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Stress proteins concentration in caged *Cyprinus carpio* as a tool to monitor ecological stability in a model dam reservoir

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Abstract: Variability of stress proteins concentration in caged carp exposed to transplantation experiment model dam reservoir was caused only by natural (climatic and biological) conditions. Thus, the reference data of stress proteins concentration range in young carp individuals were obtained. Metallothionein, HSP70 and HSP90 protein concentrations as biomarkers were assayed in the livers, gills and muscles of six-month-old (summer) or nine-month-old (autumn) carp individuals in relation to the site of encaging, season (summer or autumn), the term of sampling (1, 2 or 3 weeks after the transplantation) and tissue. Physicochemical analyses of the condition of water as well as pollution detection were conducted during each stage of the experiment. As the result of this study, the range of the variability of the stress protein concentration in young carp individuals was obtained. According to the analyses of the aquatic conditions of a reservoir with no detectable pollutants, we conclude that the variability in the stress protein concentration levels in the groups that were compared is solely the result of the natural conditions. Future regular monitoring of the reservoir using the transplantation method and young carp individuals will be both possible and reliable. Moreover, the range of variability in the stress protein concentrations that were measured in the young *C. carpio* individuals acquired from the model dam reservoir in relation to all of the studied factors may be applied in the monitoring of any other similar reservoir.

Introduction

Transplantation experiments, have advantages over laboratory models since they cover all of the complex factors that are usually neglected in controlled laboratory experiments (Ellis et al. 2002). By utilizing transplantation experiments, researchers can acquire the data that enables them to optimize the exploitation of economically important species (Wu and Shin 1998), to validate the data gathered from laboratory studies (Ellis et al. 2002), to assess the environmental pressure on bioindicator species (Shaw et al. 2002, Todd et al. 2007, Klobučar et al. 2010, Traven et al. 2013) or to estimate the range of tolerance to natural environmental factors, which is especially important in coping with invasive species (Schofield et al. 2010). For ecotoxicological purposes, caged animals can be used as biosensors of the potential toxicity of various kinds of pollutants (Bernet et al. 2000, Fåhræus-Van Ree and Payne 2005, Allert et al. 2013). Biosensors, apart from other biotests utilizing plant or animal organisms (Zgórska et al. 2011; Załęska-Radziwiłł et al. 2011; Hybská et al. 2018) are an important element of environmental engineering which is becoming increasingly popular. Its task is to assure the safety of engineering solutions for the ecosystem (Esyakova and Voronin 2020). This definition of ecoengineering seems especially important for anthropogenic reservoir of strategic significance, whose functioning results from and depends on a strict balance between ecological and hydrotechnical roles they play. The biosensors which, in fact, are the fish in the transplantation experiment may be useful for monitoring of early response of the organisms to stressors that cannot be easily measured using classical monitoring based on the detection of substances in the water. Moreover, the response obtained in this method reflects real interactions between the stressors affecting the body, including synergism and antagonism of the stressors of various character (e.g. chemical and physical; Gandar et al. 2016). Also, transplantation experiments are useful to understand and explain the mechanisms of tolerance of pollutants (Bougas et al. 2016). Mobile species are encaged before the transplantation

and after a specified period of exposure, they are subjected to biomarker measurements. This kind of investigation is useful in monitoring of the environmental conditions of ecosystems that are of special interest (Shi et al. 2018, Kazour and Amara 2020). Dam reservoirs belong to habitats of this kind since they play an important role also as potable water reservoirs, retention plants or even contribute to the energy safety of a community or country. As such, their sustainability should be strictly monitored. The subject of the present project, the Goczałkowice Reservoir, was created in 1955 when the dam was built on the upper Vistula River. Its main role is to provide the Upper Silesian Agglomeration with potable water. It also plays an important role in flood prevention, and ensures the water supplies that protect the lower courses of the Vistula River against the accumulation of pollutants (Bilnik et al. 2004). Recently, dam reservoirs are also expected to mitigate the effects of global climate changes, including drought threat (Absalon et al. 2020, Ulanczyk et al. 2021). In this project, the common carp was chosen as the bioindicator species and potential changes in the metallothionein (Mts) and heat shock protein 70 (HSP70, HSP90) concentration were chosen as the non-specific biomarkers of stress. Mts, HSP70 and HSP90 are a group of low-molecular proteins whose role is to eliminate any excess of potentially harmful metal ions and to store a pool of biogenic ones as well as to act as chaperoning proteins (Coyle et al. 2002, Osman et al. 2019). Changes in Mts, HSP70 and HSP90 concentrations are often regarded as biomarkers of exposure to metals in the environment, including aquatic ecosystems (Cretì 2010, Walker et al. 2014, Yukawa et al. 2014, El-Khayat et al. 2020) These proteins' concentration in animal organisms also changes in response to other factors. It changed significantly in the livers of lake trout (*Salvelinus namaycush*) exposed to waterborne ethinyl estradiol, benzo[a] pyrene or bacterial challenges (Werner et al. 2003, Ming et al. 2014). Similar effect was found in experimental exposure of young *C. carpio* exposed to a mixture of pharmaceuticals and household chemicals (Tarnawska et al. 2019). The aim of the project was to assess the quality of the water in the reservoir basing on the levels of stress proteins of encaged fish in relation to the season, the time of the sampling, the site of encaging and the organ. Moreover, we wanted to obtain the background level of Mts, HSP70 and HSP90 for future biomonitoring purposes.

Material and methods

Common carp (Cyprinus carpio) characteristics

The common carp, *Cyprinus carpio*, belongs to the group of freshwater fish species that are important in human nutrition. It is cultured in many Asiatic countries as well as in most parts of Europe, including Poland (Köprücü and Rahmi 2004). According to FAO reports, it contributes to 11% of the total world freshwater aquaculture production (FAO 2007). The common carp is generally omnivorous and prefers macroinvertebrates although in its early developmental stages it also consumes zooplankton. This species easily switches to an artificial diet and adapts to changing environmental conditions relatively easily by altering its behavior (Rahman et al. 2010). Although the thermal optimum for its growth and development is 23–30°C, it is able to survive cold winter periods (http://www.fao.org/fishery/culturedspecies/Cyprinus_carpio/en). Its preferred oxygen water concentration is 3 to 5 mg/l and pH 7–8. The patterns of its life history parameters differ in relation to geographical and climatic aspects (Crivelli 1981, Fernández-Delgado 1990). In the Polish climatic zone, male carp reach their maturity at the age of 3, while female at the age of 4 years. Spawning takes place when the water temperature is 18–20°C, i.e. in May and June. Carp is a long-lived fish and can live up to 45 years (Brylińska 2000).

One-year-old individuals of carp (*Cyprinus carpio* L., Lithuanian strain B, parr stadium) were acquired from the Fish Culture Experimental Station of the Polish Academy of Science in Gołysz. For the experiment, fish with an average body weight of 0.17 kg were transferred to six mesocosm cages that were placed at three different study sites in the Goczałkowice Reservoir for three weeks twice in 2012. Each cage was constructed as an equilateral prism with an octagonal base (a = 45.5 cm, h = 1 m; v = 999605.7 cm^3) in the Experimental Workshop of the Institute of Physics, University of Silesia in Katowice. This was the first successful usage of these cages for environmental studies. The utility model of the cages is protected by Polish Patent Office (exclusive right number: RWU.068708; A cage for monitoring the health condition in animals and for experiments, preferably on fish in the mesocosms; https://ewyszukiwarka.pue.uprp.gov.pl/search/pwp-details/W.123278).

Characteristics of the study sites

Two of the cages were placed in an estuary of the Bajerka River (N 49°54'28.8468"; E 18°52'9.084"; referred to as 'Bajerka'), two – in the yacht port near the Dam Management Office (N 56'14.8776"; E 18°55'52.176", 'metalimnion'); both localizations are within the area of the Goczałkowice Dam Reservoir. The control site was localized in a carp culture pond in the Fish Culture Experimental Station in Gołysz (N 49°52'2.3268"; E 18°47'49.146", 'Gołysz') (Fig. 1). The water temperatures at the Gołysz, metalimnion and the Bajerka sites at the beginning of the exposure in the summer were 24.4°C, 23.2°C and 23.2°C, respectively, while at the beginning of the exposure in the autumn, they were 18.1°C, 12.6°C and 11.8°C, respectively.

Experimental model

Two cages containing 25 individuals each were placed at the study sites and submerged 1–1.5 m below the water surface for three weeks in the summer (July) 2012. Reference fish (five individuals) were collected at the beginning of experiment, then, after 1, 2 and 3 weeks, a sample of five fish was collected from each site. This sample quantity was limited by the minimum necessary for statistical purposes according to ethical restrictions for studies on vertebrates. This experimental model was repeated in the autumn (October) 2012.

Sample preparation

On the day of sampling, randomly selected carps were collected from each experimental site and immediately transported to the laboratory in appropriate large barrels under optimal, controlled conditions of temperature and aeration of the water. To ensure the welfare of the fish during the transport as well as to neutralize potential differences in transportation duration from each of the sites, the fish from each site were collected synchronically by three teams of participants to assure the time of holding

them in the barrels was the same. Water in the transportation barrels originated from the respective experimental site and its temperature was maintained at the same level as in the reservoir water (i.e., in autumn no additional action was taken up while in summer it was necessary to gradually add ice cubes to the barrels). Immediately after arrival to the laboratory, quick morphometric measurements were performed and then, each fish was quickly stunned and decapitated and fragments of the selected organs, i.e., the liver, gills and muscles were dissected. The samples were then weighed, homogenized on ice in a 0.1 M Sorensen buffer pH 7.4 and then centrifuged at 15,000 g for 10 min at 4°C. The aliquots of the supernatant were stored at 70°C until the immunodetection of Mts, HSP70 and HSP90 using the ELISA technique.

ELISA test

Indirect ELISA was performed according to standard protocol (Crowther 2009) optimized for carp samples (Tarnawska et al. 2019). Briefly, 100 µl of each supernatant aliquot was used to coat the microplate wells (96-well Corning, transparent flat bottom). After coating overnight at 4°C, the potentially remaining sites of the non-specific binding of antibodies were blocked with a 1% bovine serum albumin solution (protein content 95%, Fluka; 1 h, 37°C). Anti-Mts antibody (fish metallothionein-reactive mouse metallothionein monoclonal antibody, Stressgen; 1:1000 for Mts), mouse anti-Heat Shock Protein Monoclonal antibody (Sigma-Aldrich; 1:1000) for HSP70 and rabbit anti-HSP90a, polyclonal antibody (Calbiochem; 2.5 h, 37°C), as secondary antibodies: goat anti-mouse IgG Polyclonal Antibody AP Conjugate (Stressgen) and Goat Anti-Rabbit IgG, H & L Chain Specific Alkaline Phosphatase Conjugate Adsorbed (Calbiochem), 1:1000, 2 h, 37°C were subsequently used. Finally, the secondary antibody was replaced with a 100 µL pNpp (p-nitrophenyl phosphate, Sigma) solution in a 10 mM diethanolamine buffer, pH 9.8. The absorbance of the reaction product was measured at 405 nm using a Tecan Infinite M200 Microplate Reader and was calculated based on the standard curve created using the standard Mts, HSP70 or HSP90 solutions that had been subjected to the above-mentioned protocol. The final stress protein concentration was expressed as a percentage of total protein. The total protein concentration in the samples was determined using the dye-binding method of Bradford (1976) with bovine serum albumin (protein content 95%, Fluka) as the standard.

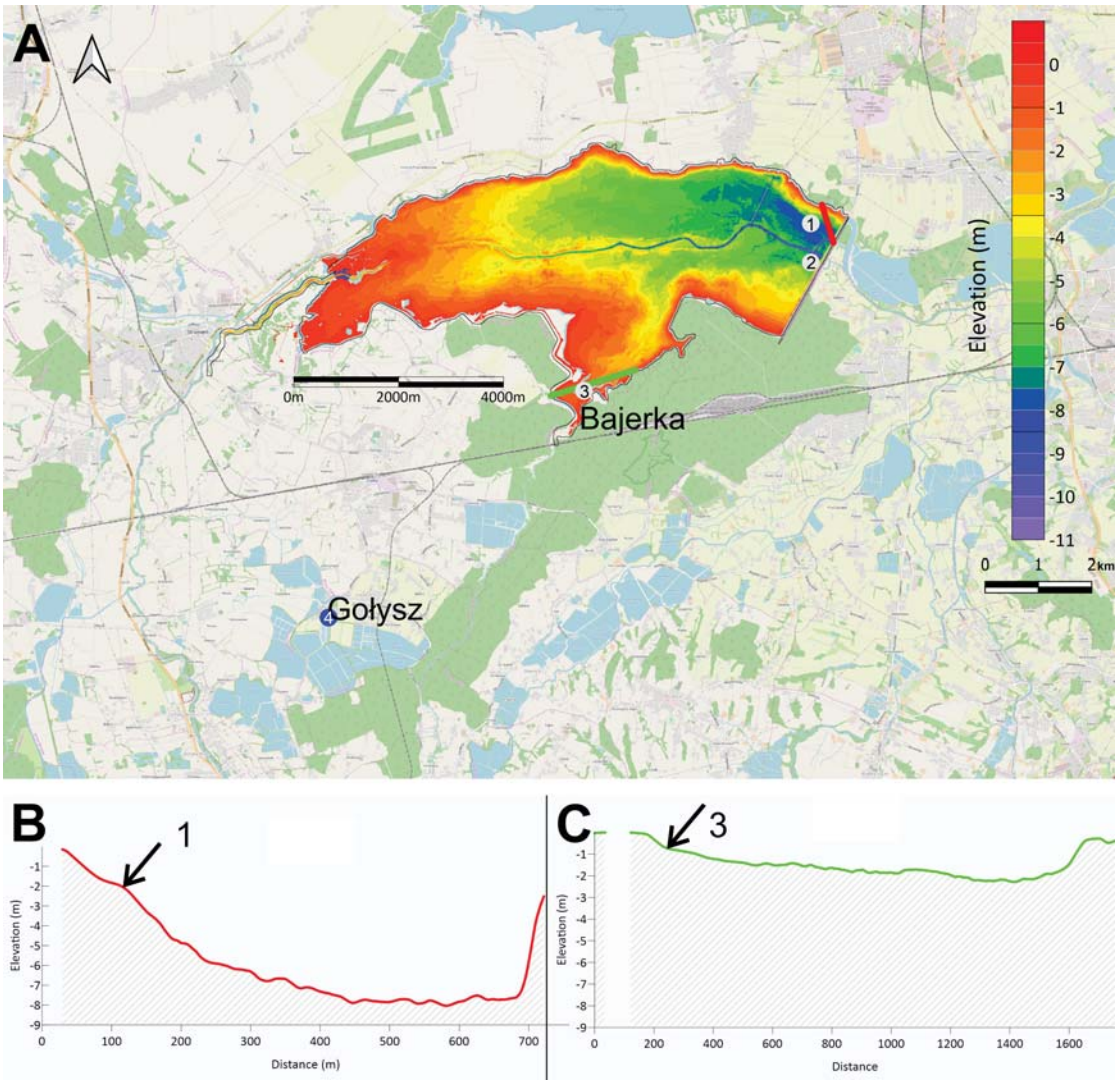

Fig. 1. The localization of the study sites within the Goczałkowice dam reservoir (1 – metalimnion, 2 – localization of the buoy, 3 – Bajerka) and the reference site, Gołysz pond (4) – A, characteristics of the bottom profile in the area of point 1 (red line) – B and point 3 (green line) – C

Physicochemical parameters of water

Water samples were analyzed for physicochemical properties in the certified laboratories (Accreditation Certificate PCA Nr AB 950) at the Institute of Environmental Engineering, Polish Academy of Sciences in Zabrze (Poland), according to the standard methods for water examination as described in the Polish Standards (PL: Polskie Normy). Insolation, water temperature, pH, water conductivity and the concentration of dissolved oxygen, nitrate and chlorophyll were continuously monitored using online-operating automatic probes (OTT Messtechnik Gmbh & Co Germany), which were mounted on a buoy.

Statistical procedures

The data were tested for the homogeneity of variance using Levene's test. Normality was checked using the Kolmogorov--Smirnov test. Having confirmed a normal distribution, post--hoc comparisons of statistical significance of differences (LSD, $p \leq 0.05$) were performed using MANOVA tests with sampling (=duration of the exposure), season, site and tissue as factors. To assess the relations between data, clustering based data aggregation techniques were applied (Euclidean distance tree). Correlations describing relationships between stress protein concentrations were established using r-Pearson correlation.

All of the tests including correlation and similarities analysis were performed using the Software Inc. (2017) STATISTICA (data analysis software system).

Results

The analyses of correlations between the stress proteins concentrations revealed high significant correlation coefficients (Fig. 2), therefore, to avoid redundancy, the general stress protein concentration will be discussed instead of each protein separately. Tests of significance revealed that each of the factors (season, sampling=duration of exposure, site and tissue) as well as their combinations were significant for the level of the parameter studied and should be taken into account in the interpretation of the results (Table 1). However, due to specificity of the organs, the obvious differences in the values of Mts concentration between gill, muscle and liver samples will not be discussed.

The effect of the site

In general, the stress protein concentrations were slightly different between the sites. Significant differences, however, were found among the samples from the autumn part of the experiment. In the majority of cases, when there were differences, the Mts concentration was lower in the samples from sites of transplantation than those from the Gołysz site (control individuals). This tendency was less pronounced in the samples of muscles in comparison with the other tissues. However, a higher concentration of Mts was found in the samples of each organ from the individuals that were kept at the 'metalimnion' site that were collected during the third sampling (Figs. 3, 4, 5).

The effect of season

In general, the stress protein that was measured in the organs of fish differed in relation to the season. Whenever the differences were significant, the concentration was usually lower in the autumn than in the summer, irrespective of the site of encaging (MANOVA, $p \leq 0.05$). These differences were independent of the factors of 'site' and 'tissue'; however, in the samples of muscles these differences were less pronounced.

The effect of the duration of exposure

The stress protein concentration was also responsive to the factor of sampling (Table 1). The sensitivity of the stress protein concentration to the duration of encaging was also conjugated with the aspect of season and site. According to this conjugation, the samples that were collected in the autumn and then transplanted to the metalimnion (all of the tissues) and the 'Bajerka' sites (liver and muscle) had a higher stress protein concentration at the third time point than at the beginning of the experiment.

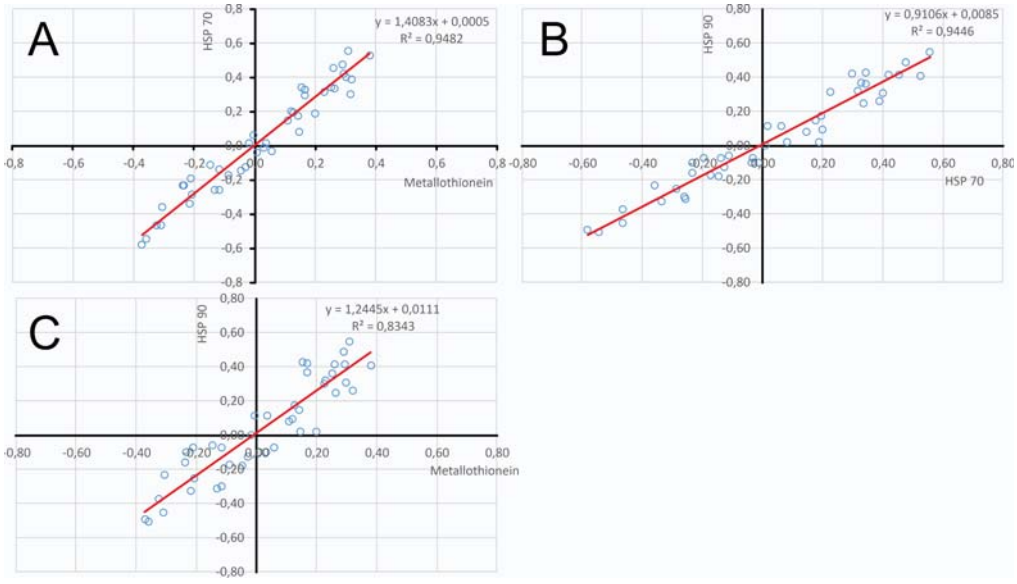

Fig. 2. Correlation between the values of stress proteins (HSP70, SP90 and Mts) concentration in the transplantation experiment

Effects of water characteristics

Water characteristics analyses revealed some correlations between stress protein concentrations and physicochemical parameters of water (Fig. 6). The factors that appeared to positively influence stress protein levels include, among others, chlorophyll, metals or Enterococci. Moreover, as it was mentioned above, the response of all the stress proteins is similar. However, the values of the chemical parameter measurements in this study suggest that the variability of basic site-to-site environmental conditions during the experiment is negligible, in the scale of two seasons.

Discussion

The effect of the site

According to the results of the stress protein concentration in the fish from various sites of encaging, it can be summarized that the slight variability, irrespective of the tissue, should not be attributed to the differences in the specific biochemical conditions or water quality at each site. The values of the chemical parameter measurements in this study suggest that the variability of basic site-to-site environmental conditions during the experiment is negligible. In addition,

Table 1. Analysis of variance (ANOVA/MANOVA) for Mts concentration in *C. carpio* from different transplantation sites (with site, sampling, season and organ as categorical factors); significant differences at $p \leq 0.05$

Source of variation	F	p
season	16.767	<0.001
duration of exposure	12.757	<0.001
site	8.177	<0.001
organ	167.129	<0.001
season * duration of exposure	9.292	<0.001
season * site	11.002	<0.001
duration of exposure * site	6.958	<0.001
season * organ	16.541	<0.001
duration of exposure * organ	2.639	0.034
site * organ	2.299	0.034
season * duration of exposure * site	13.262	<0.001
season * duration of exposure * organ	1.208	0.307
season * site * organ	0.735	0.622
duration of exposure * site * organ	1.564	0.100
season * duration of exposure * site * organ	0.918	0.529

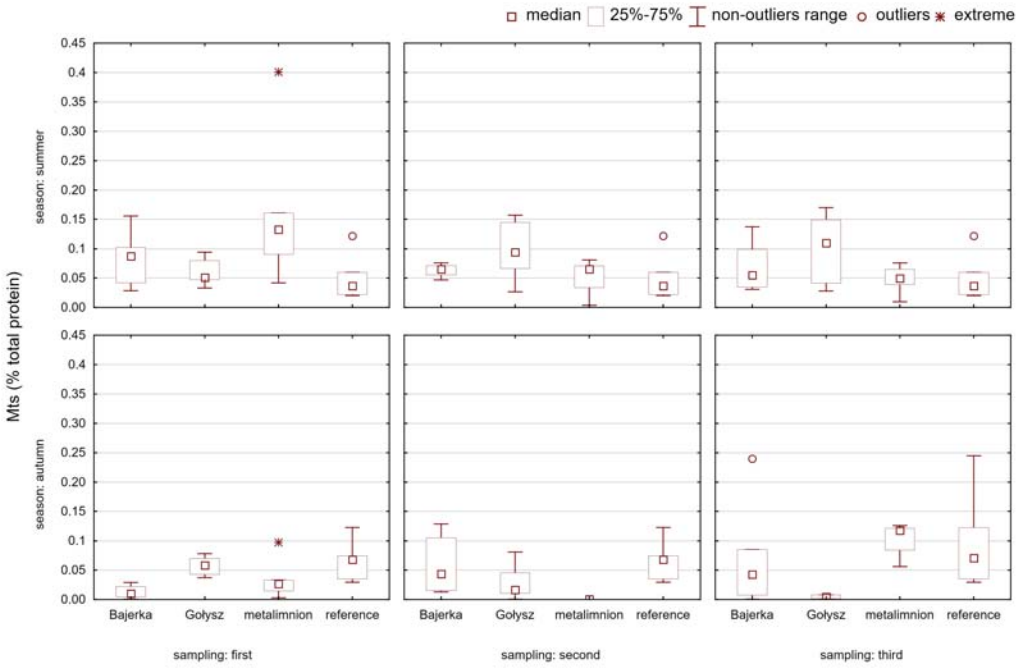

Fig. 3. Metallothionein (Mts) concentration (% of total protein) in the gills of *C. carpio* from the transplantation experiment in relation to the site of exposure, sampling and season

the concentrations of potentially toxic chemicals, including priority substances (such as PAHs), were below detection levels. Moreover, no signs of massive blooms were observed. Therefore, taking into account the results of the water quality measurements, it has to be concluded that the site-related diversity is probably only connected with the differences in the water temperature between the Gołysz site and the sites of encaging. According to the data, the temperature at each study site in autumn differed by more than 6°C with the highest temperature at the 'Gołysz' site. The metalimnion and the Bajerka sites do not differ significantly from each other in relation to water conditions, however, incidental difference

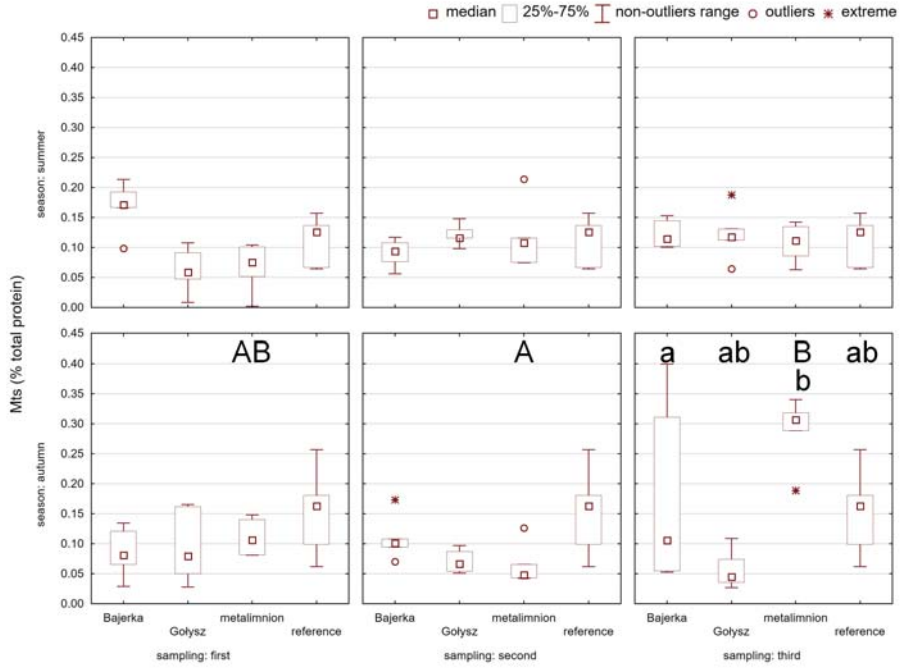

Fig. 4. Metallothionein (Mts) concentration (% of total protein) in the muscles of *C. carpio* from the transplantation experiment in relation to the site of exposure, sampling and season; a, b – different letters indicate statistically significant differences between sites within season and sampling; A, B – different capital letters indicate statistically significant differences between sampling within the site and season (Kruskal-Wallis test, $p \leq 0.05$)

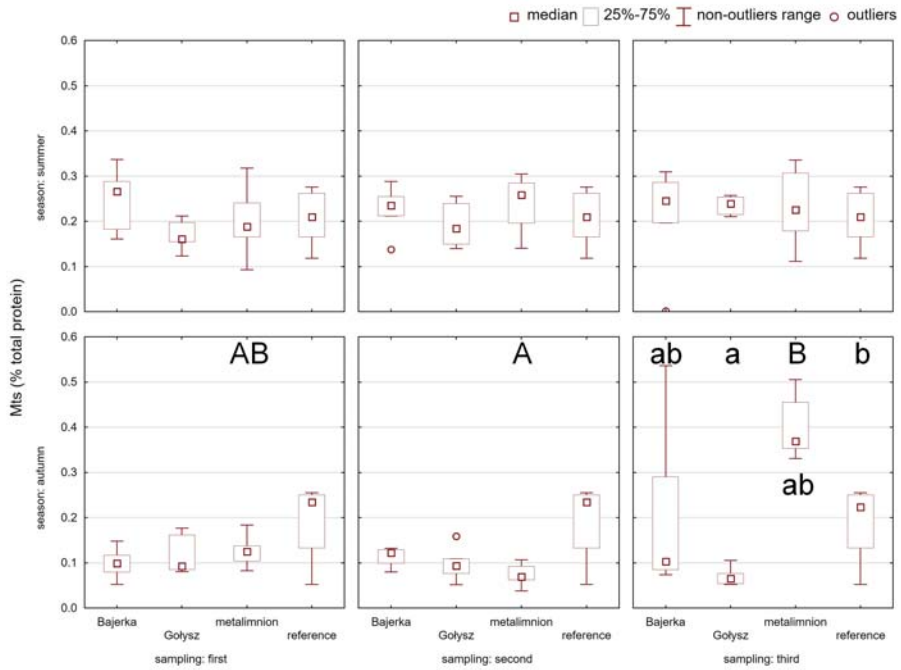

Fig. 5. Metallothionein (Mts) concentration (% of total protein) in the livers of *C. carpio* from the transplantation experiment in relation to the site of exposure, sampling and season; a, b – different letters indicate statistically significant differences between sites within a season and sampling; A, B – different capital letters indicate statistically significant differences between sampling within the site and seasons (Kruskal-Wallis test, $p \leq 0.05$)

in Mts concentration appeared. The "Bajerka" is a site where, periodically, after strong rainfall, the Bajerka river transports larger water volume what changes local environmental conditions. Here may be the explanation for wide range of values of the protein concentrations in muscles and livers of the individuals. From the analyses of the values we can state that maximum Mts concentrations were measured in the Bajerka site in comparison with all other sites in the third sampling. Temperature is not the only factor affecting the metabolism of animals. In this kind of experiment we study the resultant of all environmental factors as well as their interactions. However, the stress protein concentration

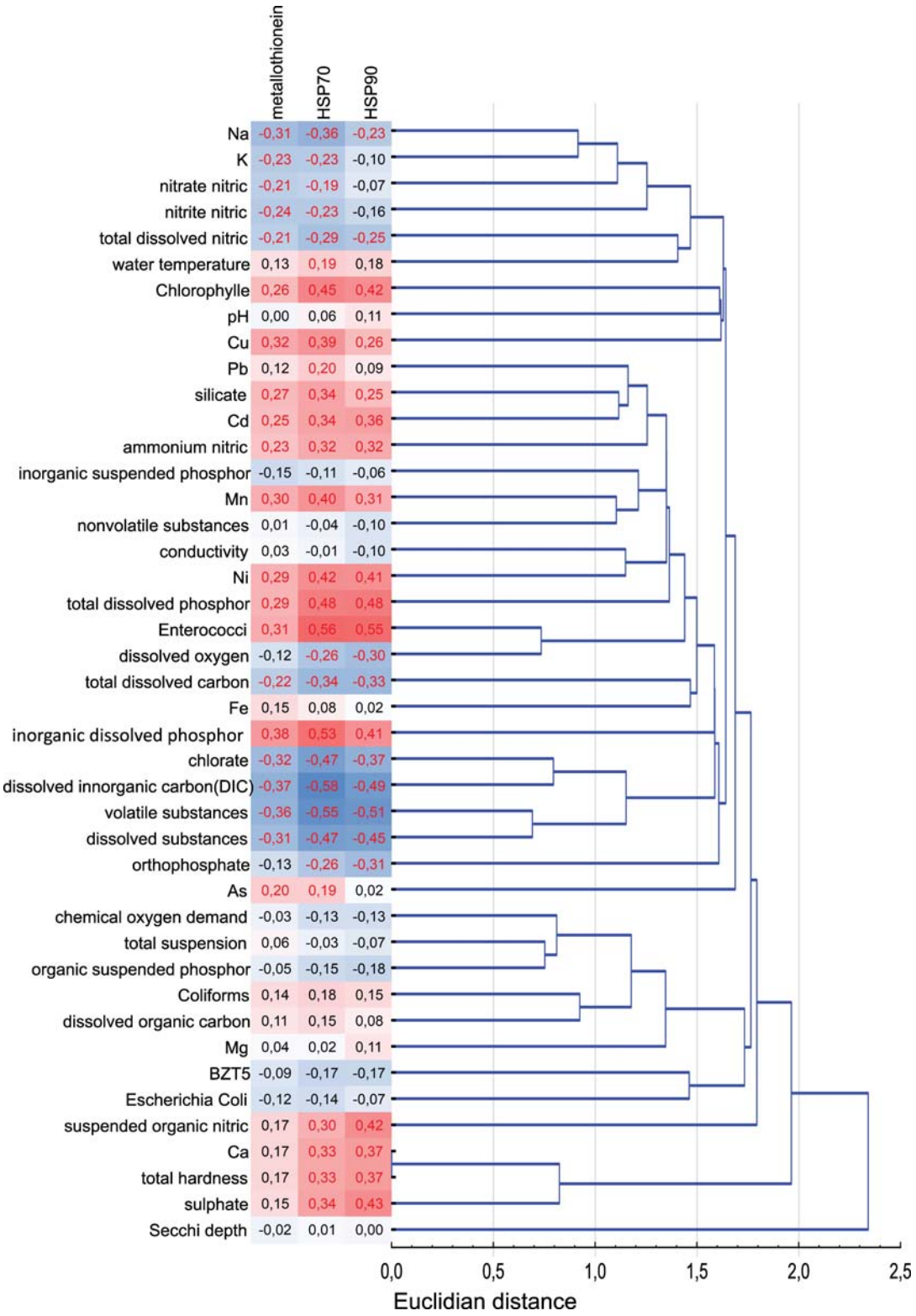

Fig. 6. Cluster analysis based on Euclidean distance of the levels of physical and chemical properties of Goczałkowice dam reservoir water and their correlations with the stress protein concentrations measured in the organs of *C. carpio* from the transplantation experiment; red font – significant correlation coefficients ($p \leq 0.05$), blue background – negative and red background – positive correlations with the stress protein levels

appears to be sensitive to the thermal conditions. A detailed analysis of the temperature-dependence of the concentration of these proteins is given below in the next section.

The effect of the season

The stress protein concentration that was measured in the tissues of the fish that were randomly collected from the cages at various times appeared to be sensitive to the factor of 'season'. This statement may be related to the possible differences in the water temperature in the hotter and cooler parts of the year, which vary due to the climatic conditions in the southern part of Poland. The sensitivity of the metallothionein concentration to temperature was recorded in several aquatic and terrestrial animals. From among fish species, the Mts concentration in the liver of *Sparus aurata* increased four-fold after the exposure to 30°C in comparison to the control conditions (22°) (Guinot et al 2012). Rope mussels (*Mytilus galloprovincialis*), which are aquatic invertebrates, that were collected from their habitat for biomarker measurements in various months also demonstrated a temperature-dependent Mts response. An increase of water temperature by 10°C was found to cause a two-fold increase in the Mts concentration in rope mussels (Kamel et al. 2014). Moreover, in the frog *Rana ridibunda*, the Mts concentration that was measured in individuals that were collected in the summer was higher than in the frogs that were sampled in other seasons (Falfushynska et al. 2008). The same authors also studied the variability in the Mts concentration in the livers and gills of the carp *Cyprinus carpio* in relation to the season and pollution level and revealed that it is the pollution that strongly influences the Mts synthesis, although significant season-related differences were also found, especially in rural areas (Falfushynska and Stolyar 2009). The statement that the Mts concentration reflects the pollution status rather than variability in the seasonal temperature was also suggested earlier by Langston et al. (2002), who studied this biomarker in the eel *Anguilla anguilla* from a variety of polluted sites. Also, in various thermal conditions (17–26°C) HSP70 and HSP90 levels in the sea fish *Argyrosomus regius* were studied. The highest HSP70 levels were found in the liver and intestine, but in the former organ the highest level was found at 17°C while in the latter – at 26°C. For HSP90 no significant difference was detected (Antonopoulou et al. 2020). Other results for the fish *Labeo rohita* and *Catla catla* revealed that both low temperature (12°C) and high temperature (37°C) is the reason of increase of expression of HSP70 and HSP90 genes (Ahmad et al. 2020).

In our study in the Goczałkowice Reservoir, which has no detectable industrial, municipal or agricultural sewage input, the highest temperature differences between summer and autumn were found at the 'Bajerka' and 'Gołysz' sites (11.5°C and 10.6°C, respectively). The differences at the remaining sites and in the summer seasons were between 4–6°C. If there is a similar relationship in fish, the temperature variability in the Goczałkowice Reservoir may be enough to be reflected by the metallothionein concentration in the caged carp. The results of Guinot et al. (2012) confirm such a correlation in the case of *Sparus aurata* – the Mts concentration in the liver of that fish increased four-fold after exposure to 30°C in comparison to the control conditions (22°C). In general, all of the authors cited above stress the applicability of the changeability of the Mts concentration as a biomarker for the condition of aquatic ecosystems due to the local and species-specific background concentration of this protein. In this study, we provide this kind of background for carp as being useful and applicable for caging experiment for sedentary fish species in the seasonal aspect.

Effect of the duration of exposure

Statistical analyses of the stress protein concentration in relation to the 'sampling' factor, which in reality means the period of encaging, did not reveal any regular pattern of variability. In general, however, the stress protein concentration increased slightly with the duration of encaging. This increase might be partly attributed to the stress that is connected with handling. The stress resulting from fish handling and transport may demonstrate itself by acute or prolonged changes in the corticosteroid-dependent metabolism (Barton and Iwama 1991, Barton 2002). Acute physiological symptoms of stress in fish may take from six hours to one day to completely disappear (Conte 2004); however, due to their dependence on the intensity and duration of the stress, the secondary effects may remain for a longer period. Although, according to some experiments, which have mainly been performed on mice, corticosterone and related hormones induce metallothionein synthesis (Mocchegiani et al. 2002, Beltramini et al. 2004), there are also reports demonstrating that the concentration or supplementation of cortisol is positively correlated with metallothionein induction (Hyllner et al. 1989, Park et al. 2010) although an opposite conclusion can also be found (Dang et al. 2001, de Fátima Mazon et al. 2007). Concerning the time interval, the effects of the application of psychological stress to rats caused an increase in the Mts level in the liver (Tian et al. 2014) and a decrease in the hippocampus (Dou et al. 2014) 14 days after the application of stress. The increase in the Mts concentration at the later time points of encaging may also be due to food deficiency, which was not necessarily connected with the availability of food but with the inability to dig in the bottom sediments. Scientific reports that connect starvation and the Mts level are scarce. According to Higashimoto et al. (2002) and Sogawa et al. (2003), who tested Mts concentration in fasting mice, the hepatic level of these proteins becomes elevated in starving animals. However, in the fish *Fundulus heteroclitus*, starvation itself did not cause any significant changes in the Mts concentration in the liver or intestine, while the Mts concentration in the gills was higher in starving non-spawning individuals in comparison with the ones that had been fed (Cleef-Toedt et al. 2000). In the present paper, thus, the significantly higher stress protein concentrations should be attributed to a combination of the two factors, but should still be treated as the background level for the potential application in possible pollution biomonitoring. It is worth stressing here that the effect of the increased stress protein concentration in the third week of encaging was independent of the site of transplantation. The question remains of whether offering an additional, artificial food source could minimize the effect of stress and provided more reliable biomarker responses. The analyses of Oikari (2006) suggest, however, that the moderate food deficiency, identical in each experimental group, accompanying the caging experiment, is a typical procedure.

Conclusions

The question of the utilization of any potential biomarker should always be preceded by recognizing the background – reference – conditions, which are specific to the animal species and its habitat. As the result of this study, the range of the variability of the stress protein concentration in young carp individuals was obtained. The analyses took into account four significant environmental and biological factors including the possible differences in water conditions (the aspect of 'site'), seasonal variability, tissue specificity as well as physiological (handling) and biological (food deprivation) stress (the aspect of 'sampling'). The results provide a complete reference material. According to the analyses of the aquatic conditions of a reservoir with no detectable pollutants, we conclude that the variability in the stress protein concentration levels in the groups that were compared is solely the result of the natural conditions. Thus, future regular monitoring of the reservoir using the transplantation method and young carp individuals will be both possible and reliable, confirming its application in ecoengineering. Moreover, the range of variability in the stress protein concentrations that were measured in the young *C. carpio* individuals acquired from the model dam reservoir in relation to all of the studied factors may be applied in the monitoring of any other similar reservoir.

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