



**Research Article** 

Volume 1; Issue 1

# Aquaponics as Promising Tool on Rearing Ohrid Lake Trout (Salmo Letnica) for Conserving the Wild Individuals and Generating Incomes

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Received Date: September 24, 2018; Published Date: October 16, 2018

# Abstract

In the Ohrid Lake the Ohrid trout (*Salmo letnica*) is an endemics species, which is not found anywhere else in Albania, FYROM or in other countries world. The fish is symbolic of the lake and of Pogradec, the biggest Albanian city on the lakeshore. The aim of this review is to give a description of potential applications of Aquaponics for the Ohrid trout rearing with the aim of reducing the wild capture fisheries and providing additional incomes to the farmers from the vegetable marketing. Furthermore on this review are presented the future perspectives of this species growing on a sustainable way, while all the region will face water scarcity problems.

Keywords: Ohrid Lake Trout; Aquaponics; Recirculating Aquaculture System; Abiotic Parameter; Biotic Parameter

**Abbreviations:** RAS: Recirculating Aquaculture System; TAA: Total Amino Acids; NEAA: Non-Essential Amino Acids.

# Introduction

Several *Salmo* taxa have been reported to inhabit Albanian rivers and neighbouring drainages in FYROM and Greece. Examples include *S. farioides*, proposed by Karaman [1], and *S. ohridanus*, *S. letnica*, *S. letnica lumi*, *S.*  *trutta, S. macrostigma, S. peristericus, S. marmoratus* and *S. montenegrinus* [2,3]. Unfortunately, confirmation of these observations and the continued existence of such trout in these waters, as well as their taxonomic status, remain uncertain, representing an absence from any comprehensive overview of Balkan trout demography, evolution and classification.

The data that do exist on trout in Albania are very scarce and mostly stem from an inventory of fishes undertaken

Citation: Bakiu R and Durmishaj S. Aquaponics as Promising Tool on Rearing Ohrid Lake Trout (*Salmo Letnica*) for Conserving the Wild Individuals and Generating Incomes. Pro Aqua Farm Marine Biol 2018, 1(1): 180003. in the country in the 1950s [4], or are restricted to certain areas (e.g. Stanković [3] on Lake Ohrid; [5] on the River Shkumbini). Rakaj [6] extended and brought up to date the work of Poljakov, et al. [4] on Albanian ichthyofauna. He described trout from the rivers Shala and Valbona (Ohrid Drin-Shkodra system; see also Schöffmann [7] results) as well as from the lakes Shkodra and Ohrid, while trout have also been reported to exist within the rivers Bistrica [6], Cemit [6], Mati [2] and Shkumbini [5].

Very few genetic analyses of Albanian trout have been performed so far and all are restricted to lakes Ohrid and Prespa [8-10].

As inferred from several previous studies on Balkan trout [11] anthropogenically induced hybridisation, particularly with introduction of non-native trout lineages, has had a considerable impact on many indigenous trout stocks and has blurred the picture of the original genetic structure and phylogeography of Balkan trout. However, because of Albania's past political isolation and low level of economic development, it is probable that stocking with non-native strains of brown trout (e.g. Atlantic line age) has not been performed here; therefore, despite any impact of overfishing and intense poaching (authors' personal observations) on the population sizes of native trout, the present distribution and composition of trout in Albania may relatively faithfully reflect the natural situation, a rare situation for salmonid rivers in Europe given the widespread practice of stocking.

Based on microsatellite and mtDNA sequence variation, the endemic Ohrid trout represents a monophyletic lineage isolated from other Adriatic basin populations, but nonetheless most likely evolving from within the Adriatic lineage of brown trout (*Salmo trutta*). Sušnik and colleagues [9] results didn't support the existence of population structuring within Lake Ohrid, even though samples included two putative intra-lacustrine forms. In the interests of protecting the unique biodiversity of this ancient ecosystem, they also recommend retaining the taxonomic epithet *Salmo letnica* for the endemic Ohrid trout. All these information were reported in order to show the probable genetic similarities and common origin of *Salmo trutta* with *Salmo letnica*.

Considering the literature in conservation aquaculture, freshwater fish farming and the importance of the Ohrid trout (*Salmo letnica*) in the region, the aim of this paper is to give a description of potential applications for the Ohrid trout rearing in integrated aquaculture production systems (represented by Aquaponics technologies) and future perspectives of this species growing on a sustainable way, while all the region will face water scarcity problems. Thus, the main purpose would be to give advices based on several research group results and consideration for reducing the wild capture fisheries of the Ohrid lake trout fish and providing additional incomes to the farmers from the vegetable marketing.

## Discussion

#### **Ohrid Trout Ecosystems**

Lake Ohrid is certainly the best-known in Europe, located 685 m above sea level and lies between 40 and 41°N. It has an area of 369 km<sup>2</sup>, of which about 90 km<sup>2</sup> belongs to Albania. The maximum depth is 285.5 m while the average depth is 145 m. The lake shore line is 83 km in length and its watershed is 680 km<sup>2</sup>. It discharges into the Adriatic Sea via the Drin River. The maximum variation in the water level of the lake does not exceed 29-30 cm, because it is constantly fed by karstic surface and sublacustrine springs which bring more water to the lake than do the surface and often temporary inflow streams [2].

The watershed of Lake Ohrid includes steep mountains, as well as both Macro and Micro Prespa Lakes. The total area of the watershed is about 3,921 km<sup>2</sup>. A little less than half of the water in Lake Ohrid comes from its tributaries. On the Macedonian side, the Sateska and Koselska Rivers are the largest contributors [12].

On the Albanian side, river flow is substantially less, but the Pogradec and Verdova Rivers are the largest contributors. The remaining inflow comes from the springs that flow into the southern part of the lake, at St. Naum, Drilon and Tushemisht. These springs are fed by water flowing out of the porous karst mountains to the east, Galicica and Mali i Thate. Over thousands of years, holes and channels have formed within the mountain rock. These channels carry water that originates in the Prespa watershed to Lake Ohrid.

Macro and Micro Prespa Lakes are filled mostly by the rivers flowing into them. About every 11 years, all the water in Lake Prespa is replaced by new water. In contrast, it takes about 70 years for all the water in Lake Ohrid to be replaced. Water flows out of Lake Ohrid near Struga, into the Black Drim River. This river eventually runs all the way to Lake Shkodra and the Adriatic Sea [12].

It is very clear that the fisheries in Lake Ohrid are in immediate danger and rapid management action is required. All the data suggest that the trout populations are severely stressed and the bleak and carp populations are also threatened. Overfishing seems to be the major cause of the decline of the trout population. The socio-economic pressures that have led to overfishing have impacted the trout more than other fish stocks because of the greater demand and higher economic value of this fish. Although the overall catch of trout has only declined slightly in the last several years, there has been a dramatic shift in the harvest. Beginning in 1992, the landings in Albania increased dramatically, while those in Macedonia began to fall. The differences in fishing pressures in the two countries are the results of differences in the social and political situation in each country and the fishing regulations in each country [12].

While there have been limits on the catch in Macedonia for the last decade through concessions and licenses granted by the government, in Albania, such limits have just begun with the establishment of the Association for Fishery Management in Pogradec in 2002 [12].

Based on Report on Development of Fisheries Harvest Statistics and a Transboundary Fisheries Stock Assessment Protocol for Lake Ohrid, for the period from 1969 to 1991 catch figures are of reliable quality as all catches were recorded, particularly weight by species for individual fishermen at certain landing sites. Data collection was not conducted in a standardized manner from the early nineties up to present and officials had to resort to estimates of the fish production. In recent years fishermen were obliged to complete catch return forms, which were collected by the resident fisheries inspector [12]. The latter monitored the quality of the returns, which were processed by the Fisheries Inspectorate in Tirana and based on these data maximum Ohrid trout production was registered in 2000, represented by 98 tons of fish, while actually the production is continuously decreasing.

In addition to harvest pressures and habitat loss, especially of the reed beds, the native fish of Lake Ohrid are also threatened by the introduction of non-native species into the lake.

Rainbow trout (*Oncorhynchus mykiss*) represents a particular concern because it may displace the native trout. Although this fish was first introduced in the 1970s, the development of fish farms in the basin offer new potential threats. There is also some preliminary evidence that the pesticides used by farmers in the watershed may threaten fish in the lake. These pesticides have been found in the tissues of fish collected from the lake. Not only are these pesticides harmful to the fish themselves, but they also pose hazards to the people who eat the fish, especially women of childbearing age and small children [12].

#### **Ohrid Trout and the Subspecies**

Smalo letnica [13] – Korani (in Albanian language; Figure 1)

The teeth on the vomer are arranged in two circles, in the form of a V over the jaws and in a single row around the whole perimeter of the mouth. The males are quite different from the females. Their scales are very firmly attached and their upper and lower jaws are prolonged. There are four or five dark spots on the operculum, seven to eight red spots along the lateral line and two or three over the pectoral fin (in the male). There are some black spots on the back, which are more distinct on the dorsal fin. The scales in general are white, except the distal anal scales, which are darker in color. The extremities of the forked tail are dark grey in colour. The dorsal scales are reddish and the other scales silvery. The males are darker in colour than the females. In the spawning season the head is prolonged and sharpened. Along the whole of the lateral line there is a number of large red spots. The head of the female is more rounded and not as large as that of the male. They are a silver colour but the red spots are larger and denser in the males. The scales are prolonged and elliptical. In summer the colour becomes lighter, being somewhat yellow to silvery [2].



Figure 1: Examples of Lake Ohrid trout (*Salmo letnica*). The Albanian name for this fish is Koran and in Macedonia it is known as letnica (photo below by Iain Wilson [9] and Skerdi Cinari).

The 'Korani' trout usually occurs at depths of 60-80 m. It approaches the coast only in the breeding season. Korani trout reach sexual maturity at the age of 4 years, having a length of about 32-35 cm, the males weighing less than females. Reproduction takes place in January- February, in areas with underground springs. Sexually mature males and females approach the coast and spawn at depths of 5-50 m. At spawning time, the male: female ratio is 1.7:1 and the water temperature varies from about 7 to 9°C. The eggs take from 58 to 74 days to hatch. The fecundity of the females is 2200-2500 eggs per kg body weight.

The eggs are light orange, with a diameter of 3.5-5.2 mm. The fry feed on entomostracan crustaceans, and later on insect larvae and benthic invertebrates (Gammaridae, Phryganidae, Perlidae, etc.) [2].

After a few months the fry withdraw to deeper water and only approach the coast when they breed for the first time. After spawning, the females move to deep water and cannot be captured until the next autumn while the males are only captured in small quantities during the summer. They feed mainly on fish such as bleak [2]. Accoding to Rakaj and Flloko [2], the Ohrid trout growth is faster than the 'Belushka' trout (*Salmothymus ohridanus*)

The older females tend to become sterile. The 'Korani' or Orhid lake trout makes up the bulk of catches of salmonids in the Albanian waters of Lake Ohrid.

In 1990s, the annual yield was 15-30 tonnes per year. The majority of the catch consists of individuals with a length of 30-50 cm, weighing 500-1300 g. The introduction of midwater trawling brought a marked increase in the fishery yield but this has since reduced from year to year. The 'Korani' trout is of considerable economic value and it is much in demand. Since 1955-56, artificial reproduction has been carried out on this species or on hybrids with *Salmothymus ohridanus* as the female [2].

#### Salmo letnica lumi [4] -- Korani i lumit

This species differs from the 'Korani' trout by having a longer, more powerful body. The tail is a little longer and higher than that of the typical 'Korani' trout. The bottom jaw is a little shorter than the upper jaw. There are always black spots on the head and on the operculum. The body is covered with black and red spots. In winter the colour of the body of the males is dark, or with a grey background. Males grow longer and broader than females. The fins are generally yellow, the unpaired fins in the male being a little higher and the paired fins a little shorter than in the female [2].

This subspecies lives in Lake Ohrid at the same depths as the 'Korani' trout. It differs from the latter by running up rivers (e.g. the River Krekes near Pogradec) to spawn in January-February. They become sexually mature at the age of 4 years, having reached a length of 20-22 cm. The females produce 3000 eggs per kg body weight. The eggs are laid in 15 cm of water and are 4.3-4.5 mm in diameter. The eggs account for up to 12% of body weight. The eggs hatch after 47-52 days (c. 400°C-days). The average temperature of the water in the spawning sites is 7-7.8°C. The fry stay in the stream until the end of June, and feed mainly on *Gammaridae*. The temperature of the water in June is c. 13°C. The depth of the stream is never greater than 30 cm. A 4-month old trout is 6 cm long and weighs 3 g. After June they move to the lake and will not return to the stream until they have reached sexual maturity [2].

Due to its rarity, it is not a commercial species, although it can be caught in small quantities in streams with traps. The stock of this subspecies has diminished tremendously [2], mainly because of habitat destruction in the streams used for spawning. Now, it is impossible to find it in Albanian waters, but anyway in the Drini river waters of Kosovo and Montenegro it can be found now-days.

#### **Aquaculture of Ohrid Trout**

It was brought into the United States by the U.S. Fish and Wildlife Service. The species was first stocked in Colorado in 1969 and subsequently into several small lakes in northern Minnesota and in Tennessee since 1971. Introductions into most of these states failed. Repeatedly stocked in Tennessee with no evidence of reproduction yet. Courtenay and Hensley [14] report that even though there has been no reproduction, spawning has been observed in Tennessee.

In the Ohrid Lake the koran (Salmo letnica) is an autochthonous species that is not found anywhere else either in Albania or in the world. The fish is symbolic of the lake and of Pogradec, the biggest Albanian city on the lakeshore. Efforts to restock the lake with koran are the responsibility of the local government hatchery, Stacioni i Linit Pogradec, run by Ms Celinike Shegani, an aquaculture specialist. The hatchery is located a few meters from the edge of the lake, but the water that is used in the hatchery comes from a spring. This ensures that the temperature of the water is a constant 10 °C. The water in the hatchery is tested at regular intervals for different elements, including nitrite, nitrate, phosphates, oxygen, pH, and ammonia. The testing is done at the laboratory attached to the hatchery (personal communication, Bakiu R.). During the breeding season in January and February local fishermen are tasked with stripping the wild spawners of eggs and sperm in the lake and then collecting the eggs and bringing them to the hatchery (personal communication, Bakiu R.). The eggs are placed in incubators and hatch after 40-45 days after which the larvae are kept in the hatchery for 2.5 months and then moved to the nursery.

The nursery is equipped with 20 tanks of which currently two are in use [15]. According to the data reported from project implementation about the re-building the hatchery of Lin during 2003-2004 by a famous Aquaculture Technology Company named Scubla, it resulted that vertical hatching cabinets, where the eggs are placed in drawers or racks above each other were installed in the Hatchery of Lin. Furthermore, it was also used the Californian method and incubation bottles of Mac Donald type were used for beginning the work on genetic research analyses. Particular substrates of incubation were used in order to create the proper conditions especially during one of the most critical phase of production cycle, the hatching and they were able to minimize the stress of fish larvae, improve the growth performance and prevent deformities of vitellus [16]. Furthermore, the applied technologies provided such conditions, which were able to produce fish larvae 20-30% bigger than the normal ones and consequently they were able to improve their feeding and increase the survival rates. The Californian method (represented by multiple trays) is perfect/ideal for salmonid species, which are easily subject to stressful conditions or tend to stay in crowding conditions, consequently increase the mortality rate [16].

In fact the fingerlings are maintained in 7 separated groups in each of the trays and they can be further maintained in optimal conditions even after the vitellus absorption and for the first 20-30 days of exsogenous feeding. During the project implementation by Scubla Company, it was shown that better results could be reach if the fingerlings were maintained in higher densities than normal conditions, because they were more obliged to search for food. After about three weeks, the fingerling were transferred in bigger tanks in order to prevent their death from crowding. Thus, 20 tanks (modular installations of tanks with dimensions 2x6 m) were installed in the Hatchery of Lin and they represent an important innovation, because they represent a middle way of using a circular and a rectangular tank at the same time. They support a sufficient water flow and they are able to create the auto-clean effect, but they are not able to provide sufficient level of oxygen to the fish or fingerlings. These tanks are also ideal for brood stock and for those species (salmonids or sturgeon), who prefer a strong water flow [16].

Every year about 1 million eggs are collected which results ultimately in about 700,000 to 800,000 fingerlings. In the fall the fish, now with a weight of 3-4 g are introduced into the lake. Based on Report on

Development of Fisheries Harvest Statistics and a Transboundary Fisheries Stock Assessment Protocol for Lake Ohrid, stocking densities have been presented in Table 1. It should be noted that larvae of Ohrid trout were sometimes untimely released for hatchery capacity reasons.

Year	Larvae	Fingerlings
1995	1,200,000	50,000
1996	815,000	168,000
1997	1,000,000	200,000
1998	1,000,000	200,000
1999	1,000,000	260,000
2000	700,000	360,000
2001	1,000,000	360,000
2002	1,230,000	260,000
2003	1,100,000	200,000
2004	1,200,000	600,000
2017	1,000,000	800,000

Table 1: Albanian stocking data of Ohrid trout (Source: Fisheries Research Institute, Pogradec).

Cutbacks in funding have forced the hatchery to concentrate on its core objective of breeding koran fingerlings to restock the lake. In 2007, the hatchery carried out a project, whereby it tagged some 120,000 fingerlings that were released into the lake in order to study the lifestyle of the fish. The results would have contributed to a more focused restocking effort, but after two years the funding dried up and the project was forced to stop [15].

### **Aquaponics Technology**

Aquaponics is the integration of recirculating aquaculture system (RAS) and hydroponics in one production system. In an aquaponic unit, water from the fish tank cycles through filters, plant grow beds and then back to the fish. In the filters, the fish wastes are removed from the water, first using a mechanical filter that removes the solid waste and then through a biofilter that processes the dissolved wastes. The biofilter provides a location for bacteria to convert ammonia, which is toxic for fish, into nitrate, a more accessible nutrient for plants. This process is called nitrification. As the water (containing nitrate and other nutrients) travels through plant grow beds the plants uptake these nutrients, and finally the water returns to the fish tank purified. This process allows the fish, plants, and bacteria to thrive symbiotically and to work together to create a healthy growing environment for each other, provided that the system is properly balanced. In aquaponics, the aquaculture effluent is diverted through plant beds and not released to the environment, while at the same time the nutrients for the plants are supplied from a sustainable, cost-effective and non-chemical source [17].

This integration removes some of the unsustainable factors of running aquaculture and hydroponic systems independently. Beyond the benefits derived by this integration, aquaponics has shown that its plant and fish productions are comparable with hydroponics and recirculating aquaculture systems. Aquaponics can be more productive and economically feasible in certain situations, especially where land and water are limited. Although the production of fish and vegetables is the most visible output of aquaponic units, it is essential to understand that aquaponics is the management of a complete ecosystem that includes three major groups of organisms: fish, plants and bacteria [17].

Aquaponics combines two of the most productive systems in their respective fields. Recirculating aquaculture systems and hydroponics have experienced widespread expansion in the world not only for their higher yields, but also for their better use of land and water, simpler methods of pollution control, improved management of productive factors, their higher quality of products and greater food safety [17].

Aquaponics is a technique that has its place within the wider context of sustainable intensive agriculture, especially in family-scale applications [17]. It offers supportive and collaborative methods of vegetable and fish production and can grow substantial amounts of food in locations and situations where soil-based agriculture is difficult or impossible. The sustainability of aquaponics considers the environmental, economic and social dvnamics. Economically, these systems require substantial initial investment, but are then followed by low recurring costs and combined returns from both fish and vegetables. Environmentally, aquaponics prevents aquaculture effluent from escaping and polluting the watershed. At the same time, aquaponics enables greater water and production control. Aquaponics does not rely on chemicals for fertilizer, or control of pests or weeds which makes food safer against potential residues. Socially, aquaponics can offer quality-of-life improvements because the food is grown locally and culturally appropriate crops can be grown. At the same time, aquaponics can integrate livelihood strategies to secure food and small incomes for landless and poor households. Domestic production of food, access to markets and the acquisition of skills are invaluable tools for securing the empowerment and emancipation of women in developing countries, and aquaponics can provide the foundation for fair and sustainable socioeconomic growth. Fish protein is a valuable addition to

the dietary needs of many people, as protein is often lacking in small-scale gardening [17].

Aquaponics is most appropriate where land is expensive, water is scarce, and soil is poor. Deserts and arid areas, sandy islands and urban gardens are the locations most appropriate for aquaponics because it uses an absolute minimum of water. There is no need for soil, and aquaponics avoids the issues associated with soil compaction, salinization, pollution, disease and tiredness. Similarly, aquaponics can be used in urban and peri-urban environments where no or very little land is available, providing a means to grow dense crops on small balconies, patios, indoors or on rooftops. However, this technique can be complicated and small-scale units will never provide all of the food for a family. Aquaponic systems are expensive; the owner must install a full aquaculture system and a hydroponic system, and this is the single most important element to consider when starting an aquaponic system [17].

Moreover, successful management requires holistic knowledge and daily maintenance of the three separate groups of organisms involved. Water quality needs to be measured and manipulated. Technical skills are required to build and install the systems, especially in the case of plumbing and wiring. Aquaponics may be impractical and unnecessary in locations with land access, fertile soil, adequate space and available water. Strong agricultural communities may find aquaponics to be overly complicated when the same food could be grown directly in the soil. In these cases, aquaponics can become an expensive hobby rather than a dedicated food production system.

Owing to the high initial start-up cost and limited comprehensive experience with this scale, commercial and/or semi-commercial aquaponic systems are few in number. Many commercial ventures have failed because the profits could not meet the demands of the initial investment plan. Most of those that do exist use monoculture practices, typically the production of lettuce or basil. Although many academic institutes in the United States of America, Europe and Asia have constructed large units, most have been for academic research rather than food production, and are not intended or designed to compete with other producers in the private sector. There are several successful farms throughout the world. One group of experts in Hawaii (United States of America) has created a fully-fledged commercial system [17]. They have also been able to obtain organic certification for their unit, enabling them to reap a higher financial return for their output. In Albania, the owner (Engr. Jani Taci) of the first aquaponics system in Albania designed and built this integrated aquaculture system by himself in 2012. In this Aquaponics system, which is located in Kashar village (near Tirana, Albania), the most used fish species are represented by Koi carp and goldfish (*Carassus auratus*), which is known to be the most used in aquaponics systems. Both species produce high levels of ammonia, which is good for maintaining nutrient levels for the aquaponic process. Both fish are also very resilient to changes in pH, pollutants, and temperature. As it is shown in the Figure 2, the used method is floating raft method. The gold fish (*C. auratus*) and Koi carp (*C. carpio*) were bought from the Hatchery of Tapiza (property of Agricultural University of Tirana) and they (fingerlings with an average weight of 30g) were transferred in the tanks shown in Figure 2.

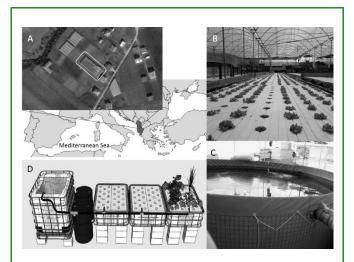


Figure 2: Location of aquaponics system farm in Albania (A) and some pictures (B and C) showing the components of the farms, together with a graphical representation of the used Aquaponic method (D).

In order to have a sufficient flux (2500 l/h) of water in the aquaponics system, a powerful pond water pump of 620W was installed and immerged in the plants tank. In these farm were performed several experiment, which results were published by Bakiu, et al. [18]. In Albania and Macedonia there are commercial production farms of trouts, but none of them used the Aquaponic technologies for growing trout fish species. Based on these observations, in these review we are presenting the important research results coming from several research groups in Europe related to the aquaculture technologies and fish feeding focused on trout species. No Ohrid lake fish intensive production farm exists and it could be a really excellent opportunity to build some in Albania and FYROM for conservation purposes of the wild individuals present in the Ohrid lake, which are at continuous risk from the fishing activities operating at both sides of the

Ohrid lake, though there are active national programs for populating the lake with fingerlings of Ohrid lake trout fish.

#### **Recirculating Aquaculture Systems**

Remaining focused on the aquaculture activities realized on using Aquaponics technologies for growing endemic species like Ohrid lake trout, it would be necessary to base our research papers analyses on RAS papers, where it is crucial to mention that disease transmission and requirements for environmentally sound management of aquaculture wastewater have led to development of RAS, as an ecologically and economically acceptable means of production [19-21]. In recent decades, RAS has progressed from unsuccessful pilot systems to becoming an important sector of freshwater as well as marine aquaculture [20,22].

Hence, RAS for trout culture rapidly came into use to maximize aquaculture productivity while maintaining environmental standards [23]. This successful RAS application, with fairly simple technology, low water requirements, minimal wastewater and high production, spurred efforts to build new systems elsewhere. In many cases, this led to a rapid increase in aquaculture production as well as to serious problems connected with disregard for local conditions. Parameters that could seriously influence the success of transferring RAS technology to other locations are the amount and quality of source water, fluctuations and range of temperature, and the risk of disease transmission from the source water or surface water [24].

A sufficient and reliable water source (drainage or borehole water) is necessary where the need for additional water supply arises, for example in the event of an acute system malfunction, such as decrease in biofilter activity, sudden turbidity or aeration or oxygenation failure [25]. That could represent a serious problem in locations with insufficient groundwater availability. The wider range of temperature fluctuations seen in temperate climate may affect biofilter efficacy [26], and perhaps reduce feeding and affect fish health [27]. In the past [28], were detected few problems with operation of open RAS in temperate climate (e.g. temperature-induced limitation of biofiltration, nitrogen supersaturation), which lead to its further re-evaluating. Norwegian scientists considered that in intensive aquaculture fish were kept at too high densities. High stocking densities can have a detrimental impact on the health and welfare of rainbow trout as well. In particular, high densities can lead to increased stress [29], increased susceptibility to disease, increased incidence of physical injuries [30], poor

body condition [31] and reduced growth, feed intake and feed conversion efficiency [31].

#### **Abiotic and Biotic Factors Related Issues**

Ellis, et al. [31] reviewed the scientific literature concerning the relationship between stocking density and welfare in farmed rainbow trout. These authors examined 43 papers that studied the effects of stocking density on productivity, health, body condition and stress level. They found that commonly reported effects of increasing stocking density include an increase in fin erosion and reductions in growth, feed intake, feed conversion efficiency plus body and liver condition.

They concluded that such changes are indicative of a reduced welfare status. Many authors proved influence on stocking density on the welfare of trout fish which affects survival, growth and feeding ratio, but the dates of these studies were contradictory.

The recommended stocking density in Bulgaria (for example) for intensive cultivation of rainbow trout is between 80 - 100 individual/m<sup>3</sup> [32]. In Bulgaria there are researchers who have investigated the influence of stock density on trout grown in cages, but publications concerning that problem are almost missing on the cultivation of trout in a recirculation system.

Based on the possible physiological requirements similarities between brown trout (Salmo trutta) and Ohrid lake trout (Salmo letnica), which have been shown to be genetically correlated [9], according the authors results showing that probably Ohrid trout have evolved from within the Adriatic lineage of brown trout (Salmo *trutta*), below are reported the results of scientific paper [33], where it was investigated the influence of stocking density on surviving, growth intensity and FCR of the rainbow trout and brown trout cultivated in a recirculation system with very efficient pelleted dry feeds (0.6-1.1 FCR) [34]. In the recirculation system, at the end of the 42 days trial period the final weight of rainbow trout was higher than that of the trout from the lowest stocking density. This tendency of average final body weight to decrease with the increasing of stocking density was valid for the trial of brown trout, too. The advantage in final body weight was on the hand of trout from low stocking density. Average individual growth rate of rainbow trout was higher with 6.32% higher than that of trout of high stocking density and for the brown trout this difference was 24.25 %. In the analyzed trials, the influence of stocking density to weight gain was better manifested in brown trout than in rainbow trout. The influence of stocking density on average values of feed conversion ratio was significantly manifested in brown

trout. This difference in rainbow trout was significant, but just 9 % to the advantage of FCR of trout from the lowest stocking density (P<0.05).

The temperature at the time of trial was in optimal borders for the two cultivated species. Ammonia and nitrites had optimal values for the cultivated fish, but nitrates had high rates, because recirculation system the paper authors use does not have a gentrification block that would have decreased high nitrate levels. Many other authors [35] remarked the influence of stocking density on the growth of trout. Reduced growth and an increase food conversion ratio with the increasing of stocking density were found in 70% of the studies investigated by Ellis [31]. Rainbow trout growth and feeding ratio was better at low stocking density compared to the growth and feeding ratio of fish at high stocking density in our trial. Brown trout in reported study showed higher differences of growth and feeding ratio at the examined density than these of rainbow trout to the advantage of fish at low stocking density too. Some studies reported that high densities lead to aggression and some welfare problems that authors suggest may be due to the existence of a dominance hierarchy [30]. Sirakov and Ivancheva [33] didn't investigate the behavior of cultivated fish species especially, but when they observed them they didn't see so many cases of aggression and they considered that the general reason for high survival, growth and feeding ratio in tanks at low density is a result of better quality of the water in them. Ellis, et al. [31] concluded that stocking density is "an important factor for fish welfare, but cannot be considered in isolation from other environmental factors".

Sirakov and Ivancheva [33] suggested that level of removal waste products was higher in tanks of high stocking density and combination with high level of nitrates in used recirculation system decreased growth of fishes and assimilation of food much more in tank with high density than this tank with low density. The most important reason is that the influence of stocking density on growth and feeding intake of brown trout is higher than in the trial with rainbow trout is the higher sensitiveness of brown trout to environmental conditions. The high number of individual/m<sup>3</sup>, which reduced the ability of fish to see and access food is another possible reason for the low growth and high feeding conversion ratio of fish in high stocking density.

Obviously, fish are in contact with their water environment for their whole life. So the water quality which is connected with production technology could influence not just the growth of the raised hydrobionts, but also the quality of fish. The numerous advantages of RAS will apparently be the reason for the increased development of this ecologically friendly technology in several European countries, but questions connected with the quality of the raised fish still remain open.

One of the few publications about this issue is represented by the published scientific work of Sirakov in 2015. The aim of his research was to study the influence of cultivation technology on the body composition of two trout species rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta m. fario*) raised in recirculation aquaculture system.

The study was conducted in a RAS located at the experimental aquaculture base of the Trakia Univesrity, Stara Zagora. The system used for the experiment consisted of a fish tank with a total volume of  $1 \text{ m}^3$  and a working volume of 800 l. The out let water from the fish tanks is directed into the filtration system. The treatment of the water in the filter was assured with a mechanical filter (settling tank) and a bio filter (moving bed bio filter).

During the cultivation in RAS, dry matter, protein, lipid and ash increased in flesh of rainbow trout respectively with 6.6%, 3.9%, 35.8% and 4.52%. At the same time the content of dry matter and protein increased in flesh of brown trout respectively with 2.28% and 6.76%, but lipid and ash decreased respectively with 3.7% and 16.1%. The analysis of differences in chemical content of flesh between the two cultivated trout species was statistically significant ( $P \le 0.05$ ) just for lipid content and it was in favour of rainbow trout's flesh.

The data received from us for the content of protein in the flesh of rainbow trout were in confirmation with the data received from Savi, et al. [36] and Bud, et al. [37] for rainbow trout being received from aquaculture. Some scientists found a higher level (20% and higher) of protein for trout reared in aquaculture. The lipid content found out in rainbow trout's fillet in the current research was lower compared with those received from different scientists [36,37], but the water content and ash were similar.

The content of protein in fillet of brown trout raised in RAS at Trakia University was much closer to the results received for wild brown trout (higher than 18%) than those received for cultured brown trout (17.24%) [38]. The content of lipids was lower in these trials compared with those received for both wild and cultured brown trout in the experiment conducted by Kaya, et al. [38], but in turn the dry matter and ash were similar to those received from Sirakov [39].

The analysis of data concerning content of mineral and microelements in rainbow trout's flesh showed that

phosphorus, sodium, iron, copper and zinc increased respectively with 28.04%, 9.67%, 7.07%, 24.6% and 64.4%, but calcium, potassium and magnesium decreased with 13.04%, 25.3% and 29.5% during the experiments. The content of phosphorus, sodium, copper and zinc increased respectively with 13.21% and 17.5%, 44.18% and 56.6%, calcium, magnesium, potassium and iron decreased respectively with 15.7%, 38.9%, 25.8%, and 38.9% in the experimental brown trout's flesh. The analysis of differences in mineral and microelements content in flesh between the start and end of experiment in two cultivated trout species was statistically significant for phosphorus, iron and zinc content.

Different studies in aquaculture are connected with mineral content in fish fillet, because of two reasons: first, to satisfy the needs of the cultivated hydrobionts with essential minerals [40] and second, the always high interest of consumers in mineral content, because of their concern for the presence of heavy metals in aquaculture products [41].

The concentration of Fe, Cu, Zn in the Sirakov [39] study were lower compared with their concentration in fillet of yellow perch cultivated in RAS [40]. The content of three amino acids (asparagine, glutamic acid and lysine) was higher in the fillet of both tested trout species compared with the quantity of the other amino acids. The same basic amino acids content for brown trout fillet were determined by Ozyurt and Polat [42], but Sabetian, et al. [43] found out that the basic amino acids in rainbow trout fillet are asparagine, glutamic acid and leucine. The content of essential amino acid threonine increased significantly in rainbow trout at the end of the experiment, the same tendency was determined for amino acid cysteine for brown trout.

In the Sirakov [39] study, the total amino acids (TAA) amount was found to be lower at the end of experiment when compared with its value at the beginning of the trial for both tested trout species. TAA from the current research was similar to the value received for cultivated brown trout in the studies of Kaya, et al. [38]. The content of EAA (valine, methionine, isoleucine, leucine, phenylalanine and histidine) increased and non-essential amino acids (NEAA) (aspartic acid, glutamic acid, alanine, glycine and proline) decreased in rainbow trout's fillet at the end of experiment. In the flesh of brown trout EAA and TNEAA decreased with time.

Nevertheless in the respect of the EAA/NEAA ratio at the end of the experiment, those calculated for brown trout kept higher value (0.81) than those found out for the flesh of rainbow trout (0.78). The results obtained from this study showed that both tested trout species in RAS have a

# **Progress in Aqua Farming and Marine Biology**

well-balanced and high quality protein source in the respect of the EAA/NEAA ratio and the received data are in confirmation of data received for brown trout by Kaya, et al. [38] and for rainbow trout by Sabetian, et al. [43].

The ratio of E/NE was determined as 0.71 for cod (*Gadus morhua*) by Jhaveri, et al. (1984), 0.77 for sea bream (*Pagrus major*), 0.69 for sardine (*Sardina melonosticta*), 0.74 for herring (*Clupea pallasi*) and 0.75 chum salmon (*Oncorhynchus keta*) by Iwasaki and Harada [44].

The Sirakov [39] study showed that the quality of fillet received from rainbow and brown trout cultivated in RAS was higher. The flesh samples of rainbow trout showed a higher lipid and better essential amino acids content in the end of the trial compared with those found out for brown trout, but differences in the other exanimate parameters for both tested trout species were not significant, which showed that both tested fish species are appropriate for cultivation in RAS in relation to their fillet quality.

Sirakov [39] recommended recirculation aquaculture technology as appropriate for the cultivation of rainbow and brown trout in Bulgaria, because it is environmentally friendly and it allows an intensification of the process while being a source for good products for human consumption in terms of nutritional value for the population at the same time. All these results could be a good starting point for performing similar experiments with Ohrid lake trout.

### Conclusion

In conclusion, the best alternative for growing *S. letnica* in a aquaponic system would be to follow the recommendations of Sirakov [39] scientific paper, because based on these results, RAS (as component of Aquaponics technologies) resulted to be appropriate for the cultivation of brown trout, which by the genetic point of view resulted to be similar to Ohrid brown trout.

Another suggestion would be to integrate the RAS (based on the abiotic and biotic parameters values reported previously regarding the RAS based on-growing systems for brown trout production) with the hydroponic technology, where several endemic plants and economically interesting plants can be grown in the integrated aquaculture system.

Anyway, it would be strictly recommended to perform some trials in a small-scale aquaponics system with Ohrid trout, based on the information reported previously regarding brown trout. If, every trial is going to be successful, on that case it would be advisable to extend the aquaponics system into a medium-scale system and perform analyses regarding its economic feasibility for at least 6 months period.

According Sirakov [39] results, the flesh samples of rainbow trout showed a higher lipid and better essential amino acids content in the end of the trial compared with those found out for brown trout, but differences in the other exanimate parameters for both tested trout species were not significant, which showed that both tested fish species are appropriate for cultivation in RAS in relation to their fillet quality. Nevertheless in the respect of the EAA/NEAA ratio at the end of the experiment those calculated for brown trout kept higher value (0.81) than those found out for the flesh of rainbow trout (0.78). This means that it would be necessary to measure and consider all these parameters for adding more value to the final product, the Ohrid trout flesh.

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#### 11

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