# The quality of rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) from technologies applied in Poland

Testing of trout production technologies applied in Poland in the light of the Commission Regulation (WE) 710/2009

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# Contents

# Introduction

Trout farming is a specific branch of agriculture, requiring a wide range of knowledge of fish behavior and the aquatic environment. Solid knowledge in this case is the most important factor for success. Only on the basis of a broad knowledge of specific breeding technology and its final product can trout breeders manage and develop their farms. The interdisciplinary database presented hereby follows this direction based on the scientific research of rainbow trout production in the Polish reality.

Currently, there is a need for expertise in the production of this sector of the economy because aquaculture is the most intensively developing branch of agricultural production in the world. This increase is largely due to increased salmonid production. This is possible on one hand due to health-related properties of fish meat, and on the other hand to the significant potential for agricultural production. Rainbow trout is in second place (after Mediterranean Blue Mussel) among the 10 main species produced in European aquaculture and in first, when it comes to fish. In Poland in 2011, the total sales volumes of all species of trout were high. The rainbow trout ratio of 96.3% amounted to 11 663 tons.

Trout farms retain the character of the river environment. The basic requirement for the production of these fish is a strong flow of cold (up to 20°C), well-oxygenated (over 6 mg/dm$^3$) and pure water (first grade purity). Currently there are two commonly used technologies. The so-called open breeding facilities (OS) with a single use of water, and water recirculation technology (RAS). The second technology, because of limited water resources, is becoming more widely deployed. The research results presented provide knowledge to farmers on both rainbow trout breeding technology and the quality of the final product. They also support the legislation, in particular Regulation (EC) No 710/2009, laying down the detailed rules on organic aquaculture production, according to which, „does not allow the use of such systems (i.e., recirculation system – Authors postscript) in organic production until obtaining greater knowledge."

By presenting information and promotional materials to trout farmers we encourage them to study the issue. Following words of Ovid: „*Hoc tibi proderit olim*" – This is used to bring you a profit.

Janusz Guziur, Krzysztof Goryczko

# 1. History of rainbow trout farming

## 1.1. Rainbow trout biology

### 1.1.1. Taxonomic features

The body of rainbow trouts is tightened laterally. Their oral cavity is large and filled with numerous teeth. The rear edge of the maxillary bone extends beyond the rear edge of the eye. The scales are cycloidal, small, similar to the scales of salmon and trout. The tail is moderately indented, and in older fish almost straight. In relation to the total length of the body their height is 23.3%, the head is 20.6% and the width of the body is 10% (Kalala 1972).

### 1.1.2. Coloration

The back of rainbow trout is gray-blue with numerous spots, occurring also on the dorsal fin, tail and fat fin. There is pink-red (rainbow) wide streak running along the sideline.

### 1.1.3. Countable features

The number of pyloric eggers in rainbow trout varies from 27 to 80. The plowshare corpus is composed of two rows of strong teeth.

Selected range of computable features of rainbow trout from Polish waters, Eagle Lake (USA) and Verde Lake (Mexico) is summarized in Table 1.1.

**Table 1.1.** The range and mean values of computable features of rainbow trout (Brylińska 1986 – own modification)

| Country | Author | Number of rays in the fins | | | | l.l s/i | Sp. branch |
|---|---|---|---|---|---|---|---|
| | | D | A | P | V | | |
| Poland | Gąsowska (1962) | III–IV 10–11 | III 10 | I 12 | I 8 | 135–150<br>21 / 20 | 17 |
| Eagle Lake (USA) | Needham, Gard (1959) | – | – | – | – | 119–125<br>28-33 / 0 | 11–12 |
| Lake Verde (Mexico) | | – | – | – | – | 105–109<br>21–24 / 0 | 10–11 |

### 1.1.1.4. Reproduction

Sexual maturity of rainbow trout largely depends on their growth rate. Females (with roe) usually reach maturity at the age of 2–4 years, and males 1–3 years. Within their natural habitat at the western edge of North America breeding period of various forms of rainbow trout is quite extended in time: from December to May, and the majority of fish have their spawning season in the spring.

In nature trout reproduction befalls in clean and well-oxygenated streams and partly in larger rivers. The optimum temperature for trout spawning shall be within 5.6–13.0°C, up to 16.0°C. According to some authors, rainbow trout reproduction is possible even at the lower temperature: 0.3–12.8°C.

Trout fertility ranges 900–3000 of grain spawn. On average it is about 1400 grains per 1 kg of body mass of the female. Egg diameter, depending on the age and size of fish (body mass) is in the range 3.5–6.2 mm.

### 1.1.5. Food and nutrition

Rainbow trout, as a carnivorous predator has adapted the gastrointestinal tract to digest animal protein and vegetable protein to a small degree. In natural waters its feed consists on crustaceans and larvae of adult forms of aquatic insects. With age, food share of fish increases.

Due to numerous studies, conducted especially in the Eastern Fish Nutrition Laboratory (Cortland, NY) there has been significant progress in the production of high-energy and environmentally friendly feed. It has revolutionized the production and breeding of rainbow trout and other salmonids. Currently, feeding salmonids with pelleted feed is one of the most important breeding operations, requiring experienced breeders and significantly influencing the final outcome and cost-effective production.

### 1.1.6. Growth

The growth of rainbow trout fluctuates, that is largely the result of environmental influences. The fastest growth of trout (and other salmonids) is noted in the sea and brackish lagoons. In lakes it is somewhat slower, and the slowest growth is in small streams and rivers. Table 1.2 shows the growth of rainbow trout under different conditions of the natural environment and culture, found in the last half-century. As the example, it is rainbow trout from Titicata Lake weighing 22.7 kg, while in the 1990's angling world record was a specimen from Pend Orelle Lake, weighing 16.8 kg. However, most authors are of the opinion that the potential for growth of this species of fish is much greater, especially as a result of further selection work. After 10 years of breeding – breeders obtained a selection of fish maturing at the age of 3 years and reaching almost 3 kg of body mass, while in the Avington farm there is strain of rainbow trout selected and obtained that after 1 year achieves weight in the range 0.75–1.0 kg, after 2 years it is 4–5 kg, and after 4 years – a record weight of 20 kg.

**Table 1.2.** The increase in body mass (g) of rainbow trout in different environments (Brylińska 1986; Guziur 1982–1991; Goryczko 2004 – own modification)

| Reservoir (Country) | Author | Age of fish | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| Streams of USA and Canada | Mc Crimmon 1971 | 42 | 193 | 224 | 312 | 498 |
| Great Lakes (USA) | Mc Crimmon 1971 | 139 | 498 | 907 | 1512 | – |
| I`Estibere river (Pyrenees) | Guziur 1991 | 2.2 | 15.7 | 45.0 | 96.0 | 156.0 |
| Carp ponds (Czech Republic) | Guziur 1982 – after: Oliwa 1959 | 60–80 | 250–400 | 1200–2000 | – | – |
| Cultures (ca 1930) | Enger, 1934 | 10 | 50 | 129 | 157 | – |
| Cultures (1961–1973)<br>Cultures (1993–1998) | Goryczko,<br>data not published | 30<br>40 | 280<br>514 | 947<br>1470 | 1404<br>2420 | 2099<br>– |
| Baltic Sea<br>(smolt stocking) | Bartel 1973, 1988 | 90*<br>50–90* | 1071<br>1043 | 2520<br>2632 | 3193<br>3060 | –<br>7300 |
| Babine (Canada) anadromous | Goryczko 2004<br>– after: Sedgwick 1973 | *smolt* | – | 2300 | 5200 | 9200 |

Comments: * rearing in ponds.

### 1.2. Origin of species

Trout as a specie was first described in 1792 by Walbaum in East Asia (Kamchatka) as *Oncorhynchus mykiss*, and individuals from the Columbia River in 1836 by Richardson, as *Salmo gairdneri*. In 1855 Gibbson described the rainbow trout as *Salmo iideus*. With time, many other authors have described rainbow trout, assigning it a dozen other Latin names.

Since 1988, decision of the American Fisheries Society eventually has included rainbow trout *Oncorhynchus* to type, adding the original name *mykiss* species. This long history of changes in a systematic classification of these fish testifies to the enormous variability and plasticity of the species. This is mainly due to a very wide range of its occurrence. Just in the 1930's, based on the different coloration, body shape and countable features there were 15 species of rainbow trout enumerated. Now the separate species are:

- Cutthroat trout (*Oncorhynchus clarki*),
- Golden trout (*Oncorhynchus aguabonita*),
- Mexican golden trout (*Oncorhynchus chrysogaster*).

In the environment this specie (*Oncorhynchus mykiss*) creates two main forms: a traveling and settled. Travelling rainbow trout's called steelhead spend their juvenile life in the river, and the period of feeding and rapid growth in the sea, then return to fresh water to spawn. Settled forms (*shasta*) spend their whole lifetime in fresh water.

### 1.3. Range of the occurrence

The range of naturally occurring rainbow trout includes the western part of North America – from the Kuskokwim River (Alaska – 61° latitude) to the basin of Del Presidio River in Mexico (24° latitude), and Asian rivers of western and eastern Kamchatka.

Due to human activities rainbow trout currently occurs on all continents except Antarctica. Their distribution range extends from the Arctic Circle in the north (Alaska, Sweden, Norway), through the equator (Kenya, Uganda, Ecuador), up to 55° south latitude in Argentina.

### 1.4. World history of the introduction of rainbow trout

The origin of trout, which were the subject of the first acclimatization and breeding operations in the states of North America, according to many authors is still controversial.

First introduction of rainbow trout was made in 1875, carrying roe trout from California to the State of New York. The study of the reports of U.S. Fish Commission and the California Acclimatization Society showed that the first batches of rainbow trout eggs did not come from McCloud River (which was only in 1878) but the rivers flowing into the Bay of San Francisco. In the years 1888–1900 the U.S. Fish Commission organized obtaining eggs from the steelhead from Klamath River, Willamette River and Rogue River in Oregon and from Redwood Creek Stream in California. Intensification of obtaining eggs probably stemmed from the huge demand, which was the result of intensive acclimatization activities.

By the end of the nineteenth century the attempts of the introduction of rainbow trout were mostly successful, and included all U.S. states (excluding Florida), as well as most European countries.

Starting from 1875, staggering career of this specie has begun. Two years later, the trout was brought to Japan, in 1882 to Germany, in 1883 to New Zealand, and in 1884 to England. In the beginning (1882 and 1886) settled and itinerant form of this species was brought to Europe, and in 1889 – a separate specie Cutthroat trout (*Salmo clarki*, *Oncorhynchus clarki* called today). In Europe these forms were the base for creation of the local form of rainbow trout.

Conducted restocking until the late 1920's were designed to acclimate the species mainly due to its fast pace of growth, reached size (weight) and the values of fishing. Data of Bradford (1982) indicates that fishing in a number of lakes (eg. Taupo Lake and rivers of New Zealand), resulted in the increased number of caught trouts. After 1911, more and more rainbow trouts were caught in place of the smaller brown trout (*Salmo trutta* m. *fario*, L.).

Rainbow trouts were probably imported to Poland in the years 1881–1889, on the grounds of the then Prussian, and Austrian part of Galicia – in the years 1891–1910, although many authors contend here with the date (Kołder 1948; Hurt 1960; Matlak 1960; Szczygielski 1967; Stok 1979; Śliwiński 2012). According to Goryczko it is the most probable that the first batch of rainbow trout eggs from overseas came to the Prussian area early in 1882 thanks to the active naturalist, angler and ichthyologist – Max von Borne (1826–1894), who brought stream trout and probably rainbow trout to his pond center in Barnówek (West Pomerania).

At that time of acclimatization, both in Poland and other European countries, all activities were based on a much earlier achievements of salmon and brown trout farming. There are XV century records of the French monk Don Pinchon who knew the way of trout reproduction, but did not give it to others. It is known that the landowner Stephen Ludwig Jacobi from Hohenhausen (1709–1784) fertilized trouts artificially since 1725. In 1733, a number of scientists (eg. Benecke 1880) reported the successful trials of the artificial spawning in trout. It was only in 1837, John Shaw in Scotland, and in 1843, Remy and Gehin in La Brasse in the Vosges and Jacob Sandungen in Ecker (Norway), independently of one another tried artificial insemination of trout eggs.

Already in 1852 all these achievements (or even in 1850 – according to Goryczko) with the financial support of the Emperor Napoleon III, resulted in the creation of Hünningen (eastern France) the center for stocking of material for salmonids production, including rainbow trout. In the years 1852–1905, after the Franco-German War the property region was turned to Germany and the Center was successfully headed by a Director Haak. He had great merit in the spring trout first import to Europe (1870), and in 1881 – the American rainbow trout. Haak was also famous as a promoter and director of fishing training courses organized for the first time in Germany. Academic supervisor of the center was known embryologist professor Coste, French member of the College and the author of many publications, including *The fisheries*. As a result all above mentioned actions Europe was launched in the rapid development of the production of salmon and rainbow trout in the second half of the nineteenth century.

The first trout hatcheries and fishing facilities then called "trout farms" aroused also on Polish land, especially in the area belonging to Austria (Galicia) during this period. The first hatchery was founded in Dubiu near Krakow in 1850 on Szklarka creek (a tributary of the Rudawa and Vistula Rivers) by a medicine doctor Jan Radziwoński. According to Dr. Kołder (Phot. 1.1), the author of the first Polish textbook on rainbow trout in ponds (1948), hatchery capacity in Dubiu initially was 200 000 grains eggs and the total pond area did not exceed 1 ha.

In the years 1870–1877 there were created another trout hatcheries, including facilities in Lipowa, Złatne, Rycerka (region of Beskid Żywiecki), in 1879 on the outskirts of Krakow near Rudawa, and in 1881 – in Złoty Potok near Janowo Częstochowskie. Small trout hatcheries, with a few stakes, were also created in the late XIX century on Podbeskidzie of Cieszyn Silesia. The oldest, was founded by the Habsburg Chamber Cieszyn, in Brenna (1872), the hatchery in Wisła-Czarne (1877) was a bit younger, which, thanks to Beskid Gorals, provided royal and imperial court in Budapest and Vienna with trouts until the First World War.

A bit later there were created small hatcheries (no longer existing) in Tyr (1890) in Zaolzie, in Istebna (1892) and Ustroń (1896). Around 1898, for a short time there was also little known hatchery trout in

Strumień so-called Country Frog (Silesia), the owner and breeder of which – Karol Weigel was together with prof. Maksymilian Siła-Nowicki (Jagiellonian University) and Tomasz Dubisch, one of the first honorary members of the Moravian Land Fisheries Society. In the 1980's and 1990's there were created another hatcheries in Kamesznica and Rycerka near Soła, Sucha Beskidzka, Czorsztyn, Myślenice, Poronin, Ruska Wieś, Poręba, Sanok, Ruda Różaniecka and distant Stryj.

More dynamic development of Polish trout culture was only after 1904, when another batch of eyed rainbow trout eggs was imported from Sweden to Złoty Potok. The eggs imported were intended mainly for the production of trout for stocking streams. With the construction of centers of hatchery and trout stocking in the late XIX century the exclusive Polish clubs and sports fishing in the region of Małopolska (Galicia) and Austrian Silesia began to arise. On the initiative of Zygmunt Fischer, the first modern sport-fishing club, saved as Krakowski Fishing Clubwas was founded in 1896. Since 1906, in Krakow, there was a Society of Friends of Fishing Sport, promoting hatchery and trout restocking to the streams, in 1907 – Fishing Club in Czarny Dunajec, in 1914 – in Rzeszów in 1917 – Nowy Sącz and Warsaw, and in 1921 – Hunting and Fishing Society in Cieszyn.

### 1.5. The period of the 1930's and 1940's development of trout farming in Poland

The years of 1930's have resulted in the construction of the following restocking facilities in Folusz (1930), Zawada (1932), Ojców and Czatkowice (1934), Olszówka (1936) and Zawoja near Babia Góra (1937). At this time in 1936 at the initiative of prof. Bronisław Romaniszyn, the musician of Music Conservatory in Katowice and also the president of the National Fisheries Society in Krakow there emerged the idea to built House of Culture Stocking and fishing in Łopuszna near Dunajec (Phot. 1.2). It was supposed to be compensation for future losses in salmonid fish stocks (!) after the planned dams in Rożnów and Czorsztyn crossing the canyon of Dunajec despite the anticipated construction of fish ladders.

In spite of the outbreak of the Second World War and the occupation, the resort, created in the style of highlander (architect B. Treter) was opened on July 18, 1942 and is currently celebrating its 70-years anniversary. The foundation act of this center is preserved to this day. It has been dedicated to the liberation on 9 August, 1948, and its opening ceremony was attended by renowned professors: Valery Goetel, Stanisław Żarnecki, Franciszek Hendzel and Bronisław Romaniszyn.

Increased production of the consumption rainbow trout (mark) was probably only in the 1940's with the construction of a flagship trout project in the Dolina Będkowska (Małopolska province, district of Krakow), foreseen also as a training center. The resort owned hatchery and set of ponds with full production cycle, which amounted to 10 tons of store fish.

### 1.6. Period after the Second World War

Country production of trout was relatively small to the years 1950's and 1960's (up to 200–300 tons per year) and traditionally focused on upland areas – Polish mountainous southern areas (mainly Małopolska voivodeship).

However, at the end of the 1960's there was rapid growth of rainbow trout farming in the Polish northwest territory, in sparsely populated areas, with no industry and with its clear waters and rivers flowing into the Baltic Sea. This action successfully developed there, especially thanks to the enthusiasm of the State Farm Fisheries ichthyologists and Polish Anglers Association in Słupsk and Koszalin. The precursors to the development of Polish trout farming are: Bernard Gliszczyński (1914–1979) – an excellent practicing farmer (Phot. 1.3), Janusz M. Latanowicz (1928–2011) – for many years director of PGRyb in Słupsk and

Dr. Konstanty Stefan Bortkiewicz (1919–2010) – Director of PGRyb in Bydgoszcz and many other ichthyologists practitioners.

In 1955, at the initiative of prof. Stanisław Sakowicz the Wheeling River Laboratory in Gdańsk Oliwa was opened. It belonged to the Inland Fisheries Institute in Olsztyn (Phot. 1.4). It was located in the place of the old hatchery (XIX century), belonging to the local forest. Its task was to conduct research and implement technologies of rearing trout stocking material and rainbow trout.

Its first director was Jan Jokiel, and since 1959 Ryszard Bartel. Till the end of mid-eighties, the Laboratory developed and implemented the first in Poland trout feed pellets, the method of controlling rainbow trout spawning season, and the method of winter incubation and rearing of rainbow trout obtained from autumn spawning.

### 1.7. Modern precursors

Important role in the construction of Polish trout farming was played by Polish Angling Association (PZW). In the 1960's, the organization had the most modern facilities destined for salmonids farming in Łopuszna, Rożnów, Porąbka (Małopolskie voivodeship), Czarci Jar (Masuria) and Rumia near Gdynia. The engineers: Ryszard Maliszewski, Marek Bartusch, Kazimierz Krasowski and Mieczysław Kowalewski (Phot. 1.5), Jerzy Palladino and Dr. Wojciech Brudzinski gained practical knowledge there. They are also those to whom we owe today's success of Polish salmonids breeding.

The anglers' initiative of development of trout farming was taken by the team of the State Farm Fishery workers and so-called private owners. Among these early pioneers there were already mentioned Bernard Gliszczyński, Janusz Latanowicz and Konstanty Bortkiewicz all actively assisted by Stefan Kosmulski, Józef Tylenda, Edward Kraus, Marek Bartusch, Józef Wandtke and Andrzej Marczyński. The Olsztyn ichthyologists like Lech Kotowicz, Jan Stafiniak and Władyslaw Gilarski followed their example. All actions undertaken were also supported by Andrzej Galli from Warsaw, and later by Witold Milczarzewicz (1940–2010).

The activities of people enumerated above were mentioned mainly by graduates of the former Faculty of Fisheries WSR-ART in Olsztyn (1951–1999): Roman Aszyk, Jacek Niewęgłowski, Piotr Gumowski, Jerzy Szarkowski, Bogusław Karaś, Antoni Pirtań, Krzysztof Grecki, Jacek Farenholc, and Andrzej Marczyński, Lidia Pirtań, Halina Wiśniewska, Bożena Kacperska and others.

This also applies to private farming pioneers, including Tadeusz Nowicki, Jan Łabęcki, Zenon Krysiński, Janusz Skołysz, Antoni Wawer, Piotr Abako, Piotr Gabriel, Jacek Juchniewicz, Marek Piszczała (Phot. 1.6), Józef Łempio and Dariusz Gorbaczow (the latter is present president of SHRŁ).

The successful development of Polish trout farming has been mainly due to exemplary cooperation of all the participants in this emerging industry. Thus, theoretical and difficult basis of the construction of the farming buildings are due to Dr. Julian Wieniawski (IRS) and the rules of culture were described in accessible manner by already mentioned Bernard Gliszczyński, Jerzy Łękawski and Adam Piller – animator of rainbow trout (and carp) culture in Galicia, a longtime fisheries inspector WRN in Krakow .

This period was characterized by dynamic growth in trout production till the end of the 1980's. In 1978 it exceeded the magical threshold of 1 000 tones, in 1986 – 3 000 tons and in 1998 – 9 000 tones. The years 2005–2008 were record-breaking. The production of trout then in Poland exceeded 17 000 tons and Poland become a leader among nineth trout producers in Europe.

According to prof. Krzysztof Goryczko (Phot. 1.7), a longtime president of Trout Farmers Association, founder and manager of scientific Trout Breeding Department in Rutki belonging to the Inland Fisheries Institute in Olsztyn named after Stanisław Sakowicz, respected educator and researcher at UWM in

Olsztyn: "The main factor in the development of the industry was the enthusiasm, knowledge and consistent action of surprisingly high number of people who have laid the foundation and built up Polish trout farming and breeding".

They were both, all named above and much larger number of farmers and friends, which should be honored collectively, and especially and personally – aged professor Stanisław Bontemps'a (†1925) who was always with trout farmers creating the atmosphere of kindness and camaraderie.

The dissemination of knowledge on fishing was also due to invaluable professional magazine "Fish Economy" (1949–1991), the continuation of which can be found in "Fishing Review" (Poznań) and "Magazine of Fishing Industry" (Gdynia) as well as Self-dependent Laboratory of IFI Development Dissemination in Olsztyn, led by many years by Jerzy Waluga (†1929).

The graduates and employees of the Faculty of Fisheries WSR-ART in Olsztyn have also a significant contribution to teaching and training, since the inception of the Faculty in 1951 to the present. The Faculty has been the part of UWM structure since 1999. Exchange of professional experience, integration and strengthening the friendly relationship bonds are also possible during the annual Trout Farmers Conference, initiated by prof. Ryszard Bartel (IFI), organized within the actions of NOT, and later organized by the Inland Fisheries Institute in Olsztyn.

Krystyna A. Skibniewska, Jan Kłobukowski, Joanna Łuczyńska

# 2. ROLE AND IMPORTANCE OF TROUT MEAT IN THE HUMAN DIET

The development of science in the 20th century has shed a new light on the role of fish consumption in supporting human health. Currently, the theory on the impact of marine-origin food on the evolution of a species is accepted. Archaeological studies have confirmed that Homo sapiens evolved from the primates in the coastal regions of Africa and Anatolia. The unique composition of diet, particularly a high content of polyunsaturated fatty acids, has enabled an expansion of brain volume and intellectual development of our ancestors in a relatively short period of time. These acids are now still used by human organism to construct cell membranes, especially the neurons and the brain, which is composed in app. 60% of lipid components.

Since lipids are not only a source of energy, but also a substrate for the construction of many components of the body, they should be supplied with food throughout life. Their supply is particularly important during growth, i.e. during pregnancy and the intensive development of a child. It has been shown that intellectual development of children is strongly and positively correlated with the volume of fish consumed by their mother during pregnancy. The authors strongly recommend listening to a very interesting lecture on these issues delivered by Prof. Michael Cawford, Institute of Brain Chemistry and Human Nutrition in London, which can be found at http://www.seafood.net.au/printerfriendly/?pid=1003&nid=403#4.

### 2.1. Characteristics of trout fat

It is generally accepted that lipids are natural, substances that are insoluble in water and mainly composed of glycerin and fatty acid esters. These glycerols may be bound to other compounds/substances to form complex lipids, such as glycolipids (with sugars) or phospholipids (with residues of phosphoric acid). They have a variety of different functions in the body and are a source of concentrated and readily-available energy as well as being substrates for many important structures in the body.

Fatty acids with different structures are the components of cell membranes. Saturated acids form simple and rigid chains, ensuring proper cell shape, whereas unsaturated acids loosen the structure, allowing for exchange through the cell membrane and for changes in its shape. Lack of an appropriate volume of fatty acids forces the body to use another acid to construct the cell, usually with the same number of carbon atoms, although this compromises its functions. This knowledge confirms the importance of supplying food with an adequate ratio of fatty acids.

Fatty acids are the components of many other biologically-active compounds, such as tissue hormones (prostaglandins), neurotrasmitters (serotonin and dopamine) and eicosanoids with anti-inflammatory and

anti-thrombotic activity. Fatty acids constitute the structure of cholesterol and it is well-known that, depending on the type of acid (saturated or unsaturated), "good" HDL or "bad" LHL cholesterol is formed. It is clearly a mistake to eliminate lipids from the human diet. Instead, the level and composition of fat should be adjusted to individual physical activity level to ensure a supply of all essential fatty acids, particularly those found in fish.

The international scientific literature constantly (practically each month) provides further evidence on the beneficial impact of fish consumption on the physical and mental state of the human body; this effect may sometimes be termed therapeutic. The first reports concerned the prophylactic and therapy-supporting impact of ω-3 fish fatty acids on the condition of the cardiovascular system. This discovery has resulted in a wide variety of available preparations based on fish liver oil. Currently, there is growing evidence confirming the role of ω-3 fatty acids in decreasing the frequency of deaths due to cardiac failure by preventing arrhythmia related to acute cardiac ischemia that leads to heart attacks.

There are numerous clinical publications that have documented the role of polyunsaturated fatty acids in reducing hypertension. This metabolic disease affects a significant fraction of the adult population in well-developed countries. Furthermore, this condition is being more commonly seen even in children. The possible reasons include decreased physical activity and inadequate diet composition: excessive salt and fat with an inadequate ratio of fatty acids (a high proportion of saturated acids, an excess of monounsaturated and diunsaturated acids, together with a deficiency in polyunsaturated fatty acids). Population studies have confirmed a significantly lower incidence of hypertension in groups of people frequently consuming fish and seafood despite a high incidence of other risk factors, such as severe stress or smoking.

Arteriosclerotic vascular disease (ASVD) is another metabolic disease with increasing incidence which can be prevented with systematic consumption of fish. Polyunsaturated fatty acids alleviate the inflammatory reaction of the endothelium by reducing the volume of free radicals, increasing the concentration of HDL cholesterol and significantly decreasing the concentration of triglycerides in the blood. The occurrence of ASVD lesions is mainly associated with the presence of "bad" cholesterol, whereas most recent studies have indicated that free radicals and pro-inflammatory factors, as well as an excess of triglycerides are the predominant cause. A diet rich in fish provides a source of polyunsaturated fatty acids which counteract these unfavourable factors.

There are several publications which have confirmed the beneficial impact of ω-3 fatty acids on patients with mental and neurological diseases (schizophrenia, depression, Alzheimer's disease and Parkinson's disease), rheumatic diseases or skin conditions (e.g. psoriasis). Administration of ω-3 preparations is commonly used to enhance general immunity. However, it would be more beneficial to introduce more fish into the daily Polish diet instead of occasional supplementation with such preparations.

### 2.2. Characteristics of trout protein

While discussing the importance of "trout meat", the role of protein cannot be omitted. It is one of the essential nutrients in the human diet. The most recent Polish standards indicate a dose of 0.8g/kg BW/day for an adult, which corresponds to app. 50g of (pure) protein per day. It is emphasized that not only the quantity, but also the quality of protein is fundamental to the proper functioning of the body. Fish protein is much higher quality than standard protein, i.e. chicken egg protein. Despite the well-known nutritional values of fish protein, there are a limited number of publications on its composition.

The nutritional value of protein in a food product, including fish protein, is determined by its amino acid composition. The main attention is focused on the amino acids that cannot be synthesized by

the human body; these are called "essential" amino acids and include histidine (essential for children), treonine, lysine, leucine, isoleucine, phenylalanine, methonine, tryptophan and valine.

The total content of essential and semi-essential amino acids in fish usually exceeds the content in the standard protein, i.e. 26.5 g in 100 g of protein. Fish protein has a high concentration of lysine, leucine, aromatic amino acids (phenylalanine and tyrosine), sulphur amino acids (methionine and cysteine) and histidine.

The basic parameter used for evaluation of the nutritional value of protein in food products is the chemical score (CS) which describes the lowest content of a given essential amino acid in relation to its content in the standard protein. Such amino acid is termed as "limiting the nutritional value of a given protein". In the case of fish, valine is a limiting amino acid although the CS values for this amino acid are very high. It is important since a valine deficiency may cause motor incoordination, loss of body mass and inappetance, whereas an adequate amount of this amino acid exerts a beneficial impact on the functioning of the dendritic cells, especially in persons with hepatic cirrhosis. The calculated CS values indicate that almost 100% of essential amino acids from fish protein may be used to synthesise the proteins of the body.

From the perspective of using fish protein in human nutrition, it seems beneficial because of the high content of lysine that is found in small amounts in cereal proteins. It should be emphasized that cereals and cereal food products are the basis of all food-guide pyramids which have been developed to date. Since it is recommended to frequently consume cereals, lysine should be supplied in daily rations with other food products. This is important because a lysine deficiency in diets may lead to muscle atrophy and bone decalcification and putrescine and cadaverine synthesis (due to decay processes in the large bowel). The lysine supply in diets should not be too high, although it helps to reduce the risk of heart diseases and neoplastic conditions which result from aberrated metabolism.

It has been found that an optimal level of leucine in the human body prevents neurological disorders. Moreover, leucine helps to maintain optimal body weight since it reduces body fat mass by 25% and improves the indices of glucose and cholesterol metabolism. A high content of aromatic amino acids (phenylalanine and tyrosine) in the protein of trout should not pose any risk in the individuals who do not show any abnormalities in the process of oxidation of phenylalanine to tyrosine. It has been shown that a diet deficient in, or without tyrosine, results in an increased demand for phenylalanine above the current nutritional recommendations.

The consumption of sulphur amino acids and its volume has attracted interest in the context of the prevalence of chronic diseases, such as cardiovascular conditions, Alzheimer's disease and diabetes. In the body, methionine is transformed into homocysteine, which elevates blood levels and leads to hyperhomocysteinaemia, a risk factor of development of sclerotic and thrombotic changes in blood vessels, infarct and stroke. Furthermore, a high consumption of proteins containing methionine and cysteine increase calcium losses, leading to a significant reduction in bone mineral density and bone mass. Methionine deficiencies may lead to degeneration of the liver and impaired immunity. This does not present a problem because of the volume of fish consumption in the world and in the countries of Eastern Europe.

### 2.3. Other advantages of trout meat

The content of undesirable substances in fish and seafood is a major obstacle to increasing the consumption of these products. For instance, in countries with a high consumption of fish and seafood, the allowed levels of daily mercury intake are exceeded. The average Polish diet has only a small percentage of the allowed amount of this very dangerous element. This is explained by the low consumption

of sea fish. Moreover, fish are an important source of contamination with dioxins. These compounds are carcinogenic, teratogenic and have estrogenic properties, even in microdoses. Their wide prevalence leads not only to increased incidence of neoplastic diseases and congenital disorders in children, but it is also associated with problems with maintaining pregnancy, a reduction in the number of live sperm cells and even with an increase in the severity of osteoporosis.

Fish from aquaculture, particularly from farms operating in Poland, are a counterbalance for fish and seafood. The strict environmental laws and the knowledge of fish producers and diligence in their daily work contribute to the fact that Polish trout are a high quality source of food (in terms of the level of environmental contamination). The attention paid to the quality of feed generates a product with many healthy properties as well as good economic effects.

### 2.4. Summary

Trout consumption in Poland should be increased since it contains highly valuable, easily digestible protein and lipids with unique compositions and beneficial properties for the human body. An increase in fish consumption, including trout, would support the activities of public health and hygiene campaigns aimed at preventing obesity and metabolic diseases. Fish consumption should be promoted, particularly among pregnant women and children, since epidemiological studies clearly indicate a better development of the nervous system and higher intellectual level in children, who may benefit from fish fatty acids starting from the foetus. The health qualities of trout and the possibility to direct it for consumption shortly after catching are additional arguments for increasing the consumption of this fish in Poland.

Janusz Guziur, Anna Wiśniewska, Stefan Dobosz, Krzysztof Goryczko

# 3. Rearing and biometric parameters of the trout

### 3.1. Methodology

For the purpose of this research, two types of trout farms were distinguished according to the applied fish production technology: OS – fish farms with extensive production (single use of water, that is open flow systems), and RAS – fish farms with a high level of water recirculation (farms using closed, recirculating aquaculture systems).

Two groups of parameters concerning rainbow trout were analyzed:

1. Rearing parameters: fish catch in kg/m$^3$, individual body gain (g/indiv.), survivability of fish stocks (S) and feed conversion ratio (FCR);
2. Biometric parameters – length in cm (Lc), weight of fish in g (W) and Fulton's condition index.

The fact that analyses of particular parameters were scheduled in two rearing seasons (by convention called autumn and spring) was justified during the preliminary stage of the research, when data collected during that phase were analyzed. This analysis (presented in this chapter) confirmed that such an approach would be necessary. As a result, it became possible to accomplish comparative analysis of data, which in turn enabled the researchers to draw more objective conclusions, both about the underlying assumptions and the technologies.

According to the adopted methodology, in three research seasons, at 6 farms located in different parts of Poland (3 farms representing each technology, i.e. OS and RAS) biometric measurements of commercial trout originating from two groups: small (S) from 350 to 500 g and big (B) from 501 to 800 g, were performed twice (in each season in spring and autumn). Based on the results of these measurements, classical rearing and condition indices were calculated according to Fulton.

### 3.2. Results of the preliminary research

The preliminary research was conducted at a fish farm owned by the Institute of Inland Fisheries in Olsztyn, the Department of Salmonid Fish Rearing . The fish farm is located in Rutki and represents fish farms with extensive type of production (OS). This was a pilot study and its main aim was to test the measurement methods, to establish logistics of collecting samples from the other model fish farms and to indicate the most important aspects of technology and rearing in aquaculture.

During this initial phase of the research, in October to November 2009 and May to June 2010, measurements were taken and the achieved parameters were analyzed (Table 3.1).

**Table 3.1.** Summary of analyzed rearing and technological parameters during the preliminary research

| Rearing and technological parameters | May – June 2010 (spring) | October – November 2009 (autumn) |
|---|---|---|
| Surface area ($m^2$) | 56.00 | 56.00 |
| Water capacity ($m^3$) | 56.00 | 56.00 |
| Water flow ( l/s) | 8.00 | 8.00 |
| STOCK | | |
| Month | April 2010 | July 2009 |
| Number of individuals | 2058 | 1987 |
| Weight (kg) | 769.00 | 705.00 |
| Population density ($kg/m^3$) | 13.73 | 12.59 |
| Body mass (g/indiv.) | 380.00 | 345.00 |
| CATCH | | |
| Month | June 10 | November 2009 |
| Number of individuals | 1997 | 1885 |
| Weight (kg) | 1192.00 | 1225.00 |
| Population density ($kg/m^3$) | 21.29 | 21.88 |
| Body mass (g/indiv.) | 572.00 | 607.00 |
| Survivability (%) | 0.97 | 0.95 |
| Gain in total (kg) | **423.00** | **520.00** |
| Individual body gain (g/indiv.) | 192.00 | 262.00 |
| Average daily gain (%) | 0.77 | 0.62 |
| FCR | 1.05 | 1.09 |

Comparison of the rearing and technological parameters recorded during the preliminary phase of the research, using *t*-Student test, enabled us to conclude that the differences observed within particular parameters were statistically significant ($t = 0.544$; $p = 0.682$). The differentiating factor proved to be the rearing season, hence it was agreed to repeat same measurements twice in each year, in spring and autumn.The values of the basic biometric parameters identified during the preliminary study are contained in Table 3.2.

**Table 3.2.** Summary of basic biometric parameters analyzed during the preliminary research at the fish farm in Rutki

| Lc | Body mass of trout | Fulton's condition index | Season | Lc | Body mass of trout | Fulton's condition index | Season |
|---|---|---|---|---|---|---|---|
| 32.0 | 458 | 1.39770508 | autumn 2009 | 31.0 | 351.0 | 1.17820818 | spring 2010 |
| 32.5 | 438 | 1.27592171 | | 32.3 | 366.0 | 1.08610926 | |
| 33.0 | 434 | 1.20766898 | | 33.0 | 404.0 | 1.12418955 | |
| 35.0 | 444 | 1.03556851 | | 34.2 | 431.0 | 1.07745453 | |
| 34.5 | 490 | 1.19327020 | | 34.6 | 454.0 | 1.09604291 | |
| 34.0 | 476 | 1.21107266 | | 32.4 | 424.0 | 1.24661063 | |
| 32.0 | 354 | 1.08032227 | | 33.8 | 456.0 | 1.18090440 | |
| 32.5 | 397 | 1.15648612 | | 32.5 | 386.0 | 1.12444242 | |
| 33.0 | 434 | 1.20766898 | | 33.7 | 478.0 | 1.24893028 | |
| 35.0 | 456 | 1.06355685 | | 33.6 | 438.0 | 1.15466574 | |
| 36.0 | 496 | 1.06310014 | | 33.0 | 423.0 | 1.17705985 | |
| 34.0 | 454 | 1.15509872 | | 33.1 | 405.0 | 1.11678878 | |
| 32.0 | 450 | 1.37329102 | | 32.0 | 387.0 | 1.18103027 | |

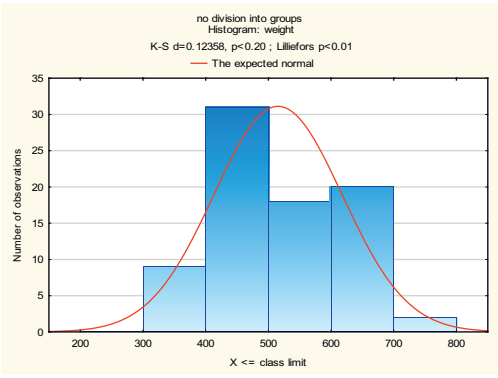
| Lc | Body mass of trout | Fulton's condition index | Season | Lc | Body mass of trout | Fulton's condition index | Season |
|---|---|---|---|---|---|---|---|
| 32.5 | 430.0 | 1.25261721 | | 33.6 | 424.0 | 1.11775861 | |
| 33.0 | 434.0 | 1.20766898 | | 34.3 | 476.0 | 1.17957237 | |
| 35.0 | 445.0 | 1.03790087 | | 32.5 | 344.1 | 1.00238507 | |
| 34.5 | 480.0 | 1.16891775 | | 32.0 | 357.3 | 1.09039307 | |
| 34.0 | 471.0 | 1.19835131 | | 32.5 | 417.8 | 1.21707783 | |
| 32.0 | 440.0 | 1.34277344 | | 34.6 | 331.3 | 0.79982162 | |
| 32.0 | 436.0 | 1.33056641 | | 33.4 | 458.4 | 1.23028353 | |
| 37.5 | 630.0 | 1.19466667 | | 38.5 | 595.1 | 1.04281618 | |
| 36.5 | 545.0 | 1.12077364 | | 36.5 | 570.2 | 1.17259657 | |
| 37.5 | 620.0 | 1.17570370 | | 39.8 | 788.7 | 1.25101531 | |
| 35.0 | 502.0 | 1.17084548 | | 37.5 | 575.7 | 1.09169778 | |
| 37.1 | 635.0 | 1.24351777 | | 37.9 | 570.0 | 1.04702542 | |
| 36.0 | 610.0 | 1.30744170 | | 35.5 | 600.7 | 1.34268016 | |
| 34.5 | 545.0 | 1.32720869 | autumn 2009 | 36.6 | 527.7 | 1.07632602 | spring 2010 |
| 37.5 | 643.0 | 1.21931852 | | 35.0 | 543.0 | 1.26647230 | |
| 36.5 | 630.0 | 1.29557320 | | 35.0 | 509.3 | 1.18787172 | |
| 37.5 | 640.0 | 1.21362963 | | 37.5 | 608.2 | 1.15332741 | |
| 37.5 | 645.0 | 1.22311111 | | 39.5 | 693.5 | 1.12526595 | |
| 36.5 | 621.0 | 1.27706501 | | 38 | 635.0 | 1.15723866 | |
| 37.5 | 647.0 | 1.22690370 | | 39.3 | 678.1 | 1.11716184 | |
| 35.0 | 510.0 | 1.18950437 | | 36.3 | 529.1 | 1.10615984 | |
| 37.1 | 623.0 | 1.22001822 | | 38.3 | 727.2 | 1.29436735 | |
| 38.0 | 692.0 | 1.26111678 | | 36.9 | 598.1 | 1.19040489 | |
| 34.5 | 555.0 | 1.35156114 | | 38.1 | 588.3 | 1.06371166 | |
| 37.5 | 620.0 | 1.17570370 | | 37 | 577.7 | 1.14050500 | |
| 36.5 | 611.0 | 1.25650036 | | 37.8 | 521.2 | 0.965003764 | |
| 37.5 | 630.0 | 1.19466667 | | 37 | 518.5 | 1.02363137 | |

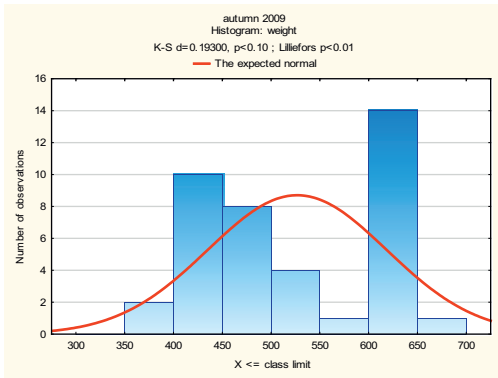
An intravital form of research was adopted in the preliminary study. Therefore, slaughter weight was not assessed.

### 3.2.1. Results of the analysis of data collected during the preliminary research

Because of the great dispersion of values in weight of caught fish, histograms were drawn up to achieve the best division into groups.

The observations suggested an uneven distribution of variables, depending on the rearing season. In further study, it was decided to take samples in two weight groups, i.e. S from 350 g to 500 g and B from 501 g to 800 g. Next, it was checked whether the observed differences in values of the analyzed parameters in the analyzed seasons were statistically significant. Table 3.3 shows results of *t*-Student test for the basic parameters, both with and without division into the two weight groups.



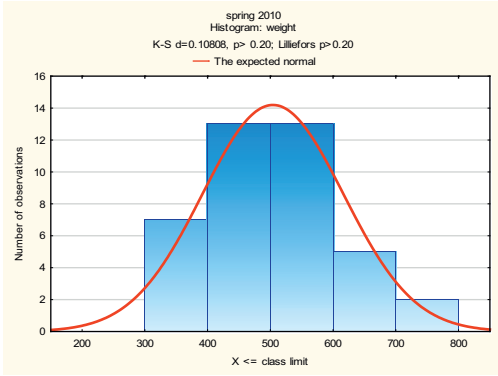




**Table 3.3.** Analysis of differences between values of analyzed parameters (*t*-Student test, significant statistically in red)

| Parameters | Mean | | *t* | *df* | *p* | SD | |
|---|---|---|---|---|---|---|---|
| | autumn 2009 | spring 2010 | | | | autumn 2009 | spring 2010 |
| No division | | | | | | | |
| Lc (cm) | 35.0425 | 35.2525 | -0.416 | 78 | 0.677 | 2.015 | 2.468 |
| Weight (g) | 526.7750 | 504.1800 | 0.985 | 78 | 0.327 | 91.680 | 112.418 |
| Fulton's condition index | 1.2151 | 1.1090 | 3.019 | 78 | 0.003 | 0.087 | 0.204 |
| S | | | | | | | |
| Lc (cm) | 33.4250 | 33.1050 | 0.897 | 38 | 0.375 | 1.269 | 0.963 |
| Weight (g) | 445.8500 | 410.5950 | 2.896 | 38 | 0.006 | 31.787 | 44.195 |
| Fulton's condition index | 1.1980 | 1.0772 | 1.841 | 38 | 0.073 | 0.108 | 0.272 |
| D | | | | | | | |
| Lc (cm) | 36.6600 | 37.4000 | -1.883 | 38 | 0.067 | 1.105 | 1.365 |
| Weight (g) | 607.7000 | 597.7650 | 0.496 | 38 | 0.622 | 49.553 | 74.496 |
| Fulton's condition index | 1.2322 | 1.1408 | 3.636 | 38 | 0.000 | 0.058 | 0.096 |

Comments: *t* – value of *t*-Student test statistics, *df* –degrees of freedom, *p* – probability, SD – standard deviation.

Without dividing the fish into two weight groups, statistically significant differences were observed only in the case of Fulton's condition index. Once the data had been divided into S and B fish, statistical

significance of differences was verified for body weight among S fish and Fulton's condition index for B fish. Since the latter index, which demonstrates condition of fish, depends on the fish body mass, it was recommended to continue collecting data divided between two rearing seasons, commonly used in trout aquaculture.

#### 3.2.2. Summary of the preliminary research

1. The results indicated that the adopted assumptions, such as division of sample collection between commonly accepted fish rearing seasons, were correct as they accounted for possible, statically significant differences between values of particular parameters depending on the season.
2. The results suggested that it is necessary to divide the material into two weight groups: B > 500 g and S < 500 g, which meant that more samples had to be collected. The minimum size of a sample from each weight group is 20 fish.

In order to present both the direction of research on the rearing and biometric parameters of trout and achieved results, data from investigations carried out in two seasons: spring and autumn, were analyzed.

### 3.3. Results of the research in the spring season

The information on technological parameters of fish production was obtained at the sampling sites, by analyzing fishpond logbooks and readings from reading devices. These data are set in Table 3.4 while Table 3.5 presents statistical results.

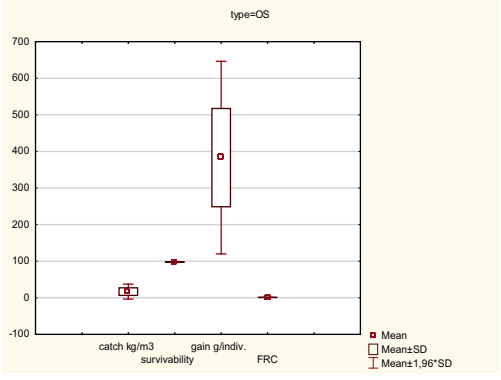
**Table 3.4.** Specification of analyzed fish rearing and technological parameters from the six fish farms

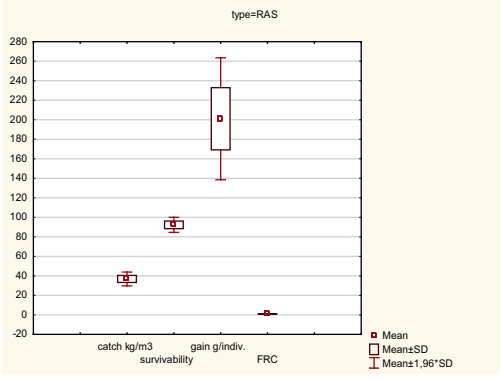
| Parameters | FISH FARM | | | | | |
|---|---|---|---|---|---|---|
| | 1-OS | 2-OS | 3-OS | 1-RAS | 2-RAS | 3-RAS |
| Surface ($m^2$) | 56 | 2 700 | 156 | 210 | 250 | 125 |
| Water capacity ($m^3$) | 56 | 3 500 | 156 | 210 | 470 | 100 |
| Water flow (l/s) | 8 | 10 | 8 | 28 | 300 | 14 |
| Recirculation (*n* steps) | – | – | – | 3 | 5 | 6 |
| No of steps with fish production | – | – | – | (II) | (IV) | (V) |
| STOCK | | | | | | |
| Number of individuals | 2 549 | 12 400 | 7 200 | 20 230 | 48 980 | 10 500 |
| Weight (kg) | 745 | 1 020 | 1 300 | 3 357 | 12 000 | 1 650 |
| Population density ($kg/m^3$) | 13.30 | 0.29 | 8.33 | 15.99 | 25.53 | 16.50 |
| Body mass (g/indiv.) | 292 | 82 | 180 | 166 | 245 | 157 |
| CATCH | | | | | | |
| Number of individuals | 2 514 | 12 100 | 7 415 | 18 625 | 47 610 | 9 200 |
| Weight (kg) | 1 364 | 7 863 | 3 805 | 6 798 | 19 425 | 3 660 |
| Population density ($kg/m^3$) | 24.36 | 2.25 | 24.39 | 32.50 | 41.33 | 36.60 |
| Body mass (g/indiv.) | 542 | 649 | 513 | 365 | 408 | 398 |
| Survivability (%) | 98.60 | 97.60 | 0.97 | 92.00 | 97.20 | 0.88 |
| Gain in total (kg) | 619 | 6 843 | 2 504 | 3 441 | 7 425 | 2 010 |
| Individual body gain (g/indiv.) | 250.00 | 567.00 | 333.00 | 199.00 | 163.00 | 241.00 |
| Average daily gain (%) | 0.62 | 0.83 | 1.05 | 0.86 | 0.65 | 1.01 |
| FCR | 0.97 | 1.08 | 1.09 | 1.13 | 0.98 | 1.03 |

**Table 3.5.** Specification of parameters achieved in the spring season and used for statistical analyses

| Parameters | 1-OS | 2-OS | 3-OS | MEAN | 1-RAS | 2-RAS | 3-RAS | Mean |
|---|---|---|---|---|---|---|---|---|
| Fish catch (kg/m$^3$) | 24.36 | 2.25 | 24.39 | ***17.01*** | 32.50 | 41.33 | 36.60 | ***36.81*** |
| Survivability (P%) | 98.6 | 97.6 | 97.1 | ***97.8*** | 92.0 | 97.2 | 87.6 | ***92.3*** |
| Individual gain (g/indiv.) | 250 | 567 | 333 | ***383*** | 199 | 163 | 241 | ***201*** |
| FCR | 0.97 | 1.08 | 1.09 | ***1.05*** | 1.13 | 0.98 | 1.03 | ***1.05*** |

At the first stage of the research, due to a modest amount of data, it was decided to check whether the preliminary noticed differences between values of the analyzed parameters were statistically significant, with the measurements performed on the OS and RAS groups taken as components of a sample.




The highest dispersion of measured values and the biggest standard deviations were observed for the parameter of gains in g/indiv. Such results were often noticed in the OS group of fish farms. In order to verify the statistical significance of the observed differentiation, an analysis was run using *t*-Student test. The results are shown in Table 3.6.

**Table 3.6.** Results of the analysis of technological and rearing parameters (*t*-Student test, statistically significant differences in red)

| Parameters | Mean | | *t* | *p* | SD | |
|---|---|---|---|---|---|---|
| | OS | RAS | | | OS | RAS |
| Catch (kg/m$^3$) | 17.0025 | 36.8100 | -3.589 | 0.011 | 10.429 | 3.607 |
| Survivabilty (%) | 97.7750 | 92.2750 | 2.768 | 0.032 | 0.623 | 3.923 |
| Gain (g/indiv.) | 383.2500 | 201.0000 | 2.642 | 0.038 | 134.219 | 31.874 |
| FCR | 1.0475 | 1.0475 | 0.000 | 1.000 | 0.054 | 0.062 |

The analysis confirmed that the observed differences in the volume of catches expressed in kg/m$^3$ were significant and at that stage of research suggest higher values achieved in the recirculatory aquaculture system. The differences in survivability and gain in g/indiv. proved to be significant, with higher values attained in the OS system. The results for FCR were rather astonishing. Our statistical analysis did not prove any differentiation – the values of this parameter did not differ significantly between the two aquaculture technologies.

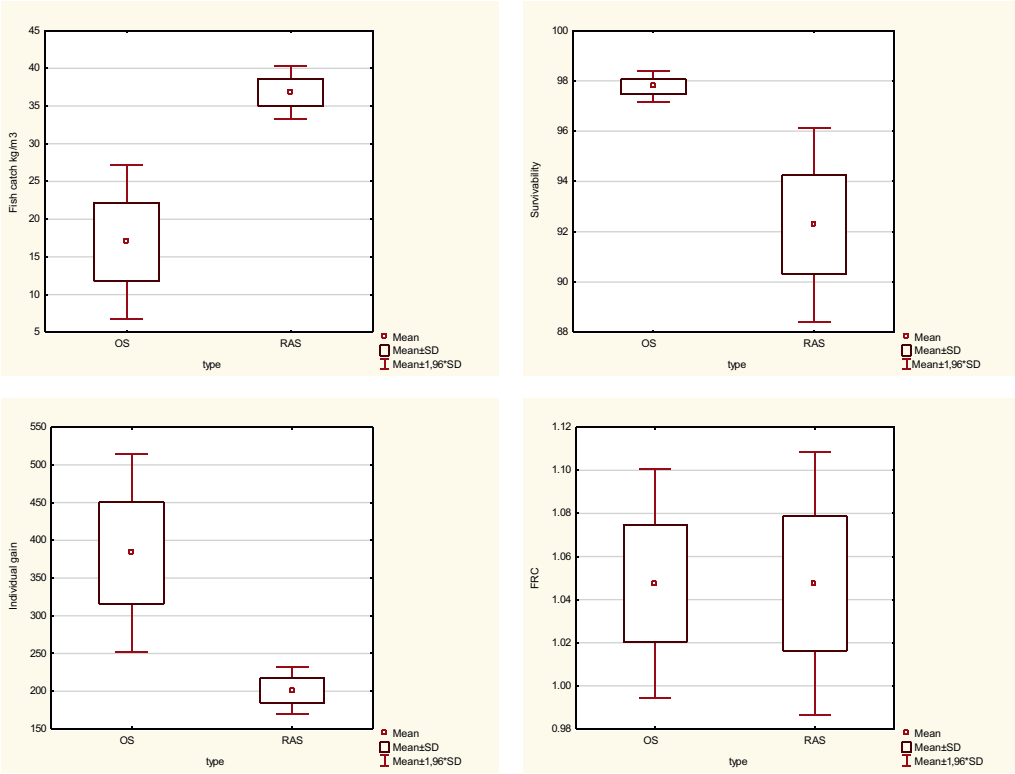



Table 3.7 sets values of biometric parameters achieved during the spring season, both with and without distinction between the two fish rearing technologies. Table 3.8 shows basic descriptive statistics of the data gathered from tests on samples obtained during the spring season, including the division into types of aquaculture technologies and size of fish.

**Table 3.7.** Basic descriptive statistics of data gathered from the spring season sample

| Parameters | *N* of valid | Mean | Minimum | Maximum | SD |
|---|---|---|---|---|---|
| Lc (cm) | 240 | 34.7900 | 26.8000 | 42.4000 | 2.7706 |
| Weight (g) | 240 | 502.9163 | 234.0000 | 825.0000 | 124.1636 |
| Slaughter ratio (g) | 240 | 429.7197 | 200.0000 | 727.0000 | 106.2284 |
| % Of slaughter ratio (%) | 240 | 85.5354 | 71.8310 | 90.8000 | 2.1450 |
| Fulton's index | 240 | 1.1756 | 0.8071 | 1.7088 | 0.1353 |
| 1-RAS | | | | | |
| Lc (cm) | 40 | 34.1975 | 26.8000 | 40.1000 | 3.7213 |
| Weight (g) | 40 | 510.5500 | 238.0000 | 825.0000 | 181.3385 |
| Slaughter ratio (g) | 40 | 440.8500 | 204.0000 | 727.0000 | 157.8082 |
| % Of slaughter ratio (%) | 40 | 86.2422 | 82.5397 | 90.0000 | 1.5202 |
| Fulton's index | 40 | 1.2228 | 1.0110 | 1.7088 | 0.1432 |
| 2-RAS | | | | | |
| Lc (cm) | 40 | 33.2450 | 28.8000 | 38.8000 | 2.6054 |
| Weight (g) | 40 | 445.0000 | 234.0000 | 696.0000 | 138.8689 |

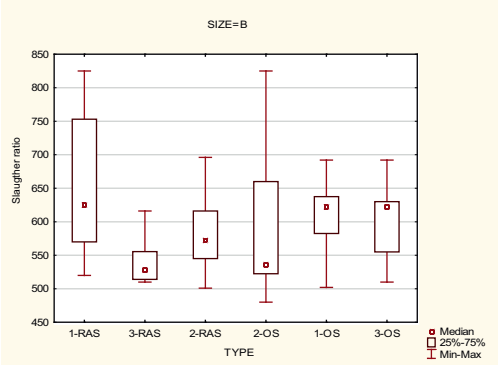
| Parameters | *N* of valid | Mean | Minimum | Maximum | SD |
|---|---|---|---|---|---|
| Slaughter ratio (g) | 40 | 379.1000 | 200.0000 | 578.0000 | 113.0402 |
| % Of slaughter ratio (%) | 40 | 85.6374 | 71.8310 | 89.6296 | 2.9128 |
| Fulton's index | 40 | 1.1688 | 0.9399 | 1.4672 | 0.1363 |
| 3-RAS | | | | | |
| Lc (cm) | 40 | 33.4950 | 29.5000 | 36.1000 | 1.75440 |
| Weight (g) | 40 | 473.7750 | 320.0000 | 616.0000 | 79.81340 |
| Slaughter ratio (g) | 40 | 402.2250 | 282.0000 | 515.0000 | 61.08820 |
| % Of slaughter ratio (%) | 40 | 85.1529 | 80.9524 | 89.2183 | 2.06759 |
| Fulton's index | 40 | 1.2503 | 1.0238 | 1.3793 | 0.07934 |
| 1-OS | | | | | |
| Lc (cm) | 40 | 35.0425 | 32.0000 | 38.0000 | 2.01595 |
| Weight (g) | 40 | 526.7750 | 354.0000 | 692.0000 | 91.68074 |
| Slaughter ratio (g) | 40 | 445.6000 | 296.0000 | 592.0000 | 82.29740 |
| % Of slaughter ratio (%) | 40 | 84.5638 | 71.8310 | 89.3728 | 2.38641 |
| Fulton's index | 40 | 1.2151 | 1.0356 | 1.3977 | 0.08792 |
| 2-OS | | | | | |
| Lc (cm) | 40 | 37.5225 | 35.8000 | 42.4000 | 1.5310 |
| Weight (g) | 40 | 538.7500 | 450.0000 | 825.0000 | 111.4402 |
| Slaughter ratio (g) | 40 | 458.6000 | 377.0000 | 710.0000 | 95.3893 |
| % Of slaughter ratio (%) | 40 | 85.1038 | 83.6364 | 86.9697 | 1.0944 |
| Fulton's index | 40 | 1.0095 | 0.8071 | 1.2699 | 0.1065 |
| 3-OS | | | | | |
| Lc (cm) | 40 | 35.2487 | 32.0000 | 38.0000 | 2.09107 |
| Weight (g) | 40 | 523.1538 | 354.0000 | 692.0000 | 90.81982 |
| Slaughter ratio (g) | 40 | 452.5128 | 307.0000 | 585.0000 | 81.13954 |
| % Of slaughter ratio (%) | 40 | 86.5376 | 83.6957 | 90.8000 | 1.83406 |
| Fulton's index | 40 | 1.1871 | 0.9871 | 1.3733 | 0.09705 |

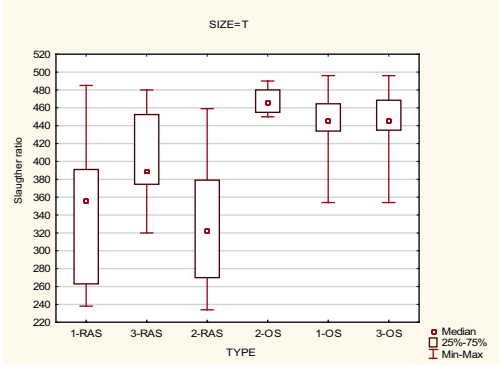
**Table 3.8.** Basic descriptive statistics of the data from the spring season sample, including the division into aquaculture technologies and size of fish

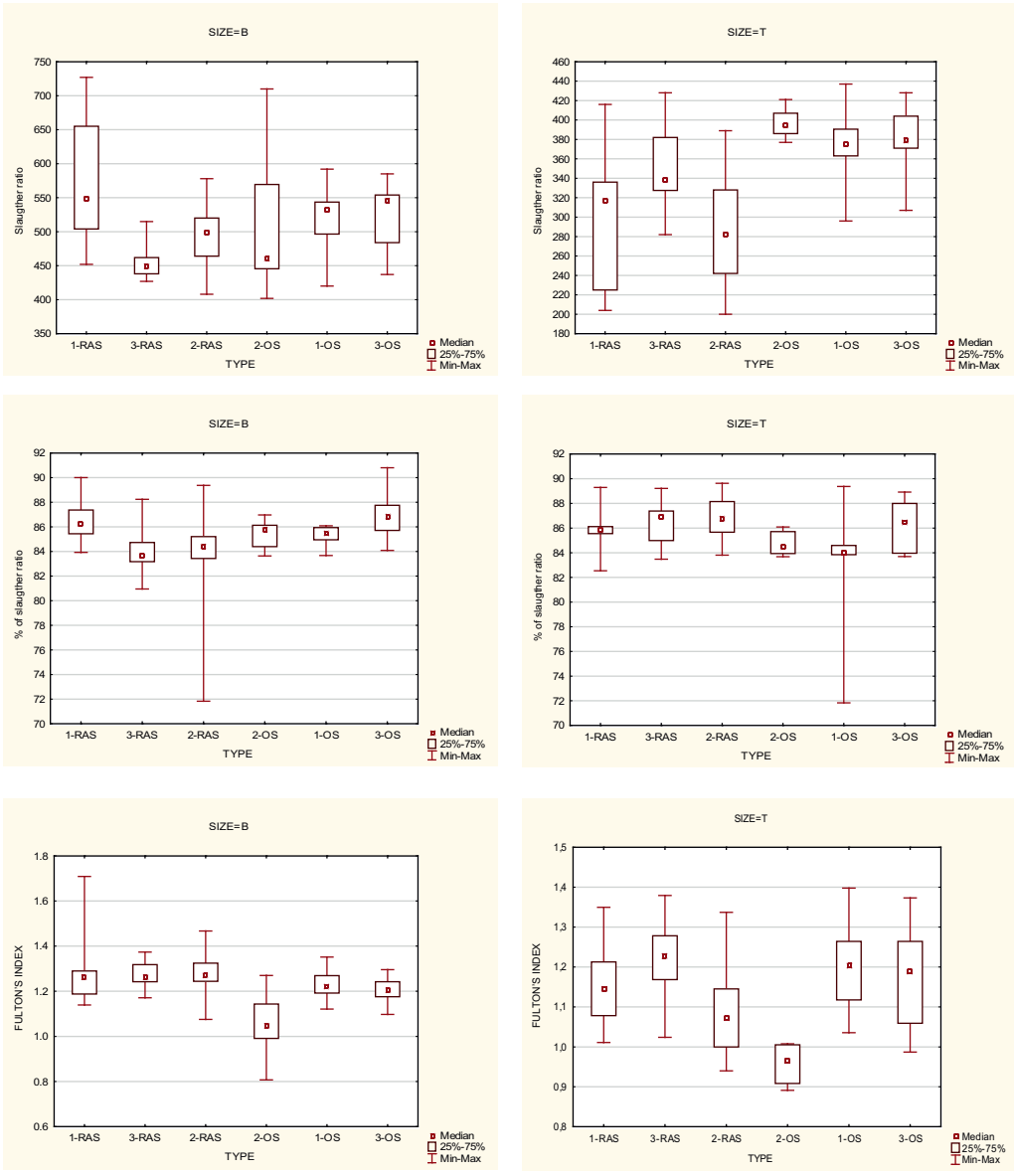
| Parameters | Size B | | | | Seize S | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean | Min. | Max. | SD | Mean | Min. | Max. | SD |
| 1-RAS | | | | | | | | |
| Lc (cm) | 37.16 | 32.80 | 40.10 | 1.87 | 30.91 | 26.80 | 33.30 | 2.11 |
| Weight (g) | 659.04 | 520.00 | 825.00 | 101.09 | 346.42 | 238.00 | 485.00 | 75.70 |
| Slaughter ratio (g) | 570.14 | 452.00 | 727.00 | 87.71 | 297.94 | 204.00 | 416.00 | 65.96 |
| % Of slaughter ratio (%) | 86.51 | 83.92 | 90.00 | 1.53 | 85.93 | 82.53 | 89.29 | 1.48 |
| Fulton's index | 1.28 | 1.13 | 1.78 | 0.15 | 1.15 | 1.01 | 1.34 | 0.09 |
| 2-RAS | | | | | | | | |
| Lc (cm) | 35.71 | 34.00 | 38.80 | 1.125 | 31.23 | 28.80 | 33.50 | 1.451 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Weight (g) | 581.61 | 501.00 | 696.00 | 48.156 | 333.23 | 234.00 | 459.00 | 69.659 |
| Slaughter ratio (g) | 489.17 | 408.00 | 578.00 | 42.896 | 289.05 | 200.00 | 389.00 | 57.976 |
| % Of slaughter ratio | 84.14 | 71.83 | 89.37 | 3.499 | 86.87 | 83.81 | 89.63 | 1.527 |
| Fulton's index | 1.28 | 1.08 | 1.47 | 0.090 | 1.08 | 0.94 | 1.34 | 0.097 |
| 3-RAS | | | | | | | | |
| Lc (cm) | 34.91 | 33.60 | 36.10 | 0.876 | 32.08 | 29.50 | 33.50 | 1.142 |
| Weight (g) | 541.95 | 510.00 | 616.00 | 34.827 | 405.60 | 320.00 | 480.00 | 45.583 |
| Slaughter ratio (g) | 454.20 | 427.00 | 515.00 | 24.941 | 350.25 | 282.00 | 428.00 | 36.752 |
| % Of slaughter ratio | 83.87 | 80.95 | 88.24 | 1.577 | 86.44 | 83.48 | 89.22 | 1.673 |
| Fulton's index | 1.27 | 1.17 | 1.37 | 0.055 | 1.23 | 1.02 | 1.38 | 0.094 |
| 1-OS | | | | | | | | |
| Lc (cm) | 36.7 | 34.5 | 38.0 | 1.11 | 33.42 | 32.00 | 36.00 | 1.270 |
| Weight (g) | 607.7 | 502.0 | 692.0 | 49.55 | 445.85 | 354.00 | 496.00 | 31.788 |
| Slaughter ratio (g) | 517.6 | 420.0 | 592.0 | 44.32 | 373.55 | 296.00 | 437.00 | 31.766 |
| % Of slaughter ratio (%) | 85.2 | 83.7 | 86.1 | 0.85 | 83.89 | 71.83 | 89.37 | 3.166 |
| Fulton's index | 1.2 | 1.1 | 1.4 | 0.06 | 1.20 | 1.04 | 1.40 | 0.109 |
| 2-OS | | | | | | | | |
| Lc (cm) | 38.47 | 36.00 | 42.40 | 1.59 | 36.57 | 35.80 | 37.50 | 0.621 |
| Weight (g) | 610.00 | 480.00 | 825.00 | 120.72 | 467.50 | 450.00 | 490.00 | 15.174 |
| Slaughter ratio (g) | 521.10 | 402.00 | 710.00 | 101.45 | 396.10 | 377.00 | 421.00 | 12.715 |
| % Of slaughter ratio (%) | 85.47 | 83.64 | 86.97 | 1.16 | 84.73 | 83.67 | 86.09 | 0.907 |
| Fulton's index | 1.06 | 0.81 | 1.27 | 0.12 | 0.96 | 0.89 | 1.01 | 0.051 |
| 3-OS | | | | | | | | |
| Lc (cm) | 36.93 | 35.00 | 38.00 | 0.823 | 33.65 | 32.00 | 36.50 | 1.607 |
| Weight (g) | 605.26 | 510.00 | 692.00 | 48.786 | 445.15 | 354.00 | 496.00 | 33.089 |
| Slaughter ratio (g) | 525.53 | 437.00 | 585.00 | 45.927 | 383.15 | 307.00 | 428.00 | 27.814 |
| % Of slaughter ratio (%) | 86.87 | 84.08 | 90.80 | 1.737 | 86.22 | 83.70 | 88.92 | 1.911 |
| Fulton's index | 1.20 | 1.10 | 1.30 | 0.056 | 1.18 | 0.99 | 1.37 | 0.125 |

Comparison of the two aquaculture technologies in the context of two fish sizes is illustrated by the following diagrams.






Because differences were noted between values of the parameters, their statistical significance was analyzed. For this purpose, non-parametric analysis of variance, Kruskal–Wallis test, was applied. Results of a model analysis for Lc parameter are shown in Table 3.9. Based on an analysis performed with no division into two sizes of fish, it was concluded that the values of the Lc parameter at the RAS farms did not differ statistically significantly. However, statistically significant results were attained when the data from the fish farm 2-OS were juxtaposed with the results from the other farms (these values were marked in red) and from the fish farm 3-OS versus 2-RAS and 3-RAS. When the fish were divided into two groups according to their body size (S and B groups), statistically significant differences between Lc values measured in spring were noticed, and our analyses imply distinct differentiation (statistically

significant differences in red). Similar analyses were performed for all the examined parameters. Values of the achieved *H* statistics are comprised in Table 3.9.

**Table 3.9.** Values of statistics z (lower part of the table) and probability p (upper part of the table, bolded italics) for multiple comparisons of the Lc parameter. Non-parametric analysis of variance – Kruskal–Wallis test: $H$ (5, $N$ = 240) = 67.4048; $p$ = 0.0000 (statistically significant values in red)

| Fish farm/ weight group | Fish farm | | | | | |
|---|---|---|---|---|---|---|
| | 1-RAS | 3-RAS | 2-RAS | 2-OS | 1-OS | 3-OS |
| Total | | | | | | |
| 1-RAS | – | ***0.889*** | ***0.822*** | ***0.000*** | ***1.000*** | ***1.000*** |
| 3-RAS | 1.886 | – | ***1.000*** | ***0.000*** | ***0.073*** | ***0.019*** |
| 2-RAS | 1.920 | 0.034 | – | ***0.000*** | ***0.065*** | ***0.017*** |
| 2-OS | 5.096 | 6.983 | 7.017 | – | ***0.000*** | ***0.003*** |
| 1-OS | 0.931 | 2.817 | 2.851 | 4.166 | – | ***1.000*** |
| 3-OS | 1.351 | 3.226 | 3.259 | 3.713 | 0.426 | – |
| Size D | | | | | | |
| 1-RAS | – | 0.000 | 0.061 | 0.527 | 1.000 | 1.000 |
| 3-RAS | 4.527 | – | 1.000 | 0.000 | 0.003 | 0.000 |
| 2-RAS | 2.871 | 1.515 | – | 0.000 | 0.515 | 0.130 |
| 2-OS | 2.107 | 6.554 | 4.865 | – | 0.071 | 0.378 |
| 1-OS | 0.751 | 3.730 | 2.116 | 2.824 | – | 1.000 |
| 3-OS | 0.186 | 4.231 | 2.625 | 2.239 | 0.549 | – |
| Size S | | | | | | |
| 1-RAS | – | 0.046 | 1.000 | 0.043 | 0.002 | 1.000 |
| 3-RAS | 2.964 | – | 1.000 | 1.000 | 1.000 | 0.013 |
| 2-RAS | 1.205 | 1.759 | – | 1.000 | 0.110 | 1.000 |
| 2-OS | 2.979 | 0.015 | 1.774 | – | 1.000 | 0.012 |
| 1-OS | 3.886 | 0.922 | 2.681 | 0.907 | – | 0.000 |
| 3-OS | 0.393 | 3.338 | 1.590 | 3.353 | 4.254 | – |

### 3.3.1. Recapitulation of analyses

Statistically significant differentiation in terms of the type of aquaculture technology appeared for the following fish rearing parameters: Lc, % of slaughter index and Fulton's index. No statistically significant differentiation was confirmed for the parameters of weight or slaughter index. The results of analyses change when an additional parameter is introduced, such as the body size of fish. Then, statistically significant differentiation appears for all the parameters – Table 3.10.

**Table 3.10.** *H* statistics from Kruskal–Wallis test – for analyses performed with the division of fish into two size groups

| Parameter | Size B | Size S |
|---|---|---|
| Lc | $H$ ( 5, $N$ = 120) = 53,49580; $p$ = 0.0000 | $H$ (5, $N$ = 120) = 70,54591; $p$ = 0.0000 |
| Weight | $H$ (5, $N$ = 120) = 23,21431; $p$ = 0.0003 | $H$ (5, $N$ = 120) = 58,94110; $p$ = 0.0000 |
| Slaughter index | $H$ (5, $N$ = 120) = 32,29371; $p$ = 0.0000 | $H$ (5, $N$= 120) = 56,66255; $p$ = 0.0000 |
| % Of slaughter index | $H$ (5, $N$ = 120) = 42,06658; $p$ = 0.0000 | $H$ (5, $N$ = 120) = 31,59334; $p$ = 0.0000 |
| Fulton's index | $H$ (5, $N$ = 120) = 43,39992; $p$ = 0.0000 | $H$ ( 5, $N$ = 120) = 60,07633; $p$ = 0.0000 |

As for analyses including both parameters: type of aquaculture and size of fish (in the first season of the investigations), the highest differentiation occurred for the 2-OS fish farm, followed by the 3-RAS and 2-RAS farms.

### 3.4. Results of the research in the autumn season

The information on technological parameters of fish production was obtained at the sampling sites, by analyzing fishpond logbooks and readings from reading devices.

The results of measurements of the rearing and technological parameters characterizing the analyzed fish farms are set in Table 3.11. The values of the indices used for statistical analysis are set in Table 3.12.

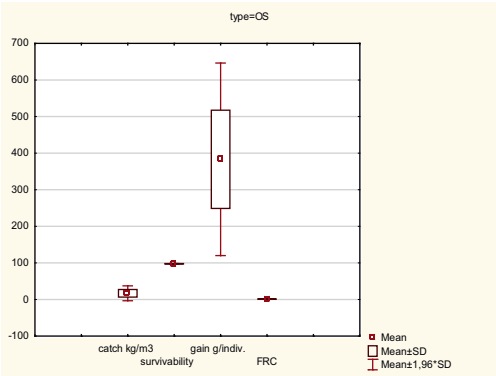
**Table 3.11.** Values of the analyzed rearing and technological parameters at the fish farms

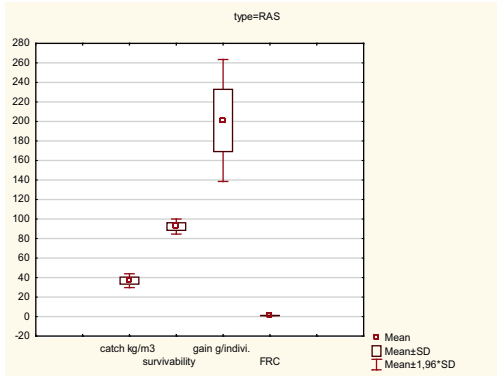
| Parameters | Fish farms | | | | | |
|---|---|---|---|---|---|---|
| | 1-OS | 2-OS | 3-OS | 1-RAS | 2-RAS | 3-RAS |
| Surface area ($m^2$) | 56 | 2 700 | 156 | 210 | 250 | 125 |
| Water capacity ($m^3$) | 56 | 3 500 | 156 | 210 | 470 | 100 |
| Water flow (l/s) | 8 | 10 | 8 | 28 | 300 | 14 |
| Recirculation (*n* steps) | – | – | – | 3 | 5 | 6 |
| No of sites with fish production | – | – | – | (II) | (IV) | (V) |
| STOCK | | | | | | |
| Number of individuals | 2 549 | 12 400 | 7 200 | 20 230 | 48 980 | 10 500 |
| Weight (kg) | 745 | 1 020 | 1 300 | 3 357 | 12 000 | 1 650 |
| Population density ($kg/m^3$) | 13.30 | 0.29 | 8.33 | 15.99 | 25.53 | 16.50 |
| Body mass (g/indiv.) | 292 | 82 | 180 | 166 | 245 | 157 |
| CATCH | | | | | | |
| Number of individuals | 2 514 | 12 100 | 7 415 | 18 625 | 47 610 | 9 200 |
| Weight (kg) | 1 364 | 7 863 | 3 805 | 6 798 | 19 425 | 3 660 |
| Population density ($kg/m^3$) | 24.36 | 2.25 | 24.39 | 32.50 | 41.33 | 36.60 |
| Body mass (g/indiv.) | 542 | 649 | 513 | 365 | 408 | 398 |
| Survivability (%) | 98.60 | 97.60 | 0.97 | 92.00 | 97.20 | 0.88 |
| Gain in toltal (kg) | 619 | 6 843 | 2 504 | 3 441 | 7 425 | 2 010 |
| Individual body gain (g/indiv.) | 250.00 | 567.00 | 333.00 | 199.00 | 163.00 | 241.00 |
| Average daily gain (%) | 0.62 | 0.83 | 1.05 | 0.86 | 0.65 | 1.01 |
| FCR | 0.97 | 1.08 | 1.09 | 1.13 | 0.98 | 1.03 |

**Table 3.12.** Values of indices obtained in spring and used for statistical analysis

| Index | 1-OS | 2-OS | 3-OS | Mean | 1-RAS | 2-RAS | 3-RAS | Mean |
|---|---|---|---|---|---|---|---|---|
| Fish catch ($kg/m^3$) | 24.36 | 2.25 | 24.39 | ***17.01*** | 32.50 | 41.33 | 36.60 | ***36.81*** |
| Survivability (%) | 98.6 | 97.6 | 97.1 | ***97.8*** | 92.0 | 97.2 | 87.6 | ***92.3*** |
| Individual gain (g/indiv.) | 250 | 567 | 333 | ***383*** | 199 | 163 | 241 | ***201*** |
| FCR | 0.97 | 1.08 | 1.09 | ***1.05*** | 1.13 | 0.98 | 1.03 | ***1.05*** |

In the first stage of the research, due to a small number of analyzed data, it was decided to test whether the observed differences in values of the analyzed parameters are statistically significant, taking the measurements in groups OS and RAS as constituents of a sample.



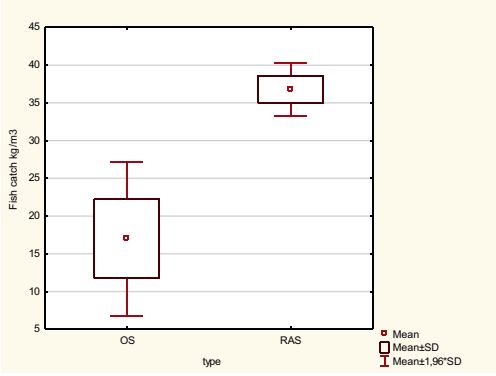



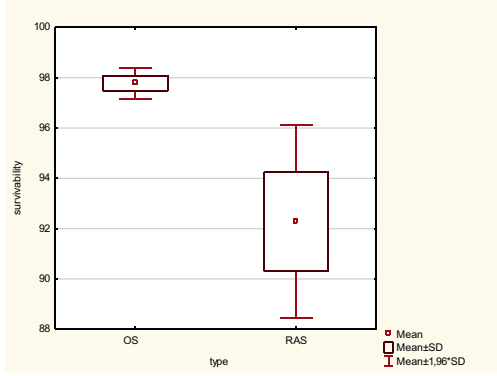

The greatest dispersion of values and the highest standard deviation were recorded for the parameter gains (g/indiv.). These observations were often made within the group of fish farms called OS. In order to verify the statistical significance of the observed differentiation, *t*-Student test was applied. The results of this analysis are contained in Table 3.13.

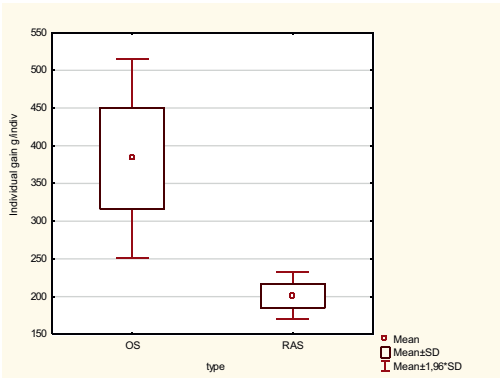
**Table 3.13.** Results of the analysis of technological and rearing parameters (*t*-Student test, statistically significant differences in red)

| Parameters | Mean | | *t* | *p* | SD | |
|---|---|---|---|---|---|---|
| | OS | RAS | | | OS | RAS |
| Catch (kg/m$^3$) | 17.0025 | 36.8100 | -3.589 | 0.011 | 10.429 | 3.607 |
| Survivability | 97.7750 | 92.2750 | 2.768 | 0.032 | 0.623 | 3.923 |
| Gains (g/indiv.) | 383.2500 | 201.0000 | 2.642 | 0.038 | 134.219 | 31.874 |
| FCR | 1.0475 | 1.0475 | 0.000 | 1.000 | 0.054 | 0.062 |

The above analysis verified that the observed differences in the volume of fish catch expressed in kg/m$^3$ were statistically significant and imply that during the autumn season higher values were achieved at the RAS fish farms. The differences in survivability and gain (g/indiv.) proved to be significant as well, and higher values were obtained at the OS farms. The values achieved for the parameter FCR were astonishing because our statistical analysis did not evidence significant differences – values of this parameter did not differ in a statistically significant manner in both types of aquaculture technology.





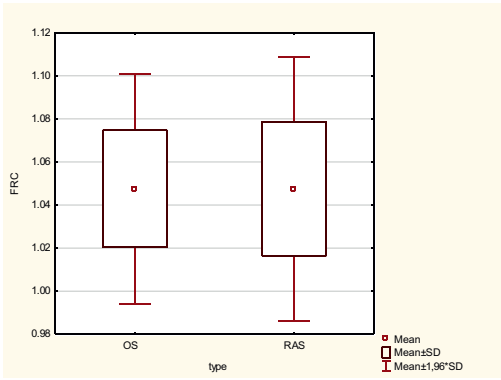




Table 3.14 contains values of the biometric parameters obtained during the research conducted in autumn, both with and without the division of the fish farms into two technological types. Table 3.15 sets basic statistical data describing the data achieved from the samples collected in autumn, including the division into the two technologies and two groups of fish according to their body mass.

**Table 3.14.** Basic descriptive statistics based on samples collected in autumn

| Parameters | *N* valid | Mean | Minimum | Maximum | SD |
|---|---|---|---|---|---|
| Lc (cm) | 240 | 34.7900 | 26.8000 | 42.4000 | 2.7706 |
| Weight (g) | 240 | 502.9163 | 234.0000 | 825.0000 | 124.1636 |
| Slaughter ratio (g) | 240 | 429.7197 | 200.0000 | 727.0000 | 106.2284 |
| % Slaughter ratio (%) | 240 | 85.5354 | 71.8310 | 90.8000 | 2.1450 |
| Fulton's index | 240 | 1.1756 | 0.8071 | 1.7088 | 0.1353 |
| 1-RAS | | | | | |
| Lc (cm) | 40 | 34.1975 | 26.8000 | 40.1000 | 3.7213 |
| Weight (g) | 40 | 510.5500 | 238.0000 | 825.0000 | 181.3385 |
| Slaughter ratio (g) | 40 | 440.8500 | 204.0000 | 727.0000 | 157.8082 |
| % Slaughter ratio (%) | 40 | 86.2422 | 82.5397 | 90.0000 | 1.5202 |
| Fulton's index | 40 | 1.2228 | 1.0110 | 1.7088 | 0.1432 |
| 2-RAS | | | | | |
| Lc (cm) | 40 | 33.2450 | 28.8000 | 38.8000 | 2.6054 |
| Weight (g) | 40 | 445.0000 | 234.0000 | 696.0000 | 138.8689 |
| Slaughter ratio (g) | 40 | 379.1000 | 200.0000 | 578.0000 | 113.0402 |
| % Slaughter ratio (%) | 40 | 85.6374 | 71.8310 | 89.6296 | 2.9128 |
| Fulton's index | 40 | 1.1688 | 0.9399 | 1.4672 | 0.1363 |
| 3-RAS | | | | | |
| Lc (cm) | 40 | 33.4950 | 29.5000 | 36.1000 | 1.75440 |
| Weight (g) | 40 | 473.7750 | 320.0000 | 616.0000 | 79.81340 |
| Slaughter ratio (g) | 40 | 402.2250 | 282.0000 | 515.0000 | 61.08820 |
| % Slaughter ratio (%) | 40 | 85.1529 | 80.9524 | 89.2183 | 2.06759 |
| Fulton's index | 40 | 1.2503 | 1.0238 | 1.3793 | 0.07934 |
| 1-OS | | | | | |
| Lc (cm) | 40 | 35.0425 | 32.0000 | 38.0000 | 2.01595 |
| Weight (g) | 40 | 526.7750 | 354.0000 | 692.0000 | 91.68074 |
| Slaughter ratio (g) | 40 | 445.6000 | 296.0000 | 592.0000 | 82.29740 |
| % Slaughter ratio (%) | 40 | 84.5638 | 71.8310 | 89.3728 | 2.38641 |

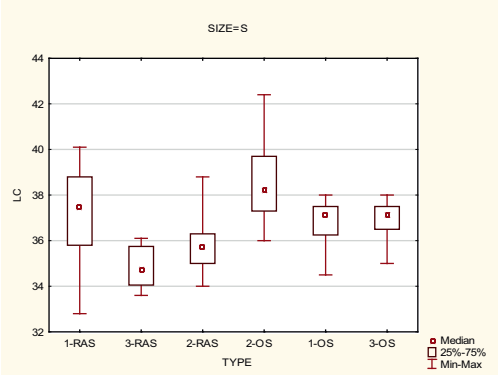
| Parameters | *N* valid | Mean | Minimum | Maximum | SD |
|---|---|---|---|---|---|
| Fulton's index | 40 | 1.2151 | 1.0356 | 1.3977 | 0.08792 |
| 2-OS | | | | | |
| Lc (cm) | 40 | 37.5225 | 35.8000 | 42.4000 | 1.5310 |
| Weight (g) | 40 | 538.7500 | 450.0000 | 825.0000 | 111.4402 |
| Slaughter ratio (g) | 40 | 458.6000 | 377.0000 | 710.0000 | 95.3893 |
| % Slaughter ratio (%) | 40 | 85.1038 | 83.6364 | 86.9697 | 1.0944 |
| Fulton's index | 40 | 1.0095 | 0.8071 | 1.2699 | 0.1065 |
| 3-OS | | | | | |
| Lc (cm) | 40 | 35.2487 | 32.0000 | 38.0000 | 2.09107 |
| Weight (g) | 40 | 523.1538 | 354.0000 | 692.0000 | 90.81982 |
| Slaughter ratio (g) | 40 | 452.5128 | 307.0000 | 585.0000 | 81.13954 |
| % Slaughter ratio (%) | 40 | 86.5376 | 83.6957 | 90.8000 | 1.83406 |
| Fulton's index | 40 | 1.1871 | 0.9871 | 1.3733 | 0.09705 |

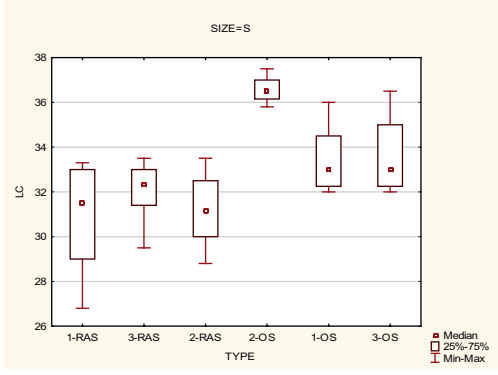
**Table 3.15.** Basic descriptive statistics based on samples collected in autumn including the division between types of aquaculture technology and groups of fish according to weight

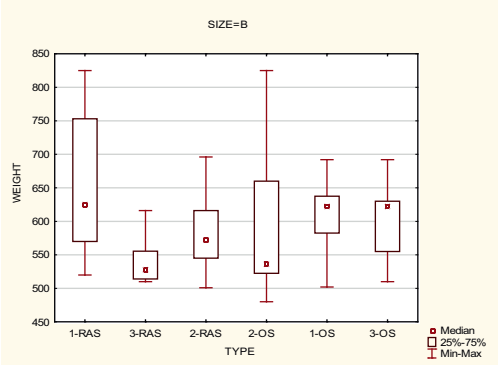
| Parameters | Size B | | | | Size S | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean | Min. | Max. | SD | Mean | Min. | Max. | SD |
| 1-RAS | | | | | | | | |
| Lc (cm) | 37.16 | 32.80 | 40.10 | 1.87 | 30.91 | 26.80 | 33.30 | 2.11 |
| Weight (g) | 659.04 | 520.00 | 825.00 | 101.09 | 346.42 | 238.00 | 485.00 | 75.70 |
| Slaughter ratio (g) | 570.14 | 452.00 | 727.00 | 87.71 | 297.94 | 204.00 | 416.00 | 65.96 |
| % Slaughter ratio (%) | 86.51 | 83.92 | 90.00 | 1.53 | 85.93 | 82.53 | 89.29 | 1.48 |
| Fulton's index | 1.28 | 1.13 | 1.78 | 0.15 | 1.15 | 1.01 | 1.34 | 0.09 |
| 2-RAS | | | | | | | | |
| Lc (cm) | 35.71 | 34.00 | 38.80 | 1.125 | 31.23 | 28.80 | 33.50 | 1.451 |
| Weight (g) | 581.61 | 501.00 | 696.00 | 48.156 | 333.23 | 234.00 | 459.00 | 69.659 |
| Slaughter ratio (g) | 489.17 | 408.00 | 578.00 | 42.896 | 289.05 | 200.00 | 389.00 | 57.976 |
| % Slaughter ratio (%) | 84.14 | 71.83 | 89.37 | 3.499 | 86.87 | 83.81 | 89.63 | 1.527 |
| Fulton's index | 1.28 | 1.08 | 1.47 | 0.090 | 1.08 | 0.94 | 1.34 | 0.097 |
| 3-RAS | | | | | | | | |
| Lc (cm) | 34.91 | 33.60 | 36.10 | 0.876 | 32.08 | 29.50 | 33.50 | 1.142 |
| Weight (g) | 541.95 | 510.00 | 616.00 | 34.827 | 405.60 | 320.00 | 480.00 | 45.583 |
| Slaughter ratio (g) | 454.20 | 427.00 | 515.00 | 24.941 | 350.25 | 282.00 | 428.00 | 36.752 |
| % Slaughter ratio (%) | 83.87 | 80.95 | 88.24 | 1.577 | 86.44 | 83.48 | 89.22 | 1.673 |
| Fulton's index | 1.27 | 1.17 | 1.37 | 0.055 | 1.23 | 1.02 | 1.38 | 0.094 |
| 1-OS | | | | | | | | |
| Lc (cm) | 36.7 | 34.5 | 38.0 | 1.11 | 33.42 | 32.00 | 36.00 | 1.270 |
| Weight (g) | 607.7 | 502.0 | 692.0 | 49.55 | 445.85 | 354.00 | 496.00 | 31.788 |
| Slaughter ratio (g) | 517.6 | 420.0 | 592.0 | 44.32 | 373.55 | 296.00 | 437.00 | 31.766 |
| % Slaughter ratio (%) | 85.2 | 83.7 | 86.1 | 0.85 | 83.89 | 71.83 | 89.37 | 3.166 |
| Fulton's index | 1.2 | 1.1 | 1.4 | 0.06 | 1.20 | 1.04 | 1.40 | 0.109 |
| 2-OS | | | | | | | | |
| Lc (cm) | 38.47 | 36.00 | 42.40 | 1.59 | 36.57 | 35.80 | 37.50 | 0.621 |
| Weight (g) | 610.00 | 480.00 | 825.00 | 120.72 | 467.50 | 450.00 | 490.00 | 15.174 |
| Slaughter ratio (g) | 521.10 | 402.00 | 710.00 | 101.45 | 396.10 | 377.00 | 421.00 | 12.715 |
| % Slaughter ratio (%) | 85.47 | 83.64 | 86.97 | 1.16 | 84.73 | 83.67 | 86.09 | 0.907 |
| Fulton's index | 1.06 | 0.81 | 1.27 | 0.12 | 0.96 | 0.89 | 1.01 | 0.051 |

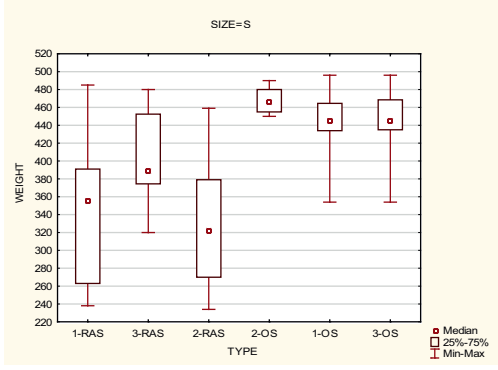
| Parameters | Size B | | | | Size S | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean | Min. | Max. | SD | Mean | Min. | Max. | SD |
| 3-OS | | | | | | | | |
| Lc (cm) | 36.93 | 35.00 | 38.00 | 0.823 | 33.65 | 32.00 | 36.50 | 1.607 |
| Weight (g) | 605.26 | 510.00 | 692.00 | 48.786 | 445.15 | 354.00 | 496.00 | 33.089 |
| Slaughter ratio (g) | 525.53 | 437.00 | 585.00 | 45.927 | 383.15 | 307.00 | 428.00 | 27.814 |
| % Slaughter ratio (%) | 86.87 | 84.08 | 90.80 | 1.737 | 86.22 | 83.70 | 88.92 | 1.911 |
| Fulton's index | 1.20 | 1.10 | 1.30 | 0.056 | 1.18 | 0.99 | 1.37 | 0.125 |

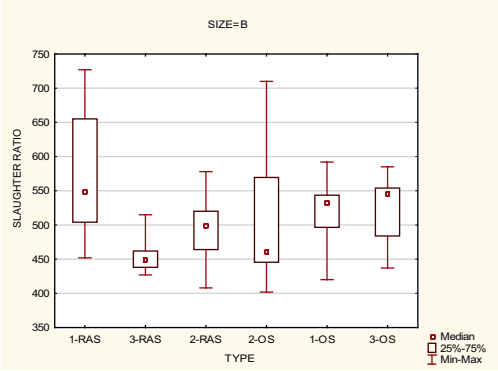
Comparisons of types of aquaculture systems including the division of fish according to body mass – diagrams

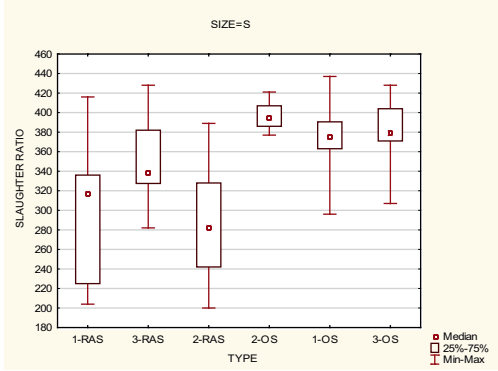


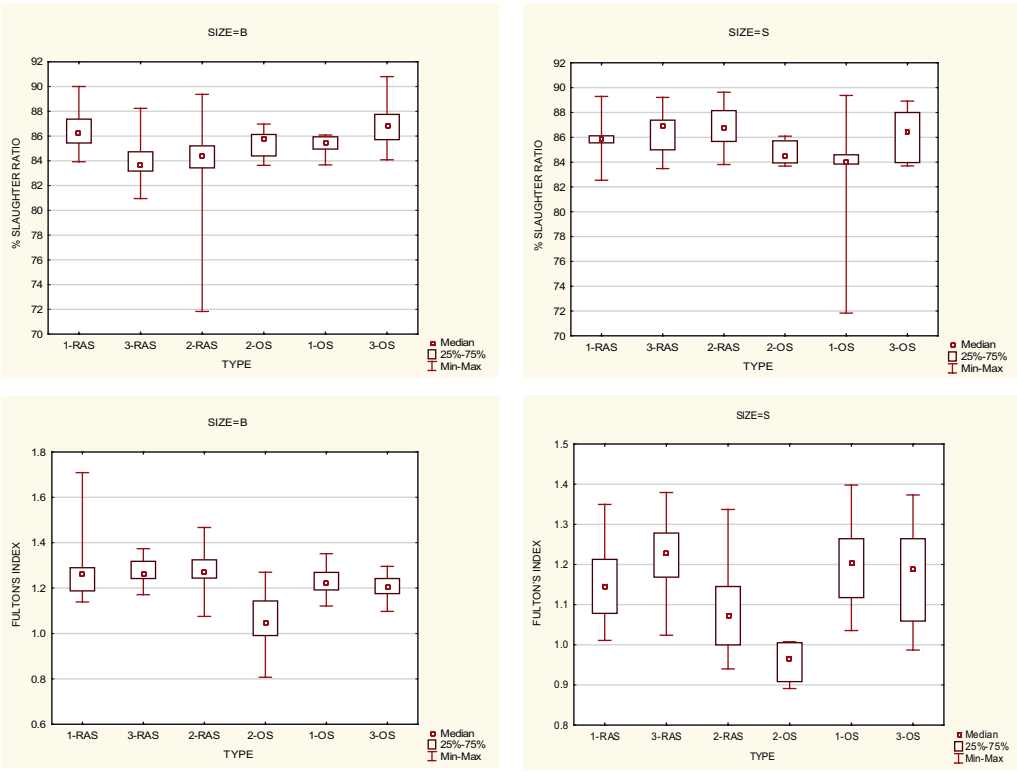






Because of the observed differences in the values of the analyzed parameters, their statically significance was tested. To this end, a non-parameteric analysis of variance, that is Kruskal–Wallis test, was applied. The results of a model analysis of the Lc parameter are presented in Table 3.16. Based on the analysis of the data not divided between the two weight groups of fish, it was concluded that the values of the Lc achieved in the RAS fish farms do not differ significantly. However, significant differences were confirmed when comparing the results obtained for farm 2-OS and the other farms (data marked in red) and for farm 3-OS versus farms 2-RAS and 3-RAS when the data were divided between the two groups of fish according to their weight. At this stage of the study, statistically significant differences were determined for the parameter Lc, but it was impossible to identify any clear tendency. Moreover, the analyses suggest distinct differentiation (statistically significant differences marked in red). Similar analyses were performed for all the analyzed parameters. The values of the achieved statistics *H* are presented in Table 3.17.

**Table 3.16.** Value of the statistics *z* (lower part of the table) and probability *p* (upper pat of the table, bolded italics) for multiple comparisons of the parameter Lc. Non-parameteric analysis of variance – Kruskal–Wallis test: $H\ (5, N = 240) = 67.74048$; $p = 0.0000$ (statistically significant values in red)

| Fish farm/ weight group | Fish farm | | | | | |
|---|---|---|---|---|---|---|
| | 1-RAS | 3-RAS | 2-RAS | 2-OS | 1-OS | 3-OS |
| Total | | | | | | |
| 1-RAS | – | *0.889* | *0.822* | *0.000* | *1.000* | *1.000* |
| 3-RAS | 1.886 | – | *1.000* | *0.000* | *0.073* | *0.019* |

| Fish farm/ weight group | Fish farm | | | | | |
|---|---|---|---|---|---|---|
| | 1-RAS | 3-RAS | 2-RAS | 2-OS | 1-OS | 3-OS |
| 2-RAS | 1.920 | 0.034 | – | ***0.000*** | ***0.065*** | ***0.017*** |
| 2-OS | 5.096 | 6.983 | 7.017 | – | ***0.000*** | ***0.003*** |
| 1-OOH | 0.931 | 2.817 | 2.851 | 4.166 | – | ***1.000*** |
| 3-OOH | 1.351 | 3.226 | 3.259 | 3.713 | 0.426 | – |
| D size | | | | | | |
| 1-RAS | – | 0.000 | 0.061 | 0.527 | 1.000 | 1.000 |
| 3-RAS | 4.527 | – | 1.000 | 0.000 | 0.003 | 0.000 |
| 2-RAS | 2.871 | 1.515 | – | 0.000 | 0.515 | 0.130 |
| 2-OOH | 2.107 | 6.554 | 4.865 | – | 0.071 | 0.378 |
| 1-OOH | 0.751 | 3.730 | 2.116 | 2.824 | – | 1.000 |
| 3-OOH | 0.186 | 4.231 | 2.625 | 2.239 | 0.549 | – |
| S size | | | | | | |
| 1-RAS | – | 0.046 | 1.000 | 0.043 | 0.002 | 1.000 |
| 3-RAS | 2.964 | – | 1.000 | 1.000 | 1.000 | 0.013 |
| 2-RAS | 1.205 | 1.759 | – | 1.000 | 0.110 | 1.000 |
| 2-OOH | 2.979 | 0.015 | 1.774 | – | 1.000 | 0.012 |
| 1-OOH | 3.886 | 0.922 | 2.681 | 0.907 | – | 0.000 |
| 3-OOH | 0.393 | 3.338 | 1.590 | 3.353 | 4.254 | – |

### 3.4.1. Summary of the analyses

Statistically significant differentiation in respect of the aquaculture technology was observed for the rearing parameters Lc, % slaughter index and Fulton's index. No statistically significant differences were determined for the following parameters; body mass and slaughter index.

The above results change when an additional aspect is introduced, namely weight groups of fish. Then, differences occur for all the parameters (cf. Table 3.17).

**Table 3.17.** Results of *H* statistics of Kruskal–Wallis test performed after the fish had been divided into two weight groups

| Parameter | B size | S size |
|---|---|---|
| Lc | $H$ (5, $N$ = 120) = 53.49580; $p$ = 0.0000 | $H$ (5, $N$ = 120) = 70,54591; $p$ = 0.0000 |
| Weight | $H$ (5, $N$ = 120) = 23.21431; $p$ = 0.0003 | $H$ (5, $N$ = 120) = 58,94110; $p$ = 0.0000 |
| Slaughter index | $H$ (5, $N$ = 120) = 32.29371; $p$ = 0.0000 | $H$ (5, $N$ = 120) = 56,66255; $p$ = 0.0000 |
| % Slaughter index | $H$ (5, $N$ = 120) = 42.06658; $p$ = 0.0000 | $H$ (5, $N$ = 120) = 31,59334; $p$ = 0.0000 |
| Fulton's index | $H$ (5, $N$ = 120) = 43.39992; $p$ = 0.0000 | $H$ (5, $N$ = 120) = 60,07633; $p$ = 0.0000 |

In respect of the differentiation observed when both parameters, aquaculture technology and fish weight group, were applied, the biggest differences (in the autumn season) were demonstrated within the results obtained for fish farm 2-OS, followed by 3-RAS and 2-RAS.

### 3.5. Preliminary conclusions

The fish production technological parameters were measured at the sampling sites, by analyzing the information from fishpond logbooks and reading from reading devices.

The results are set in Table 3.4 above, but the values of analyzed parameters used for statistical analyses are comprised in Tables 3.18–3.21.

**Table 3.18.** Technological parameters in the two groups of fish farms, OS and RAS, in the spring season

| Parameter | Extensive technology OS | | | | Intensive technology RAS | | | |
|---|---|---|---|---|---|---|---|---|
| | 1-OS | 2-OS | 3-OS | mean | 1-RAS | 2-RAS | 3-RAS | mean |
| Fish catch (kg/m$^3$) | 19.50 | 2.25 | 23.49 | 11.68 | 34.74 | 37.72 | 32.00 | 34.82 |
| Survivability (%) | 97.8 | 96.8 | 97.0 | 96.3 | 88.0 | 96.1 | 86.00 | 90.0 |
| Individual gain (g/indiv.) | 332 | 516 | 368 | 433 | 225 | 165 | 222 | 204 |
| FCR | 1.06 | 1.14 | 1.06 | 1.10 | 1.19 | 0.99 | 1.05 | 1.08 |

**Table 3.19.** Technological parameters at farms from the OS and RAS groups during the autumn season

| Parameter | Extensive technology OS | | | | Intensive technology RAS | | | |
|---|---|---|---|---|---|---|---|---|
| | 1-OS | 2-OS | 3-OS | mean | 1-RAS | 2-RAS | 3-RAS | mean |
| Fish catch (kg/m$^3$) | 20.30 | 2.30 | 15.90 | 10.22 | 35.50 | 41.05 | 38.10 | 38.22 |
| Survivability (%) | 98.0 | 94.5 | 96.8 | 96.2 | 86.1 | 95.2 | 91.9 | 91.1 |
| Individual gain (g/indiv.) | 262 | 454 | 129 | 321 | 250 | 200 | 231 | 227 |
| FCR | 1.09 | 1.11 | 1.08 | 1.10 | 1.18 | 1.02 | 1.05 | 1.08 |

**Table 3.20.** Basic descriptive statistics for data collected during the spring season with no distinction between technologies or fish sizes

| Parameter | *N* of valid | Mean | Median | Minimum | Maximum | SD |
|---|---|---|---|---|---|---|
| Lc (cm) | 240 | 34.225 | 34.400 | 26.800 | 41.500 | 3.154 |
| Weight (g) | 240 | 484.987 | 496.000 | 220.000 | 825.000 | 145.071 |
| Slaughter ratio (g) | 240 | 421.619 | 429.000 | 190.000 | 727.000 | 125.924 |
| % Slaughter ratio (%) | 240 | 86.464 | 86.912 | 81.120 | 92.545 | 6.131 |
| Fulton's index | 240 | 1.1735 | 1.176 | 0.843 | 1.708 | 0.117 |

**Table 3.21.** Basic descriptive statistics for data collected during the autumn season with no distinction between technologies or fish sizes

| Parameter | *N* of valid | Mean | Median | Minimum | Maximum | SD |
|---|---|---|---|---|---|---|
| Lc (cm) | 240 | 35.125 | 34.000 | 28.500 | 45.200 | 4.294 |
| Weight (g) | 240 | 536.156 | 475.000 | 276.000 | 1120.000 | 202.960 |
| Slaughter ratio (g) | 240 | 457.939 | 393.000 | 238.000 | 990.000 | 172.848 |
| % Slaughter ratio (%) | 240 | 85.505 | 85.531 | 65.681 | 90.909 | 2.604 |
| Fulton's index | 240 | 1.185 | 1.181 | 0.983 | 1.497 | 0.092 |

The statistical processing of the technological parameters described in Tables 3.18 and 3.19 proved that the results achieved in spring and autumn can be treated as homogenous. The analysis was performed with t tests at the level of significance $\alpha = 0.05$. No statically significant differences were found for any of the parameters.

**Table 3.22.** Results of t test for comparison of values of technological parameters obtained in spring and autumn season

| Parameter | Mean | | *t* | *p* | SD | |
|---|---|---|---|---|---|---|
| | spring | autumn | | | spring | autumn |
| Catch (kg/m³) | 24.0933 | 23.2933 | 0.119764 | 0.907042 | 12.0367 | 11.0831 |
| Survivability (%) | 93.9500 | 93.8167 | 0.052060 | 0.959506 | 4.5623 | 4.3060 |
| Gain (g/indiv.) | 348.6767 | 418.3333 | -0.563242 | 0.585677 | 190.1347 | 235.8302 |
| FCR | 1.0800 | 1.1067 | -0.736460 | 0.478372 | 0.0710 | 0.0532 |

In our further analyses, the technological parameters were compared only in respect of the two aquaculture technologies. In that case, the expected differences in the catch and survivability parameters did occur, but no effect of the fish rearing technologies on values of gain and FCR was revealed (Table 3.23).

**Table 3.23.** Results of t test for comparison of technological parameters obtained in both types of aquaculture. Statistically different results are marked in red

| Parameter | Mean | | *t* | *p* | SD | |
|---|---|---|---|---|---|---|
| | OS | RAS | | | OS | RAS |
| Catch (kg/m³) | 15.02 | 32.37 | -4.544 | 0.0011 | 9.13 | 2.02 |
| Survivability (%) | 96.72 | 91.05 | 3.096 | 0.0113 | 1.19 | 4.32 |
| Gain (g/indiv.) | 480.61 | 286.40 | 1.772 | 0.1068 | 245.59 | 108.32 |
| FCR | 1.10 | 1.09 | 0.361 | 0.7256 | 0.03 | 0.08 |

Analogously to the initial stage of the research, differences were noticed in values of biometric parameters, which were then tested for their statistical significance. Same as above, at first statistical significance was tested in respect of the spring and autumn research season and then relative to the fish rearing technologies and fish sizes. The first step of statistical analysis employed *t*-test at $\alpha = 0.05$ (Tables 3.24–3.26).

**Table 3.24.** Results of t test for comparison of values of biometric parameters obtained in the spring and autumn research season. Values of statistically significant differences in red

| Parameter | Mean | | *t* | *p* | SD | |
|---|---|---|---|---|---|---|
| | autumn | spring | | | autumn | spring |
| Lc (cm) | 34.9134 | 34.2337 | 2.127393 | 0.033899 | 3.8143 | 3.1540 |
| Weight (g) | 523.7950 | 484.8667 | 2.549841 | 0.011088 | 188.2554 | 143.1753 |
| Slaughter index (g) | 462.9183 | 420.8625 | 3.199778 | 0.001467 | 161.0111 | 124.6392 |
| % Of slaughter index (%) | 91.8445 | 86.8101 | 2.522032 | 0.011992 | 30.8220 | 2.5136 |
| Fulton's index | 1.0860 | 1.1731 | 1.098402 | 0.272582 | 0.1429 | 0.1118 |

**Table 3.25.** Results of t test for comparison of values of biometric parameters obtained for both types of aquaculture. Values of statistically significant differences in red

| Parameter | Mean | | *t* | *p* | SD | |
|---|---|---|---|---|---|---|
| | OS | RAS | | | OS | RAS |
| Lc (cm) | 35.00 | 33.81 | 2.6652 | 0.0085 | 2.63 | 2.99 |
| Weight (g) | 526.09 | 473.28 | 2.2934 | 0.0231 | 125.47 | 163.35 |
| Slaughter index (g) | 459.87 | 408.73 | 2.5208 | 0.0127 | 109.63 | 144.64 |
| % Of slaughter index (%) | 87.44 | 86.07 | 3.3073 | 0.0012 | 2.55 | 2.65 |
| Fulton's index | 1.11 | 1.08 | 0.8265 | 0.4097 | 0.08 | 0.22 |

**Table 3.26.** Results of t test for comparison of values of biometric parameters obtained for two groups of fish size (B > 500 g, S < 500 g). Values of statistically significant differences in red

| Parameter | Mean | | *t* | *p* | SD | |
|---|---|---|---|---|---|---|
| | B size | S size | | | B size | S size |
| Lc (cm) | 37.13 | 32.02 | 23.169 | 0.0000 | 2.82 | 1.92 |
| Weight (g) | 630.71 | 377.95 | 24.955 | 0.0000 | 135.10 | 79.80 |
| Slaughter index (g) | 526.84 | 356.94 | 15.770 | 0.0000 | 101.78 | 132.27 |
| % Of slaughter index (%) | 84.46 | 94.20 | -4.970 | 0.0000 | 8.98 | 29.00 |
| Fulton's index | 1.12 | 1.04 | 7.365 | 0.0000 | 0.11 | 0.13 |

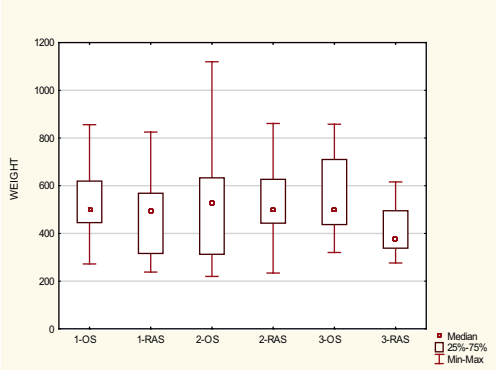
Because of the above differentiation, and in order to perform more precise analyses, non-parametric analysis of variance, Kruskal–Wallis test, was applied, taking into consideration all dependences. Regarding the six farms, it was suggested that statistically significant differences in values of Lc may have been caused by the results obtained at the fish farm coded as 3-RAS. Basically, the results from that farm both in spring and autumn were statistically different from the results obtained from the other farms. Moreover, statistically significant differences appeared between the farms coded as 1-RAS and 3-OS. No statistically significant differences were observed between the other farms (Table 3.27).

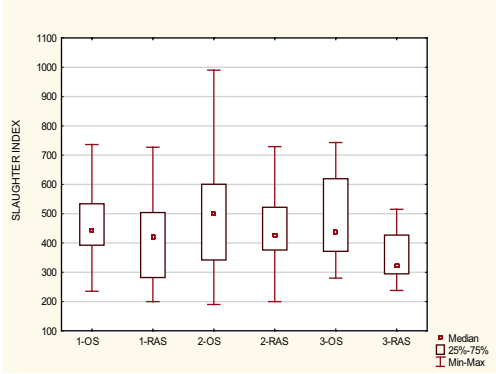
**Table 3.27.** Model results of Kruskal–Wallis analysis of variance for the parameter Lc, for the six fish farm in the spring season

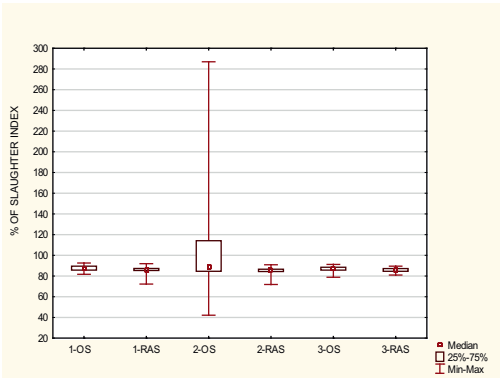
| Fish farm | 1-OS R:269.64 | 1-RAS R:217.81 | 2-OS R:247.66 | 2-RAS R:263.17 | 3-OS R:306.09 | 3-RAS R:138.63 |
|---|---|---|---|---|---|---|
| 1-OS | – | 2.363 | 1.002 | 0.295 | 1.662 | 5.973 |
| 1-RAS | 2.363 | – | 1.361 | 2.068 | 4.025 | 3.610 |
| 2-OS | 1.002 | 1.361 | – | 0.707 | 2.665 | 4.971 |
| 2-RAS | 0.295 | 2.068 | 0.707 | – | 1.957 | 5.678 |
| 3-OS | 1.662 | 4.025 | 2.665 | 1.957 | – | 7.636 |
| 3-RAS | 5.973 | 3.610 | 4.971 | 5.678 | 7.636 | – |

Similar analyses were performed for all the examined biometric parameters, and similar results were achieved. The results of our analyses for the other parameters are illustrated in the following diagrams.

Values of *H* statistics are presented in Table 3.28.





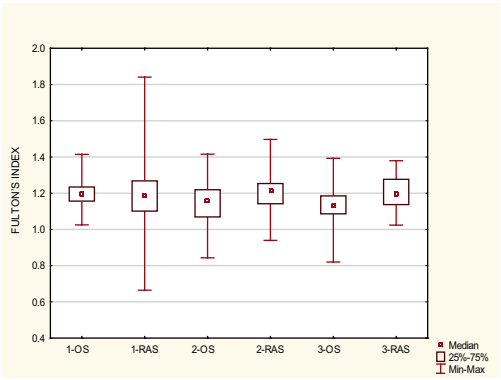




**Table 3.28.** Results of *H* statistics of Kruskal–Wallis test – for analysis of biometric parameters

| Parameter | Kruskal-Wallis Test |
|---|---|
| Lc | $H$ (5, $N$ = 480) = 69.07560; $p$ = 0.0000 |
| Weight | $H$ (5, $N$ = 480) = 45.33375; $p$ = 0.0000 |
| Slaughter index | $H$ (5, $N$ = 480) = 56.53545; $p$ = 0.0000 |
| % Of slaughter index | $H$ (5, $N$ = 480) = 45.37242; $p$ = 0.0000 |
| Fulton's index | $H$ (5, $N$ = 480) = 37.26878; $p$ = 0.0000 |

### 3.6. Summary and conclusions

The results of the fish production technological parameters in both groups of fish farms at the presented research stages (preliminary, spring and autumn) were highly reproducible. The observed differences were a result of farm-specific characteristics and values of input parameters rather than a consequence of differences between the two technologies. For comparison, parametrization of results accounting for input values and levelling input differences was applied in statistical analyses.

The results available at the moment seem to indicate that production effects, including biometric parameters and fish condition index, are independent from the type of aquaculture. Obviously, the RAS technology is much more efficient and ensures a much higher production return at a lower water consumption. During the present study, the final fish density (kg/m$^3$) in ponds and tanks during the last fish catch at the farms employing the intensive fish rearing technology (RAS) was 2- to 4-fold higher (36.81 kg/m$^3$ as an average for the whole duration of the research) than in the fish farm with the extensive system OS (17.01 kg/m$^3$ on average). A higher growth rate, determined according to the per cent daily gain, was attained by fish from the RAS farms (0.65–1.34%), which was associated with their short but more intensive fattening period, and the fluctuations noticed in this study were most probably due to the changing temperature of water. In the OS fish farms the per cent daily gain was from 0.40 to 0.77. The FCR values achieved in the fish tanks from which samples were taken were estimated to equal 0.97–1.19. The assessed FCR in both technologies was similar (differences statistically non-significant at $p$ = 0.9026) and reached 1.08 in the extensive technology (OS) and 1.07 in the intensive technology (RAS). This result is consistent with the previously calculated levelled Fulton's index for both aquaculture technologies.

Noteworthy is the question of survivability. High survivability was recorded in both aquaculture technologies at the end of the fattening period (up to 98.6%). The results attained at particular farms, however, were diverse and eventually shaped the final results of our comparisons. For example,

the survivability rate was approximately 5.7% higher at the OS farms than at the RAS farms (91.1%). The fish farms with the open flow of water (OS) had more stable rates of survivability and eventually generated an average rate of 95%, which was statistically different ($p = 0.0001$) from the wide-range survivability determined at the RAS farms ($P = 86.1 – 97.2\%$). Such variation, and more precisely the occurrence of a statically significantly different result of 86.1%, affected the final outcome of the whole analysis. If that result was to be taken as divergent, than one might claim that the survivability at both types of farms is similar and does not differ in a significant way.

Production cycles at the intensive RAS farms were reproducible (weight of stocked fish, length of rearing cycle) and some small differences in the duration of fish rearing were connected with the season of the year. Production cycles at the OS farms were more changeable and depended on the season for the year. The final fattening of fish in the OS lasted 5 months on average, and a mean body gain of an individual fish equalled 379 g. In turn, at the RAS farms fish were fattened for just 2.5 months and the average body gain per fish was about 211 g. The research carried out at six trout farms in Poland seems to indicate that despite a variety of fish stock densities and water flow intensities, the final catch and individual body gain as well as FCR are similar (statistically non-significant differences, p-0.9026). This held true for both small (S group, up to 500 g/indiv.) and large fish (B, up to 850 g/indiv.). It has been found out that an average length of fish classed as small ones was 32.4818 cm, and 37.7364 cm for large fish; the body mass was 396.01 g for S fish and 646.1088 g for B fish. The relative slaughter index of fish (%) in both technological groups was statistically similar, reaching on average 86.29% (84.8–87.8%) for fish from the OS farms and 85.32% (83.9–86.6%) for fish from the RAS farms, but this parameter was not statically differentiated between the two fish size group (S and B).

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# 4. Chemical composition of trout muscle tissue

## 4.1. Introduction

The food industry is one of the major branches in the Polish economy. Recently, the fish production has become one of the fastest growing branches in the food sector. At present, the fish and processed fish market is worth around 4.5 billion Polish zloty, which corresponds to about 3% of the value of the whole market of food products. Among the major activities within this branch of food industry, next to aquaculture production and fishing, is fish processing. There are about 450 business enterprises, including 150 industrial companies, in this sector. Most of the fish processing firms (*ca* 75%) are located in the Provinces of West Pomerania (*województwo zachodniopomorskie*) and Pomerania (*województwo pomorskie*); the remaining ones are scattered all over Poland.

Both in Poland and in the whole European Community, food safety is taken very seriously, which is why fish industry companies, both manufacturing and processing ones, make every effort to ensure raw material and final products of excellent parameters. Section 5 paragraph 3 of the Act on Food and Nutrition Safety, of 25 August 2006, defines food safety as "the whole set of conditions which must be satisfied, pertaining in particular to: a) additives and flavours added to food, b) levels of contaminants, c) pesticide residues, d) food irradiation, e) organoleptic properties, and all activities which must be undertaken at all the stages of food production and trade – in order to assure human health and life". Owing to its chemical composition, fish meat is an excellent produce for preparation of highly valuable dishes, but it is also easily perishable foodstuff.

The chemical composition of fish meat depends on the fish species and diet. The biggest differences, reaching tens of per cent, are noticed in the content of fat, especially between herbivorous fish and predators. Some differences in the profile of fatty acids and the content of certain mineral components (content of iodine or selenium) appear between sea and freshwater fish. Like the meat of big slaughter animals and poultry, fish meat contains above 10% of protein, but it is a better source of this nutrient than other animal products; fish provides us with much of high-value protein together with a low amount of energy. The high nutritive value of fish is confirmed by the index of nutritional quality (INQ) which equals 7.61 for fish and fish products, which makes it even higher than the value calculated for eggs and twice as high as determined for meat and dairy products.

Certainly, the content of particular nutrients in muscular tissues of fish is affected by other factors, such as the age, sexual maturation stage and the health of an individual fish. Regarding the external

factors, the following should be mentioned: type of feed (which affects the content of total fats and profile of fatty acids) and the aquatic environment quality (especially the risk of accumulating environmental pollutants like heavy metals, pesticides or dioxins in fish meat). The nutritive value of fish has been already discussed in chapter 2 ("Role and importance of trout meat in human diet"). This chapter deals with the chemical composition of the trout's muscle tissue.

### 4.2. Material and methods

This chapter contains results of determinations of the basic components found in the muscle tissues of trout obtained from 6 fish farms, which employ different trout aquaculture technologies: a technology which resembles the natural conditions, that is with open flow of water (OS), and a technology with water recirculation (RAS). The trout belongs to predatory fish, so all the fish from the six farms are fed artificial granulated feeds: the OS farms use the feeds called SKRETING and AQUA-FISH, whereas most of the RAS farms use feed manufactured by the company ALLER. The basic composition of the feeds made by different manufacturers of feeds for table trout is similar.

The material for analyses was sampled four times: twice in spring and twice in autumn. The material for the chemical analysis consisted of samples of muscle tissue about 5 cm in width, dissected from the central part of a fillet from the dorsal (back) to the abdominal side of a fish, without fish bones or skin. The following determinations were made: dry matter, crude ash, total protein, total fat and profile of fatty acids, and concentration of some heavy metals.

### 4.3. Results

#### 4.3.1. Content of dry matter

The fish analyzed in 2010–2012 contained on average from 24.41% to 26.89% of dry matter. No significant differences were found between fish produced at the six farms, located in different parts of Poland.

Greater diversity was observed between samples of fish from the subsequent sampling dates in the two seasons. The lowest content of dry matter (25.70% for the OS farms and 25.87% for the RAS farms) (Fig. 4.1) was determined in the meat of fish from spring catches, e.g. 25.74% at the 3-OS farm and 25.74% at the 3-RAS farm (Fig. 4.2). Among the spring specimens, the highest content of dry matter was determined in the muscle tissue of fish from the 2-OS farm (26.11%); for comparison, in the RAS technology, the highest dry matter content was achieved by fish from the 1-RAS farm (26.05%). Higher values of the dry matter content were determined for samples collected in autumn, which resulted from the fish foraging calendar and a higher concentration of nutrients in muscle tissues. The highest content of dry matter was determined in samples originating from the 3-RAS farm (26.47%), followed by 26.38% at 2-RAS, 26.17% at 1-RAS, 26.31% at1-OS, 26.16% at 2-OS and 26.37% at 3-OS (Fig. 4.2).

In our analyses of samples from the two different technologies, a higher content of dry matter in aggregated spring catches was found for the RAS technology: 25.87% (median – 25.93%; SD – 0.299); for comparison, in the OS technology, the analogous values were 25.70% d.m. (median – 25.86%, SD – 0.487). For the autumn catches, a higher content of dry matter was also determined in samples from fish originating from the RAS farms: 26.34% (median – 26.24%, SD – 0.213). In samples from fish cultured at the OS farms, the determined dry matter content was 26.28% (median – 26.22%, SD – 0.318) (Fig. 4.1). When analyzing all the samples from all the catches, slightly higher results were achieved in the RAS group: 26.10% (median – 26.14%, SD – 0.349) than in the OS group: 25.99% (median – 26.12%, SD – 0.050).

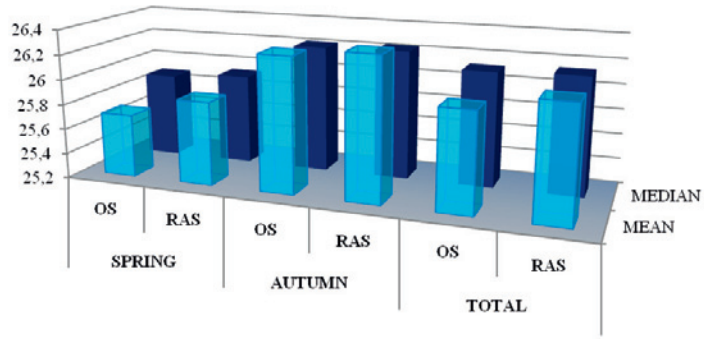


Fig. 4.1. Content of dry matter in muscle tissue of trout from two technologies

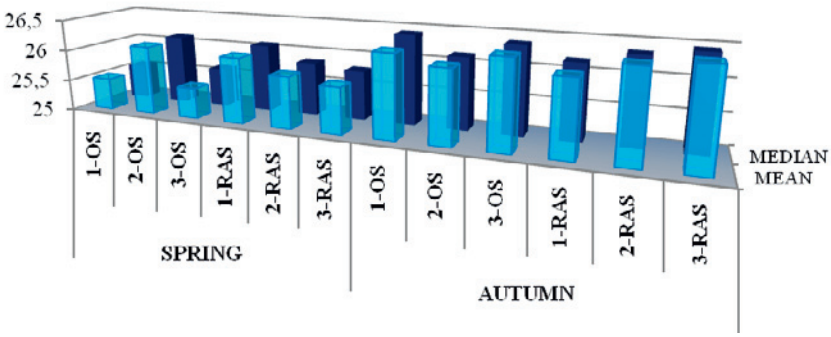


Fig. 4.2. Content of dry matter in muscle tissue of trout from six fish farms

### 4.3.2. Ash content

The content of total ash remaining after incineration of a product sample is a measure of the total content of mineral constituents in foods. The composition and quantities of mineral substances in meat of farm animals, including the trout, depends primarily on the availability of these elements in feeds, on the species of animals, their physiological condition and age.

Significant difference has been demonstrated in meat of trout fish depending on the date of sampling. The content of crude ash in samples from the six farms is presented in figure 4.3. When analyzing the results obtained for samples from the different fish farms, it was found out that the highest content of crude ash in samples collected in the spring season occurred at the 2-OS farm: 1.18% (median – 1.21%, SD – 0.074) and in samples from the 1-RAS farm: 1.16% (median – 1.14%, SD – 0.045). The mean crude ash content in samples from the other farms was as follows: 1.09% at 1-OS (median – 1.09%, SD – 0.019), 1.13% at 3-OS (median – 1.12%, SD – 0.046), 1.07% at 2-RAS (median – 1.07%, SD – 0.025), 1.06% at 3-RAS (median – 1.07%, SD – 0.045) (Fig. 4.3). The results obtained from the autumn catch were higher than those from the spring sampling at all the farms. The highest content of crude ash in muscle tissue of trout fish caught in autumn was determined at the following fish farms: 2-OS – 1.34%, 1-RAS – 1.28% and 3-OS – 1.26%. The content of crude ash in samples from the other farms was as follows: 1.17% at 1-OS, 1.20% at 2-RAS and 1.22% at 3-RAS (Fig. 4.3).

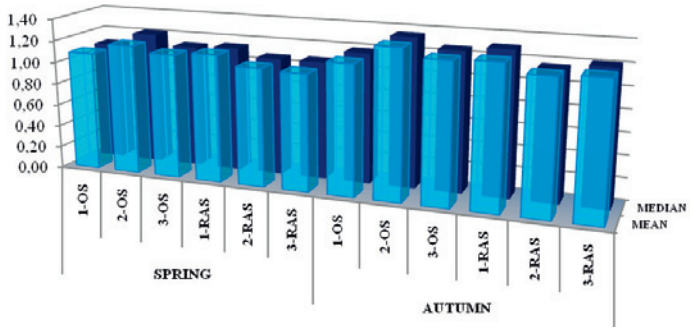


Fig. 4.3. Total ash content in samples from the six fish farms

Our analysis of all the specimens in the context of the fish rearing technologies showed a higher content of total ash in trout muscle tissues sampled in the spring (Fig. 4.4): 1.13% in samples from the OS technology (median – 1.10%, SD – 0.063) and 1.09% from the RAS technology (median – 1.08%, SD – 0.060). Samples from the fish captured in the autumn contained identical amounts of crude ash at both types of aquaculture technology (OS and RAS), i.e. mean 1.24%, median 1.22% and SD 0.077 (OS) and 0.067 (RAS).

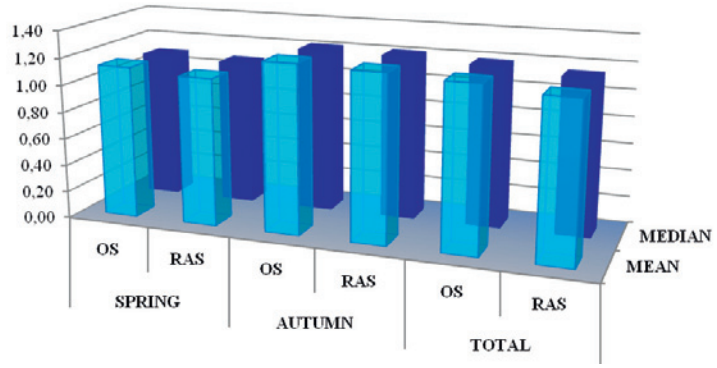


Fig. 4.4. Content of crude ash in samples from the two aquaculture technologies

Direct comparison of all the samples from the two technologies revealed a higher crude ash content in OS samples: mean 1.20%, median 1.19% and SD 0.091 versus RAS samples: mean 1.15%, median 1.15% and SD 0.092 (Fig. 4.4).

### 4.3.3. Protein content

High quality protein is one of the essential characteristics of fish neat. The analyzed fish contained on average about 17.00–20.22% total protein, which does not diverge from determinations of this nutrient in meat of other fish species or slaughter animals. Statistical analysis did not confirm differences between groups of results although a slightly higher mean was observed for the OS group (19.10%) than for the RAS group (18.81%). In general, it ca be said that aggregating results over a longer period of time minimizes the small differences between particular farms or sampling dates.

In the spring catch, the content of protein determined in particular samples was: mean 18,93% at 1-OS (median – 18.90%, SD – 0.721), mean 19.06% at 2-OS (median – 19.05%, SD – 0.630), mean 19.17% at 3-OS

(median – 19.25%, SD – 0.576), mean 18.64% at 1-RAS (median – 18.60%, SD – 0.544), mean 18.32% at 2-RAS (median – 18.20%, SD – 0.692), mean 18.73% at 3-RAS (median – 18.80, SD – 0.731). Slightly higher values were determined in meat from the autumn catch: 19.06% at 1-OS, 19.24% at 2-OS, 19.14% at 3-OS, 19.23% at 1-RAS, 19.14% at 2-RAS and 18.79% at 3-RAS (Fig. 4.5).

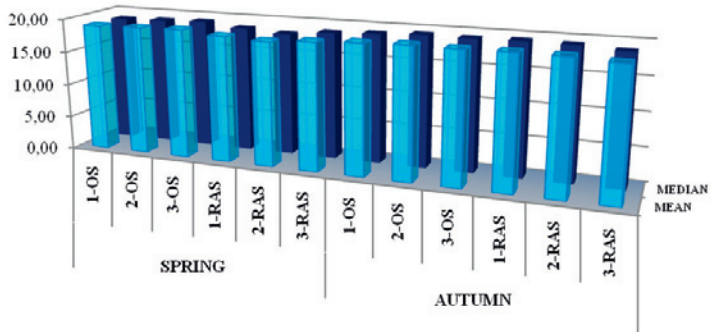


Fig.4.5. Content of total protein in samples from the six farms

The comparison of samples from the RAS and OS technologies (Fig. 4.6) obtained in the spring revealed higher determination values in the OS group: mean 19.05% (median – 19.10% and SD – 0.650) than in the RAS group: mean 18.56% (median – 18.50% and – SD 0.682) (Fig. 4.6). Also, samples from the autumn catch contained more protein if obtained from the OS system: mean 19.15% (median – 19.30%, SD – 0.556) rather than from the RAS technology: mean 19.05% (median – 19.30%, SD – 0.729).

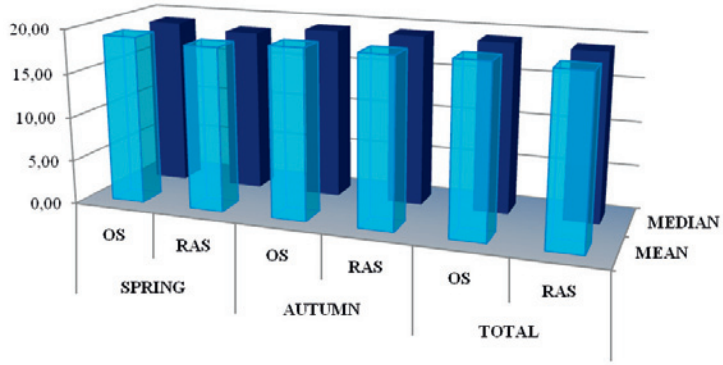


Fig. 4.6. Content of total protein in samples from the two technologies

### 4.3.4. Total fat content

The role of fats in human nutrition is among the core areas researched in contemporary nutrition science, and possible resolution of controversies is of great importance for food manufacturers and technologists as well as for nutritionists and, above all, for consumers. It is claimed that high level of fat consumption and wrong composition of fats in a diet may contribute to an increased risk of developing civilization diseases, such as obesity, cardiovascular disorders, colorectal cancer, breast cancer and

weaker immunity. Fish fats are sources of vitamins A and D. Unlike fat from slaughter animals, fish fat has a more complex composition, which has a beneficial influence on the human health.

The results quite clearly demonstrate that fish captured in the spring contained less fat than those caught in the autumn (Fig. 4.7). Fish sampled in the autumn had gone through a period of intensive foraging and it was predictable that they would contain more fat. In the winter and early spring, fish are given little feed or no feed at all when temperatures fall low, which makes them burn stored fat, e.g. intestinal fat. In this research, muscle tissue (and not a fillet) was analyzed, hence no large losses in the fat content were detected in fish caught in the spring. When comparing the content of fat in fish caught in the spring at particular fish farms, the highest value was found in samples from the 2-RAS farm: on average 2.47%.

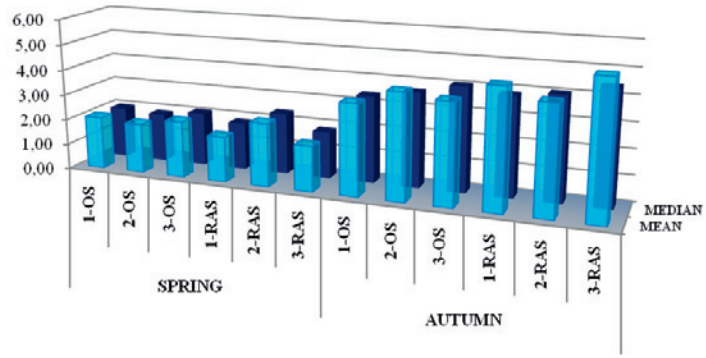


Fig.4.7. Content of total fat in muscle tissue at the six farms

In the other samples obtained in the spring, the following total fat content was determined: 2.06% at 1-OS, 1.95% at 2-OS, 2.21% at 3-OS, 1.78% at1-RAS and 1.78% at 3-RAS.

Much higher values of the total fat content in trout muscle tissue were determined in samples obtained in the autumn (Fig. 4.7), and the highest content was determined in samples from the 3-RAS fish farm: mean 5.25%. The other samples of muscle tissue contained the following amounts of total fat: 3.56% at 1-OS, 4.13% at 2-OS, 3.94% at 3-OS, 4.63% at 1-RAS and 4.19% at 2-RAS.

Our comparison of the spring samples from the two fish rearing technologies demonstrated the same results of total fat determinations (ca 2%); in the autumn catches, a higher total fat content was

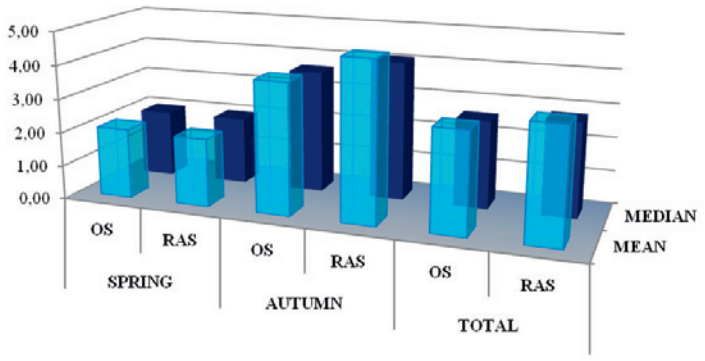


Fig.4.8. Content of total fat in muscle tissue of trout from the two aquaculture technologies

determined in samples originating from the RAS technology – on average 4.69%. The percentage of total fat in samples from the OS technology was lower: on average 3.87% (Fig. 4.8). Comparison of all the samples from either of the two technologies showed a higher result for the RAS fish farms (on average 3.35%) than for the OS farms (on average 2.98%).

### 4.3.5. Profile of fatty acids

Higher incidence of obesity and metabolic diseases in populations living in developed countries has ignited broad discussions about factors which favour such undesirable developments. Much attention has been devoted to fats, crudely divided into plant fats (healthy) and animal fats (unhealthy). The latter category, however, contains an exception, that is fish fats, which are said to be not only nutritionally valuable but also good for our health. Particularly valuable are the fats containing the so-called polyunsaturated fatty acids (PUFAs), which practically speaking appear in adult diet only in fish. Their availability from other sources, e.g. blackcurrant or tomato seeds, is questionable, while another possible source, that is flax oil, is too rare on Polish tables.

Hydrolysis of fats was performed on fat samples from the trout fish and a profile of fatty acids was established. The dominant acid was oleic acid $C_{18:1}$, whose contribution to the total pool of fatty acids was about 21–28%. Slightly more of this acid was determined in big than in small fish specimens. The second most abundant acid was saturated palmitic acid $C_{16:0}$ (15–18%), next was linoleic acid $C_{18:2}$, whose content ranged 8–12%, and linolenic acid $C_{18:3}$ (2–6%). The muscle tissue of trout also contained considerable amounts (over 5%) of eicosapentaenoic acid (EPA), which belongs to an important group of ω-3 polyunsaturated acids.

Figure 4.9 presents the ratios of fatty acids in muscle tissue from trout reared at each of the six fish farms. The average total content of monounsaturated fatty acids (MUFA) in samples from the 1-OS farm was 37.66%, 2-OS – 38.02%, 3-OS – 39.63%, 1-RAS – 33.79%, 2-RAS – 36.91% and from 3-RAS – 39.03%. The determined total of polyunsaturated fatty acids (PUFA) was 37.01% at 1-OS, 39.72 % at 2-OS, 33.61% at 3-OS, 39.01% at 1-RAS, 38.77% at 2-RAS and 34.58% at 3-RAS (Fig. 4.9).

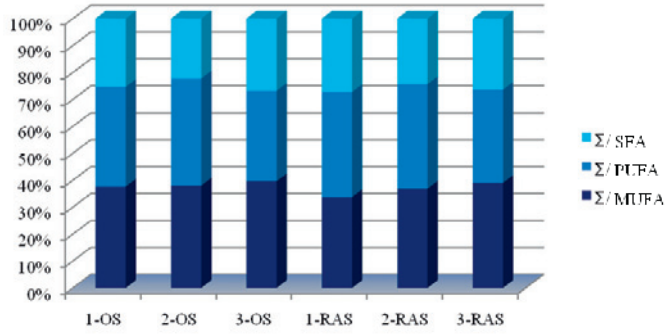


Fig. 4.9. Composition of fatty acids in muscle tissue of trout from the six farms

Our comparison of the samples from both aquaculture systems showed that the total MUFA for the OS technology was 38.44% and for the RAS technology equaled 36.58%. The total PUFA for the OS system was 36.78% and for the RAS reached 37.45%. The total of saturated fatty acids (SFA) in the OS system was 24.78% and in the RAS – 25.97% (Fig. 4.10).

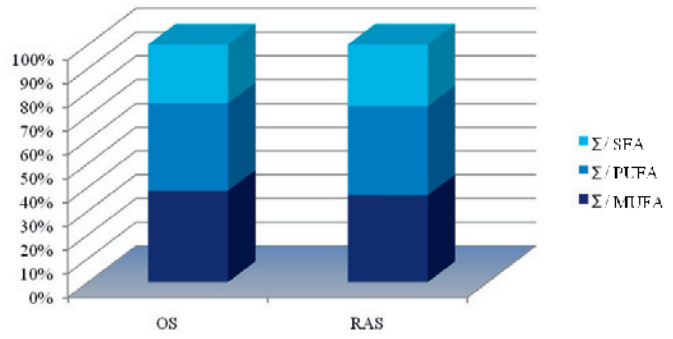


Fig. 4.10. Composition of fatty acids in muscle tissue of trout from the two technologies

### 4.3.6. Content of heavy metals

The content of lead and cadmium in plant and animal tissues (as well as in the human body) is an indicator of the quality of an environment in which these organisms live. The concentration of lead (Figs. 14.11 and 4.12) in the analyzed muscle tissues of trout was low and ranged from less than detectable amount to a few tens of µg/kg (the determined values did not exceed 0.10 mg/kg). Figure 4.11 shows the maximum allowable concentration (MAC), which is 0.30 mg/kg.

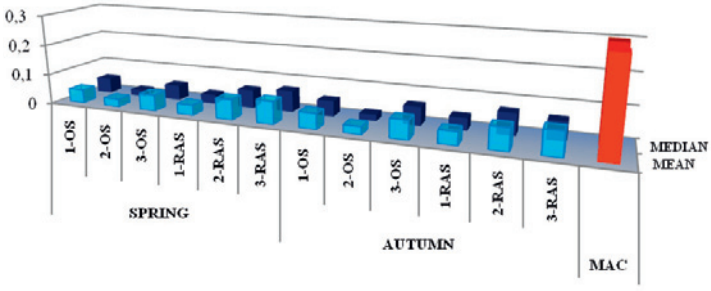


Fig. 4.11. Concentration of lead in muscle tissue of trout fish from the ix fish farms

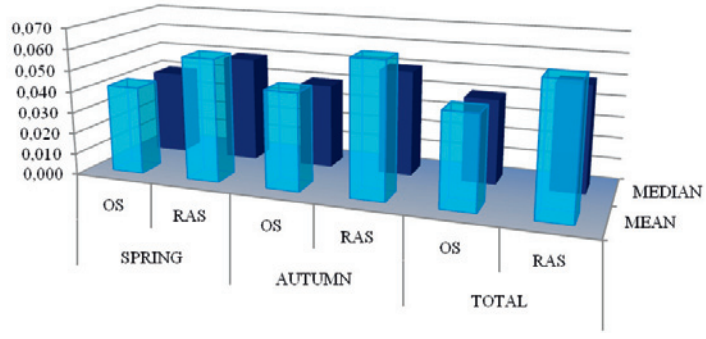


Fig. 4.12. Concentration of lead in muscle tissue of trout from the two technologies

When analyzing samples from the two technologies, it was detected that fish from the RAS farms had slightly more lead in their muscle tissues (0.061 mg/kg) than the ones from the OS farms (0.044 mg/kg), at the MAC of 0.30 mg/kg. All the analyzed samples contained small amounts of lead, much below the MAC.

Analogously to lead, the concentrations of cadmium determined in all the samples from the particular fish farms were from less than detectable to a few µg/kg. The concentrations of cadmium in muscle tissue of trout from the particular fish farms together with the MAC are shown in figure 4.13.

With respect to the two technologies, lightly lower concentrations were determined for the OS system (mean – 0.0109 mg/kg, median – 0.009 mg/kg) than for the RAS technology (mean – 0.0122 mg/kg, median – 0.009 mg/kg) (Fig. 4.14).

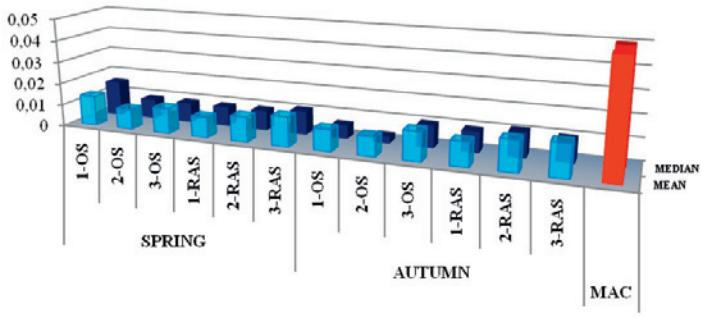


Fig. 4.13. Concentration of cadmium in muscle tissue of trout from the six fish farms

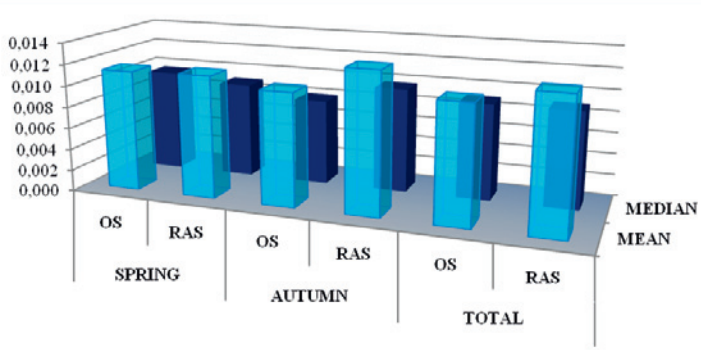


Fig. 4.14. Concentration of cadmium in muscle tissue of trout from the two technologies.

### 4.4. Summary

When analyzing the results of our determinations, it should be concluded that the chemical composition of muscle tissue sampled from trout fish was affected not only by the composition of granulated feed they were given but also by the season of the year when the fish were sampled. Samples obtained in the spring season were characterized by a lower fat content than samples derived from autumn catches. It has been observed that the content of dry matter as well as total crude ash are

similar in both groups of fish. The content of total protein varies slightly depending on the season. All the analyzed samples contained very little lead or cadmium (on the threshold of their detectablity). In short, the present study has confirmed the high health-promoting and nutritional value of fish meat from trout reared in Poland, and no distinction needs to be made between extensively and intensively cultured fish in this context.

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# 5. Consumer value of trout meat

## 5.1. Introduction

If the statement "Man is what he eats" written by Ludwig Andreas Feuerbach in 1863 is true, then trout meat is, according to the studies discussed below, the ideal component of human diet. Dynamic development of food industry has generated an increase in food supplies on the markets thanks to which a consumer can select, driven by his needs, a product that will meet his requirements to the highest degree possible. Until recently, fish producers thought that each fish, either produced or caught, was good raw material. Nowadays, all fish producers are paying increasing attention to the chemical composition and nutritional value of offered raw materials or products. A growing number of people involved in the fish industry are searching for solutions to increase the aesthetical and taste values of offered products. The expectations of a modern consumer are clearly directed at slow food, which includes low-processed, traditional, regional as well as organic food. In Poland, the rules of production and control over ecological agricultural products have been developed, but there is a lack thereof that would be applied in aquaculture and its production. Moreover, there is a lack of definition of which fish fit the standards of ecological production, a lack of knowledge of the quality of these food products and a lack of requirements for producers. In order to lay the foundations of general assumptions for the quality of trout, it was decided to carry out sensory analyses of fish originating from different production technologies.

Sensory analyses are mainly based on people and their abilities. Selection of members for a sensory team is not coincidental. Such persons must meet the requirements specified in the standards PN-ISO 8586-1:1996, PN-ISO 5496:1997 and PN-ISO 3972:1998. People applying for employment in a sensory laboratory are subjected to sensory verification allowing for the assessment of candidates' predispositions. Sensory evaluation is performed by a team of sensory-verified evaluators who have undergone tests checking their sensory capabilities (e.g. a test for taste, smell and vision blindness; a test for taste sensitivity thresholds, etc.) (Olszewska-Siemaszko et al. 2009).

People are one of the elements necessary for a reliable sensory analysis with the second factor being the environment, i.e. the conditions in which an evaluation is performed. A sensory analysis laboratory is essential for evaluations. According to the standards it must meet a series of requirements, such as appropriate temperature, humidity, lighting and should be odourless, properly sound-insulated and provide evaluators with comfortable working places. Proper recruitment of a team of evaluators and provision of an adequate laboratory generate correct sensory evaluations.

## 5.2. Materials and methods

### 5.2.1. Formation of the evaluation team

The sensory analysis was performed as a consumer test. The selection of candidates for the evaluation team was carried out. This selection was made in accordance with the standard PN-ISO 8586-1:1996 specifying the method of recruitment, initial selection and introduction, general rules and methods of training, selection of people with specific predispositions, monitoring of results, and possibilities for training of evaluators. Twenty respondents were finally qualified to the evaluation group.

### 5.2.2. Materials

The evaluation was performed on the fish fillets which originated from six farms located in different regions of Poland. Three farms operated on open system (OS) and the other three – recirculation aquaculture systems (RAS).

### 5.2.3. Preparation of materials for evaluation

Immediately after catching, fish were anaesthetized, slaughtered, gutted and washed. The fish were then cooled and transported to the laboratory. Until analyses, fish were stored at approximately 4°C. The preparation of trout samples for the evaluation of muscle tissue was performed in accordance with the methodology consistent with the recommendations by the German Fishery Association with the modifications by Białowąs and Zakrzewski. In the laboratory, the long bone and the backbone were removed from the washed fillets. The fillets were then thermally processed by steam cooking, under the cover, for 10 minutes. Spices were not added, so as not to distort the natural taste of muscle tissue.

### 5.2.4. Methods of sensory evaluation

A 9-degree hedonic scale in conformity with the publication by Peryam and Pilgrim (1957) was used to assess the degree of acceptance (desire). An assessment on the scale from 1 to 9 was assumed, where:

1 – "Dislike extremely",
2 – "Dislike very much"
3 – "Dislike moderately",
4 – "Dislike slightly",
5 – "Neither like or dislike"
6 – "Like slightly",
7 – "Like moderately",
8 – "Like very much",
9 – "Like extremely".

### 5.2.5. The evaluated determinants

In accordance with art. 3, sec. 3 of the Food Safety Act of 25 August 2006 the following determinants were selected for the sensory analysis: colour, smell, texture, juiciness, and taste. In addition, the respondents made a subjective general assessment. In order to verify the subjective general assessment, the evaluation of individual determinants was calculated which included the importance of individual sensory features according to Table 5.1.

Table 5.1. Importance indices of individual determinants used to calculate the evaluation of the determinants

| Importance of Individual Determinants | | | | | Subjective Assessment |
|---|---|---|---|---|---|
| Colour | Smell | Texture | Juiciness | Taste | General |
| 15% | 25% | 13% | 12% | 35% | 100% |

## 5.3. Colour

### 5.3.1. Colour – spring samplings

The highest mean assessment of colour from the spring samplings were attributed to the samples that originated from two farms: 2-OS (mean – 8.67, median – 9, standard deviation (SD) – 0.475) and 1-RAS (mean – 8.56, median – 9, SD – 0.521). The samples from the 1-OS farm were given the mean result of 8.08 (median – 8, SD – 0.534), from the 3-OS farm – 7.85 (median – 8, SD – 0.463), from the 2-RAS farm – 7.87 (median – 8, SD – 0.64), and from the 3-RAS farm – 7.05 (median – 7, SD – 0.897) (Fig. 5.1).

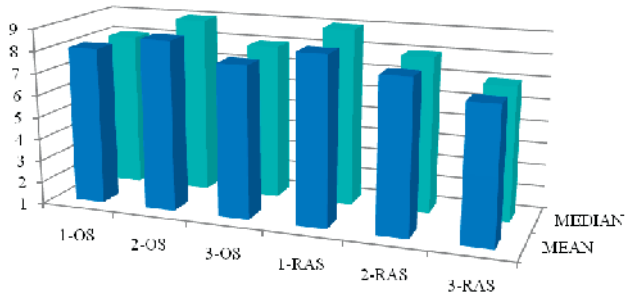


Fig. 5.1. The results of an assessment of the color – spring samplings

### 5.3.2. Colour – autumn samplings

In the evaluations generated for the autumn samplings, the samples from the 2-OS farm were given the highest scores (mean – 8.91, median – 9, SD – 0.290). The slightly lower result, i.e. 8.73, was recorded for the samples from the 1-RAS farm (median – 9, SD – 0.446). The other samples were given the following scores: 1-OS – mean 7.55 (median – 8, SD – 0.781); 3-OS – mean 7.53 (median – 8, SD – 0.697); 2-RAS – mean 7.40 (median – 8, SD – 0.722); and 3-RAS – mean 7.68 (median – 8, SD – 0.538) (Fig. 5.2).

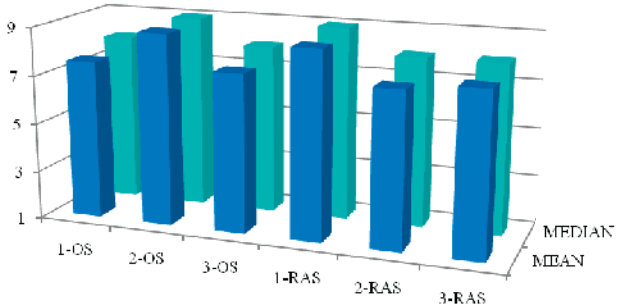


Fig. 5.2. The results of an assessment of the color – autumn samplings

### 5.3.3. Colour – all samplings, comparison of technologies

The average evaluation of colour for all samples from all samplings from the OS technology was 8.10 (median – 8, SD – 0.772). Slightly lower scores were given to the samples from the RAS technology, i.e. 7.88 (median – 8, SD – 0.880). These results did not differ statistically. Within the samples from the spring samplings, the higher scores were recorded for the samples that originated from the OS farms, i.e. 8.20 (median – 8, SD – 0.602), whereas the samples from the RAS farms were given the average score of 7.83 (median – 8, SD – 0.939). For the autumn samplings, the results were more homogenous: the mean of 8.00 for OS (median – 8, SD – 0.899) and 7.94 for RAS (median – 8, SD – 0.814) (Fig. 5.3).

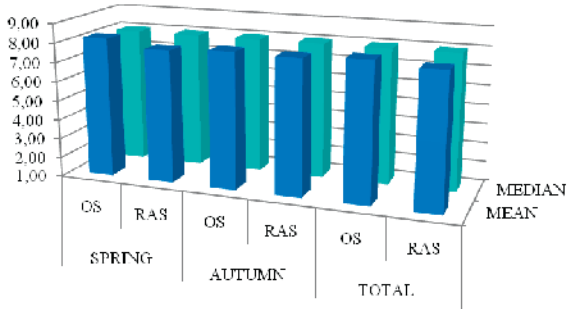


**Fig. 5.3.** The results of an assessment of the color

### 5.3.4. Colour – a summary

Colour is one of the most important indices of food. Vision is a measuring device and is the most complex sense used by humans. Vision enables an experienced consumer to gain information on the freshness of a product or raw material since the colorants in food change during maturation and decomposition. The ideal colour of a given product is difficult to define, but, as opposed to other determinants, it is easy to measure accurately. The evaluation of colour allows for drawing initial conclusions on a given product and they usually have a considerable impact on acceptance of a product and its further evaluation with other senses. IEvaluation team came across this phenomena during taste examination of the natural colour of muscle tissue (shades of grey) as well as the stained trout by carotenoids (orange). In order to avoid evaluation of taste being influenced by evaluation of colour this test was carried out with closed eyes. It was found that in the "blind evaluation" the respondents gave significantly higher scores to the samples with natural colour, whereas in the standard evaluation higher scores were attributed to the samples coloured with carotenoids. Taking the acquired knowledge into consideration, it was decided to lower the importance of colour determinant for the purpose of evaluation calculated from the determinants. Such approach resulted in better objectification of the results.

All results recorded in the evaluation of colour indicate a lack of differences in the colour of muscle tissue in the samples from different culture technologies.

## 5.4. Smell

### 5.4.1. Smell – spring samplings

In the spring samplings, the highest scores for smell were attributed to the samples that originated from the following farms: 2-OS – mean 8.88 (median – 9, SD – 0.331) and 1-RAS – mean 8.72 (median – 9, SD – 0.451). The other samples were given the following scores: 1-OS – mean 8.25 (median – 8,

SD – 0.533), 3-OS – mean 7.89 (median – 8, SD – 0.601), 2-RAS – mean 8.13 (median – 8, SD – 0.560), and 3-RAS – mean 7.81 (median – 8, SD – 0.579) (Fig. 5.4).

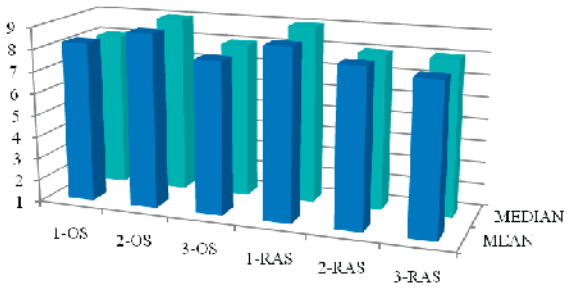


Fig. 5.4. Results of an assessment of the smell: spring samplings

### 5.4.2. Smell – autumn samplings

Within the autumn samplings, the highest scores for smell were given to the samples from the 2-OS farm, i.e. 8.89 (median – 9, SD – 0.313) and the 1-RAS farm, i.e. 8.78 (median – 9, SD – 0.416). The other samples were attributed the following scores: 1-OS – mean 8.26 (median – 8, SD – 0.514), 3-OS – mean 7.92 (median – 8, SD – 0.606), 2-RAS – mean 8.03 (median – 8, SD – 0.585), and 3-RAS – mean 7.89 (median – 8, SD – 0.582) (Fig. 5.5).

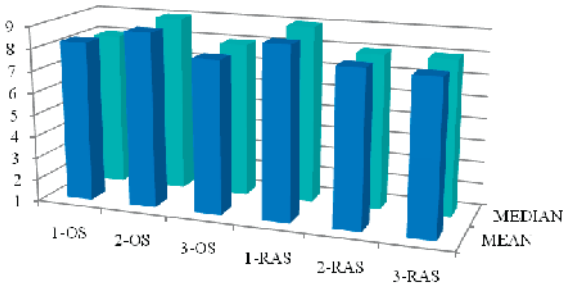


Fig. 5.5. Results of an assessment of the smell: autumn samplings

### 5.4.3. Smell – all samplings, comparison of technologies

The average assessment of smell for all samples from all samplings in the OS technology was 8.35 (median – 8, SD – 0.641). A slightly lower average score was given to the samples from the RAS technology, i.e. 8.23 (median – 8, SD – 0.656). These scores did not differ statistically. The evaluation of smell for the samples from the spring (mean – 8.34, median – 8, SD – 0.646) and autumn (mean – 8.36 , median – 8, SD – 0.635) samplings in the OS technology were almost identical. The results recorded for the samples in the RAS technology were also almost identical for the spring and autumn samplings, i.e. 8.22 (median – 8, SD – 0.652) and 8.23 (median – 8, SD – 0.661) (Fig. 5.6).

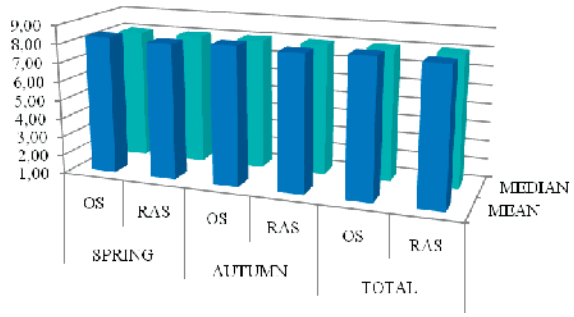


Fig. 5.6. Results of an assessment of the smell

### 5.4.4. Smell – a summary

Smell is the second (after colour) determinant that influences the pre-consumptive stimulation of behaviours associated with the acceptance of a product or raw material. For analyzing smells, humans use the olfactory sense that is thought to be the oldest and most sensitive. The "heart" of this sense is the inconspicuous olfactory epithelium located in the upper nasal cavity. There are approximately 5 million receptor cells on the surface of about 5 $cm^2$. In order to evaluate the smell of a product, a substance must be volatile and access the nostrils. The impression of smell is created by very complex mixtures. The evaluation of this determinant was thus limited to the assessment of the specificity of smell conventionally linked to the muscle tissue in trout and to the detection of other undesired odours.

All samples were given very high scores and in none of them were any other odours detected. The analyses of all samples from all technologies and samplings did not reveal any differences.

## 5.5. Texture

### 5.5.1. Texture – spring samplings

The highest scores for texture of the muscle tissue in trout that originated from the spring samplings were given to the samples from the 2-OS farm, i.e. 8.67 (median – 9, SD – 0.475), and from the 1-RAS farm, i.e. 8.56 (median – 9, SD – 0.521). The other samples from the spring samplings were attributed the following scores: 1-OS – mean 8.08 (median – 8, SD – 0.534); 3-OS – mean 7.86 (median – 8, SD – 0.452); 2-RAS – mean 7.88 (median – 8, SD – 0.631); and 3-RAS – mean 7.66 (median – 8, SD – 0.725) (Fig. 5.7).

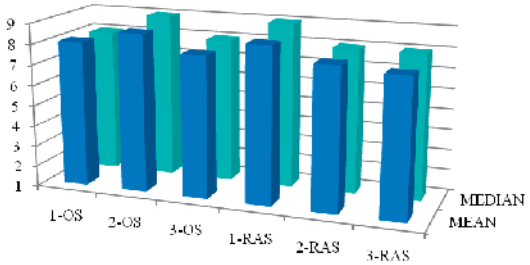


Fig. 5.7. Results of an assessment of the texture: spring samplings

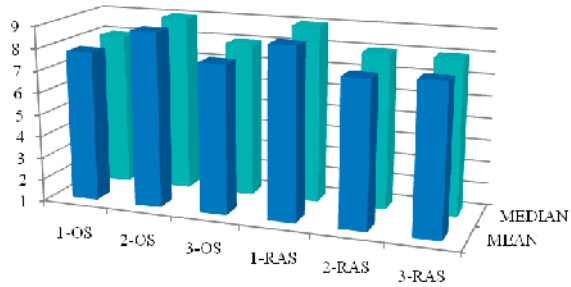


Fig. 5.8. Results of an assessment of the texture: autumn samplings

### 5.5.2. Texture – autumn samplings

The samples from the 2-OS farm, i.e. 8.91 (median – 9, SD – 0.290), and the 1-RAS farm, i.e. 8.73 (median – 9, SD – 0.446), were given the highest scores of texture for the autumn samplings. The other samples from these samplings were attributed the following scores: 1-OS – mean 7.81 (median – 8, SD – 0.699); 3-OS – mean 7.72 (median – 8, SD – 0.605); 2-RAS – mean 7.59 (median – 8, SD – 0.594); and 3-RAS – mean 7.71 (median – 8, SD – 0.585) (Fig. 5.8).

### 5.5.3. Texture – all samplings, comparison of technologies

All the scores given to the samples that originated from different culture technologies differed slightly and were, in total, for all samplings: OS – 8.17 (median – 8, SD – 0.693) and RAS – 8.02 (median – 8, SD – 0.743) (Fig. 5.9). For the spring and autumn samplings, the scores given to the samples from the individual technologies were similar to all samplings assessed in total.

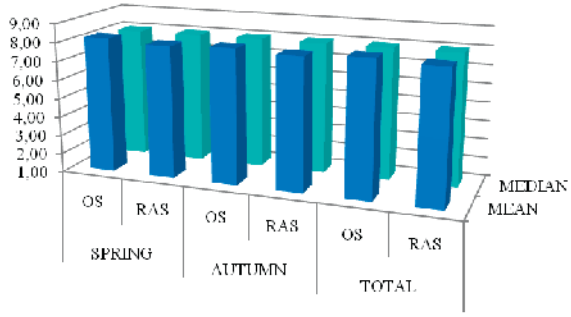


Fig. 5.9. Results of an assessment of the texture

### 5.5.4. Texture – a summary

Texture includes all rheological and structural features of a food product that may be recorded by humans with the receptors of touch, mechanical and visual as well as those related to hearing (if possible). Texture is one of the most complex determinants of food. The structure-building and sensory-stimulating substances include the basic food components: carbohydrates, proteins and lipids. Their quantity and quality and their transformation during thermal processing determine the structure that is specific to each food product.

All scores given to the samples that originated from different culture technologies differed slightly and were not statistically different.

### 5.6. Juiciness

#### 5.6.1. Juiciness – spring samplings

The highest scores for the spring samplings were given to the samples from the 2-OS farms, i.e. 8.67 (median – 9, SD – 0.475) and from the 1-RAS farm, i.e. 8.56 (median – 9, SD – 0.521). The other samples were attributed the following scores: 1-OS – 8.08 (median – 8, SD – 0.534); 3-OS – 7.85 (median – 8, SD – 0.463); 2-RAS – 7.87 (median – 8, SD – 0.645); and 3-RAS – 7.05 (median – 7, SD – 0.897) (Fig. 5.10).

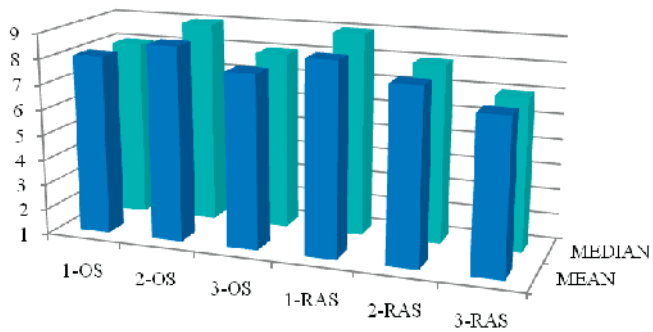


Fig. 5.10. Results of an assessment of the juiciness: spring samplings

#### 5.6.2. Juiciness – autumn samplings

The samples from the autumn samplings from the 2-OS farm were given the highest scores, i.e. 8.91 (median – 9, SD – 0.290). The equally high scores were attributed to the samples from the farm 1-RAS, i.e. 8.73 (median – 9, SD – 0.446). The other samples were given the following scores: 1-OS – 7.55 (median – 8, SD – 0.781); 3-OS – 7.53 (median – 8, SD – 0.697); 2-RAS – 7.40 (SD – 0.722, median – 8); 3-RAS – 7.68 (SD – 0.538, median – 8) (Fig. 5.11).

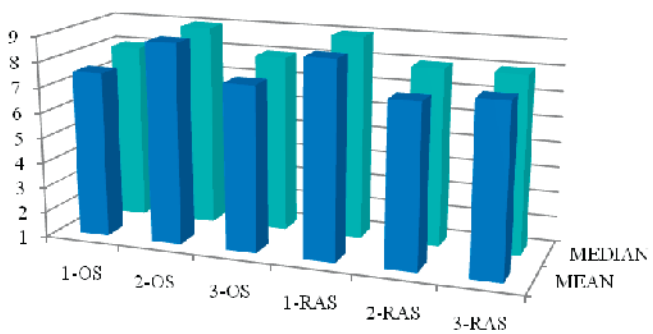


Fig. 5.11. Results of an assessment of the juiciness: autumn samplings

### 5.6.3. Juiciness – all samplings, comparison of technologies

The summary of all results from all samplings reveals that slightly higher scores were given to the samples from the OS technology: 8.10 (median – 8, SD – 0.772), whereas the samples from the farms operating on an RAS system were assessed as 7.88 (median – 8, SD – 0.880) (Fig. 5.12). For the spring and autumn samplings, the evaluations of samples from the individual technologies were comparable to all samples in total.

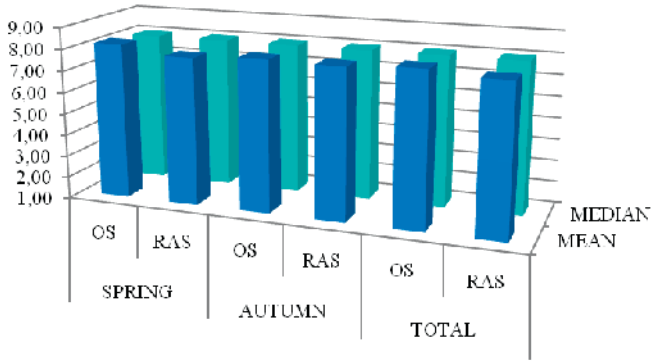


Fig. 5.12. Results of an assessment of the juiciness

### 5.6.4. Juiciness – a summary

Juiciness of fish meat and muscle tissue that have been cooked is determined by two factors: the first is the feeling of moisture in the initial stage of mastication due to the release of fluids from the muscle tissue and the second is the maintenance of juiciness probably due to the slow release of serum and the stimulating impact of intermuscular and intramuscular fat on the production of saliva. Since the maintenance of the juiciness sense during mastication leaves a longer impression than the initial sense of fluid release, it becomes understandable that the majority of studies on the juiciness of fish meat and muscle tissue reveal a closer correlation between the juiciness of meat and the content of fat than between the juiciness of meat and the volume of fluid released from meat. Juiciness of meat from different animal species and from different structural elements is extremely variable. Since the sense of juiciness is closely correlated to the content of intramuscular fat, the conditions influencing the volume of intramuscular fat in meat are reflected in its juiciness. Therefore, more marbled meat of an adult animal with a relatively high degree of maturity will be juicier than less-marbled meat from young animals. Meat from young animals (e.g. veal) initially leaves a feeling of wateriness, but the final sense is dryness. In the case of fish muscle tissue, dryness or wateriness is also evaluated. Both extreme features decrease its price and influence the total impression felt by consumers.

All samples from all farms were given high scores, which indicates the excellent juiciness of muscle tissue in trout cultured in Poland.

## 5.7. Taste

### 5.7.1. Taste – spring samplings

From the spring samplings, the highest scores for taste were given to the samples from the farms: 1-RAS – 8.75 (median – 9, SD – 0,432) and 2-OS – 8.66 (median – 9, SD – 0.475). The other samples were attributed the following scores: 1-OS – 7.85 (median – 8, SD – 0.549), 3-OS – 7.25 (median – 8, SD – 0.720), 2-RAS – 8.05 (median – 8, SD – 0.490), and 3-RAS – 7.37 (median – 8, SD – 0.990) (Fig. 5.13).

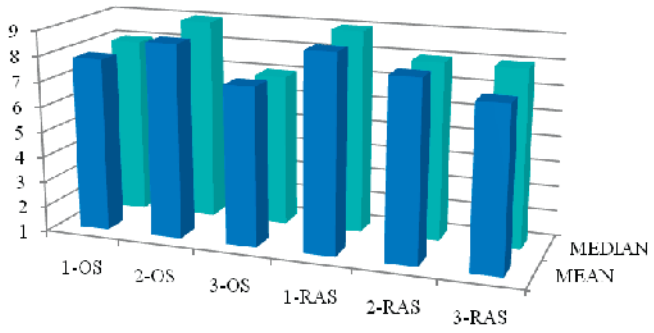


Fig. 5.13. Results of an assessment of the taste: spring samplings

### 5.7.2. Taste – autumn samplings

The highest scores were given to the samples from the autumn samplings that originated from the farm 2-OS, i.e. 8.94 (median – 9, SD – 0.242), and from the farm 1-RAS, i.e. 8.88 (median – 9, SD – 0.331). The other samples were attributed the following scores: 1-OS – mean 7.77 (median – 8, SD – 0.601), 3-OS – mean 7.09 (median – 7, SD – 0.623), 2-RAS – mean 6.47 (median – 6, SD – 0.700), and 3-RAS – mean 7.27 (median – 7, SD – 0.766) (Fig. 6.14).

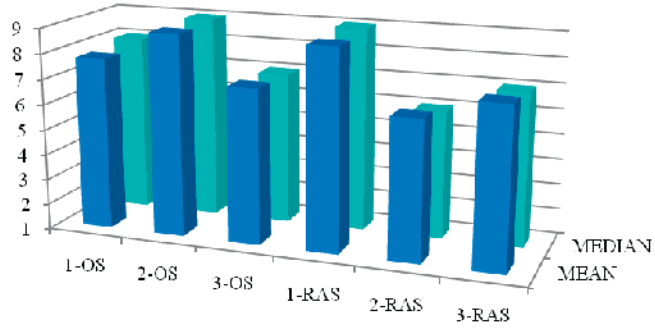


Fig.5.14. Results of an assessment of the taste: autumn samplings

### 5.7.3. Taste – all samplings, comparison of technologies

The average evaluation of taste for all samples from all samplings from the OS technology was 7.93 (median – 8, SD – 0.875). The mean score for the samples from the RAS technology was 7.80 (median – 8, SD – 1.076). These evaluations did not differ statistically. For the samples from the spring samplings, higher scores were given to the samples from the RAS cultures were evaluated as 8.06 (median – 8, SD – 0.888), whereas the samples from the OS technology, i.e. 7.92 (median – 8, SD – 0.825). For the autumn samplings, the results were more homogenous – amounting to on average 7.93 for the OS system (median – 8, SD – 0.923) and 7.54 for the RAS technology (median – 8, SD – 1.181) (Fig. 5.15).

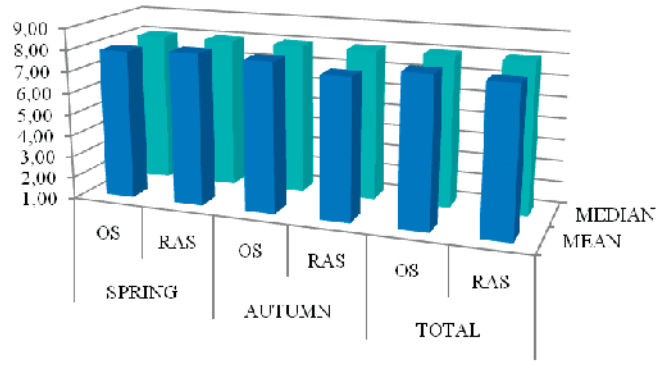


Fig.5.15. Results of an assessment of the taste

### 5.7.4. Taste – a summary

Taste is a sense which plays a very significant role in human nutrition. It should be remembered that, during mastication, other sensory impressions occur in the oral cavity and it is practically impossible to separate them from taste. Therefore the term "tastefulness" is more often used in the sensory analysis. Tastefulness is a sensory feature which is composed of taste, smell and touch impressions. This feature is final and most important. It determines the usefulness of a given product in human nutrition. A bad or unacceptable taste eliminates a given product or raw material from a consumer's diet.

All tested samples were given high scores for their taste. The individual farms from the individual samplings differed significantly, but an analysis of individual technologies generated scores that did not differ statistically. It should be remembered that, in the case of fish, particularly the salmonidae, taste is largely influenced by the type of pelleted feed that is used in the production cycle and, to a lesser extent, by environmental factors.

## 5.8. General subjective assessment (GSA) and assessment calculated from the determinants (AFD)

### 5.8.1. GSA and AFD – spring samplings

The highest average subjective assessments for the spring samplings were attributed to the samples from two farms: 2-OS (mean – 8.84, median – 9, SD – 0.365) and 1-RAS (mean – 8.73, median – 9, SD – 0.446). The samples from the farm 1-OS were on averaged assessed as 8.00 (median – 8, SD – 0.283),

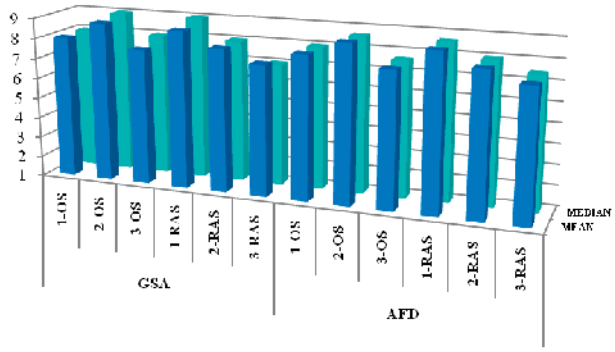


Fig. 5.16. Results GSA and AFD: spring samplings

from the farm 3-OS – 7.68 (median – 8, SD – 0.468), from the farm 2-RAS – 8.02 (median – 8, SD – 0.378), and from the farm 3-RAS – 7.41 (median – 7, SD – 0.523) (Fig. 5.16). The total evaluation based on the importance of individual determinants was calculated for all samples. The AFD results were comparable with the GSA values and did not differ significantly or statistically.

### 5.8.2. GSA and AFD – autumn samplings

For the autumn samplings, the samples from the 2-OS farm were given the highest mean score, i.e. 8.98 (median – 9, SD – 0.140), whereas a slightly lower score, i.e. 8.89, was attributed to the samples from the 1-RAS farm (median – 9, SD – 0.313). The other samples were given the following scores: 1-OS – 7.85 (median – 8, SD – 0.426); 3-OS – 7.52 (median – 8, SD – 0.520); 2-RAS – 7.24 (median – 7, SD – 0.477); and 3-RAS – 7.59 (median – 8, SD – 0.488) (Fig. 5.17). The AFD assessment for the samples from the autumn samplings was slightly lower for the best farms and slightly higher for the other farms in relation to GSA.

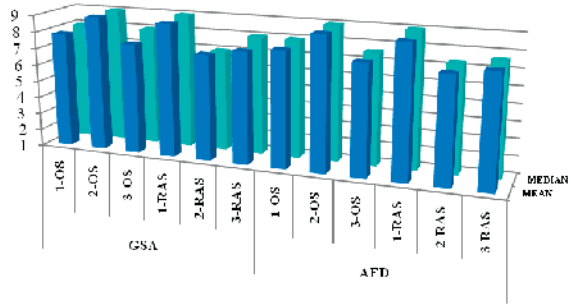


Fig. 5.17. Results GSA and AFD: autumn samplings

### 5.8.3. GSA and AFD – all samplings, comparison of technologies

All average GSAs for the individual technologies and for all samplings analysed together and separately were very similar and within the range from 7.98 (RAS – all samplings in total) to 8.15 (OS – all samplings in total). The AFD assessment was also comparable to GSA and ranged between 7.96 (RAS) and 8.11 (OS) (Fig. 5.18).

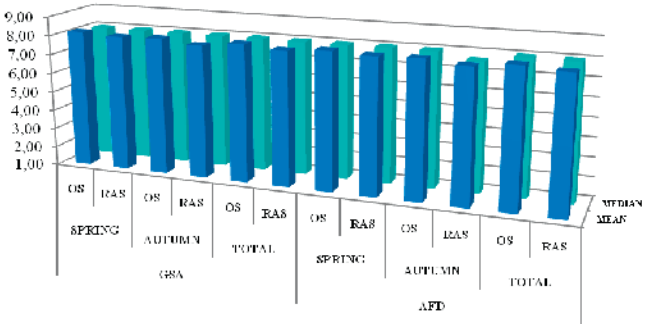


Fig. 5.18. Results GSA and AFD

#### 5.8.4. GSA and AFD – a summary

Sensory quality of a product is one of its most important features, if not the most important. It determines the attractiveness and acceptability of a given food product perceived by a consumer. Based on the GSA and AFD results, it may be concluded that fish producers pay considerable attention to the sensory quality of trout from Polish cultures. The results discussed above prove the very high acceptability of the samples from all technologies and samplings by the evaluating team.

### 5.9. Conclusions

All the results indicate that the technologies of trout production used in Poland ensure the high sensory quality of trout and therefore provide safe raw materials in terms of sensory features.

All tested samples were given high or very high acceptability in all determinants and in the general subjective assessment, which was further confirmed by the general evaluation calculated from the determinants.

Elżbieta Terech-Majewska, Andrzej K. Siwicki

# 6. Microbiological and immunological assessment of the rainbow trout from fish rearing technologies used in Poland

### 6.1. Clinical examination

This research has dealt with the assessment of health of fish from 6 fish farms representing different production levels: 3 farms with an open water flow system (OS) and 3 farms with a high degree of water recirculation, that is the recirculatory aquaculture system (RAS).

At each farm, the health of 40 rainbow trout individuals divided into two groups according to body weight (in the same manner as presented in the other chapters, i.e. S and B) was evaluated. The assessment was performed in May–June 2011 and 2012 and in October-November 2011, that is at the time when the water temperature changes, which causes stress to fish.

Evaluation of the condition and health of rainbow trout required developing an innovative approach to the diagnostic process and procedures, which consisted of the following tests: clinical, bacteriological, viral, immunological assays and biochemical.

On each occasion, the clinical tests of rainbow trout fish were performed before commencing other analyses. In order to control the effect of polyethiological stress (manipulation), the fish were given general anaesthesia using Propiscin (manufactured by the Institute of Inland Fisheries in Olsztyn) in a concentration of 1 mL of the preparation per 1 L of water, and maintained in aerated plastic containers until the examination procedures began, Propiscin is a 0.2% solution of etomidate, which anaesthetizes fish for up to half an hour. This is a low toxicity substance and has been tested on many fish species, mainly salmonids (Kazuń et al. 2001). Blood for immunological assays was sampled with a needle from the caudal fin and kept in plastic containers at 4°C.

The S and B group trout fish were analyzed clinically by evaluating their actual condition. The results demonstrated lack of any significant divergence from the physiological condition. The fish were in good condition and showed no changes that would indicate any pathology. Such observations were made with respect to a given individual farm and to the type of fish rearing system, i.e. OS and RAS.

### 6.2. Bacteriological assays

The bacteriological tests were performed using routine biological methods, which enable isolation, culture and identification of bacteria collected from the skin, fins, gills and from internal organs of the

rainbow trout. Each time, the material for bacteriological assays was sampled from 40 specimens at each farm and inoculated onto transport substrates until delivered to the bacteriological laboratory. Next, the samples were cultured on solid media and maintained under optimum conditions. Afterwards, pure cultures were isolated and identified with the API test (BioMerieux, Poland). The results of the bacteriological tests performed at the set time periods are presented in tables 6.1–6.3.

**Table 6.1.** Results of bacteriological assays on the rainbow trout covered by the investigations in the spring of 2011

| Origin of fish (fish farm, group) | Site of isolation of bacteria | | | |
|---|---|---|---|---|
| | skin | fins | gills | internal organs |
| 1-OS | NG | NG | NG | NG |
| 2-OS | NG | *A. hydrophila* | *P. fluorescens* | *A. salmonicida*<br>*P. fluorescens* |
| 3-OS | NG | NG | NG | NG |
| 1-RAS | NG | NG | NG | NG |
| 2-RAS | *A. hydrophila* | NG | *A. hydrophila* | NG |
| 3-RAS | NG | NG | NG | NG |

Comments: *A. hydrophila – Aeromonas hydrophila, A. salmonicida – Aeromonas salmonicida, P. fluorescens – Pseudomonas fluorescens,* NG – no growth of bacteria pathogenic to fish.

**Table 6.2.** Results of bacteriological assays on the rainbow trout covered by the investigations in the autumn of 2011

| Origin of fish (fish farm, group) | Site of isolation of bacteria | | | |
|---|---|---|---|---|
| | skin | fins | gills | internal organs |
| 1-OS | *A. hydrophila* | *A. hydrophila* | *A. hydrophila* | NG |
| 2-OS | *A. hydrophila*<br>*F. oryzihabitans* | *A.hydrophila*<br>*F. oryzihabitans* | *A.hydrophila*<br>*F. oryzihabitans* | NG |
| 3-OS | *A. hydrophila* | *A. hydrophila* | *A. hydrophila* | NG |
| 1-RAS | *A. hydrophila* | *A. hydrophila* | *A. hydrophila* | NG |
| 2-RAS | *A. hydrophila* | *A. hydrophila* | *A. hydrophila* | *P. fluorescens* |
| 3-RAS | *P. fluorescens*<br>*A. hydrophila* | *P. fluorescens*<br>*A. hydrophila* | *P. fluorescens*<br>*A. hydrophila* | NG |

Comments: *A. hydrophila – Aeromonas hydrophila, F. oryzihabitans – Flavimonas oryzihabitans, P. fluorescens – Pseudomonas fluorescens,* NG – no growth of bacteria pathogenic to fish.

**Table 6.3.** Results of bacteriological assays on the rainbow trout covered by the investigations in the spring of 2012

| Origin of fish | | Site of isolation of bacteria | | | |
|---|---|---|---|---|---|
| fish farm | group | skin | fins | gills | internal organs |
| 1-OS | S | *P. fluorescens*<br>*S. aureus* | *P. fluorescens*<br>*S. aureus* | *P. fluorescens*<br>*S. aureus* | *P. fluorescens*<br>*H. alvei* |
| | B | *P. fluorescens*<br>*S. aureus* | *P. fluorescens*<br>*S. aureus* | *P. fluorescens*<br>*S. aureus* | *P. fluorescens*<br>*S. aureus* |
| 1-OS | S | *A. hydrophila*<br>*S. maltophilia*<br>*F. oryzihabitans*<br>*Ch. luteola* | *A. hydrophila*<br>*S. maltophilia*<br>*F. oryzihabitans*<br>*Ch. luteola* | *A. hydrophila*<br>*S. maltophilia*<br>*F. oryzihabitans*<br>*Ch. luteola* | NG |
| | B | *A. hydrophila*<br>*S. maltophilia*<br>*F. oryzihabitans*<br>*Ch. luteola* | *A. hydrophila*<br>*S. maltophilia*<br>*F. oryzihabitans*<br>*Ch. luteola* | *A. hydrophila*<br>*S. maltophilia*<br>*F. oryzihabitans*<br>*Ch. luteola* | NG |

| Origin of fish | | Site of isolation of bacteria | | | |
|---|---|---|---|---|---|
| fish farm | group | skin | fins | gills | internal organs |
| 3-OS | S | *A. salmonicida*<br>*P. fluorescens*<br>*Staphylococcus* sp. | *A. salmonicida*<br>*P. fluorescens*<br>*Staphylococcus* sp. | *A. salmonicida*<br>*P. fluorescens*<br>*Staphylococcus* sp. | *A. salmonicida*<br>*P. fluorescens*<br>*Staphylococcus* sp. |
| | B | *A. salmonicida*<br>*P. fluorescens*<br>*S. pneumotica*<br>*K. oxytoca* | *A. salmonicida*<br>*P. fluorescens*<br>*S. pneumotica*<br>*K. oxytoca* | *A. salmonicida*<br>*P. fluorescens*<br>*S. pneumotica*<br>*K. oxytoca* | NG |
| 1-RAS | S | *A. hydrophila,*<br>*P .fluorescens,*<br>*V. fluvialis,*<br>*S. aureus,*<br>*Ervinia sp.,* | *A. hydrophila*<br>*P. fluorescens*<br>*V. fluvialis*<br>*S. aureus*<br>*Ervinia* sp. | *A. hydrophila*<br>*P. fluorescens*<br>*V. fluvialis*<br>*S. aureus*<br>*Ervinia* sp. | *H. alvei* |
| | B | *A. hydrophila*<br>*P. fluorescens*<br>*V. fluvialis*<br>*S. aureus*<br>*Ervinia* sp. | *A. hydrophila*<br>*P. fluorescens*<br>*V. fluvialis*<br>*S. aureus*<br>*Ervinia* sp. | *A. hydrophila*<br>*P. fluorescens*<br>*V. fluvialis*<br>*S. aureus*<br>*Ervinia* sp. | *H. alvei* |
| 2-RAS | S | *P. fluorescens*<br>*A. salmonicida*<br>*Staphylococcus* sp. | *P. fluorescens*<br>*A. salmonicida*<br>*Staphylococcus* sp. | *P. fluorescens*<br>*A. salmonicida*<br>*Staphylococcus* sp. | *P. fluorescens*<br>*Staphylococcus sp.*<br>*P. pneumotica* |
| | B | *P. fluorescens*<br>*A. salmonicida*<br>*Staphylococcus* sp. | *P. fluorescens*<br>*A. salmonicida*<br>*Staphylococcus* sp. | *P. fluorescens*<br>*A. salmonicida*<br>*Staphylococcus* sp. | *H. alvei* |
| 3-RAS | S | *P. fluorescens*<br>*S. aureus*<br>*V. fluvialis* | *P. fluorescens*<br>*S. aureus*<br>*V. fluvialis* | *P. fluorescens*<br>*S. aureus*<br>*V. fluvialis* | *P. fluorescens*<br>*S. aureus*<br>*V. fluvialis*<br>*H. alvei* |
| | B | *V. fluvialis* | *V. fluvialis* | *V. fluvialis* | *P. fluorescens*<br>*V. fluvialis* |

Comments: *A. hydrophila – Aeromonas hydrophila, A. salmonicida – Aeromonas salmonicida, Ch. luteola – Chryseomonas luteola, F. oryzihabitans – Flavimonas oryzihabitans, H. alvei – Hafnia alvei, K. oxytoca – Klebsiella oxytoca, P. fluorescens – Pseudomonas fluorescens, S. pneumotica - Serratia pneumotica, S. aureus – Staphylococcus aureus, S. maltophilia – Stenotrophomonas maltophilia, V. fluvialis – Vibrio fluvialis,* NG – no growth of bacteria pathogenic to fish.

The results of bacteriological assays have revealed no differences in the pathogenic flora infesting fish from the same farm but of different body weight. At all the farms with either OS or RAS rearing systems, the dominant bacterial flora consisted of saprophytic, conditionally pathogenic bacteria of the genera *Aeromonas* and *Pseudomonas*, which are widespread in open waters and on external integuments of farmed and wild fish. However, in the spring of 2012, presence of *Aeromonas salmonicida* was detected at the fish farm 3-OS on S and B fish and at the fish farm 2-RAS on S and B fish. These bacteria are indicated in the aetiology of furunculosis in salmonid fish. The absence of the disease symptoms or deaths is a result of the high anti-infective potential of the fish on those farms, which was evidenced through immunological tests. In turn, isolation and identification of another type of bacterial flora on the fish's integumental coverings and gills, such as cells of *Staphylococcus* or *Vibrio* genera, did not pose a health threat to the examined fish.

### 6.3. Viral assays

The viral assays were executed to test possible presence of viruses which are pathogenic to the rainbow trout. The tests focused on the following diseases, which cause the largest loss in the aquaculture of this species:

- viral haemorrhagic septicaemia (VHS) caused by a virus of the family *Rhabdoviridae*,
- infectious haematopoietic necrosis (IHN) caused by a virus of the family *Rhabdoviridae*,
- infectious pancreatic necrosis (IPN) caused by a virus of the family Birnaviridae,
- herpesvirus disease of salmonids caused by *Salmonid Herpesvirus* Type 2 (SalHV-2), of the family *Herpes*.

The material for the assays consisted of gills, kidneys, liver and brain. The material was collected under sterile conditions into transport containers and maintained frozen (-20°C). Isolation and identification of viruses were performed using PCR and Nested-PCR molecular methods according to the procedures used by the Department of Pathology and Immunology of Fish at the Institute of Inland Fisheries, and recommended by reference laboratories. As for the diagnosis of VHS, IHN and IPN viruses, the following procedures were adopted:

- isolation of the viral RNA from the material (organs and gills) with Tri-reagent,
- optimization of reverse transcription for the viral RNA,
- Mutiplex PCR method using primers specific for the genes of VHS, IHN and IPN viruses.

In respect of the diagnostic tests towards the SalHV-2, the following procedures were adopted:

- isolation of DNA from the analyzed material (organs, gills) using Tri-reagent or a QIAamp Virus Spin kit,
- PCR method with primers specific for the gene of virus SalHV-2.

The results of the viral assays conducted at the set time periods are presented in tables 6.4–6.6.

Analysis of the results of viral assays showed that in none of the fish originating from the OS and RAS farms and examined in the two research periods of 2011 and 2012, presence of the reportable VHS and IHN viruses or SalHV-2 virus was detected. In just two farms, one with the open water flow (2-OS) and the other one with the recirculatory system (3-RAS), presence of IPN virus in two research period was determined. However, the high anti-infective potential found in these fish prevented development of clinical manifestation of the disease and possibly fatal anatomopathological changes.

**Table 6.4.** Results of viral l assays on the rainbow trout covered by the investigations in the spring of 2011

| Origin of fish (fish farm) | Isolated viruses | | | |
|---|---|---|---|---|
| | VHS | IHN | IPN | SalHV-2 |
| 1-OS | – | – | – | – |
| 2-OS | – | – | – | – |
| 3-OS | – | – | + | – |
| 1-RAS | – | – | – | – |
| 2-RAS | – | – | – | – |
| 3-RAS | – | – | + | – |

**Table 6.5.** Results of viral l assays on the rainbow trout covered by the investigations in the autumn of 2011

| Origin of fish (fish farm) | Isolated viruses | | | |
|---|---|---|---|---|
| | VHS | IHN | IPN | SalHV-2 |
| 1-OS | – | – | – | – |
| 2-OS | – | – | – | – |
| 3-OS | – | – | + | – |
| 1-RAS | – | – | – | – |
| 2-RAS | – | – | – | – |
| 3-RAS | – | – | + | – |

Table 6.6. Results of viral l assays on the rainbow trout covered by the investigations in the spring of 2012

| Origin of fish (fish farm) | Isolated viruses | | | |
|---|---|---|---|---|
| | VHS | IHN | IPN | SalHV-2 |
| 1-OS | – | – | – | – |
| 2-OS | – | – | – | – |
| 3-OS | – | – | – | – |
| 1-RAS | – | – | – | – |
| 2-RAS | – | – | – | – |
| 3-RAS | – | – | – | – |

### 6.4. Immunological assays

The aim of these tests was to determine the potential of non-specific cellular and humoral defence mechanisms in the rainbow trout in two different aquaculture systems. For this purpose, procedures and methods had been developed to ensure objective evaluation of the impact of the different aquaculture systems on the non-specific defence mechanisms and anti-infective resistance. The fish were fist anaesthetized with Propiscin and then blood for immunological tests was sampled. After blood centrifugation, blood serum was obtained, which, until submitted to assays, was kept frozen at -70°C in plastic vials labelled according to the system adopted in this research: fish farm symbol, individual number assigned to a given fish, date of sampling. At the same time, when the clinical examination had been completed and material for bacteriological and viral assays had been collected, the spleen was isolated and put into sterile plastic containers with RPMI-1640 medium (Sigma) medicated with antibiotics and cooled to 4°C. The containers were then transported to the laboratory for further immunological tests. Determinations of the selected parameters reflecting non-specific cellular and humoral resistance were made with the aid of published methods used for assessment of the defence potential in fish in different types of aquaculture facilities (Siwicki et al. 2010, 2011). The cellular activity of defence mechanisms including blood phagocytes and T and B lymphocytes was determined using the following methods:

a) RBA (Respiratory Burst Activity) – metabolic activity of phagocytes (capability of intercellular oxygen burst), isolated from blood and from the spleen, was determined by spectrophotometry after PMA (Phorbol Myristate Acetate) stimulation of cells, as described by Siwicki et al. (2010). Blood phagocytes and macrophages from the spleen were isolated after centrifugation of cells in Gradisol G gradient (Polfa);
b) PKA (Potential Killing Activity) – assessment of the killing activity of blood phagocytes and macrophages isolated from the spleen was made by spectrophotmetry after stimulation of cells with *Aeromonas hydrophila* bacteria, according to the method described by Siwicki and Anderson (1993), Blood phagocytes and macrophages from the spleen were isolated after centrifugation of cells in Gradisol G gradient (Polfa);
c) Activity of lymphocytes was determined on the basis of proliferative response of T lymphocytes (LyTP) after stimulation with concanavalin A (ConA, Sigma) and application of the MTT method first adapted for fish by Siwicki et al. (2010). Lymphocytes from blood and from the spleen were isolated after centrifugation of cells in Gradisol G gradient (Polfa).

Activity of non-specific humoral mechanisms in blood serum was determined using the following methods:

a) activity of lysozyme in serum was established using the turbidimetric method with *Micrococcus lysodeikticus* bacteria, as modified by Siwicki and Anderson (1993);
b) total protein level was determined by spectrophotometry with the biuret method using commercially available Diagnostic Kits – Protein Total Reagents (Sigma), as described by Siwicki and Anderson (1993);

c) levels of gamma-globulins were measured by spectrophotometry with the biuret method (Diagnistic Kits – Protein Total reagents; Sigma) and polyethylene glycol 10 000 (Sigma), as described by Siwicki and Anderson (1993);
d) levels of ceruloplasmin, an acute phase protein, were measured by spectrophotometry with the methods described for fish for the first time by Siwicki et al. (1986).

The results of the above assays underwent statistical analysis for determination of means and standard deviation (SD), employing Statistica for Windows 7.1 software (StatSoft, Inc. 2004), while significance of differences at $P > 0.05$ was demonstrated by one-factor analysis of variance (ANOVA).

The results of immunological assays for determination of metabolic activity and killing activity of phagocytes and macrophages of the fish examined in the different seasons of 2011 and 2012 are presented in tables 6.7–6.9.

**Table 6.7.** Results of immunological assays for determination of metabolic activity and killing activity of blood phagocytes and macrophages of the rainbow trout covered by the investigations in the spring of 2011 (means ± SD)

| Origin of fish | | Determined parameters | | | |
|---|---|---|---|---|---|
| fish farm | group | RBA f | RBA m | PKA f | PKA m |
| 1-OS | S | 0.38 ± 0.05 | 0.36 ± 0.03 | 0.30 ± 0.03 | 0.30 ± 0.03 |
| | B | 0.42 ± 0.05 | 0.37 ± 0.04 | 0.31 ± 0.03 | 0.31 ± 0.04 |
| 2-OS | S | 0.37 ± 0.04 | 0.34 ± 0.05 | 0.28 ± 0.03 | 0.27 ± 0.04 |
| | B | 0.40 ± 0.06 | 0.36 ± 0.05 | 0.27 ± 0.04 | 0.29 ± 0.05 |
| 3-OS | S | 0.32 ± 0.03 *1, 2 | 0.32 ± 0.03 *1 | 0.24 ± 0.04 *1 | 0.25 ± 0.03 *1 |
| | B | 0.33 ± 0.04 *1, 2 | 0.31 ± 0.05 *1 | 0.25 ± 0.03 *1 | 0.24 ± 0.04 *1, 2R |
| 1-RAS | S | 0.35 ± 0.04 | 0.34 ± 0.04 | 0.28 ± 0.04 | 0.27 ± 0.04 |
| | B | 0.36 ± 0.04 | 0.33 ± 0.05 | 0.29 ± 0.04 | 0.29 ± 0.05 |
| 2-RAS | S | 0.37 ± 0.05 | 0.35 ± 0.04 | 0.28 ± 0.04 | 0.28 ± 0.03 |
| | B | 0.38 ± 0.05 | 0.37 ± 0.04 | 0.29 ± 0.05 | 0.29 ± 0.04 |
| 3-RAS | S | 0.31 ± 0.05 *1, 2, 2R | 0.31 ± 0.03 *1 | 0.24 ± 0.03 *1, 2 | 0.23 ± 0.02 *1, 2R |
| | B | 0.32 ± 0.03 *1, 2, 2R | 0.32 ± 0.04 *1 | 0.22 ± 0.04 *1, 2 | 0.24 ± 0.03 *1, 2R |

Comments: RBA f – metabolic activity (RBA) of blood phagocytes (OD 620 nm), RBA m – metabolic activity (RBA) of macrophages (OD 620 nm), PKA f – killing activity (PKA) of blood phagocytes (OD 620 nm), PKA m – killing activity (PKA) of macrophages (OD 620 nm); * – differences statistically significant at $P < 0.05$, relative in 1 (1-OS), 2 (2-OS), 1R (1-RAS), 2R (2-RAS).

**Table 6.8.** Results of immunological assays for determination of metabolic activity and killing activity of blood phagocytes and macrophages of the rainbow trout covered by the investigations in the autumn of 2011 (means ± SD)

| Origin of fish | | Determined parameters | | | |
|---|---|---|---|---|---|
| fish farm | group | RBA f | RBA m | PKA f | PKA m |
| 1-OS | S | 0.40 ± 0.05 | 0.38 ± 0.05 | 0.32 ± 0.05 | 0.30 ± 0.04 |
| | B | 0.43 ± 0.05 | 0.39 ± 0.05 | 0.34 ± 0.05 | 0.31 ± 0.04 |
| 2-OS | S | 0.40 ± 0.05 | 0.38 ± 0.04 | 0.32 ± 0.05 | 0.29 ± 0.05 |
| | B | 0.42 ± 0.05 | 0.40 ± 0.04 | 0.33 ± 0.05 | 0.30 ± 0.04 |
| 3-OS | S | 0.35 ± 0.04 *1, 2 | 0.32 ± 0.03 *1, 2 | 0.26 ± 0.04 *1, 2 | 0.24 ± 0.05 *1, 2R |
| | B | 0.34 ± 0. 03 *1, 2, 1R, 2R | 0.33 ± 0.04 *1, 2, 2R | 0.25 ± 0.05 *1, 2, 1R, 2R | 0.23 ± 0.05 *1, 2, 1R, 2R |

| | | | | | |
|---|---|---|---|---|---|
| 1-RAS | S | 0.38 ± 0.05 | 0.35 ± 0.05 | 0.30 ± 0.05 | 0. 29 ± 0.04 |
| | B | 0.41 ± 0.05 | 0.36 ± 0.04 | 0.31 ± 0.05 | 0. 30 ± 0.04 |
| 2-RAS | S | 0.40 ± 0.05 | 0.37 ± 0.04 | 0.30 ± 0.05 | 0. 30 ± 0.04 |
| | B | 0.41 ± 0.05 | 0.38 ± 0.04 | 0.32 ± 0.05 | 0.31 ± 0.04 |
| 3-RAS | S | 0.35 ± 0.03 *1, 2 | 0.34 ± 0.03 *1, 2 | 0.27 ± 0.04 *1, 2 | 0.25 ± 0.04 *1, 2R |
| | B | 0.36 ± 0.03 *1, 2 | 0.32 ± 0.04 *1, 2, 2R | 0.26 ± 0.05 *1, 2 | 0.26 ± 0.03 *1, 2R |

Comments: key cf. table 6.7.

**Table 6.9.** Results of immunological assays for determination of metabolic activity and killing activity of blood phagocytes and macrophages of the rainbow trout covered by the investigations in the spring of 2012 (means ± SD)

| Origin of fish | | Determined parameters | | | |
|---|---|---|---|---|---|
| fish farm | group | RBA f | RBA m | PKA f | PKA m |
| 1-OS | S | 0.45 ± 0.04 | 0.43 ± 0.05 | 0.36 ± 0.05 | 0.35 ± 0.05 |
| | B | 0.43 ± 0.04 | 0.42 ± 0.05 | 0.34 ± 0.05 | 0.33 ± 0.05 |
| 2-OS | S | 0.45 ± 0.05 | 0.38 ± 0.05 | 0.34 ± 0.04 | 0.31 ± 0.03 |
| | B | 0.46 ± 0.05 | 0.41 ± 0.03 | 0.35 ± 0.04 | 0.33 ± 0.05 |
| 3-OS | S | 0.39 ± 0.04 *1, 2 | 0.37 ± 0.04 *1 | 0.32 ± 0.05 | 0.31 ± 0.04 |
| | B | 0.41 ± 0.03 | 0.35 ± 0.04 *1 | 0.30 ± 0.04 | 0.30 ± 0.05 |
| 1-RAS | S | 0.39 ± 0.04 | 0.36 ± 0.05 *1 | 0.31 ± 0.05 | 0.30 ± 0.05 |
| | B | 0.42 ± 0.04 | 0.38 ± 0.04 | 0.33 ± 0.05 | 0.31 ± 0.05 |
| 2-RAS | S | 0.39 ± 0.05 *1, 2 | 0.32 ± 0.05 *1, 2 | 0.30 ± 0.05 *1 | 0.31 ± 0.03 |
| | B | 0.37 ± 0.05 *1, 2 | 0.31 ± 0.03 *1, 2 | 0.29 ± 0.05 *1 | 0.30 ± 0.04 |
| 3-RAS | S | 0.36 ± 0.04 *1, 2 | 0.35 ± 0.04 *1 | 0.28 ± 0.05 *1, 2 | 0.28 ± 0.04 *1 |
| | B | 0.37 ± 0.05 *1, 2 | 0.36 ± 0.03 *1 | 0.29 ± 0.05 *1, 2 | 0.30 ± 0.05 |

Comments: key cf. table 6.7.

The results of immunological assays on activity of lymphocytes isolated from the spleen and stimulated with mitogens: concanavalin A and lipopolysaccharide as well as the activity of lysozyme and ceruloplasmin in the serum of the fish examined in the different seasons of 2011 and 2012 are presented in tables 6.10–6.12.

**Table 10.** Results of immunological assays for determination of activity of lymphcoytes stimulated with mitogens: concanavalin A and lipopolysaccharide and the activity of lisozyme and ceruloplasmin in the rainbow trout covered by the investigations in the spring of 2011 (means ± SD)

| Origin of fish | | Determined parameters | | | |
|---|---|---|---|---|---|
| fish farm | group | LyP ConA | LyP LPS | LZM | Cp |
| 1-OS | S | 0.48 ± 0.05 | 0.30 ± 0.05 | 19.5 ± 2.5 | 75.5 ± 5.0 |
| | B | 0.49 ± 0.05 | 0.32 ± 0.04 | 20.5 ± 3.0 | 77.0 ± 4.5 |
| 2-OS | S | 0.47 ± 0.05 | 0. 27 ± 0.04 | 18.5 ± 3.5 | 72.4 ± 4.8 |
| | B | 0.45 ± 0.05 | 0. 30 ± 0.05 | 16.5 ± 4.0 | 75.5 ± 4.5 |

| | | | | | |
|---|---|---|---|---|---|
| 3-OS | S | 0.38 ± 0.03 *1, 2, 1R | 0. 23 ± 0.03 *1, 2 | 10.5 ± 2.5 *1, 2, 1R, 2R | 85.5 ± 3.5 *1, 2, 1R, 2R |
| | B | 0.39 ± 0.04 *1, 2, 1R | 0. 26 ± 0.04 *1, 2 | 11.5 ± 2.0 *1, 2, 1R, 2R | 84.5 ± 3.5 *1, 2, 1R, 2R |
| 1-RAS | S | 0.45 ± 0.04 | 0. 30 ± 0.04 | 20.5 ± 6.5 | 75.0 ± 6.0 |
| | B | 0.44 ± 0.03 | 0. 29 ± 0.05 | 19.5 ± 5.0 | 78.0 ± 5.5 |
| 2-RAS | S | 0.43 ± 0.05 | 0. 28 ± 0.04 | 18.5 ± 6.5 | 75.0 ± 6.0 |
| | B | 0.44 ± 0.05 | 0. 30 ± 0.05 | 19.0 ± 5.0 | 78.0 ± 5.5 |
| 3-RAS | S | 0.35 ± 0.05 *1, 2, 1R, 2R | 0. 21 ± 0. 03*1, 2, 1R, 2R | 9.5 ± 1.5 *1, 2, 1R, 2R | 83.5 ± 3.0 *1, 2, 1R, 2R |
| | B | 0.33 ± 0.05 *1, 2, 1R, 2R | 0. 23 ± 0.04 *1, 2, 1R, 2R | 10.5 ± 1.5 *1, 2, 1R, 2R | 84.0 ± 2.5 *1, 2, 1R, 2R |

Comments: LyP ConA – proliferative activity of T lymphocytes stimulated by ConA (OD 620 nm), LyP LPS – proliferative activity of B lymphocytes stimulated by LPS (OD 620 nm), LZM – activity of lisozyme in serum (mg/l), Cp – activity of ceruloplasmin in serum (IU); * – differences statistically significant at $P < 0.05$, relative in 1 (1-OS), 2 (2-OS), 1R (1-RAS), 2R (2-RAS).

**Table 6.11.** Results of immunological assays for determination of activity of lymphcoytes stimulated with mitogens: concanavalin A and lipopolysaccharide and the activity of lisozyme and ceruloplasmin in the rainbow trout covered by the investigations in the autumn of 2011 (means ± SD)

| Origin of fish | | Determiend parameters | | | |
|---|---|---|---|---|---|
| fish farm | group | LyP ConA | LyP LPS | LZM | Cp |
| 1-OS | S | 0.47 ± 0.05 | 0.31 ± 0.05 | 22.5 ± 4.5 | 72.5 ± 10.0 |
| | B | 0.48 ± 0.05 | 0.30 ± 0.05 | 21.5 ± 3.5 | 75.0 ± 10.5 |
| 2-OS | S | 0.45 ± 0.06 | 0.29 ± 0.05 | 20.7 ± 3.4 | 73.2 ± 8.7 |
| | B | 0.47 ± 0.05 | 0.31 ± 0.04 | 22.0 ± 2.7 | 76.0 ± 8.2 |
| 3-OS | S | 0.41 ± 0.04 *1 | 0.26 ± 0.04 *1 | 15.0 ± 3.8 *1, 2 | 69.5 ± 8.0 |
| | B | 0.38 ± 0.05 *1, 2, 1R, 2R | 0.27 ± 0.03 *1, 2 | 16.8 ± 3.6 *1, 2 | 68.5 ± 5.8 |
| 1-RAS | S | 0.44 ± 0.05 | 0.30 ± 0.04 | 22.3 ± 5.3 | 75.1 ± 8.5 |
| | B | 0.43 ± 0.05 | 0.29 ± 0.04 | 22.1 ± 6.1 | 75.4 ± 9.3 |
| 2-RAS | S | 0.48 ± 0.05 | 0.30 ± 0.05 | 21.7 ± 5.4 | 70.5 ± 6.2 |
| | B | 0.49 ± 0.05 | 0.31 ± 0.06 | 20.3 ± 4.0 | 77.2 ± 8.6 |
| 3-RAS | S | 0.40 ± 0.05 *1 | 0.26 ± 0.04 *1 | 13.6 ± 3.8 *1, 2 | 70.1± 6.4 |
| | B | 0.41 ± 0.05 *1, 2, 1R, 2R | 0.25 ± 0.04 *1, 2 | 15.0 ± 3.2 *1, 2 | 69.8 ± 7.4 |

Comments: key cf. table 6.10.

**Table 6.12.** Results of immunological assays for determination of activity of lymphcoytes stimulated with mitogens: concanavalin A and lipopolysaccharide and the activity of lisozyme and ceruloplasmin in the rainbow trout covered by the investigations in the spring of 2012 (means ± SD)

| Origin of fish | | Determined parameters | | | |
|---|---|---|---|---|---|
| fish farm | group | LyP ConA | LyP LPS | LZM | Cp |
| 1-OS | S | 0.47 ± 0.05 | 0.35 ± 0.05 | 20.9 ± 4.7 | 81.1 ± 6.3 |
| | B | 0.45 ± 0.05 | 0.34 ± 0.05 | 20.5 ± 5.7 | 76.2 ± 4.2 |
| 2-OS | S | 0.46 ± 0.05 | 0.32 ± 0.04 | 20.1 ± 4.7 | 81.4 ± 7.1 |
| | B | 0.45 ± 0.04 | 0.34 ± 0.05 | 20.2 ± 7.3 | 77.5 ± 8.3 |
| 3-OS | S | 0.43 ± 0.05 | 0.34 ± 0.05 | 20.9 ± 7.9 | 85.7 ± 10.3 |
| | B | 0.42 ± 0.05 | 0.32 ± 0.04 | 19.0 ± 5.8 | 80.5 ± 13.0 |
| 1-RAS | S | 0.43 ± 0.05 | 0.34 ± 0.05 | 19.2 ± 5.4 | 75.1 ± 8.9 |
| | B | 0.44 ± 0.05 | 0.35 ± 0.05 | 20.9 ± 3.2 | 82.0 ± 9.6 |

| | | | | | |
|---|---|---|---|---|---|
| 1-RAS | S | 0.42 ± 0.05 | 0.34 ± 0.05 | 19.4 ± 8.7 | 76.7 ± 8.3 |
| | B | 0.43 ± 0.05 | 0.31 ± 0.05 | 20.9 ± 4.7 | 78.5 ± 7.7 |
| 1-RAS | S | 0.42 ± 0.05 | 0.32 ± 0.05 | 20.5 ± 4.8 | 80.1± 9.4 |
| | B | 0.44 ± 0.05 | 0.31 ± 0.05 | 21.4 ± 3.5 | 78.9 ± 10.9 |

Comments: key cf. table 6.10.

The results of immunological assays on levels of total protein and immunoglobulins in the rainbow trout individuals covered by the tests conducted in the spring and autumn of 2011 and in the spring of 2012 are presented in tables 6.13–6.15.

**Table 6.13.** Results of immunological assays for determination of total protein and immunoglobulins in rainbow trout covered by the investigations in the spring of 2011 (means ± SD)

| Origin of fish | | Determined parameters | |
|---|---|---|---|
| fish farm | group | Bc | Ig |
| 1-OS | S | 54.5 ± 4.5 | 14.5 ± 2.5 |
| | B | 56.0 ± 4.0 | 15.8 ± 2.8 |
| 2-OS | S | 52.5 ± 3.5 | 14.8 ± 2.4 |
| | B | 53.5 ± 3.0 | 14.5 ± 2.5 |
| 3-OS | S | 52.5 ± 2.5 | 18.5 ± 6.5 |
| | B | 54.5 ± 4.0 | 18.0 ± 5.5 |
| 1-RAS | S | 55.8 ± 4.2 | 18.5 ± 5.5 |
| | B | 56.0 ± 3.0 | 19.6 ± 6.4 |
| 2-RAS | S | 55.5 ± 3.5 | 19.5 ± 7.5 |
| | B | 52.0 ± 4.0 | 19.0 ± 6.0 |
| 3-RAS | S | 51.5 ± 3.5 | 17.5 ± 4.5 |
| | B | 52.5 ± 3.0 | 18.0 ± 5.0 |

Comments: Bc – level of total protein in serum (g/L), Ig – total level of immunoglobulins in serum (g/L); * differences statistically significant at $P < 0.05$.

**Table 6.14.** Results of immunological assays for determination of total protein and immunoglobulins in rainbow trout covered by the investigations in the autumn of 2011 (means ± SD)

| Origin of fish | | Determined parameters | |
|---|---|---|---|
| fish farm | group | Bc | Ig |
| 1-OS | S | 52.5 ± 5.5 | 11.4 ± 2.8 |
| | B | 54.0 ± 6.0 | 12.5 ± 2.5 |
| 2-OS | S | 54.4 ± 5.2 | 12.9 ± 3.1 |
| | B | 51.8 ± 4.8 | 12.5 ± 6.4 |
| 3-OS | S | 42.7 ± 4. 2 *1, 2, 2R | 14.9 ± 4.5 |
| | B | 41.6 ± 5.8 *1, 2, 1R, 2R | 15.0 ± 5.7 |
| 1-RAS | S | 48.9 ± 7.7 | 11.4 ± 6.0 |
| | B | 50.1 ± 3.2 | 14.4 ± 6.4 |
| 2-RAS | S | 48.2 ± 3.9 | 12.0 ± 2.1 |
| | B | 47.3 ± 4.4 | 11.1 ± 2.7 |
| 3-RAS | S | 43.7 ± 6.8 *1, 2 | 11.4 ± 2.3 |
| | B | 42.1 ± 5.3 *1, 2, 1R | 10.4 ± 2.5 |

Comments: key cf. table 6.13; * – differences statistically significant at $P < 0.05$, relative in 1 (1-OS), 2 (2-OS), 1R (1-RAS), 2R (2-RAS).

**Table 6.15.** Results of immunological assays for determination of total protein and immunoglobulins in rainbow trout covered by the investigations in the spring of 2012 (means ± SD)

| Origin of fish | | Determined parameters | |
|---|---|---|---|
| fish farm | group | Bc | Ig |
| 1-OS | S | 43.6 ± 7.4 | 10.6 ± 2.6 |
| | B | 44.5 ± 6.1 | 10.3 ± 2.3 |
| 2-OS | S | 44.6 ± 5.0 | 10.2 ± 3.1 |
| | B | 46.9 ± 5.0 | 9.8 ± 2.8 |
| 3-OS | S | 45.1 ± 4.5 | 11.6 ± 3.4 |
| | B | 47.0 ± 9.5 | 11.4 ± 3.0 |
| 1-RAS | S | 42.5 ± 4.3 | 11.6 ± 1.6 |
| | B | 43.5 ± 6.3 | 10.7 ± 2.9 |
| 2-RAS | S | 44.3 ± 6.4 | 10.5 ± 2.7 |
| | B | 44.8 ± 5.4 | 10.4 ± 2.9 |
| 3-RAS | S | 50. 0 ± 6.9 | 9.6 ± 2.1 |
| | B | 50.3 ± 4.9 | 9.3 ± 2.8 |

Comments: key cf. table 6.13.

Our preliminary analysis of the results of immunological assays from the two periods of a breeding season evidently suggests that increased activity of non-specific cellular defence mechanisms occurred during the second cycle of a rearing season (October–November) in all the fish from the three OS farms and three RAS farms. Simultaneously, an increase is noticed in the activity of lysozyme as well as a decrease in the level of gamma-globulins (Ig) in blood serum of all the fish originating from the examined farms. This development is indicative of a depressed level of the negative polyetiological (environmental) stress on fish. Higher activity of immunocompetent cells, which are responsible for anti-infective resistance, is a very positive event, observed at both OS and RAS farms. The highest cellular and humoral resistance level was determined at 1-OS farm during all the periods of analyses in both 2011 and 2012. At the same time, a statistically significant decrease in the activity of cellular defence mechanisms in S and B fish was recorded at two farms (3-OS and 3-RAS), where the presence of IPN virus was detected in both periods of investigations in 2011, in contrast to the other farms, where this virus was never found. The fish from 3-OS and 3-RAS farms were also determined to have a depressed activity of lysozyme in the two examination periods in 2011. The research results clearly suggest that the IPN virus produced strong influence, depressing the activity of blood macrophages and T and B lymphocytes, which causes the observed depression in the activity of lysozyme. In contrast, in the spring of 2011, a statistically significant increase in the activity of ceruloplasmin, an acute phase protein, was noticed, which is an evident manifestation of the activation of hepatocytes due to viral infection. The levels of total protein and Ig were similar in the fish from both OS and RAS farms. However, a statistically significant decrease in total protein was recorded during the autumn assays on fish from 3-OS and 3-RAS farms, where the presence of the IPN virus was evidenced.

In conclusion, it should be stated that no significant differences in the parameters of non-specific cellular and humoral resistance in rainbow trout were noticed due to the different aquaculture system, i.e. OS and RAS. The high anti-infective potential determined in all the fish did not allow for manifestation of disease symptoms in fish individuals. The occurrence of the IPN virus proven through this research enabled us to make an objective assessment of its effects on the cellular ad humoral defence mechanisms in fish at farms equipped with different aquaculture systems. This finding is of much cognitive and practical importance and it allows researchers to develop effective methods for prevention of diseases.

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# 7. Macroscopic and microscopic evaluation of the liver, spleen and kidneys in rainbow trout

## 7.1. Introduction

Increase in growing and breeding of the rainbow trout using traditional methods (increase of water utilization) is difficult, and even unreal, to implement in the light of current regulations. Trout breeders are more and more often using techniques of water treatment, such as aeration, oxygenation, sediments separation, biological treatment (Ciereszko et al 2007; Goryczko et al. 2003; Molony et al. 1999), as well as partial (semi RAS), or full water recirculation (RAS) enabling re-use of the spent water.

Unfortunately, these modern technological processes are lacking the full scientific evaluation making possible their acceptation by bioethicists and promoters of the "ecological" food. The effect of this situation is the EC directive 98/58 "Minimal standards of protection of animals grown and kept for commercial reasons, including fish" and the European Council recommendation for fish welfare. In Poland, as in other countries of EU, the program of sustained development of rural areas is being implemented, which includes aspects of aquaculture. This program is putting some stress on the promoting and supporting the development of ecological production.

In the light of the described aims of fish growing and breeding, including rainbow trout, the Regulation of the Commission 710/2009 was issued on 5 August 2009 which established the detailed regulations of the ecological production in the aquaculture sector. According to this regulation the application of such systems (water recirculation systems) is prohibited in the ecological production until the sufficient knowledge is collected.

## 7.2. Morphological studies as a tool for assessment of the influence of the rainbow trout production technology on the quality of fish

The presented results of the morphological studies are collected to obtain the detailed knowledge about the influence of the environment on the organism of the rainbow trout, as well as the final product being the outcome of both the extensive rearing in the water free-flow system, as well as the intensive growing in the water recirculation (RAS) system.

The pathomorphological methods used in the present study are aimed at the diagnostics of morphological changes both in fish and in other animals. They allow to detect even subtle disturbances in the organism and, in the case of animal death, to learn about the cause of death (Barlas 1999; Cengiz 2006; Fischer et al. 2000; Peyghan et al. 2002). It must be mentioned that it is not widely known that the morphological studies are becoming the tool for the assessment of the environmental influence on the vital processes of animals, including fish (Ayas et al. 2007; Gul et al. 2004; Ozmen et al. 2006; Sopińska et al. 2000; Szarek et al. 2007).

The presented study, utilizing the mentioned research techniques, was designed as the innovative attempt to assess the influence of two different technologies of the rainbow trout growing on the morphology of the liver, spleen and kidneys, reflecting the overall condition and health status of these fish.

## 7.3. Materials and methods

The morphological studies of the rainbow trout were conducted for 3 years, twice a year, in spring and autumn. The morphological examinations were done on 40 specimens from each fish catch from all six fish farms. The animals were every time divided into two groups according to the body mass: 350–500 g ($n = 20$) and 501–850 g ($n = 20$). The specimens were collected in six fish farms, i. e. in 3 farms using the free-flow water system (described as OS) and in 3 farms using water recirculation (denoted as RAS). The detailed description of growing technology is to be found in the chapter "Influence of growing technology on the water quality". The specimens were always collected in the particular farm during one day, after one-day fasting.

The macroscopic examination consisted of visual inspection with the special attention paid to the scales behind eyes, eyes themselves, gills and fins, as well as the internal organs.

For microscopic examination samples from each liver were taken from five sites. Specimens from the spleen and kidneys were also collected. Histological sections were stained with hematoxylin/eosin (Bancroft et al. 2000). Additionally, sections from the liver were stained with PAS method to assess the content and distribution of polysaccharides (Bancroft et al. 2000). The content of polysaccharides was evaluated taking into consideration recommendations by Pearse (1968) and the semiquantitative method described by Szarek et al. (1985).

Results of macroscopic and microscopic observations are collected in Tables 7.1–7.10. For all examined parameters the basic descriptive statistics are shown. The collected data were statistically analyzed using U-Mann-Whitney and Friedman's ANOVA (nonparametric analysis of variance) tests. U-Mann-Whitney test was used to determine whether the observed differences are statistically significant and whether they depend on the rearing system. Friedman's test is a non-parametric equivalent of a one-way analysis of variance for repetitive measurements. It was used when, besides the rearing system, another parameter was introduced making possible to differentiate the results of microscopic analyses. The differentiating variable in the present study was the rearing system. In case of the statistically significant variability additional variables were used describing the size of fish (S and B) and the sampling period (spring and autumn).

## 7.4. Results of studies

### 7.4.1. Results of macroscopic morphological studies

It was found that the macroscopic pattern was normal in virtually all fish. Abnormalities were found comparatively seldom. This conclusion regards both the variability associated with the period of sampling (spring, autumn) and with body size (S, B). The character and frequency of macroscopic lesions is

illustrated with an example showing results from trout's coming from different growing systems in autumn sampling (Table 7.1). The results of statistical analysis are shown in Table 7.2.

**Table 7.1.** The collective data on the number of macroscopic changes in 160 rainbow trouts* collected for studies in autumn from four fish farms

| Localization and kind of macroscopical lesions | OS | | | | RAS | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 | | 3 | | 2 | | 3 | |
| | S | B | S | B | S | B | S | B |
| Skin and scales – mechanical damage | 0 | 2 | 1 | 1 | 1 | 2 | 0 | 0 |
| Skin and scales – other changes | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Fins – mechanical damage | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Fins – other changes | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Circulatory disturbances | 0 | 0 | 1 | 2 | 0 | 2 | 0 | 1 |
| Eye | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Liver – circulatory disturbances | 1 | 1 | 2 | 1 | 1 | 2 | 1 | 1 |
| Liver – retrogressive changes | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| Spleen – developmental malformations | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Spleen – circulatory disturbances | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Other changes | 0 | | 0 | 0 | 0 | 0 | 0 | 0 |
| Total – body mass/rearing technology/fish farm | 4 | 5 | 5 | 7 | 4 | 10 | 4 | 6 |
| Total – rearing technology | 21 | | | | 24 | | | |

Comments: * changes concern 20 trouts in each weight group (S and B).

**Table 7.2.** The results of U-Mann-Whitney test for the studied parameters of macroscopic assessment

| Localization and kind of macroscopical lesions | Sum. rang | | *U* | *Z* | *p* |
|---|---|---|---|---|---|
| | OS | RAS | | | |
| Skin and scales – mechanical damage | 19.50000 | 16.50000 | 6.500000 | 0.28868 | 0.772830 |
| Skin and scales – other changes | 14.00000 | 22.00000 | 4.000000 | -1.01036 | 0.312322 |
| Fins – mechanical damage | 18.00000 | 18.00000 | 8.000000 | 0.14434 | 0.885234 |
| Fins – other changes | 18.00000 | 18.00000 | 8.000000 | 0.14434 | 0.885234 |
| Gills – circulatory disturbances | 18.00000 | 18.00000 | 8.000000 | 0.14434 | 0.885234 |
| Eye | 18.00000 | 18.00000 | 8.000000 | 0.14434 | 0.885234 |
| Liver – circulatory disturbances | 18.00000 | 18.00000 | 8.000000 | 0.14434 | 0.885234 |
| Liver – retrogressive changes | 14.00000 | 22.00000 | 4.000000 | -1.01036 | 0.312322 |
| Spleen – developmental malformations | 18.00000 | 18.00000 | 8.000000 | 0.14434 | 0.885234 |
| Spleen – circulatory disturbances | 18.00000 | 18.00000 | 8.000000 | 0.14434 | 0.885234 |
| Other changes | 12.00000 | 16.00000 | 6.000000 | 0.17678 | 0.859684 |
| Total – body mass/rearing system | 18.00000 | 18.00000 | 8.000000 | 0.14434 | 0.885234 |

The results of the U-Mann-Whitney test confirmed the hypothesis of the lack of the statistically significant variability regarding the observed macroscopic changes. In the light of this we didn't find any

statistically significant differences associated with the studied rearing systems. It was found that in all trouts pathognomonic changes indicating the occurrence of diseases were absent. At this stage no further analyses were done. It was accepted that in both rearing systems similar macroscopic changes occur.

The skin and scales in examined fish were of sustained integrity and only sporadically, and in restricted areas, lesions were seen. The injuries to skin and scales were comparatively more frequent in the fish from RAS system (Fig. 7.1). These changes were more intensely pronounced in this technological system. The fin injuries were seen twice as seldom as skin and scale lesions. The pattern of eyeballs was normal for trouts. In gills congestion was seen in several cases and extravasations were seen sporadically.

The congestion was seen most often in the liver, less often in the spleen and it was rarest in kidneys. In some individuals small point-like petechiae, and/or ecchymotic extravasations were present under the serous membrane. The described macroscopic circulatory disturbances were regarded being sporadic alterations, however, they were seen more frequently than other lesions.

In several trouts the steatosis of the liver was found. In these animals the organ was brown with different amount of a yellow tint. Such organ was showing decreased tenderness and sometimes was of a clay-like consistency. This change, of a physiological character, was more frequent in B-size trouts (body mass 501–800 g) than in S-size ones (body mass 350–500 g). It was also more often seen in the fish coming from the systems with high degree of water recirculation (semi RAS), or closed-circuit systems (RAS). Sporadically, the parenchymatous degeneration of the liver was found, however, this lesion was usually weakly, or very weakly pronounced.

Sometimes alterations in spleen shape were visible and sometimes they were of a character of developmental malformation.

The variability of macroscopic lesions shown in Table 7.1 in relation to the body mass (S and B) and growing technology is shown in Fig. 7.2.

Macroscopic lesions characteristic for trouts examined are shown in Phots. 7.1–7.5.

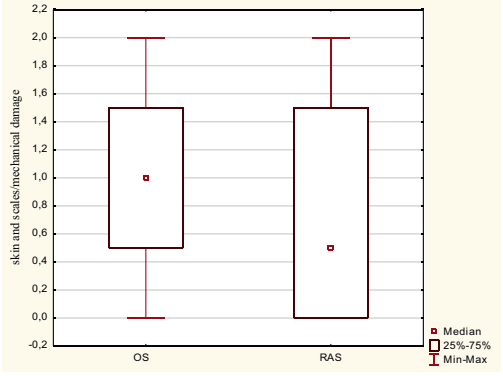


Fig. 1. Mechanical damage to skin and scales in rainbow trouts in relation to rearing technology (OS, RAS)

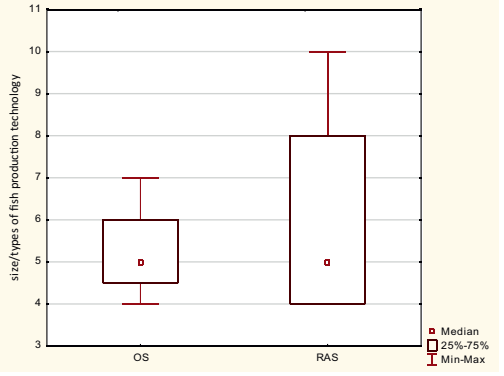


Fig. 2. Macroscopic changes in rainbow trouts in relation to body mass (S and B) and growing technology (OS, RAS)

### 7.4.2. Results of microscopic and histochemical examinations

#### 7.4.2.1. Results of microscopic examination of the liver

The microscopic picture of the liver of the rainbow trouts, both of size S and B, independently from the season of sampling, was usually concordant with the accepted criteria (Phot. 7.6). The character and

the frequency of microscopic lesions is presented using an example of the results from rainbow trouts reared in OS and RAS technology coming from the autumn sampling in 4 fish farms (Table 7.3). The statistical analysis of the results is shown in Table 7.4. The results of the U-Mann-Whitney test confirmed the hypothesis of the absence of statistically significant variability of the liver microscopic lesions. It was accepted that in both rearing technologies similar microscopic lesions occur. Because the statistically significant differences were detected regarding the rearing technology/fish farm parameter additional analysis was conducted using the type of the fish farm as a criterion (Fig. 7.3). The performed non-parametric analysis of variance (Kruskall–Wallis test) allowed to confirm the conclusion drawn on the basis of the former test that there is no statistically significant variability of the observed microscopic liver lesions associated with the rearing technology: Kruskall-Wallis test: $H$ (3, $N$ = 8) = 6.533333; $p$ = 0.0884.

**Table 7.3.** The frequency of selected microscopical changes in the liver of 160 rainbow trouts* sampled in autumn from four fish farms

| Kind of macroscopic lesion | OS | | | | RAS | | | |
|---|---|---|---|---|---|---|---|---|
| | 1-OS | | 2-OS | | 1-RAS | | 2-RAS | |
| | S | B | S | B | S | B | S | B |
| Necrosis | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Adipose degeneration | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 |
| Parenchymatous degeneration | 2 | 4 | 2 | 3 | 2 | 4 | 4 | 5 |
| Congestion | 3 | 5 | 3 | 4 | 3 | 6 | 3 | 5 |
| Extravasation | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| Melanomacrophages infiltration** | 2 | 3 | 1 | 2 | 2 | 2 | 1 | 3 |
| Lymphoid cell infiltration | 1 | 1 | 1 | 3 | 2 | 3 | 2 | 3 |
| Total – body mass/rearing technology | 9 | 15 | 7 | 14 | 10 | 17 | 12 | 19 |
| Total – rearing technology /fish farm | 24 | | 21 | | 27 | | 31 | |
| Total – rearing technology | 45 (22.5 – mean for one farm) | | | | 58 (29.0 – mean for one farm) | | | |

Comments: * given changes regard 20 rainbow trout's in each group; ** assessment in the view field using objective 20x (Nikon Eclipse 80i microscope). Number of analysed slides: 1 liver = 5 samples (parafin blocks), 1 section = 1 slide hematoxylin and eosin stained, 20 trouts in S group ($n$ = 20) and 20 trouts in B group ($n$ = 20) = 40 trouts × 5 sections = 200 sections × 1 slide = 200 slides from fish with place of origin × 4 fish farms = 800 slides.

**Table 7.4.** Results of the U–Manna-Whitneya test for examined parameters of the microscopic evaluation of the liver (statistical significant value in red color)

| Kind of macroscopic lesion | Sum. rang | | $U$ | $Z$ | $p$ | $Z$ corected | $p$ |
|---|---|---|---|---|---|---|---|
| | OS | RAS | | | | | |
| Necrosis | 20.00000 | 16.00000 | 6.000000 | 0.43301 | 0.665006 | 0.51235 | 0.608408 |
| Adipose degeneration | 14.00000 | 22.00000 | 4.000000 | -1.01036 | 0.312322 | -1.15752 | 0.247062 |
| Parenchymatous degeneration | 14.00000 | 22.00000 | 4.000000 | -1.01036 | 0.312322 | -1.06221 | 0.288141 |
| Congestion | 16.50000 | 19.50000 | 6.500000 | -0.28868 | 0.772830 | -0.30966 | 0.756818 |

| Kind of macroscopic lesion | Sum. rang | | *U* | *Z* | *p* | *Z* corected | *p* |
|---|---|---|---|---|---|---|---|
| | OS | RAS | | | | | |
| Extravasation | 14.00000 | 22.00000 | 4.000000 | -1.01036 | 0.312322 | -1.33658 | 0.181359 |
| Melanomacrophages infiltration | 18.00000 | 18.00000 | 8.000000 | 0.14434 | 0.885234 | 0.15590 | 0.876110 |
| Lymphoid cell infiltration | 13.00000 | 23.00000 | 3.000000 | -1.29904 | 0.193932 | -1.37477 | 0.169203 |
| Total – body mass / rearing technology | 14.00000 | 22.00000 | 4.000000 | -1.01036 | 0.312322 | -1.01036 | 0.312322 |
| Total – rearing technology / fish farm | 10.00000 | 26.00000 | 0.000000 | -2.16506 | 0.030384 | -2.21853 | 0.026519 |

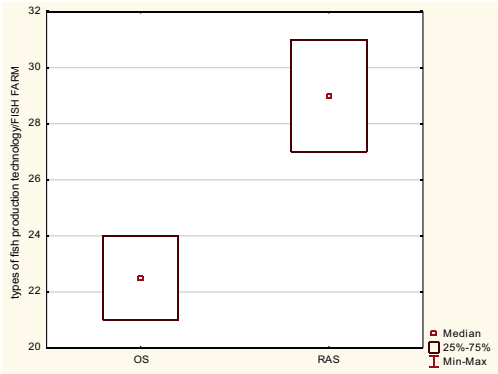


**Fig. 3.** Microscopic lesions in the liver of the rainbow trouts in relation to the fish farm and rearing technology.

Among the observed degenerative changes the hepatocyte necrosis was observed comparatively seldom. In such cases it was affecting single cells (Phot. 7.7). Only sporadically the necrosis was affecting small cell clusters. The necrosis was slightly more frequent in the fish of size B. The same tendency was observed regarding the parenchymatous degeneration. This kind of change was rare in younger fish and more frequent in the older ones, especially in RAS systems.

Among the circulatory disturbances the congestion was the most frequent one (Phot. 7.8). It is noteworthy to mention that it was observed more often than any of the regressive changes. The liver congestion was observed in 3 to 5 individuals from every size group. Sometimes the passive haemostasis was observed. Small extravasations, mainly point-like petechiae, were seen only sporadically.

The variable degree of the simple steatosis (reversible change) was seen in all groups of trouts in many individuals (sometimes in more than half of the group) (Phots. 7.9, 7.10). Lipid droplets were of different size and they were packed in the hepatocytes cytoplasm pushing the nucleus to the periphery. Such hepatocytes were of a ring-like shape. The damage to the nucleus, and the consequent adipose degeneration was seen very seldom. It must be mentioned that the steatosis was detected more frequently in the fish of size B in the autumn in fish farms using the RAS technology.

In ca 5% of the studied trouts brown-black melenomacrophages were present in the liver (Phot. 7.11). Comparatively frequently that were forming clusters called "centers". Melanomacrophage centers were usually localized in the vicinity of blood vessels and bile ducts. They were more frequently observed in the fish coming from farms using RAS technology.

The similar frequency was seen in the case of lymphoid cell infiltration. Usually these infiltration was seen as clusters consisting of several cells (Phot. 7.12).

### 7.4.2.2. Results of the histochemical examination o the liver

In the cytoplasm of liver parenchymal cells variable amount of polysaccharides was observed. The detailed assessment of the polysaccharide level in the livers of trouts from two fish farms in two seasonal samplings is shown in the Tables 7.5 and 7.6. The collective data on the polysaccharide content in the liver of the rainbow trout's were presented using an example data encompassing two seasonal samplings in four fish farms (Tables 7.7 and 7.8). The numerical data (scores) were in the range 1–5. They concerned the semiquantitative assessment criteria by Szarek et al. (1985). Similarly to the presented example (Tables 7.5 and 7.6) the most common score observed during the whole study were grades 3 and 4. In such cases the polysaccharide levels were denoted as slightly higher and high (Phots. 7.13–7.15). The luminal scores were observed very seldom – then they were denoted as very low and very high. It shows that the disturbances in glycogen distribution were observed sporadically.

**Table 7.5.** Results of the semiquantitative evaluation of polysaccharides content (range 1–5)* in the liver of 80 rainbow trouts** sampled in the spring from two fish farms

| No. of fish | 2-OS | | 1-RAS | |
|---|---|---|---|---|
| | S | B | S | B |
| 1 | 3 | 5 | 4 | 5 |
| 2 | 5 | 4 | 5 | 3 |
| 3 | 1 | 5 | 4 | 5 |
| 4 | 3 | 4 | 5 | 4 |
| 5 | 3 | 4 | 1 | 5 |
| 6 | 5 | 5 | 5 | 5 |
| 7 | 2 | 4 | 5 | 4 |
| 8 | 4 | 2 | 4 | 5 |
| 9 | 5 | 4 | 5 | 4 |
| 10 | 4 | 4 | 3 | 1 |
| 11 | 5 | 4 | 5 | 5 |
| 12 | 3 | 1 | 4 | 4 |
| 13 | 3 | 4 | 3 | 4 |
| 14 | 4 | 5 | 5 | 5 |
| 15 | 3 | 4 | 4 | 5 |
| 16 | 5 | 4 | 3 | 4 |
| 17 | 3 | 4 | 5 | 5 |
| 18 | 3 | 4 | 2 | 5 |
| 19 | 4 | 4 | 5 | 5 |
| 20 | 5 | 5 | 4 | 5 |
| Total – body mass / rearing system / fish farm | 73 | 80 | 81 | 88 |
| Total – rearing system / fish farm | 153 | | 169 | |

Comments: * given scores regard 1 trout in each group; ** assessment in the view field using objective 20× (Nikon Eclipse 80i microscope). Number of analysed slides: 1 liver = 1 sample (block) = 1 slide, 20 fish in the group S ($n$ = 20) and 20 fish in the group B ($n$ = 20) = 40 fish × 1 sample = 40 samples × 1 slide = 40 slides from the fish from particular location × 2 fish farms = 80 slides – staining for polysaccharides.

**Table 7.6.** Results of the semiquantitative evaluation of polysaccharides content (range 1–5)* in the liver of 80 rainbow trouts* sampled in the autumn from two fish farms

| No. of fish | 2-OS | | 1-RAS | |
|---|---|---|---|---|
| | S | B | S | B |
| 1 | 3 | 4 | 5 | 3 |
| 2 | 5 | 5 | 4 | 5 |
| 3 | 4 | 5 | 3 | 4 |
| 4 | 2 | 5 | 4 | 5 |
| 5 | 5 | 4 | 5 | 4 |
| 6 | 4 | 3 | 2 | 5 |
| 7 | 3 | 5 | 5 | 4 |
| 8 | 5 | 2 | 4 | 5 |
| 9 | 5 | 4 | 3 | 3 |
| 10 | 4 | 5 | 4 | 5 |
| 11 | 5 | 4 | 4 | 5 |
| 12 | 4 | 1 | 5 | 3 |
| 13 | 3 | 4 | 4 | 5 |
| 14 | 4 | 5 | 3 | 4 |
| 15 | 3 | 5 | 4 | 5 |
| 16 | 3 | 4 | 3 | 4 |
| 17 | 3 | 5 | 4 | 5 |
| 18 | 3 | 5 | 4 | 5 |
| 19 | 5 | 3 | 4 | 5 |
| 20 | 4 | 5 | 4 | 3 |
| Total – body mass / rearing system / fish farm | 76 | 83 | 78 | 87 |
| Total – rearing system / fish farm | 159 | | 165 | |

Comments: markings as in Table 7.5.

**Table 7.7.** Results of the semiquantitative evaluation of polysaccharides content (range 1–5)* in the liver of 240 rainbow trouts** sampled in the spring from six fish farms

| Rearing parameters | OS | | | | | | RAS | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 1 | | 2 | | 3 | |
| | S | B | S | B | S | B | S | B | S | B | S | B |
| Body mass / rearing system / fish farm | 75 | 84 | 73 | 80 | 74 | 84 | 81 | 88 | 78 | 86 | 74 | 85 |
| Rearing system / fish farm | 159 | | 153 | | 158 | | 169 | | 164 | | 159 | |
| Rearing technology | 470 (156.67 – mean for fish farm) | | | | | | 492 (164.00 – mean for fish farm) | | | | | |

Comments: markings as in Table 7.5.

**Table 7.8.** Results of the semiquantitative evaluation of polysaccharides content (range 1–5)* in the liver of 240 rainbow trouts** sampled in the autumn from six fish farms

| Rearing parameters | OS | | | | | | RAS | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 1 | | 2 | | 3 | |
| | S | B | S | B | S | B | S | B | S | B | S | B |
| Body mass / rearing system / fish farm | 71 | 85 | 76 | 83 | 75 | 76 | 78 | 87 | 72 | 81 | 71 | 80 |
| Rearing system / fish farm | 156 | | 159 | | 151 | | 165 | | 153 | | 151 | |
| Rearing technology | 466 (155.34 – mean for fish farm) | | | | | | 469,00 (156.34 – mean for fish farm) | | | | | |

Comments: markings as in Table 7.5.

The statistical analysis done with the U-Mann-Whitney test in the presented examples confirmed the absence of the statistically significant differences as regards the polysaccharide levels. Because no statistically significant differences were related to the studied rearing technologies no further analyses were performed and it was assumed that the similar polysaccharide contents occur in both rearing technologies of the rainbow trout. However, the Friedman's non-parametric variance analysis (ANOVA) was used to check whether the statistically significant differences between spring and autumn sampling could be seen. The results of the test (Chi-square ANOVA ($N = 4$, $df = 39$) = 38.53424; $p = 0.49095$) allow to confirm the assumption of the absence of variability associated with the sampling season.

Nevertheless, the tendency for more intense storage of the polysaccharides in the liver in the fish farms 2-OS and 1-RAS could be noticed. On the other hand, in the liver of the fish coming from the fish farms 1-OS and 1-RAS the level of the above-mentioned compounds was similar, but lower than in trouts from the fish farm 2-OS. The liver of the fish from the farms 3-OS and 3-RAS contained lower amounts of polysaccharides in comparison to the above-mentioned, but it was slightly higher than in the fish from 2-RAS. Analogical relations were seen in the liver of the body mass groups S and B. Additionally, it was noticed that in the fish sampled in the spring the liver contained higher amount of polysaccharides (Phot. 7.15).

#### 7.4.2.3. Microscopic examination of the spleen

In the spleen, in comparison to other studied organs, microscopic lesions were observed comparatively seldom (Table 7.9). The organ displayed normal microscopic appearance in the majority of the studied rainbow trouts (Phot. 7.16). The changes were observed more often in the rainbow trouts reared using RAS system than in rainbow trouts grown in the OS system. The difference in the frequency of lesions sometimes reached 20%.

The higher intensity of changes could be also seen in the fish grown in the intensive rearing system. Especially clear increase in the number of melanomacrophages could be seen in the fish grown in RAS system in comparison to those grown in OS technology (Phot. 7.17).

Among other morphological lesions circulation disturbances could be seen comparatively often. The most frequent change was hyperaemia, then small extravasations and the hemostasis was detected only sporadically.

The number of observed microscopic lesions in the studied fish was small – in 40 examined fish it was in the range 0–2. The trouts of B size displayed more of such changes than those of S size.

#### 7.4.2.4. Microscopic examination of the kidney

##### 7.4.2.4.1. Microscopic examination of the anterior kidney

The anterior ("head") kidney, being a hematopoietic organ, displayed a reticulo-endothelial structure. In the mesh of this organ blood cells in different stages of maturation were seen (Phot. 7.18). The high number of erythrocytes, leukocytes and heamoglobin crystals could be seen outside the blood vessels. The features described indicate that the microscopic pattern of the anterior kidney was normal and it was predominant in the rainbow trout's studied in all groups and sampling seasons.

Among scarce histological lesions the congestion was comparatively predominant (Table 7.10, Phot. 7.19) and other circulatory changes were sporadic.

Melanomacrophages were present in every section of the organ studied (Phots. 7.18–7.20). They were more frequently observed in the anterior kidney of the trouts sampled from the intensive system (RAS) than in those from OS system. They were most often forming small "centers" consisting of a few cells (Phot. 7.20).

Other changes were seen only sporadically.

**Table 7.9.** The frequency of the selected microscopic changes in the spleen of 160 rainbow trouts* sampled in autumn from four fish farms

| Kind of microscopic change | OS | | | | RAS | | | |
|---|---|---|---|---|---|---|---|---|
| | 2-OS | | 3-OS | | 1-RAS | | 2-RAS | |
| | S | B | S | B | S | B | S | B |
| Necrosis | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Parenchymatous degeneration | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hyperaemia | 3 | 4 | 4 | 5 | 3 | 5 | 4 | 5 |
| Extravasations | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| Melanomacrophage infiltration** | 5 | 7 | 6 | 7 | 6 | 6 | 6 | 7 |
| Total – body mass / rearing system | 8 | 12 | 10 | 14 | 10 | 13 | 11 | 14 |
| Total – rearing system / fish farm | 20 | | 24 | | 23 | | 25 | |
| Total – rearing technology | 44 (22.0 – mean for one fish farm) | | | | 57 (28.5 – mean for one fish farm) | | | |

Comments: * given changes regard 20 rainbow trouts in each group; ** in the viewing field under the objective 20× (Nikon Eclipse 80i microscope). The number of analysed preparations: 1 spleen = 1 sample, 1 sample = 1 slide stained with HE, 20 trouts in the group S ($n = 20$) and 20 trouts in the group B ($n = 20$) = 40 trouts × 1 sample = 40 samples × 1 slide = 40 slides from the fish from the particular location × 4 fish farms = 160 slides.

##### 7.4.2.4.2. Microscopic examination of the posterior kidney

The microscopic pattern of the posterior kidney of the rainbow trouts of both size B and S, independently from the sampling season, was concordant with the morphologic criteria accepted for this organ (Phot. 7.21).

The microscopic lesions observed in the discussed organ were 30% more frequent than in the anterior kidney (Table 7.10). Among the regressive changes, which were altogether infrequent, the degeneration of the cells of the renal tubules was seen most often (Phot. 7.22), and the necrosis affecting very restricted

areas was seen only sporadically. The congestion was seen much more frequently and extravasations were less frequent (Phot. 7.23). Usually they were seen as point-like petechiae or small ecchymoses.

Of all the studied organs of the rainbow trout the posterior kidney was the one most often affected by the melanomacrophages infiltration. They were observed usually as vast gatherings forming "centers" located most frequently in the vicinity of blood vessels (Phots. 7.22–7.24).

Sporadically the lymphoid cells infiltration could be seen (Phot. 7.25).

The microscopic lesions noticed in the posterior kidney were more frequent in the rainbow trouts sampled from RAS systems. They were also more pronounced in these fish. It was associated especially with the degree of affected organ areas.

**Table 7.10.** The frequency of the selected microscopic changes in the kidney (anterior – A and posterior – P) in 160 rainbow trouts* sampled in autumn

| Kind of microscopic changes | OS | | | | | | | | RAS | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | | | | 3 | | | | 1 | | | | 2 | | | |
| | S | | B | | S | | B | | S | | B | | S | | B | |
| | A | P | A | P | A | P | A | P | A | P | A | P | A | P | A | P |
| Necrosis | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Adipose (a) / vacuolar (v) degeneration | 0 | 0 | 0 | 1a | 0 | 1v | 0 | 1a 1v | 0 | 0 | 0 | 1v | 0 | 1v | 0 | 1v |
| Parenchymatous degeneration | 0 | 3 | 0 | 3 | 0 | 3 | 0 | 2 | 0 | 2 | 0 | 3 | 0 | 2 | 0 | 4 |
| Hyperaemia | 3 | 4 | 4 | 6 | 1 | 1 | 0 | 2 | 4 | 4 | 6 | 6 | 4 | 6 | 5 | 5 |
| Extravasations | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 0 | 1 | 1 | 1 |
| Melanomacrophages infiltration** | 5 | 7 | 8 | 8 | 7 | 6 | 8 | 8 | 6 | 8 | 8 | 9 | 7 | 8 | 8 | 9 |
| Lymphoidal infiltration | 1 | 2 | 0 | 1 | 1 | 2 | 1 | 2 | 0 | 1 | 1 | 3 | 2 | 1 | 1 | 2 |
| Total – body mass / rearing system | 9 | 17 | 12 | 21 | 9 | 13 | 10 | 18 | 11 | 16 | 16 | 24 | 13 | 19 | 15 | 22 |
| Total – rearing system / fish farm | 59 | | | | 50 | | | | 67 | | | | 69 | | | |
| Total – rearing technology | 109 (54.5 – mean for one fish farm) | | | | | | | | 136 (68.0 – mean for one fish farm) | | | | | | | |

Comments: markings as in Table 7.6. The number of analysed slides = 320.

The statistical analysis using Friedman's test (non-parametric analysis of variances) showed that the microscopic lesions found in the trouts were not specific neither for any of the studied rearing technologies (OS and RAS), nor for the specific fish farm. However, the changes were statistically significantly more frequent in the fish weighting 501–800 g than in smaller studied trouts from group S and in fish taking to examination in autumn.

### 7.5. Summary

The microscopic morphological examination allowed for the assessment of the outside and inside of the studied fish and the microscopic examination delivered the data on the status of the liver, spleen and anterior ("head") and posterior kidney of the rainbow trouts coming from two different rearing systems.

The first, and the easiest, evaluation method showed that in the both studied technologies the fish were of good health condition. This finding is crucial for the conducted studies and suggests the good quality of the rainbow trouts coming from the two rearing systems most popular in Poland. Moreover, the macroscopic examination of the interior of the body allowed for the preliminary assessment of the occurrence of the absence of such changes as regressive lesions and circulatory disturbances. It is also important that in this way the mutual relation of these morphological lesions was determined. In the described studies the gross morphologic lesions were comparatively rare, however, the circulatory disturbances were slightly more common.

It is noteworthy that the determination of the occurrence of steatosis simplex, the reversible change, was comparatively easy to perform. This can be done by the farmer himself. Having the elaborated criteria in hand it is possible to determine the degree of the steatosis and to modify the diet accordingly. The liver free form steatosis is the pattern more favorable.

Doing the analysis of the results of morphological examinations it must be kept in mind that the number of morphological lesions increases with age, what was stressed by many authors (Ayas et al. 2007; Barlas 1999; Fontagne et al. 1998; Kong et al. 2002; Korwin-Kossakowski et al 2003; Ozmen et al. 2006; Szarek et al. 2007). Such phenomenon could be seen also in this study – B-size fish displayed more microscopic lesions than those weighting 350 -500 grams. However, while assessing the occurrence of the changes it must be stressed that the most interesting thing is the clarification of the genesis and the dynamics of the detected changes.

The described studies showed the differences in the intensity of the microscopic morphological lesions, especially in the liver, of the rainbow trout. It was noted that the found anomalies, although with very similar frequencies, were differing in the intensity degree between the analysed rearing technologies – OS and RAS. In this second rearing system they were more pronounced – they were more intensive. Moreover, it was shown that the fish from the RAS system displayed more regressive changes than the fish from the open systems. This notice is important, both from veterinary and rearing point of view, for the planning of the undertaken rearing steps and prophylaxis.

The studies on the presence of melanomacrophages in the liver, spleen, anterior and posterior kidney are very important. In the first organ they were present in 5% of the studied fish. In the spleen they were present in 20–30% of trouts. They were most frequent in the anterior and posterior kidney. In such cases they were present in the double number of trouts in comparison to the spleen. The occurrence of melanomacrophages in the fish organs is a normal phenomenon. Macrophages absorb, among others, melanin, lipofuscin, ceroid and haemosyderin forming centers stained in brown-black. They are a form of biological cleaning stations (Prost 2004). Their presence in the fish organs is, on one hand beneficial, but on the other hand it may indicate the level of environment pollution (Dobsikova et al. 2006; Gul et al. 2004; Joerink et al. 2006; Poleksic et al. 1999; Szarek et al. 2008). The melanomacrophage infiltration is related with the aging and the stress associated with environmental xenobiotics.

It is also noteworthy that the obtained results dealing with the microscopic structure of the internal organs give the knowledge on the influence of OS and RAS technology on the condition and health of the rainbow trouts for human consumption. Using these research methods it was decided to undertake an innovative attempt to assess the influence of two different rearing technologies on the microscopic and gross morphological structure of the internal organs of these fish and, indirectly, on their condition and health.

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# 8. Ultrastructural examination of the liver of the rainbow trout

## 8.1. Introduction

An ultrastructural examination delivers more information in comparison to the microscopic observation. Observation using a light microscope allows for the observation of a larger area showing the structure of the organ. In this study the main goal of the ultrastructural analysis is the structure of hepatocytes. Nevertheless, an insight into the hepatocyte structure, and, when it is needed, into its vicinity, allows for the examination of more details than in the microscopic observation of a whole organ. Additionally, in the ultrastructural study the object of the observation is a direct cause of morphological changes. Because of that the hepatocyte is a frequent object of the interest in the ultrastructural analysis (Alvarez et al. 2006; Braunbeck 1998; Li et al. 2003; Szarek et al. 2000).

Recently, the ultrastructural analysis is used as a tool for studying the influence of various xenobiotics on the animal organism (Alvarez et al. 2006; Benedeczky et al. 1986; Saez 1984; Schmidt et al. 2005; Schramm et al. 1998; Szarek et al. 2000, 2007; Triebskorn et al. 2007; Vera et al. 1993). It is a very sensitive method, because the structures of a cell react to exogenous factors and they change due to the alteration or adaptation (Braunbeck 1998; Miyazaki et al. 2005). Although it is more complicated than the microscopic examination it gives, in result, more possibilities (Imagawa 1994; Kovalchuk 1990; Li et al. 2007; Miyazaki et al. 2005; Szarek et al. 2008).

On the basis of the above-mentioned facts the presented studies were aimed at the assessment of the influence of two technologies of the rainbow trout rearing on the substructural pattern of the liver in these fish reflecting their condition and health.

## 8.2. Materials and methods

The ultrastructural studies in the rainbow trouts (*Oncorhynchus mykiss*, Walbaum) were conducted during three years, in autumn and in spring from 2010 till 2012. The detailed description of the research subject and the rearing technology is given in the chapter 7 and 9. Here, we only mention that the trouts for studies were sampled in six fish farms: from three farms with the open water system

(free flow systems – denoted as OS), and from three farms using recirculating systems (recirculates – denoted as RAS).

Immediately after the sacrifice the sample of the liver was taken for ultrastructural studies. Collected samples were fixed in 2.5% paraformaldehyde and 2% glutaraldehyde in a phosphate buffer (pH 7.4) for two hours. Postfixation was performed in 2% osmium tetraoxide in the phosphate buffer (pH 7,4). The samples were then rinsed in a Ringer solution, dehydrated in a series of alkohol dilutions and in acetone. The tissues were then embedded in Epon 812. The polymerisation was performed at 45 Centigrades for 2 hours and at 60 Centigrade's for 48 hours. From the blocks semithin sections were prepared, which were then stained with the method of Levis and Knight (1977) and observed under the light microscope to determine the proper site for making ultrathin sections. The semithin sections were observed under an Opton microscope (Zeiss, Germany).

Statistical examination was conducted in this same way as in 7 chapter.

### 8.3. Results and Discussion

Studying the structure of the liver of the rainbow trouts in the electron microscope only small abnormalities were found concerning both their number and intensity (Tables 8.1–8.4, Phots. 8.1–8.8). However, the absolute number of these changes was higher than when studied in the light microscope. This is associated with the fact that the ultrastructural observation is a much more precise procedure.

**Table 8.1.** The frequency of ultrastructural lesions in the liver of 80 rainbow trouts* sampled in spring from two fish farms

| Kind of ultrastructural lesions | 2-OS | | 1-RAS | |
|---|---|---|---|---|
| | S | B | S | B |
| Necrosis | 0 | 1 | 1 | 1 |
| Meylin-like structure | 2 | 3 | 2 | 3 |
| Retrogressive changes in mitochondria | 3 | 4 | 3 | 4 |
| Numerous macrophages/lysosomes | 5 | 5 | 3 | 3 |
| Congestion | 4 | 6 | 5 | 6 |
| Extravasation | 0 | 1 | 2 | 3 |
| Melanomacrophages infiltration** | 2 | 4 | 5 | 6 |
| Proliferation of mitochondria | 7 | 8 | 5 | 7 |
| Lymphoid cell infiltration | 2 | 3 | 3 | 3 |
| Total – body mass / rearing technology | 25 | 35 | 29 | 36 |
| Total – rearing technology / fish farm / rearing technology | 60 | | 65 | |

Comments: * given changes regard 20 rainbow trouts in each group; ** in the viewing field under magnification 10 000×. The number of analysed ultramicroscopic preparations: 1 liver = 1 sample (block) = 1 preparation, 20 trouts in the S group ($n$ = 20) and 20 trouts in the group B ($n$ = 20) = 40 trouts × 1 sample = 40 samples × 1 slide = 40 slides from the fish from particular location × 2 fish farms = 80 slides.

**Table 8.2.** The frequency of the ultrastructural lesions in the liver of 80 rainbow trouts* sampled in autumn from two fish farms

| Kind of ultrastructural lesions | 2-OS | | 1-RAS | |
|---|---|---|---|---|
| | S | B | S | B |
| Necrosis | 0 | 3 | 1 | 2 |
| Meylin-like structure | 3 | 5 | 3 | 5 |
| retrogressive changes in mitochondria | 5 | 5 | 4 | 6 |
| Numerous macrophages/lysosomes | 4 | 5 | 3 | 5 |
| Congestion | 4 | 5 | 5 | 8 |
| Extravasation | 1 | 2 | 2 | 2 |
| Melanomacrophages infiltration** | 3 | 4 | 3 | 4 |
| Proliferation of mitochondria | 6 | 7 | 8 | 7 |
| Lymphoid cell infiltration | 4 | 4 | 4 | 5 |
| Total – body mass / rearing technology | 30 | 40 | 33 | 44 |
| Total – rearing technology / fish farm / rearing technology | 70 | | 77 | |

Comments: markings as in Table 8.1.

The majority of hepatocytes in every of analysed sample displayed normal structure (Phot. 8.1). Both mature cells with lighter cytoplasm and of bigger size (Phots. 8.5, 8.6A), as well as young cells, with darker cytoplasm, sometimes with a doubled nucleus were seen (Phots. 8.6B, 8.8B). The latter were especially often seen in trouts from fish farms with OS rearing system. Parenchymatous liver cells were confluent and connected to each other with microvili (for example Phot. 8.1). The nuclei were usually round or oval, with definite borders and big nucleoli (for example Phots. 8.1, 8.5, 8.6). The canals of the rough endoplasmic reticulum were numerous, narrow and with regular course (for example Phot. 8.1). Mitochondria were usually rod-like with a normal structure (for example Phots. 8.7B, 8.8B). The Golgi apparatus was usually well developed and without any damage, usually with scarce lysosomes in the vicinity (Phots. 8.4, 8.6B). Numerous glycogen granules were dispersed in the cytoplasm (for example Phots. 8.5, 8.8B) or were forming small clusters (for example Phots. 8.1, 8.7C), especially in young cells. Those granules occurred in variable numbers and correlated with values expressed in micrograms obtained with PAS staining according to McManus.

Ultrastructural lesions most often concerned single hepatocytes (for example Phot. 8.2). They were seldom seen in clusters of hepatocytes (for example Phots. 8.6–8.8).

One of the most unwanted changes found in the examined rainbow trouts was the liver necrosis. It was observed seldom (Tables 8.1, 8.2). More often it was located in the fragments of single hepatocytes (Phot. 8.2). Sporadically it was encompassing whole cell or clusters of cells. In such cases the mentioned lesion was seen in the fish from RAS rearing system, especially of B size. Comparatively often the early symptoms of necrosis – the rarefication of the cytoplasm (Phots. 8.3, 8.4) and the myelin-like structures (Phots. 8.4, 8.5, 8.7B) were seen. In this second instance it was also the symptom of apoptosis (the natural cell death).

It is known that the appropriate mitochondrial structure is an indicator of a normal cellular respiration, what is pointed out by some researchers. In the majority of examined electronmicrographs the examined organelles were of a normal structure. However, in some farms, especially 3-OS (Phot. 8.4) and 2-RAS and 3-RAS the mitochondria were damaged, especially their crest structures were blurred. Comparatively often the polymorphy of mitochondrial pattern was seen (Phots. 8.2, 8.4, 8.6, 8.7, 8.8B). More seldom dense bodies were seen in their matrix (Phots. 8.1A, 8.4). Besides the destructive lesions the adaptive changes were also seen in the mitochondria. Comparatively often the mitochondrial proliferation was seen (Phots. 8.6, 8.7B, 8.8B). This change was more frequent in the fish from the intensive rearing system.

**Table 8.3.** The frequency of ultrastructural lesions in the liver of 240 rainbow trouts* sampled in the spring from six fish farms

| Rearing parameters | OS | | | | | | RAS | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 1 | | 2 | | 3 | |
| | S | B | S | B | S | B | S | B | S | B | S | B |
| Body mass / rearing technology | 29 | 36 | 25 | 35 | 30 | 36 | 29 | 36 | 30 | 37 | 32 | 37 |
| Rearing technology / fish farm | 65 | | 60 | | 66 | | 65 | | 67 | | 69 | |
| Rearing technology | 191 (63.67 – mean for one fish farm) | | | | | | 201 (67.00 – mean for one fish farm) | | | | | |

Comments: * given changes regard 20 trouts in each group.

**Table 8.4.** The frequency of ultrastructural lesions in the liver of 240 rainbow trouts* sampled in autumn from six fish farms

| Rearing parameters | OS | | | | | | RAS | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 1 | | 2 | | 3 | |
| | S | B | S | B | S | B | S | B | S | B | S | B |
| Body mass / rearing technology | 33 | 41 | 32 | 39 | 35 | 44 | 32 | 43 | 36 | 44 | 32 | 46 |
| Rearing technology / fish farm | 74 | | 71 | | 79 | | 75 | | 80 | | 78 | |
| Rearing technology | 224 (74.67 – mean for 1 fish farm) | | | | | | 233 (77.67 – mean for 1 fish farm) | | | | | |

Comments: markings as in Table 8.3.

Sporadically the widening of the canals of the rough endoplasmatic reticulum, undulate or concentric course, eventually its proliferation was seen in the parenchymatous liver cells (Phot. 8.8). In few cases the fragmentation of this structure was seen in the fish from a few fish farms of RAS type.

It is noteworthy to point out the presence of lipid vacuoles in the hepatocyte cytoplasm (Phots. 8.1B, 8.7, 8.8B). Most often they were localized in the periphery of the mentioned cells. Hepatocytes containing the lipid vacuoles usually possessed a normal nucleus. It may be assumed that the lipids in their cytoplasm are symptoms of steatosis. This change is totally reversible, in contrast to the adipose degeneration – an irreversible process, occurring in the examined fish only sporadically. It may be added that the frequency of lipid occurrence was increasing with age. Trouts of S size had less lipids than older fish. In these cases the lipid droplets were small and most often occurred a singularly in the hepatocyte (Phot. 8.8B). Slightly higher degree of steatosis was found in the fish from fish farms of RAS type (Phots. 8.7A, 8.7B). In these cases lipid droplets were bigger and more numerous, even bigger than mitochondria.

The ultrastructural pattern of the trout liver steatosis was correlated with this lesion detected in this organ with light microscope.

Morphological abnormalities of hepatocyte nuclei were seen comparatively seldom. These were abnormalities of the shape of the nucleus (Phot. 8.7C) or abnormalities in heterochromatin distribution. Those changes were seen only in the fish from intensive rearing systems, especially from the fish farm 3-RAS.

The sinuses were usually normal with narrow Disse spaces and numerous microvili on the surface of hepatocytes. They usually didn't show any signs of damage. Nevertheless, in the lumen of some sinuses some cellular organelles or fragments of disrupted cells were seen. Then the Disse spaces were widened

and the microvili located on the sinusal surface of hepatocytes were irregularly distributed. Sometimes fragments of hepatocyte cytoplasm invaginated into the Disse space. Such patterns were seen in the trouts from fish farms 3-OS, 2-RAS and 3-RAS.

Slight proliferation of collagen fibers, especially in older fish was seen (Phots. 8.5, 8.7C). This unwanted change was clearly visible in the fish from RAS systems. Sometimes, especially when other destructive lesions were present, myelin-like structures were visible in sinuses. The lymphoidal infiltration was seen seldom and melanomacrophages infiltration was seen more often in hepatocytes.

### 8.4. Statistical examinations

Presented results of ultrastructural studies were statistically analyzed. The observed submicroscopic lesions in the trout liver were compared between two rearing technologies (independently from the sampling season). All parameters were analyzed because it was assumed that showing the statistical significance of differences between parameters: body size/rearing system and rearing system / fish farm will be additionally verified in farther analyses. The analyses were done at the confidence level being $p = 0.05$. The obtained results confirm the lack of variability of studied parameters, analyzed in relation to the rearing technology (Table 8.5).

**Table 8.5.** The analysis of differences between values of the studied parameters (*t*-Student test)

| Analyzed parameter | Mean | | *t* | *p* | SD | |
|---|---|---|---|---|---|---|
| | OS | RAS | | | OS | RAS |
| Body mass of trouts / rearing technology | 34.5833 | 36.1667 | -0.71101 | 0.484548 | 5.28219 | 5.62193 |
| Rearing technology / fish farm | 69.1667 | 72.5000 | -1.33360 | 0.195978 | 6.53429 | 5.68091 |
| Kind of rearing technology | 207.5000 | 217.0000 | -1.37088 | 0.184236 | 17.23369 | 16.71145 |

Then the statistical analysis was done on the number of the ultrastructural lesions observed in the liver of the trout's in relation to the rearing technology (OS, RAS). The results suggest that the observed differences are of a random character and are not related to the rearing technology (Fig. 8.1).

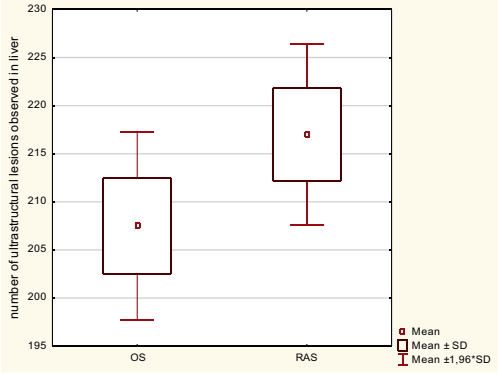


**Fig. 8.1.** The number of observed lesions in the liver of rainbow trout's in relation to the rearing system.

It was decided also to perform more detailed analysis to find out whether it may be stated that there are statistically significant differences between observed ultrastructural changes related to the sampling season (and, thus, the time of rearing cycle) and if there are differences related to rearing system.

Subsequent analyses were performed with the use of the Kruskall–Wallis test, treating the analyzed parameters as linked variables, while the code of particular fish farm was chosen as a grouping variable (1-3-OS, 1-3-RAS). This analysis also did not show any statistically significant differences (Fig. 8.2).

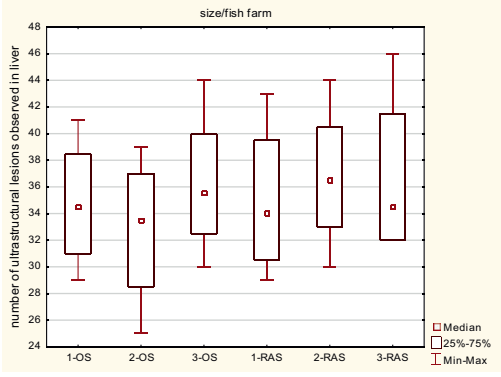


**Fig. 8.2.** The number of observed ultrastructural lesions in the liver of trout's in relation to the particular fish farm (the sampling season – spring, autumn - was not considered)

Slightly different results were obtained when the data were analyzed considering the sampling season – autumn and spring. The analysis where the grouping variable was the rearing system no statistically significant differences were found, however, the trend was observed showing that the number of changes was higher in size B group in autumn than in other fish (Figs. 8.3, 8.4).

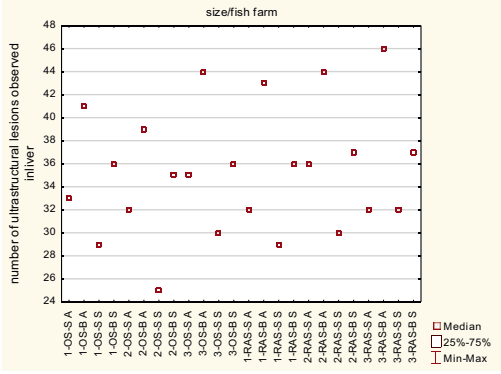


**Fig. 8.3.** The number of observed ultrastructural lesions in the trout liver in the particular studied fish farms (S – spring, A – autumn)

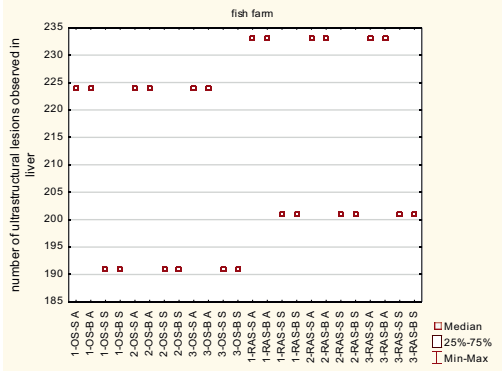


**Fig. 8.4.** The number of ultrastructural lesions observed in the liver of trouts in relation to all the analyzed parameters with the sampling seasons considered (S – spring, A – autumn): fish farm codes: S A – S body size group in autumn; S S – body size group S in spring; B A – body size group B in autumn; B S – body size group B in spring

It is significant that after choosing the sampling season as a grouping variable statistically significant differences were found showing the higher number of the observed changes in the liver of trouts in autumn (Figs. 8.5–8.7).

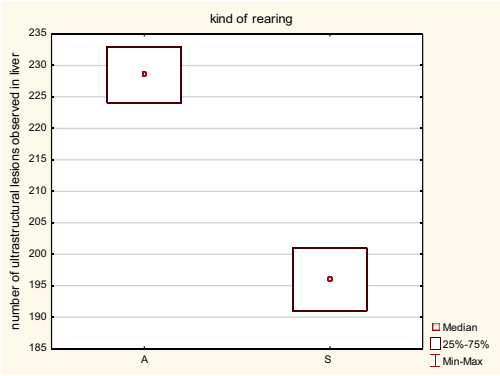


Fig. 8.5. The differences in the number of the ultrastructural lesions in the liver of trouts related to the rearing system (A – autumn, S – spring)

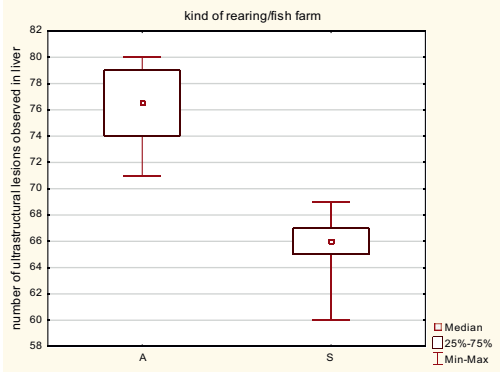


Fig. 8.6. Season-related differences in the number of ultrastructural lesions observed in the liver of trouts (divided according to the particular fish farm and rearing technology) (A – autumn, S – spring)

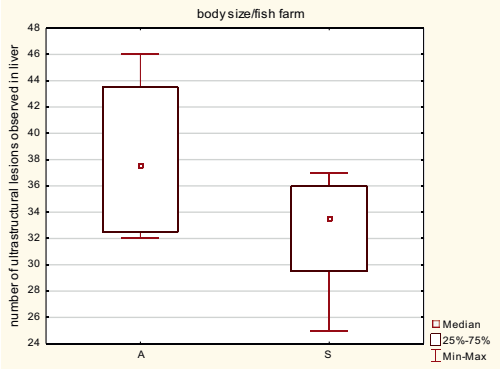


Fig. 8.7. Season-related differences between the numbers of ultrastructural lesions in the trout liver (divided according to the body size S and B in the particular fish farm and the rearing system) (A – autumn, S – spring)

## 8.5. Summary

The observed structural abnormalities in the liver if the trouts are a response to the direct influence of the fish environment, as well as other factors being the stress for the organism (Schwaiger et al. 1997). Presented studies conducted in this direction allow to differentiate the changes in the organs in relation to stimuli evoking these abnormalities. It must be kept in mind that frequently the response of the cell is in the limits of its physiological abilities. In such cases the ultrastructural pathology, linking the morphological structure with the function on the subcellular level, allows to determine the kinetics of the

pathological process. This may reveal subcellular lesions even at the earliest stages of the pathological process (Szarek et al. 1999).

The presented database, derived from the ultrastructural studies, indicates that the subcellular morphological abnormalities occurred in the studied trouts comparatively seldom. Both their character and intensity allows to point in dynamic aspect (size groups S and B) to the direct influence of fish environment and the rearing technology on the response of the trout organism at the level of cellular organelles in the analysed organ.

The conducted ultrastructural liver examination allows to claim that the most frequent changes were seen in mitochondria and less frequent in the rough endoplasmic reticulum. The location of the mentioned abnormalities suggest that the lesions were both of alterative and adaptive nature. The alterative changes are associated with the hepatocyte necrosis and its early symptoms. Such pattern is a basis for the claim that the critical point in the trout rearing is the provision of the adequate cellular respiration – water temperature and oxygen concentration. This suggests the necessity of very frequent oxygen concentration measurements (for example with oxymeters).

Both the number of ultrastructural lesions and the degree of their intensity were increasing with the age of the fish. B-size trouts showed more abnormalities than S-size ones. It was especially visible in case of the regressive changes – their frequency and intensity. The differences related to ultrastructural changes in the liver of the studied fish under two rearing technologies (OS and RAS) regarding the number of changes were similar. There were differences in the degree of intensity of changes expressed as the broadness of structural damage. In the light of obtained results it must be stated that the intensive rearing caused the higher number of liver abnormalities in comparison to OS systems. However, in RAS-type fish farms the occurrence of the lesions was variable. The intensity of changes in 1-RAS fish farm was lower than in the other two farms. Similar relation, albeit visible to the lower degree, occurred in the fish farm using the other technology. Trouts coming from farms 1-OS and 2-OS had changes expressed to the lower degree than in the farm 2-OS.

The presented facts indicate that the rainbow trouts coming from the OS and RAS technology show a few abnormalities in the liver ultrastructure. The observed changes being characteristic for the different intensity of the rearing system give new knowledge about the possibility of ingerence into the rearing process both from the point of view of the breeder and of the veterinarian.

Józef Koc, Marcin Sidoruk

# 9. Effect of a trout aquaculture technology on quality of waters

## 9.1. Introduction

Human economic activity often competes with the natural environment conservation and protection demands. Our drive towards the most intensive use of natural resources as well as the transformation of landscapes and original habitats drastically depress the quality of the latter and the biodiversities (Zając et al. 2004).

The dynamic development of aquaculture means its growing influence on the purity of surface water bodies. Fish farms generate water soluble contaminants, whose high concentrations can pose a serious threat to the aquatic environment (Sikora et al. 2009). The main sources of water pollution from fish ponds are fish secretions and residues of fish feed, either not ingested or undigested. Contamination of water in fish ponds affects the development of aquatic organisms. Moreover, contaminated pond water is discharged to other water bodies, where it produces adverse effects on fish and other water animals. Pond aquaculture has direct impact on the biocenoses of fish ponds and water reservoirs which receive pond water. Ponds are most often located in the upper part of drainage basins, which means that even small rates of pollutants can depress the quality of waters in the whole watercourse and have a negative effect on trout production, which requires the best quality water. Fish production causes changes in the quality of water due to fish feeds, mineral and organic fertilizers, pharmaceuticals and disinfectants used in aquaculture (Bieniarz et al. 2003).

The principle of rational water management is to maintain or restore the highest quality of water in natural ecosystems. This argument is often raised in discussions on the influence of fish rearing on the natural environment. Obviously, fish farms produce certain amounts of waste and pollutants discharged to the environment together with used water, but their harmfulness depends on the type of a given fish farm and its actual condition (Karakassis 2001).

The Regulation of the Minister for the Natural Environment Protection, Natural Resources and Forestry of 14 July 1998 (Journal of Law no 93 item 589) classifies fish ponds as a type of investment which can cause deterioration of the environment, hence it is required that a rational assessment of the impact of such enterprises on nature be made. At present, such evaluation seems even more important because pond aquaculture becomes increasingly popular and is one of the most dynamically growing branches of agriculture in Poland (Prądzyńska 2004).

The quality of waters discharged from fish farms and the load of pollutants they carry depend on a series of factors. The following should be considered: quality of water supplied to a fish farm, fish species, fish rearing technology, fish stand, amounts and quality of fish feeds as well as meteorological and physiographic conditions (Kolasa-Jamińska 2004).

Pond aquaculture involves breeding or rearing fish that takes into account its production potential, profitability and environmental impact. Thus, it is essential to identify threats and conflicts which occur in the natural environment due to fish pond production (Prądzyńska 2004).

### 9.2. Methods

This 3-year study dealt with the effect of trout rearing on quality of water. Six farms were selected for the study and divided into two groups in respect of water management technologies. One group comprised farms where water was used once (open flow farms) and was assigned the symbol OS. The other group gathered farms where water was reused several times (recirculated); these farms were marked with the symbol RAS. In open systems, water flows through ponds once and then is discharged outside. This way water supplies fish with oxygen and removes suspended solids as well as dissolved residue feeds. Water supplying ponds is collected from a river, flows through fish ponds and is treated before it is discharged back to the river. The total water volume at a farm is exchanged at least once daily. In contrast, a recirculatory system means that water is used several times, each time purified mechanically and biologically. The purpose is to lower the water and energy demand and to reduce emission of nutrients to the natural environment. Such systems offer many advantages, e.g. they save water and energy, ensure accurate control of water quality and have a limited effect on nature (Bardócz 2009).

Water assessment measuring points were established in all the examined farms, so as to check the quality of water supplied to fish ponds, water in ponds and water discharged from farms.

### 9.3. Physical parameters of water in trout ponds

Water warms and cools much more slowly than air. The reason is that water has high heat capacity, which means that it takes long for water to warm up but it takes just as long to give off heat to the ambient environment. Sudden changes in the thermal properties of water deprive water organisms from a chance to adapt to a new situation, which often end in their death (Chojnacki 1998; Ntengwe et al. 2008). To a large extent, changes in water temperature depend on the depth of water, surface area and winds. Ponds belong to polymictic water bodies so that daily temperature oscillations are higher in the topmost layer of water, but tend to weaken in deeper ponds (Kajak 2001). Fish are ectothermic, which means that water temperature affects indirectly the growth rate of fish by accelerating or retarding their metabolism. Although trout is said to be able to stand a temperature up to 25°C, the optimum temperatures for salmonids are between 14°C and 18°C (Goryczko 1999).

During the whole period of observations, the average temperature of waters supplied to the ponds was 9.41 ± 1.43°C to 13.14 ± 3.48°C, and the maximum temperature did not exceed 18.24°C. Once the supplied water had been used for fish rearing, the average temperature of water released from farms 2-OS and 3-RAS increased by about 3°C and 2°C, but at the other farms it fell by about 1°C. The lowest temperature variation was observed at fish farm 2-RAS, where the coefficient of variation ranged from 10% to 12%, but the widest range of water temperatures occurred at fish farm 3-RAS (26–41%) (Table 9.1).

Temperature and oxygen content in water are important parameters which influence life in water bodies. Oxygen solubility in water depends on temperature: the higher the water temperature increases, the lower the content of oxygen in water. The changeability of aerobic and thermal conditions in water are closely connected with phyto- and zooplankton, which also affect the amount of light penetrating into the water depths.

**Table 9.1.** Temperature of supplied and released waters at trout farms (°C)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 13.14 | 13.06 | 13.12 | 9.41 | 11.49 | 12.50 | 10.83 | 10.30 | 10.29 | 12.34 | 12.01 | 11.81 | 11.21 | 11.32 | 11.26 | 10.44 | 10.26 | 11.91 |
| Min | 8.25 | 8.38 | 8.23 | 8.09 | 7.76 | 8.49 | 7.51 | 7.47 | 7.55 | 8.70 | 7.80 | 7.60 | 9.55 | 9.22 | 9.26 | 6.71 | 6.08 | 5.78 |
| Max | 18.24 | 18.62 | 18.87 | 12.44 | 14.91 | 16.58 | 13.57 | 13.59 | 13.15 | 17.82 | 18.40 | 17.21 | 12.57 | 13.25 | 13.34 | 14.54 | 14.50 | 18.21 |
| Median | 12.79 | 12.50 | 12.64 | 9.20 | 11.40 | 12.50 | 10.97 | 10.01 | 10.06 | 11.26 | 10.85 | 10.88 | 11.27 | 11.43 | 11.27 | 10.76 | 10.63 | 11.95 |
| SD | 3.48 | 3.59 | 3.72 | 1.43 | 2.63 | 3.21 | 2.01 | 2.11 | 1.97 | 3.69 | 3.95 | 3.63 | 1.18 | 1.34 | 1.37 | 2.73 | 3.00 | 4.86 |
| Coefficient of variation | 27 | 27 | 28 | 15 | 23 | 26 | 19 | 20 | 19 | 30 | 33 | 31 | 10 | 12 | 12 | 26 | 29 | 41 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

The concentration of oxygen produced via photosynthesis by phytoplankton in a pond ecosystem is shaped by several factors, including temperature, water transparency, insolation, and content of nutrients. Oxygen produced by phytoplankton may reach up to 80% of total oxygen supplied to a pond. It is therefore extremely important to maintain appropriate temperature in a fish pond because its increase by 10°C doubles the intensity of chemical and biochemical processes and depresses the solubility of oxygen in water. The same amount of oxygen in water at a different water temperature corresponds to a different degree of water saturation in oxygen (Jawecki et al. 2008).

Changes in the content of oxygen are proportional to the fertility of a fish pond. The content of oxygen in water depends on such factors as the water temperature, water transparency, content of nutrients and insolation. Pond-specific characteristics and all fish production measures carried out in a given pond are important as well (Jawecki 2008). The changeability of oxygen conditions is also affected by meteorological conditions, for example insolation, cloud cover, wind velocity or ambient temperature. Other factors involved are certain local conditions, e.g. shading of the water surface by plants growing along banks or depth of a pond (Jawecki 2006, 2011).

During the whole study, waters supplied to the fish ponds were well aerated and their average oxygen content was around 8.77 ± 0.54 mg/dm$^3$ (2-OS) to 10.31 ± 1.22 mg/dm$^3$ (3-OS) (Tables 8.2, 8.3). At the fish farms where water was used once (1-OS and 2-OS), the oxygen concentration in waters discharged from the farms was about 0.21 mg/dm$^3$ and 1.04 mg/dm$^3$ higher, but at farms with a closed water circulation system the discharged water was poorer in oxygen by about 0.26–1.91 mg/dm$^3$. The same situation was documented in respect of water saturation with oxygen, because discharge waters from fish farms 1-OS and 2-OS contained about 3–20% more oxygen, but the water discharged from the RAS fish farms was poorer in oxygen by 1–20%. In the fish farms with water recirculation, despite intensive fish production, the loss of oxygen in waters discharged from the ponds was small, an effect achieved owing to artificial water aeration (with mechanical aerators or pure oxygen) carried out to create optimal living conditions for fish.

**Table 9.2.** Oxygen saturation of supplied and released waters at trout farms (%)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 92.9 | 79.6 | 95.3 | 77.1 | 81.4 | 92.3 | 93.4 | 86.7 | 91.1 | 98.4 | 74.9 | 78.8 | 86.6 | 76.9 | 70.9 | 82.5 | 66.8 | 81.9 |
| Min | 71.0 | 54.3 | 67.8 | 71.9 | 72.0 | 84.0 | 75.2 | 68.9 | 74.0 | 92.7 | 67.8 | 70.0 | 69.6 | 61.2 | 49.9 | 60.4 | 32.2 | 48.3 |
| Max | 127.7 | 132.5 | 135.3 | 91.1 | 89.3 | 110.3 | 111.5 | 107.9 | 114.5 | 110.0 | 91.5 | 99.7 | 116.6 | 103.2 | 106.1 | 98.0 | 102.7 | 116.8 |
| Median | 89.9 | 73.1 | 89.4 | 74.1 | 81.9 | 91.5 | 91.2 | 84.9 | 90.9 | 97.0 | 72.6 | 74.5 | 83.4 | 72.5 | 63.5 | 86.4 | 69.0 | 86.1 |
| SD | 23.4 | 34.4 | 28.5 | 7.3 | 6.1 | 8.7 | 11.6 | 12.3 | 12.5 | 5.7 | 7.3 | 9.8 | 16.4 | 13.6 | 17.0 | 12.6 | 28.4 | 23.6 |
| Coefficient of variation | 25 | 43 | 30 | 10 | 7 | 9 | 12 | 14 | 14 | 6 | 10 | 12 | 19 | 18 | 24 | 15 | 43 | 29 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

**Table 9.3.** Dissolved oxygen content in supplied and released waters at trout farms (mg/dm$^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 9.70 | 8.28 | 9.91 | 8.77 | 8.84 | 9.81 | 10.31 | 9.67 | 10.21 | 10.27 | 7.91 | 8.36 | 9.46 | 8.40 | 7.76 | 9.19 | 7.43 | 8.93 |
| Min | 8.16 | 6.29 | 7.98 | 8.36 | 7.59 | 8.38 | 9.00 | 8.26 | 8.39 | 9.92 | 6.87 | 7.08 | 7.94 | 6.64 | 5.43 | 7.38 | 3.99 | 4.74 |
| Max | 12.70 | 13.24 | 13.52 | 9.71 | 9.64 | 11.33 | 12.21 | 11.20 | 12.92 | 10.75 | 8.59 | 9.59 | 12.71 | 11.31 | 11.69 | 11.51 | 11.07 | 14.02 |
| Median | 9.62 | 7.78 | 9.50 | 8.44 | 9.01 | 9.82 | 10.06 | 9.51 | 9.98 | 10.21 | 8.26 | 8.69 | 9.02 | 8.07 | 7.18 | 9.38 | 7.34 | 8.76 |
| SD | 1.63 | 2.57 | 2.08 | 0.54 | 0.75 | 1.07 | 1.22 | 1.29 | 1.54 | 0.37 | 0.78 | 1.03 | 1.80 | 1.64 | 2.14 | 1.51 | 2.94 | 3.16 |
| Coefficient of variation | 17 | 31 | 21 | 6 | 8 | 11 | 12 | 13 | 15 | 4 | 10 | 12 | 19 | 20 | 28 | 16 | 40 | 35 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

Electrolytic conductivity (EC) expresses the capacity of water for conducting electric current and is an indirect indicator of concentrations of ions dissolved in water, but it is also dependent on temperature. The value of EC increases by about 1.8–2.5% when water temperature rises by 1°C (Macioszczyk et al. 2002).

Electrolytic conductivity of water corresponds to its content of mineral contaminants, and increases as their content rises. Thus, the value of EC enables researchers to determine salinity of water, content of dissolved substances and dry residue (Macioszczyk et al. 2002).

Our analysis of EC in waters supplied to ponds demonstrated that the average EC values at all the farms were similar and ranged from 291.8 ± 79.7 µS/cm (1-RAS) to 384.7±69.0 µS/cm (3-OS) (Table 9.4). The median varied from 317.5 µS/cm (1-RAS) to 407 µS/cm (3-OS). The technologies implemented at all the farms were found to produce no effect on the EC in discharged waters. At fish farms 1-OS and 2-RAS, the EC value in spent water decreased by 1 µS/cm and 25 µS/cm, respectively. At the other farms, it increased by 2–34 µS/cm. The assessment of changes in the EC revealed similar values at both types of farms: with single water use (OS) and with water recirculation (RAS), but its average increase was about 9 µS/cm among the former and less than 4 µS/cm in the latter group of farms.

**Table 9.4.** Proper electrolytic conductivity in supplied and released waters at trout farms (μS/cm)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 361.5 | 356.5 | 360.5 | 360.7 | 358.4 | 362.6 | 384.7 | 412.3 | 411.2 | 291.8 | 324.0 | 325.3 | 376.3 | 351.0 | 351.2 | 373.7 | 361.0 | 376.8 |
| Min | 331.0 | 335.0 | 332.0 | 326.0 | 312.0 | 330.0 | 239.0 | 347.0 | 356.0 | 154.0 | 282.0 | 285.0 | 318.0 | 322.0 | 323.0 | 344.0 | 329.0 | 332.0 |
| Max | 401.0 | 400.0 | 401.0 | 388.0 | 385.0 | 393.0 | 459.0 | 446.0 | 445.0 | 402.0 | 345.0 | 351.0 | 479.0 | 383.0 | 383.0 | 415.0 | 391.0 | 413.0 |
| Median | 359.0 | 349.5 | 361.5 | 369.0 | 371.0 | 368.0 | 407.0 | 438.5 | 433.0 | 317.5 | 338.0 | 336.0 | 364.5 | 349.5 | 349.0 | 375.5 | 363.0 | 378.0 |
| SD | 25.1 | 23.7 | 22.4 | 25.5 | 29.8 | 23.5 | 69.0 | 45.2 | 41.8 | 79.7 | 23.2 | 22.4 | 48.2 | 26.8 | 26.8 | 22.0 | 28.2 | 32.1 |
| Coefficient of variation | 7 | 7 | 6 | 7 | 8 | 6 | 18 | 11 | 10 | 27 | 7 | 7 | 13 | 8 | 8 | 6 | 8 | 9 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

Water reaction is another factor which conditions presence of life in water and is specific for given organisms. On the other hand, organisms living in water change water pH. The value of water pH is also affected by photosynthesis, respiration and nitrogen assimilation processes. However, when water pH is less than 6.3, respiration and photosynthesis have little effect on water reaction, unlike nitrogen assimilation (Lampert et al. 1996). Water reaction plays a decisive role in most of biological and chemical processes which take place in water. It also affects live organisms which dwell in water. In ponds with alkaline or neutral reaction, biodiversity is much higher than in ponds with acidic water (Bieniarz et al. 2003). Water pH is a factor which readily reflects changes in environmental conditions. It is assumed that water used for trout farms should possess pH within 6.5–8.2, with 7.2 being the optimum reaction. At pH below 6.5 or above 9.0, the growth of fish is retarded, and when it falls below 4.0 or exceeds 11.0, fish die rapidly (Bieniarz et al. 2003). The value of pH also influences the toxicity of ammonia in aquatic environment. High alkaline reaction leads to an increase in the number of non-ionized ammonia molecules, which have toxic properties. For salmonid fish, the toxic concentration of non-ionized ammonia is just 0.0125 mg/dm$^3$ (Goryczko 1999; Ntengwe et al. 2008).

Water supplied to fish ponds was characterized by neutral reaction shifting towards slightly alkaline one, and the median pH was 7.70 (2-OS) up to 8.20 (1-OS) (Table 9.5). Our analysis of the reaction of used water showed a slight effect of the applied water management technology on the value of pH. When water was recirculated, its pH decreased by 0.07-0.36, but when it flew through fish ponds, its pH rose by 0.10–0.48.

**Table 9.5.** Values of pH in supplied and released waters at trout farms

| | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| min | 6.13 | 7.85 | 7.61 | 6.73 | 7.05 | 7.62 | 7.35 | 7.01 | 7.41 | 7.98 | 7.12 | 7.35 | 6.39 | 7.26 | 7.40 | 6.88 | 6.80 | 6.96 |
| max | 8.48 | 8.32 | 8.34 | 8.48 | 8.35 | 8.33 | 8.27 | 8.16 | 8.12 | 8.66 | 8.19 | 8.33 | 8.25 | 8.02 | 7.97 | 8.40 | 8.12 | 7.84 |
| median | 8.20 | 8.15 | 8.16 | 7.70 | 7.90 | 8.23 | 7.90 | 8.05 | 8.06 | 8.12 | 8.00 | 7.96 | 8.07 | 7.81 | 7.76 | 7.86 | 7.73 | 7.69 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

### 9.4. Organic matter indices in waters at trout fish farms

Dry residue gives an insight into the total content of organic and inorganic compounds in water (Hermanowicz et al. 1999). The average concentration of dry residue in waters supplied to the fish farms ranged from 199.5 ± 22,0 mg/dm$^3$ (1-RAS) to 376.7±215.5 mg/dm$^3$ (3-OS), and the median was from 198.5 mg/dm$^3$ (1-RAS) to 288 mg/dm$^3$ (3-OS). Most of the dry residue consisted of mineral substances, and

their percentage in dry residue found in waters supplied to ponds ranged from 66.6% at fish farm 2-RAS to 85.7% at fish farm 1-OS (Tables 9.6, 9.7). After water had been used for fish production, the dry residue content in water discharged from the farms with a recirculation system increased by 21.7–32.7 mg/dm$^3$. However, dry residue was observed to have decreased by 4.7 mg/dm$^3$ and 90.7 mg/dm$^3$ in water discharged from farms 1-OS and 3-OS (flow-through systems), respectively. It was only at one open flow farm (2-OS) that the content of dry residue in discharged water increased slightly, by about 10.3 mg/dm$^3$. In discharged waters from most of the fish farms, an increase in the content of organic matter relative to supplied waters was noticed, except for fish farm 3-OS, where it was reduced by ca 5%.

**Table 9.6.** Content of dry residue in supplied and released waters at trout farms (mg/dm$^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 236.7 | 246.7 | 232.0 | 240.6 | 240.0 | 250.9 | 376.7 | 286.7 | 286.0 | 199.5 | 207.3 | 221.2 | 233.3 | 241.3 | 256.7 | 252.7 | 261.3 | 285.3 |
| Min | 192.0 | 200.0 | 188.0 | 216.0 | 212.0 | 212.0 | 248.0 | 232.0 | 220.0 | 168.0 | 184.0 | 194.0 | 200.0 | 196.0 | 228.0 | 236.0 | 240.0 | 252.0 |
| Max | 304.0 | 304.0 | 304.0 | 276.0 | 276.0 | 296.0 | 812.0 | 332.0 | 368.0 | 236.0 | 228.0 | 260.0 | 256.0 | 296.0 | 324.0 | 268.0 | 280.0 | 360.0 |
| Median | 232.0 | 242.0 | 226.0 | 240.0 | 236.0 | 256.0 | 288.0 | 288.0 | 282.0 | 198.5 | 210.0 | 210.0 | 234.0 | 236.0 | 252.0 | 252.0 | 264.0 | 276.0 |
| SD | 37.0 | 33.4 | 39.0 | 21.7 | 21.8 | 30.3 | 215.5 | 34.7 | 47.4 | 22.0 | 14.6 | 28.4 | 20.3 | 37.2 | 35.3 | 10.9 | 13.8 | 40.1 |
| Coefficient of variation | 16 | 14 | 17 | 9 | 9 | 12 | 57 | 12 | 17 | 11 | 7 | 13 | 9 | 15 | 14 | 4 | 5 | 14 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

**Table 9.7.** Content of ash substances in supplied and released waters at trout farms (mg/dm$^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 199.3 | 200.0 | 190.7 | 206.3 | 202.3 | 213.1 | 272.7 | 226.7 | 220.0 | 170.8 | 178.5 | 171.5 | 155.3 | 163.3 | 170.0 | 212.0 | 218.0 | 220.7 |
| Min | 156.0 | 156.0 | 152.0 | 160.0 | 160.0 | 180.0 | 156.0 | 164.0 | 136.0 | 124.0 | 148.0 | 132.0 | 100.0 | 112.0 | 96.0 | 168.0 | 168.0 | 176.0 |
| Max | 232.0 | 240.0 | 236.0 | 256.0 | 280.0 | 276.0 | 552.0 | 268.0 | 292.0 | 232.0 | 220.0 | 248.0 | 188.0 | 188.0 | 216.0 | 252.0 | 268.0 | 264.0 |
| Median | 206.0 | 198.0 | 190.0 | 202.0 | 208.0 | 196.0 | 232.0 | 230.0 | 230.0 | 168.5 | 177.5 | 160.0 | 160.0 | 178.0 | 172.0 | 212.0 | 222.0 | 222.0 |
| SD | 30.9 | 28.1 | 28.0 | 35.7 | 38.6 | 38.4 | 141.3 | 39.9 | 51.5 | 35.2 | 24.1 | 43.6 | 31.3 | 30.7 | 41.3 | 27.0 | 33.1 | 28.9 |
| Coefficient of variation | 16 | 14 | 15 | 17 | 19 | 18 | 52 | 18 | 23 | 21 | 13 | 25 | 20 | 19 | 24 | 13 | 15 | 13 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

The $BOD_5$ and $COD_{Cr}$ indices express the amount of oxygen needed to oxidize organic compounds found in water. The value of these indices is strongly connected with the abundance of phytoplankton in water (Bieniarz et al. 2003; Bonisławska et al. 2011).

Waters used to supply the analyzed trout farms were characterized by a relatively low $BOD_5$, and its average value during the whole period of investigations ranged from 1.60±0.62 mg/dm$^3$ (2-OS) to 3.50±2.13 mg/dm$^3$ (3-RAS) (Table 9.8). In most farms (except 2-OS), the $BOD_5$ periodically exceeded the values set for inland waters used as habitats of salmonid fish (Regulation of the Minister for the Natural Environment of 4 October 2002, Journal of Law no 176).

**Table 9.8.** Values of the $BOD_5$ in supplied and released waters at trout farms ($mg/dm^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 2.32 | 3.24 | 3.21 | 1.60 | 2.04 | 2.59 | 2.51 | 1.96 | 1.82 | 2.09 | 2.83 | 2.99 | 2.61 | 4.95 | 5.10 | 3.50 | 2.74 | 6.58 |
| Min | 0.76 | 1.71 | 2.46 | 0.96 | 1.51 | 1.60 | 1.10 | 1.10 | 0.87 | 0.70 | 0.88 | 1.13 | 0.93 | 1.52 | 1.88 | 1.01 | 1.69 | 3.86 |
| Max | 4.39 | 4.72 | 4.51 | 2.46 | 2.73 | 3.19 | 4.14 | 2.60 | 3.10 | 4.85 | 4.11 | 4.18 | 3.70 | 7.04 | 6.38 | 6.80 | 4.46 | 9.60 |
| Median | 2.24 | 3.31 | 3.19 | 1.67 | 2.09 | 2.76 | 2.23 | 2.24 | 1.83 | 1.81 | 2.97 | 3.17 | 2.73 | 5.18 | 5.67 | 3.20 | 2.38 | 6.39 |
| SD | 1.18 | 1.02 | 0.75 | 0.62 | 0.45 | 0.51 | 1.06 | 0.68 | 0.89 | 1.43 | 1.23 | 1.19 | 1.09 | 1.90 | 1.62 | 2.13 | 0.99 | 2.04 |
| Coefficient of variation | 51 | 31 | 23 | 39 | 22 | 20 | 42 | 35 | 49 | 68 | 43 | 40 | 42 | 38 | 32 | 61 | 36 | 31 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

The least favourable situation occurred at fish farm 3-RAS, where these norms were sporadically exceeded over two-fold. This may have been due to an increased inflow of contaminants, for example by leaching fields or from uncontrollable pollution above the fish farm.

Our analysis of the results shows a distinct effect of the implemented water management technology on an increased value of the $BOD_5$ in waters discharged from the trout fish farms. In used waters flowing from the farms with a flow-through system, a slight increase in the $BOD_5$ was seen, by about 0.9 $mg/dm^3$ (1-OS and 2-OS) or 0.7 $mg/dm^3$ (3-OS). In turn, at the fish farms with a recirculating aquaculture system, the increase in the $BOD_5$ was much higher, i.e. 2.49 $mg/dm^3$ and 3.08 $mg/dm^3$ (2-RAS and 3-RAS, respectively). Fish farm 1-RAS was an exception as the $BOD_5$ increment was smaller (0.90 $mg/dm^3$).

Our analysis of the mean value of $COD_{Cr}$ in waters supplied to the fish farms showed that it was low and ranged within 14.82 ± 5.88 $mg/dm^3$ (3-RAS) to 20.03 ± 12.50 $mg/dm^3$ (2-OS) (Table 9.9). A marked difference was stated between the farms with a recirculating system and the ones with an open flow system in terms of an increase in the $COD_{Cr.}$ The $COD_{Cr}$ was found to be reduced by 0.02 $mg/dm^3$ (1-OS) to 3.43 $mg/dm^3$ (3-OS) in spent waters from the open flow fish farms compared to the supplied waters, but in the farms with a recirculating aquaculture system, analogous values were found to have risen by 0.2 $mg/dm^3$ (1-RAS), 2.1 $mg/dm^3$ (2-RAS) and 6.52 $mg/dm^3$ (3-RAS).

**Table 9.9.** Values of $COD_{cr}$ in supplied and released waters at trout farms ($mg/dm^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 17.67 | 17.52 | 17.65 | 20.03 | 20.91 | 16.60 | 16.90 | 14.72 | 13.82 | 17.34 | 16.92 | 17.48 | 15.38 | 16.58 | 17.43 | 14.82 | 13.97 | 21.33 |
| Min | 13.60 | 13.20 | 10.40 | 4.30 | 7.80 | 6.00 | 6.80 | 6.40 | 8.40 | 11.20 | 8.40 | 7.60 | 11.30 | 12.00 | 12.00 | 7.60 | 9.60 | 13.70 |
| Max | 26.60 | 27.40 | 24.60 | 44.80 | 40.80 | 25.10 | 26.00 | 19.30 | 19.80 | 32.90 | 34.40 | 34.40 | 22.00 | 19.50 | 19.60 | 23.70 | 19.30 | 28.30 |
| Median | 16.10 | 15.75 | 17.35 | 18.10 | 21.70 | 16.00 | 18.15 | 15.80 | 13.85 | 15.06 | 14.76 | 15.65 | 13.30 | 16.90 | 19.00 | 13.70 | 12.25 | 21.30 |
| SD | 5.05 | 5.32 | 5.74 | 12.50 | 10.31 | 6.70 | 6.69 | 5.28 | 4.45 | 8.28 | 9.28 | 9.10 | 4.66 | 2.72 | 3.10 | 5.88 | 4.23 | 5.76 |
| Coefficient of variation | 29 | 30 | 33 | 62 | 49 | 40 | 40 | 36 | 32 | 48 | 55 | 52 | 30 | 16 | 18 | 40 | 30 | 27 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

When analyzing changes in values of the $BOD_5$ and $COD_{Cr}$ at the two types of aquaculture systems, it was demonstrated that the spent waters discharged from fish farms with an open flow system had a higher $BOD_5$ value by an average of 0.39 mg/dm$^3$ and a lower $COD_{Cr}$ by 2.18 mg/dm$^3$. However, at the RAS farms, an increase in the $BOD_5$ was higher and reached 2.15 mg/dm$^3$ on average, whereas the $COD_{Cr}$ was 2.90 mg/dm$^3$ higher.

### 9.5. Biogenic indices of water in trout farms

Phosphorus in pond water appears as phosphate ions, produced by dissociation of orthophosphate acid, and as dissolved organic phosphorus. Phosphate ions in waters of high pH and high content of calcium are precipitated and create insoluble complexes of calcium phosphate, which are deposited on the bed of a water body. The main function of phosphorus in water ecosystems is to regulate biological production as a basic biogenic substance for synthesis of organic compounds. In this way, phosphorus affects fertility of water bodies (Bieniarz et al. 2003; Raczyńska et al. 2006). An effect (an indicator) of a high mineral phosphorus content in water is massive algal growth (Dojlido 1995). This process is accompanied by an increase in water pH, depletion of free $CO_2$, increased water oxidation, decreased ammonia nitrogen concentration (Tucker et al. 1984; Lewkowicz et al. 2003).

Phosphorus in waters supplied to fish farms 1-OS, 2-OS and 2-RAS appeared mainly in the organic form, making up from 53% of $P_{total}$ in water supplied to farm 1-OS to 71% in water supplied to farm 2-OS. In waters supplied to the other fish farms, mineral phosphorus prevailed, constituting from 50.3% (3-OS) to 69% (1-RAS) of $P_{total.}$

The average concentration of total phosphorus in waters supplied to the farms equalled 0.085 ± 0.087 mg/dm$^3$ (2-OS) to 0.270 ± 0.332 mg/dm$^3$ (2-RAS) (Table 9.10). At farm 2-RAS, the supplied waters were found to contain periodically excessive amounts of $P_{total}$, above the norms set for inland waters used as a salmonid fish habitat (Regulation of the Minister for the Environment of 4 October 2002, Journal of Law No 176). The mean concentration of phosphate phosphorus in waters supplied to the fish farms ranged 0.025 ± 0.021 mg/dm$^3$ ( 2-OS) to 0.090 ± 0.075 mg/dm$^3$ (3-RAS). The median of the concentrations was from 0.21 mg/dm$^3$ (2-OS) to 0.093 mg/dm$^3$ (2-RAS) (Table 9.11). Once the water had been used for fish production, an increase in the concentration of $P_{total}$ and $P–PO_4$ was observed in most samples of discharged waters. The highest increase in the concentration of phosphorus compounds was observed at farms 3-RAS [$P_{og}$ – 0.099 mg/dm$^3$ (75%), P–PO4 – 0.033 mg/dm$^3$ (36%)] and 2-OS [($P_{og}$ – 0.034 mg/dm$^3$ (40%), $P–PO_4$ – 0.032 mg/dm$^3$ (129%)]. A reverse situation occurred at farm 2-RAS, where the discharged water was determined to contain 0.111 mg/dm$^3$ less organic phosphorus.

**Table 9.10.** Content of $P_{total}$ in supplied and released waters at trout farms (mg/dm$^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 0.128 | 0.116 | 0.146 | 0.085 | 0.080 | 0.120 | 0.087 | 0.145 | 0.111 | 0.091 | 0.121 | 0.111 | 0.270 | 0.157 | 0.159 | 0.133 | 0.128 | 0.232 |
| Min | 0.063 | 0.060 | 0.075 | 0.011 | 0.047 | 0.043 | 0.057 | 0.096 | 0.041 | 0.045 | 0.069 | 0.067 | 0.083 | 0.106 | 0.124 | 0.038 | 0.056 | 0.204 |
| Max | 0.179 | 0.184 | 0.292 | 0.273 | 0.106 | 0.195 | 0.129 | 0.210 | 0.219 | 0.129 | 0.198 | 0.167 | 0.944 | 0.228 | 0.201 | 0.344 | 0.252 | 0.289 |
| Median | 0.144 | 0.105 | 0.122 | 0.072 | 0.083 | 0.110 | 0.086 | 0.137 | 0.089 | 0.105 | 0.127 | 0.101 | 0.143 | 0.140 | 0.156 | 0.114 | 0.115 | 0.226 |
| SD | 0.044 | 0.042 | 0.080 | 0.087 | 0.019 | 0.048 | 0.026 | 0.049 | 0.064 | 0.034 | 0.048 | 0.041 | 0.332 | 0.046 | 0.037 | 0.113 | 0.068 | 0.031 |
| Coefficient of variation | 35 | 37 | 55 | 101 | 24 | 40 | 29 | 33 | 58 | 38 | 39 | 37 | 123 | 29 | 23 | 85 | 54 | 13 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

**Table 9.11.** Content of $P–PO_4$ in supplied and released waters at trout farms ($mg/dm^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 0.060 | 0.062 | 0.064 | 0.025 | 0.023 | 0.056 | 0.044 | 0.050 | 0.051 | 0.063 | 0.076 | 0.079 | 0.082 | 0.107 | 0.109 | 0.090 | 0.085 | 0.123 |
| Min | 0.032 | 0.038 | 0.036 | 0.003 | 0.007 | 0.014 | 0.024 | 0.014 | 0.030 | 0.043 | 0.040 | 0.046 | 0.028 | 0.055 | 0.047 | 0.031 | 0.040 | 0.036 |
| Max | 0.094 | 0.081 | 0.084 | 0.069 | 0.080 | 0.084 | 0.071 | 0.090 | 0.081 | 0.085 | 0.094 | 0.098 | 0.112 | 0.156 | 0.166 | 0.236 | 0.145 | 0.166 |
| Median | 0.063 | 0.064 | 0.071 | 0.021 | 0.011 | 0.075 | 0.044 | 0.049 | 0.046 | 0.061 | 0.086 | 0.087 | 0.093 | 0.107 | 0.110 | 0.079 | 0.087 | 0.134 |
| SD | 0.022 | 0.021 | 0.020 | 0.021 | 0.026 | 0.029 | 0.018 | 0.031 | 0.019 | 0.018 | 0.021 | 0.021 | 0.034 | 0.038 | 0.044 | 0.075 | 0.042 | 0.046 |
| Coefficient of variation | 37 | 34 | 32 | 85 | 113 | 51 | 42 | 62 | 37 | 28 | 28 | 26 | 42 | 35 | 40 | 83 | 49 | 38 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

Raised concentrations of phosphorus compounds in waters discharged from fish farms are due to the fact that fish farmers aim to obtain quick body gains of fish and therefore prefer intensive feeding. Only some of the compounds in feeds are incorporated into fish bodies, while most remain in water (Tucholski 1994; Orlik et al. 2005; Raczyńska et al. 2006). In fact, it is estimated that just 5–20% of the matter is used to build fish bodies while the rest stays in water and contributes to its contamination (Madeyski 2001; Sikora et al. 2009).

When analysing average increases in phosphorus at both types of fish farms, it was discovered that the farms with recirculating systems generated a much higher increase in the concentration of $P–PO_4$ in discharged waters (by 0.026 $mg/dm^3$ on average) than the farms with an open flow system (0.014 $mg/dm^3$ more). The results concerning $P_{total}$ were opposite. A much higher average increase in total phosphorus was observed in waters discharged from the farms without water recirculation (0.026 $mg/dm^3$) than from the ones which used recirculating water system (0.003 $mg/dm^3$).

Nitrogen enters waters mainly in the form of mineral compounds originating from the process of decomposition of organic nitric compounds, from atmospheric precipitation and from soils. It appears in water in the forms with different oxidation numbers, in organic and inorganic bonds and as free dissolved nitrogen. Microbiological transformations of nitrogen which take place in an aquatic environment are analogous to the ones in a soil habitat. Organic nitrogen most often occurs in water as proteins, amino acids and non-protein organic compounds, i.e. urea, amine, pyridine, purine. In natural waters, nitrogen originates from dead animal and plant organisms and from food leftovers (Piedrahita 2003).

Inorganic nitrogen is present in the form of ammonia, ammonium salts as well as nitrates, nitrites and cyanogens. Some organic nitrogen is reduced by biodegradation to ammonia, which does not accumulate in water under aerobic conditions but is oxidized to nitrites and nitrates (Łysak 1995; Avnimelech 1999). Nitrification and denitrification processes take place in water. Denitrification leads to a change in the content of nitrates and enriches water in free molecular nitrogen, which is released to the atmosphere when the conditions are suitable. Thus, reduction of nitrates causes a decrease in the total content of nitrogen in a water reservoir (Koc et al. 2006; Makuch et al. 2009).

In the waters supplying four of the analyzed fish farms, nitrogen appeared mostly in the organic form, from 52% of $N_{total}$ (2-RAS) to 72% (1-OS). In two farms (2-OS and 3-RAS), the mineral form of nitrogen prevailed, reaching 77% of the total nitrogen at farm 2-OS and 57% at farm 3-RAS. The average concentration

of total nitrogen in the waters supplied to the fish farms varied from 0.54 ± 0.04 mg/dm$^3$ (1-RAS) to 2.14 ± 0.43 mg/dm$^3$ (2-OS), and the median of the concentrations was 0.54 mg/dm$^3$ (1-RAS) to 2.11 mg/dm$^3$ (2-OS) (Tables 9.12, 9.13). Once they had been used for fish production, the spent waters from the farms with open flow systems contained less (by 0.074 mg/dm$^3$ at farm 2-OS and 0.393 mg/dm$^3$ at farm 3-OS) or slightly more total nitrogen (0.135 mg/dm$^3$ at farm 1-OS). In contrast, at the fish farms equipped with recirculating aquaculture systems, an increase in the total nitrogen concentration in used waters was higher, ranging from 0.150 mg/dm$^3$ (1-RAS) to 2.535 mg/dm$^3$ (3-RAS).

**Table 9.12.** Content of $N_{total}$ in supplied and released waters at trout farms (mg/dm$^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 0.97 | 1.15 | 1.05 | 2.14 | 2.20 | 1.75 | 1.65 | 1.39 | 1.52 | 0.54 | 0.82 | 0.69 | 1.44 | 2.18 | 2.23 | 0.76 | 1.18 | 3.29 |
| Min | 0.80 | 0.91 | 0.83 | 1.41 | 0.34 | 1.20 | 1.03 | 0.80 | 0.89 | 0.49 | 0.64 | 0.60 | 1.08 | 1.85 | 1.85 | 0.49 | 0.88 | 2.28 |
| Max | 1.41 | 1.45 | 1.34 | 2.68 | 3.33 | 2.30 | 2.77 | 1.71 | 2.03 | 0.61 | 1.49 | 0.89 | 1.93 | 2.69 | 2.82 | 0.95 | 1.43 | 3.79 |
| Median | 0.88 | 1.10 | 1.02 | 2.11 | 2.29 | 1.76 | 1.35 | 1.49 | 1.55 | 0.54 | 0.68 | 0.68 | 1.36 | 2.00 | 2.02 | 0.80 | 1.19 | 3.44 |
| SD | 0.22 | 0.20 | 0.20 | 0.43 | 1.02 | 0.40 | 0.70 | 0.33 | 0.39 | 0.04 | 0.33 | 0.11 | 0.32 | 0.37 | 0.45 | 0.18 | 0.23 | 0.58 |
| Coefficient of variation | 23 | 18 | 19 | 20 | 46 | 23 | 42 | 24 | 25 | 07 | 40 | 15 | 22 | 17 | 20 | 23 | 19 | 17 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

**Table 9.13.** Content of $N_{org.}$ in supplied and released waters at trout farms (mg/dm$^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 0.70 | 0.85 | 0.75 | 0.50 | 0.67 | 0.68 | 0.87 | 0.59 | 0.75 | 0.36 | 0.61 | 0.49 | 0.74 | 0.59 | 0.69 | 0.44 | 0.46 | 1.31 |
| Min | 0.41 | 0.70 | 0.40 | 0.22 | 0.31 | 0.30 | 0.37 | 0.48 | 0.49 | 0.27 | 0.38 | 0.38 | 0.47 | 0.26 | 0.20 | 0.16 | 0.18 | 0.64 |
| Max | 1.22 | 1.20 | 1.01 | 1.22 | 1.20 | 1.01 | 1.96 | 0.74 | 1.15 | 0.44 | 1.36 | 0.75 | 1.03 | 0.95 | 1.65 | 0.61 | 0.68 | 2.85 |
| Median | 0.63 | 0.81 | 0.79 | 0.30 | 0.67 | 0.81 | 0.57 | 0.58 | 0.74 | 0.37 | 0.45 | 0.47 | 0.74 | 0.62 | 0.47 | 0.50 | 0.51 | 0.94 |
| SD | 0.29 | 0.18 | 0.22 | 0.38 | 0.30 | 0.28 | 0.63 | 0.12 | 0.23 | 0.06 | 0.38 | 0.14 | 0.25 | 0.29 | 0.56 | 0.17 | 0.18 | 0.86 |
| Coefficient of variation | 41 | 21 | 30 | 76 | 45 | 41 | 72 | 20 | 30 | 17 | 62 | 28 | 33 | 49 | 81 | 38 | 39 | 65 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

Our analysis of the effect of both water management technologies (OS and RAS) on concentration of $N_{total}$ in discharged waters showed that the farms using flow-through systems had reduced concentrations of total nitrogen in spent waters compared to the supplied waters by an average of 0.15 mg/dm$^3$,

whereas the fish farms fitted with RAS generated a higher content of $N_{total}$ in discharged than in supplied waters, with an average increase equal to 1.16 mg/dm$^3$.

When analyzing the average content of mineral nitrogen in waters supplying the fish farms, it was determined that the lowest mineral nitrogen concentration appeared in waters at farm 1-RAS (0.18 mg/dm$^3$), while the highest one was at farm 2-OS (1.64 mg/dm$^3$). The main contributor was $N–NO_3$, whose concentration varied from 0.10±0.07 mg/dm$^3$ (1-RAS) to 1.59±0.70 mg/dm$^3$ (2-OS), while the median of the concentration varied within the range of 0.11 mg/dm$^3$ (1-RAS) to 1.79 mg/dm$^3$ (2-OS) (Tables 9.14–9.16). At farm 2-RAS and, periodically, at farm 1-OS, the $N–NO_3$ concentration threshold values were exceeded. Additionally, norms set for $N–NH_4$ were exceeded at farm 3-RAS (Regulation of the Minister for the Environment of 4 October 2002, Journal of Law No 176).

**Table 9.14.** Content of $N–NH_4$ in supplied and released waters at trout farms (mg/dm$^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 0.09 | 0.11 | 0.11 | 0.04 | 0.06 | 0.10 | 0.06 | 0.08 | 0.05 | 0.07 | 0.09 | 0.07 | 0.09 | 0.80 | 0.73 | 0.19 | 0.48 | 1.76 |
| Min | 0.05 | 0.06 | 0.05 | 0.00 | 0.00 | 0.05 | 0.00 | 0.00 | 0.00 | 0.04 | 0.04 | 0.04 | 0.05 | 0.60 | 0.24 | 0.04 | 0.23 | 0.56 |
| Max | 0.19 | 0.21 | 0.22 | 0.09 | 0.11 | 0.15 | 0.13 | 0.16 | 0.16 | 0.10 | 0.16 | 0.11 | 0.14 | 0.92 | 0.93 | 0.82 | 0.66 | 2.51 |
| Median | 0.06 | 0.08 | 0.07 | 0.05 | 0.07 | 0.10 | 0.06 | 0.06 | 0.04 | 0.06 | 0.06 | 0.06 | 0.09 | 0.82 | 0.80 | 0.07 | 0.48 | 1.97 |
| SD | 0.07 | 0.07 | 0.07 | 0.03 | 0.03 | 0.04 | 0.04 | 0.06 | 0.06 | 0.03 | 0.06 | 0.03 | 0.04 | 0.12 | 0.25 | 0.31 | 0.14 | 0.80 |
| Coefficient of variation | 82 | 59 | 66 | 75 | 51 | 42 | 67 | 75 | 112 | 42 | 64 | 43 | 45 | 15 | 34 | 167 | 30 | 45 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

**Table 9.15.** Content of $N–NO_2$ in supplied and released waters at trout farms (mg/dm$^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 0.0139 | 0.0142 | 0.0144 | 0.0081 | 0.0188 | 0.0177 | 0.0059 | 0.0086 | 0.0126 | 0.0063 | 0.0069 | 0.0080 | 0.0294 | 0.0393 | 0.0380 | 0.0073 | 0.0078 | 0.0408 |
| Min | 0.0040 | 0.0043 | 0.0052 | 0.0025 | 0.0094 | 0.0057 | 0.0001 | 0.0064 | 0.0064 | 0.0041 | 0.0051 | 0.0059 | 0.0148 | 0.0070 | 0.0070 | 0.0015 | 0.0016 | 0.0083 |
| Max | 0.0321 | 0.0297 | 0.0315 | 0.0238 | 0.0529 | 0.0315 | 0.0113 | 0.0154 | 0.0264 | 0.0081 | 0.0092 | 0.0101 | 0.0457 | 0.0784 | 0.0736 | 0.0110 | 0.0117 | 0.0618 |
| Median | 0.0068 | 0.0070 | 0.0075 | 0.0058 | 0.0127 | 0.0186 | 0.0051 | 0.0079 | 0.0097 | 0.0053 | 0.0060 | 0.0086 | 0.0270 | 0.0317 | 0.0313 | 0.0071 | 0.0074 | 0.0395 |
| SD | 0.0106 | 0.0099 | 0.0100 | 0.0072 | 0.0153 | 0.0086 | 0.0038 | 0.0034 | 0.0070 | 0.0017 | 0.0017 | 0.0015 | 0.0100 | 0.0236 | 0.0219 | 0.0033 | 0.0035 | 0.0179 |
| Coefficient of variation | 76 | 70 | 69 | 89 | 81 | 48 | 63 | 39 | 56 | 28 | 24 | 18 | 34 | 60 | 58 | 45 | 45 | 44 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

**Table 9.16.** Content $N–NO_3$ w wodach dopływających i odpływających z obiektów chowu pstrąga ($mg/dm^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 0.17 | 0.17 | 0.18 | 1.59 | 1.45 | 0.95 | 0.71 | 0.71 | 0.70 | 0.10 | 0.12 | 0.12 | 0.57 | 0.75 | 0.76 | 0.24 | 0.24 | 0.35 |
| Min | 0.12 | 0.12 | 0.11 | 0.13 | 0.02 | 0.13 | 0.35 | 0.09 | 0.07 | 0.09 | 0.09 | 0.09 | 0.10 | 0.33 | 0.40 | 0.06 | 0.17 | 0.15 |
| Max | 0.29 | 0.20 | 0.26 | 2.22 | 2.40 | 1.66 | 0.90 | 0.91 | 0.94 | 0.12 | 0.20 | 0.16 | 0.89 | 1.01 | 0.93 | 0.31 | 0.29 | 0.47 |
| Median | 0.15 | 0.18 | 0.17 | 1.79 | 1.83 | 0.89 | 0.78 | 0.84 | 0.82 | 0.11 | 0.11 | 0.12 | 0.64 | 0.77 | 0.85 | 0.27 | 0.24 | 0.37 |
| SD | 0.07 | 0.04 | 0.07 | 0.70 | 0.98 | 0.47 | 0.20 | 0.31 | 0.32 | 0.02 | 0.04 | 0.03 | 0.31 | 0.22 | 0.20 | 0.10 | 0.06 | 0.11 |
| Coefficient of variation | 38 | 24 | 36 | 44 | 67 | 49 | 29 | 44 | 46 | 15 | 32 | 20 | 54 | 30 | 26 | 41 | 24 | 32 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

In the water depths of ponds with RAS and at farm 1-OS, water quality was assessed to have deteriorated in respect of the content of $N_{min}$. In spent waters discharged from these farms, the concentration of mineral nitrogen rose by 0.03–1.72 $mg/dm^3$ and ranged from 0.20 $mg/dm^3$ (1-RAS) to 2.15 $mg/dm^3$ (3-RAS). In turn, at two farms with an open flow system (2-OS and 3-OS), the concentration of $N_{min}$ in spent waters was reduced by 0.58 $mg/dm^3$ and 0.02 $mg/dm^3$.

Among all the mineral forms of nitrogen, the highest increase in the concentration in discharged waters was observed for ammonia nitrogen. A much higher increase in its concentration was also observed at the farms using recirculating systems, for example at 3-RAS it reached 1.57 $mg/dm^3$ and at 2-RAS it equalled 0.64 $mg/dm^3$. It was only at farm 1-RAS that the concentration of $N–NH_4$ in discharged waters was on the same level as in the supplied waters. At the farms with an open-flow water system, the average increase in the concentration of $N–NH_4$ was lower and reached 0.02 and 0.06 $mg/dm^3$ at 1-OS and 2-OS, while in the water discharged from farm 3-OS it was even reduced by 0.01$mg/dm^3$.

While analyzing changes in the concentration of mineral nitrogen in waters discharged from the fish farms, it was concluded that the concentration of $N_{min}$ in water released from the farms with open water flow was reduced by an average of 0.19 $mg/dm^3$, while the water discharged from the RAS farms contained increased levels of mineral nitrogen, higher by an average 0.86 $mg/dm^3$.

### 9.6. Water salinity at trout farms

Bicarbonates ($HCO_3^-$) originate from liming of calcium and magnesium carbonates. As a result, they are widespread in waters which have contact with air, e.g. in surface waters and in shallow groundwater. In unpolluted waters, the content of bicarbonates may be as high as a few hundred of $mg/dm^3$ (Choiński 1995).

According to the dependence cited by Boyd (1982), there is a dynamic equilibrium in water between carbon dioxide $CO_2$, bicarbonate ions $HCO_3^-$, carbonate ions $CO_2^{2-}$ and reaction (pH). When the content of $CO_2$ declines, the carbonate balance is disturbed and then $CO_2$ can be taken up from carbonates.

Then, decomposition of acid carbonates $HCO_3^-$ as well as $CO_3^{2-}$ may occur (Dojlido 1995) and hydroxyl ions as well as $CO_2$ will appear. Thus, the abundance of water in bicarbonates (acid calcium bicarbonate) is an indicator suggesting a large resource of carbon dioxide stimulating primary production.

The average content of carbonates in waters supplied to the ponds ranged from 119.7 ± 1.6 mg/dm$^3$ (3-RAS) to 172.2 ± 6.9 mg/dm$^3$ (2-OS), while the median of concentrations varied from 119.0 mg/dm$^3$ (3-RAS) to 150.0 mg/dm$^3$ (2-OS) (Table 9.17). During the whole period of investigations, the content of carbonates in supplied waters was on a relatively stable level and the coefficient of variation ranged from 1% to 9%. In water depths of the ponds, a slight reduction in the concentration of $HCO_3^-$ was observed and the discharged waters contained 1.83-4.0 mg/dm$^3$ less of these compounds at most farms. Two farms, 3-OS and 3-RAS, were an exception in that their waters were found to be of inferior quality and their concentration of carbonates had risen by 1.33 mg/dm$^3$ and 10.50 mg/dm$^3$.

**Table 17.** Content of $HCO_3^-$ in supplied and released waters at trout farms (mg/dm$^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 144.0 | 144.7 | 142.2 | 172.2 | 163.8 | 169.2 | 140.3 | 140.3 | 141.7 | 140.3 | 137.0 | 136.3 | 132.2 | 131.0 | 129.8 | 119.7 | 121.3 | 130.2 |
| Min | 132.0 | 132.0 | 132.0 | 136.0 | 128.0 | 132.0 | 128.0 | 128.0 | 136.0 | 132.0 | 132.0 | 132.0 | 119.0 | 119.0 | 123.0 | 119.0 | 119.0 | 119.0 |
| Max | 163.0 | 163.0 | 154.0 | 156.0 | 150.0 | 156.0 | 158.0 | 154.0 | 154.0 | 165.0 | 149.0 | 148.0 | 154.0 | 150.0 | 145.0 | 123.0 | 125.0 | 141.0 |
| Median | 140.5 | 139.5 | 138.5 | 150.0 | 145.0 | 141.0 | 137.0 | 139.5 | 139.5 | 134.0 | 135.0 | 135.0 | 130.0 | 131.0 | 128.0 | 119.0 | 121.0 | 131.0 |
| SD | 11.8 | 12.9 | 9.6 | 6.9 | 7.5 | 8.0 | 11.4 | 9.0 | 7.2 | 13.1 | 6.4 | 6.0 | 11.7 | 10.7 | 8.2 | 1.6 | 2.3 | 8.3 |
| Coefficient of variation | 8 | 9 | 7 | 4 | 5 | 5 | 8 | 6 | 5 | 9 | 5 | 4 | 9 | 8 | 6 | 1 | 2 | 6 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

In surface waters, calcium appears as dissolved calcium carbonate and its content depends on the presence of carbon dioxide in water (Sidoruk et al. 2006; Degefu et al. 2011). Calcium, alongside some other elements and compounds such as magnesium, bicarbonates and sulphates, shapes the hydrochemical type of most waters circulating within drainage basins located in young glacial landscapes, and high calcium content in surface waters is predominantly an effect of its intensive leaching from soils (Koc et al. 2003). In a moderate climate, calcium is leached from soils, a process which is encouraged by acid rains. An adequately high concentration of calcium in water is important because of its buffering properties. It is also significant for primary production by ensuring sufficient $CO_2$ concentration for photosynthesis (Kajak 2001).

The results of our tests on the content of calcium in waters supplied to the fish farms showed that its concentration was similar at all the farms and varied from 55.3 ± 3.7 mg/dm$^3$ (1-RAS) to 67.5 ± 9.0 mg/dm$^3$ (2-OS). The median for the calcium concentration was from 53.1 mg/dm$^3$ (2-RAS) to 69.6 mg/dm$^3$ (2-OS), and the coefficient of variation was between 6% and 13% (Table 9.18). Calcium levels in waters supplied to the pond during the experiment did not exceed the norms set for water purity class I (Regulation of the Minister for the Environment of 9 November 2011, Journal of Law No 257). The content of calcium in waters used for fish aquaculture was not observed to have been changed considerably by fish rearing. In waters discharged from farms 1-OS, 2-OS and 1-RAS, a slight increase in the concentration of $Ca^{2+}$ appeared (by 0.18–0.80 mg/dm$^3$), while in waters released from the other farms, the content of this element was reduced by 0.38-1.65 mg/dm$^3$.

**Table 9.18.** Content of $Ca^{2+}$ in supplied and released waters at trout farms (mg/dm$^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 56.6 | 57.7 | 56.8 | 67.5 | 68.4 | 68.3 | 66.2 | 64.7 | 64.6 | 55.3 | 57.7 | 55.8 | 56.0 | 56.2 | 55.4 | 59.7 | 58.6 | 59.3 |
| Min | 49.2 | 49.2 | 42.0 | 49.2 | 49.2 | 42.0 | 60.4 | 60.4 | 60.4 | 49.2 | 55.2 | 49.2 | 51.6 | 51.6 | 51.6 | 54.2 | 54.2 | 54.2 |
| Max | 62.8 | 62.8 | 63.7 | 77.0 | 75.8 | 79.2 | 72.0 | 72.1 | 67.4 | 58.9 | 62.3 | 59.4 | 62.8 | 62.8 | 62.8 | 62.8 | 65.2 | 62.8 |
| Median | 54.7 | 55.4 | 54.7 | 69.6 | 70.0 | 69.6 | 66.3 | 62.8 | 67.4 | 55.2 | 55.2 | 55.2 | 53.1 | 54.2 | 53.7 | 57.9 | 55.9 | 57.9 |
| SD | 5.2 | 5.8 | 8.6 | 9.0 | 9.0 | 12.3 | 3.9 | 4.3 | 3.3 | 3.7 | 3.1 | 3.6 | 4.6 | 4.1 | 3.9 | 3.6 | 4.4 | 3.3 |
| Coefficient of variation | 9 | 10 | 15 | 13 | 13 | 18 | 6 | 7 | 5 | 7 | 5 | 6 | 8 | 7 | 7 | 6 | 8 | 6 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

Manganese compounds in water originate primarily from the processes of dissolution of such minerals as dolomites or magnesites. Changes in concentrations of magnesium in surface waters are associated, for example, with the presence of humic substances in water. These substances may occur in the dissolved or collodial forms, creating magnesium-humic complexes. The capability of humic substances to bind magnesium cations is largely dependent on the water reaction (pH) and, consequently, on the degree of dissociation of function groups (Kolanek et al. 2002). Typically, much higher concentrations of magnesium than calcium are found, which is probably because the former element is much more intensively absorbed by plants and appears in higher concentrations in atmospheric precipitations (Wróbel et al. 1990).

The average concentration of magnesium in waters supplied to most of the examined fish farms was from 5.6 ± 0.8 mg/dm$^3$ (1-RAS) to 7.7 ± 0.6 mg/dm$^3$ (1-OS), while the median of the concentrations was from 5.6 mg/dm$^3$ (3-RAS) to 7.7 mg/dm$^3$ (1-OS) (Table 9.19). The waters supplied to farm 2-OS were slightly different, as their average content of $Mg^{2+}$ was much lower than in the other waters, reaching 1.7 ± 2.7 mg/dm$^3$, and the median of the concentration was 0.7 mg/dm$^3$. This was due to completely different geological and soil conditions in the drainage basin of the watercourse supplying that fish farm. The supply of water to fish farm 2-OS was characterized by the highest coefficient of variation of the concentration of $Mg^{2+}$, which went up to 158%. For comparison, at the other farms it ranged from 8% to 14%. At most of the fish farms, deep waters of the ponds contained higher concentrations of magnesium and the increase relative to the supplied waters was from 0.083 mg/dm$^3$ (1-RAS) to 0.483 mg/dm$^3$ (3-RAS). Two farms, 3-OS and 2-RAS, were an exception, because there the concentration of magnesium in pond waters decreased by 0.55 mg/dm$^3$ and 0.30 mg/dm$^3$.

**Table 9.19.** Content of $Mg^{2+}$ in supplied and released waters at trout farms (mg/dm$^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 7.7 | 8.2 | 7.9 | 1.7 | 1.9 | 1.9 | 6.8 | 6.4 | 6.3 | 5.6 | 5.4 | 5.7 | 6.0 | 5.9 | 5.7 | 5.9 | 5.6 | 6.3 |
| Min | 6.9 | 7.5 | 7.5 | 0.5 | 0.7 | 0.7 | 5.8 | 5.6 | 5.5 | 4.3 | 4.3 | 4.8 | 5.3 | 5.5 | 4.3 | 5.1 | 4.9 | 5.0 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Max | 8.5 | 9.2 | 8.3 | 7.7 | 8.0 | 8.2 | 8.1 | 7.2 | 7.0 | 6.6 | 6.6 | 6.4 | 6.9 | 6.8 | 6.3 | 7.1 | 6.4 | 7.6 |
| Median | 7.7 | 7.8 | 8.0 | 0.7 | 0.8 | 0.9 | 6.8 | 6.4 | 6.3 | 5.7 | 5.3 | 5.8 | 6.0 | 5.8 | 6.0 | 5.6 | 5.5 | 6.2 |
| SD | 0.6 | 0.8 | 0.3 | 2.7 | 2.7 | 2.8 | 0.9 | 0.7 | 0.5 | 0.8 | 0.9 | 0.6 | 0.6 | 0.5 | 0.7 | 0.7 | 0.6 | 0.9 |
| Coefficient of variation | 8 | 10 | 4 | 158 | 144 | 145 | 13 | 11 | 8 | 14 | 17 | 11 | 10 | 8 | 13 | 13 | 10 | 15 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

Our analysis of the changes in concentrations of magnesium at the two types of fish farms enabled us to conclude that the concentration of $Mg^{2+}$ in discharged water from the farms with recirculating systems increased by 0.09 mg/dm$^3$ compared to the supplied waters.

Potassium, like magnesium compounds, occurs in the bedrock in hardly soluble forms, e.g. as a component of orthoclase or biotite. In the subterrestrial environment, migration of potassium is hindered due to its adsorption by loamy minerals.

The average concentration of potassium ions in waters supplied to the fish farms was from 0.8 ± 0.8 mg/dm$^3$ (2-OS) to 2.5 ± 1.0 mg/dm$^3$ (2-RAS). The median of the concentration varied from 0.7 mg/dm$^3$ (2-OS) to 2.4 mg/dm$^3$ (1-OS) (Table 9.20). The highest variation of the concentrations of potassium, up to 98%, was determined for the water supplied to farm 2-OS, while the most stable situation was found at farm 1-OS (14%). Passing through the fish ponds, water only slightly changed its content of potassium, for example the concentration of $K^+$ was raised by 0.23 mg/dm$^3$, 0.15 mg/dm$^3$ and 0.72 mg/dm$^3$ in waters discharged from farms 1-OS, 2-OS and 3-RAS, but the waters released from farms 3-OS, 1-RAS and 2-RAS contained 0.08 mg/dm$^3$, 0.35 mg/dm$^3$ and 0.30 mg/dm$^3$ less potassium, respectively.

**Table 20.** Content of $K^+$ in supplied and released waters at trout farms (mg/dm$^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 2.4 | 2.6 | 2.5 | 0.8 | 1.0 | 1.0 | 1.7 | 1.8 | 1.7 | 1.7 | 1.3 | 1.3 | 2.5 | 2.2 | 2.2 | 1.5 | 1.6 | 2.2 |
| Min | 1.9 | 2.2 | 2.1 | 0.3 | 0.5 | 0.7 | 1.4 | 1.4 | 1.4 | 1.3 | 1.3 | 1.3 | 2.0 | 2.1 | 2.1 | 1.3 | 1.3 | 2.1 |
| Max | 2.8 | 3.0 | 3.0 | 2.6 | 2.7 | 2.7 | 2.2 | 2.6 | 1.9 | 3.5 | 1.4 | 1.4 | 4.5 | 2.3 | 2.4 | 2.0 | 1.8 | 2.4 |
| Median | 2.4 | 2.6 | 2.5 | 7.7 | 8.0 | 8.2 | 1.7 | 1.7 | 1.7 | 1.3 | 1.3 | 1.3 | 2.1 | 2.2 | 2.2 | 1.4 | 1.6 | 2.2 |
| SD | 0.3 | 0.3 | 0.3 | 0.8 | 0.8 | 0.7 | 0.3 | 0.5 | 0.2 | 0.9 | 0.1 | 0.1 | 1.0 | 0.1 | 0.2 | 0.3 | 0.2 | 0.1 |
| Coefficient of variation | 14 | 11 | 12 | 98 | 74 | 71 | 18 | 26 | 11 | 53 | 4 | 4 | 39 | 4 | 7 | 18 | 10 | 4 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

Sodium cations in surface waters are as widespread as calcium compounds. Salt can originate from dissolved evaporates and from anthropogenic pollutants, but another common source of salt are sodium minerals. Cations of sodium, as well as potassium, are adsorbed by loamy minerals (Chełmicki 2001).

In waters supplied to the fish farms, the average concentration of sodium was on a low level, and its range was within 4.6 ± 2.3 mg/dm$^3$ (2-OS) to 9.3 ± 1.4 mg/dm$^3$ (3-OS). The highest coefficient of variation for this variable was determined at farm 2-OS (50%), while at the other farms it ranged from 14% to

24% (Table 9.21). In the waters released from farms 1-OS, 2-OS and 3-RAS, an increase in the concentration of sodium was observed (by 0.25 mg/dm$^3$ to 1.45 mg/dm$^3$), while at farms 2-OS and 2-RAS, it was reduced by 0.03 mg/dm$^3$ and 0.07 mg/dm$^3$. Finally, at farm 1-RAS, the concentration of sodium in supplied and discharged waters was the same.

**Table 9.21.** Content $Na^+$ in supplied and released waters at trout farms (mg/dm$^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 9.1 | 9.0 | 9.4 | 4.6 | 5.1 | 5.2 | 9.3 | 9.3 | 9.3 | 5.6 | 5.5 | 5.6 | 6.7 | 6.8 | 6.6 | 6.4 | 6.6 | 7.8 |
| Min | 6.0 | 5.6 | 6.0 | 2.9 | 2.9 | 3.2 | 6.8 | 7.0 | 6.8 | 4.0 | 4.0 | 4.0 | 4.8 | 4.8 | 5.0 | 5.0 | 5.0 | 5.6 |
| Max | 11.4 | 11.4 | 11.4 | 8.7 | 8.5 | 8.7 | 11.0 | 11.0 | 11.0 | 6.1 | 6.2 | 6.3 | 8.3 | 8.7 | 7.6 | 7.6 | 7.6 | 9.4 |
| Median | 9.6 | 9.4 | 10.1 | 3.6 | 4.4 | 4.3 | 9.6 | 9.5 | 9.5 | 6.0 | 5.8 | 5.8 | 6.8 | 7.0 | 7.0 | 6.2 | 6.6 | 8.0 |
| SD | 2.2 | 2.2 | 2.0 | 2.3 | 2.4 | 2.3 | 1.4 | 1.3 | 1.4 | 0.8 | 0.8 | 0.8 | 1.4 | 1.5 | 1.2 | 0.9 | 1.0 | 1.3 |
| Coefficient of variation | 24 | 24 | 21 | 50 | 46 | 45 | 15 | 14 | 15 | 14 | 15 | 15 | 21 | 21 | 17 | 15 | 15 | 17 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

When analyzing changes in the concentrations of potassium between supplied and discharged waters in the two groups of fish farms, it was noticed that a higher mean increase (by 0.10 mg/dm$^3$ on average) appeared at the farms with water recirculation, where the average rise in the potassium concentration was 0.02 mg/dm$^3$.

Juxtaposition of the increases in concentrations of sodium in discharged versus supplied waters at both types of fish farms demonstrates that farms with water recirculation produce a stronger effect on water quality than farms with open flow. At the RAS farms, the increase in the concentration of $N^+$ in released water was 0.47 mg/dm$^3$ on average, being much lower at the OS farms, namely 0.27 mg/dm$^3$ on average.

Sulphates permeate into waters primarily through decomposition of minerals, but also with atmospheric precipitation or as products of oxidation of sulphur and sulphates, which originate from decomposition of organic proteins (Koc et al. 2009). Thus, elevated concentrations of sulphates in surface waters are attributed to human activity. In waters containing high amounts of aluminium, the concentration of sulphates can go up to a few hundreds of mg/dm$^3$ (Choiński 1995).

The water used to supply the analyzed trout farms was classified as belonging to water purity class I, and the average concentration of sulphates ranged from 34.4 ± 16.1 mg/dm$^3$ (2-OS) to 109.8 ± 40.7 mg/dm$^3$ (3-RAS) (Table 9.22). The median of sulphate concentrations varied from 35.1 mg/dm$^3$ (2-OS) to 120 mg/dm$^3$ (3-RAS). In deep water of the ponds at farms 2-OS, 2-RAS and 3-RAS, an increase in the concentration of $SO_4^{2-}$ was determined and the analogous increase in the waters discharged from these farms was 8.4–34.8 mg/dm$^3$ higher. At the other farms, a reduction in the concentration of sulphates by 4.0–7.8 mg/dm$^3$ was determined. With respect to farm 3-RAS, an increase in sulphates in discharged waters was periodically so high (up to 180.8 mg/dm$^3$) that the water was classified as representing water purity class II.

**Table 9.22.** Content of $SO_4^{2-}$ in supplied and released waters at trout farms (mg/dm$^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 63.0 | 50.6 | 55.2 | 34.4 | 38.5 | 43.7 | 92.2 | 93.0 | 88.2 | 51.0 | 51.8 | 45.8 | 87.4 | 73.5 | 95.8 | 109.8 | 115.7 | 144.6 |
| Min | 35.1 | 17.6 | 12.7 | 20.9 | 14.6 | 20.9 | 48.3 | 47.3 | 44.9 | 35.6 | 45.4 | 26.8 | 68.3 | 18.6 | 48.3 | 43.4 | 62.9 | 106.3 |
| Max | 91.7 | 84.3 | 82.9 | 66.5 | 109.1 | 92.1 | 118.5 | 140.9 | 124.8 | 97.5 | 56.5 | 58.5 | 107.7 | 112.1 | 151.1 | 145.6 | 143.1 | 180.8 |
| Median | 61.0 | 48.9 | 61.4 | 35.1 | 21.2 | 36.6 | 97.9 | 100.2 | 89.7 | 39.0 | 52.4 | 49.0 | 87.9 | 85.8 | 89.9 | 120.7 | 120.2 | 147.0 |
| SD | 22.5 | 28.8 | 26.8 | 16.1 | 34.3 | 24.2 | 27.3 | 36.3 | 32.6 | 24.1 | 3.7 | 11.3 | 16.3 | 35.9 | 37.1 | 40.7 | 29.6 | 31.9 |
| Coefficient of variation | 36 | 57 | 48 | 47 | 89 | 55 | 30 | 39 | 37 | 47 | 7 | 25 | 19 | 49 | 39 | 37 | 26 | 22 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

Based on the analysis of changes in the content of sulphates in released waters relative to supplied waters in both groups of fish farms, it can be claimed that the farms with open flow systems contained reduced concentrations of $SO_4^{2-}$ in released waters (by 0.8 mg/dm$^3$ on average), whereas the farms fitted with recirculating aquaculture systems released water that contained higher concentrations of this component (by 12.6 mg/dm$^3$ on average).

Chlorides appear in all types of water bodies in nature. Water resources receive chlorides with atmospheric precipitation, but also from agricultural production and as a result of removing snow and ice from roads. If the concentration of chloride anions in wet atmospheric precipitation is within 1–4 mg/dm$^3$, it corresponds to a concentration of 6–12 mg/dm$^3$ in surface water bodies. According to Sapek (2008), chlorides reach waters mainly with atmospheric precipitations whereas anthropogenic pollution supplies almost negligible quantities of these compounds. Chlorides neither undergo transformations in soil or water nor become adsorbed by soil material. They remain completely soluble in surface waters. However, they are easily taken up by plants and are easily leached from soil, also by surface runoffs (Sapek 2008).

The content of chlorides in the waters supplying the analyzed trout farms classified them as belonging to water purity class I and the concentration of chlorides ranged from 6.7 ± 1.4 mg/dm$^3$ (1-RAS) to 21.3 ± 0.8 mg/dm$^3$ (2-RAS) (Table 9.23). The higher $Cl^-$ concentration in waters released from farm 2-RAS is because the drainage basin of the supplying watercourse lies within an impact zone of the sea. The content of chlorides in waters flowing to the farms was stable throughout the whole period of the experiment and the coefficient of variation was about 4–20%.

**Table 23.** Content of $Cl^-$ in supplied and released waters at trout farms (mg/dm$^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 11.8 | 11.7 | 11.7 | 8.0 | 8.4 | 9.0 | 21.3 | 21.0 | 20.7 | 6.7 | 6.0 | 5.8 | 12.5 | 11.0 | 10.3 | 13.8 | 14.7 | 12.8 |
| Min | 9.0 | 9.0 | 9.0 | 7.0 | 8.0 | 8.0 | 20.0 | 19.0 | 19.0 | 5.0 | 5.0 | 5.0 | 10.0 | 9.0 | 8.0 | 11.0 | 11.0 | 11.0 |
| Max | 15.0 | 14.0 | 14.0 | 11.0 | 11.0 | 11.0 | 22.0 | 22.0 | 22.0 | 9.0 | 7.0 | 7.0 | 24.0 | 16.0 | 14.0 | 17.0 | 20.0 | 14.0 |

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Median | 12.0 | 11.5 | 11.0 | 8.0 | 8.0 | 8.0 | 21.5 | 21.5 | 21.0 | 6.5 | 6.0 | 6.0 | 10.0 | 10.0 | 10.0 | 13.5 | 13.5 | 13.0 |
| SD | 2.6 | 1.8 | 2.0 | 1.4 | 1.1 | 1.4 | 0.8 | 1.3 | 1.0 | 1.4 | 0.6 | 0.8 | 5.6 | 2.7 | 2.1 | 2.3 | 3.3 | 1.0 |
| Coefficient of variation | 22 | 15 | 17 | 18 | 13 | 16 | 4 | 6 | 5 | 20 | 11 | 13 | 45 | 24 | 20 | 17 | 22 | 8 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

When the waters had been used for fish farming, a decrease in the concentration of chlorides was observed in waters discharged from most of the fish farms. The highest reduction was determined at the farms using water recirculation systems, where chlorides were depressed by 7–17%, which corresponded to concentrations lower by 0.83–2.17 mg/dm$^3$. In turn, at the farms with an open flow of water a decrease in the concentration of $Cl^-$ was equalled to 0.1 mg/dm$^3$ (1-OS) and 0.6 mg/dm$^3$ (3-OS), but at farm 2-OS it was found to have incrased by about 1.0 mg/dm$^3$.

### 9.7. Heavy metals in trout farms

The content of trace elements depends on a number of natural and man-made factors. Above all, it depends on the geological structure of a given drainage basin, the geomorphology of the landscape and the climatic conditions, which modify the course of rock weathering process and affect activation, migration and accumulation of elements in the environment.

In non-industrial areas, high values of potentially harmful trace elements in bed sediments are predominately an effect of different kinds of human economic activity, especially farming, carried out in the drainage basic area (Bojakowska et al. 2003; Cieszewski et al. 2003). Water pollution with heavy metals is particularly dangerous because these elements are not eliminated during natural water self-purification processes. As a result of various reactions, they bind to organic and inorganic compounds, they can accumulate and eventually, through the biological food chain, they can reach humans in elevated quantities and cause poisoning. The content of Cd and Pb in waters used to supply the analyzed fish farms was low, and equalled 0.0050±0.0 mg/dm$^3$ of Pb (1-OS) to 0.0065 ± 0.0023 mg/dm$^3$ of Cd (3-RAS), while the average concentrations of cadmium at all the farms were on the same level, i.e. less than 0.001 mg/dm$^3$ (Tables 9.24, 9.25). In one case only, that is at farm 3-RAS, the average concentration of cadmium was at a level of 0.0013 mg/dm$^3$. No effect of using waters by aquaculture on the content of Cd and Pb in discharged waters was observed.

**Table 24.** Content of Cd in supplied and released waters at trout farms (mg/dm$^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Min | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Max | 0.001 | 0.001 | 0.001 | 0.0013 | 0.0013 | 0.0013 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Median | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| SD | 0 | 0 | 0 | 0.0001 | 0.0001 | 0.0001 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Coefficient of variation | 0 | 0 | 0 | 11 | 11 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

**Table 25.** Content of Pb in supplied and released waters at trout farms (mg/dm$^3$)

| Parameters | Single water use | | | | | | | | | Multiple water use | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-OS | | | 2-OS | | | 3-OS | | | 1-RAS | | | 2-RAS | | | 3-RAS | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean | 0.0050 | 0.0050 | 0.0050 | 0.0054 | 0.0054 | 0.0054 | 0.0054 | 0.0054 | 0.0054 | 0.0060 | 0.0060 | 0.0060 | 0.0053 | 0.0053 | 0.0053 | 0.0065 | 0.0065 | 0.0072 |
| Min | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 |
| Max | 0.0050 | 0.0050 | 0.0050 | 0.0077 | 0.0077 | 0.0077 | 0.0073 | 0.0073 | 0.0073 | 0.0110 | 0.0110 | 0.0110 | 0.0065 | 0.0065 | 0.0065 | 0.0100 | 0.0100 | 0.0130 |
| Median | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 | 0.0050 |
| SD | 0 | 0 | 0 | 0.0010 | 0.0010 | 0.0010 | 0.0009 | 0.0009 | 0.0009 | 0.0024 | 0.0024 | 0.0024 | 0.0006 | 0.0006 | 0.0006 | 0.0023 | 0.0023 | 0.0035 |
| Coefficient of variation | 0 | 0 | 0 | 19 | 19 | 19 | 17 | 17 | 17 | 41 | 41 | 41 | 12 | 12 | 12 | 35 | 35 | 49 |

Comments: 1 – water before farm; 2 – flow from pond; 3 – flow from farm.

The concentration of cadmium in spent waters was on the same level at all the farms. However, at farm 3-RAS spent water contained 0.001 mg/dm$^3$ more lead than water flowing to that farm. At the other farms, the Pb content in supplied and discharged waters was the same.

### 9.8. Conclusions

1. The temperature of water supplied to trout ponds was within a range of temperatures optimal for salmonid fish and equalled 9.41–13.14°C, while its highest value did not exceed 18.24°C throughout the whole period of observations.
2. Waters supplied to the trout ponds were well aerated and their content of dissolved oxygen ranged between 8.77–10.31 mg/dm$^3$, i.e. 77.13–98.43% of oxygen saturation. After the water passed through the fish ponds at the farms with open flow systems, it was determined to be richer in oxygen by 0.21–1.04 mg/dm$^3$, but discharged waters from the farms with recirculating aquaculture systems were poorer in oxygen by 0.26–1.91 mg/dm$^3$.
3. The water management system had some effect on the $BOD_5$ and $COD_{Cr}$. In spent waters released from the RAS farms, a slight increase in the $BOD_5$ was noticed (by an average 0.39 mg/dm$^3$) and a reduced $COD_{Cr}$ (by about 2.18 mg/dm$^3$). At the farms with open d\flow systems, the increase in the $BOD_5$ was much higher, reaching 2.15 mg/dm$^3$ on average while the $COD_{Cr}$ rose by 2.90 mg/dm$^3$.
4. As a result of intensive feeding of fish, aiming at high body gains, an increase in the concentration of phosphorus in released waters was recorded at all the farms. At the farms with water recirculation,

discharged waters were found to be much richer in $P–PO_4$ (an average of 0.026 mg/dm$^3$) than waters released from the farms with a flow-through system (0.014 mg/dm$^3$). Regarding $P_{total}$, a much higher average increase in its concentration was observed in waters discharged from the farms with open flow system (0.026 mg/dm$^3$) than from the farms with water recirculation (0.003 mg/dm$^3$).

5. The water management systems (OS and RAS) were found to produce a clear effect on the concentration of $N_{total}$ in discharged waters. At the farms with open flow of water, the spent waters contained reduced concentrations of total nitrogen compared to the waters supplied to these farms (less by an average 0.15 mg/dm$^3$), but at the farms with recirculating systems, an increase in the concentration of $N_{total}$ was observed (by 1.16 mg/dm$^3$ on average).
6. Periodically, excessive concentrations of $P_{total}$ were observed in waters supplied to farms 2-RAS and 3-RAS, above the norms set for inland waters used as habitats for salmonid fish.
7. Trout production did not have any considerable effect on water salinity indices at either type of fish farms. A very small increase in salinity was recorded in waters released from the observed fish farms, and the concentrations of $HCO_3^-$ and $Cl^-$ were even reduced in discharged waters.
8. Waters supplied to the fish ponds met all the requirements set for inland waters used as habitats for salmonid fish. These norms, however, were periodically exceeded in the case of $BOD_5$ at most farms (except 2-OS) and $P_{total}$ and $N–NH_4$ at farms 2-RAS and 3-RAS.
9. Depressed values of water quality indices caused by trout aquaculture did not change their attributed water purity class, except farm 3-RAS, where the $BOD_5$, $N–NH_4$ and $SO_4^{2-}$ reached the values that degraded the water from water quality class I to II.

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# 10. The practical side of innovations in technologies of trout farming

Currently, the rearing of rainbow trout requires technologies that provide a high quality product that meets the requirements of consumers and the processing industry and also ensure the preservation of the welfare of these fish. In order to provide the parameters required the breeder ought to proceed in the right way. This is especially important in the case of rainbow trout. These fish are very sensitive to environmental degradation as well as all sorts of physical and chemical changes in breeding performance.

The laws of both Polish and EU rules do not specify the handling of fish. Due to specificity of fish as a specie the rules applicable for other animals are not valid.

Rainbow trout is a specie belonging to the predatory fish. This fact affects the formation of a hierarchy in a breeding flock. This results in the variation in body mass. Therefore, the rainbow trout farming is connected with the proper handling due to frequent sorting of fish and moving to other basins around the farm. Loading fish for transportation in order to sell them for further breeding or for consumption is another very common action. The specie is very sensitive to lack of oxygen and the stress associated with mechanical manipulation during catching for sorting, weighing and loading. Physical factors influencing the physiology of the fish are primarily oxygen availability, and temperature. Manipulation related specifically to harvesting, sorting, weighing and loading for transportation make fish stay out of the water environment. This situation may cause changes in body temperature and lower the fish oxygen uptake capabilities to the level of danger to their lives. Staying out of the water environment leads to deficit in oxygen intake and finally affects the number of anaerobic processes, lactic acid formation, causing acidification of the muscles and decrease of the quality of their muscle tissue. Also the extending stress causes the release of cortisol, the so-called stress hormone that leads to the fish carcass deterioration.

In order to fulfill all the needs coming from rainbow trout farming and manipulation as well as obtain high-quality materials while maintaining welfare the modern methods of production and management are required. To this end, so-called „Archimedean screw" was adapted to the raising and breeding of rainbow trout. It is one of the inventions attributed to Archimedes, which was developed in order to improve raising water used for irrigation.

Innovative use of this device in rainbow trout farming is the fact that it is used for shifting the water along with the fish between the pools and ponds, as well as loading up to a height of 4 m. It is a water screw pump, where the fluid transport is forced by rotation of worm screw. Depending on the diameter of the tube it can move the fish from 5 g to 2.5 kg average body mass along with the water (Phot. 10.1).

Transport of concentrated fish with the water without damaging the fish while the animals are pulled out of their natural aqueous environment. This ensures sufficient oxygen demand and reduces stress. The mechanical sorting of fish has also been developed. This process allows the sorting of fish in two or three assortments in the range of 10–600 g. When sorting the fish are sprayed with water, the amount of which is controlled by means of special valves (Phot. 10.2).

Fish of various sorts are transported to the respective joints (swimming pools) by means of special tubes. Performance of sorting depends on the mode of administration of fish and using the water pump – Archimedean screw can reach up to 1 000 kg/h (Phot. 10.3).

The authors of the project conclude based on preliminary observation that in order to meet the current requirements of farmed fish welfare and to ensure the production of high-quality consumer fish of the salmon family each farm should have a water pump – Archimedean screw.

The scale of Zakrzewski and Guziur (SGZ scale) was used for the first time during the project in order to asses in a practical way individual farms and trout production. The method allowed for comprehensive evaluation of production effectiveness. Using data collected during the project the following parameters were included: production – fish carcass performance, technology – determining the nutritional value of the raw material – the content of protein and total fat and fatty acid ω-3 / ω-6 ratio, and the sensory consumer test: subjective assessment of the overall and the average rating calculated using the respective weights of the different parameters. These parameters were used to create the table as a basis for evaluating the production of rainbow trout. The parameters used depended on the characteristics of fish farming technology. The studies omitted the features common for all tested farms (eg. water content, ash and heavy metal contamination). Taking into account the different meanings of selected features there were used proper parameters that affected final evaluation of the production efficiency.

Based on the observation some conclusions were formulated. The proposed SGZ scale should be a precious help for producers of fish, to facilitate the selection of the optimal technology that is receiving the maximum efficiency while maintaining the high quality of raw fish. The practice should decide what differentiators are to be applied. Along with a change in consumer preferences the already established assumptions may evolve. The proposed model will provide a useful scale in the analysis of economic indicators and marketing of production and economic efficiency as an important element of a business plan and planning of investment in fishing. The proposed method can also be used by the state institutions and non-governmental controls or statistical purposes.

Information acquired by morphological studies also deserves to be mentioned. It has been shown that they are highly sensitive methods of acting and great tool in the assessment of condition and health of rainbow trout. The breeder based on the knowledge presented in the publication of macroscopic morphological study is able to assess the degree of hepatic steatosis and correct the feeding in the right moment.

In addition, pathological assessment allows for the localization of structural deviations and to determine their level of intensity. It gives the database as a clue that allows to evaluate the level of changes as adaptive and damaging. Reading of which allows to draw conclusions about both the state of the fish itself and its environment. This study also shows that it is crucial to provide proper cellular respiration in

rainbow trout farming that is strictly connected with water temperature and oxygen level. Therefore, it is necessary to perform very frequent measurements of oxygen level (eg, using oxygen meters).

The morphological study leads to the conclusion that the observed changes being characteristic of the type of farming in terms of intensity, provide new knowledge regarding the possibility of intervention in the rearing both for the farmer and veterinarian.

**PHOTOGRAPHS**
**(see pp. 133–170)**

Fot. 1.1. Doktor Władysław Kołder (1905–1976), ur. w Zabłociu na Śląsku Cieszyńskim (Zaolziu), absolwent UJ Kraków (1928), w okresie międzywojennym jeden z prekursorów nowoczesnej hodowli pstrąga tęczowego w Polsce, wieloletni inspektor rybacki w Izbach Rolniczych w Katowicach, Kielcach i Krakowie, do emerytury pracownik naukowy Zakładu Biologii Wód AN w Krakowie. Autor ponad 80 prac, w tym pierwszej polskiej monografii (1948) o hodowli i chowie pstrągów w stawach /

Phot. 1.1. Doctor Władysław Kołder (1905–1976), born in Zabłocie in Cieszyn Silesia (Zaolzie), a graduate of the Jagiellonian University Krakow (1928), in the period between one of the pioneers of modern rainbow trout farming in Poland, for many years the fishing inspector in Agricultural Chambers in Katowice, Kielce and Krakow, to retirement employee of the Department of Water Biology Science AN in Krakow. Author of more than 80 papers, including the first Polish monograph (1948) about the breeding and rearing of trout in the ponds

Fot. 1.2. Ośrodek Zarybieniowy PZW w Łopusznej nad Dunajcem (lata 60. XX w.) / Phot. 1.2. PZW stocking center in Łopuszna over Dunajec (1960's)

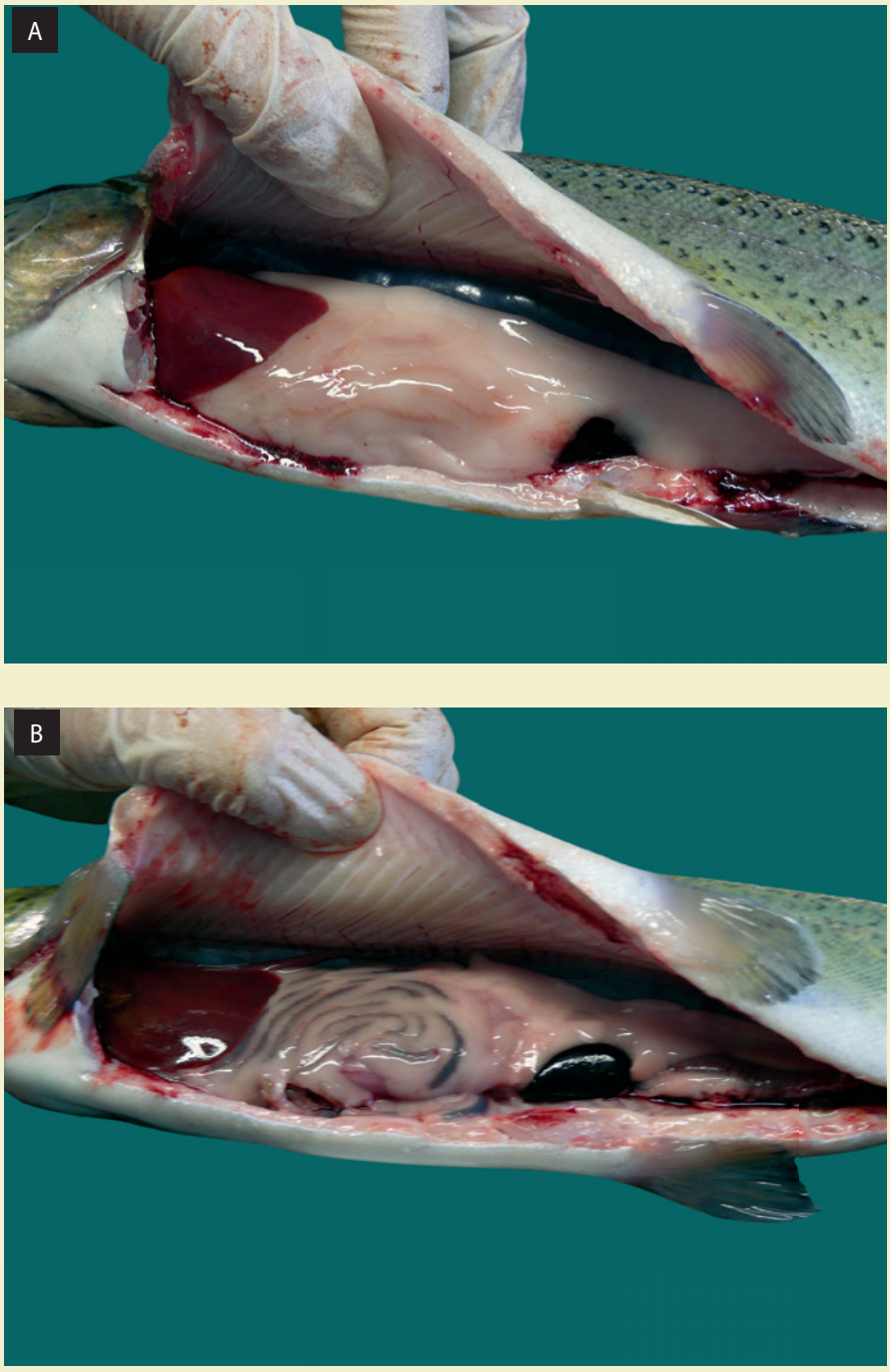
Fot. 1.3. Bernard Gliszczyński (1919–1979) – prekursor hodowli pstrągów na Pomorzu (lata 60. XX w.) / Phot. 1.3. Bernard Gliszczyński (1919–1979) – the precursor of trout culture in Pomerania (1960's)

Fot. 1.4. Terenowa Pracownia Rzeczna IRS w Gdańsku Oliwie (lata 70. XX w.) / Phot. 1.4. IRS Wheeling River Laboratory in Gdańsk Oliva (1970's)


Fot. 1.5. Magister inż. Mieczysław Kowalewski (†1943) – znany praktyk i wieloletni kierownik ośrodka pstrągowego PZW w Łopusznej i Czarnym Dunajcu / Phot. 1.5. Mieczysław Kowalewski, MSc. (†1943) – known practitioner and long-term head of the Trout Centre for PZW in Łopuszna and Czarny Dunajec

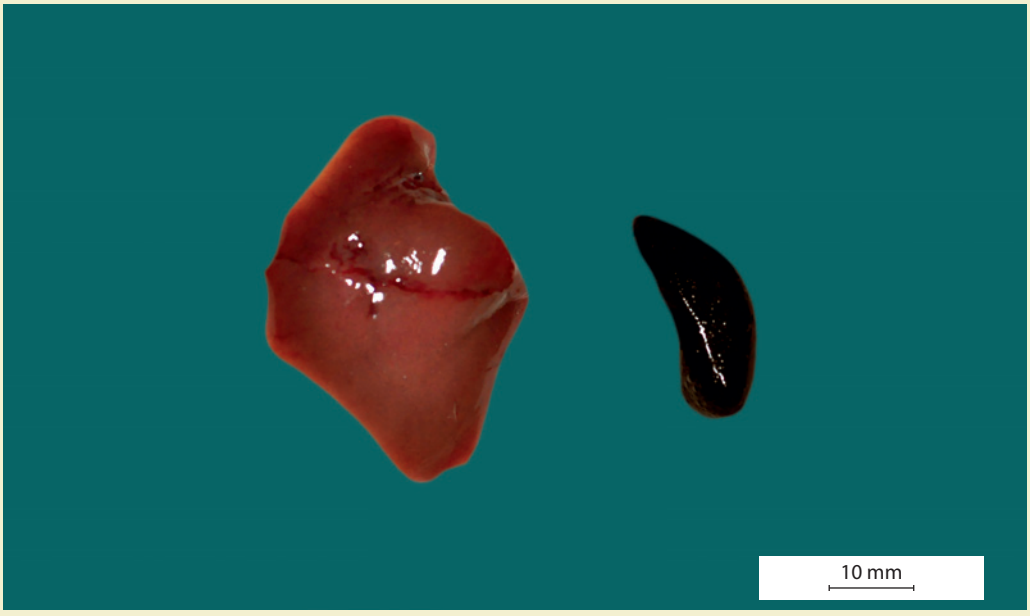
Fot. 1.6. Marek Piszczała (pierwszy z lewej) razem z prof. Januszem Guziurem na tle obiektu wylęgarniczo-podchowowego z 1881 r. w Złotym Potoku / Phot. 1.6. Marek Piszczała (first on the left side) together with prof. Janusz Guziur, in the background of hatchery and rearing facility from 1881 in Złoty Potok

Fot. 1.7. Profesor dr hab. inż. Krzysztof Goryczko (wykład na inauguracji roku akademickiego 2006/2007 na UWM w Olsztynie) / Phot. 1.7. Prof. Dr. eng. Krzysztof Goryczko (lecture at the inauguration of the academic year 2006/2007 at UWM in Olsztyn)



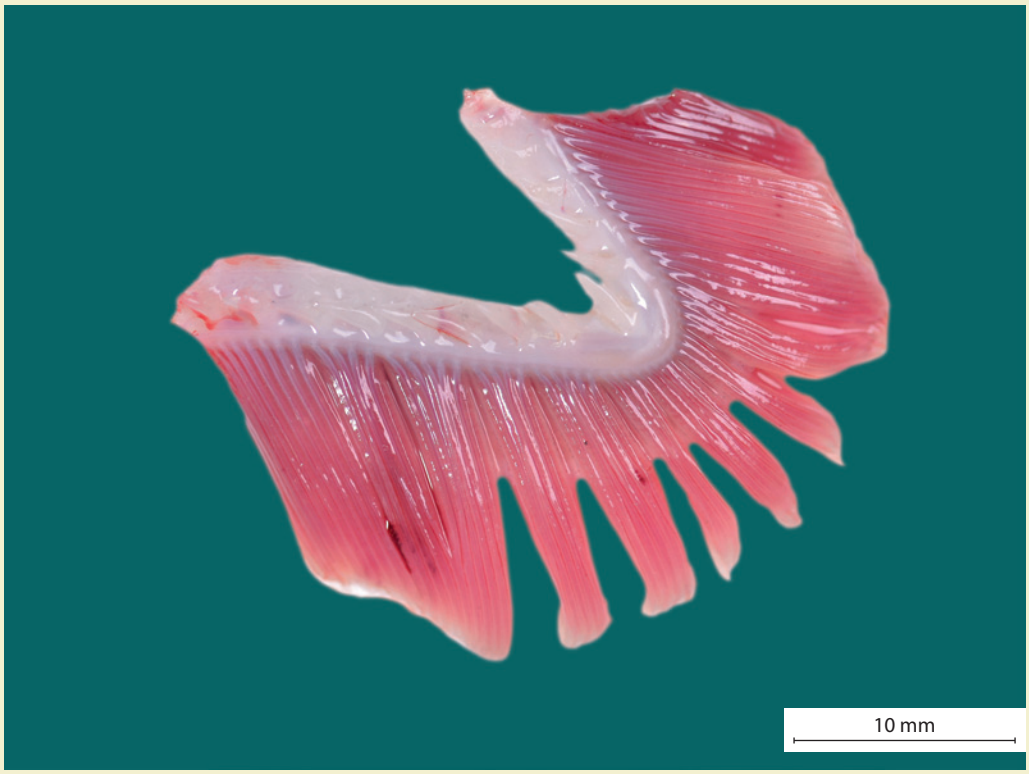

Fot. 7.1. Prawidłowy obraz makroskopowy wątroby przy znacznie (A) i miernie (B) rozwiniętym tłuszczu otrzewnowym – pstrągi tęczowe z odłowu jesiennego, 3-OOH, S / Phot. 7.1. Normal macroscopic pattern of the liver with remarkably (A) and weakly (B) developed peritoneal fat – rainbow trout's from the autumn sampling, 3-OS, S

Fot. 7.2. Stłuszczenie zwykle wątroby zauważalne po odsłonięciu jamy otrzewnowej – pstrąg tęczowy z odłowu jesiennego, 3-OOH, D / Phot. 7.2. Liver steatosis visible after peritoneal cavity opening – rainbow trout from the autumn sampling, 3-OS, B

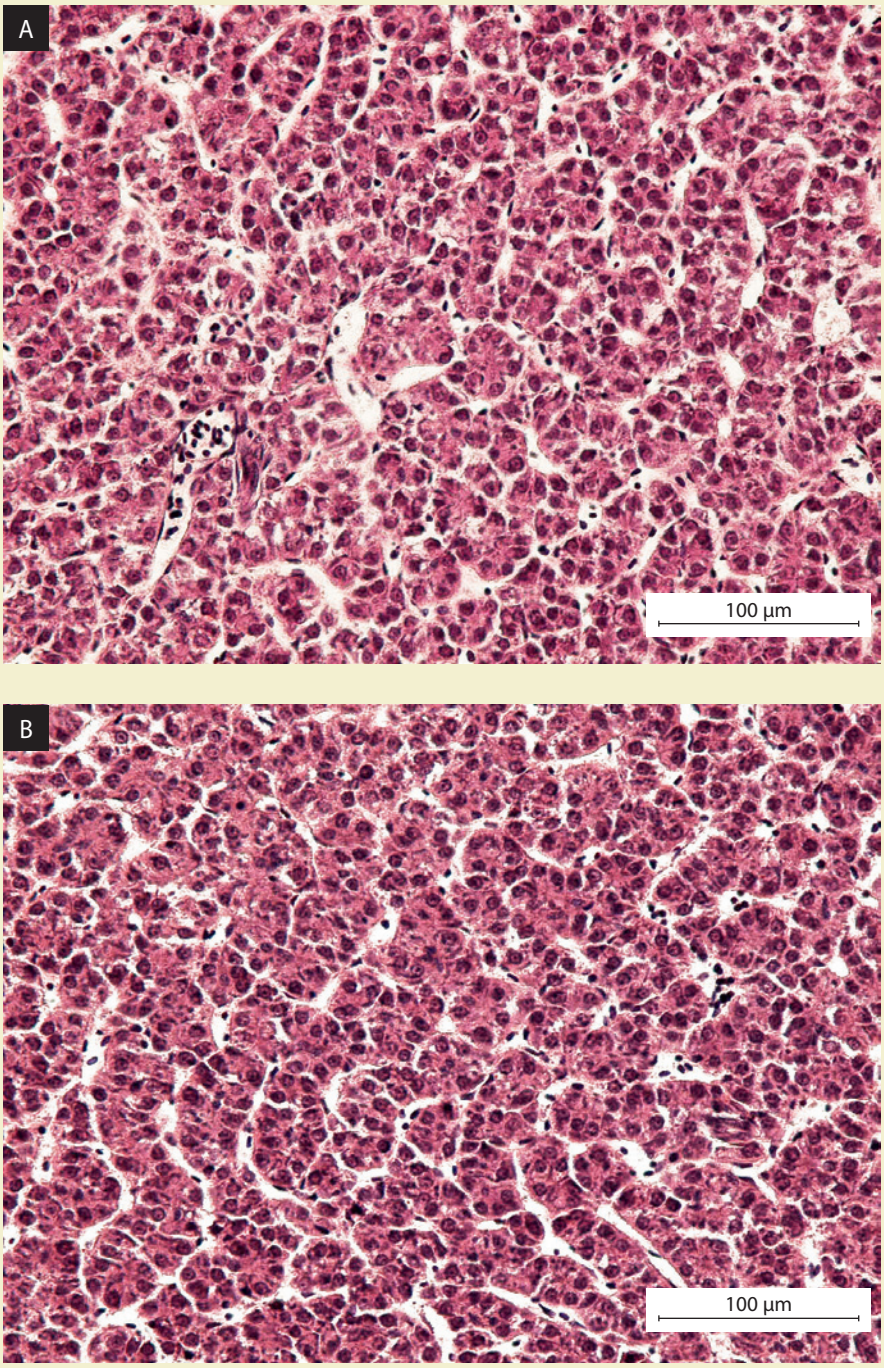



Fot. 7.3. Prawidłowy obraz makroskopowy wątroby i śledziony – pstrąg tęczowy z odłowu wiosennego, 1-RAS, S (podziałka w cm) / Phot. 7.3. Macroscopic pattern of the liver with steatosis and petechiae – rainbow trout from the spring sampling, 1-RAS, B(ruler in centimeters)

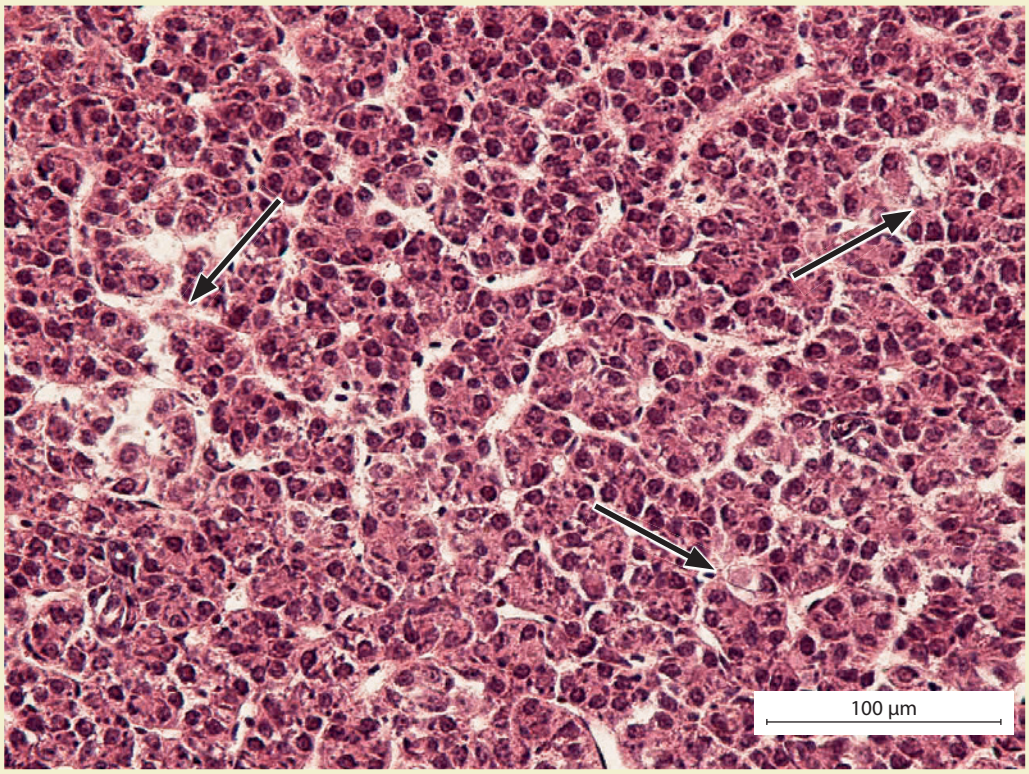

Fot. 7.4. Obraz makroskopowy stłuszczenia wątroby, pstrągi tęczowe z odłowu wiosennego: 2-OOH, D (A); 1-RAS, S (B); prawidłowa śledziona pstrąga sortymentu S (B) (podziałka w cm) / Phot. 7.4. Macroscopic pattern of the steatotis hepatis – rainbow trout from the spring sampling: 2-OS, B (A); 1-RAS, S (B); normal spleen of the S trout (B) (ruler in centimeters)



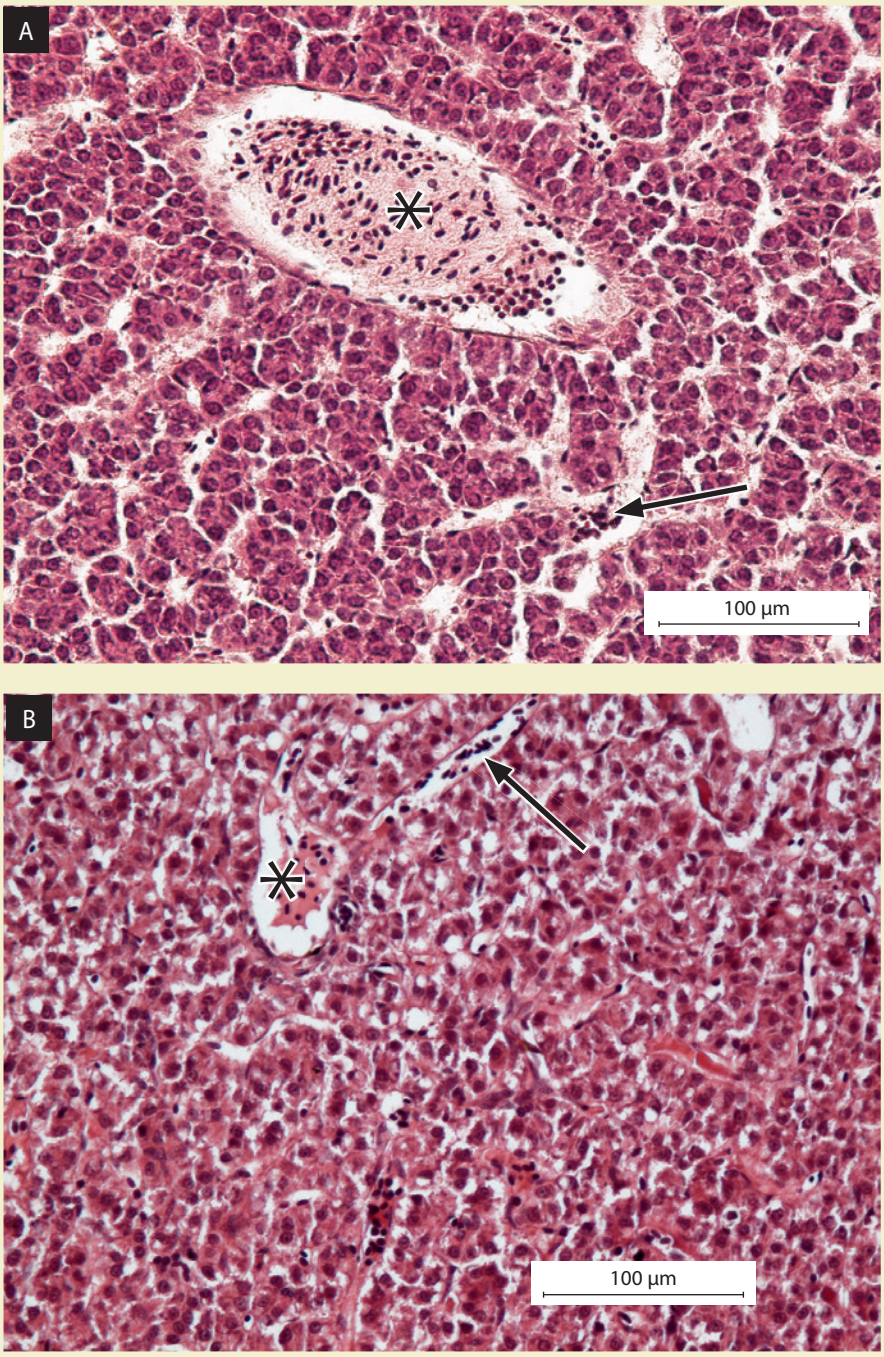

Fot. 7.5. Przekrwienie listków skrzelowych – pstrąg tęczowy z odłowu wiosennego, 3-RAS, S (podziałka w cm) / Phot. 7.5. Hyperaemia of gill foils – rainbow trout from the spring sampling, 3-RAS, S (ruler in centimeters)



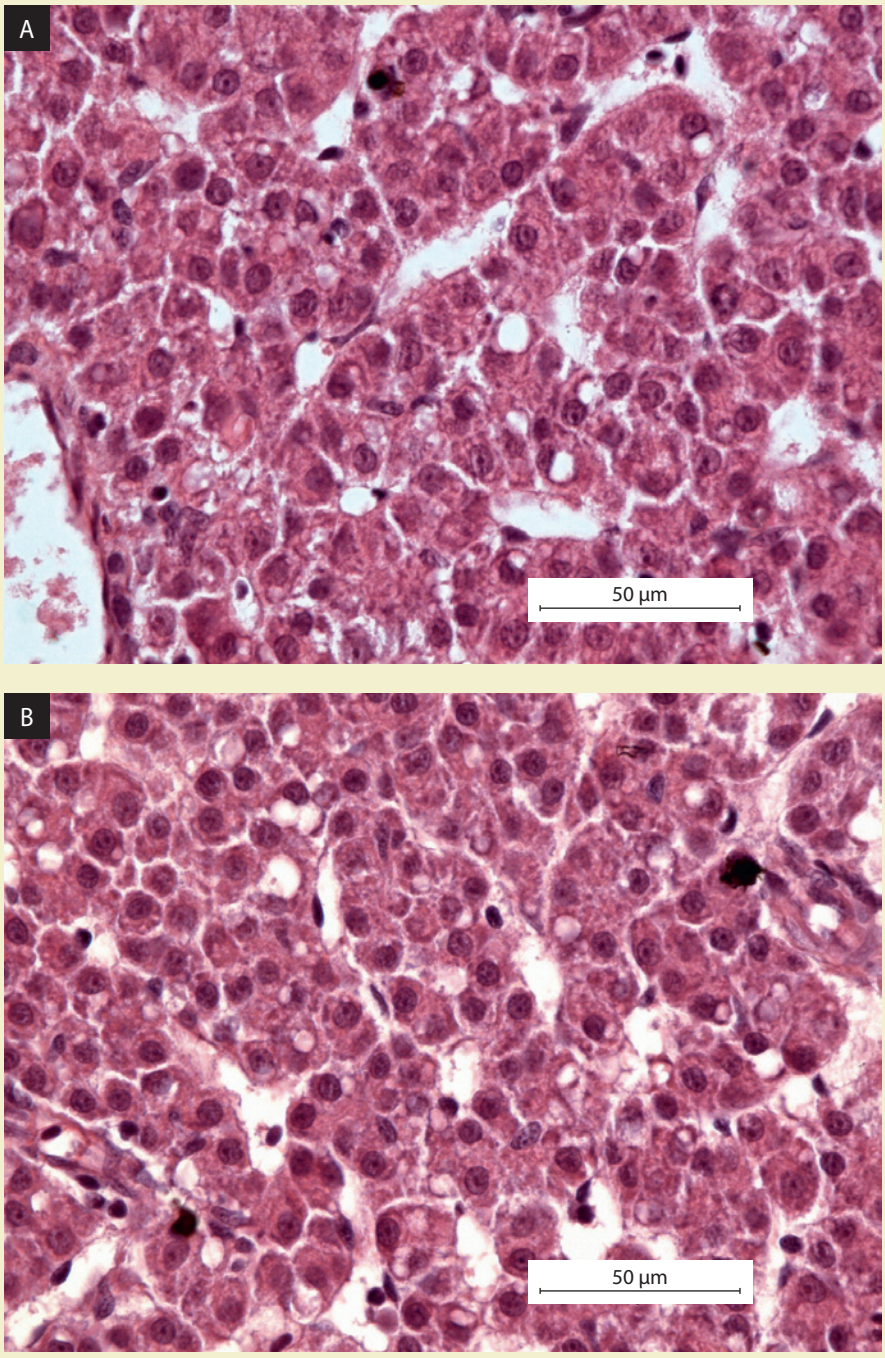

Fot. 7.6. Prawidłowy obraz mikroskopowy wątroby: pstrąg tęczowy z odłowu wiosennego, 1-OOH, S (A); pstrąg tęczowy z odłowu jesiennego, 2-RAS, S (B); barwienie HE / Phot. 7.6. The normal microscopic pattern of the liver: the rainbow trout from the autumn sampling, 1-OS, S (A); the rainbow trout from the autumn sampling, 2-RAS, T (B); HE staining



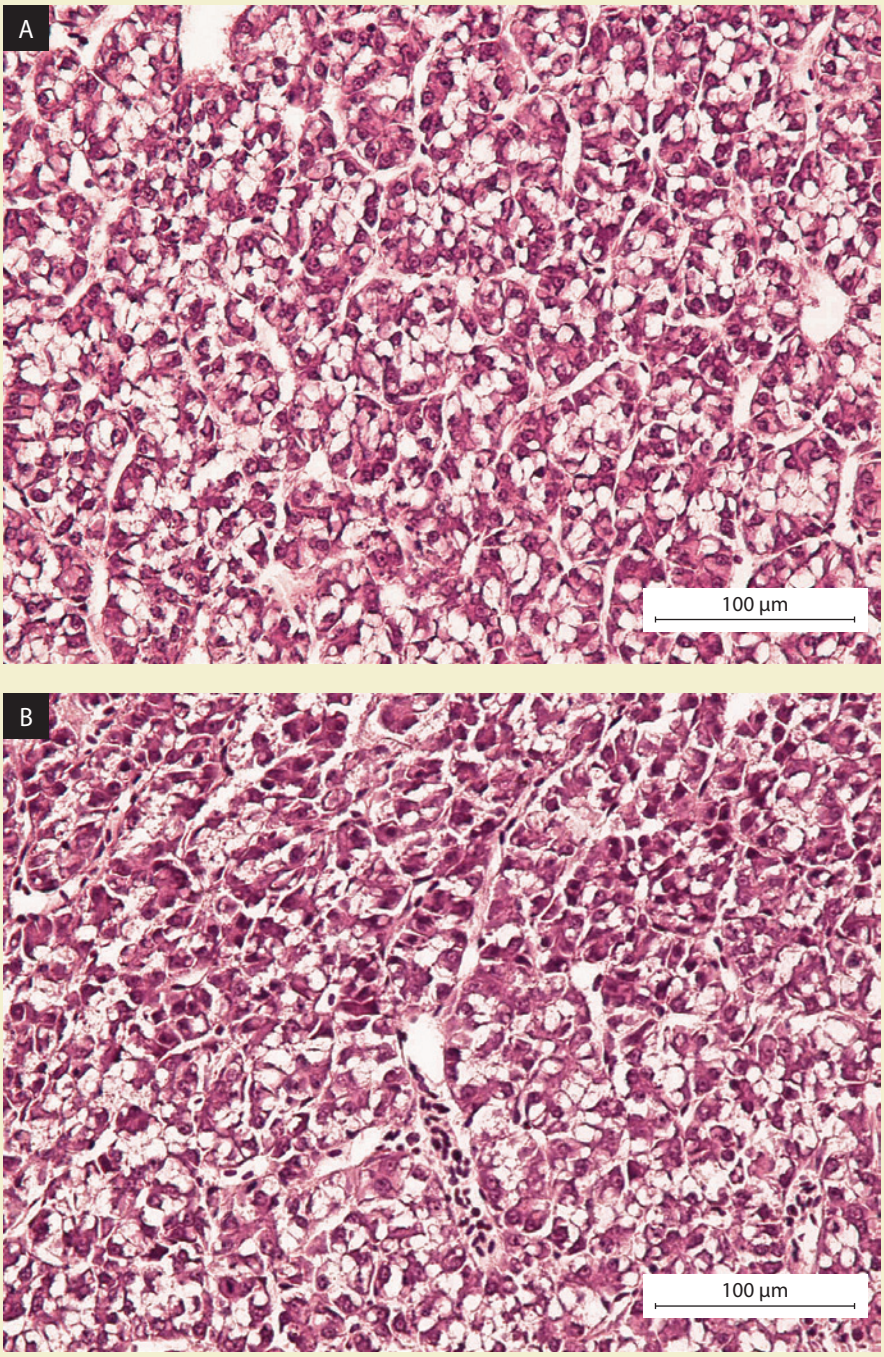

Fot. 7.7. Martwica pojedynczych hepatocytów pstrąga tęczowego z odłowu wiosennego (strzałki), 3-OOH, S; barwienie HE / Phot. 7.7. Necrosis of a single hepatocyte of the rainbow trout from the spring sampling (arrows), 3-OS, S; HE staining



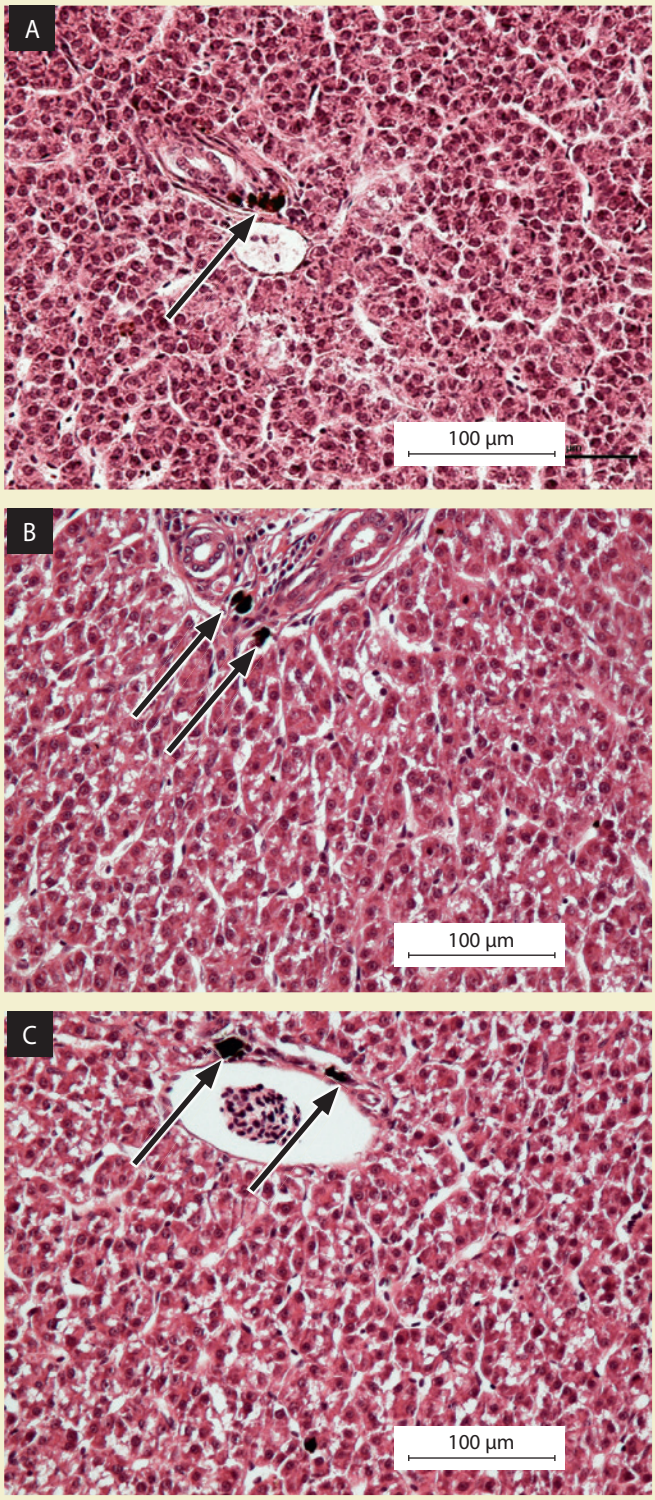

Fot. 7.8. Przekrwienie wątroby (strzałki) i zastój krwi (gwiazdki): w znacznym stopniu, pstrąg tęczowy z odłowu wiosennego, 2-OOH, D (A); w małym stopniu, pstrąg tęczowy z odłowu jesiennego, 2-RAS, D (B); barwienie HE / Phot. 7.8. Congestion of the liver (arrows) and the blood stasis (asteriks): of a marked degree, the rainbow trout from the spring sampling, 2-OS, B (A); of a slight degree, the rainbow trout from the autumn sampling, 2-RAS, B (B); HE staining



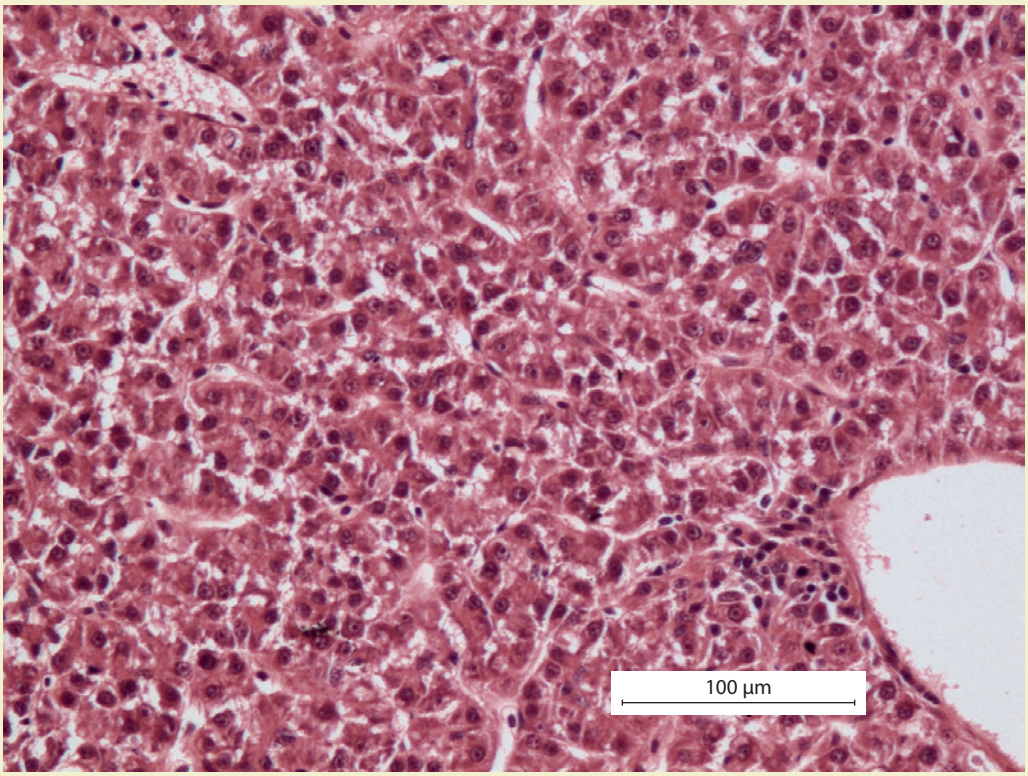

Fot. 7.9. Stłuszczenie zwykłe wątroby: w małym stopniu, pstrąg tęczowy z odłowu wiosennego, 3-OOH, D (A); w średnim stopniu, pstrąg tęczowy z odłowu jesiennego, 3-RAS, D (B); barwienie HE / Phot. 7.9. Steatosis simplex: of the low intensity, the rainbow trout from the spring sampling, 3-OS, B (A); of the medium intensity, the rainbow trout from the autumn sampling, 3-RAS, B (B); HE staining



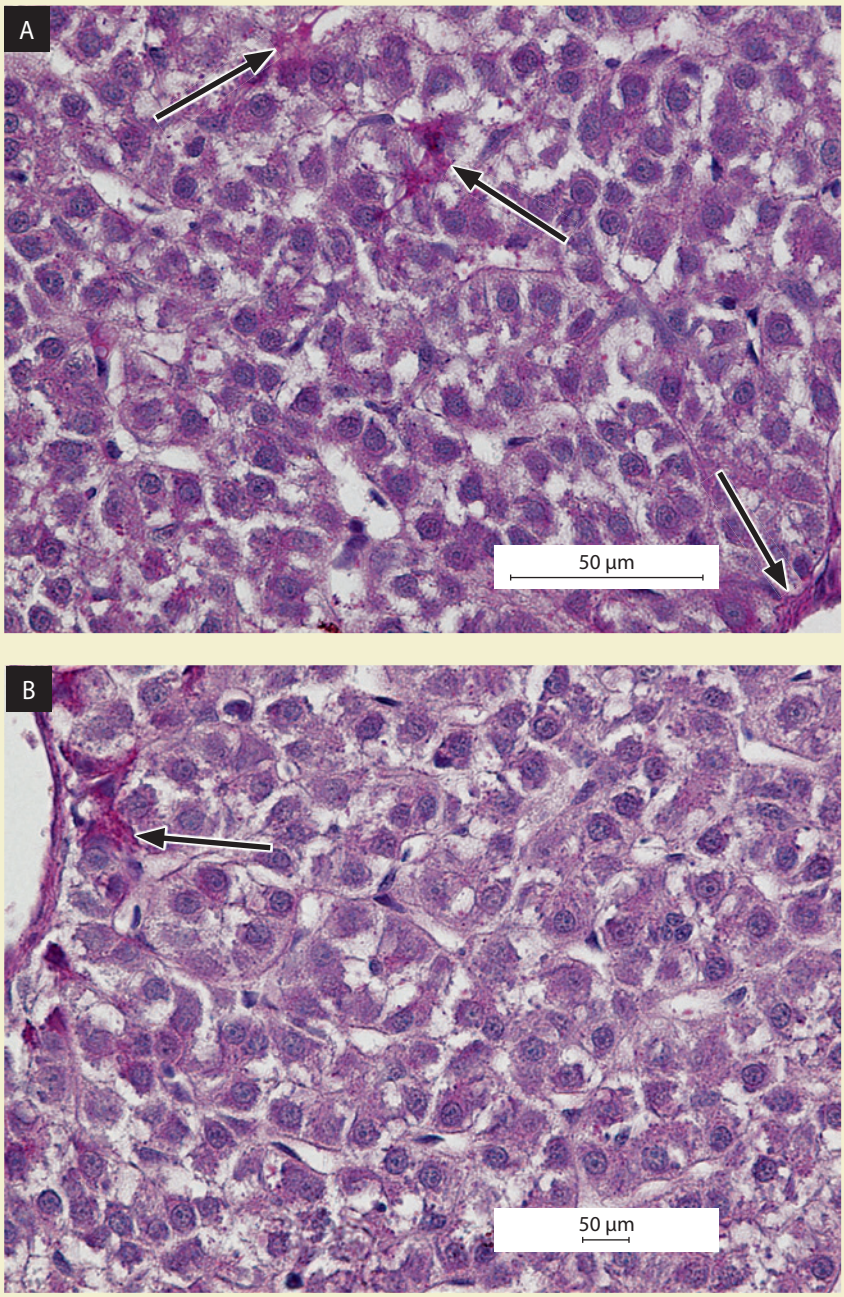

Fot. 7.10. Stłuszczenie zwykłe wątroby w bardzo dużym stopniu pstrągów tęczowych z odłowu jesiennego: 3-OOH, S (A); 2-RAS, S (B); barwienie HE / Phot. 7.10. Steatosis simplex of a very high degree in the rainbow trout's from the autumn sampling: 3-OS, S (A), 2-RAS, S (B); HE staining



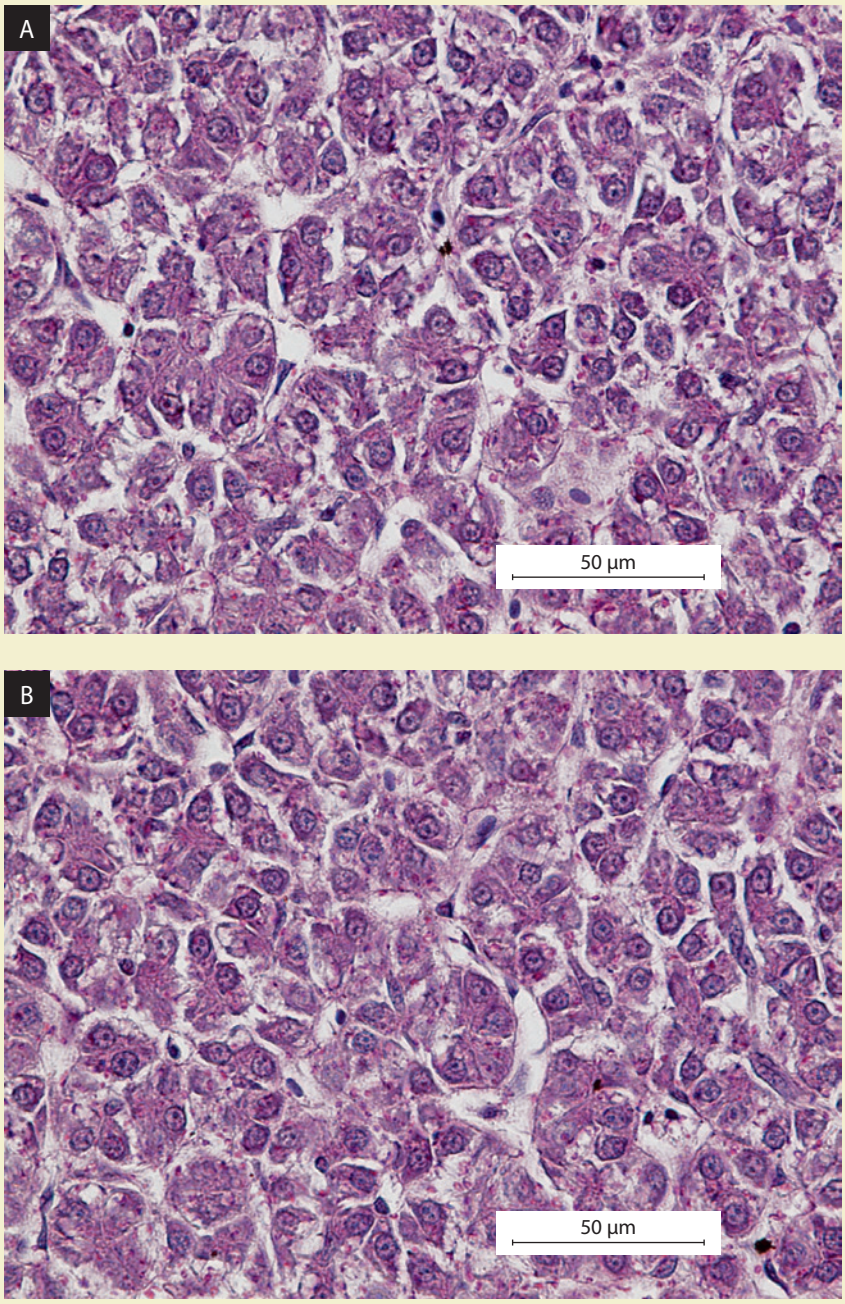

Fot. 7.11. Naciek melanomakrofagów (strzałki) w wątrobie pstrągów tęczowych w pobliżu przewodów żółciowych: odłów wiosenny, 1-OOH, S (A); odłów jesienny, 1-RAS, D (B); oraz naczynia krwionośnego: odłów jesienny, 2-OOH, S (C); barwienie HE / Phot. 7.11. Melanomacrophages infiltrating (arrows) the liver of the rainbow trout's in the vicinity of bile ducts: spring sampling, 1-OS, S (A); autumn sampling, 1-RAS, B (B); and in the vicinity of the blood vessel: autumn sampling, 2-OS, S (C); HE staining



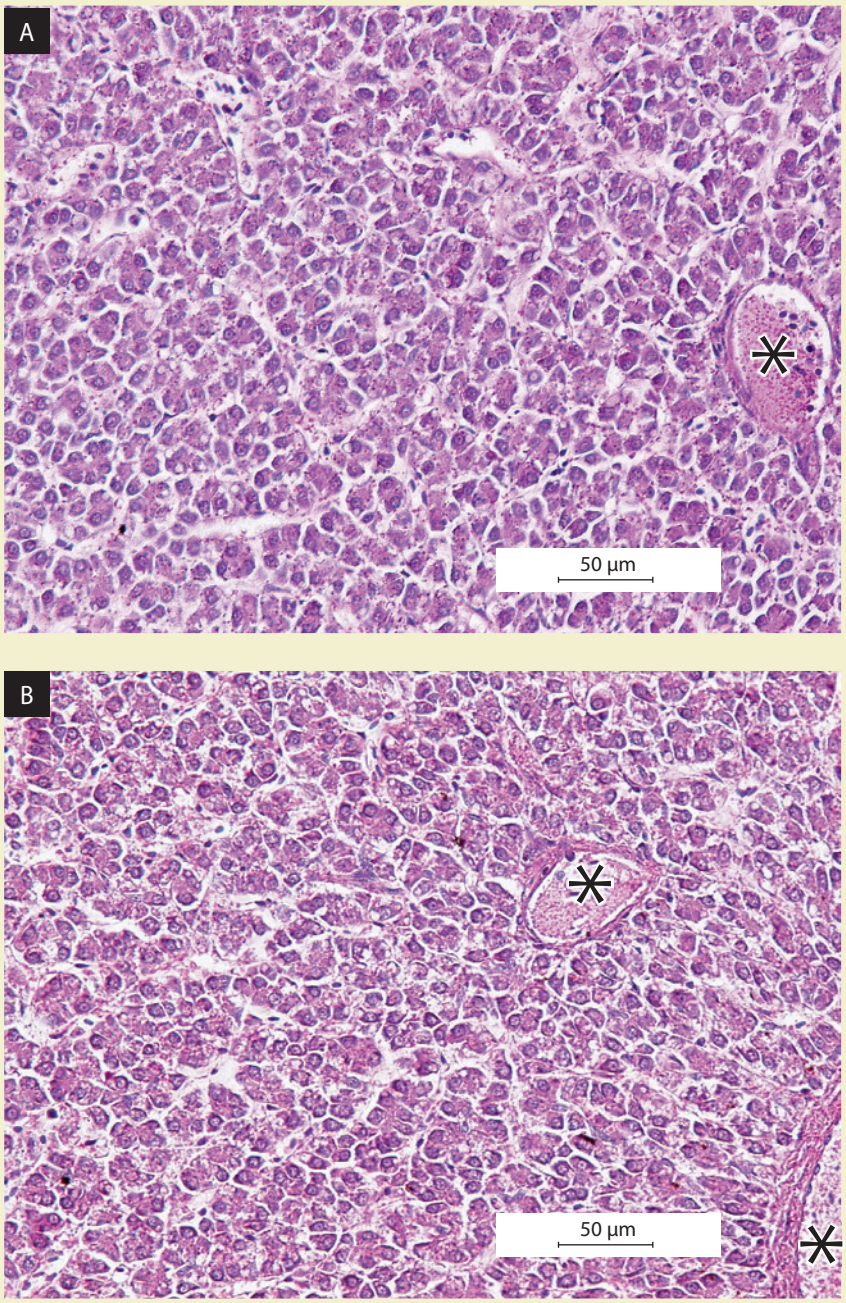

Fot. 7.12. Naciek pojedynczych komórek limfoidalnych w pobliżu naczynia krwionośnego w wątrobie pstrąga tęczowego z odłowu wiosennego, 1-RAS, D; barwienie HE / Phot. 7.12. The infiltration of the scarce lymphoid cells in the surrounding of the blood vessel in the rainbow trout from the spring sampling, 1-RAS, B; HE staining



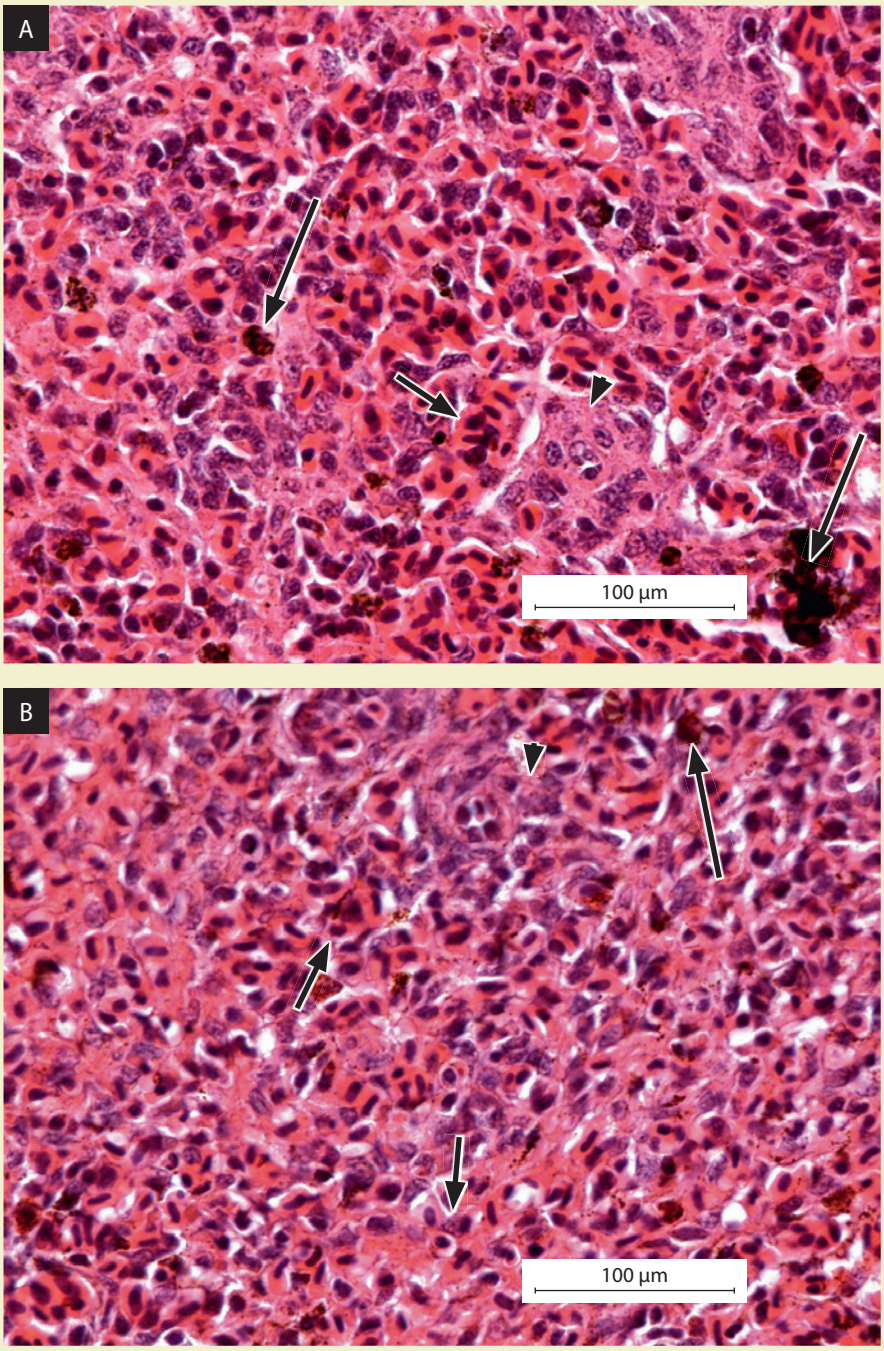

Fot. 7.13. Średnia zawartość wielocukrów (wybarwione purpurowo), miejscami znaczna (strzałki) w cytoplazmie hepatocytów pstrągów tęczowych odłowionych wiosną, stłuszczenie zwykłe: 2-OOH, S (A); 2-OOH, D (B); barwienie zgodnie z metodą PAS wg McManusa / Phot. 7.13. Slightly higher content of polysaccharides (purple), in some foci even high (arrow), in the cytoplasm of hepatocytes of the rainbow trout's sampled in the spring. Steatosis simplex: 2-OS, S (A); 2-OS, B (B); PAS staining according to McManus



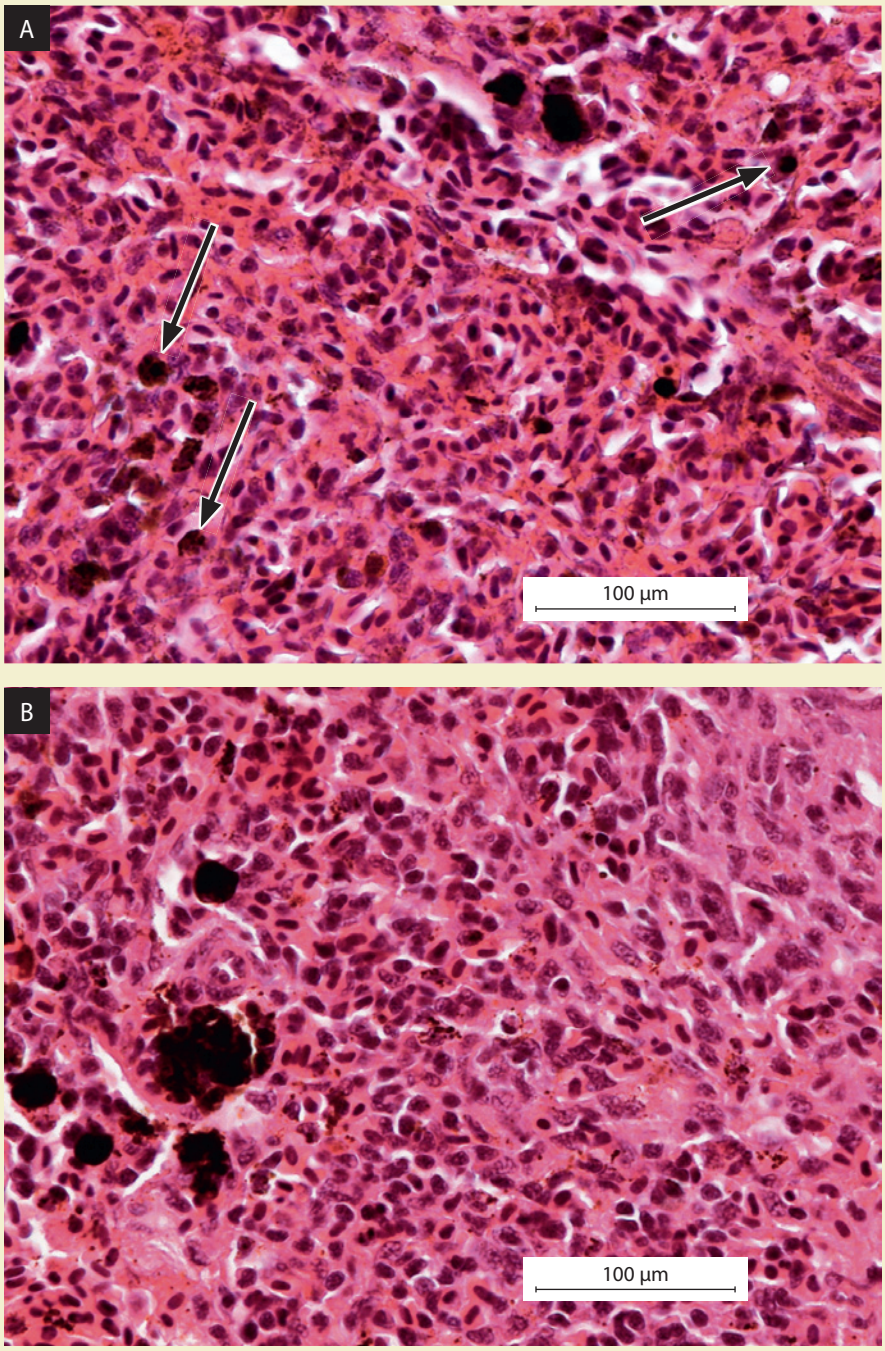

Fot. 7.14. Znaczna zawartość wielocukrów (wybarwione purpurowo) równomiernie rozmieszczonych głównie w postaci ziaren w cytoplazmie hepatocytów pstrągów tęczowych odłowionych jesienią: 1-RAS, S (A); 1-RAS, D (B); barwienie zgodnie z metodą PAS wg McManusa / Phot. 7.14. High content of polysaccharides (purple) evenly distributed as granules in the cytoplasm of hepatocytes of the rainbow trout's sampled in the autumn: 1-RAS, S (A); 1-RAS, B (B); PAS staining according to McManus



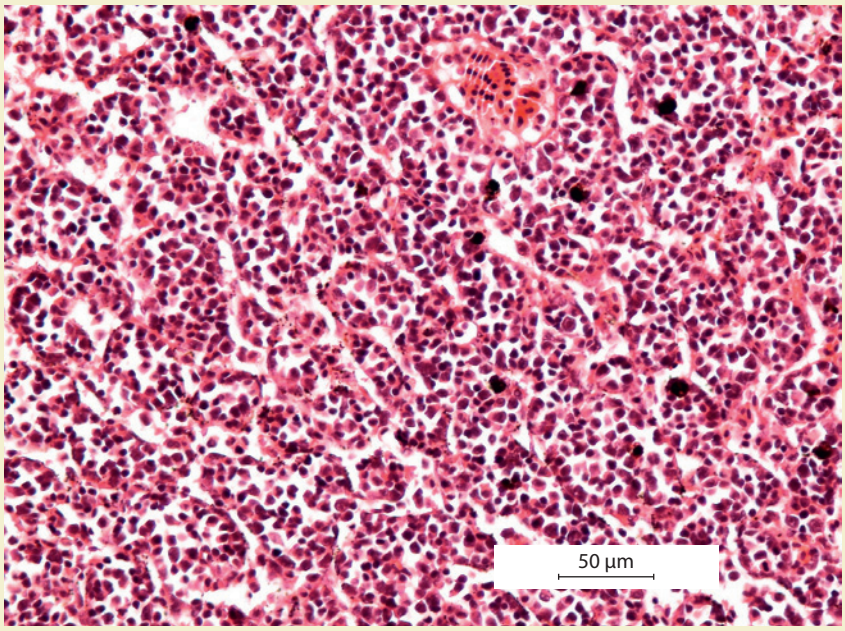

Fot. 7.15. Bardzo znaczna zawartość wielocukrów (wybarwione purpurowo) równomiernie rozmieszczonych w cytoplazmie hepatocytów pstrągów tęczowych odłowionych wiosną (gwiazdki), zastój krwi: 2-OOH, S (A); 2-OOH, D (B); barwienie zgodnie z metodą PAS wg McManusa / Phot. 7.15. Very high content of polysaccharides (purple) evenly distributed in the cytoplasm of hepatocytes of the rainbow trout's sampled in the spring, the haemostasis (asteriks): 2-OS, S (A); 2-OS, B (B); PAS staining according to McManus



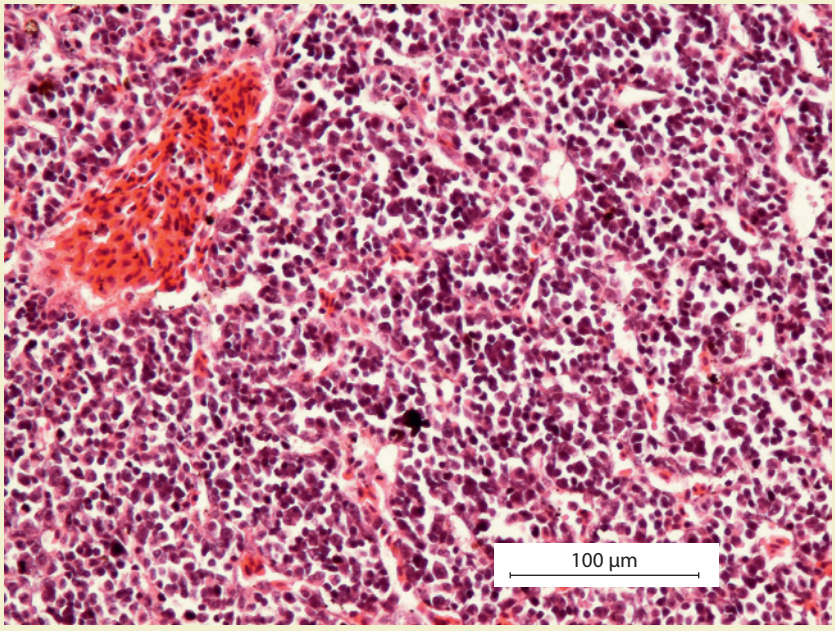

Fot. 7.16. Obraz prawidłowy śledziony pstrągów tęczowych – miazga czerwona (krótka strzałka), miazga biała (główka strzałki), melanomakrofagi (długa strzałka): odłów wiosenny, 2-OOH, D (A); odłów jesienny, 3-RAS, S (B); barwienie HE / Phot. 7.16. The normal pattern of the spleen of the rainbow trout's – red pulp (long arrow), white pulp (arrowhead), melanomacrophages (long arrow): spring sampling, 2-OS, B (A); autumn sampling, 3-RAS, S (B); HE staining



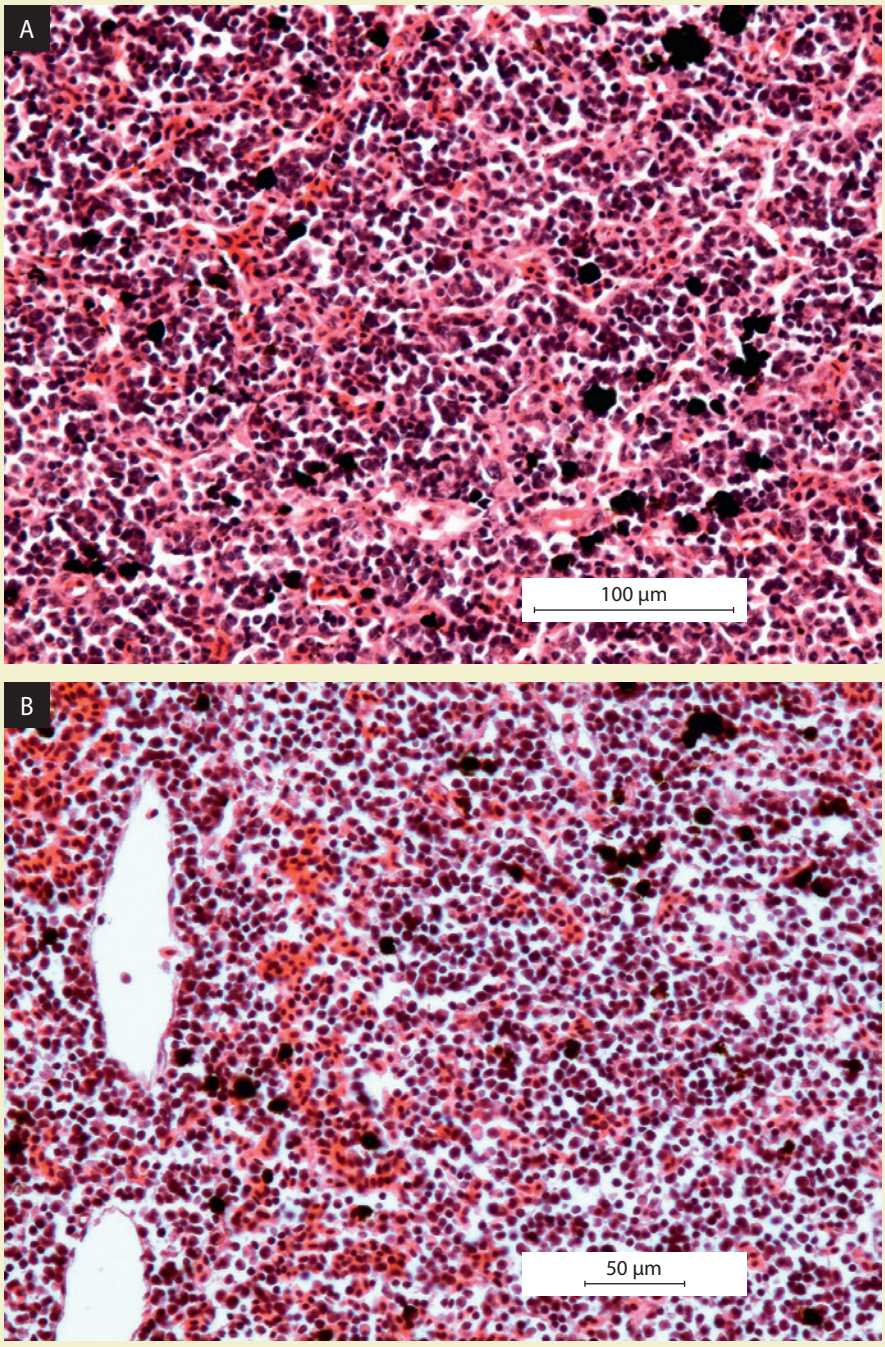

Fot. 7.17. Liczne melanomakrofagi w śledzionie pstrągów tęczowych: rozproszone (strzałki), odłów wiosenny, 2-OOH, S (A); tworzące duże skupisko (centrum melanomakrofagów), odłów jesienny, 3-RAS, D (B); barwienie HE / Phot. 7.17. Numerous melanomacrophages in the spleen of the rainbow trout's: dispersed (arrows), autumn sampling, 2-OS, S (A); forming clusters (melanomacrophage centers), autumn sampling, 3-RAS, B (B); HE staining



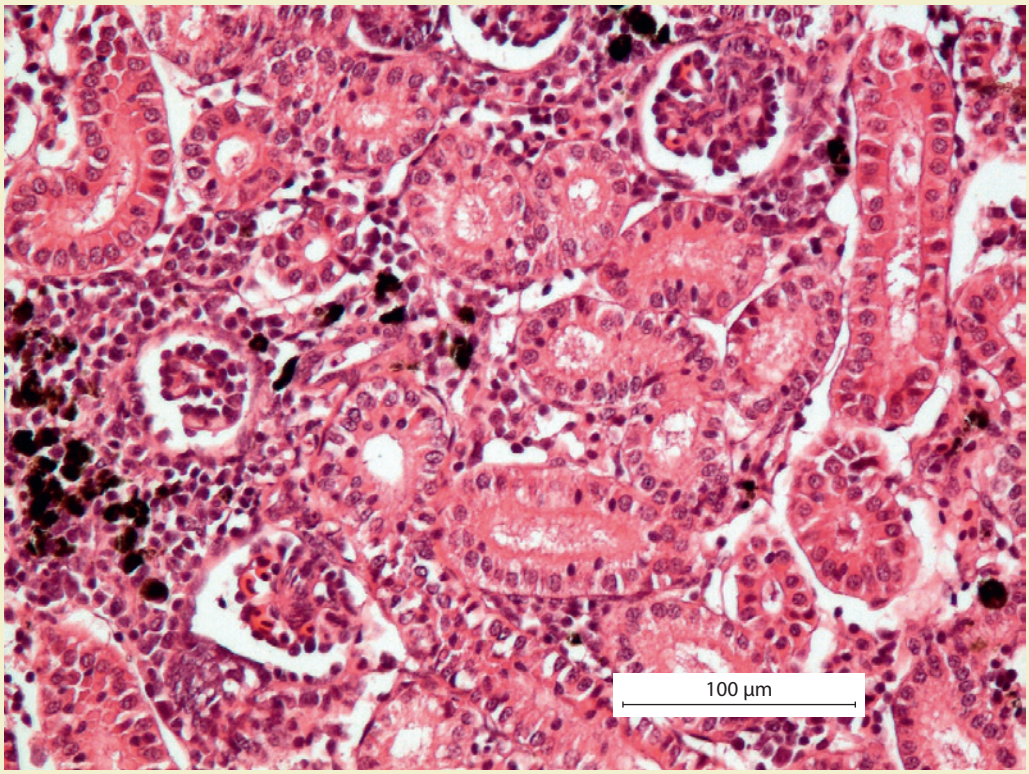

Fot. 7.18. Obraz prawidłowy nerki głowowej pstrąga tęczowego, rozproszone melanomakrofagi zabarwione na kolor brunatnoczarny, odłów wiosenny, 1-RAS, S; barwienie HE / Phot. 7.18. The normal pattern of the anterior kidney of the rainbow trout, dispersed melanomacrophages stained in brown-black, spring sampling, 1-RAS, S; HE staining



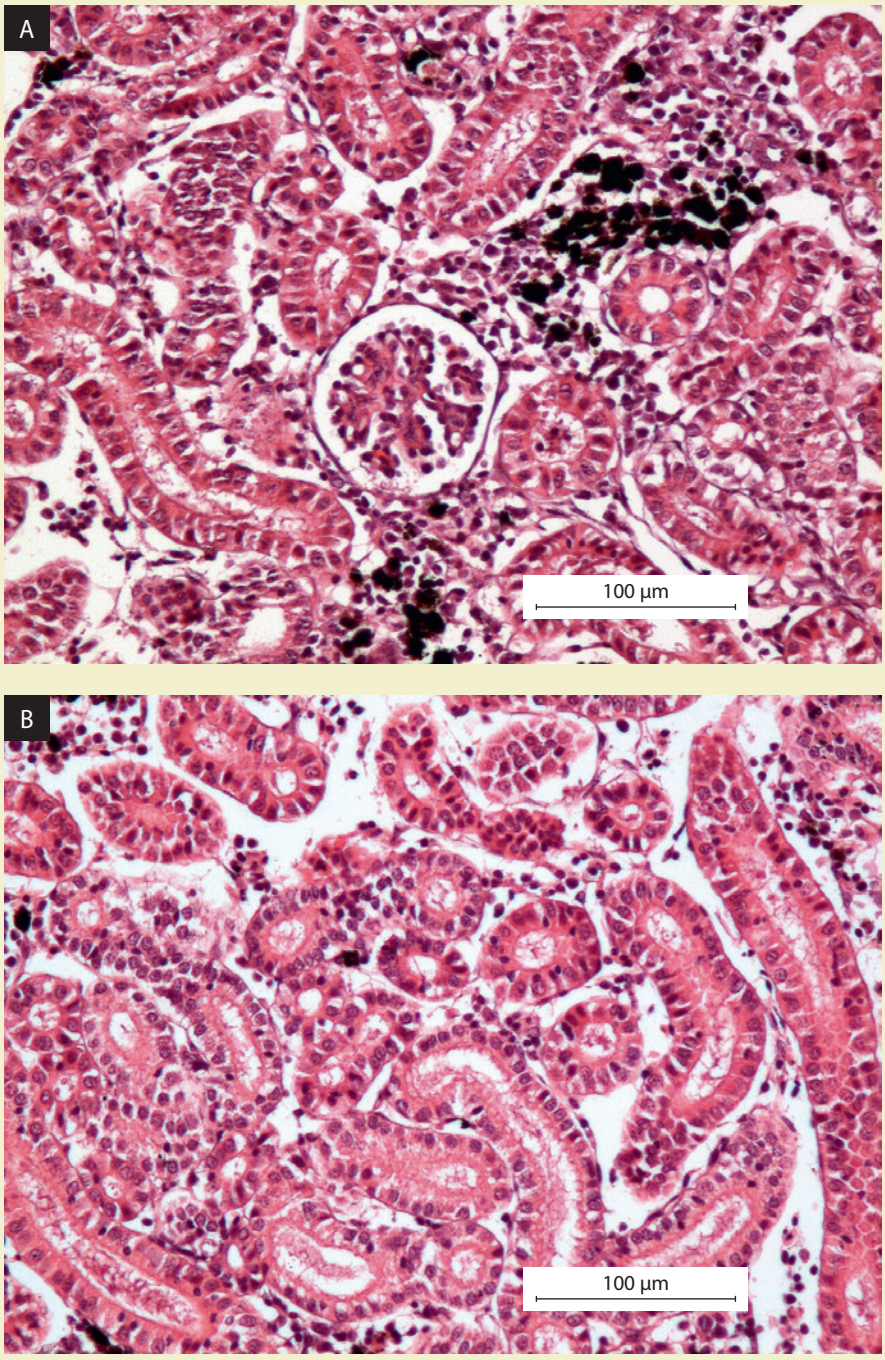

Fot. 7.19. Przekrwienie w nerce głowowej pstrąga tęczowego, melanomakrofagi zabarwione na kolor brunatno czarny, odłów wiosenny, 2-OOH, D; barwienie HE / Fig. 7.19. Congestion of the anterior kidney of the rainbow trout, melanomacrophages stained in brown-black, spring sampling, 2-OS, B; HE staining



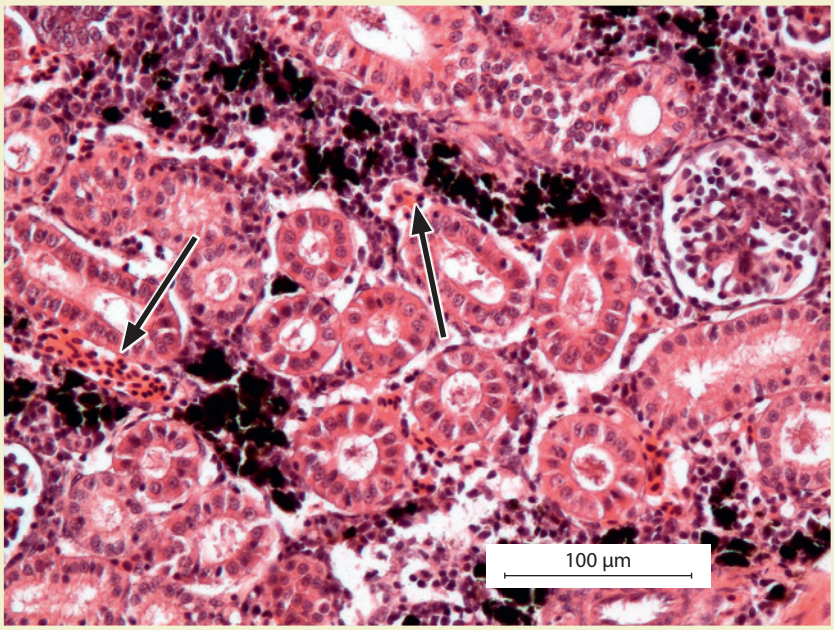

Fot. 7.20. Liczne melanomakrofagi, miejscami tworzące centra, zabarwione na kolor brunatnoczarny w nerce głowowej pstrągów tęczowych, odłów jesienny: 2-OOH, D (A), 3-RAS, D (B); barwienie HE / Phot. 7.20. Numerous melanomacrophages, locally forming centers, stained in brown-black, in the anterior kidney of the rainbow trout's, autumn sampling, 2-OS, B (A); 3-RAS, B (B); HE staining



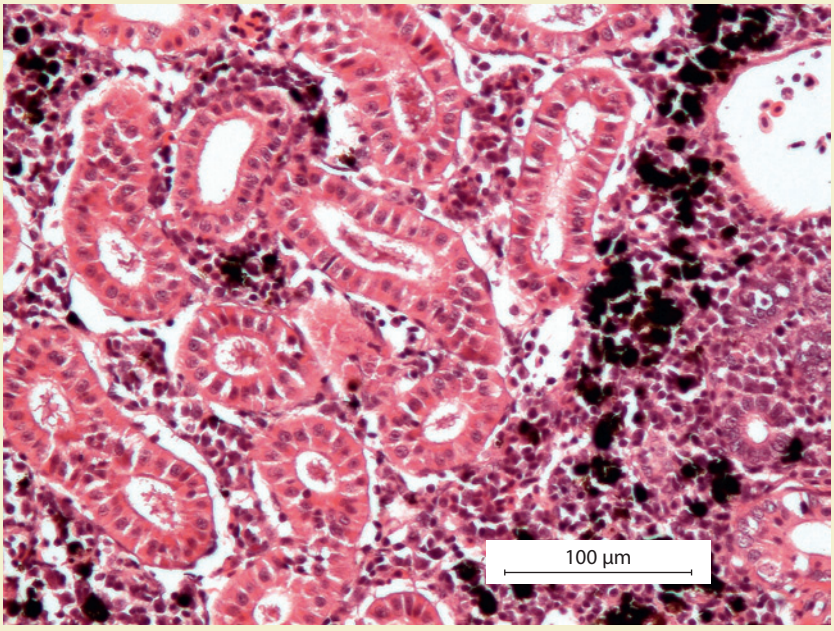

Fot. 7.21. Obraz prawidłowy nerki tułowiowej pstrąga tęczowego, liczne, o prawidłowej strukturze, kanaliki nefronu bliższe i dalsze, odłów wiosenny, 3-RAS, S; barwienie HE / Phot. 7.21. The normal pattern of the posterior kidney of the rainbow trout, numerous normal proximal and distal nephrone tubules, spring sampling, 3-RAS, S; HE staining



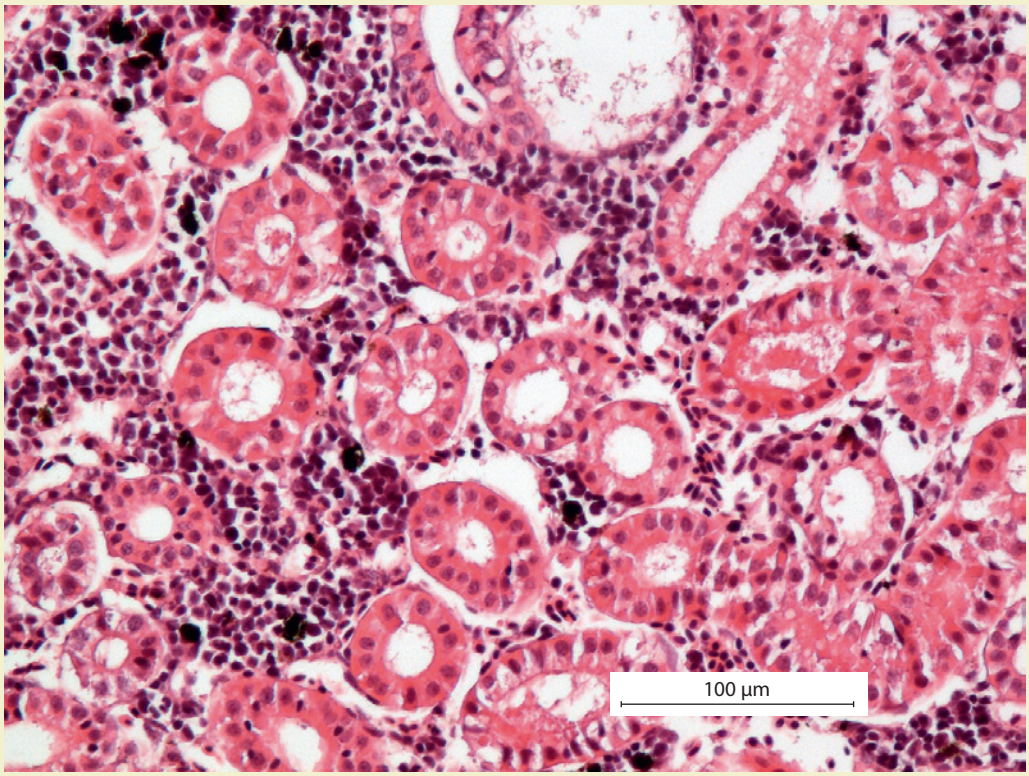

Fot. 7.22. Zwyrodnienie nerki tułowiowej pstrągów tęczowych: wodniczkowe (A), miąższowe (B), odłów jesienny, 3-RAS, D; barwienie HE / Phot. 7.22. Degeneration of the posterior kidney of the rainbow trout: vacuolar degeneration (A), parenchymatous degeneration (B), autumn sampling, 3-RAS, B; HE staining



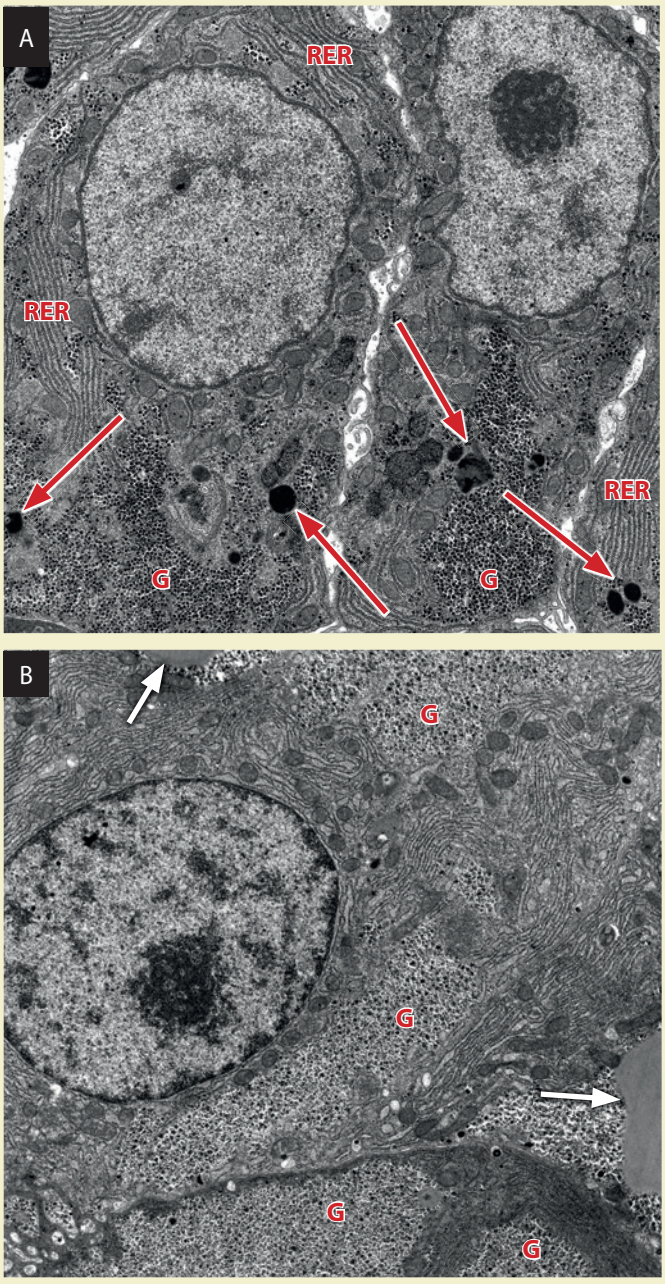

Fot. 7.23. Małe wynaczynienia (strzałki) w nerce tułowiowej pstrąga tęczowego, liczne duże centra melanomakrofagów, odłów jesienny, 3-OOH, D; barwienie HE / Phot. 7.23. Petechiae in the posterior kidney (arrows) of the rainbow trout, numerous big centers of melanomacrophages, autumn sampling, 3-OS, B; HE staining



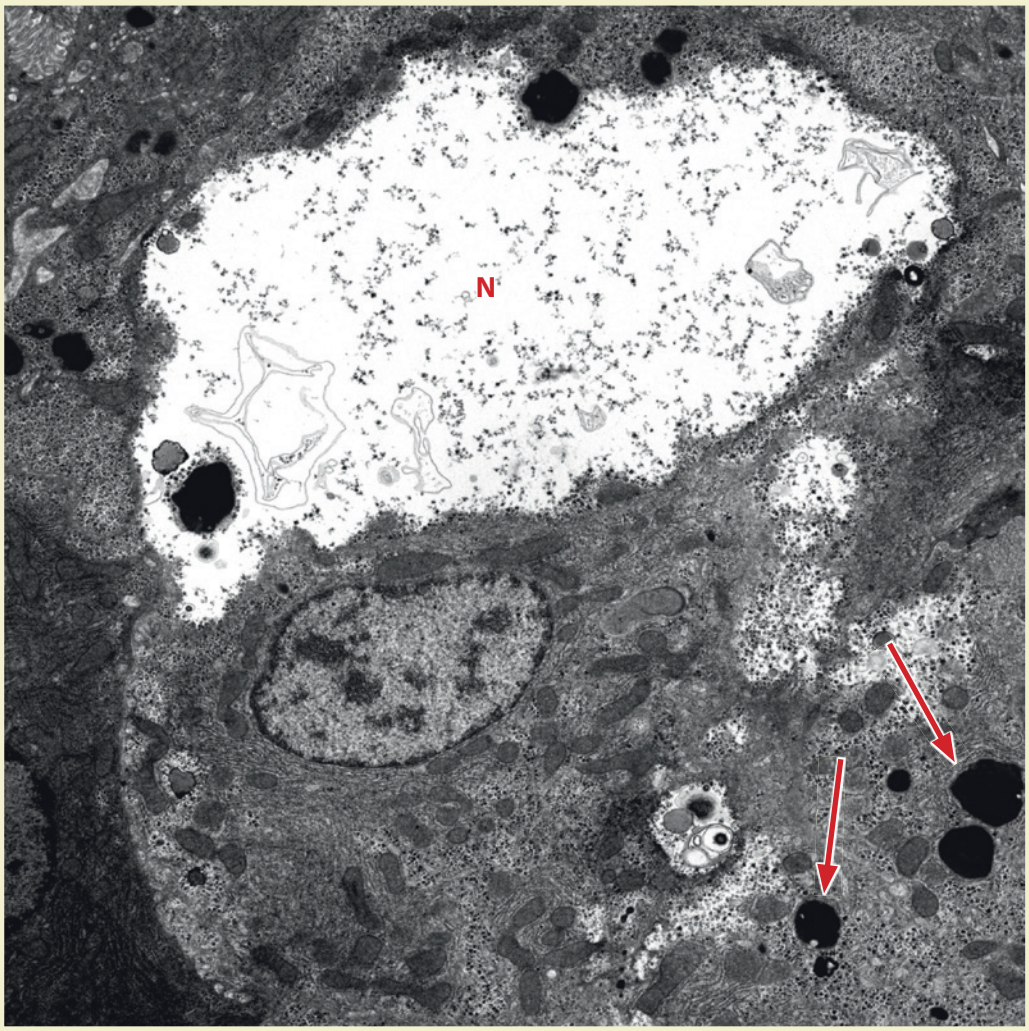

Fot. 7.24. Rozlegle centrum melanomakrofagów zlokalizowane w pobliżu naczynia krwionośnego i rozciągające w głąb miąższu, odłów wiosenny, 3-OOH, S; barwienie HE / Phot. 7.24. Vast melanomacrophage center located in the vicinity of the blood vessel and reaching the parenchyma, spring sampling, 3-OS, S; HE staining



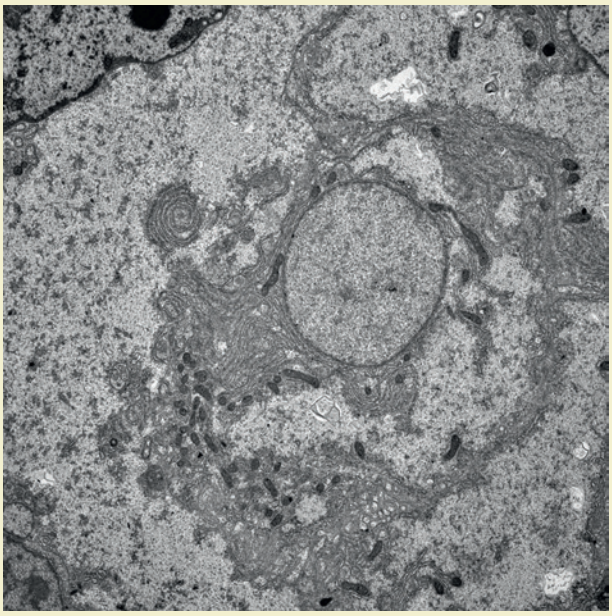

Fot. 7.25. Naciek komórek limfoidalnych okalający kanaliki nefronu bliższe, odłów jesienny, 2-RAS, D; barwienie HE /
Phot. 7.25. Infiltration of lymphoid cells surrounding the proximal tubules of the nephron, autumn sampling, 2-RAS, B; HE staining.



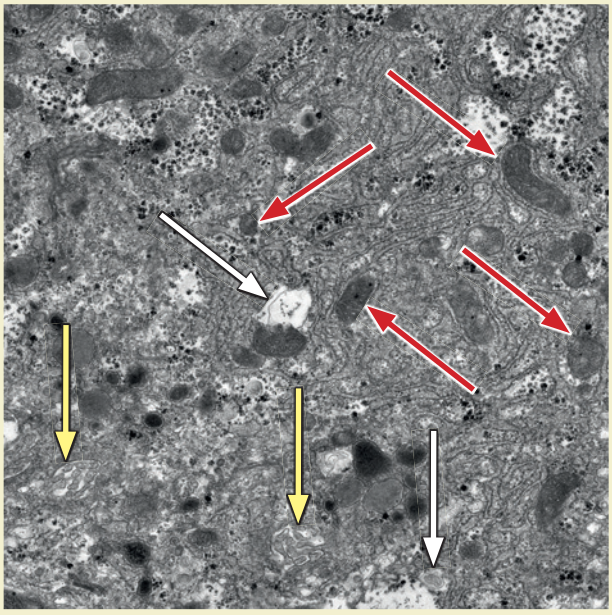

Fot. 8.1. Prawidłowy obraz ultrastrukturalny hepatocytów: liczne ziarna glikogenu (G), dobrze rozwinięta szorstka siateczka endoplazmatyczna (RER), lizosomy (czerwone strzałki) fragmenty kropel lipidowych (białe strzałki), mikrokosmki widoczne pomiędzy dwoma hepatocytami, pstrągi tęczowe z odłowu wiosennego: 1-OOH, S (A); 2-RAS, D (B) / Phot. 8.1. Normal ultrastructure of hepatocytes: numerous granules of glycogen (G), well development rough endoplasmatic reticulum (RER), lysosomes (red arrows), fragments of lipid drops (white arrows), microrilli visible between two hepatocytes, rainbow trout's from the spring sampling: 1-OS, S (A); 2-RAS, B (B).



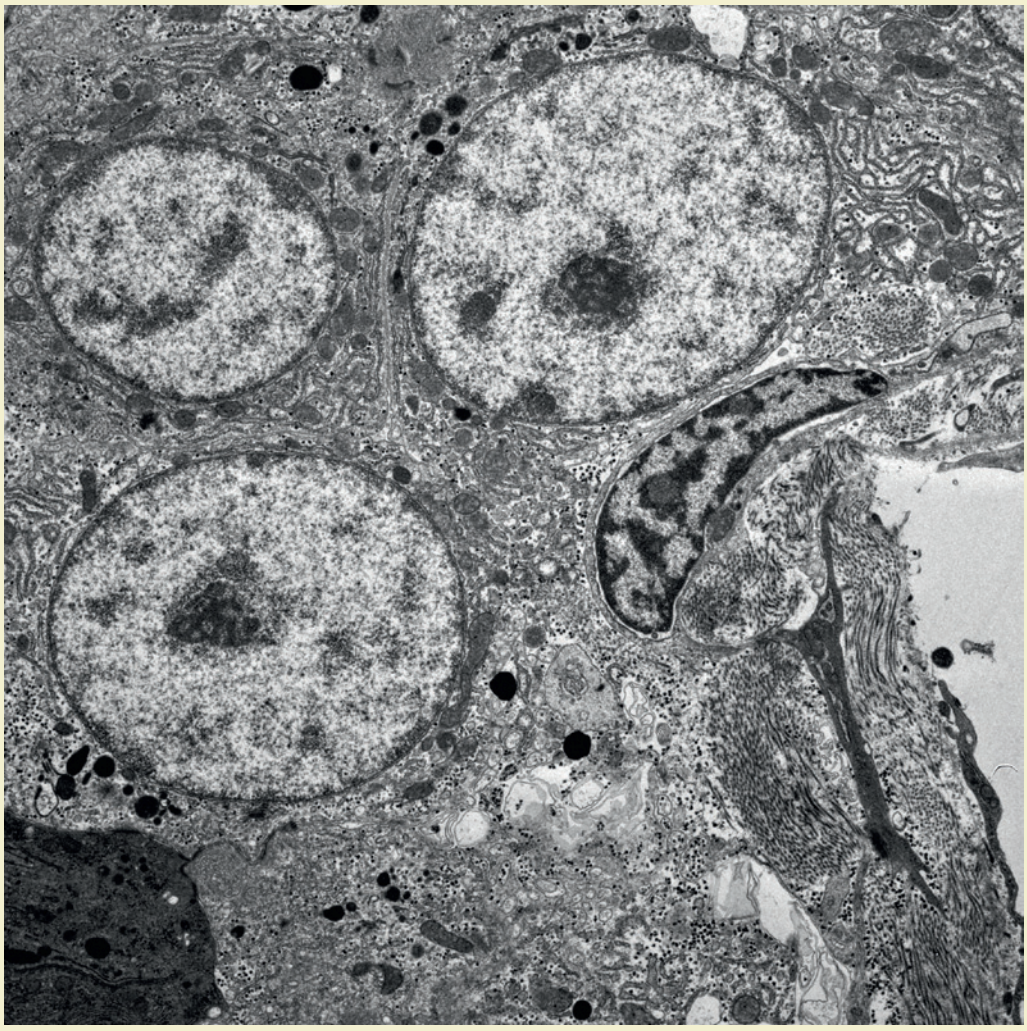

Fot. 8.2. Częściowa martwica hepatocyta (N), polimorfizm mitochondriów, lizosomy (strzałki): pstrąg tęczowy z odłowu jesiennego, 3-RAS, D / Phot. 8.2. Partial necrosis of hepatocyte (N), mitochondrial polymorphism, lysosomes (arrows): rainbow trout from the autumn sampling, 3-RAS, B



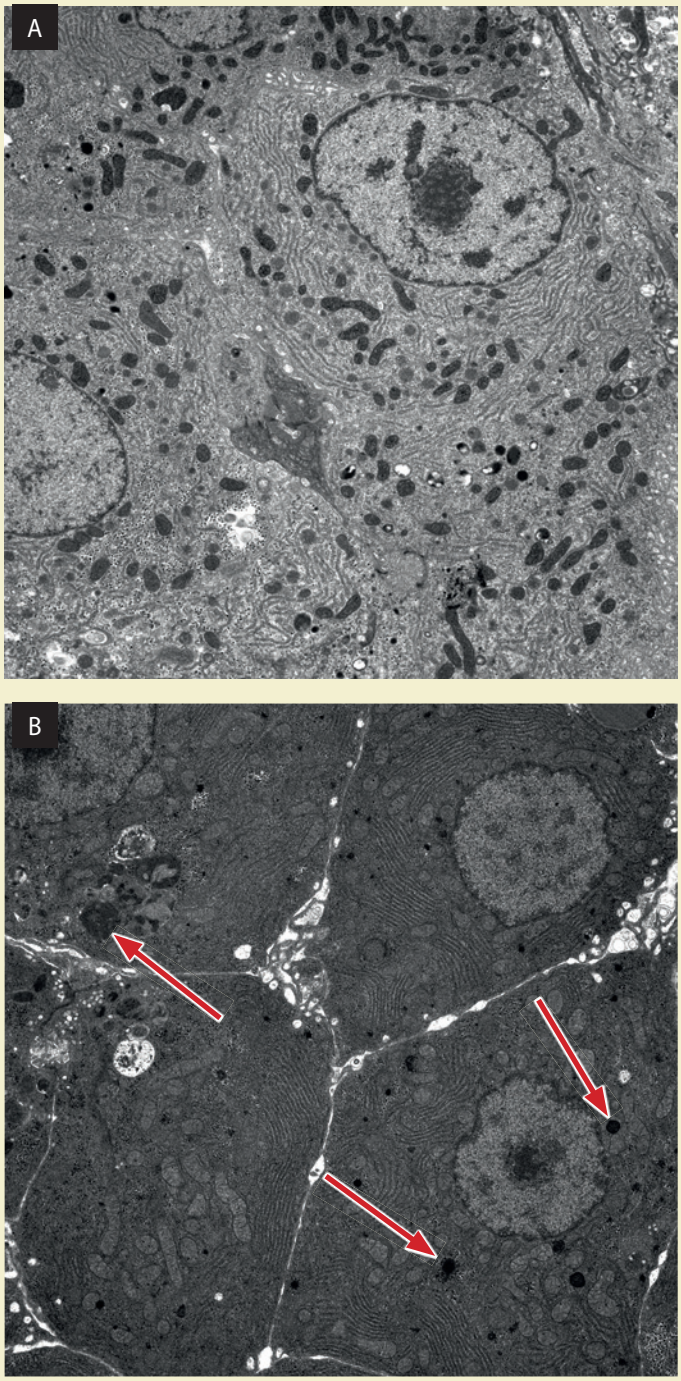
Fot. 8.3. Rozrzedzenie cytoplazmy hepatocyta z rozpadem jej struktur: pstrąg tęczowy z odłowu wiosennego, 2-RAS, S / Phot. 8.3. Cytoplasmic rarefication with the dissolution of cytoplasmic structures: rainbow trout from the spring sampling, 2-RAS, S



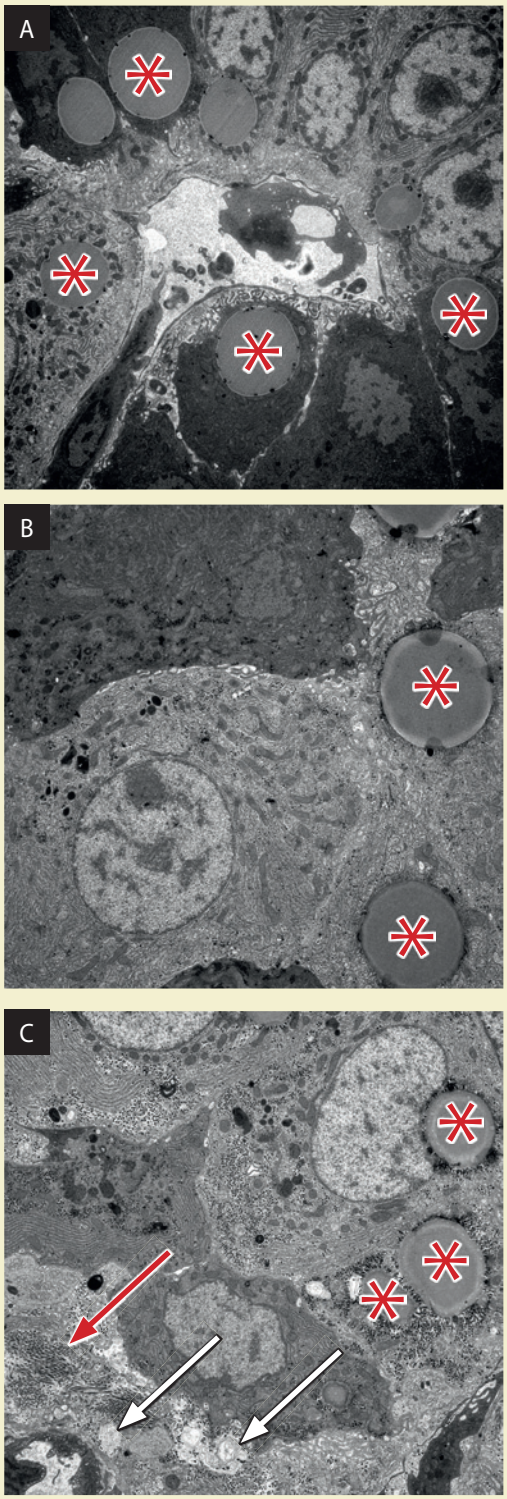
Fot. 8.4. Miejscami rozrzedzenie cytoplazmy, zatarcie struktur grzebieniastych w mitochondriach i ich polimorfizm (czerwone strzałki), struktury mielinopodobne (białe strzałki), widoczne aparaty Golgiego (żółte strzałki): pstrąg tęczowy z odłowu jesiennego, 3-OOH, S / Phot. 8.4. Local cytoplasmic rarefication, blurring of mitochondrial crest structures, mitochondrial polymorphism (red arrows), myelin-like structures (white arrows), Golgi apparatuses visible (yellow arrows): rainbow trout from the autumn sampling, 3-OS, S



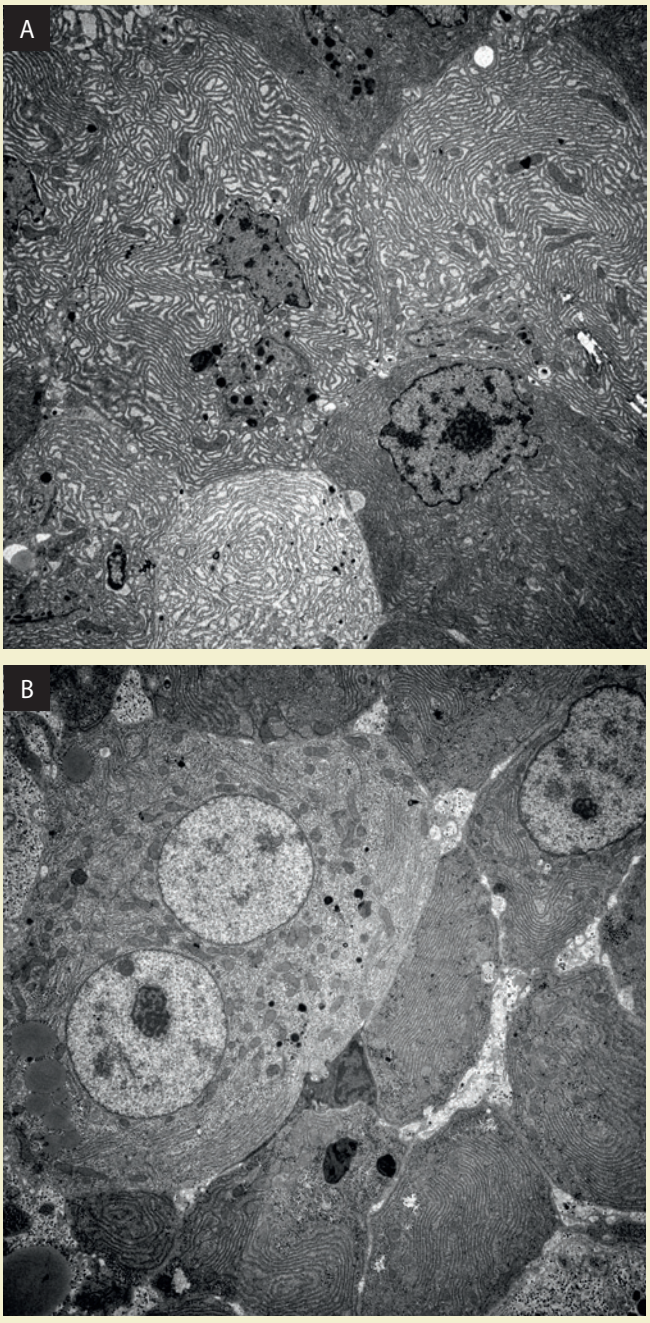
Fot. 8.5. Hepatocyty ze strukturami mielinopodobnymi, widoczne liczne włókna kolagenowe: pstrąg tęczowy z odłowu wiosennego, 3-OOH, D / Phot. 8.5. Hepatocytes with myelin-like structures, numerous collagen fibers visible: rainbow trout from the spring sampling, 3-OS, B




Fot. 8.6. Polimorfizm i rozplem mitochondriów w hepatocytach pstrągów tęczowych (A, B), miejscami lizosomy – strzałki (B), odłów jesienny: 2-OOH, S (A); 1-RAS, D (B) / Phot. 8.6. Polymorphism and proliferation of mitochondria in hepatocytes of the rainbow trout's (A, B), locally lysosomes visible – arrows (B), autumn sampling: 2-OS, S (A); 1-RAS, B (B)




Fot. 8.7. Liczne krople lipidów w cytoplazmie hepatocytów pstrągów tęczowych (gwiazdki): rozplem i polimorfizm mitochondriów w hepatocycie (A), odłów jesienny, 3-RAS, D; miejscami rozplem mitochondriów (B), odłów jesienny, 1-OOH, S; miejscami włókna kolagenowe (czerwona strzałka) i struktury mielinopodobne (białe strzałki) (C), odłów wiosenny, 1-OOH, D / Phot. 8.7. Numerous lipid droplets in the cytoplasm of hepatocytes of the rainbow trout's (asteriks): mitochondrial proliferation and polymorphism in the hepatocyte (A), autumn sampling, 3-RAS, B; local mitochondrial proliferation (B), autumn sampling, 1-OS, S; locally visible collagen fibers (red arrow) and myelin-like structures (white arrows) (C), autumn sampling, 1-OS, B




Fot. 8.8. Rozplem szorstkiej siateczki endoplazmatycznej w cytoplazmie hepatocytów pstrągów tęczowych: odłów wiosenny, 3-RAS, D (A); hepatocyt z podwójnym jądrem i rozplemem mitochondriów, odłów jesienny, 1-OOH, S (B) / Phot. 8.8. The proliferation of the rough endoplasmatic reticulum in the cytoplasm of the hepatocytes of the rainbow trout's: spring sampling, 3-RAS, B (A); hepatocyte with a double nucleus and mitochondrial proliferation, autumn sampling, 1-OS, S (B)

Fot. 10.1. Pompa wody z zastosowaniem śruby Archimedesa / Phot. 10.1. Water pump using Archimedean screw

Fot. 10.2. Automatyczna sortownica wykorzystująca pompę wody / Phot. 10.2. Automatic sorting device using water pump

Fot. 10.3. Praktyczne wykorzystanie urządzeń: pompa wody z śrubą Archimedesa i sortownica / Phot. 10.3. Practical use of equipment: water pump with Archimedean screw and sorting device