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Analysis of environmental conditions and macro-cations composition (Ca, Mg, Na, K, Sr) in the operculum bones of estuarine fishes in the Pomeranian Bay (southern Baltic Sea)

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Abstract

The aim of this study was to show the influence of environmental conditions on the ionic composition of fish bones. The analysis concerned the ionic structural composition of the operculum in fish species with different abiotic habitat preferences. Three species of fish were examined: cod, *Gadus morhua* L., 1758; sea trout, *Salmo trutta* morpha *trutta* L., 1758 and perch, *Perca fluviatilis* L., 1758. Results from hydrochemical research on salinity in the Pomeranian Bay (Southern Baltic) were also utilized. Using the determined ratios (Ca:Mg, Na:Mg, Sr:Ca and Na:Ca in the operculum and Ca:Mg, Na:Mg and Na:Ca in the waters of the Pomeranian Bay), typical correlations were determined for the analyzed habitat.

Macrocation structure in the operculum bones for each species were found to be as follows:

- cod: Ca – 32.3%, Mg – 29.8%, Na – 1.4%, K – 0.8%, Sr – 2.1%;
- sea trout: Ca – 22.8%, Mg – 13.5%, Na – 0.8%, K – 0.2%, Sr – 1.7%;
- perch, Ca – 37.9%, Mg – 15.1%, Na – 2.4%, K – 0.7%, Sr – 1.6%.

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INTRODUCTION

Estuaries, having a high variability of abiotic conditions, are characterized by their significant biodiversity resulting from, among other things, large quantities of food. They are habitats for many species of fish that benefit from conditions advantageous for reproduction, feeding and wintering. The ichthyofauna of estuary waters must be highly tolerant to changes in physical, chemical and biological factors (Elliott, Hemingway 2002). Apart from a specific species composition of ichthyofauna in estuary waters, they have high seasonal differentiation, dependent on local hydrological and climatic conditions. The basic criterion determining the possibility of different fish species' existence in estuary waters are the total dissolved solids (TDS) - the entire content of macro-ions responsible for salinity. The occurrence of individual species of fish in estuary waters depends on their physiological preferences which in turn may depend on salinity, and salinity perhaps plays a decisive part in controlling a species' range (Secor, Rooker 2000). The composition of basic macro-elements in the bones of estuary fishes may be a differentiating factor which shows the environmental preferences of individual species (Volk et al. 2000). The ionic composition of a fish skeleton can also indicate correlations between different populations - within one species, but living in niches with different hydrochemical regimes, and between different species living in the same habitat (Forrester, Swearer 2002). Determining the dependence between the ratios of macro-cation concentrations in fish skeletons and in the waters where these fish live enables the determination of their environmental preferences.

The Pomeranian Bay is a specific estuary, a complementary estuary of the Oder River, with the highest salinity of all the regions of the Oder estuary. The predominant part of the catchment area of the Pomeranian Bay is the Szczecin Lagoon (89.5%), so the main river inflow into the Pomeranian Bay is indirectly the Oder river. The Pomeranian Bay's chemism (within 3 to 9-12 marine leagues from the shore bank) is under the prevailing influence of open Baltic waters. Waters flowing from rivers into the sea play a crucial role in the coastal zone and in the area of their outlet. The predominant influence of river waters is clearly visible at the Świna river outlet. The Pomeranian Bay is quite shallow (an average depth of 13 m), has a small capacity and a relatively high inflow of river waters. Mikulski (1967) reported that in the general inflow of river waters to the Pomeranian Bay, Oder river waters constitute 83%, and the average volume of the inflowing river waters is 16.6 km^3 a year. According to Majewski (1972), such a high percentage of inflowing river waters in hydrological balance with the Pomeranian Bay makes them a major factor in the formation of the bay's hydrological, hydrochemical and biological conditions.

Ichthyofauna species living in this dynamic habitat include sea species, eg. cod; anadromous species, eg. sea trout; and fresh-water, euryhaline species, eg. perch. cod (*Gadus morhua* L., 1758) lives in the near-bottom layer and is a cryophilic species. This species has numerous sub-species and ecological races. Sea trout (*Salmo trutta* morpha *trutta* L., 1758) is an anadromous species; it returns to the river where it was born for spawning (homing instinct). Sea trout is a very adaptable species, with many resident forms. Perch (*Perca fluviatilis* L., 1758) lives in both fresh and brackish waters, and tolerates considerable fluctuations in salinity (euryhaline species) (Więcaszek et al. 2006). All the discussed species of fish are predators.

In this paper, the ionic compositions of the operculum bones of selected ichthyofauna species populating the waters of the Pomeranian Bay were examined (cod, sea trout and perch). The analyzed species are characterized by different types of adaptations to their habitats (sea-species, anadromous species and fresh-water, euryhaline species). This type of research has not been conducted before and its results can significantly contribute to a wider recognition of the environmental preferences of the analyzed species of fish.

The aim of this study was to (i) show differences in macro-elements composition in the skeletons of fish with different types of adaptations to their habitats, (ii) analyze correlations between the ionic compositions of the fish skeletons and the ionic compositions of Pomeranian Bay waters and (iii) show correlations between the ratios determined for fish bone macro-cations (Ca:Mg, Na:Mg, Sr:Ca, Na:Ca) and ratios determined for the waters populated by these species (Ca:Mg, Na:Mg, Na:Ca).

MATERIALS AND METHODS

The samples of operculum bones were prepared according to a method detailed by Rooker et al. (2003). Determinations of calcium, magnesium, sodium, potassium and strontium were conducted using a Perkin-Elmer 3100 atomic absorption spectrophotometer according to a method described in Analytical Methods for Atomic Absorption Spectrophotometry (1982). The analysis involved 20 specimens of each species caught in the Pomeranian Bay. Analysis was performed twice for each sample. The following ratios were then calculated: Ca:Mg, Na:Mg, Sr:Ca and Na:Ca. The results were averaged and the extreme values determined.

The study also used the hydrochemical results of a study conducted in the Pomeranian Bay, southern Baltic Sea, from July 2000 to August 2003 with an average bimonthly frequency (Tórz 2007). Macro-cations determinations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) were conducted in water samples after filtration through 0,45 μm pores to determine the quantity of ions in dissolved form. The cations were determined with a Perkin-Elmer 3100 atomic absorption spectrophotometer.

Standard conditions for a Perkin-Elmer 3100 atomic absorption spectrophotometer:

- sensitivity: calcium (λ=422.7 nm) – 0.092 mg dm^{-3}; magnesium (λ=285.2 nm) – 0.0078 mg dm^{-3}; sodium (λ=589 nm) – 0.012 mg dm^{-3}; potassium (λ=766.5 nm) – 0.043 mg dm^{-3} and strontium (λ=460.7 nm) – 0.11 mg dm^{-3};
- sensitivity check: calcium – 4.0 mg dm^{-3}; magnesium – 0.3 mg dm^{-3}; sodium – 0.5 mg dm^{-3}; potassium – 2.0 mg dm^{-3} and strontium – 5.0 mg dm^{-3}.

Precision of used methods with atomic absorption spectrophotometer were ±1%.

Using the obtained results, the following ratios were determined: Ca:Mg, Na:Mg and Na:Ca. Total dissolved solids (TDS) were calculated using data introduced by Tórz (2007). The results were averaged and the extreme values were determined.

The obtained results were analyzed statistically using *STATISTICA 7.1* software (StatSoft, Inc. 2006).

RESULTS

All the analyzed fish species inhabiting the waters of the Pomeranian Bay had distinctly high levels of calcium compared to the rest of the determined cations in the operculum. The highest average calcium content was observed in the operculum of perch (37.9%), the lowest in sea trout (22.8%), and medium values were observed in cod (32.3%) (Table 1). The operculum of perch also had the highest average concentration of sodium (2.4%), which was lower in cod (1.4%) and lowest in sea trout (0.8%). The remaining cations (magnesium, potassium and strontium) were found in higher quantities in cod operculum. The operculum of cod consisted of 29.8% magnesium, while the operculum of perch contained only 15.1%. Moreover, the operculum of cod contained 2.1% strontium and 0.8% potassium, while in the operculum of sea trout these cations were 1.7% and 0.2%, respectively, and in the operculum of perch they were 1.6% and 0.7% (Table 1).

The determined Ca:Mg, Na:Mg and Na:Ca ratios had the highest average values in the operculum of perch. Ca:Mg and Na:Mg ratios had the lowest levels in the cod operculum, and Na:Ca was the lowest in sea trout (Table 1). The Sr:Ca ratio had the highest values for the sea trout (0.075), lower for cod (0.064) and the lowest for perch (0.043) (Table 1).

Table 1

The percentage composition of the analyzed cations and the determined ratios in the bones of cod. sea trout and perch operculum.

Value	Ca	Mg	Na	K	Sr	Ca:Mg	Na:Mg	Sr:Ca	Na:Ca
Content at operculum bones of cod									
Average	32.3	29.8	1.4	0.8	2.1	1.11	0.05	0.064	0.045
Minimum	28.4	21.8	1.1	0.4	1.6	0.94	0.03	0.053	0.035
Maximum	37.6	34.4	1.9	1.3	2.8	1.38	0.08	0.082	0.057
Content at operculum bones of sea trout									
Average	22.8	13.5	0.8	0.2	1.7	1.80	0.06	0.075	0.033
Minimum	19.8	10.1	0.6	0.2	1.1	1.18	0.04	0.049	0.027
Maximum	24.6	20.7	0.9	0.2	2.5	2.19	0.07	0.126	0.037
Content at operculum bones of perch									
Average	37.9	15.1	2.4	0.7	1.6	2.53	0.16	0.043	0.063
Minimum	33.6	13.0	2.1	0.5	1.1	2.00	0.13	0.033	0.054
Maximum	41.1	16.8	3.0	1.0	2.5	3.00	0.19	0.067	0.073

Organic matter in the operculum of the examined species of fish had the highest levels in sea trout (53.9%) and the lowest in perch (37.0%) (Table 2).

Correlations between organic matter in the operculum and the concentrations of calcium and sodium in the bones of the operculum were statistically significant for sea trout (inversely proportional correlation for Na (r = -0.74) and directly proportional for Ca (r = 0.86)) and perch (inversely proportional correlation for Na (r = -0.76) and Ca (r = -0.83)) (Table 3). For cod, an inversely proportional correlation was observed between the amount of organic matter and concentrations of magnesium (r = -0.76) and strontium (r = -0.87) in the operculum bones, and a directly proportional correlation for organic matter and potassium (r = 0.73) in these bones (Table 3). A directly proportional correlation between the amount of organic matter and strontium concentration was observed in sea trout (r = 0.75) (Table 3).

Table 2

The organic matter content in the bones of the operculum (n=20).

Factor	Value	Cod	Sea trout	Perch
% of organic matter content in the bones of the operculum	Average	40.0	53.9	37.0
	Minimum	34.2	51.1	33.8
	Maximum	46.6	60.1	40.6

The analyzed variability of macro-cations concentration in the operculum vs. ion concentration dissolved in the waters of the Pomeranian Bay also showed significant differences for each examined fish species (Table 4).

Table 3

The examined cations' concentrations in the operculum vs. the mass of the bones, ash and organic matter content in the operculum for the examined fish species in the waters of the Pomeranian Bay, based on the Pearson's correlation coefficient (statistically significant correlations in bold).

Index	Cod			Sea trout			Perch		
	A*	B	C	A*	B	C	A*	B	C
Ca	**0.75**	**0.76**	-0.62	-0.37	-0.05	**0.86**	0.22	0.27	**-0.83**
Mg	**0.67**	**0.67**	**-0.76**	**-0.65**	-0.47	-0.46	-0.21	-0.19	0.13
Na	-0.01	-0.02	0.33	-0.11	0.15	**-0.74**	0.54	0.59	**-0.66**
K	-0.37	-0.39	**0.73**	0.32	-0.56	0.24	-0.29	-0.29	0.15
Sr	**0.83**	**0.85**	**-0.87**	0.25	-0.04	**0.75**	0.07	0.10	-0.53

* - A – mass of the operculum [g]; B – mass of the ash after combustion of the operculum [g]; C – organic mass content in the operculum bones [%]

Table 4

Correlations between the concentrations of the analyzed cations in the operculum of the examined fish species and the concentrations of the macrocations [mg dm^{-3}] and the values of the determined ratios in the waters of the Pomeranian Bay, based on the Pearson's correlation coefficient (statistically significant correlations in bold).

Index	Species	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	TDS	Ca:Mg	Na:Mg	Na:Ca
Ca	Cod	0.13	0.01	0.25	0.17	0.22	0.02	0.24	0.10
Mg		**0.54**	0.30	0.40	**0.70**	**0.43**	0.06	-0.07	-0.06
Na		-0.36	0.21	0.14	**-0.47**	0.10	-0.36	-0.28	0.34
K		**-0.54**	0.03	-0.11	**-0.75**	-0.15	-0.31	-0.23	0.28
Sr		0.03	-0.03	0.19	0.30	0.18	0.02	0.26	0.13
Ca	Sea trout	**-0.44**	-0.18	-0.10	0.14	-0.12	-0.06	0.20	0.36
Mg		**-0.44**	**-0.51**	**-0.51**	**-0.46**	**-0.53**	0.26	0.32	-0.16
Na		-0.32	**-0.68**	**-0.41**	-0.32	**-0.45**	**0.47**	0.65	-0.19
K		0.25	0.34	0.07	0.17	0.35	**-0.42**	-0.39	0.09
Sr		**0.51**	**-0.49**	-0.25	**-0.67**	-0.27	**0.71**	**0.46**	**-0.91**
Ca	Perch	-0.09	0.24	0.27	-0.01	0.24	-0.22	-0.13	0.28
Mg		**-0.73**	0.03	-0.20	**-0.85**	-0.24	**-0.42**	-0.31	**0.44**
Na		-0.29	**0.55**	0.39	-0.11	0.39	**-0.60**	**-0.62**	**0.53**
K		**-0.62**	-0.10	-0.22	**-0.66**	-0.27	-0.30	-0.09	0.31
Sr		0.34	**0.73**	**0.85**	0.16	**0.86**	**-0.41**	**-0.44**	0.34

Some of the ratios determined both for cations forming the opercula of the examined species of fish, and for the waters of the Pomeranian Bay, had statistically significant correlations (Table 4, Fig. 1-3).

Statistically significant correlations were found between ratios determined for each cation forming the opercula of the examined species of fish, and the total dissolved solids (TDS) in the waters of Pomeranian Bay (Table 4, Fig. 1-4). The Ca:Mg ratio in the operculum of sea trout directly and

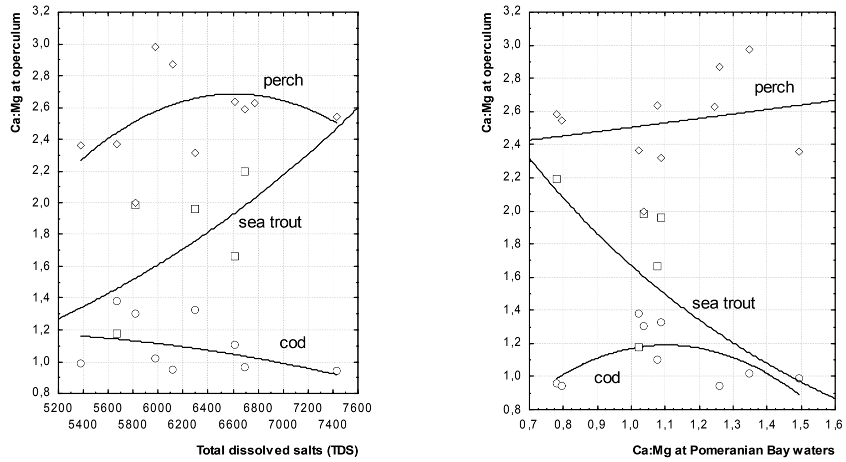

Fig. 1. Ca:Mg ratio in the operculum of the examined fish species vs. the total dissolved salts (TDS) and the Ca:Mg ratio in the waters of the Pomeranian Bay.

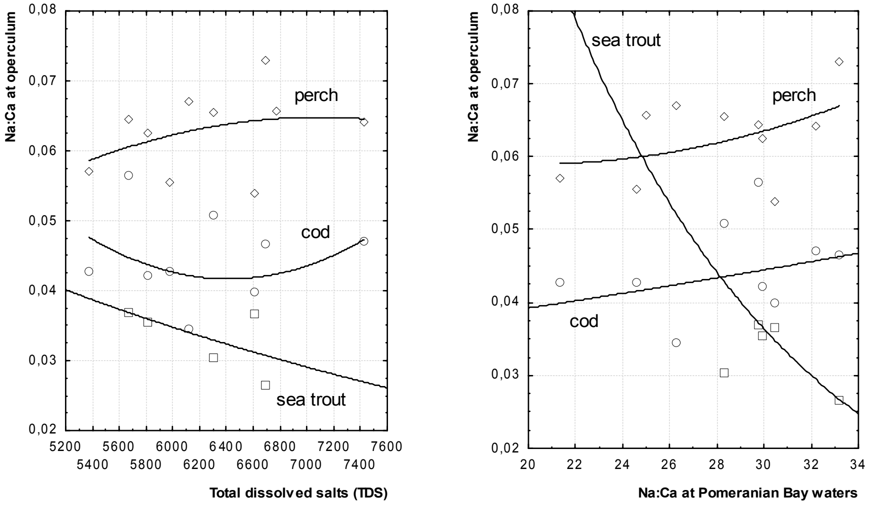

Fig. 2. Na:Ca ratio in the operculum of the fish species vs. the total dissolved salts (TDS) and the Na:Ca ratio in the waters of the Pomeranian Bay.

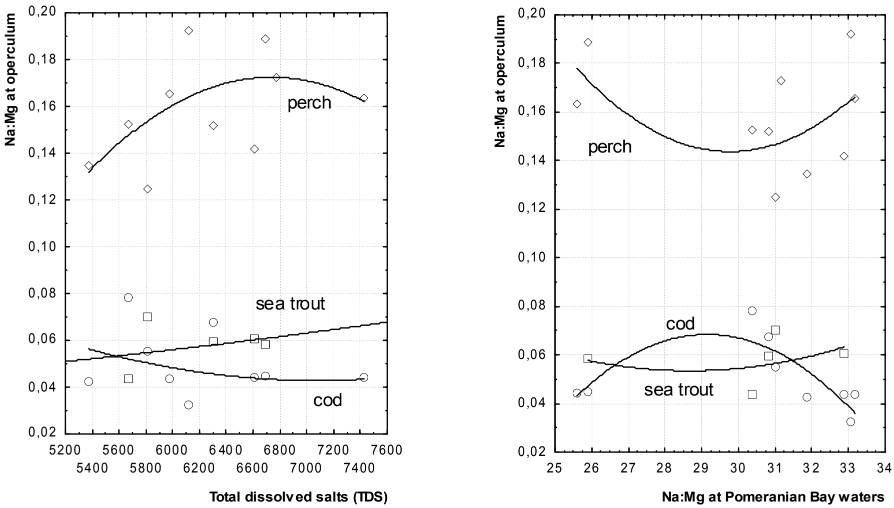

Fig. 3. Na:Mg ratio in the operculum of the examined fish species vs. the total dissolved salts (TDS) and the Na:Mg ratio in the waters of the Pomeranian Bay.

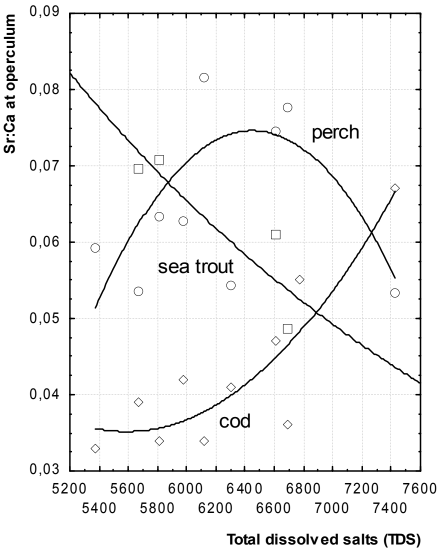

Fig. 4. Sr:Ca ratio in the operculum of the examined fish species and the total dissolved salts (TDS) in the waters of the Pomeranian Bay.

proportionally correlated with TDS (Table 4, Fig. 1). The Na:Ca ratio showed an inversely proportional correlation with TDS (Table 4, Fig. 2). The Na:Mg and Sr:Ca ratios in the operculum of perch were directly and proportionally dependent on TDS in the waters of Pomeranian Bay (Table 4, Fig. 3, 4).

DISCUSSION

Analysis of major chemical constituents in fish bones have been used to distinguish population-specific differences for stock discrimination, as indicators of pollution, and to describe the environmental adaptations of individual fish (Volk et al. 2000). Estimation of adaptations for a given species to environmental conditions should be conducted for fish populating habitats with a high variability of biotic and abiotic factors. A relatively homogeneous habitat does not have to have a distinct diversity in the composition of macroelements in the skeletons of different species of fish (Gillanders et al. 2001). Concentrations of certain elements (e.g. Ca, Mg, Na, K, Sr) in fish bones vary in different locations in which fish are collected (Gillanders and Kingsford 2003). Such differences have been found for a wide range of species inhabiting riverine (Thorrold et al. 1998), estuarine (Gillanders, Kingsford 2003), open coast (Forrester, Swearer 2002), and marine environments (Rooker et al. 2003). The waters of the Pomeranian Bay constitute a habitat with highly dynamic abiotic factors. Three species - cod, sea trout and perch - that populate this habitat, show very distinct differences in the levels of macro-cations in the bones of the operculum. The lowest concentrations of calcium and magnesium were observed in the operculum of sea trout and the highest concentrations of magnesium were found in the operculum of cod (Table 1). Similar observations for sea trout and cod were made by Thoppe et al. (2007). They ascertained that fish from the family Salmonidae, populating different estuaries, had lower levels of calcium and magnesium compared with other species in these estuaries. The highest concentration of calcium in the operculum of the perch can be explained by its preference for an abiotic habitat. Perch is a fresh-water species, with calcium as the prevailing macro-cation, thus more frequently used in its skeletal structure than magnesium, as is confirmed by a statistically significant inversely proportional correlation: $Mg_{operculum}$ and Ca_{water}. A similar correlation was observed in anadromous sea trout. Cod, in turn, showed a different tendency: toward magnesium. The correlation between $Mg_{operculum}$ and Ca_{water} is directly proportional (Table 4). A similar differentiation in the concentration of calcium in fish skeletons, depending on salinity, was reported by Humphreys et al. (2006), who examined populations of fish from the family Eletridae, living in inland estuaries.

Thoppe et al. (2007) observed bone sodium concentration levels in estuary fish which were similar to those observed in this study, with a slight decrease in Na concentrations in the bones of fish that preferred the sea. In the bones of the operculum, the highest sodium concentrations were observed in perch (Table 1), and lower ones in cod (a typical sea species). A statistically significant correlation between sodium concentration and total dissolved solids (TDS) also confirmed this dependence (Table 4).

Some other interesting observations were made by Bijvelds at al. (1997), Arai et al. (2005), and Arai, Hirata (2006) which ascertained that an increase in water salinity resulted in calcium and sodium ions being utilized less in the skeletal structure in favor of magnesium, strontium and potassium ions. Similar correlations were also observed in species inhabiting the Pomeranian Bay. The highest concentrations of calcium and sodium were observed in the operculum of a fresh-water species (perch). . The highest concentrations of magnesium, strontium and potassium were observed in the operculum of a typical sea-species (cod). A higher level of strontium was also observed in the operculum of an anadromous species of sea trout than in perch (Table 1). In most aquatic environments, strontium is a trace element whereas calcium is a major ion, so strontium is unlikely to inhibit calcium uptake. Thus, it is the concentration of strontium uptake relative to calcium in the water, and not the absolute concentration of strontium, that should determine uptake of strontium into fish bones. The strontium uptake is intensive to a wide range of strontium concentrations in the water (i.e., TDS levels), but there is a direct relationship between strontium uptake and calcium concentration in the water (Kraus, Secor 2004).

In the conducted analysis of organic matter concentration in the bones of the operculum, the highest concentration of organic matter in the operculum was observed in sea trout, the lowest in perch (Table 2). As observed by Thoppe et al. (2007), the concentration of macro-cations is strictly related to bone hardness. Higher concentrations of calcium in the bone increase its hardness. The operculum of perch had the highest calcium concentration - hence the lowest proportional participation of organic matter in these bones. This correlation was confirmed by statistical analysis (Table 3). As reported by Thoppe et al. (2007), salmon and trout are species characterized with high swimming activity and the high content of organic matter in their bones may indicate better elasticity of the bones to support that high activity. The highest observed concentration of organic matter in the bones of the operculum in sea trout is consistent with this proposition by Thoppe et al. (2007).

In this research we also examined the variability of Ca:Mg, Na:Mg, Sr:Ca and Na:Ca ratios in the bones of the operculum in the studied species of fish against the variability of Ca:Mg, Na:Mg, Na:Ca and TDS in the waters of the Pomeranian Bay (Table 4, Fig. 1-4).

An increase in the Ca:Mg ratio in the operculum accompanied by an increase in TDS was observed in perch, and an inverse correlation was observed in cod (Table 4, Fig. 1). The ascertained correlations confirm the aforementioned environmental preferences of the examined species. Cod, a sea-species, 'prefers' to use magnesium to build its skeleton, and fresh-water perch shows a preference towards calcium. This conclusion is confirmed by correlations between the Ca:Mg ratio in the operculum of these species and the Ca:Mg ratio in water (Table 4, Fig. 1). The correlations between the Ca:Mg ratio in the opercula of sea trout and the TDS, and Ca:Mg in the operculum and Ca:Mg in water were completely different (Table 4, Fig. 1). In the first case, a directly proportional correlation was observed, suggesting the use of calcium to build skeletal structures, and in the latter an increase in calcium availability in the water and a decreased Ca:Mg ratio in the bones of the operculum were observed (Table 4, Fig. 1). Such a surprising range of variability may only be explained by the high tendency of this species to build a skeleton with a high level of elasticity (Thoppe et al. 2007), and therefore neither calcium nor magnesium are fully utilized by this species.

Correlations between the $\mathrm{Na{:}Ca_{operculum}}$ ratio and TDS, and between $\mathrm{Na{:}Ca_{operculum}}$ and $\mathrm{Na{:}Ca_{waters}}$ ratios, also had various ranges in the analyzed species (Fig. 2). Both for cod and sea trout, a fall in Na:Ca content in the operculum was observed, accompanied by an increase in TDS. An inverse correlation was observed for perch (Fig. 2). In cod and perch, an increase in the $\mathrm{Na{:}Ca_{operculum}}$ ratio was observed, accompanied by an increase in the availability of sodium in the water (Fig. 2). Only in the case of perch were both the correlations directly proportional. It may be supposed that, similar to Thresher (1999), it was not so much an increase in TDS concentration, but a real-time increase in the concentration of a given macro-cation in the water that caused an increase in its concentration in fish skeletons. A higher availability of sodium caused an increase in the $\mathrm{Na{:}Ca_{operculum}}$ ratio of cod (Fig. 2).

A decrease in the $\mathrm{Na{:}Mg_{operculum}}$ ratio of the analyzed species of fish was observed in conjunction with an increase in the $\mathrm{Na{:}Mg_{waters}}$ ratio of the Pomeranian Bay. This correlation confirmed earlier observations, showing a more frequent use of magnesium in building the skeleton, regardless of the amount of available sodium (Bijvelds at al. 1997; Arai at al. 2005; Arai, Hirata 2006).

Similar correlations were observed for the analyzed species of fish. Fresh-water perch used very high assimilations of strontium (instead of calcium) in the building of their skeletal structures, which was related to an increase in TDS (Fig. 4). Of significance in strontium utilization in the building of the skeleton is the association with real-time concentrations of strontium in the water. As reported by Thresher (1999), strontium concentration in fresh waters is approximately 0.1 ppm and in sea waters approximately 8.0 ppm. –Cod, a typical sea species, did not show such a pronounced tendency to use strontium (instead of calcium) in building its skeleton.

CONCLUSIONS

The above discussion leads to the following general conclusions:

1. The structures and possibilities of macro-cation assimilation by cod (*Gadus morhua* L., 1758) (a sea species) were as follows: Ca - 32.3%, Mg - 29.8%, Na - 1.4%, K - 0.8%, Sr - 2.1%. Mean values of the examined ratios were: Ca:Mg - 1.11, Na:Mg - 0.05, Sr:Ca - 0.064, Na:Ca - 0.045. This species had the highest concentration of magnesium in the operculum.
2. The structures and possibilities of macro-cation assimilation by sea trout (*Salmo trutta* morpha *trutta* L., 1758) (an anadromous species) were as follows: Ca - 22.8%, Mg - 13.5%, Na - 0.8%, K - 0.2%, Sr - 1.7%. Mean values of the examined ratios were: Ca:Mg - 1.80, Na:Mg - 0.06, Sr:Ca - 0.075, Na:Ca - 0.033. The analysis revealed that the highest concentrations of organic matter were in the operculum of this species, which is associated with a high bone elasticity, permitting the high activity of this species.
3. The structures and possibilities of macro-cation assimilation by perch (*Perca fluviatilis* L., 1758) (a euryhaline species) weres as follows: Ca - 37.9%, Mg - 15.1%, Na - 2.4%, K - 0.7%, Sr - 1.6%. Mean values of the examined ratios were: Ca:Mg - 2.53, Na:Mg - 0.16, Sr:Ca - 0.043, Na:Ca - 0.063. This species had the highest concentration of calcium in the operculum bones, which is connected with its habitat (fresh-water), where calcium is usually a prevailing macroelement. The higher calcium concentration in the bone increases its hardness.

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