elemencie, skutkuje dużą zmiennością uzyskiwanych wyników. Świadczyć o tym może, chociażby brak zgodności danych, uzyskiwanych w czasie badań, prowadzonych przez różne osoby, które szacowały wiek dla ryb w oparciu o te same struktury twarde. Odnotowanie atomów żelaza w otolitach, może być efektem kumulacji tego metalu w organizmach żywych, a źródłem jego jest związek chemiczny, aplikowany do jeziora w celu poprawy jego stanu ekologicznego.

Abstract

The study revealed that the ray of the dorsal fin D was the most readable structure for age estimation in perch from Lake Trzesiecko. Otoliths turned out to be more problematic structures. Age estimation based on otoliths was difficult, and the structures were very brittle, which made it impossible to prepare microscope slides. This was probably related to their crystal structure. Growth rate could only be calculated based on the scale. In the case of older specimens aged 5+ and more, however, it was difficult to identify the mark related to the first year of life due to the low readability of the middle part of the scale. This results in inflated results obtained for group L1. Studies on age of fish should be conducted based on several structures due to the possibility of verification of the obtained results. Performing the analysis of only one selected element results in high variability of the obtained data, and therefore inaccurate conclusions. The recorded presence of iron atoms in the otoliths suggests the accumulation of the metal resulting from the application of chemical compounds by man in living organisms.

Słowa kluczowe:

Okoń *Perca fluviatilis*, otolity, łuski, promienie płetw, wiek, skład chemiczny w otolitach

Keywords:

perch *Perca fluviatilis*, otolith, scales, rays fins, age, chemical compositions of otolith