

Content of the calcium and phosphorus in the meat of Prussian carp (*Carassius auratus gibelio* BLOCH, 1783) from the Lake Gopło (Poland)

Zawartość wapnia i fosforu w mięsie karasia srebrzystego (*Carassius auratus gibelio* BLOCH, 1783) odłowionego z Jeziora Gopło (Poland)

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ABSTRACT

The aim of this work was to compare the concentration of calcium and phosphorus and Ca/P ratio in the meat of Prussian carp (*Carassius auratus gibelio*) caught from the Lake Gopło. The study involved 14 individuals of fish caught in autumn (October, 26 2010). The muscles samples for analyses were taken from the whole body without the head and with the bones. Calcium concentration was determined by atomic absorption spectrophotometer Solar 969, Unicam. Phosphorus concentration was analysed using colorimetric method, by spectrophotometer Lambda 25, Perkin-Elmer. Measurements were performed at the analytical wavelength 430 nm. Analyses indicated that calcium concentration in the meat of Prussian carp was $5.63 \text{ g} \cdot \text{kg}^{-1}$. The mean content of P in the meat was $2.38 \text{ g} \cdot \text{kg}^{-1}$. Ratio of calcium to phosphorus in the meat of Prussian carp was 2.37:1. As analysis of correlation indicated there was a negative and statistical significant correlation between calcium and phosphorus concentration in the meat and the body length and the body mass of analysed fish collected from the Lake Gopło.

Keywords: calcium, meat, phosphorus, Prussian carp (*Carassius auratus gibelio*)

STRESZCZENIE

Celem pracy było oznaczenie stężenia wapnia i fosforu oraz obliczenie stosunku Ca/P w mięsie karasia srebrzystego (*Carassius auratus gibelio*) odłowionego z Jeziora Gopło. Badaniami objęto 14 osobników pozyskanych jesienią (26 października 2010 roku). Mięso do badań pochodziło z całej tuszy z ością, bez głowy i wnętrzności. Stężenie wapnia oznaczono przy pomocy spektrofotometru absorpcji atomowej Solar 969, Unicam. Stężenie fosforu oznaczono metodą kolorymetryczną przy pomocy spektrofotometru Lambda 25, Perkin-Elmer. Pomiar

wykonywano przy analitycznej długości fali 430 nm. Stężenie wapnia w mięsie analizowanego karasia srebrzystego wynosiło $5.63 \text{ g} \cdot \text{kg}^{-1}$. Średnia zawartość fosforu wynosiła $2.38 \text{ g} \cdot \text{kg}^{-1}$. Stosunek Ca/P w mięsie karasia wynosił 2.37:1. Analiza korelacji wykazała negatywną i statystycznie istotną zależność pomiędzy zawartością wapnia i fosforu a długością ciała i masą ciała ryb odłowionych jesienią z Jeziora Gopło.

Słowa kluczowe: fosfor, karaś srebrzysty (*Carassius auratus gibelio*), mięso, wapń

STRESZCZENIE SZCZEGÓLWE

Celem pracy było oznaczenie stężenia wapnia i fosforu oraz obliczenie stosunku Ca/P w mięsie karasia srebrzystego (*Carassius auratus gibelio*) odłowionego z Jeziora Gopło. Badaniami objęto 14 osobników pozyskanych jesienią (26 października 2010 roku). Mięso do badań pochodziło z całej tuszy z ością, bez głowy, ogona, łusek i wnętrzności. Dla każdej ryby dokonano pomiarów długości ciała (Lc) ($\pm 0.1 \text{ cm}$), długości całkowitej (Lt) ($\pm 0.1 \text{ cm}$) oraz masy ciała (BW) ($\pm 0.01 \text{ g}$). Masa ciała ryb mieściła się w zakresie od 25.14 do 48.87 g, a długość ciała wynosiła od 9.0 do 12.0 cm. Pobrane próbki mięsa na wstępie liofilizowano w liofilizatorze Finn-Aqua Lyovac GT2 a następnie mineralizowano w mineralizatorze mikrofalowym Ethos Plus, Milestone. W tym celu odważano 0.1g liofilizatu i dodawano HNO_3 i H_2O_2 w stosunku 4:1. Stężenie wapnia oznaczono przy pomocy spektrofotometru absorpcji atomowej Solar 969, Unicam. Stężenie fosforu oznaczono metodą kolorymetryczną przy pomocy spektrofotometru Lambda 25, Perkin- Elmer (przy długości fali 430 nm). Oznaczenia pierwiastków wykonano zgodnie z normami PN-EN 13805/2003, PN-EN 15505/2009 i PN-ISO 13730. Analizę statystyczną danych dokonano przy pomocy programu Statistica 8.0 (StatSoft, USA). Obliczono wartości średnie stężenia wapnia i fosforu i stosunek Ca/P oraz dokonano analizy korelacji pomiędzy stężeniem wapnia i fosforu a długością ciała ryb i masą ciała. Stężenie wapnia w mięsie analizowanego karasia srebrzystego wynosiło $5.63 \text{ g} \cdot \text{kg}^{-1}$. Analizy wykazały, że średnia zawartość fosforu wynosiła $2.38 \text{ g} \cdot \text{kg}^{-1}$. Stosunek Ca/P w mięsie karasia wynosił 2.37:1. Analiza korelacji wykazała negatywną i statystycznie istotną zależność pomiędzy zawartością wapnia i fosforu a długością i masą ciała ryb odłowionych jesienią z Jeziora Gopło.

INTRODUCTION

Fish meat is a source of amino acids, proteins, vitamins dissolved in lipids, long chain fatty acids and lipids as a valuable energy source. Through their high nutritive values fish meat should be very significant part of human diet. Fish are located at the end of the aquatic food chain and may accumulate metals and pass them to human organism (Al-Yousuf, et al., 2000). Metals can be accumulated by fish through the food chain and water (Mendil and Uluözlü, 2007). The level and intensity of the accumulation depends on many factors, such as: species of fish, size, sex, seasonal changes and environmental factors (Yilmas, et al., 2010). Trace elements are introduced into the aquatic system (lakes, rivers) through atmospheric fallen dumping wastes and geological weathering (Al-Yousuf, et al., 2000).

Phosphorus is a mineral essential for normal growth, bone mineralization, reproduction and energy metabolism in fish (Albrektsen, et al., 2009). Ca/P ratio is the most important indicator for good bone health (Celik and Oehlenschläger 2004). These elements play a very important roles in several physiological processes and are directly involved in the development and maintenance of the skeletal system. In vertebrates calcium is complexed with phosphorus in hydroxyapatite to form the principal crystalline of bone (Ye, et al., 2006). The availability of phosphorus on the organism depends on the chemical form and solubility of the mineral. Albrektsen, et al., (2009) analysed Atlantic salmon and they indicated that primary inorganic salts of phosphorus are more available than secondary salts, whole phosphorus bound to calcium in the bone tissue is the last available.

Daily dose of phosphorus in the diet of an adult should be 800 mg (Sapek, 2009), and approximately 1000 mg of calcium. The ideal would be to consume the same amount of phosphorus as calcium (calcium/phosphorus balance). The ratio between phosphorus and calcium greater than 3:2 may cause metabolic disorders. Fish meat is a rich source of phosphorus and those can be the order of 150-200 mg·100g⁻¹ of product. This element occurs in almost all species of fish. Species which are rich in calcium are sardines, herring, sprat and salmon (Gawęcki and Hryniewiecki, 2004).

The aim of this work was to determine calcium and phosphorus content and Ca/P ratio in the meat of Prussian carp caught in the Lake Gopło during autumn. In the literature, there are very little information about the content of phosphorus and calcium in the meat of Prussian carp.

MATERIAL AND METHODS

STUDY AREA

The Lake Gopło is located in the southern part of Kuyavia-Pomeranian province (fig 1). The western part of this lake it's a strict nature reservuar. The main morphometric indicators of the Lake Gopło are as follows; surface area: 22 km², maximum depth: 16 m, average depth ca 4.7 m and length of shoreline: 90 km (Łuczyńska, et al., 2008). The Lake Gopło is an eutrophic reservoir, based on the limnological classification and it is a zander type of the lake, based on the fishing classification. Among the zooplankton of the Lake Gopło, there are identified 65 species of *Rotiferas*, 34 species of *Copepode* and 8 species of *Cladocera*. The main group of benthos it's *Chironomid* (dominated by *Chironomus plumosus*). The ichthyofauna is dominated by white bream (*Abramis bjoerkna* L.). Among the predatory fish, a significant part of the fishing are eel (*Anguilla anguilla* L.) and zander (*Sander lucioperca* L.). Analyses revealed that the waters are qualified as unclassified (Raport WIOŚ, 2008).

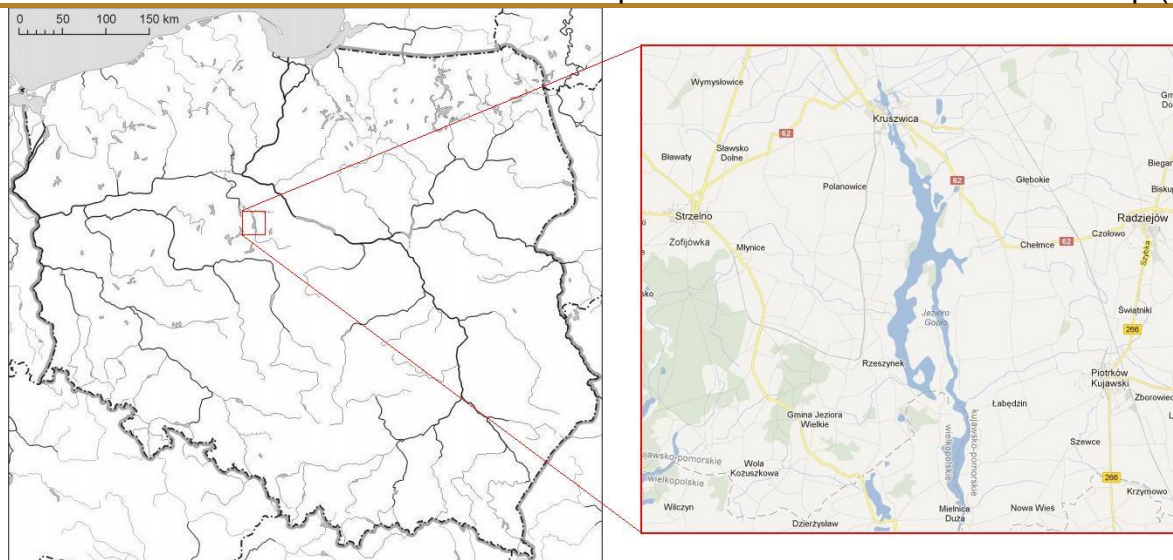


Figure 1. The location of the Lake Gopło in relation to Poland

FISH SAMPLES

The study involved 14 individuals of Prussian carp (*Carassius auratus gibelio* Bloch) caught in autumn (October, 26 2010). Prussian carp is a freshwater fish in the family of the *Cyprinidae*, feed on a variety of food. The most frequent diet include planktonic crustaceans, insect larvae, mollusks and plant (Rybczyk, 2006). As Litwińczuk, et al., (2006) indicated, Prussian carp caught in autumn showed highest carcass meat content. And this meat was characterized with higher parameters of physicochemical quality: lower electrical conductance, higher water binding capacity and darker color. The experimental fish were obtained in natural condition from the Lake Gopło. Measurements of the mass of the fish body (BW) (± 0.01 g) and body length (Lc) (± 0.1 cm) and the total length (Lt) (± 0.1 cm) were taken on the each individuals. The meat samples for analyses were taken from the whole fish body with the bones, and without the head, tail, fins and scales. The internal organs were removed. There were chosen for analysis individuals with the similar biometric measurements. The body weight ranged from 25.14 to 48.87 g, and the body length was from 9.0 to 12.0 cm.

The samples of fish meat were immediately frozen after preparation and kept in the deep freezer before analyzing. All frozen samples were freeze dried in a Finn-Aqua Lyovac GT2 freeze drier (parameters: temperature -40°C , pressure $6 \cdot 10^{-2}$ mbar, duration at least 48h).

The freeze dried samples were mineralized in microwave mineralizator Ethos Plus, Milestone. For the mineralization 0.1g of the tissue was weighted and then HNO_3 and H_2O_2 were added in ratio 4:1. During the first 10 minutes, the temperature was increased to 190°C . During the next 7 minutes the temperature was kept at a level of $190 \pm 5^{\circ}\text{C}$. The mineralized samples have been carried quantitatively to the measuring flask with a capacity of 50 cm^3 .

Calcium concentration was determined by atomic absorption spectrophotometer Solar 969, Unicam. Phosphorus content was analyzed with colorimetric method, by spectrophotometer Lambda 25, Perkin-Elmer (at wavelength 430 nm). Analysis were

carried out according to PN-EN 13805/2003, PN-EN 15505/2009 and PN-ISO 13730. Tissue concentrations of minerals have been reported as $\text{g}\cdot\text{kg}^{-1}$ dry weight ($\text{g}\cdot\text{kg}^{-1}$ d. w.).

The accuracy of the analyses was controlled by adding standard solutions. As a standards were used calcium standard solution $\text{Ca}(\text{NO}_3)_2$ in HNO_3 (Merck, Germany) and KH_2PO_4 (POCH S.A., Poland) dissolved in water.

Data analyses were performed by using the Statistica 8.0 software (StatSoft, USA). Significance of differences in the average content of calcium and phosphorus in the meat of Prussian carp were calculated. Correlation between calcium and phosphorus concentration and the body length and body mass were analysed. Pearson rank correlation coefficient (r) was calculated.

RESULTS AND DISCUSSION

Analyses indicated that the mean content of calcium in the meat of Prussian carp was $5.63 \text{ g}\cdot\text{kg}^{-1}$ of wet weight. As previous analyses indicated calcium concentration in the large side muscle of the fish body above the lateral line of roach (*Rutilus rutilus* L.) caught from the Brda River in spring was $1.82 \text{ g}\cdot\text{kg}^{-1}$ (in females) and $1.93 \text{ g}\cdot\text{kg}^{-1}$ (in males). In the meat of individuals collected during autumn it was 0.83 and $1.10 \text{ g}\cdot\text{kg}^{-1}$, respectively (Stanek and Janicki, 2011). These analyses indicated that calcium concentration accumulate in the large amounts in the whole body of fish than in the various parts of fish body. A higher concentrations of those minerals accumulate in the bones, therefore the fish eaten with the bones (for example, sardines) are the best source of calcium. Content of the calcium differed statistically significant between individuals caught in a different seasons, but there were not statistically significant differences between samples taken from females and males caught within one season. Meat of the analysed roach were not a rich source of calcium.

Table 1. The mean content of calcium and phosphorus ($\text{g}\cdot\text{kg}^{-1}$ dry weight) in the meat of Prussian carp (*Carassius auratus gibelio*) from the Lake Gopło and roach (*Rutilus rutilus*) from Brda River

Tabela 1. Średnia zawartość wapnia i fosforu ($\text{g}\cdot\text{kg}^{-1}$ suchej masy) w mięsie karasia srebrzystego (*Carassius auratus gibelio*) odłowionego z Jeziora Gopło i płoci (*Rutilus rutilus*) z Rzeki Brdy

Species	Place of catch	n	Ca ($\text{g}\cdot\text{kg}^{-1}$)	P ($\text{g}\cdot\text{kg}^{-1}$)	Ca:P ratio
Prussian carp	Lake Gopło	14	$5.63 \pm 1,24$	$2.38 \pm 0,31$	2.37:1
Roach *	Brda River	40	$1.41 \pm 0,76$	$2.11 \pm 0,27$	0.67:1

* Stanek and Janicki, 2011

The present studies indicated that the mean concentration of phosphorus in the whole body of Prussian carp was $2.38 \text{ g}\cdot\text{kg}^{-1}$ (tab.1). Two-way analyses of

variance indicated, that the mean value of phosphorus in the meat of roach caught from Brda River collected in spring was higher than in the meat of fish from autumn, and it was $2.24 \text{ g}\cdot\text{kg}^{-1}$ in females and $2.30 \text{ g}\cdot\text{kg}^{-1}$ in males from spring, respectively. There were no statistical significant differences between those values. In the meat of fish caught in autumn the mean value of phosphorus was $1.89 \text{ g}\cdot\text{kg}^{-1}$ in the tissue of females and $2.01 \text{ g}\cdot\text{kg}^{-1}$ in males. There were no statistically significant differences between these values (Stanek and Janicki, 2011). Eastwood and Couture (2002) investigated seasonal variations of metal contamination in the liver of yellow perch (*Perca flavescens*) caught from seven northeastern Ontario Lakes. There were much higher concentration of metals in the spring. It may be due to increased metal input or bioavailability caused by snowmelt events or lake turnover that affect water quality parameters. The same results were observed by Laitinen (1994).

Analysis carried out on Prussian carp and those previous about roach indicated that the muscle tissue are not considered to the specific physiological sites for calcium and phosphorus (Al-Yousuf, et al., 2000). Phosphorus and calcium accumulate in the largest amounts in bones. Brucka-Jastrzębska, et al. (2009) determined micro- and macroelements concentration in the different tissues of freshwater fish (rainbow trout, common carp and Siberian sturgeon (*Acipenser baeri* B.)). And they reported that calcium distribution followed the same pattern for all the three analyzed species in decreasing order: gills > muscles > skin > liver > kidney > blood. As Perkowska and Protasowicki (2000) indicated cadmium and lead accumulate in a different ratio in the liver and kidney. In all analyzed species the content of these metals were higher in the gills than in the muscles. It proves that the respiratory system is the main way of acquisition of this metals by fish. Higher cadmium concentrations were found in the liver of red gurnard (*Trigla cuculus*), whole lowest cadmium levels were always found in muscles tissues of the analyzed fish. Lead concentration were much higher than cadmium and the liver and gills accumulated the great amounts of this metal (Canli and Atli, 2003).

Ca/P ratio in the meat of analyzed fish was 2.37:1 (tab.1). Ratio of calcium to phosphorus in the meat of wild roach from Brda River was ranged from 0.43:1 to 0.82:1 (Stanek and Janicki, 2011). As numerous studies show the value of this ratio should be 1:1 in the consumed products, because when there is an excess of calcium over phosphorus, phosphorus is not absorbed, because this form calcium phosphates is not biologically available (Chavez-Sanchez, et al., 2000). Approximate share of phosphorus and calcium in the fish meat should be at 1.8 % (dry weight) (Kabata-Pendias and Pendias, 1999). Porn-Ngan, et al. (1993) investigated which portion of calcium phosphate and ratio phosphorus to calcium inhibits zinc availability in rainbow trout by giving diets with different amounts of P and Ca. They observed that increase Ca levels slightly reduced the average body weight. Nakamura (1982) investigated a negative relationship between the amount of P absorbed by carp and the dietary Ca content. And numerous studies show that excess phosphorus in the body causes calcium malabsorption, which can lead to decalcification of the bones.

Analysis of correlation indicated that there were a negative and statistically significant correlations between the body length and calcium ($r = -0.867235$, $p < 0.05$) and phosphorus ($r = -0.814923$, $p < 0.05$) content in the meat (fig. 2). The same results were obtained for correlation between the body mass and calcium ($r = -0.812297$, $p < 0.05$) and phosphorus ($r = -0.807769$, $p < 0.05$) concentration. The same results were obtained for roach collected from Brda River. A negative correlation between metal concentration and body length may be due to dilution of these elements with

the increase of fish body, reducing the rate of nutrition and food ratios and mineral excretion by the gills, skin, and feces. Investigation have show that the bioaccumulation of heavy metals (Cr, Mn, Ni) decreased as fish body length increased (negative correlation) (Canli and Atli, 2003). The main explanation of this correlation is a higher ability to metabolize compounds for the younger fish than for the older individuals. The next explanation is the fact that the mechanism of the neutralization of harmful metals are not developed sufficiently in the young organisms. Therefore, larger amounts of minerals can be accumulate in their body (Kljaković, et al., 2002). The analysis of the dependence of the content of mineral elements in particular organs and the body length of the fish from the Żnin Duże Lake indicated a positive correlation for Cu, Fe and Ni, and negative correlation for Zn and Mn (Stanek, et al., 2005).

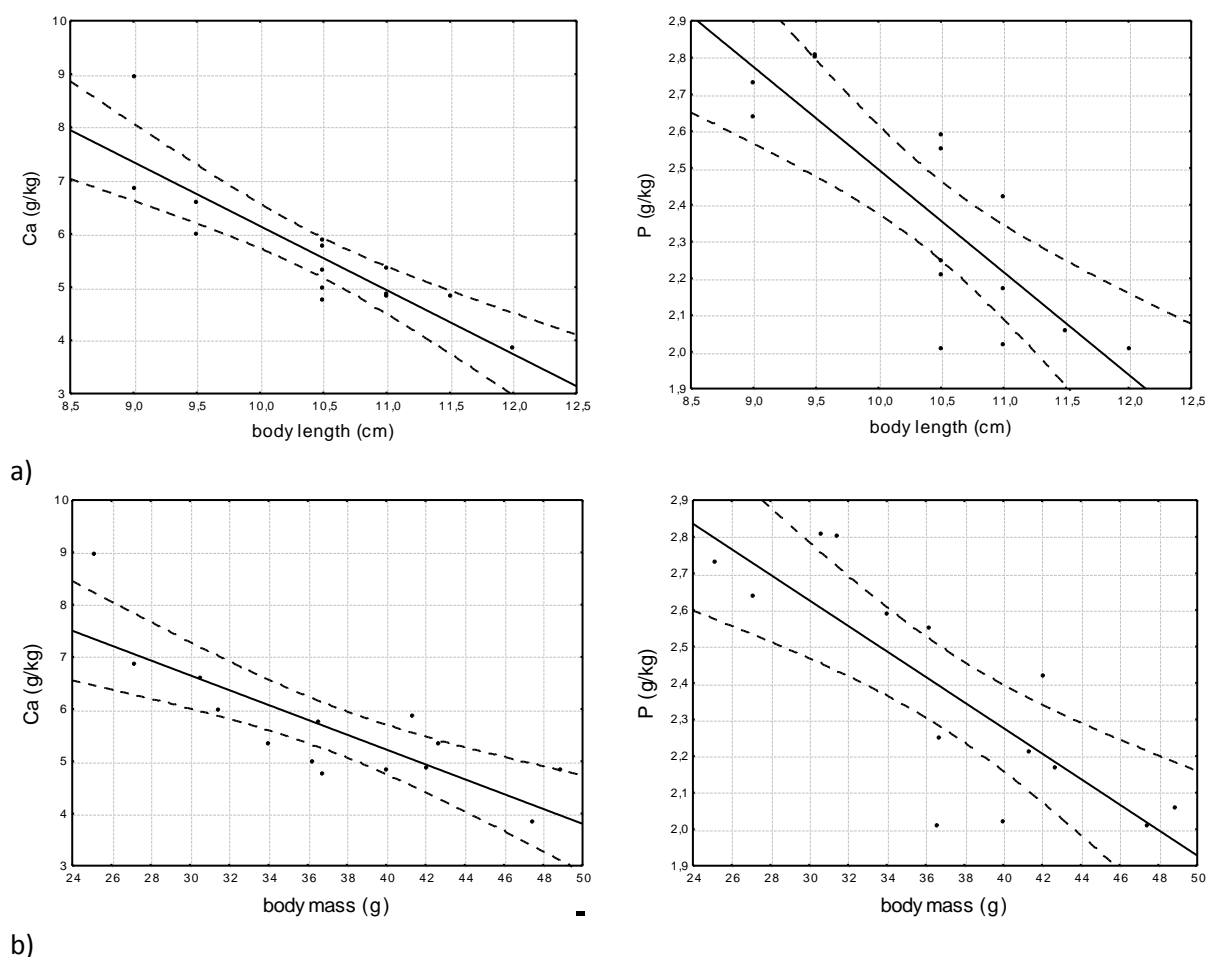


Figure 2. Correlations between fish body length (cm) (a) and the body mass (b) and calcium and phosphorus concentration ($\text{g}\cdot\text{kg}^{-1}$ dry weight) in the meat samples of Prussian carp (*Carassius auratus gibelio*) from the Lake Gopło.

Rysunek 1. Korelacje pomiędzy długością ciała ryb (cm) (a) i masą ciała (b) a zawartością wapnia i fosforu ($\text{g}\cdot\text{kg}^{-1}$) w mięsie karasia srebrzystego (*Carassius auratus gibelio*) odłowionego z Jeziora Gopło

Tissue metals concentrations are influenced by environmental contamination. Fish from more contaminated or cooler lakes had lower indicators of physical condition than individuals from cleaner reservoirs (Eastwood and Couture, 2002). There were carried out analyses on variations in the heavy metals pollution of the Lake Balaton and accumulation capacity especially for Zn, Cu, Cd and Pb in common bream. There were observed significant positive correlations between the level of heavy metals accumulated in the organs of fish and the pollutant load of the water (Farkas, et al., 2003). As Chen and Chen (2001) indicated, the metal content in fish tissues are related to the pollution status of the environment. Except for Zn and Mn concentrations in the muscles of *Sardinella lemuru* being higher than those of the slightly polluted Chi-Ku Lagoon, the heavy metal concentration in the fish of Ann-Ping coastal were similar to those of slightly polluted regions.

CONCLUSION

1. Analysis carried out on Prussian carp indicated, that the muscle tissue were not considered to the specific physiological sites for calcium and phosphorus content.
2. A correlation analysis indicated a negative and statistically significant correlation between the body length and mass and calcium and phosphorus content in the meat.

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