Università degli Studi di Milano

GRADUATE SCHOOL OF VETERINARY SCIENCES
FOR ANIMAL HEALTH AND FOOD SAFETY
Director: Prof. Valentino Bontempo

Doctoral Program in Animal Nutrition and Food Safety

Academic Year: 2013-2014

FRESHWATER FISH OF NORTHERN ITALY: FROM THE VALORIZATION OF WILD UNEXPLOITED SPECIES TO NEW FARMING PERSPECTIVES

Mauro Vasconi

Tutor:

Prof. Vittorio Maria Moretti

Coordinator:

Prof. Giovanni Savoini

Index

CHAPTER 1

Foreword

1.1. Italian situation of freshwater fisheries

1.1.1. Italian inland waters and fisheries

Italy has an extension of more than 300000 km^2, with 7458 km of sea coast. Inland waters occupy 7230 km^2. Freshwater lakes, located mainly in the northern and central regions, have an extensions of 2500 km^2, including water reservoirs. Rivers present a total length of 7700 km. The remaining inland waters consist in lagoons and coastal lakes with brackish waters. For these reasons the freshwaters commercial catch represents only the 0.7% of total Italian wild fish production. The activity of commercial fishing in inland waters is a production based on ancient traditions, passed down from father to son, almost miraculously survived to the progressive modification of lacustrine environment. The EU "Intervention in inland fisheries" reports that Italian inland fishermen are 3600, with 1000 operating in freshwaters and estuaries and 2600 in coastal salted lagoons. The larger part of them works in the north lake district, located in Piemonte, Lombardia and Veneto, where there are the large glacial lakes of Orta, Maggiore, Como, Iseo and Garda. This lakes, associated with other smaller lakes positioned in the same regions, represents more than the 80% of total Italian lacustrine volume (Premazzi et al., 2003). Regione Lombardia provides the more accurate data of inland fisheries in its document "Piano Triennale della Pesca professionale e dell'acquacoltura 2009-2011". In that region 196 professional fishermen are active, the larger part operating in the Como lake, with 69 operator. Forty-seven fishermen work in Garda lake, 29 in Iseo lake, 15 in Maggiore lake. The remaining fishermen work in smaller lakes or fluvial waters. In Piemonte 22 fishermen are active on the Maggiore lake. In Veneto 23 fishermen are active on the Garda lake. The trend of the number of fishermen is in a steady decline in recent years and the fishermen population in becoming older. There is a lack of young and new workers who want to enter in this traditional business.

1.1.2. Analysis of capture and economic value

In 2005 inland (freshwater) fisheries production in Italy totaled 3823 tons; 2136 tons from the northern regions, 1610 tons from the central regions and 77 tons from the southern regions. The catch comprised of whitefish and trout (820 tons), eel (75 tons), perch and pike (407 tons), bleak, carp and tench (374 tons) and big-scale sand smelt, landlocked shad and others minor species (2147 tons) (Colombari, 2007)

In table 1.1 are shown data of mean capture of Maggiore, Lugano, Como, Varese and Comabbio lake during five years (2007-2011). The specie more abundant is whitefish, followed by landlocked shad and roach. Table 1.2 shows the mean

price of freshwater fish catch in the same lake during the same period. Fish with high price is trout, followed by perch, charr and whitefish. Therefore the most important economic source of fishermen is whitefish, with 1.28 million € per year of mean gain, followed by landlocked shad and perch with a mean gain of 0.40 and 0.28 million €.

Table 1.1 Mean value of capture during the period 2005-2010, divided by species of five lake (kg x 100). Source :Analisi di Mercato, Aquaprogram, 2012 in La valorizzazione dei prodotti ittici tradizionali ed innovativi dei laghi insubrici.

Species		Maggiore		Como		Lugano		Varese		Comabbio	
Landlocked shad	*Alosa fallax lacustris*	289	±165.7	340.4	±80	5.3	±1.6				
Bleak	*Alburnus alburnus alborella*			3.7	±0.3						
Eel	*Anguilla anguilla*	0.9	±1	16.5	±14.7	0.2	±0.1			0.1	±0
Burbot	*Lota lota*	10.5	±4.8	81	±5.5	4.7	±0.6				
Carp	*Cyprinus carpio*	19.8	±5.3			8	±2.2	2.6	±1	0.7	±1
Whitefish	*Cregonus lavaretus*	591.7	±117.4	1172.3	±262.6	6.1	±3				
Northern Pike	*Esox lucius*	16	±5.3	4.3	±3.1	5.5	±0.7	1.5	±0.5	0.1	±0.2
Pikeperch	*Sander lucioperca*	30.3	±6.2	12.1	±1.8	61.2	±13.6	2.9	±0.1	0.3	±0
European Perch	*Perca fluviatilis*	58.3	±18	207.1	±24.1	56.9	±18.4	6.8	±4.5	0.7	±1
Largemouth Black Bass	*Micropterus salmonides*					4.9	±1.3				
Black Bullhead	*Aimerus melas*							66.5	±89.8		
Artich Charr	*Salvelinus alpinus*	1.6	±0.8	20.6	±1.6	0.2	±0.1				
Tench	*Tinca tinca*			23.2	±16.7	13.8	±5.8	1.8	±0.9	0.5	±1.6
Trout	*Salmo trutta*	19.3	±7.9	9.7	±1.9	5.9	±2.8				
Chub	*Leuciscus cephalus*	72	±26.8	78.9	±9.8	28.5	±28				
Roach	*Rutilus rutilus*	389.6	±86.8			171.2	±90.9	68	±12.5	8	±75.3
Crucian Carp	*Carassius carassius*							109	±72.1	6	±0
Rudd	*Scardinius erytrophtalmus*							42.9	±38.3	6.2	±63.0
Wels Catfish	*Silurus glanis*							5.3	±5.4	13.6	±15.2
Total		1492.5		1969.6		372.3		307.2		31.2	

Table 1.2. Mean price of the more abundant species catch in Lombardia lakes. Source :Analisi di Mercato, Aquaprogram, 2012 in La valorizzazione dei prodotti ittici tradizionali ed innovativi dei laghi insubrici.

Species	Price (€/kg)
Landlocked shad	6.31
Burbot	4.92
Crucian Carp	0.95
Tench	3.65
Chub	2.61
Whitefish	7.24
Roach	2.13
Northern Pike	6.33
Pikeperch	9.5
European Perch	8.6
Italian Roach	4.3
Artich Charr	9.85
Rudd	5.35
Trout	11.82
Other	2.01

The Veneto region, through its publication "La pesca e l'acquacoltura nel Veneto" (2006), provides data about the catch of inland waters of the triennium 2002-2004. Total catch amounted on average about 350 tons, with the prevalence of the catches of landlocked shad, sand smelt and other species, with 223 tons of average production, followed by the group of pike and perch with 77 tons and salmonids (trout, whitefish and charr and Carpione del Garda) with 43 tons. The proceeds of fishermen showed a positive growth trend, due more to the increase of price of some species (perch and pike) than to an increase in catches. The gain of Veneto inland fisheries has gone from 1318000 € in 2002 to 141000 € in 2004.

In Trentino fish catches are quite stable during the period from 2001 to 2010. The more abundant production came from salmonids fish, with an average production of 145 tons, followed by landlocked shad, sand smelt and other species with 120 tons, perch and pike with 77 tons and cyprinids with 28 tons (Provincia autonoma di Trento, www. provincia.tn.it).

1.1.3. Productivity of North Italy lakes

Lakes of north Italy could be divided in two groups. The bigger lakes, with a glacial origin has a high depth, and the flat ones, characterized by a small surface and a minimum depth. This difference is also reflected in the trophic status of their water. Larger lakes, like Maggiore, Como, Garda and Iseo are mesotrophic , while the others tend to the eutrophication, as shown in table 1.3. Trophic status of water bodies is determined by the presence of nutrients, mainly phosphorus and nitrogen, which tend to be the limiting factors for the plant growth. Lakes with an high level of nutrients have a great productivity of algae, with could also cause problems to the environment and fish life. Especially during summer, when the light intensity is higher, there could be an algal bloom, with an explosion of algal biomass. The consequent decomposition of this biomass produce a large oxygen consumption, that could generate a lack of oxygen in waters. This anoxia represent a potential risk for the survival of fish species with high oxygen demand, like fishes belonging to the salmonidae family. Fish like trout cannot find in small and eutrophic lakes the environment where survive during the hot season, when the cold water is localized only in the bottom of lakes, where the water has the minimum level of oxygen.

Table 1.4 show the fish population of lakes where professional fishermen work. Lakes characterized by a trophic status of mesothrophia host an important population of fish with an high economic value, like whitefish and perch, while flat and small lakes have a population based on cyprinid species, which are less popular with consumers. Selective fishing of certain fish species could generate a high pressure of that species population, causing their decline. It is important to determinate the sustainable fishing effort in order to ensure the future of this activity. In Valutazione della produttività ittica dei laghi e quantificazione dello sforzo di pesca sostenibile, part of the program "Valorizzazione sostenibile dei prodotti ittici tradizionali e innovativi dei laghi insubrici", is analyzed the productivity of Como lake and the sustainability of the professional fisheries exerted on it , where there is the higher number of fishermen. Based on the results of the density of plankton and the amount of fish captured during the last 10 years the study shows that the population of whitefish and landlocked shad is quite stable and in equilibrium with the fishing effort. The fishing effort is more or less at the 80% of the maximum sustainable.

Table 1.3. Morphometric characteristics and productivity of the lakes object of professional fisheries

Lake	Area (km^2)	Maximum depth (m)	Mean depht (m)	Throphic status
Alserio	1.44	8	5.4	Eutrophic
Comabbio	3.59	8	4.3	Eutrophic
Como	145	410	155	Meso-eutrophic
Garda	368	350	133	Mesotrophic
Garlate	4.64	35	15	Mesotrophic
Iseo	61	251	125	Eutrophic
Lugano	48.9	288	85	Eutrophic
Maggiore	213	370	176	Mesotrophic
Mezzola	5.85	69	26	Oligo-mesotrophic
Varese	14.8	26	11	Eutrophic

Table 1.4. Occurrence and abundance of fish species in the lakes (+ unusual; ++ scarce; +++ common; ++++ abundant).

			Lakes									
Family	**Common name**	***Scientific name***	**Como**	**Maggiore**	**Lugano**	**Varese**	**Garda**	**Iseo**	**Mezzola**	**Alserio**	**Comabbio**	**Garlate**
Acipenseridae	Adriatic sturgeon	*Acipenser naccarii*	+	+	-	-	-	-	-	-	-	+
Angullidae	European eel	*Anguilla anguilla*	+	+	+	++	+++	+++	+	+	++	+
Blennidae	Freshwater blenny	*Salaria fluviatilis*	+	+++	-	-	+	-	-	-	-	-
Centrarchidae	Pumpkinseed	*Lepomis gibbosus*	++	+++	-	++	+++	+	++	+++	+++	+
	Largemouth blackbass	*Micropterus salmoides*	++	+++	-	++	+	+	+	+++	+++	-
Ciprinidae	Bleak	*Alburnus alburnus alborella*	+++	++	+	+	+++	++	+	-	-	+
	Padanian balbel	*Barbus plebejus*	+	++	-	-	+++	+	+	-	-	+
	Crucian carp	*Carassius carassius*	++	++++	-	++++	+	+	+++	+++	++++	+
	Common carp	*Cyprinus carpio*	++	++++	+++	+++	+++	+	++	+++	++++	+
	Chub	*Leuciscus cephalus*	+++	++++	+++	+	+++	++++	++	-	-	++
	Roach	*Rutilus rutilus*	++	++++	++++	-	-	-	-	-	++++	-
	Gudgeon	*Gobio gobio*	+	+	-	-	+	-	+	-	-	+
	Italian roach	*Rutilus pigus*	+++	++	-	-	-	+	+++	-	-	-
	Stone moroko	*Pseudorasbora parva*	-	+	+	-	-	-	-	-	-	+
	Bitterling	*Rhodeus sericeus*	-	+	+	-	-	-	-	-	-	+
	Eurasian minnow	*Phoxinus phoxinus*	+	++	-	-	++	+	-	-	-	+
	Savetta	*Chondrostoma soetta*	+	+	-	-	+	+	-	-	-	+
	Rudd	*Scardinius erythrophthalmus*	+++	+++	+++	+	+++	++++	-	++++	++++	+++
	Tench	*Tinca tinca*	++	+++	+++	+++	+++	+++	-	+++	+++	++
	Gardon des pauvres	*Rutilus erythrophthalmus*	+	++	-	++++	+++	++	++	+++	+	++
	Vairone	*Leuciscus souffia muticellus*	+	++	-	++	+	+	+	-	-	+
Clupeidae	Landlocked shad	*Alosa fallax lacustris*	++++	+++	+++	-	++++	+++	++	-	-	-
Cobitidae	Spined loach	*Cobitis taenia*	+	++	-	+++	+++	+	+	-	++	-
Cottidae	European bullhead	*Cottus gobio*	+	++	-	-	+	-	+	-	-	-
Esocidae	Northern pike	*Esox lucius*	++	+++	+++	++	++	++++	++++	+	+	+
Gadidae	Burbot	*Lota lota*	+++	+++	++	-	++	++++	++++	-	-	-
Gobidae	Goby	*Padogobius martensii*	+	-	-	++	+++	-	+	-	++	+
Ictaluridae	Black bullhead	*Ameiurus melas*	+	+	-	+++	+	++	-	++++	+	-
Percidae	Pikeperch	*Sander lucioperca*	++	+++	+++	+++	-	-	++	+++	++	+
	European Perch	*Perca fluviatilis*	++++	+++	+++	+++	++	+++	+++	++++	+++	+
Salmonidae	European whitefish (bondella)	*Coregonus macrophtalmus*	+	++++	-	-	-	-	-	-	-	+
	European whitefish (lavarello)	*Coregonus lavaretus*	++++	++++	+++	+	++	++++	++++	-	-	+
	Arctic charr	*Salvelinus alpinus*	++	+	+	-	-	+	++	-	-	-
	Brook trout	*Salvelinus fontinalis*	+	-	-	-	-	-	+	-	-	-
	Grayling	*Thymallus thymallus*	+	-	-	-	-	-	+	-	-	-
	Brown trout	*Salmo trutta fario*	+	+	-	+	+	+	+	-	-	+
	"Carpione del Garda"	*Salmo carpio*	-	-	-	-	++	-	-	-	-	-
	Brown trout	*Salmo trutta lacustris*	+++	++	++	+	++	++	++	-	-	-
	Marble trout	*Salmo trutta marmoratus*	+	-	-	-	-	-	-	-	-	-
Siluridae	Wels catfish	*Silurus glanis*	++	+	++	+++	-	-	-	-	++++	+

There are no data concerning the sustainability of the professional fisheries in the others lakes, but analyzing the abundance of fish population of the last 20 years is clear that there are some species of fish that are in spread of abundance. Some of these species are not object of professional fishing due to their poor economic value. In relatively recent years, there has been a rapid change in composition of lakes fish populations. Eutrophication in many lakes, especially smaller ones, has favored species more resistant to poor environmental conditions, such as rudd, often dominant over other fish species. The introduction, voluntary or accidental, of many exotic species, which are in some cases becoming perfectly acclimatized sometimes even dominant on the existing fish community, has contributed on fish population traditional composition. These new species have accordingly made their massive appearance also in caught, sometimes strongly decreasing the commercial value, mainly due to lack of historical knowledge of processing and use of such fish. One of these species, whose presence is increasing in the last decade, becoming the most abundant species in the lake Maggiore, Lugano and Comabbio, is the roach, an exotic species for the Italian freshwater. The introduction of this fish has been demonstrated to be dangerous for the perch population, one of the most profitable fish. Roach shows an high competition for the food and habitat use with the juvenile perch, especially in small lakes (Persson and De Ross, 2012, Kahal et Radke, 2006). This competition could be artificially increased in a lake where perch is object of professional fishing while roach could escape from human pressure. Another fish species which is increasing on abundance of its population is the crucian carp. This species is especially abundant in lake where the eutrophication has changed the water quality from the natural status. Crucian carp could survive also in nearly anoxic waters for a short period, enduring living condition that may be impossible for the survival of other fish. Crucian Carp is one of the most farmed fish species in the word, popular in Ester Europe and Asia, but it is not appreciated by Italian consumer.

Following the indications of some authors (Catalano et al 2006) it is possible to reduce the eutrophication of a lake acting by biomanipulation. The elimination from the aquatic environment of a large amount of omnivorous fish could help to reduce the abundance of nutrients that had caused the environmental alteration. In Varese, Comabbio, and Pusiano lakes a trial was carried to control the crucian carp population by selective fishing, in order to reduce the population of this species. Crucian carp in eutrophic lakes reach early a size that it can not be preyed by any other fish, so their population can grow without control. The project had been interrupted because the crucian carps captured didn't found a market.

1.2. Valorization of fish product through processing

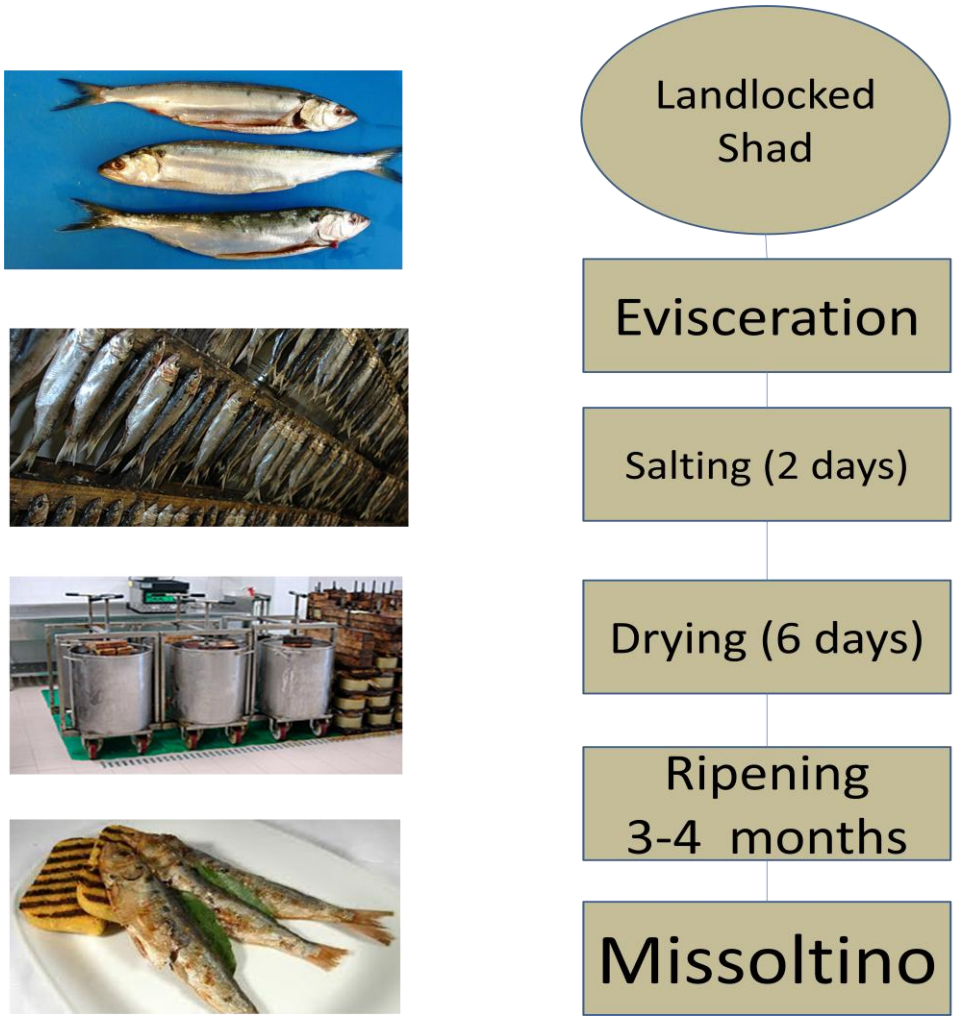
The fish that are caught in lakes are in part intended to be consumed fresh, whole or filleted, and partly processed and stored in accordance with techniques handed down by local tradition. Processing is a way to buffer the fluctuation of catches. In some periods there could be a high availability of some fish species and their price could decrease due to the lack of flexibility of the demand. If a fisherman process a fresh product into a stable one he could keep the price of fresh product high and sell preserved fish for the period in which the catches are low or during the rest of the year. The majority of the fishermen who work in North Italy held a own laboratory where they process the fish, after having caught and landed on earth from their boats. Others fishermen confer their fish to cooperatives, where there is the processing of fish into fish products. The price of preserved fish is also more economic attractive for the fishermen compared to price of the same species sold as a fresh product. Landlocked shad has an average price at 6.3 €/kg when it is sold as a fresh fish, but when it is transformed in Missoltino his price increases to 30.9 €/kg. Made 100 the total catch of this species, slightly more than 35% is used for the preparation of missoltini. Other fish with a low value, like chub and Italian roach, are used for the preparation of based on fish products, like meatball or pate. Lately some fishermen are starting to use the same technique that they use for the production of missoltino, to process other similar fish product, starting from roach, rudd and chub. Another fish product that has been introduced in the last years is the bottarga, that is the product obtained by salting and drying of fish roe. To prepare this product fishermen use mainly the ovary of whitefish, but some test were carried also with the ovary of roach, crucian carp and chub.

1.2.1. The Missoltino

The most common practice of transformation is that of the so-called "Missoltino" the processing of the shad is an ancient practice, founded in the nineteenth century along the banks of the lake. This product allowed to the coastal population the preservation of a valuable product, otherwise available only in the summer months. The tradition dictates that missoltini are prepared with the shad caught in the June, at the end of the breeding season. Obviously, the tradition must then deal with the regulation, that fixes the term of prohibition during which there can be neither caught nor held of the landlocked shad at the place fishing. The transformation process involves, after evisceration and washing, fish-salting and then drying; this is

followed by pressing for 60-120 days, until " maturity " of the flesh that take a red color has come. Briefly the freshly-caught landlocked shads, weighing about 80g, are eviscerated and then salted using fine sea salt, about 80g/kg of fresh fish, for 2 days. The exceeding salt is removed by water washing and the fish are naturally dried in a room for 3-5 days, according to the humidity and temperature. Afterwards fish are arranged in layers in metallic containers and pressed for a long period, nearly 3 month. These containers are closed with a wooden lid and the pressure is progressively increased with a crank handle. During this period fish lose water and lipid. The maximum loss occurs in the first twenty days. A schematic overview of the production process in shown in figure 1.1. Due to its traditional value and its quality this product is now recognized as a Slow Food presidium.

Figure 1.1. Schematic resume of the processing of Missoltino



1.3. Freshwater aquaculture in northern Italy

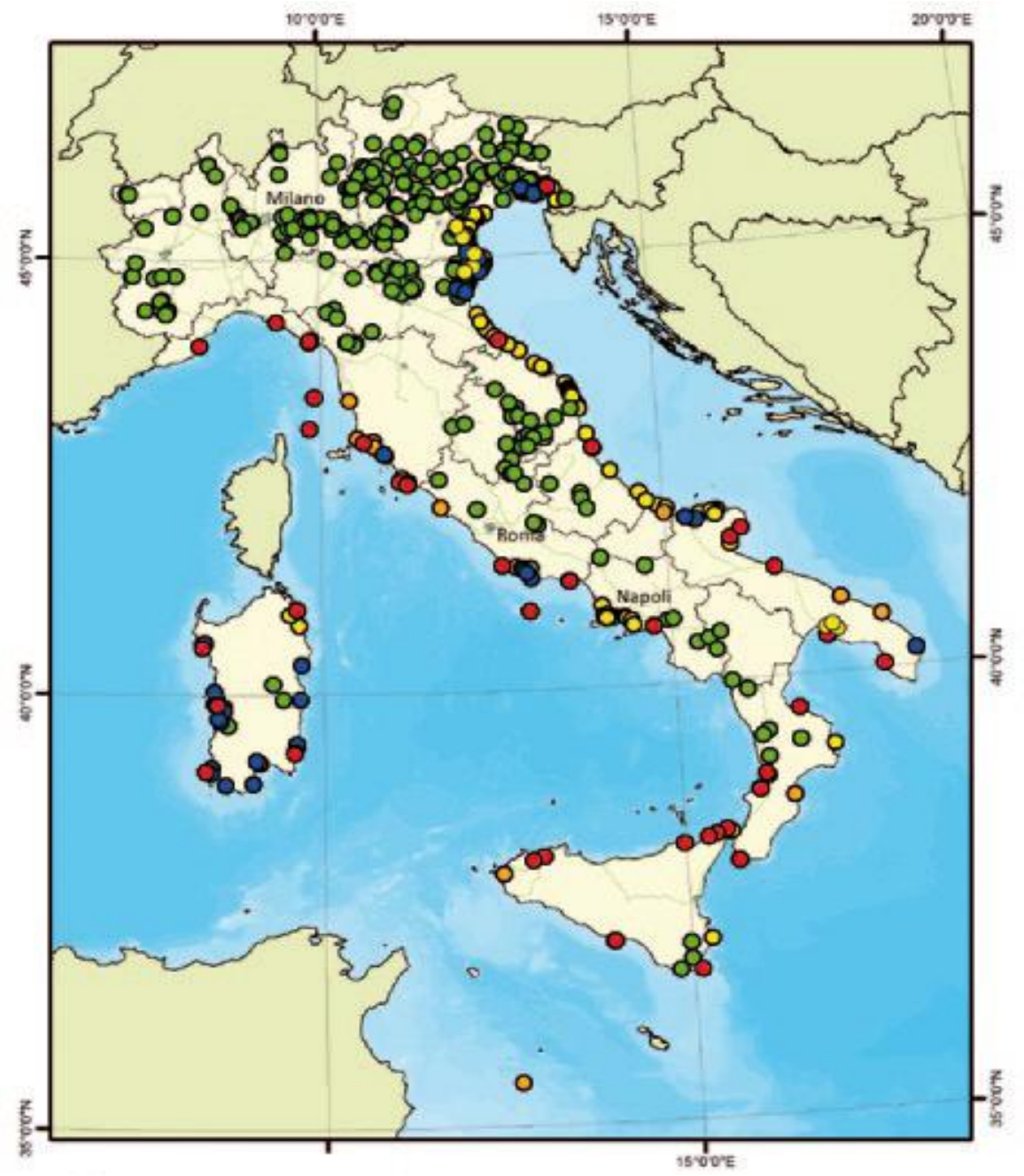
Italian aquaculture has a total fish production of 68330 tons in 2010 (API 2011). Freshwater aquaculture plays the dominant role, with the production of more than 43000 tons, due mainly to the trout production, which is the highest Italian fish production. Others freshwater production include, in order of importance, sturgeons, eels, catfish, carp and other cyprinids and other species, including stripped perch. Sturgeons have a small production, if we consider the tons farmed, but with an high economic value, due to the price of caviar, the product derived from the processing of roe of female sturgeons. Eel production shows a constant decline, common in all Europe, due to the problem of the farming of this species, which depends on the recruitment from the natural population, which is in steady decline. Carps and other cyprinids, which include grass carp and tench, and catfish farms are concentrated in the Po valley. Their productions have suffered health problems, with the outbreak of some viral disease. As shown in figure 1.2, freshwater sites of production are located mainly in northern regions, with few farm in central and south Italy.

1.3.1. Freshwater aquaculture market

Freshwater farmed fish product finds some market problems, due in part to the high level of competition of marine and foreign production and to the lack of variety. Freshwater farmed fish have also seafood, that are more popular in Italy, as a competitor for the same market. Italian consumption of fish is covered only for the 30% by national production, the rest of national consumption is based on imported products. Imports into Italy come mainly from the EU (FAO 2012). During the past few years, due to a marked rise in volumes, the aquaculture sector has registered a decrease in production prices. The growth in national and foreign output has caused a reduction in the prices and this negative trend has been characteristic over the past five years. The cost of production of the trout, associated with the sale price makes profit margins of this product very limited. Different strategies have put in place to valorize Italian production, such as diversification of production with the breeding of new species and the further improvement of the quality of the product, the marketing and promotion of production, together with the extension of the offer through transformation processes and conservation. Some interest is manifested, in fact, also for other

species, such as tench, common carp and perch, that are already known of Italian consumers and have a better value in the market.

Figure 1.2. Localization of Italian aquaculture. Green circle Freshwater farm. Blu circle Lagoons, valli and Brackish lakes. Orange circle Inland saltwater farm. Red circle cage farming, Yellow circle shellfish production. From "Lo stato della pesca e dell'acquacoltura dei mari italiani" S. Cautadella and M. Spagnolo, 2012.



1.3.2. Tench farming

A possible way for Italian freshwater aquaculture to find a new profitable market is to diversify its production, with new species. Tench, which was the only Italian fish with the Protected Designation of Origin, Tinca Gobba Dorata del Pianalto di Poirino, could be the candidate of this diversification process (Wang et al, 2006; Turchini et Da Silva, 2010). Intensifying tench production would be a response to the need for promoting and diversifying freshwater aquaculture, thus increasing the sustainable production of a wide range of species.

The tench (*Tinca tinca*) is species of great interest in Europe aquaculture and highly valued by consumers (Wedekind et al, 2003). Europe production of tench has got decreasing trend in the last ten years (1997–2008) from 2023 tons to 1262 tons (Slozka, 2011). Traditional rearing of tench is made in ponds systems, especially in Central and Eastern Europe. This way of farming depends on the climate variation of the seasons, so it is difficult to plan the production. Also the reproduction of tench depends on the seasonal changes, with natural spawning that occurs between June and August, according to water temperature, so the availability of tench fry is limited to the end of summer. Tench growing performances are unsatisfactory, mainly due to high mortality of the early life stages and poor growth rate of the tench during first weeks of the exogenous nutrition. Poor growth rate continue during all the fish life when reared in ponds, due also to the stop of feeding than tench have during winter period. For these reasons the commercial size is reached only after three years of life (Horvàth et al 1984).

A possible way to improve the tench rearing techniques could be the use of recirculation aquaculture system (RAS), where it is possible to give to fish the optimal termical condition during all the year, exploiting the optimal growing temperature for this species. The use of RAS has also the advantage of creating the possibility to have off-of-the season spawning of tench, anticipating their reproductive season. The possibility to have tench fry, available for pond use, at the beginning of warm season could increase the growing performance during the first year of live of fish, where the fish have the high growing deficiency problems and where there is the higher mortality.

1.3.3. Fish farming in Recirculation Aquaculture System

RAS culture systems are typically land-based, using containment systems such as tanks or raceways for the fish. A percentage of the water in RAS flows from a fish tank through a treatment process of wastes removal and is then returned to the tank. The level of waste treatment and water reuse depends largely on the

requirements of the fish, the environmental parameters and the technology available. Reusing water gives the farmer a greater degree of control over the environment, reduces water consumption and waste discharge and enables production close to markets. Various methods can be used to clean the water from the fish tanks and make it reusable. Some RAS fish farms incorporate aquaponics, the practice of growing herbs and vegetables in water, into their system. Plants need 13 elements to grow; the wastewater from the fish tanks naturally provides 10 of these elements (Rakocy, 2009). Owing to relatively high capital costs, high energy dependencies and more complex technology, RAS is largely restricted in its use to higher value species or life stages (especially hatcheries where control over environmental conditions is more critical and unit values higher). However, it could become a more competitive approach if economic factors change (Bostock et al, 2010). To compete with traditional farming techniques RAS cultivation needs a larger productivity, that could be obtained increasing the density of fish in tanks.

1.3.4. Use of alternative protein ingredients in fish feed formulation

Historically the artificial feeding of fish was based on the fishmeal. Fishmeal is an excellent source of protein and other essential nutrients for aquaculture species but it is a limited natural resource, since it takes its origin mainly from wild catch of fish that are not used for direct human consumption. Only a small amount of fishmeal came from by-products of fish processing. As reported in many FAO reports the status of many fish population is under an excessive fishing pressure, endangering the future of fisheries. The need of fishmeal, which is largely absorbed by the aquaculture feeding industry, contribute to this ecological problem, making some fish production not sustainable. For this reason nowadays there is a significantly decrease of use of fishmeal in aquaculture feeds. Feeding company continue to search amino acid sources and other essential nutrients in co-products to continue our journey in replacing fishmeal in aquaculture diets. To reach a sustainable aquaculture and to reduce the feed prize, new alternative protein sources including cheaper plant or animal origin proteins are needed to be introduced for stable aquafeed production (Higgs et al., 1995). There are several protein sources that have the potential of replacing fishmeal in aquaculture feeds without affecting the growth performance of fish (Tacon et Metian, 2008). The complete or partial replacement of fishmeal with plant based protein is possible without loss of growth performance (Gomes et al, 2005; Webster et al, 1999; Gómez-Requenia et al 2004). Microalgae are already known as a food for aquatic animals, they were used mainly in shellfish and shrimps cultivation, or in fish hatchery. Recently some studies have investigated

the potential of microalgae as proteins and lipids source in the formulation of fish feeds. Microalgae appear to be nutritionally suitable as a resource for mariculture feed because many species have a high protein content and because their lipids may have a high polyunsaturated fatty acids content (Gonzáles López et al. 2010).

1.3.5 Spirulina meal

Spirulina is a cyanobacterium belonging primarily to two genera of cyanobacteria, *Arthrospira* and *Spirulina*. *Arthrospira platensis* is the most common species available in the word. Arthrospira are free-floating filamentous cyanobacteria characterized by cylindrical, multicellular trichomes in an open left-hand helix. They occur naturally in tropical and subtropical lakes with high pH and high concentrations of carbonate and bicarbonate. *Arthrospira platensi*s is naturally present in Africa, Asia and South America (Habib et al, 2008). Spirulina was historically used by pre-Columbian populations and in Africa, around lake Chad (Abdulqader et al, 2000) as a food resource for human consumption. Spirulina cultivation has increased significantly in recent years, and its use as animal feed has been studied in various species, due to the high protein content, the presence of pigments that act as antioxidant and to the good content of polyunsaturated fatty acids.
The chemical composition of spirulina meal reported a protein content that range from the 55 to the 70 %, depending of the source. This proteic meal is superior to all terrestrial protein plant product. Amino acid composition includes all essential amino acids (Habib et al, 2008). The Agricultural Research Service of United States, Department of Agriculture provides standard composition of spirulina meal, reported in Table 1.5. Spirulina meal is also rich in lipids, whose composition could vary depending of many conditions, as reported by several author (Othles and Pire, 2001; Muhling et al, 2005; Diraman et al, 2009). Average spirulina meal contains mostly palmitic acid(16:0, 44.6–54.1 percent), oleic acid (18:1 *n*-9, 1–15.5 percent), linoleic acid (18:2 *n*-6, 10.8–30.7 percent) and γ -linolenic acid (18:3 *n*-6, 8.0–31.7 percent) (Habib et al, 2008). Some authors reported also the presence of the *n*-3 series of PUFA (Diraman et al, 2009), especially in oil extract from Chinese strain of *Arthrospira platensis*.

Table 1.5 Chemical composition of standard Spirulina meal

Proximates			**Lipids**		
Water	g	4.68	Fatty acids, total saturated	g	2.650
Energy	kcal	290	4:0	g	0
Energy	kJ	1213	6:0	g	0
Protein	g	57.47	8:0	g	0
Total lipid (fat)	g	7.72	10:0	g	0
Ash	g	6.23	12:0	g	0
Carbohydrate, by difference	g	23.9	14:0	g	0.075
Fiber, total dietary	g	3.6	16:0	g	2.496
Sugars, total	g	3.1	18:0	g	0.077
Minerals			Fatty acids, total monounsaturated	g	0.675
Calcium, Ca	mg	120	16:1 undifferentiated	g	0.328
Iron, Fe	mg	28.5	18:1 undifferentiated	g	0.347
Magnesium, Mg	mg	195	20.01	g	0
Phosphorus, P	mg	118	22:1 undifferentiated	g	0
Potassium, K	mg	1363	Fatty acids, total polyunsaturated	g	2.080
Sodium, Na	mg	1048	18:2 undifferentiated	g	1.254
Zinc, Zn	mg	2	18:3 undifferentiated	g	0.823
Copper, Cu	mg	6.100	18.04	g	0
Manganese, Mn	mg	1.900	20:4 undifferentiated	g	0
Selenium, Se	µg	7.2	20:5 n-3 (EPA)	g	0
Vitamins			22:5 n-3 (DPA)	g	0
Vitamin C, total ascorbic acid	mg	10.1	22:6 n-3 (DHA)	g	0
Thiamin	mg	2.380	Cholesterol	mg	0
Riboflavin	mg	3.670	**Amino Acids**		
Niacin	mg	12.820	Tryptophan	g	0.929
Pantothenic acid	mg	3.480	Threonine	g	2.970
Vitamin B-6	mg	0.364	Isoleucine	g	3.209
Folate, total	µg	94	Leucine	g	4.947
Folic acid	µg	0	Lysine	g	3.025
Folate, food	µg	94	Methionine	g	1.149
Folate, DFE	µg	94	Cystine	g	0.662
Choline, total	mg	66	Phenylalanine	g	2.777
Vitamin B-12	µg	0	Tyrosine	g	2.584
Vitamin B-12, added	µg	0	Valine	g	3.512

(Continue)

Vitamin A, RAE	µg	29	Arginine	g	4.147
Retinol	µg	0	Histidine	g	1.085
Carotene, beta	µg	342	Alanine	g	4.515
Carotene, alpha	µg	0	Aspartic acid	g	5.793
Cryptoxanthin, beta	µg	0	Glutamic acid	g	8.386
Vitamin A, IU	IU	570	Glycine	g	3.099
Lycopene	µg	0	Proline	g	2.382
Lutein + zeaxanthin	µg	0	Serine	g	2.998
Vitamin E (alpha-tocopherol)	mg	5			
Vitamin E, added	mg	0			
Vitamin D (D2 + D3)	µg	0			
Vitamin D	IU	0			
Vitamin K (phylloquinone)	µg	25.5			

References

Abdulqader, G., Barsanti, L. and Tredici, M. (2000) Harvest of Arthrospira platensis from Lake Kossorom (Chad) and its household usage among the Kanembu. *Journal of Applied Phycology*. 12: 493-498.

Bostock J., McAndrew B., Richards R., Jauncey K., Telfer T., Lorenzen K., Little D., Ross L., Handisyde N., Gatward I.,and Corner R., (2010) Aquaculture: global status and trends. *Philosophical Transactions of the Royal Society London B Biological Science* ; 365(1554): 2897–2912.

Catalano M.J, Allen M.S., Schaus M.H., Buck D.G. and Beaver J.R. (2006) Evaluating short-term effects of omnivorous fish removal on water quality and zooplankton at a subtropical lake. *Hydrobiologia* **.** *655*, 1, 159-169

Cileček, M., Baranek, V., Vitek, T., Kopp,R. and Mareš J. (2011) Production effect of different commercial feeds on juvenile tench (*tinca tinca* l.) under the intensive rearing conditions. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis.* LIX, 6, 93−98

Colombari P. T. (2007). Inland fisheries management in the Bolsena lake, Central Italy. Presentation. Conference on EFF, inland fishing, and aquaculture production methods enhancing the environment, 27-29 June 2007, Bucharest, Romania.

Diraman H., Koru E. and Dibeklioglu H. (2009) Fatty acids profile of *Spirulina platensis* used as a food supplement. *The Israeli Journal of Aquaculture* 62 (2): 134-142

Gomes E.F , Paulo Rema P., Kaushik S.J. (1995) Replacement of fish meal by plant proteins in the diet of rainbow trout (*Oncorhynchus mykiss*): digestibility and growth performance. *Aquaculture* 130, I 2−3, 177−186

Gómez-Requenia P., Mingarroa M., Calduch-Ginera J.A., Médaleb F., Martinc S.M.A., Houlihanc D.F., Kaushikb S. and Pérez-Sánchez J. (2004) Protein growth performance, amino acid utilisation and somatotropic axis responsiveness to fish meal replacement by plant protein sources in gilthead sea bream (*Sparus aurata*). *Aquaculture* 232 I1-4, 493-510

Gonzáles López CVG, Garcia MCC, Fernandez FGA, Bustos CS, Chisti Y, Sevilla JMF (2010) Protein measurements of microalgal and cyanobacterial biomass. *Bioresource Technology* 101:7587–7591

Habib, M. Ahsan B.; Parvin, Mashuda; Huntington, Tim C.; Hasan, Mohammad R. (2008). A Review on Culture, Production and Use of Spirulina as Food dor Humans and Feeds for Domestic Animals and Fish ". Food and Agriculture Organization of The United Nations. Retrieved November 20, 2011

Higgs, D.A., Dosanjh, B.S., Prendergast, A.F., Beams, R.M., Hardy, R.W. Riley, W. and Deacon, G. 1995. Use of rapeseed/canola protein products in finfish diets. In: D.J. Sessa (Ed.), Nutrition and utilization of technology in aquaculture. AOCS Press, Champaign USA: 130-156

Horvàth L., Tamàs G. and Tölg I. (1984). Special methods in pond fish husbandry. Halver Corporation, Seattle, p. 148

Kahl U., Radke, R.J. (2006). Habitat and food resource use of perch and roach in a deep mesotrophic reservoir: enough space to avoid competition? *Ecology of freshwater fish.* ,*15*, 1, 48-56

Lennart Persson and Andre´ M. De Roos ,(2012) Mixed competition–predation: potential vs. realized interactions. *Journal of Animal Ecology. 81*, 483−493

Mühling M., Belay A. and Whitton B.A. (2005) Variation in fatty acid composition of *Arthrospira* (Spirulina) strains. *Journal of Applied Phycology* 17: 137- 146

Öthles S. and Pire R. (2001). Fatty acid composition of *Chlorella* and *Spirulina* microalgae species. *Journal of AOAC International* 84: 1708-1714

Premazzi, G.; Dalmiglio, A.; Cardoso, A. C.; Chiaudani, G., (2003) Lake management in Italy: the implications of the Water Framework Directive. *Lakes & Reservoirs: Research & Management. 8*, 41-59

Rakocy J (2009). "The UVI Aquaponic System." Clean, Green,Sustainable Recirculating Aquaculture Summit. Washington D.C.: hosted by Food and Water Watch. January 2009.

Steffens W. (1995). The tench (Tinca tinca L.) a neglected pond fish species. *Polskie Archiwum Hydrobiologii* 42: 161–180.

Tacon A. G.J. and Metian M. (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture 285 (2008) 146–158

Turchini G.M., De Silva S.S., (2008). Bio-economical and ethical impacts of alien finfish culture in European inland waters. *Aquaculture International* 16:243–272

Wang, J., Min, W., Guan M., Gong, L., Ren J., Huang, Z., Zheng, H., Zhang, J., Liu, H., Han, Y., (2006). Tench farming in China. Present status and future Prospects. *Aquaculture International* . 14:205-208

Webster, C. D., L. G. Tiu, and A. M. Morgan. 1999. Effect of partial and total replacement of fishmeal on growth and body composition of sunshine bass (*Morone chrysops* x *M. saxatilis*) fed practical diets. Journal of the World AquacultureSociety. 30: 443-453.

Wedekind H., Rennert B. and Kohlmann K. (2003). Product quality in different strains of tench (*Tinca tinca*) tested under controlled environmental conditions. *Journal of Applayed Ichthyology* 19 : 174–176

Regione Lombardia, 2010. Piano Regionale della pesca professionale e dell'acquacoltura 2009-2011.

Regione Veneto . 2006 La pesca e l'acquacoltura in Veneto. Osservatorio socio economico della pesca nell'alto Adriatico.

FAO 2011. National Aquaculture Sector Overview, Italy.

CHAPTER 2

Objectives

2 Objectives

The aim of these studies was to increase the knowledge and the value of some freshwater fish and fish product produced the North of Italy. Nowadays we are witnessing a drop of the number of employment in the inland fisheries and aquaculture sector, due to the difficulty of obtaining an economic livelihood from these activities. The productivity of inland freshwater of Northern Italy, although we have seen a change in the natural environment due to human activity, is almost at good levels, so there are enough resources to ensure catch for the fish industry. The only way to stimulate a new generation of fisherman, in order to not lose this ancient and traditional craft, is to increase the economic incoming. To reach this objective there are some fishermen who have bounded fishing with tourism, allowing their customers to participate in their activities and to taste the results of their working at the end of the day. Another and more practical solution is to promote the consumption of freshwater fish, emphasizing its nutritional value. It is also necessary to focus our attention not only to the fish species who have a stable and profitable market, but also to that species which have difficulty in finding an appreciation by the consumers. The fishing of these species, like rudd, crucian carp and roach, could have also an environmental benefit to the ecology of some eutrophic lakes, balancing the population of these fish that is becoming prevalent with other species which are submitted to the fishing pressure. The removal of a large amount of fish is also a way to reduce the presence of nutrients in the aquatic environment and to promote a biological purification of water.

Freshwater aquaculture is stable in their production during the last 10 years, except for the eel production which is in steady decline. Profits of freshwater aquaculture industry are menaced by price stagnation and rising farming costs, due to the increase of price of energy and aquafeed. Price of trout, the principal aquaculture products of Northern Italy is now near to its production cost; Sturgeons have a high value with the caviar production, but this niche market is quite unpredictable and there are now other countries, China and Vietnam, Eastern Europe country, which are starting a large caviar production. One possibility for Italian fish farmer to play on a new and profitable market is to find some new productions, with fish that are not completely unknown for the Italian consumers, but whose supply on the market is low.

Chemical composition and fatty acid profile of freshwater fish species of commercial interest harvested in northern Italian lakes

In this study we analyzed the chemical composition of 15 species of freshwater fish, caught in some lakes of northern Italy, where there is professional fishing. The aim was to give a complete information on their nutritional quality, comparing the composition of the species more common and valued by the market to the new species, which are in some case more abundant but not exploited because of their poor commercial value. Furthermore we divided fish species according to their dietary habits. We identified the ichthyophagous, omnivorous and planktovorous group, observing how their feeding behavior affects their fatty acids composition.

Chemical composition, histamine content and histamine-forming bacteria in missoltino, a salted and dried landlocked shad (*Alosa fallax lacustris*) product

Missoltino is the most popular and known fish product obtained by a freshwater species in northern Italy. During the summer of 2013 it has been recognized as a traditional product, worthy of protection and recognition, with the registration as a Slow Food presidium. The aim of the work was to follow the fish during all the phases of processing, recording the nutritional characteristic and how they changed during ripening. In a second step we investigated on the health aspect of this product, in order to give indications to the producers to how reduce the hazard of histamine formation, the biggest problem noticed by Italian veterinary services.

Use of spirulina (*Arthrospira platensis*) as protein source for the nutrition of juvanile tench (*Tinca tinca*)

Tench is a potential candidate as a new species for intensive aquaculture. This fish is now farmed only in extensive pond system, with a low production. In this study we analyzed the growing performances of tench in recirculating aquaculture system,

feeding the fish with five diets, where a part of fishmeal was substituted with an increasing amount of Spirulina meal.

CHAPTER 3

Chemical composition and fatty acid profile of freshwater fish species of commercial interest harvested in northern Italian lakes

3.1 Introduction

In Italy there are more than 2000 lakes. Of these, 389 are freshwater and 104 are coastal with brackish water. The most important lake district is located in northern Italy and includes the large lakes of glacial origin (Como, Garda, Maggiore, Lugano and Iseo) and some small medium insubrian lakes. Together these lakes represent more than 80 % of the total Italian lacustrine volume (Premazzi et al, 2003).
Commercial fishing activities carried out on these lakes play a significant role in the economy of the region and contribute to the support of local food services and fish market. The estimated total amount of fish caught in the northern lake district is more than 1000 tons per year (Dill, 1990). The main fish species of commercial interest are, sorted by descending order of economic value, perch (*Perca fluviatilis*), whitefish (*Coregonus sp.*), shad (*Alosa fallax lacustris*), eel (*Anguilla Anguilla*), brown trout (*Salmo trutta*), bleak (*Alburnus alburnus alborella*), pikeperch (*Stizostedion lucioperca*), pike (*Esox lucius*), tench (*Tinca Tinca*), burbot (*Lota lota*), roach (*Rutilus pigus*) and carp (*Cyprinus carpio*). In terms of living biomass harvested the most representative fish are whitefish, landlocked shad and perch. Most of these species are sold as fresh fish on local market. However, some fish species such as whitefish, tench or roach, are subjected to drying, salting and smoking according to traditional methods (Paleari et al, 1993), in order to increase their market value and to improve the shelf-life of the product.
In recent years the inland small-scale traditional fishing sector has been affected by a loss of competitiveness, followed by a progressive lowering of incomes. Commercial fishing continue in a traditional manner, but with the composition of the catches changing toward less valued species, i.e., from pelagic to littoral species and gradual diminution of the practice. The large rise on the market in supply of aquaculture products such as trout, sea bass and sea bream at lower prices has pushed the prices of these lake fish towards their lowest level, with a consequent decline in profit for fishermen. Moreover, the introduction in inland water of alien species have contributed to the loss of many high value native species and had led to negative influences on trophic quality of lakes (Volta et al, 2011). To address these problems, local fishermen have reacted by diversifying their products towards gutted and filleted fish and to adapt their production to market demand, applying more modern processing techniques and better integration among the production, processing, and marketing sectors.
The chemical composition of fish varies from species to species and between specimens of the same species, determined by numerous factors such as diet, age, environment and season (Huss, 1995). Protein content is considered to be a rather

stable component among the principal constituents of fish muscle. The amino acids profile is influenced by intrinsic factors such as species but seemed to be unaffected by temperature, feeding rate and fish size (Halver et al., 2002) Conversely, the lipid amount is strictly correlated to the feed intake and spawning time, showing a specific seasonal trend.

Fish are an important source of polyunsaturated fatty acids. The consumption of these fatty acids involves high benefits for human health. These fatty acids prevent human coronary artery disease and autoimmune disorders (Mozaffarian et al., 2005; Belluzzi, 2001; Connor, 2000; Simopoulos, 1991), improve retina and brain development (Crawford, 1993), decrease incidence of cancer (Larsson et al., 2004). Among polyunsaturated fatty acids (PUFA), fish lipids contain high level of eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA) (Ackman, 1989). Compared to marine fish, freshwater fish muscle are characterized by high levels of C18 PUFA and low levels of n-3 PUFA (Ackman, 1967; Ackman, 1999) Besides, freshwater fish show a greater ability to elongate and desaturate short fatty acids into EPA and DHA (Bell et al., 1986; Henderson et al., 1987). Some factors such as geographical location, season and the nature and the availability of food can influence the fatty acids profile of fish muscle. In general, herbivorous fish feed on algae containing higher levels of C18 than C20 or C22 PUFA and the fatty acid composition of muscle reflects the fatty acids pattern of their dietary lipid. The lipids of carnivorous fish, preying on small fish, is influenced by the fatty acids composition of their prey.

Few works concerning the chemical composition and fatty acid profile of freshwater species harvested from Italian lakes had been published. Paleari et al. (1993) analyzed the microbiological and chemical composition of a typical product from *Alosa fallax lacustris*. Luzzana et al. (1996) studied the seasonal variations of fat content and fatty acid composition of landlocked shad from Como lake. Orban et al. studied the nutritional quality of European perch (Orban, 2007) and whitefish (Orban, 2006) from the lakes of central Italy. Serrini et al. (1996) reported the fatty acid composition of some coregonids caught in European lakes.

Considering the importance of these edible fish on local market, some groups of producers started to think of a better enhancement of their production. This has led them to set up quality initiatives, thus promoting the freshness certification, the quality and the safety of their products. With this respect, the aim of this study is to assess the nutritional quality of most of freshwater species harvested in the Northern Italian lake by commercial fishing.

3.2 Materials and methods

3.2.1 Aquatic environments

Lake Garda is the largest Italian lake with a surface area of 368 km^2 and it is located 65 m asl. Lake Maggiore is the second Italian lake in terms of surface area and volume. Its drainage basin occupies an area of 6600 km^2, of which 50% is in the Swiss territory. Lake Como is the deepest Italian lake and the third in terms of surface area (145 km^2) and volume. Its drainage basin occupies an area of 4552 km^2, of which 487 km^2 is in the Swiss territory. Lake Iseo is the fourth Italian lake with a surface area of 61 km^2. Lake Varese is a relatively small lake, with a surface area of 14.8 km^2. The composition of the phytoplankton communities of these lakes register marked similarities from one lake to another, as regards density, biomass and species composition. A number of species are ubiquitous in these lakes. The most important taxa of the phytoplankton communities are represented by the bluegreens and the diatoms, followed by the cryptophytes and the dinoflagellate (Premazzi et al, 2003). The zooplankton found in the deep Italian subalpine lakes is typically represented by copepoda, cladocera and rotatoria.

3.2.2 Sampling and biometric measurements

The study was carried out in two successive years, from 2010 to 2011. Fifteen fish species were sampled on 16 catches in 10 different Northern lakes by different commercial inland fishermen, using gill nets. The collected and analyzed fish are shown in Table 3.1.
After catching the fish were immediately stored under ice in polystyrene boxes until the arrival in the laboratory, where fish were accurately weighed and measured. Fulton's K condition index was calculated using the formula $K=weight/length^3$. This morphometric index assumes that heavier fish for the same length are in better nutritional conditions. All fish were then filleted by hand, with the exception of bleaks that, due to their small size and to the way that they are generally eaten, were analyzed as whole eviscerated fish. Gender difference was evaluated, when evident, but not considered in the present study. After dissection and filleting, the head, axial skeletal bones, viscera, liver, gonads (when evident and separable), perivisceral fat (when present) and right and left fillets were weighed. Fillet yield were calculated as (fillet weight/weight)x100. Fillet samples were vacuum packed and stored at - 20°C until analysis. Biometrics measurements of wells catfish were not recoded because fish fillets were directly collected by fishermen

Table 3.1 Feeding behavior, habitat, and reproductive season of fish analyzed during the trial

Species	Common name	Feeding Behaviour	Lacustrine habitat	Food items	Reproductive season	Origin (lake)	Fish sampled n.	Month of capture
Alosa fallax lacustris	Landlocked shad	Planktivorous	Pelagic	cladocerans and copepods and small fish	Early summer	Como	17	September
						Iseo	16	November
						Como	4	October
Alburnus arborella	Bleak	Planktivorous	Pelagic	plankton, insects larvae and invertebrates	Summer	Iseo	100	November
Lota lota	Burbot	Carnivorous	Benthonic	crayfish, molluscs, fish and other invertebrates	Winter	Iseo	11	November
Carassius carassius	Crucian carp	Omnivorous	Littoral	plankton, benthic invertebrates, plant materials and detritus	Late spring	Alserio	7	March
						Comabbio	10	April
						Mezzola	10	May
						Varese	10	May
Cyprinus carpio	Common carp	Omnivorous	Littoral	benthic organisms and plant material	Late spring	Varese	5	June
Leuciscus cephalus	Chub	Omnivorous	Littoral	aquatic and terrestrial animal, plant material and fish	Spring	Como	10	November
						Garlate	9	April
						Iseo	3	November
						Maggiore	5	March
						Como	10	April
Rutilus rutilus	Roach	Omnivorous	Littoral	benthic invertebrates, zooplankton, plant material and detritus	Spring	Ceresio	10	April
						Maggiore	10	March
Coregonus macrophi	European whitefish (bondella)	Planktivorous	Pelagic	Insects larvae and plankton	Winter	Como	10	September
Esox lucius	Pike	Ichthyophagous	Littoral	invertebrates, terrestrial vertebrates	Late winter	Iseo	3	November
Perca fluviatilis	European perch	Ichthyophagous	Littoral	benthic prey and fish	Spring	Garda	19	November
						Iseo	11	November
Ictalurus melas	Black bullhead	Omnivorous	Benthonic	insects, leeches and crustaceans, clams, snails, plant material and fishes	Spring	Alserio	10	April
Rutilus pigus	Italian Roach	Phytophagous	Littoral	benthic macroinvertebrates, macrophytes	Spring	Como	4	February
						Como	10	April
Scardinius erythroph	Rudd	Omnivorous	Littoral	plankton, terrestrial insects and plant material	Spring	Alserio	10	April
						Comabbio	10	April
						Garlate	10	April
Tinca tinca	Tench	Omnivorous	Littoral	detritus, molluscs, benthic animals and plant materials	Summer	Comabbio	7	June
						Como	5	September
						Iseo	5	November
						Mezzola	7	May
						Varese	3	May
Silurus glanis	Wels Catfish	Ichthyophagous	Benthonic	Invertebrates, fish and other aquatic vertebrates	Early summer	Comabbio	8	April

3.2.3 Proximate composition and fatty acid analysis

All assays for proximate composition analysis were performed using standard methods (A.O.A.C., 1996). Moisture content of fillets was determined by drying samples in an oven at 60 °C to constant weight. Total protein was determined by Kjeldahl method, by which the concentration of nitrogen is measured. A conversion factor of 6.25 was used to convert total nitrogen to crude protein. For the analysis an automated distillation unit (Büchi 339, Switzerland) was used.

The extraction and determination of total lipids was performed according to Bligh & Dyer (Bligh and Dyer, 1959). Ash was determined by incineration of sample in a muffle furnace at 550 °C for 18 h.

The preparation of fatty acid methyl esters (FAMEs) was performed according to Christie (Christie, 2003). Briefly, the lipid sample (20 mg) was dissolved in 1 mL of

toluene and 10% methanolic hydrogen chloride (2 mL) was added. A 0.1 mL solution of tricosanoic acid (10 mg/mL) was added as internal standard. The sample was sealed and heated at 50 °C overnight and then 5 mL of a NaCl 5% (w/v) solution was added to each sample. The FAMEs were extracted with 2 × 2 mL of hexane. The hexane layer was washed with 2 mL of a potassium carbonate solution (1M), dried over anhydrous sodium sulphate and 1 μL was injected into the gas chromatograph, in split mode (split ratio 1:50). Fatty acid analysis was performed on an Agilent gas chromatograph (model 6890), equipped with an automatic sampler (model 7683) and a flame ionization detector (FID). The carrier gas was helium with a flow at 1.0 mL/min and an inlet pressure of 16.9 psi. The column was an HP-Innowax fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) (Agilent Technologies, Palo Alto, CA).
The oven temperature program was from 100 to 180 at 3 °C/min, then from 180 to 250 at 2.5 °C/min, then held for 10 min. Fatty acids were identified using external standards and quantified using tricosanoic acid as internal standard. Peak areas were corrected according to the theoretical relative FID response correction factors (TRFs) published by Ackman (Ackman, 2002). The results are presented as g/100 g of fatty acids (% by weight). All analyses were done in duplicate.

3.2.4 Statistical analysis

Firstly, data were checked for normal distribution and variance homogeneity. Homogeneity of variance was confirmed and comparison between means was performed by ANOVA. Student-Newman-Keuls was used as *post hoc* test for comparison of the means among different groups of fish. Significance was accepted at probabilities of 0.05 or less. Principal component analysis was performed in order to compare different samples and to detect the most important variables affecting the distribution of fish samples. All the statistical analysis were performed by SPSS version 19.0 (SPSS Inc. Chicago, Illinois) and The Unscrambler version 9.7 (Camo, Norway). Data in the tables are reported as mean values ± standard error of mean (SEM).

3.3 Results and discussion

3.3.1 Biometric measurements
The fish can be distinguished, according to their feeding behavior, in planktophagous (landlocked shad, bleak and whitefish), omnivorous (crucian carp, common carp, chub, roach, black bullhead, Italian roach, rudd and tench) and

carnivorous/ichthyophagous (burbot, pike, perch and wels catfish) fish. Each species occupies a particular habitat, designated as pelagic, littoral or benthonic, that for some species could change during the reproductive season. Biometric measurements taken on each captured fish were listed in Table 3.2. All harvested fish were adult specimens of commercial size, according to fishermen practices and to the regulations enforced for the access to the lakes resources. The fillet yield of commercial fish varied from 42.3 to 69.3% with a mean yield of 53.8%.

3.3.2 Proximate composition

Table 3.3 shows the proximate composition of the edible flesh of freshwater fish collected in this study. The lipid content varied from 0.3% in burbot to 9.7% in shad, with most of fish having lipid content lower than 1.0%. Ackman (1989) (Ackman, 1989) classified fish into four categories according to their lipid content: lean fish (<2%), low fat (2-4%), medium fat (4-8%) and high fat fish (>8%). According to this classification most analyzed fish can be considered as lean fish. Two exceptions are represented by bleak and landlocked shad, which presented a lipid content of 4.5 and 9.7%, respectively. Luzzana et al. (1996), in a study on seasonal variation in fat content and fatty acid composition of landlocked shad from lake Como, reported the lipid content for this fish caught in October as 9.9% for the female specimens and 8.5% for the male. These data were comparable with this study.
Protein in fish flesh ranged from 18.1% of burbot to 21.4% of whitefish ($p<0.05$). It's known that fish are a good protein source ranging from 16 to 21 g/100 g. (Huss, 1988). Protein is considered to be a rather stable component of fish body, depending on fish size and genetic factors. The differences in protein content showed in Table 3.3 were presumably related to genetic factors. The ash level of the fish fillets was similar and was approximately 1-1.5%. Bleak samples, due to their small size (adult maximum length ~10 cm), were analyzed as whole eviscerated fish and presented the ash amount higher ($p<0.05$) then fish fillets from the other species.
The moisture content ranged from 69.4% of shad to 80.7 of burbot. It is well recognized in fish an inverse relationship between the water and the lipid content. As a rule, to an higher lipid content in flesh corresponds a lower water content.

Table 3.2 . Biometric measurements of fish species collected in northern Italian lakes

Fish	Origin (lake)	Length (cm)		Weight(g)		Eviscerated weight (g)		Fillet weight(g)		Yield (%)		Fulton index (K)	
Landlocked shad	Como	22.5	±0.90	89.1	±9.55	78.0	±8.63	53.7	±5.93	60.0	±0.72	0.75	±0.01
	Iseo	24.4	±0.33	111.8	±3.18	98.9	±2.83	66.5	±1.77	59.6	±0.57	0.77	±0.01
Italian Bleak[a]	Iseo	9.3	±0.15	8.5	±0.42	89.6	±2.52		-		-	1.05	±0.04
Burbot	Iseo	34.1	±0.90	318.5	±17.32	255.0	±16.24	134.9	±7.77	42.3	±0.78	0.80	±0.03
Crucian carp	Alserio	35.1	±1.91	1379.6	±212.15	1026.4	±148.67	648.6	±103.33	46.9	±0.89	3.05	±0.14
	Comabbio	38.7	±0.53	1437.4	±61.37	990.1	±106.91	674.7	±33.29	46.8	±0.49	2.50	±0.13
	Mezzola	35.5	±0.81	956.8	±82.22	774.1	±48.24	415.3	±24.17	44.1	±1.43	2.11	±0.05
	Varese	37.0	±1.37	1160.5	±129.16	924.7	±89.72	559.2	±53.15	49.3	±1.49	2.20	±0.08
Common carp	Varese	26.0	±1.46	406.1	±62.11	366.2	±55.49	222.7	±32.47	54.9	±1.32	2.25	±0.08
Italian Chub	Como	34.5	±0.87	559.0	±35.10	483.1	±28.02	313.7	±18.24	56.5	±0.55	1.34	±0.04
	Garlate	32.2	±0.79	483.3	±45.33	408.6	±41.94	269.4	±27.38	55.5	±0.64	1.43	±0.04
	Iseo	54.3	±0.91	1938.2	±98.70	1696.3	±121.13	1127.9	±110.72	58.1	±3.86	1.21	±0.04
	Maggiore	35.3	±1.70	632.1	±102.96	563.5	±89.79	369.7	±64.29	58.0	±1.40	1.38	±0.04
Roach	Ceresio	22.7	±0.79	184.8	±21.77	157.6	±17.25	103.0	±10.88	56.4	±1.62	1.52	±0.03
	Maggiore	26.5	±1.25	317.9	±52.93	242.4	±38.42	156.4	±30.17	47.5	±4.00	1.63	±0.17
European whitefish	Como	27.6	±0.39	186.9	±3.44	165.0	±2.65	129.6	±2.77	69.3	±0.70	0.89	±0.04
Northern pike	Iseo	50.7	±9.35	1053.6	±581.32	922.1	±482.50	572.6	±289.97	57.3	±2.75	0.65	±0.04
European perch	Garda	23.8	±0.86	181.5	±20.37	162.8	±17.96	95.6	±11.46	52.3	±0.64	1.25	±0.03
	Iseo	24.0	±0.45	167.5	±7.36	148.6	±6.17	83.8	±3.81	50.1	±0.85	1.21	±0.03
Black bullhead	Alserio	20.3	±0.41	140.0	±9.40	120.6	±6.12	53.0	±2.76	38.9	±2.58	1.69	±0.12
Italian Roach	Como	33.1	±1.33	517.5	±65.14	449.1	±52.35	309.4	±37.92	60.4	±1.87	1.34	±0.03
Rudd	Alserio	22.7	±0.61	240.8	±13.14	213.6	±11.42	138.7	±6.96	57.8	±1.02	2.10	±0.15
	Comabbio	20.8	±1.32	225.1	±71.53	187.0	±55.29	131.1	±39.86	60.4	±0.64	1.73	±0.10
	Garlate	30.0	±0.47	594.6	±13.23	488.1	±12.16	331.0	±8.34	55.7	±0.85	2.28	±0.13

Table 3.2. Biometric measurements of fish species collected in northern Italian lakes (continue)

Fish	Origin (lake)	Length (cm)		Weight(g)		Eviscerated weight (g)		Fillet weight(g)		Yield (%)		Fulton index (K)	
Tench	Comabbio	51.2	±1.13	2513.9	±176.44	2084.9	±115.07	1261.8	±68.38	50.6	±1.22	1.86	±0.07
	Como	37.6	±2.67	874.4	±162.70	774.9	±141.18	439.5	±73.74	51.3	±1.46	1.57	±0.05
	Iseo	39.8	±1.80	1083.5	±126.45	1011.1	±107.87	532.3	±52.76	49.5	±1.32	1.71	±0.05
	Mezzola	43.8	±1.45	1773.2	±167.82	1555.9	±130.95	953.9	±75.61	54.4	±1.46	2.12	±0.18
	Varese	53.7	±1.20	2702.9	±289.06	2288.9	±176.93	1425.2	±78.95	53.3	±2.76	1.73	±0.07

[a] chemical composition of ten pools of whole eviscerated fish (ten fish per pool).

Table 3.3. Chemical composition of freshwater fish fillets captured in Northern Italian lakes

	Moisture		Protein		Lipid		Ash	
Italian bleak* (n=10)	72.91	±0.35^{c}	19.83	±0.18^{b}	4.54	±0.49^{b}	2.72	±0.23^{b}
Landlocked shad (n=26)	69.44	±0.43^{a}	19.44	±0.32^{ab}	9.69	±0.49^{d}	1.43	±0.02^{a}
Crucian carp (n=21)	78.04	±0.25^{d}	19.57	±0.15^{ab}	0.84	±0.06^{a}	1.55	±0.24^{a}
European whitefish (n=10)	71.38	±0.45^{b}	21.37	±0.17^{c}	5.99	±0.54^{c}	1.26	±0.02^{a}
Common carp (n=5)	78.77	±0.29^{de}	19.26	±0.26^{ab}	0.63	±0.04^{a}	1.35	±0.39^{a}
Northern pike (n=3)	79.23	±0.54^{de}	18.93	±0.49^{ab}	1.50	±0.32^{a}	1.34	±0.07^{a}
Black bullhead (n=5)	78.15	±0.25^{d}	19.29	±0.32^{ab}	1.13	±0.09^{a}	1.43	±0.25^{a}
Burbot (n=10)	80.71	±0.18^{e}	18.07	±0.21^{a}	1.28	±0.04^{a}	0.96	±0.02^{a}
European perch (n=10)	79.03	±0.21^{de}	19.16	±0.18^{ab}	1.57	±0.15^{a}	1.23	±0.07^{a}
Italian roach (n=9)	78.73	±0.35^{de}	19.85	±0.36^{b}	0.72	±0.16^{a}	1.46	±0.16^{a}
Roach (n=10)	78.49	±0.32^{d}	19.32	±0.23^{ab}	0.94	±0.03^{a}	1.24	±0.12^{a}
Rudd (n=14)	78.68	±0.26^{de}	19.07	±0.16^{ab}	0.87	±0.04^{a}	1.39	±0.14^{a}
Wels catfish (n=5)	78.24	±0.99^{d}	19.16	±0.35^{ab}	0.62	±0.08^{a}	1.98	±0.69^{a}
Chub (n=25)	78.38	±0.21^{d}	19.16	±0.24^{ab}	1.00	±0.12^{a}	1.45	±0.17^{a}
Tench (n=23)	77.46	±0.53^{a}	18.93	±0.29^{ab}	2.01	±0.45^{a}	1.60	±0.13^{a}

* chemical composition of ten pools of whole eviscerated fish (ten fish per pool).
Value within the same column not sharing a common letter are significantly different ($P<0.05$)

3.3.3 Fatty acids profiles.

The fatty acid composition of edible fillet of fifteen freshwater fish species is presented in Table 3.4 A total of 19 major fatty acids, including 3 saturated fatty acids (SFA), 5 monounsaturated fatty acids (MUFA) and 11 polyunsaturated fatty acids (PUFA) were identified and quantified. All fatty acids varied significantly among fish species.
Total SFA ranged from 22.9 to 38.0%, MUFA ranged from 12.2 to 47.4% and PUFA from 29.7 to 61.2%. The most abundant SFA in all fish studied was palmitic acid (16:0), which ranged from 16.7% in bleak to 21.5% in shad, followed by 18:0,

which varied from 3.8% in bleak to 15.7% in pike. These fatty acids have been reported by many authors as the major SFAs in freshwater fish (Rahmanet al., 1995).

In all freshwater species analyzed, Kinsella et al. (1978) found that 16:0 was one of the major fatty acids of fillets. Guler et al.(2007) reported that 16:0 was the major saturated fatty acid in zander fillets (*Sander lucioperca*), ranging from 14.2 to 17.9%. Celik et al.(2005) found similar results in the same species harvested from two different Turkey lakes, with a 16:0 content of 19.6 and 20.8%. Ozogul et al. (2007) reported a 16:0 content varying from 15.9% to 20.5% in some freshwater fishes.

The most represented MUFA in our freshwater fish were 18:1n-9 (6.3% in roach to 30.7% in bleak), 16:1n-7 (1.9% in pike to 11.9% in bleak) and 18:1n-7 (2.4% in pike to 6.7% in tench). Other minor MUFA were 20:1n-9 (0.1% in perch to 1.7% in shad) and 22:1 (0.03% in bleak to 0.3% in common carp).

Among PUFA, 22:6n-3 (DHA) and 20:5n-3 (EPA) varied significantly among fish species. DHA ranged from 3.6% in bleak to 29.7% in roach, whereas EPA ranged from 3.7% in bleak to 15.9% in burbot. Linoleic (18:2n-6) and arachidonic (20:4n-6) acids were also present in moderate proportions and varied in fish fillets from 1.9% in burbot fillet to 10.1% in bleak and from 3.4% in bleak to 11.2% in common carp fillet, respectively. Total n-3 fatty acids ranged from 14.4% in bleak to 47.3 in roach, whereas total n-6 fatty acids ranged from 11.0% in whitefish to 20.0% in common carp. The fish with the highest content of n-3 fatty acids was roach (47.2%), whereas the species with the highest content of n-6 fatty acids was common carp (20.0%).

Fatty acid composition of wild freshwater fish is influenced by the lipid pattern of their natural food (Henderson, et Tocher, 1987) and this is strictly linked to other factors such as water temperature, season and place of capture. In general, planktivorous freshwater fish analyzed in this study where characterized by a lower content of PUFA and a higher content of MUFA, when compared to omnivorous and ichthyophagous fish. Fatty acids of freshwater fish growing on plankton showed two main features differentiating them from fatty acids found in omnivorous or ichthyiophagous fish. One was the presence of substantial proportion of 18:3n-3 and 18:4n-3 fatty acids (~ 5-10%) and the other was the occurrence of higher amount of 16:1n-7 and 18:1n-7. This can be understood on the basis of the fatty acid composition of freshwater microalgae (Ahlgren, et al., 1992; Caramujo et al., 2008), plankton feed (Desvilettes et al., 1997) an freshwater insects (Ghioni et al, 1996) Ghioni et al., in a work on the fatty acid composition of neutral lipids and phospholipids of freshwater insects, reported that SFA and MUFA together represented up to 85% of the fatty acids of total neutral lipids, with

16:0 being the most abundant SFA and 16:1n-7, 18:1n-9 and 18:1n-7 the most abundant MUFA. Linko et al. (1992), when studying the relationship between vendace (*Coregonus albula*) fatty acids and its available plankton feed, found that plankton fatty acids (mainly 16:0, 18:1n-9, 16:1n-7, 18:1n-7, 20:4n-6, 20:5n-3) were transferred to vendace flesh lipids without major modifications. Fatty acid composition of whitefish of this study resemble those reported in literature for coregonids by other authors (Luzzana et al, 1996; Serrini, 1996).

As regards planktivorous freshwater fish of this study, the predominant fatty acids in bleak were oleic acid (18:1*n*-9), palmitic acid (16:0), linoleic acid (18:2*n*-6) and 16:1*n*-7, the predominant fatty acid in shad were oleic acid, palmitic acid, EPA (20:5*n*-3) and linolenic acid (18:3*n*-3), whereas the predominant fatty acids in whitefish were palmitic acid, oleic acid, EPA and DHA.

Among omnivorous fish presented here, SFAs content varied in the range of 24.4-27.9%, MUFAs varied in the range of 13.0-32.0%, whereas PUFAs varied from 40.0 to 61.3% of total fatty acids. When compared to planktivorous, omnivorous fish contained a higher amount of n-3 fatty acids, in particular DHA and EPA. DHA content was 8.2% in tench, 14.4% in black bullhead, 16.4% in Italian roach, 18.1% in common carp, 18.6% in crucian carp, 21.9% in rudd, 22.2% in chub and 29.7% in roach flesh. EPA content was 7.6% in tench, 12.5% in black bullhead, 10.4% in Italian roach, 6.7% in common carp, 9.4% in crucian carp, 12.5% in rudd, 6.8% in chub and 11.4% in roach flesh. Palmitic acid was the major SFA, DHA was the major n-3 PUFA, oleic acid was the predominant MUFA and arachidonic acid was the predominant n-6 PUFA in all freshwater omnivorous fish analyzed.

Freshwater ichthyophagous fish such as pike and perch were characterized by high proportion of DHA (26.7 and 30.3%), 16:0 (18.0 and 20.9%), 18:0 (15.7 and 16.1%), 20:4n-6 (6.4 and 9.5%) and EPA (5.7 and 6.9%, respectively). Total n-3 fatty acid accounted for 41.6 and 35.3% of total fatty acids in pike and perch, respectively.

Wels catfish (*Silurus glanis*) is a very aggressive large carnivore fish that takes advantage from an incredible different sources of food, such as fish, frog or aquatic birds (Küçükgülmez et al., 2010). It is generally consumed fresh in Eastern European countries or marketed after smoking. Interestingly, catfish flesh contained 50.9% of PUFA, 22.5% MUFA and 26.6% of SFA. Most abundant fatty acids were DHA, 16:0, 18:1n-9 and arachidonic acid (20:4n-6). These results were rather different to those obtained by other authors, who reported fatty acid profile of smoked catfish meat (Küçükgülmez et al., 2010) or catfish reared under different conditions (Jankowska et al., 2004). The difference with our study were probably due to the difference in fish size and environmental conditions.

In the present study a strong relationship between feeding behavior and fatty acid composition of fish meat was observed, as showed in Table 3.5. Planktivorous fish (bleak, whitefish and shad) had the lowest total PUFA, *n*-3 LC-PUFA and *n*-3 fatty acids, but had the highest MUFA content, in particular oleic acid (18:1*n*-9). Carnivorous and ichthyophagous fish (pike, perch, burbot, wels catfish) had the highest *n*-3 LC-PUFA and *n*-3 fatty acids, but the lowest MUFA content, whereas omnivorous fish, which eat both plant and animal food, had a substantial proportion of *n*-3 fatty acids and the highest content of *n*-6 LC-PUFA and *n*-6 fatty acids, when compared to the other fish (Table 3.5).

Recently, the importance of the *n*-6/*n*-3 fatty acids ratio in the diet has been seriously addressed in human nutrition (Simopoulos, 2002). During the past half-century, the increase in dietary vegetable oils has resulted in unbalanced *n*-6/*n*-3 ratio, with intake of *n*-6 PUFA increased whereas *n*-3 intake decreased. A ratio of *n*-6 PUFA/*n*-3 PUFA of around 5:1 is now regarded as optimal and formerly *n*-6 PUFA/*n*-3 PUFA ratio has increased to about 10:1 or higher (Leaf, 1987). All the analyses fish presented a *n*-6/*n*-3 ratio ranging from 0.3 to 0.9, typical of similar freshwater fish (Steffens et al., 2007).

Table 3.4. Fatty acid composition of freshwater fish fillets captured in northern Italian lakes

Fish	Fatty acid									
	14:0	**16:0**	**16:1*n*-7**	**18:0**	**18:1*n*-9**	**18:1*n*-7**	**18:2*n*-6**	**18:3*n*-6**	**18:3*n*-3**	**18:4*n*-3**
Italian bleak (n=10)	2.4 ±0.1 b	16.7 ±0.4 a	11.9 ±0.6 e	3.9 ±0.3 a	30.7 ±1.5 e	3.6 ±0.4	10.1 ±0.2 d	0.4 ±0.0 cd	6.5 ±0.2 d	0.3 ±0.1 ab
Landlocked shad (n=26)	4.4 ±0.1 d	21.5 ±0.2 d	4.5 ±0.1 abc	4.5 ±0.1 ab	26.6 ±0.9 e	3.9 ±0.1	4.6 ±0.2 abc	0.6 ±0.0 d	5.3 ±0.2 cd	2.6 ±0.2 c
Crucian carp (n=21)	1.5 ±0.1 a	16.6 ±0.4 a	6.0 ±0.3 bc	7.1 ±0.3 cd	11.6 ±0.9 abc	5.3 ±0.1	3.6 ±0.2 abc	0.2 ±0.0 ab	2.9 ±0.2 ab	1.0 ±0.1 b
European whitefish (n=10)	3.6 ±0.2 c	19.6 ±0.3 bcd	7.2 ±0.4 cd	4.3 ±0.1 a	19.3 ±0.7 d	3.6 ±0.1	4.2 ±0.1 abc	0.3 ±0.0 bc	5.4 ±0.2 cd	3.3 ±0.2 d
Common carp (n=5)	0.8 ±0.1 a	17.8 ±0.7 ab	4.4 ±0.3 abc	9.1 ±0.3 d	10.3 ±0.6 abc	4.9 ±0.2	5.0 ±1.0 bc	0.2 ±0.0 ab	2.3 ±0.8 a	0.2 ±0.0 ab
Northern pike (n=3)	1.3 ±0.4 a	18.1 ±0.5 ab	1.9 ±0.8 a	15.7 ±1.3 f	7.6 ±1.5 ab	2.4 ±0.5 a	3.4 ±0.6 abc	0.2 ±0.2 ab	2.1 ±0.6 a	0.4 ±0.4 ab
Black bullhead (n=5)	1.2 ±0.2 a	18.4 ±0.5 ab	3.2 ±0.4 ab	7.2 ±0.2 cd	13.7 ±1.0 bcd	5.4 ±0.3	3.8 ±0.2 abc	0.1 ±0.0 ab	3.4 ±0.2 abc	0.7 ±0.1 ab
Burbot (n=10)	0.9 ±0.1 a	18.0 ±0.5 ab	3.7 ±0.3 abc	11.3 ±0.6e	12.1 ±0.3 abc	5.3 ±0.2	1.9 ±0.2 a	0.2 ±0.0 ab	1.7 ±0.2 a	0.6 ±0.3 ab
European perch (n=10)	1.0 ±0.1 a	20.9 ±0.4 cd	2.8 ±0.3 ab	16.1 ±0.5 f	6.7 ±0.6 ab	2.8 ±0.2	2.9 ±0.2 ab	0.0 ±0.0 a	1.2 ±0.1 a	0.1 ±0.1 a
Italian roach (n=9)	0.8 ±0.3 a	18.1 ±0.8 ab	7.3 ±1.2 cd	5.5 ±0.4 abc	16.0 ±2.8 cd	4.1 ±0.4	2.8 ±0.4 ab	0.2 ±0.0 ab	2.2 ±0.5 a	0.3 ±0.1 ab
Roach (n=10)	0.5 ±0.1 a	18.5 ±0.8 ab	2.6 ±0.3 ab	6.8 ±0.1 bcd	6.3 ±0.4 a	3.4 ±0.1	2.0 ±0.3 a	0.1 ±0.0 a	1.2 ±0.1 a	0.4 ±0.1 ab
Rudd (n=14)	0.9 ±0.1 a	19.7 ±0.2 bcd	3.8 ±0.2 abc	6.5 ±0.2 bc	8.0 ±1.0 ab	2.7 ±0.1	3.5 ±0.7 abc	0.2 ±0.0 ab	5.0 ±1.1 bcd	0.7 ±0.1 ab
Wels catfish (n=5)	1.1 ±0.2 a	18.1 ±0.5 ab	4.5 ±0.6 abc	7.4 ±0.3 cd	10.8 ±1.5 abc	5.9 ±0.2	4.3 ±0.5 abc	0.1 ±0.0 ab	2.3 ±0.4 a	0.3 ±0.1 ab
Chub (n=25)	1.1 ±0.1a	18.5 ±0.3 ab	3.6 ±0.3 abc	7.7 ±0.6 cd	13.0 ±1.1 abc	3.9 ±1.1	6.2 ±0.6 c	0.3 ±0.1 bc	1.6 ±0.2 a	0.4 ±0.1 ab
Tench (n=23)	2.3 ±0.3 b	19.0 ±0.4 abc	9.7 ±1.2 de	6.6 ±0.6 bc	15.0 ±1.4 cd	6.7 ±0.8 b	6.0 ±0.6 c	0.1 ±0.0 ab	3.5 ±0.5 abc	0.6 ±0.1 ab

Continued

Fish	Fatty acid								
	20:1*n*-9	**20:2*n*-6**	**20:3*n*-6**	**20:4*n*-6**	**20:5*n*-3**	**22:1**	**22:4*n*-6**	**22:5*n*-3**	**22:6*n*-3**
Italian bleak (n=10)	1.1 ±0.1 d	0.6 ±0.0 abcd	0.5 ±0.0 abc	3.4 ±0.3 a	3.7 ±0.4 a	0.0 ±0.0 a	0.2 ±0.0 a	0.3 ±0.2 a	3.6 ±0.6 a
Landlocked shad (n=26)	1.7 ±0.1 e	0.3 ±0.0 ab	0.3 ±0.0 a	4.6 ±0.2 ab	6.8 ±0.3 bc	0.3 ±0.0 d	0.8 ±0.0	2.1 ±0.2bc	4.7 ±0.6 a
Crucian carp (n=21)	1.1 ±0.1 d	0.4 ±0.0abc	0.6 ±0.0 abc	8.9 ±0.4 cde	9.4 ±0.3 cd	0.1 ±0.0 abc	1.0 ±0.1	4.0 ±0.2defg	18.6 ±0.7 bc
European whitefish (n=10)	0.7 ±0.1 c	0.4 ±0.0 ab	0.2 ±0.0 a	5.2 ±0.2 ab	11.2 ±0.4 de	0.1 ±0.0 abc	0.8 ±0.0	2.7 ±0.1 cde	8.0 ±0.7 a
Common carp (n=5)	0.8 ±0.1 c	1.1 ±0.1 ef	1.0 ±0.1 e	11.2 ±0.7 e	6.7 ±0.5 bc	0.3 ±0.0 d	1.5 ±0.1 b	4.3 ±0.3 efg	18.1 ±1.0 bc
Northern pike (n=3)	0.2 ±0.2 a	0.3 ±0.2 ab	0.2 ±0.2 a	6.4 ±0.3 bc	5.7 ±0.7 ab	0.1 ±0.1 ab	0.7 ±0.2	3.1 ±1.5 cdef	30.3 ±4.7 f
Black bullhead (n=5)	0.8 ±0.0 c	0.7 ±0.1 bcde	0.6 ±0.1 abc	7.8 ±0.5 cd	12.5 ±0.2 e	0.1 ±0.0 abc	0.9 ±0.1	5.3 ±0.2 g	14.4 ±1.2 b
Burbot (n=10)	0.5 ±0.1 bc	0.5 ±0.1 abc	0.3 ±0.0 ab	9.1 ±0.3 cde	15.9 ±0.4 f	0.2 ±0.0 d	0.4 ±0.1 a	1.1 ±0.6 ab	16.3 ±0.5 bc
European perch (n=10)	0.1 ±0.0 a	0.2 ±0.1 a	0.2 ±0.0 a	9.5 ±0.2 cde	6.9 ±0.5 bc	0.0 ±0.0 ab	1.5 ±0.2 b	0.5 ±0.4 a	26.7 ±1.2 ef
Italian roach (n=9)	0.6 ±0.1 bc	0.8 ±0.1 cdef	0.5 ±0.1 abc	9.3 ±0.9 cde	10.4 ±1.0 de	0.1 ±0.0 abc	0.8 ±0.1	4.0 ±0.3 defg	16.4 ±2.2 bc
Roach (n=10)	0.5 ±0.1 bc	0.8 ±0.1 cdef	0.4 ±0.1 abc	9.9 ±0.4 de	11.4 ±1.1 de	0.1 ±0.0 abc	0.7 ±0.1	4.6 ±0.3 fg	29.7 ±1.0 f
Rudd (n=14)	0.3 ±0.1 ab	0.5 ±0.1 abc	0.5 ±0.1 abc	8.6 ±0.7 cde	12.5 ±0.9 e	0.1 ±0.0 bc	0.8 ±0.1	3.8 ±0.2 cdefg	21.9 ±0.7 cd
Wels catfish (n=5)	1.3 ±0.1 d	1.1 ±0.1 f	0.8 ±0.0de	11.1 ±1.0 e	5.9 ±0.3 ab	0.0 ±0.0 ab	1.5 ±0.1 b	3.5 ±0.1 cdef	20.3 ±1.7 bc
Chub (n=25)	0.6 ±0.1 bc	0.9 ±0.1 cdef	0.8 ±0.1 de	9.5 ±0.5 cde	6.8 ±0.3 bc	0.1 ±0.0 abc	0.7 ±0.0	2.2 ±0.3 bcd	22.2 ±1.2 cd
Tench (n=23)	0.6 ±0.1 bc	1.0 ±0.1 def	0.7 ±0.1 cd	8.1 ±0.8 cde	7.6 ±0.5 bc	0.2 ±0.0 c	1.4 ±0.3 b	2.8 ±0.4 cde	8.2 ±1.4 a

Continued

Fish	Fatty acid							
	SFA	**MUFA**	**PUFA**	***n*-3**	***n*-6**	***n*-6/*n*-3**	***n*-3 LC PUFA**	***n*-6 LC PUFA**
Italian bleak (n=10)	22.9 ±0.5 a	47.4 ±1.6 g	29.7 ±1.3 a	14.4 ±0.8 a	15.3 ±0.5 bcd	1.1 ±0.0	7.6 ±0.9 a	4.7 ±0.4 a
Landlocked shad (n=26)	30.3 ±0.3 c	36.9 ±0.8 f	32.7 ±0.8 a	21.5 ±0.8 b	11.2 ±0.3 ab	0.6 ±0.0	13.6 ±0.7 b	6.1 ±0.2 a
Crucian carp (n=21)	25.2 ±0.4 ab	24.2 ±1.2 bcde	50.6 ±1.2 de	36.0 ±0.9 cd	14.6 ±0.6 abc	0.4 ±0.0	32.1 ±1.0 def	10.9 ±0.5 bcd
European whitefish (n=10)	27.5 ±0.3 bc	30.9 ±1.1 def	41.6 ±0.9 bc	30.7 ±0.8 c	11.0 ±0.2 a	0.4 ±0.0	21.9 ±1.1 c	6.5 ±0.2 a
Common carp (n=5)	27.7 ±0.6 bc	20.7 ±0.5 abc	51.7 ±0.8 de	31.7 ±0.6 c	20.0 ±1.3 e	0.6 ±0.1	29.2 ±1.4 d	14.9 ±0.9 e
Northern pike (n=3)	35.1 ±0.7 d	12.2 ±2.9 a	52.8 ±2.3 de	41.6 ±3.0 de	11.2 ±0.7 ab	0.3 ±0.0	39.1 ±4.0 f	7.7 ±0.1 ab
Black bullhead (n=5)	26.9 ±0.6 bc	23.2 ±1.4 bcde	49.9 ±1.8 de	36.2 ±1.3 cd	13.7 ±0.5 abc	0.4 ±0.0	32.2 ±1.5 def	9.9 ±0.6 bc
Burbot (n=10)	30.3 ±0.9 c	21.8 ±0.7 abcd	48.0 ±0.5 cd	35.5 ±0.5 cd	12.4 ±0.4 ab	0.4 ±0.0	33.3 ±0.7 def	10.3 ±0.3 bc
European perch (n=10)	38.0 ±0.8 e	12.5 ±0.9 a	49.5 ±0.6 de	35.3 ±0.8 cd	14.2 ±0.4 abc	0.4 ±0.0	34.1 ±0.8 def	11.3 ±0.4 bcde
Italian roach (n=9)	24.4 ±1.0 ab	28.0 ±4.2 cde	47.6 ±3.3 cd	33.3 ±3.0 c	14.3 ±0.7 abc	0.5 ±0.0	30.8 ±3.3 de	11.4 ±0.9 bcde
Roach (n=10)	25.8 ±0.8 ab	13.0 ±0.7 a	61.3 ±1.3 f	47.3 ±1.5 e	14.0 ±0.5 abc	0.3 ±0.0	45.7 ±1.3 g	11.9 ±0.5 cde
Rudd (n=14)	27.1 ±0.3 bc	14.9 ±1.2ab	58.0 ±1.0 ef	43.9 ±1.5 e	14.1 ±0.6 abc	0.3 ±0.0	38.2 ±1.5 ef	10.5 ±0.7 bc
Wels catfish (n=5)	26.6 ±0.7 bc	22.5 ±2.3 bcd	50.9 ±1.9 de	32.2 ±1.4 c	18.7 ±0.7 e	0.6 ±0.0	29.7 ±1.8 d	14.3 ±1.1 de
Chub (n=25)	27.2 ±0.9 bc	21.1 ±1.5 abcd	51.7 ±1.1 de	33.3 ±1.3 c	18.4 ±0.7 de	0.6 ±0.1	31.3 ±1.4 def	11.9 ±0.5 cde
Tench (n=23)	27.9 ±0.7 bc	32.1 ±2.5 ef	40.0 ±2.2 b	22.7 ±1.6 b	17.3 ±1.0 cde	1.1 ±0.3	18.6 ±1.6 bc	11.1 ±1.1 bcde

within the same column not sharing a common letter are significantly different ($P<0.05$)

Table 3.5. Fatty acid composition of freshwater fish grouped according to their feeding behaviour

Fatty acid	**Planktovorous**[a] (n=46)		**Omnivorous**[b] (n=112)		**Ichthyophagous**[c] (n=28)	
C14:0	3.75	±0.13 a	1.30	±0.09 b	1.01	±0.07 b
C16:0	20.05	±0.32 a	18.32	±0.19 b	19.08	±0.35 b
C16:1n-7	6.69	±0.47 a	5.54	±0.36 a	3.32	±0.23 b
C18:0	4.32	±0.09 a	7.00	±0.20 b	12.80	±0.70 c
C18:1n-9	25.93	±0.84 a	12.08	±0.55 b	9.46	±0.58 c
C18:1n-7	3.76	±0.10	4.68	±0.32	4.20	±0.29
C18:2n-6	5.72	±0.37 a	4.51	±0.25 b	2.86	±0.22 c
C18:3n-6	0.47	±0.02 a	0.19	±0.02 b	0.12	±0.03 c
C18:3n-3	5.58	±0.16 a	2.78	±0.21 b	1.63	±0.15 c
C18:4n-3	2.25	±0.19 a	0.58	±0.04 b	0.31	±0.12 b
C20:1n-9	1.34	±0.07 a	0.65	±0.03 b	0.47	±0.09 b
C20:2n-6	0.36	±0.02 a	0.76	±0.04 b	0.47	±0.07 a
C20:3n-6	0.34	±0.02 a	0.65	±0.03 b	0.30	±0.05 a
C20:4n-6	4.48	±0.16 a	9.00	±0.24 b	9.31	±0.31 b
C20:5n-3	7.05	±0.42 a	9.11	±0.29 b	9.82	±0.91 b
C22:1	0.16	±0.02 a	0.12	±0.01 b	0.10	±0.02 b
C22:4n-6	0.68	±0.04 a	0.96	±0.06 b	1.02	±0.12 b
C22:5n-3	1.88	±0.17 a	3.46	±0.14 b	1.51	±0.36 a
C22:6n-3	5.17	±0.43 a	18.30	±0.75 b	22.21	±1.22 c
SFA	28.12	±0.48 a	26.62	±0.30 b	32.89	±0.95 c
MUFA	37.89	±1.04 a	23.08	±0.94 b	17.55	±1.10 c
PUFA	33.99	±0.84 a	50.30	±0.88 b	49.56	±0.55 b
n-3	21.94	±0.96 a	34.24	±0.91 b	35.49	±0.67 b
n-6	12.05	±0.33 a	16.06	±0.35 b	14.07	±0.51 c
n-6/n-3	0.62	±0.04	0.58	±0.06	0.40	±0.02
n-3 LC-PUFA	14.10	±0.87 a	30.87	±0.94 b	33.54	±0.77 b
n-6 LC-PUFA	5.86	±0.18 a	11.36	±0.30 b	11.10	±0.42 b

Value within the same raw not sharing a common letter are significantly different ($P<0.05$)

[a] *Alburnus arborella, Alosa fallax lacustris, Coregonus macrophtalmus*
[b] *Carassius carassius, Cyprinus carpio, Leuciscus cephalus, Rutilus rutilus, Ichtalurus melas, Rutilus pigus, Scardinius erythrophtalmus, Tinca tinca*
[c] *Lota lota, Esox lucius, Perca fluviatilis, Silurus glanis*

Principal Component Analysis was used to provide an overview of the capacity of the fatty acid composition to discriminate fish of different species, irrespectively of

lake of origin and month of capture. After applying PCA to our data set, three PCs were extracted. The percentage of variance explained by first and second PC were 83% and 6%, respectively. According to the loading of the fatty acids in the first PC (Figure 3.1), the most contributing descriptors were 18:1n-9, 16:1n-7, 22:6n-3 and 20:4n-6. Furthermore, the correlation loadings showed strong correlation between 18:1n-9, 14:0, 16:1n-7 and between 20:4n-6 and 22:6n-3. When representing the scores of the fish samples on the two dimensional space defined by the calculated PCs (Figure 3.2), fish species appeared well distinguished from each other according to their habitat and food habits, especially when considering planktivorous versus carnivorous fish (Figure 3.3).

3.4 Conclusion

The present study contributed to the chemical characterization of fifteen species of freshwater fish caught in northern Italian lakes, particularly for fatty acid composition. To the authors' knowledge, it is the first time that an accurate assess of lipid composition of these species has been performed.

Fatty acid composition has been demonstrated as a suitable tool to distinguish feeding behavior of caught fish and to provide their position in the food web of the aquatic environment. Predatory fish show the tendency to accumulate in their flesh substantial amount of *n*-3 LC-PUFA, especially DHA, although the lipid content of flesh is low. Planktivorous fish are generally medium or high fat fish and their fatty acid profile could be considered of a lower quality. Omnivorous fish of our study are the leanest and their fatty acid profile is in between.

From a nutritional point of view, due to their general low fatty tissue and high content of polyunsaturated fatty acid, these freshwater fish represent an appropriate source of healthy components, especially long chain *n*-3 fatty acids, EPA and DHA. In addition, emphasizing the nutritional properties of these fish represents a feasible way to support the demand for this products and thereby could help in sustaining the professional fishing activities.

Figure 3.1. Principal component analysis: loading plot of the fatty acids in the bidimensional space of the first two PCs.

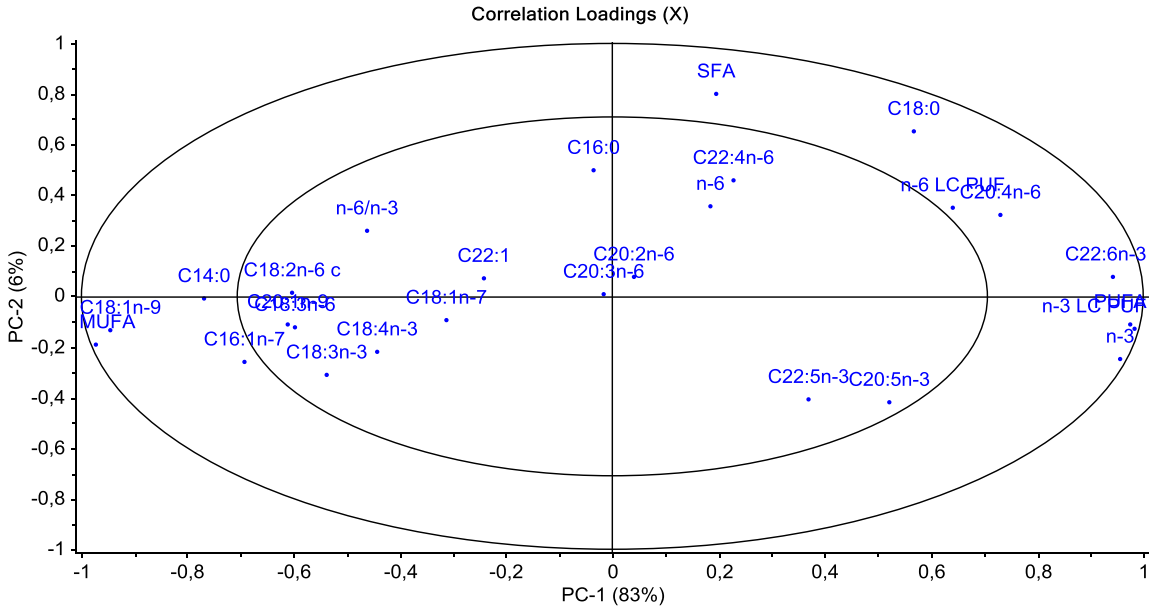
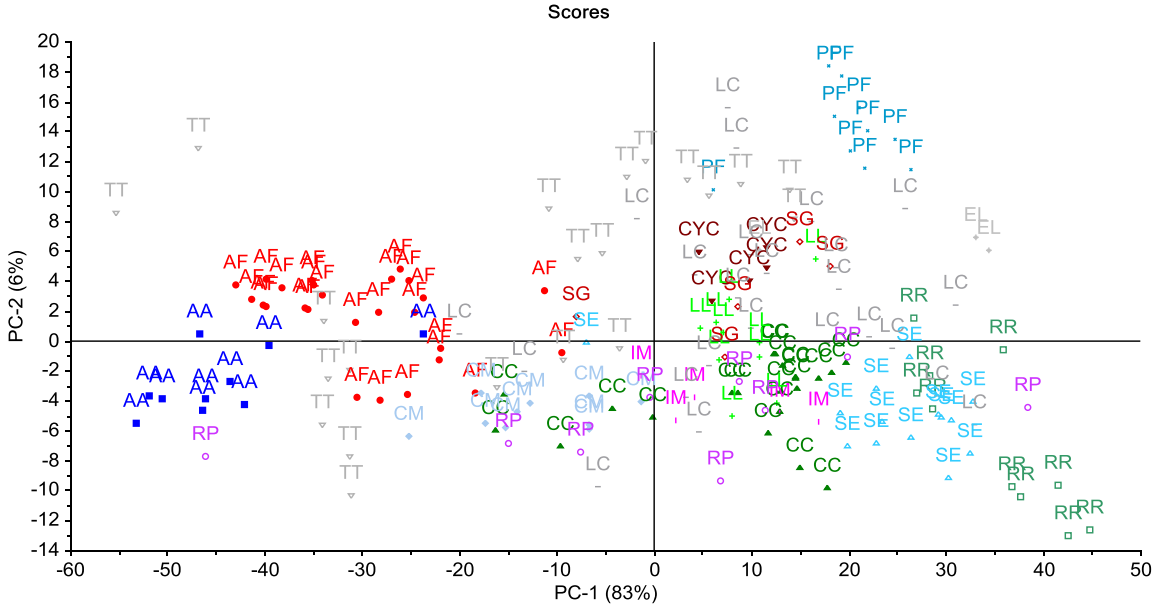
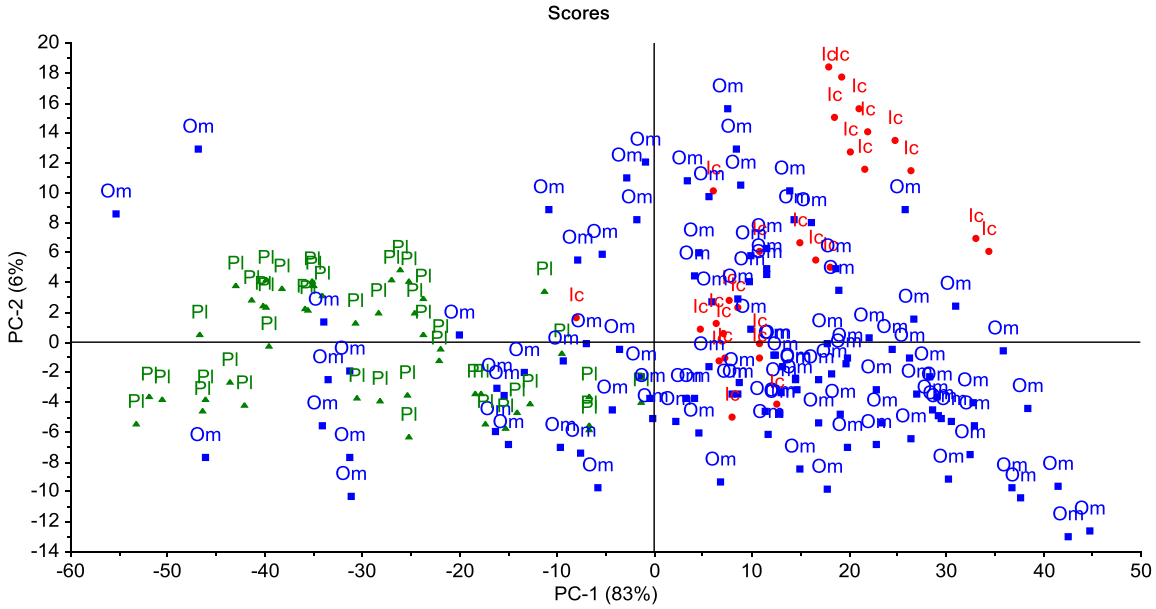


Figure 3.2. Principal component analysis: score plot of the fish samples grouped by species in the bidimensional space of the first two PCs.



Legenda: AF: *Alosa fallax lacustris*, AA: *Alburnus arborella*, LL: *Lota lota*, CC: *Carassius carassius*, CYC: *Cyprinus carpio*, LC: *Leuciscus cephalus*, CM: *Coregonus macroftalmus* EL: *Esox lucius*, PF: *Perca fluviatilis*, IM: *Ictalurus melas*, RP: *Rutilus pigus*, RR: *Rutilus rutilus*, SE: *Scardinius erythrophthalmus*, TT: *Tinca tinca*, SG: *Silurus glanis*

Figure 3.3. Principal component analysis: score plot of the fish samples grouped by feeding behavior in the bidimensional space of the first two PCs.



Legenda: Pl: Planktovorous fish, Om: Omnivorous fish, Ic: ichthyophagous fish

References

A.O.A.C. (1996) Official methods of analysis of the Association of Official Analytical Chemists. *Association of Official Analytical Chemists, Arlington (U.S.A.)*

Ahlgren G., Gustafsson I.-B. and Boberg M. (1992) Fatty acid content and chemical composition of freshwater microalgae. *Journal of Phycology* 28, 37-50.

Ackman R. G. (1967) Characteristic of the fatty acid composition and biochemistry of some freshwater fish oils and lipids in comparison with marine oils lipids. *Comparative Biochemistry Physiology 22* 907–922.

Ackman R. G (1989) Nutritional composition of fats in seafoods. *Progress in food & nutrition science* 13, 161-289.

Ackman R. G. (1999) Comparison of lipids in marine and freshwaters organisms. In *Lipids in freshwater ecosystems*, Arts, M. T.; Wainman, B. V., Eds. Springer: New York, 1999; pp 263-298.

Ackman R. G. (2002) The gas chromatograph in practical analyses of common and uncommon fatty acids for the 21st century. *Anal Chim Acta* 465, 175-192.

Bell M., Henderson R. and Sargent J. (1986) The role of polyunsaturated fatty acids in fish. *Comparative Biochemistry and Physiology. B, Comparative Biochemistry & Molecular Biology* 83, 711-9

Belluzzi A. (2001) N-3 and n-6 fatty acids for the treatment of autoimmune diseases. *European Journal of Lipid Science and Technology* 103, 399-407.

Bianco, P. G. (1995) Mediterranean endemic freshwater fishes of Italy. *Biological Conservation* 72, 159-170

Bligh E. G. and Dyer W. Y. (1959) A rapid method of total lipid extraction and purification. *Canadian Journal Biochemistry Physiology* 37, 911-917

Caramujo M.-J., Boschker H. T. S. and Admiraal W. I. M. (2008) Fatty acid profiles of algae mark the development and composition of harpacticoid copepods. *Freshwater Biology* 53, 77-90

Celik M., Diler A. and Kucukgulmez A. A. (2005) comparison of the proximate compositions and fatty acid profiles of zander (Sander lucioperca) from two different regions and climatic conditions. *Food Chemistry* 92, 637-641.

Christie W. W. (2003) Preparation of derivatives of fatty acid. In *Lipid Analysis. Isolation, Separation, Identification and Structural Analysis of Lipids*, 3th ed.; Christie, W. W., Ed. The Oily Press: Bridgwater England, 2003; Vol. 15, pp 205-224.

Connor W. E.(2000) Importance of n-3 fatty acids in health and disease. *American Journal of Clinical Nutrition* 71, 171-175.

Crawford M. A. (1993) The role of essential fatty acids in neural development: implications for perinatal nutrition. *American Journal of Clinical Nutrition* 57, 703-709

Desvilettes C. H., Bourdier G., Amblard C. H. and Barth B. (1997) Use of fatty acids for the assessment of zooplankton grazing on bacteria, protozoans and microalgae. *Freshwater Biology* 38, 629-637

Dill W. A.(1990) *Inland fisheries in Europe*. FAO: Rome; Vol. 52

Ghioni C., Bell J. G. and Sargent J. R. (1996) Polyunsaturated Fatty Acids in Neutral Lipids and Phospholipids of Some Freshwater Insects. *Comparative Biochemistry and Physiology -- Part B: Biochemistry and Molecular Biology* 114, 161-170

Godefroot M., Sandra P. and Verzele M., (1981) New method for quantitative essential oil analysis. *Journal of Chromatography A* 203, 325-335.

Guler G. O., Kiztanir B., Aktumsek A., Citil O. B. and Ozparlak H. (2008) Determination of the seasonal changes on total fatty acid composition and omega3/omega6 ratios of carp (*Cyprinus carpio* L.) muscle lipids in Beysehir Lake (Turkey). *Food Chemistry* 108, 689-694.

Halver J. E. and Hardy R. W (2002) *Fish nutrition*. 3rd ed. ed.; Academic Press: San Diego, California p xiv, 824 p

Henderson R. J. and Tocher, D. R. (1987) The lipid composition and biochemistry of freshwater fish. *Prog Lipid Res* **1987,** *26*, 281-347.

Huss H. H. (1988) Fresh fish quality and quality changes. *FAO Fisheries Series***,** 29, 132.

Jankowska B., Zakes Z., Zmijewsky T., Ulikowski D. and Kowalska A. (2004) Impact of diet on the fatty acid profile of European catfish (*Silurus glanis* L.). *Archives of Polish Fisheries* 12, 99-110.

Kinsella J., Shimp J. and Mai J. 0 The Proximate and Lipid Composition of Several Species of Freshwater Fishes. *New York's Food and Life Sciences Bulletin* **1978,** *69*.

Küçükgülmez A., Eslem Kadak A. and Celik M. (2010) Fatty acid composition and sensory properties of Wels catfish (*Silurus glanis*) hot smoked with different sawdust materials. *International Journal Food Science and Technology* 45, 2645-2649

Larsson S. C., Kumlin M., Ingelman-Sundberg M. and Wolk A (2004) Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. *American Journal of Clinical Nutrition* 79, 935-945

Leaf A. and Weber P. C. (1987) A new era for science in nutrition. *American Journal of Clinical Nutrition* 45, 1048-1053

Linko R. R., Rajasilta M. and Hiltunen R. (1992) Comparison of lipid and fatty acid composition in vendace (*Coregonus albula* L.) and available plankton feed. *Comparative Biochemistry and Physiology Part A: Physiology* 103, 205-212.

Luzzana U. Serrini G., Moretti V. M., Grimaldi P., Paleari M. A. and Valfre F. (1996) Seasonal variations in fat content and fatty acid composition of male and female coregonid 'bondella' from Lake Maggiore and landlocked shad from Lake Como (northern Italy). *Journal of Fish Biology* 48, 352-366

Mozaffarian D., Bryson C. L. and Lemaitre, R. (2005), Fish Intake and Risk of Incident Heart Failure. *ACC Current Journal Review* 14, 8-9

Orban E., Masci M., Nevigato T., Di Lena G., Casini I., Caproni R., Gambelli L., De Angelis P. and Rampacci M. (2006) Nutritional quality and safety of whitefish (*Coregonus lavaretus*) from Italian lakes. *Journal of Food Composition and Analysis* 19, 737-746

Orban E., Nevigato T., Masci M., Di Lena G., Casini I., Caproni R., Gambelli L., De Angelis P. and Rampacci M. (2007) Nutritional quality and safety of European perch (*Perca fluviatilis*) from three lakes of Central Italy. *Food Chemistry* 100, 482-490.

Özogul Y., Özogul F., Alagoz S.,(2007) Fatty acid profiles and fat contents of commercially important seawater and freshwater fish species of Turkey: A comparative study. *Food Chemistry* 103, 217-223

Paleari M. A., Soncini G., Beretta G., Grimaldi P. and Tava A. (1993) Microbiology and chemical composition of a typical product from Alosa fallax lacustris. *Journal of Applied Ichthyology* 9, 222-9

Premazzi G., Dalmiglio A., Cardoso A. C. and Chiaudani G. (2003) Lake management in Italy: the implications of the Water Framework Directive. *Lakes & Reservoirs: Research & Management* 8, 41-59.

Rahman S. A., Teh Sing H., Hassan O. and Daud N. M., (1995) Fatty acid composition of some Malaysian freshwater fish. *Food Chemistry* 54, 45-49.

Serrini G., Luzzana U., Grimaldi P., Paleari M. A. and Valfre F.(1996) Fatty acid composition of some coregonid species (Coregonus spp.) from different European lakes. *Journal of Applied Ichthyology* 12, 57-59.

Simopoulos A. P. (1991) Omega-3 fatty acids in health and disease and in growth and development. *American Journal of Clinical Nutrition* 54, 438-463.

Simopoulos, A. P. (2002) The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy* 56, 365-379

Steffens W. and Wirth M. (2007) Influence of nutrition on the lipid quality of pond fish: common carp (*Cyprinus carpio*) and tench (*Tinca tinca*). *Aquaculture International* 15, 313-319.

Volta P., Oggioni A., Bettinetti R. ans Jeppesen E. (2011) Assessing lake typologies and indicator fish species for Italian natural lakes using past fish richness and assemblages. *Hydrobiologia* 671, 227-240

CHAPTER 4

Chemical composition, histamine content and histamine-forming bacteria in missoltino, a salted and dried landlocked shad (*Alosa fallax lacustris)* product.

.

4.1 Introduction

Missoltino is an Italian typical product obtained by salted and dried landlocked shad (*Alosa fallax lacustris*), a migratory anadromous species of Clupeidae family. Probably this species originated long time ago, when groups of shad became trapped in waterbasins (Berg and Grimaldi, 1996). Actually, shad is a common species of large northern Italian lakes such as Maggiore, Como, Iseo and Garda. The salted and dried shad, called "*missoltino*", is produced especially along the coastal zone of Lake Como, by professional fishermen and according traditional methods. After catching, fish are eviscerated through a cut close to the branchial operculum and stacked with alternating layers of coarse salt for two days (80 g/kg fish). After salting the fish receive a quick rinse with fresh running water. Then fish are dried for 3-5 days in a ventilated room and then are ripened under physical pressure for a period which could vary from 90 to 120 days, depending of the judgment of the producer. For pressing process, shad are placed in layers in metal cans, with bay leaves interspersed between each layer of fish. Once a container is filled, it is closed with a wooden lid slightly smaller in diameter than that of the can, then stacked with other prepared cans in a special press. The press is controlled by a crank handle and causes a loss of water and fat. The pressing is then increased progressively so that excess liquids are slowly eliminated (Paleari et al., 1993).

Shad belongs to fish species characterized by a high level of histidine and according to European Regulation shad and their products have to be monitored for histamine hazard. In literature, there are no information about the occurrence of histamine in fresh shad and missoltino, and related bacteria in this product, with the exception of preliminary data published by Pirani et al. (2010). Moreover, no reports are available on the occurrence of scombroid syndrome in Italy caused by consumption of missoltino. This is probably due to the lack of reports to the medical authority by patients, also because in the most cases the poisoning is solved in short time without treatment and symptoms resemble those of other food allergies (Attaran and Probst, 2002).

Histamine is the biogenic ammine responsible of the scombroid syndrome, formed through decarboxylation of the amino acid histidine, by histidine decarboxylase (HDC), enzyme present on the membrane of several bacterial species. The activity of HDC could continue also when the HDC positive bacteria are no longer viable. (Hungerford, 2010). Histamine intoxication could be associated with the consumption of meat, cheese, wine and fish product (Taylor 1986). Scombroid and other fishes have high level of free histidine in their flesh and they are often involved in scombroid syndrome if they are not processed and stored following the proper hygienic conditions.

Several bacteria have shown the ability to produce histamine: in fresh products the production of histamine is caused mainly by the proliferation of enteric

Gram negative bacteria. like *Morganella morganii, Proteus vulgaris, Citrobacter freundii* and *Enterobacter aerogenes* which have been isolated from fish with high levels of histamine in their flesh (Kim et al., 2003; Ferrario et al 2012; Lee et al 2012).
Other bacteria, common in the aquatic environment, have also been reported as histamine producers, like *Clostridium* spp., *Vibrio* spp., *Aeromonas* spp. and *Pseudomonas* spp. (Hsu et al 2009).
Although some bacteria are present in the normal microbial flora of live fish, others seem to be derived from post-catching contamination on board fishing vessels, at the processing plant or in the distribution system, or in restaurants or homes (Lehand and Olley, 2000) In salted products also halotolerant and halophile bacteria were isolated as histamine forming bacteria: Hernandez Herrero et al. (1999) found *Staphylococcus epidermidis, Staphylococcus xylosus*, *Klebsiella oxytoca, Enterobacter cloacae, Pseudomonas cepaciae* and *Bacillus* spp. in salted Spanish anchovies. Hsu et al. (2009) isolated *S. xylosus*, *S. sciuri*, *Bacillus thuringiensis*, *Citrobacter freundii* and *Klebsiella pneumonia* as weak histamine formers, while *Enterobacter aerogenes* and *Citrobacter* spp. were identified as strong histamine formers bacteria in dried milkfish (*Chanos chanos*). Recently, in a study on histamine content of different salted fish products in Taiwan, Lin et al. (2012) found that *Bacillus megaterium* shows the ability to grow and produce histamine in elevated NaCl concentrations.
According to the European Regulation 2073/2005, the maximum mean value of histamine in fresh fish products is 100 mg/kg. Among nine samples, only two can have a histamine content higher than 100 mg/kg but lower than 200 mg/kg. In enzyme matured products, within 9 samples only two can have a histamine content higher than 200 mg/kg, but lower than 400 mg/kg. EU regulation specifies also fish family associated with high amounts of histidine: Scomberidae (like tuna and mackerel), Engraulidae (anchovy), Clupeidae (pilchard), Coryfenidae (mahi mahi), Pomatomatidae (bluefish) and Scombresosidae (saury).
The scombroid syndrome is spread all over the world and it can be considered one of the most common intoxication caused by fish consumption. Histamine has an important vasoactive effect in human body (Taylor 1985). Symptoms are variable according to histamine concentration and individual susceptibility. They include gastrointestinal symptoms such as nausea, vomiting, abdominal cramps and diarrhea (Gilbert et al., 1980); problems concerning the central nervous system such as dizziness, anxiety, headache (Sabroe and Black, 1998; Specht, 1998; Hungerford, 2010) and the cutaneous apparatus with rush. Patients can present metallic or peppery taste, palpitations, oral numbness, difficulty in swallowing and thirsty (Hungerford, 2010). Rarely, severe respiratory and cardiac problems may occur. In most cases, symptoms of scombroid syndrome take place between 10 minutes to few hours after the ingestion of the incriminated seafood. Disorders, usually mild, resolve within 24 hours.

The aim of the present research was to investigate the chemical composition of missoltino, following the entire process, and determine its histamine content, in order to better understand the histamine formation process and to give to the producers useful indications on how minimize the hazard.

4.2 Materials and Methods

4.2.1 Sampling

Two sampling campaigns were performed. The first was made in autumn 2010 and the second during summer 2011. Missoltini used for the chemical characterization of the product, were collected from one producer during the autumn 2010. Four samples of missoltini were collected at the end of drying period, and then at 30, 60 and 90 days of ripening. At the same time we collected also four fresh shad from the lot used for the preparation of missoltini. All Samples were collect from the same lot of production, transported to the laboratory and then stored at -25°C until analysis.
Missoltini used for the determination of histamine and bacterial analysis were collected from seven traditional producers, named producer from A to G, during the summer 2011. Sampling was performed at each stage of processing: fresh fish, salting, drying and after 15, 60 and 120 days of ripening. Nine samples, obtained from each producer, were collected from the same lot, transported to the laboratory aseptically in ice for the next analyses, where they were divided in three sub samples. From the nine samples collected from each producers three were used for water, salt and A_w determination, three for histamine determination and three for the bacteriology analysis.

4.2.2 Proximate and fatty acid composition

Proximate composition of fish and missoltini was performed using standard methods (A.O.A.C. 1996). Moisture content was determined by drying samples in an oven at 60 °C to constant weight. Protein content was determined by Kjeldahl method, by which the concentration of nitrogen is measured. A conversion factor of 6.25 was used to convert total nitrogen to crude protein. For the analysis an automated distillation unit (Büchi 339, Switzerland) was used. The total lipid analysis was performed by a modified method of Folch et al. (1957). About 2 grams of muscle were suspended in 2x30 ml of chloroform:methanol (2:1) solution and homogenized. 30 ml of 0,44 KCl in Methanol water 3:1 was added, favouring a two-phase solution. The lower phase was collected after the two phases were well separated overnight. The solution collected was divided into two aliquots for quantification (60%) and analysis of

fatty acid composition (40%). Ash was determined by incineration of sample in a muffle furnace at 550 °C for 18 h. The preparation of fatty acid methyl esters was performed according to Cristie (1982). Briefly, the extracted lipid solution was concentrated by a rotary evaporator (BUCHI CH-9230) and dissolved in acetyl chloride:methanol (1:9) solution in a sealed cap, and incubated at 50°C overnight. Tricosanoic acid was dissolved in 1 ml of toluene and added as an internal standard. The acetyl chloride-methanol solution trans-esterifies the fatty acids and forms fatty acid methyl esters (FAME). Then 5 ml NaCl solution 5% was added to allow the separation between the hydrophobic and the aqueous phases. 2 ml of 1M potassium carbonate solution was added to buffer the solutions. The FAME's were extracted in 2x2 ml of hexane, and the mixture was evaporated to dryness under a stream of nitrogen. Fatty acid methyl esters were recovered with hexane and analyzed in an Agilent gas-chromatograph (model 6890), equipped with an automatic sampler (model 7683) and a flame ionization detector (FID). The carrier gas was helium with a flow at 1.0 ml/min and an inlet pressure of 16.9 psi. The injection volume was 1 μl, and a split ratio of 1:80 was used. A HP-Innowax fused silica capillary column (30 m × 0.25 mm i.d.) was used to separate fatty acid methyl esters (Agilent Technologies). The oven temperature program for separation was from 100 to 180 °C at 3 °C/min, then from 180 to 250 °C at 2.5 °C/min and held for 10 min. All analyses were done in duplicate.

4.2.3 Salt content, water content and water activity determination

Salt, water content and water activity were performed on three samples for each sampling point. Salt and water content was determined according to the AOAC. Briefly for salt determination sample (5–10 g) was burnt at 500 °C, the ash solubilised in distilled water and the NaCl content was titrated with 0.1 M AgNo3 using 10% w/v K2CrO4 solution as an indicator. Water content was determined by difference after oven heating at 110°C for 48 h. Water activity of samples was determined by the mean of a hygroscopic apparatus type Rotronic DT (Switzerland) at 25.5±1°C.

4.2.4 Histamine determination

Histamine was determined on fresh shad and missoltini by HisQuick® Histamine Rapid test (Labor Diagnostika Nord LDN, Germany). Three samples were analyzed at each stage of sampling. HisQuick test contains all the reagents and the detailed instructions required for histamine analysis on fish samples. Analysis of each single sample were done in duplicate. First, the histamine was extracted from the fish sample. Briefly, 2 grams of ground sample were added to 20 ml of 70% isopropanol, shacked for 4 minutes, centrifuged and 200 μl of

supernatant were collected and placed into an ion exchange column. The histamine was bound to the ion-exchanger column and separated from impurities. After washing histamine was eluted. Results were read at 450 nm with a microtiter plate reader. The calibration curve for the determination of histamine was linear over the tested concentration range of 0-2000 ppm (6 standards points: 0, 125, 250, 500, 1000, 2000) with a correlation coefficient of 0.9992 (six replicates).

4.2.5 Microbiological analysis

At each step of missoltino process, from fresh shad to 120 days of pressing, three samples for microbiological analysis were collected from every producer. 10g of each sample was diluted 10-fold in physiological saline (0.85% NaCl) with 0.1% peptone (PS), homogenized for 60 s in a Stomacher (400 Lab-Blender) and spread in duplicate onto Plate Count Agar (PCA, Oxoid, I) for the enumeration of Total Viable Count and incubated at 30°C for 48 h.

4.2.6 Isolation of histamine forming-bacteria

Randomly from PCA plates, a total number of 99 colonies were isolated, subcultured on sheep blood agar (Oxoid, Italy) and evaluated the main macroscopic characteristics. Catalase, oxidase and urease tests were performed, Gram was determined for the identification of the bacterial Genus, different cultural media were used (i.e., for *Enterobacteriaceae,* MacConkey Agar, Brilliant Green Agar, Eosin Methylene Blue Agar, for *Staphylococcus* spp. Baird Parker Agar and Mannitol Salt Agar),finally biochemical tests were performed using API system. After identification, all the strains were transferred on Niven's medium (Niven, 1981; Mavromatis, 2002) for the evaluation of histamine production on this cultural medium, bacteria determined a different colour depending on the quantity of histamine they produce (Mavromatis, 2002).

4.2.7 Statistical analysis

All data are reported as mean values ± the standard error of the mean (SEM.). Homogeneity of variance was confirmed and comparison between means was completed by one-way ANOVA (analysis of variance). The significance was accepted at probabilities of 0.05. These statistical analyses were performed by SPSS 20.0 (SPSS Inc., Chicago, IL, U.S.A.).

4.3 Results

In order to give some information about missoltino, a niche Italian fish product, in the present research its nutritional characteristics are shown. Proximate composition of fresh shad and missoltino is showed in Table 4.1. The higher loss of moisture occurs during the salting and drying period, when moisture decrease from the value of 73.5g/100g of the fresh fish to the 52.5g/100g of the missoltino at the beginning of ripening. Then there is a further decrease to the value of 46.9 g/100g till the stable value of 46.2 that we found in the final product. This loss of water is due to the pressure that acts on missoltini during ripening. Lipids have an initial increase due to the dehydration of the product, then they start to decrease. During ripening the product lost an amount of oil. The increase of lipid concentration that there is from the 60th day of ripening and the final product, that is at the 90th day, is due to the producer, who use to add an amount of the same oil that missoltino lost if he repute that missoltini have become too low in lipid (personal communication). Protein value is the most stable, and follows the dehydration process. Missoltino shows a protein content of 33.8 g/100g while the raw fish has a value of 18.6 g/100g. Ash value has the maximum increase during the first stage of processing, due to the amount of salt added during salting, going from the value of 1.2 of fresh fish to 8.6g/100g at the end of drying. Then they reach the final value of 12.9 g/100g at the end of processing, when missoltino is considered ready for the final consumers.

Table 4.1. Proximate composition of missoltino collected at different stage of processing (n=4). Data are expressed as g/100g of product

	Fresh	Drying	30 days ripening	60 days ripening	90 days ripening
Moisture	73.5 ±0.85^{c}	52.5 ±1.54^{b}	46.9 ±1.12^{a}	46.2 ±0.65^{a}	46.2 ±1.10^{a}
Protein	18.6 ±0.45^{a}	28.1 ±1.45^{b}	35.6 ±1.22^{c}	35.6 ±0.56^{c}	33.8 ±0.94^{c}
Lipid	6.7 ±1.20^{a}	10.9 ±0.83^{b}	6.5 ±0.58^{a}	5.9 ±0.07^{a}	7.1 ±0.49^{a}
Ash	1.2 ±0.05^{a}	8.6 ±0.53^{b}	11.1 ±0.27^{c}	12.2 ±0.44^{d}	12.9 ±0.45^{d}

Data are reported as mean values ± standard error. Values in the same column with different letters are statistically different ($P < 0.05$).

Considering the total fatty acid composition, presented in Table 4.2, palmitic (16:0) and oleic (18:1n-9) acids are the most representative among saturated fatty acid (SFA) and monounsaturated fatty acids (MUFA) (21.63% and 24.08% respectively). Missoltino is characterized by a high presence of polyunsaturated fatty acids (PUFA, 34.32%), especially those belonging to the n-3 series (25.46%). Among PUFA n-3 series, eicosapentaenoic (EPA, 20:5n-3; 8.44%) and

docosahexaenoic acids (DHA, 22:6n-3; 6.35%) are most representative. These two fatty acids are known to be important fatty acids in the protection of the cardiovascular system. In particular, EPA plays a significant role in the anti-inflammatory response. The fatty acids profile of missoltino is quite similar to the one of fresh shad. We can only note a small decrease of the MUFA, due mainly to the variation of the presence of oleic acid.

Table 4.2. fatty acids composition of missoltino collected at different stage of processing (n=4). Data are expressed as g/100g of total fatty acids**.**

	Fresh	Drying	30 days ripening	60 days ripening	90 days ripening
14:0	3.75 ±0.136	3.55 ±0.097	3.88 ±0.151	4.05 ±0.217	3.81 ±0.064
16:0	19.36 ±0.249^{b}	18.36 ±0.364^{a}	23.94 ±0.303^{d}	24.29 ±0.202^{d}	21.64 ±0.337^{c}
16:1 n7	4.60 ±0.192	4.34 ±0.164	4.11 ±0.150	4.23 ±0.297	4.35 ±0.115
18:0	4.38 ±0.170^{a}	4.43 ±0.160^{a}	5.43 ±0.147^{b}	5.08 ±0.193^{b}	5.05 ±0.155^{b}
18:1 n9	26.53 ±1.215^{b}	26.90 ±0.734^{b}	23.54 ±0.518^{a}	23.09 ±0.821^{a}	24.08 ±0.342^{ab}
18:1 n7	3.57 ±0.090	3.77 ±0.103	3.55 ±0.037	3.48 ±0.097	3.79 ±0.071
18:2 n6	2.99 ±0.058^{b}	2.86 ±0.037^{b}	2.46 ±0.062^{a}	2.60 ±0.057^{a}	2.83 ±0.100^{b}
18:3 n6	0.42 ±0.031^{ab}	0.46 ±0.019^{b}	0.34 ±0.006^{a}	0.36 ±0.019^{a}	0.39 ±0.017^{ab}
18:3 n3	4.85 ±0.187^{c}	4.56 ±0.108^{bc}	3.59 ±0.098^{a}	4.04 ±0.187^{ab}	4.22 ±0.191^{b}
18:4 n3	2.68 ±0.136^{c}	2.71 ±0.069^{c}	1.84 ±0.043^{a}	2.08 ±0.098^{ab}	2.22 ±0.121^{b}
20:0	0.27 ±0.010^{ab}	0.30 ±0.006^{b}	0.25 ±0.004^{a}	0.27 ±0.009^{ab}	0.27 ±0.012^{ab}
20:1 n9	1.68 ±0.068^{a}	1.95 ±0.118^{b}	1.54 ±0.058^{a}	1.51 ±0.081^{a}	1.53 ±0.101^{a}
20:2 n6	0.11 ±0.037	0.07 ±0.003	0.06 ±0.003	0.06 ±0.003	0.06 ±0.003
20:3 n6	0.26 ±0.014	0.28 ±0.011	0.25 ±0.009	0.26 ±0.006	0.26 ±0.004
20:4 n6	4.22 ±0.245	4.09 ±0.146	4.48 ±0.204	4.32 ±0.204	4.49 ±0.180
20:3 n3	1.27 ±0.028^{b}	1.28 ±0.077^{b}	1.02 ±0.015^{a}	1.09 ±0.011^{a}	1.10 ±0.027^{a}
20:5 n3	8.05 ±0.613	8.61 ±0.682	8.55 ±0.340	8.04 ±0.289	8.45 ±0.423
22:0	0.19 ±0.015	0.23 ±0.023	0.19 ±0.006	0.20 ±0.007	0.20 ±0.007
22:1 n9	0.27 ±0.008^{ab}	0.34 ±0.029^{b}	0.27 ±0.021^{ab}	0.25 ±0.014^{a}	0.26 ±0.009^{ab}
22:4 n6	0.82 ±0.063	0.82 ±0.063	0.84 ±0.039	0.89 ±0.082	0.82 ±0.058
22:5 n3	3.19 ±0.143	3.39 ±0.159	3.07 ±0.056	2.91 ±0.129	3.12 ±0.107
22:6 n3	5.94 ±0.441	5.95 ±0.441	6.08 ±0.295	6.19 ±0.397	6.36 ±0.519
24:1 n9	0.59 ±0.039	0.76 ±0.074	0.69 ±0.017	0.72 ±0.055	0.71 ±0.030
SFA	27.96 ±0.198^{a}	26.87 ±0.476^{a}	33.70 ±0.411^{b}	33.88 ±0.210^{b}	30.96 ±0.443^{b}
MUFA	37.24 ±1.295^{b}	38.05 ±0.594^{b}	33.71 ±0.645^{a}	33.29 ±0.834^{a}	34.72 ±0.547^{a}
PUFA	34.80 ±1.105	35.08 ±0.886	32.59 ±0.703	32.84 ±0.914	34.32 ±0.293
n-3	25.98 ±0.841	26.50 ±0.793	24.16 ±0.454	24.35 ±0.654	25.46 ±0.208
n-6	8.82 ±0.270	8.58 ±0.168	8.42 ±0.253	8.49 ±0.290	8.86 ±0.148

Data are reported as mean values ± standard error. Values in the same column with different letters are statistically different ($P < 0.05$).

Table 4.3 shows the difference of the processing technique used by the seven producers. The mainly differences are represented by the evisceration technique, the amount of salt used and the drying stage. Concerning evisceration two producers use the abdominal incision, while the other five practice the operculum incision. Even if this second technique has an higher hazard of microbiological contamination there is no correlation with the evisceration technique and the presence of histamine. The amount of salt used during the salting phase seem to be no correlated with the presence of salt at the end of drying. Producers who add the larger quantity of salt have not the higher salt value in dried shad, so this parameter is more dependent of the drying technique than the quantity of salt used. The seven producers used three different drying technique. Two dried their product in a oven, three in a ventilated room and two outside, under the sun, as provided the old traditional technique. Both the oven and the outside technique seems to be more effective, as the producers who use the ventilated room are the ones who have the higher water activity and histamine level.

Table 4.3 Processing technique for the preparation of missoltini

	Evisceration	**Salting**			**Drying**		
Producer		**Salt (g/kg)**	**Temperature °C**	**Time (hour)**	**Location**	**Temperature °C**	**Time (days)**
A	abdominal incision	65	4	36	ventilated room	27	4
B	operculum incision*	70	4	40	oven	27	5-6
C	abdominal incision	150	1-4	48	oven	26	3
D	operculum incision*	50	6	40	ventilated room	20	-
E	operculum incision*	70	2	48	ventilated room	18	4
F	operculum incision*	65	14	48	outside	25	8
G	operculum incision*	80	10	24	outside	18	5

*a small incision at the operculum, followed by abdominal pressure and emission of viscera from the cutting

Values of water content, water activity (a_W), salt content and histamine are presented in Table 4. Salt content in all samples ranges from 2.93g/100g to 12.85g/100g. Significant differences in the salt content of samples from different producers are evident during drying phase and at 15, 60 and 120 days of pressing. At the end of the process (after 120 days of ripening) missoltino of producer D presents the lower salt content (8.27 g/100g) and samples from producer B the higher salt content (12.51 g/100g).

Water content in all samples of missoltino ranges from 39.48 g/100g to 68.47g/100g. Statistically significant differences are recorded in samples from different producers during salting and at 15, 60 and 120 days of ripening. At the end of the process, samples of producer C show the lower amount (39.07g/100g) and producer A the higher water amount (44.79g/100g). Water activity in all samples ranges from 0.710 to 0.948. Similarly to water content, water activity shows statistical differences between samples at salting stage and at days 15, 60 and 120 of pressing.
Histamine content ranges from 6 mg/Kg to a maximum level of 1977,33 mg/Kg. The level of histamine tends to increase during the process. First appearance of elevated levels of histamine (606.71 mg/Kg) occurs during the drying stage for producer D. At 60 days of ripening producers A and E present levels that exceed legal limit value for EU (1782.50 and 913.13 mg/Kg respectively).
Pearson correlation was applied to evaluate potential relationship among the salt, water content, water activity and histamine amount for all the samples tested. A negative correlation was found between histamine and water content in 105 samples analyzed ($r= -268$ $p<0.01$).
A total number of 99 isolates were identified from PCA plates (the Total Viable Count ranged from 3.1 to 8.0 Log CFU/gin fresh, salting, drying and ripening 15 days): considering these phases, the most representative genus were *Citrobacter* spp. (36 identified), *Staphylococcus* spp. (22 identified), *Proteus* spp. (10 identified), *Micrococcus* spp. (8 identified) and *Enterobacter* spp. (8 identified). The 71.7% (71 bacteria on the total of 99) of the identified microorganisms resulted as medium producing histamine bacteria while only the 7.1% (7 bacteria on the total of 99) resulted as high histamine producing bacteria. Table 4.5
The microbial population identified in the first phases, thanks to the selective pressure exerted by the increase of WPS and the reduction of a_w, changed into halotolerant genera like *Staphylococcus* spp., *Micrococcus* spp. and *Enterococcus* spp.. Since 60 days of ripening until 120 days, the extreme environment of missoltini (mean a_w at 60 days and 120 days: 0.76 and 0.74 respectively) inhibit microbial growth (TVC resulted below the detection limit of 2 Log CFU/g), not allowing the isolation and subsequent identification of any bacteria

4.4 Discussion

In the present study, samples of missoltino exceed the maximum level of histamine content based on EU regulation. Although histamine content is higher than 400 mg/Kg for 24 samples on a total of 126, not scombroid poisoning incidents were reported among consumers in relation to the consumption of

missoltino. This result can be attributed to the not specific symptoms of histamine poisoning from fish, that can be confused with an allergic reaction.
Three producers on seven present these unacceptable levels of histamine in missoltino they produced. Presumably the different process and processing conditions may have caused a different trend of increase of histamine in the product.
Different kind of fish and fish product can be involved in scombroid syndrome due to high level of histamine. Hsu et al. (2009) found high content of histamine in thirty-two dried milkfish products collected from five retail market of southern Taiwan. Beside the biogenic amine, they analyzed the water content, water activity and salt content too. The traditional process for dried milkfish presents some common steps with missoltino, as degutting, salting and drying the product for several days. Authors found a water content, water activity and salt content in all samples ranged from 20.5-57.9%, 0.72-0.93 and 1.2-11.6% respectively. The amount of histamine was more than 400 ppm for three samples of one of the retails.
Huang et al. (2010) studied the water content, water activity, salt and histamine content in forty-six dried fish products, from different species, sold in Penghu Island of Taiwan. In their research, authors demonstrated a presence of histamine higher than EU limits of 400 ppm for nine samples of dried Yellowstripe scad (*Selariodes leptolepis*) product.
Considering missoltino specifically, actually there are no studies on the prevalence of histamine in this Italian fish product, as we mentioned before.

4.5 Conclusion

According to our results missoltini have a good nutritional value, with an high protein content and a valuable lipid quote, where PUFA and especially the n-3 series, are well represented. Missoltino appears to be a stable product at the end of its processing, due to the low a_w that it reach, produced by salt presence and by the water loss that occurred during drying and ripening, which inhibits the microbial growth. Based on histamine quantification and histamine-producing bacteria identified was not possible understand which was the most critical phase where the main production occurred: Further studies are necessary to clear the phenomenon of histamine production in these products. Anyway, we could hypothesize that the suspect phase involved in histamine production is likely between 15 and 60 days of ripening where we found the highest increase of histamine. The best way to reduce the risk of histamine formation seems to be the reduction of a_w to value below 0.8 during the drying stage of processing. This aspect could be identify as a critical control point in the hygienic supervision of the production process.

Table 4.4. Salt content, water content, water activity and histamine concentration in fresh shad (*Alosa fallax lacustris*) and in missoltino at salting and drying stages

	Fresh	Salting				Drying			
	Histamine mg/kg	Salt%	Aw	Water%	Histamine mg/kg	Salt%	Aw	Water%	Histamine mg/kg
Producer									
A	44.9±20.18	4.1±0.58	0.94±0.008^{ab}	63.3±1.37^{a}	15.8±2.60	9.8±0.40^{ab}	0.83±0.014	43.2±3.43	20.3±3.84^{a}
B	23.8±23.10	4.2±0.88	0.92±0.007^{ab}	65.0±1.32^{a}	6.3±7.41	10.0±3.33^{ab}	0.77±0.097	42.3±7.91	82.1±55.08^{a}
C	22.1±5.67	4.2±0.20	0.91±0.017^{a}	61.2±0.74^{a}	16.5±22.23	8.3±1.94^{ab}	0.80±0.017	50.1±13.96	47.0±19.84^{a}
D	24.6±10.51	3.4±0.45	0.94±0.010^{b}	68.5±0.75^{b}	19.9±17.36	6.7±3.50^{a}	0.85±0.077	46.2±7.69	606.7±310.45^{b}
E	22.0±8.86	2.9±0.76	0.94±0.002^{b}	68.4±2.85^{b}	22.1±13.13	5.3±3.65^{a}	0.88±0.053	58.0±6.23	54.2±22.75^{a}
F	17.8±13.44	3.7±1.28	0.94±0.015^{ab}	62.9±1.80^{a}	11.3±14.85	14.7±3.65^{b}	0.79±0.029	46.4±0.54	20.7±8.13^{a}
G	120.1±164.34	3.2±0.84	0.95±0.006^{b}	63.6±0.93^{a}	21.0±8.22	11.4±1.79^{ab}	0.82±0.025	50.6±2.58	51.4±54.63^{a}

Data are reported as mean values ± standard deviation (S.D.). Values in the same column with different letters are statistically different ($P < 0.05$).

Table 4.4. (Continue) Salt content, water content, water activity and histamine concentration in missoltino at 15, 60, 120 days of ripening

	15 days ripening				60 days ripening				120 days ripening			
	Salt%	**Aw**	**Water%**	**Histamine mg/Kg**	**Salt%**	**Aw**	**Water%**	**Histamine mg/Kg**	**Salt%**	**Aw**	**Water%**	**Histamine mg/Kg**
Producer												
A	8.9±0.55^{a}	0.83±0.017	49.6±0.23^{c}	55.6±11.41^{a}	9.8±0.19^{a}	0.80±0.003^{c}	44.6±0.50^{b}	1782.5±234.99^{c}	9.8±0.11^{b}	0.77±0.012^{c}	44.8±1.33^{b}	1977.3±33.83^{d}
B	10.0±0.40^{ab}	0.79±0.002	45.6±1.82^{b}	36.4±12.58^{a}	12.2±0.23^{c}	0.74±0.009^{a}	42.6±0.74^{ab}	193.2±6.33^{a}	10.9±0.22^{c}	0.72±0.006^{a}	40.5±1.67^{a}	153.2±31.83^{a}
C	9.8±2.21^{ab}	0.79±0.042	43.4±0.99^{a}	79.2±64.10^{a}	10.7±0.68^{b}	0.75±0.003^{a}	40.0±1.81^{a}	136.6±80.00^{a}	10.5±0.89^{bc}	0.73±0.006^{a}	39.1±2.20^{a}	65.9±51.09^{a}
D	8.1±0.66^{a}	0.77±0.019	41.4±0.61^{a}	515.6±269.51^{b}	9.1±0.38^{a}	0.77±0.009^{b}	39.5±1.21^{a}	493.6±153.10^{a}	8.3±0.20^{a}	0.75±0.008^{b}	39.6±0.57^{a}	418.8±46.83^{b}
E	8.2±0.66^{a}	0.82±0.024	48.8±0.53^{c}	118.5±77.26^{a}	9.0±0.52^{a}	0.80±0.009^{c}	44.8±2.45^{b}	913.1±367.25^{b}	8.3±0.64^{a}	0.79±0.008^{c}	44.2±0.35^{b}	1644.1±238.31^{c}
F	11.8±0.68^{b}	0.79±0.034	46.2±0.94^{b}	63.5±23.69^{b}	12.4±0.20^{c}	0.74±0.010^{a}	42.7±0.85^{ab}	77.2±14.19^{a}	12.2±0.20^{d}	0.71±0.008^{a}	42.4±1.35^{ab}	45.3±6.77^{a}
G	12.1±0.50^{b}	0.76±0.014	46.5±1.82^{b}	35.8±5.01^{b}	12.8±0.56^{c}	0.75±0.004^{a}	42.3±0.59^{ab}	85.0±16.46^{a}	12.5±0.25^{d}	0.71±0.008^{a}	42.3±0.80^{ab}	124.0±129.26^{a}

Data are reported as mean values ± standard deviation (S.D.). Values in the same column with different letters are statistically different ($P < 0.05$)

Table 4.5 Identification of microorganisms in missoltini products and their ability to produce histamine in during fresh, salting, drying and ripening phases

		Number of strains identified	Percentage of strain identified	Number of high histamine producer	Number of medium histamine producer	Number of no histamine producer
Fresh	*Citrobacter* spp.	10	45,5%	0	8	2
	Enterobacter spp.	5	22,7%	1	4	0
	Flavobacterium spp.	3	13,6%	0	2	1
	Pseudomonas spp.	2	9,1%	0	0	2
	Escherichia coli	1	4,5%	0	1	0
	Klebsiella spp.	1	4,5%	0	1	0
	Total	22		1	16	5
Salting	*Proteus* spp.	5	23,8%	0	5	0
	Serratia spp.	4	19,0%	0	4	0
	Citrobacter spp.	4	19,0%	1	3	0
	Enterobacter spp.	3	14,3%	0	3	0
	Micrococcus spp.	3	14,3%	0	0	3
	Flavobacterium spp.	1	4,8%	0	1	0
	Escherichia coli	1	4,8%	0	1	0
	Total	21		1	17	3
Drying	*Staphylococcus* spp.	9	37,5%	0	8	1
	Citrobacter spp.	8	33,3%	1	6	1
	Proteus spp.	5	20,8%	0	3	2
	Micrococcus spp.	1	4,2%	0	0	1
	Klebsiella spp.	1	4,2%	0	1	0
	Total	24		1	18	5
Ripening 15 days	*Citrobacter* spp.	14	43,8%	4	9	1
	Staphylococcus spp.	13	40,6%	0	11	2
	Micrococcus spp.	4	12,5%	0	0	4
	Enterococcus spp.	1	3,1%	0	0	1
	Total	32		4	20	8

Acknowledgements

The study was supported by the ERDF: European Regional Development Fund – INTERREG program

References

A.O.A.C. (1996), *Official methods of analysis of the Association of Official Analytical Chemists*, Association of Official Analytical Chemists, Arlington, U.S.A.

Ababouch, L., Afila, M. E., Rhafiri, S. and Busta, F. F. (1991), Identification of histamine-producing bacteria isolated from sardine (Sardina pilchardus) stored in ice and at ambient temperature (25°C). *Food Microbiological*, 8, 127-136.

Attaran, R. R. and Probst, F. (2002) Histamine fish poisoning: a common but frequently misdiagnosed condition . *Emergency Medicine Journal*, Vol. 19 No. (5), pp. 474-475.

Berg A. and Grimaldi E. (1965). Study on the biology of the fish in the Lago Maggiore with the aim of collecting data as a basis for forecasting the possible interactions between these fish and the radioactivity present in the water. II° Genus alosa. - III° Census of the commercial fish catches.

Bjornsdottir, K., Bolton, G. E., McClellan-Green, P. D., Jaykus, L. A. and Green, D. P. (2009), "Detection of Gram-Negative Histamine-Producing Bacteria in Fish: A Comparative Study". *Journal of Food Protection*, Vol. 72 No. (9), pp. 1987-1991.

Cristie, W. (1982), *Lipid analysis. Isolation, separation, identification and structural analysis of lipids*, Pergamon Press, Oxford

Ferrario, C., Pegollo, C., Ricci, G., Borgo, F. and Fortina, M. G. (2012), PCR Detection and Identification of Histamine-Forming Bacteria in Filleted Tuna Fish Samples. *Journal of Food Science* 77: 115-120.

Folch, J., Lees, M. and Sloane-Stanley, G. H. (1957), "A simple method for the Isolation and purification of total lipides from animal tissues". *Journal of biological chemistry*, Vol. 226 No., pp. 497–509.

Gilbert, R. J., Hobbs, G., Murray, C. K., Cruickshank, J. G. and Young, S. E. (1980) Scombrotoxic fish poisoning: features of the first 50 incidents to be reported in Britain (1976-9). *British Medical Journal* 5. 71-72.

Hernandez-Herrero, M. M., Roig-Sagues, A. X., Rodriguez-Jerez, J. J. and Mora-Ventura, M. T. (1999), Halotolerant and halophilic histamine-

forming bacteria isolated during the ripening of salted anchovies (*Engraulis encrasicholus*)". *Journal of Food Protection*, Vol. 62 No. (5), pp. 509-514.

Hsu, H. H., Chuang, T. C., Lin, H. C., Huang, Y. R., Lin, C. M., Kung, H. F. (2009) Histamine content and histamine-forming bacteria in dried milkfish (*Chanos chanos*) products. *Food Chemistry*, 114 : 933-938.

Huang, Y. R., Liu, K. J., Hsieh, H. S., Hsieh, C. H., Hwang, D. F. and Tsai, Y. H. (2010) Histamine level and histamine-forming bacteria in dried fish products sold in Penghu Island of Taiwan. *Food Control*, 21 1234-1239.

Hungeford J.M. (2010) Scombroid poisoning: A review. *Toxicon* 56, 231-243

Kim S. H., Barros-Velazquez J., Ben-Gigrey B., Eun J. B., Jun S. H., Wie, C. I. (2003) Identification of the main bacteria contributing to histamine formation in seafood to ensure product safety. *Food Science and Biotechnology*, 12 : 451-460.

Lee Y. C., Huang T. C., Lin C. S., Lin C. M. and Tsai Y. H. (2012), Determination of Histamine and Histamine-forming Bacteria in Striped Marlin Fillets (*Tetrapturus audax*) Implicated in a Food-borne Poisoning. *Toxicon*, 60 :161-162.

Lehane, L. and Olley, J. (2000) Histamine fish poisoning revisited. *International Journal of Food Microbiology*, Vol. 58 No. (1-2), pp. 1-37.

Lin, C. S., Liu, F. L., Lee, Y. C., Hwang, C. C. and Tsai, Y. H. (2012) Histamine contents of salted seafood products in Taiwan and isolation of halotolerant histamine-forming bacteria. *Food Chemistry*, Vol. 131 No. (2), pp. 574-579.

Mavromatis P & Quantick P.C. (2002), Modification of Niven's Medium for the enumeration of histamine-forming bacteria and discussion of the parameters associated with its use. *Journal of Food Protection*, Vol. 65, No 3, pp. 546-551.

Niven C.F. Jr., Jeffrey M.B., Corlett D.A. Jr. (1981), Differential Plating medium for quantitative detection of histamine-producing bacteria. *Applied and Environmental Microbiology,* Vol. 41, No. 1, pp. 321-322.

Paleari, M. A., Soncini, G., Beretta, G., Grimaldi, P. and Tava, A. (1993), Microbiology and Chemical-Composition of a Typical Product from *Alosa Fallax Lacustris*. *Journal of Applied Ichthyology*- Vol. 9 No. (3-4), pp. 222-229.

Pirani, S., Bernardi, C. and Cantoni, C. (2010), "First signaling of high content of histamine in missoltini (Alosa agone)". *Industrie Alimentari*, Vol. XLIX No., pp. 9-12.

Sabroe R. A. and Black A. K. (1998) Scombrotoxic fish poisoning. *Clinical and Experimental Dermatology*, 23: 258-259.

Spetch, D. (1998) Scombroid fish poisoning. *Journal of emergency nursery* 24 : 118-119.

Taylor, S. L. (1986), Histamine Food Poisoning - Toxicology and Clinical Aspects. *Crc Critical Reviews in Toxicology*, 17: 91-128.

CHAPTER 5

Use of spirulina (*Arthrospira platensis*) as protein source for the nutrition of juvanile tench (*Tinca tinca*)

5.1 Introduction

The tench is a benthophagus omnivorous fish, belonging to the family of Cyprinidae, common in all Europe. This species is traditional farmed in the Central and Eastern Europe in extensive pond culture. Tench is a candidate to complement rainbow trout (*Oncorhynchus mykiss*) in commercial farms (Quiròs and Alvarino, 2000; Turchini and De Silva, 2008), as it has been introduced in intensive farm in Spain (Quiròs et al, 2003). Tench has shown high mortality during fry stage in ponds and it has a slow growth rate compared with other cyprinids (Von Lukowich et al., 1986) and so traditional ponds methods for tench production are unsatisfactory (Steffens, 1995; Wolnicki et al, 2003, Wolnicki et al, 2006). Several studies were carried to find effective techniques for rearing juvenile tench in intensive and controlled conditions, to ensure during all the year fish for stocking outdoor pond (González-Rodríguez et al, 2014). Currently there are not enough knowledge on the nutrition of tench, no commercial feeds are available for this species, so it is common to use feeds formulated for other species, which create poor growth, deformities and high mortality (Garcia et al, 2013).

The price of fish meal is under a constant trend of increase, due to the world demand of this ingredient and to the limited availability of fish stock. Several fish nutritionists are in a continuous research of new and more sustainable alternative ingredients to substitute fish meal in aquafeeds. Plant protein has become one of the more common fish meal replacement ingredients in recent years, mostly soybean meal and other terrestrial protein plants (Brown et al., 2008). Water plants, especially microalgae could be an good source of protein as a feed ingredient because of their high protein and lipids content (Hemaiswarya et al., 2011; Shields and Lupatsch, 2012). Algae could been easily cultivated and their production is increasing in all the world, due to their various potential use. Spirulina (*Arthrospira platensis*) is symbiotic, multicellular and filamentous blue-green microalgae with symbiotic bacteria that fix nitrogen from air. Spirulina contains usually high amount of protein and lipids, (2008) report an average content of 55-70 percent by dry weight, depending on source, and a fat content of 5-6 percent. Among fatty acids there is a good amount of polyunsaturated fatty acids (PUFA), with γ-linolenic acid (GLA), linoleic acid (LA, 36 percent of total). Stearidonic acid (18:3 n-4, SDA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (AA) are also present in minor amounts. Spirulina is also rich of antioxidants and photosynthetic pigments. Thanks to these characteristics spirulina has been tested as a protein source for the formulation of various fish feeds (Mustafa and Nakagawa, 1995; Nandeesha et al , 1998; Palmegiano et al 2005 and 2008; Proyma and Chitmanat, 2011).

5.2 Matherial and methods

5.2.1 Fish, facility and experimental procedure

Diet experiments were carried on juvenile tench, one summer old, provided by a local pond farmer. The fish were transported into the Aquaculture Facility located in Polo Veterinario di Lodi and kept in square tanks of 1 m^3 for a period of acclimation to the RAS conditions of two months. The fish were maintained in recirculation system at the temperature of 26 °C and fed twice a day with trout commercial feed at ratio of 2% body weight. At the beginning of experiments fish were transferred to 15 cylindrical tanks of 400 l, three tanks per diet, l00 individuals per tank, into a second RAS system, provided of mechanical and biological filtration. Water temperature was 26±1°C, controlled by a heater system. Dissolved oxygen saturation, measured in each tank with Hach HQ 30d Portable Meter, (Hach Lange, Dussendolf, Germany) was 80-85%. Total ammonia and nitrites, measured after biological filtration, were below 0.05 mg/l, and they were measured once a week by Hach 2800 Portable Spectophotometer (Hach Lange, Dussendolf, Germany). The photoperiod regime was 12L:12D with a very low level of light intensity.
At the beginning of experiment 600 fish were measured. Total lengths and body weights were respectively 5.78 ±0.62 cm and 3.06 ± 1.26 g. Experimental diets were hand administered three time a day at regular intervals during light hours, 7 days a week for 90 days. The ration level, 4% live body weight per day, was adjusted every 10 days weighting the entire biomass of each tank. All feed was eaten within 30 minutes.

5.2.2 Experimental diets

The proximate compositions of the main dietary ingredients were determined using standard methods as described below; the nitrogen-free extract value was calculated by subtracting the content of moisture, protein lipid and ash from total weight. Based on the results, five nearly isoproteic, isolipidic and isoenergetic experimental diets were formulated, following the nutrients requirement indication for the juvenile tench provided by several authors (De Petro et al, 2001; Turchini et al., 2007; Garcia et al, 2013). The five diets were formulated to test different replacement of fish meal protein by spirulina meal protein: diet A (control) has fish meal as a principal protein source, diet B has a 25% of replacement, diet C 50%, diet D 75 % and diet E has the total replacement of fish meal with spirulina meal. Different levels of soybean and wheat meal were used to balance the protein content.
Spirulina meal used in this trial had a lipid content of 10.5 g/100g of product. We analyzed the fatty acid composition of extracted lipids. Spirulina oil was

composed mainly of palmitic acid (42.7%), γ-linoleic acid (23.7%) and linoleic acid (20%).
The ingredients and proximate composition of the diets are given in Table 5.1. Fatty acid composition of the experimental diet are shown in Table 5.2.

Table 5.1 Diet formulation and proximate composition of the five experimental diets

	Replacement				
	0	0.25	0.5	0.75	1
Diet	A	B	C	D	E
Ingredients (g/kg)					
Fish meal (737 g CP/kg)[a]	500	375	250	125	0
Spirulina meal (632 g CP/kg)[b]	0	125	250	375	500
Soybean meal (432g CP/kg)[c]	100	135	170	206	240
Wheat meal (91 g CP/kg)[d]	250	215	180	144	110
Cod liver oil [e]	120	110	105	95	90
Carboxymethyl cellulose [f]	10	20	25	35	40
Vitamin premix [g]	20	20	20	20	20
Proxymate composition (g/100g)					
Moisture	1.6	1.5	1.4	1.5	1.75
Crude Protein	44.16	45.05	45.11	46.06	45.95
Crude lipid	13.02	11.22	12.87	12.57	11.11
Nitrogen-free extract	29.2	31.49	31.59	32.28	34.5
Ash	12.02	10.74	9.03	7.59	5.69
Gross energy KJ g^{-1} [h]	17.45	17.32	17.94	18.11	18.09

[a] ItalFeed, Italy ,73.7% protein, 4.1 % lipid
[b] Italfeed Italy, 63.25 protein, 10.5 % lipid
[c] Zenit Mangimi, Italy, 43.2 protein, 2% lipid
[d] Barilla, Italy, 9.1 %protein 1% lipid
[e] ItalFeed. The oil include 500000 UI/kg of vitamin A, 50000 UI/kg of vitamin D3 and 100 UI/kg vitamin E.
[f] Sodium Carboxymethyl cellulose Sigma Aldrich, Germany , used as filler and feed binder
[g] Vitamin kit, Sigma Aldrich, Germany. constituted by: p-Aminobenzoic Acid 5 g, d-Biotin 100 mg, Folic Acid 1g, Niacinamide 100 g, d-Pantothenic Acid, Calcium Salt 5 g, Pyridoxal Hydrochloride 500 mg, Pyridoxamine Dihydrochloride 250 mg, Pyridoxine Hydrochloride 5 g, Riboflavin 5 g, Thiamine Hydrochloride 5 g , DL-6,8-Thioctic Acid 500 mg. To this kit was added 50g of L-ascorbic acids and 100g of Choline chloride, Sigma Aldrich.
[h] Calculed on the basis of 19 kJg^{-1} of protein, 36 kJg^{-1} of lipids and 15 kJg^{-1} of carbohydrate

Ingredients were mixed using a blender adding an aliquot of line water to allow the blending, then pellets were formed using a pasta extruder (La Monferrina,

Imperia) with a pellet diameter of 1,8 mm. Diet were dried in ventilated oven at 40° C for two days. All the feed used in the trial was made in single processing.

Table 5.2 Fatty acid composition of the five experimental diets (g/100g fatty acids)

	A	B	C	D	E
14:0	4.05	3.73	3.04	2.89	3.03
16:0	13.14	14.06	13.72	15.27	17.32
16:1n-7	5.20	5.22	4.69	4.95	5.36
16:2n-4	0.44	0.44	0.39	0.41	0.43
16:3n-4	0.34	0.32	0.26	0.25	0.25
18:0	2.88	2.95	2.80	2.69	2.59
18:1n-9	24.95	23.87	21.92	21.56	20.70
18:1n-7	3.07	2.93	2.80	2.61	2.54
18:2n-6	9.75	10.62	11.12	12.41	13.34
18:2n-4	0.23	0.25	0.20	0.24	0.19
18:3n-6	0.13	1.29	2.36	3.70	5.22
18:3n-4	0.30	0.34	0.38	0.32	0.36
18:3n-3	2.96	2.95	2.86	2.83	2.71
18:4n-3	1.36	1.31	1.16	1.14	1.07
20:0	0.27	0.26	0.29	0.27	0.26
20:1n-9	5.05	4.86	5.04	4.71	4.24
20:1n-7	0.38	0.36	0.35	0.33	0.27
20:2n-6	0.67	0.69	0.76	0.81	0.74
20:3n-6	0.23	0.19	0.20	0.22	0.21
20:4n-6	0.68	0.64	0.60	0.52	0.43
20:3n-3	0.28	0.27	0.27	0.26	0.23
20:4n-3	0.92	0.88	0.90	0.85	0.76
20:5n-3	6.52	6.22	6.15	5.59	4.89
22:0	0.19	0.17	0.20	0.19	0.15
22:1n-11	4.72	4.50	5.35	4.75	4.19
22:1n-9	0.65	0.62	0.74	0.63	0.56
22:2n-6	0.09	0.07	0.09	0.08	0.07
22:4n-6	0.14	0.14	0.16	0.14	0.12
22:5n-6	0.05	0.03	0.18	0.16	0.14
22:5n-3	2.04	1.96	2.26	1.98	1.74
22:6n-3	8.29	7.84	8.75	7.23	5.88
SFA	20.53	21.18	20.04	21.31	23.35
MUFA	44.03	42.36	40.89	39.54	37.86
PUFA	35.44	36.46	39.07	39.15	38.79
n-6	11.74	13.68	15.48	18.05	20.28
n-3	22.38	21.43	22.35	19.88	17.28
n-6/n-3	0.52	0.64	0.69	0.91	1.17
n-3/n-6	1.91	1.57	1.44	1.10	0.85

5.2.3 Data collection and analysis

At the beginning and at the end of trial the individual weight and length of a sample of 20 fish per tank (60 fish per treatment) were determined. Fulton's coefficient, or condition factor, used to determinate the fish condition score, was expressed as $K=100\ W\ TL^{-3}$, where W and TL are the weight and total length of fish. Specific growth rate (SGR) was expressed as $SGR = 100(\ln W_f - \ln W_i) days^{-1}$ where Wf and Wi where final and initial weight. Feed conversion ratio (FCR) was expressed as $FCR= F(W_f-W_i)^{-1}$ where F is the amount of feed provided. Feed intake was calculated as FI= total feed fed/number of fish.

5.2.4 Proximate and fatty acid composition

Eighteen tench from the initial fish stock and 18 tench sampled from each tank at the end of the diet experiment were sacrificed by a bath of tricaine methanesulfonate (Finquel MS 222, Argent) in a lethal dosage. Samples used for the chemical analysis of whole fish were formed by a pool of three fish, three pool per tank. The same protocol was performed for carcass samples. Three pool per tank were formed pooling the body of three tench after the evisceration and decapitation of fish.
Proximate composition of whole fish and carcass was performed using standard methods (A.O.A.C. 1996). Moisture content was determined by drying samples in an oven at 60 °C to constant weight. Protein content was determined by Kjeldahl method, by which the concentration of nitrogen is measured. A conversion factor of 6.25 was used to convert total nitrogen to crude protein. For the analysis an automated distillation unit (Büchi 339, Switzerland) was used.
The total lipid analysis was performed by a modified method of Folch et al. (1957). About 1 g of muscle were suspended in 2x30 ml of chloroform:methanol (2:1) solution and homogenized. 30 ml of 0,44 KCl in Methanol water 3:1 was added, favouring a two-phase solution. The lower phase was collected after the two phases were well separated overnight. The solution collected was divided into two aliquots for quantification (60%) and analysis of fatty acid composition (40%). Ash was determined by incineration of sample in a muffle furnace at 600 °C for 18 h.
The preparation of fatty acid methyl esters was performed according to Cristie (1982). Briefly, the extracted lipid solution was concentrated to dryness under a stream of nitrogen and dissolved in acetyl chloride:methanol (1:9) solution in a sealed cap, and incubated at 50°C overnight. Tricosanoic acid (T6543-1G SIGMA) was dissolved in 1 ml of toluene and added as an internal standard. The acetyl chloride-methanol solution trans-esterifies the fatty acids and forms fatty acid methyl esters (FAME). Then 5 ml NaCl solution 5% was added to allow the

separation between the hydrophobic and the aqueous phases. 2 ml of 1M potassium carbonate solution was added to buffer the solutions. The FAME's were extracted in 2x2 ml of hexane, and the mixture was evaporated to dryness under a stream of nitrogen. Fatty acid methyl esters were recovered with hexane and analyzed in an Agilent gas-chromatograph (model 6890), equipped with an automatic sampler (model 7683) and a flame ionization detector (FID). The carrier gas was helium with a flow at 1.0 ml/min and an inlet pressure of 16.9 psi. The injection volume was 1 μl, and a split ratio of 1:80 was used. A HP-Innowax fused silica capillary column (30 m × 0.25 mm i.d.) was used to separate fatty acid methyl esters (Agilent Technologies). The oven temperature program for separation was from 100 to 180 °C at 3 °C/min, then from 180 to 250 °C at 2.5 °C/min and held for 10 min.
All analyses were done in duplicate.

5.2.5 Statistical analysis

All data are reported as mean values ± standard deviation (SD) or the standard error of the mean (SEM). Homogeneity of variance was confirmed and comparison between means was completed by one-way ANOVA (analysis of variance). The significance was accepted at probabilities of 0.05. These statistical analyses were performed by SPSS 20.0 (SPSS Inc., Chicago, IL, U.S.A.).

5.3 Results

Experimental fish readily accepted all diets, most of feed was consumed in few minutes from the feeding, all the feed was eaten within 30 minutes. Data on survival rate and growth are presented in Table 5.4. Survival rate ranged from 98 % to 99.6 and was not affected by treatment. Some fish died the day after the biomass measurement, probably due to the stress caused by manipulation. The highest growth (6.51 g of final weight and 7.11 cm of final length) was observed in fish fed with diet A, which was the one based on the only use of fish meal. Lower growth (4.93 g of final weight and 6.57 cm of final length) was observed in fish that received feed based on spirulina meal. Growth did not appear to have been affected by dietary treatment, as all the differences were not statistically significant. This aspects is probably due to the increase of the variability of fish, that can be noted examining the standard deviation of total weight and total length. At the end of experiment fish showed a very high size difference even within the same diet group. The condition factor ranged from 1.69 to 1.61, where those fed with diet C had the highest condition factor. FCR for the experimental diets ranged from 3.06 to 5.14, with best performance in diet B and also this parameter was not affected by dietary treatment. Also the

SGR has his highest value in diet B, with 0.96, even if also this parameter did nor show a significant difference. Feed intake was highest in diet A, with 12.11 g of feed eaten by each fish, and minimum in diet D, with 10.29 g of feed eaten by each fish.

Table 5.4 Total length, body weight, survival, condition factor, Feed conversion rate and specific growth rate of the five experimental diets. (n= 60, 20 fish for 3 tanks).

	Diet									
	A		**B**		**C**		**D**		**E**	
Overall survival	98.0		99.6		99.6		99.3		99.6	
Final weight (g)	6.51	±4.62	6.17	±5.93	5.57	±4.76	5.24	±4.49	4.93	±2.72
Final length (cm)	7.11	±1.18	6.69	±1.34	6.60	±1.22	6.45	±1.16	6.57	±0.89
Condition factor	1.56	±0.30	1.69	±0.37	1.63	±0.37	1.68	±0.32	1.61	±0.24
FI	12.11	±0.46^{b}	11.10	±0.14^{a}	10.66	±0.75^{a}	10.49	±0.08^{a}	10.54	±0.49^{a}
FCR	3.96	±0.79	3.06	±0.60	3.87	±1.09	4.57	±1.08	5.14	±1.18
SGR	0.68	±0.17	0.96	±0.21	0.78	±0.26	0.62	±0.10	0.55	±0.15

Values are means ± SD; value in the same row having different superscripts are significantly different (P>0.05)

Data of proximate composition of carcass and whole fish at the beginning and at the end of the experiment are given in table 5.5. Fish fed with diet A had the higher lipid content, both for the carcass composition, both for the whole fish. This diets had affected the fat content of fish, which passed from the initial value of starting fish of 12.7 to 15.5 g/100 g, when compared to the other experiment diets. Moisture content follows the trend of lipid content, since this two parameter are linked by a reverse proportion. Protein content was not influenced by dietary treatment. Its value ranged from the 17 to the 15.9 g/100g in the carcass, while in whole fish ranged from 15 to 13.9 g/100g. Ash content was higher in fish fed with diet E, lower in fish fed with diet A. This variation could be linked to the abundance of fat tissue compared to skeletal part that was found in fish.

Tables 5.6 shows the fatty acid composition of fish at the beginning and at the end of experiment. Since there were no significant difference between the fatty acid composition of the carcass and whole fish the data presented are the average of the two values. The major fatty acids category of fish body of all treatment was the mono unsaturated fatty acids (MUFA), followed by polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA). In order of amount, oleic acid (18:1*n*-9; OA) was the more abundant. Fish fed with diet A showed the highest amount of this fatty acids, while the lowest was found in fish fed with diet D. The second fatty acids in order of amount was palmic acid

(16:0), then linoleic acid (18:2*n*-6; LA) or docosahexaenoic acid (22:6*n*-3; DHA) depending on dietary treatment.

Table 5.5 Carcass and whole fish proximate composition (g/100g) of tench at the beginning of trial and after 90 days of dietary treatment. Data are expressed as mean ± SEM. (n= 3 pool of 3 fish, 3 tanks/treatment).

			Diet									
	Starting fish		A		B		C		D		E	
Carcass												
Moisture	69.4	±1.51	66.9	±0.57	68.6	±0.76	69.8	±0.60	69.7	±0.58	69.7	±0.53
Protein	15.9	±0.87	15.9	±0.46	16.7	±0.35	16.3	±0.27	17.0	±0.26	16.8	±0.20
Lipid	12.7	±1.16^{a}	15.5	±0.73^{b}	12.9	±0.85^{a}	11.7	±0.60^{a}	11.2	±0.66^{a}	11.2	±0.61^{a}
Ash	1.9	±0.08^{ab}	1.7	±0.08^{a}	1.8	±0.09^{ab}	2.1	±0.09^{abc}	2.1	±0.10^{bc}	2.2	±0.09^{c}
Whole fish												
Moisture	70.6	±0.90^{c}	63.1	±1.21^{a}	66.3	±1.14^{ab}	67.4	±0.64^{bc}	66.2	±0.73^{ab}	68.8	±0.65^{bc}
Protein	13.4	±0.24	13.5	±0.42	13.9	±0.48	15.0	±0.34	14.3	±0.39	15.0	±0.25
Lipid	13.3	±0.73^{a}	21.7	±1.47^{b}	17.6	±1.19^{a}	15.1	±0.71^{a}	16.8	±0.86^{a}	13.3	±0.63^{a}
Ash	2.6	±0.04^{bc}	1.8	±0.09^{a}	2.2	±0.12^{b}	2.5	±0.14^{bc}	2.7	±0.13^{c}	2.9	±0.13^{c}

Values are means ± SEM; value in the same row having different superscripts are significantly different (P>0.05)

The amount of LA and GLA were strongly influenced by treatment. The higher value of LA was found in fish fed with diet E, 14.47 g/100g of total lipids, and this value decreased, following the presence of spirulina meal in feed, till the lowest value, 9.87 g/100g of total lipids, found in diet A, where no spirulina meal was used. The same trend was found following the presence of GLA, which amount in groups was directly linked to the presence of spirulina meal in diets. The variation in presence of these fatty acids influenced the sum of *n*-6 fatty acids which followed the same trend of LA and GLA.

n-3 fatty acids were almost constant in all fish, and they were not many influenced by diet. DHA was the larger of this category, followed by eicosapentaenoic acid (20:5*n*-3; EPA). The *n*-3/*n*-6 ratio in fish follow the trend of LA, but it was always greater than 1, even in fish fed with diet E where this value was 0.85.

Fatty acids found in fish partially respect the fatty acid composition of diets. OA increase its value in all the experiment fish. At the beginning of the trial the value of OA was 25.28 g/100g of total fatty acids, while at the end its amount increased in all groups, even if the amount of this fatty acid was lower than 25% in all diets. The increase followed the progressive trend of the presence of OA in diets, diet A had the highest amount of OA and fish fed with this diet had the higher content of OA. LA had it highest value at the beginning of the trial, due

to the diet provided by the farmer and the adaptation diet administered before the beginning of trial. At the end of the experiment its amount was related to the presence of LA in diets. GLA content followed the trend of diets, even if its amount in all groups was lower than the amount of this fatty acid in diets. For instance the higher amount was found in group E, with 1.06 %, which was fed with a diet where GLA was 5.22 %. α-linolenic acid (18:3*n*-3, ALA) had the same value in all diets, higher when compared to its content in starting fish. Its value in fish during the feeding trial remains constant, and was not affected by dietary treatment.

Table 5.7 Fatty acids composition (g/100g of fatty acids) of tench after 90 days of dietary treatment. Data are expressed as mean ± SEM. (n= 6 pool of 3 fish, 3 tanks/treatment).

		Diet				
	Starting fish	**A**	**B**	**C**	**D**	**E**
14:0	2.96 ±0.069	2.65 ±0.028^{c}	2.77 ±0.045^{c}	2.50 ±0.059^{b}	2.43 ±0.050^{b}	2.30 ±0.038^{a}
16:0	16.25 ±0.206	15.22 ±0.165	14.80 ±0.191	14.21 ±0.151	14.20 ±0.478	14.09 ±0.348
16:1*n*-7	9.37 ±0.125	8.44 ±0.135	8.02 ±0.148	8.04 ±0.132	8.19 ±0.085	8.10 ±0.158
16:2*n*-4	0.30 ±0.011	0.29 ±0.004^{a}	0.33 ±0.008^{b}	0.32 ±0.011^{b}	0.32 ±0.007^{b}	0.27 ±0.007^{a}
16:4*n*-1	0.25 ±0.010	0.26 ±0.007^{ab}	0.31 ±0.011^{c}	0.29 ±0.009^{bc}	0.26 ±0.009^{ab}	0.24 ±0.012^{a}
18:0	2.12 ±0.129	2.14 ±0.039^{ab}	2.17 ±0.043^{b}	2.07 ±0.032^{ab}	2.13 ±0.028^{ab}	2.01 ±0.031^{a}
18:1*n*-9	25.28 ±0.444	30.05 ±0.215^{b}	27.08 ±0.763^{a}	26.78 ±0.377^{a}	25.25 ±1.320^{a}	26.53 ±0.217^{a}
18:1*n*-7	3.23 ±0.123	3.21 ±0.020	3.19 ±0.043	3.04 ±0.036	4.58 ±1.331	3.39 ±0.050
18:2*n*-6	13.38 ±0.325	9.87 ±0.141^{a}	11.32 ±0.190^{b}	12.37 ±0.175^{c}	13.10 ±0.257^{d}	14.17 ±0.197^{e}
18:3*n*-6	0.25 ±0.012	0.19 ±0.003^{a}	0.55 ±0.013^{b}	0.70 ±0.019^{c}	0.88 ±0.030^{d}	1.06 ±0.069^{e}
18:3*n*-4	0.36 ±0.014	0.28 ±0.008^{d}	0.24 ±0.006^{c}	0.20 ±0.006^{b}	0.18 ±0.008^{b}	0.16 ±0.010^{a}
18:3*n*-3	1.80 ±0.052	1.82 ±0.023^{a}	2.00 ±0.031^{b}	1.95 ±0.026^{b}	1.97 ±0.037^{b}	1.97 ±0.039^{b}
18:4*n*-3	0.30 ±0.010	0.64 ±0.017^{abc}	0.71 ±0.026^{c}	0.67 ±0.022^{bc}	0.60 ±0.018^{ab}	0.58 ±0.015^{a}
20:1 (*n*9+*n*11)	1.75 ±0.022	3.59 ±0.055^{c}	3.50 ±0.089^{c}	2.78 ±0.046^{b}	2.47 ±0.045^{a}	2.29 ±0.081^{a}
20:2*n*-6	0.59 ±0.018	0.69 ±0.015^{a}	0.74 ±0.029^{ab}	0.78 ±0.019^{bc}	0.86 ±0.021^{d}	0.84 ±0.020^{cd}
20:3*n*-6	0.66 ±0.029	0.52 ±0.012^{a}	0.75 ±0.013^{b}	0.83 ±0.018^{c}	0.98 ±0.022^{d}	1.01 ±0.022^{d}
20:4*n*-6	0.77 ±0.031	0.68 ±0.016^{a}	0.78 ±0.023^{b}	0.76 ±0.026^{b}	0.77 ±0.021^{b}	0.72 ±0.024^{b}
20:3*n*-3	0.16 ±0.006	0.24 ±0.004^{a}	0.29 ±0.008^{b}	0.27 ±0.014^{b}	0.27 ±0.007^{b}	0.26 ±0.007^{ab}
20:4*n*-3	0.88 ±0.034	0.86 ±0.014	0.89 ±0.019	0.88 ±0.014	0.88 ±0.014	0.87 ±0.015
20:5*n*-3	5.63 ±0.254	4.38 ±0.066^{ab}	4.65 ±0.130^{b}	4.59 ±0.105^{b}	4.33 ±0.083^{ab}	4.17 ±0.065^{a}
22:1 (*n*11+*n*9)	0.70 ±0.019	1.87 ±0.142^{a}	2.08 ±0.211^{a}	3.26 ±0.381^{b}	2.67 ±0.372^{ab}	2.32 ±0.261^{ab}
22:5*n*-3	2.14 ±0.096	2.25 ±0.041	2.29 ±0.056	2.23 ±0.041	2.08 ±0.083	2.14 ±0.046
22:6*n*-3	10.84 ±0.516	9.86 ±0.216	10.55 ±0.249	10.50 ±0.252	10.59 ±0.254	10.50 ±0.193
SFA	21.34 ±0.239	20.00 ±0.180^{c}	19.73 ±0.228^{bc}	18.78 ±0.193^{ab}	18.77 ±0.469^{ab}	18.40 ±0.361^{a}
MUFA	40.34 ±0.494	47.15 ±0.243^{b}	43.89 ±0.631^{a}	43.89 ±0.443^{a}	43.16 ±0.322^{a}	42.63 ±0.367^{a}
PUFA	38.32 ±0.658	32.84 ±0.395^{a}	36.38 ±0.607^{b}	37.32 ±0.407^{bc}	38.07 ±0.549^{cd}	38.96 ±0.414^{d}
***n*6**	15.65 ±0.340	11.95 ±0.154^{a}	14.14 ±0.211^{b}	15.43 ±0.179^{c}	16.60 ±0.251^{d}	17.81 ±0.246^{e}
***n*3**	21.75 ±0.860	20.06 ±0.297	21.37 ±0.445	21.08 ±0.360	20.72 ±0.395	20.49 ±0.283
***n*6/*n*3**	0.72 ±0.041	0.60 ±0.009^{a}	0.66 ±0.012^{b}	0.74 ±0.015^{c}	0.80 ±0.016^{d}	0.87 ±0.015^{e}
***n*3/*n*6**	1.39 ±0.080	1.68 ±0.024^{e}	1.51 ±0.027^{d}	1.37 ±0.029^{c}	1.25 ±0.024^{b}	1.15 ±0.021^{a}

Values are means ± SD; value in the same row having different superscripts are significantly different (P>0.05)

Stearidonic acid (18:4*n*-3 SDA) increased in all groups compare to the fish at the beginning of trial although its value never reached the amount of this fatty acids in diets. EPA was found higher in diets respect to fish. Its value decrease also if it is compared to the value found in fish at beginning of trial. DHA value remains stable in fish, without following the presence of this fatty acid in the diets. DHA progressively decrease from diet A to diet E, and all values are lower in diets respects of fish.

5.4 Discussion

The growth rates of juvenile tench of this study resulted poor in all the diets treatment. The SGR ranges from 0.55 to 0.96, value lower than those found by other authors in juvenile tench. Mareš et al (2007) reported a SGR ranging from 1.49 to 0.8 in fish of two size classes during a 63 days trial. Gonzalez Rodriguez et al (2014) had a SGR of 1.74 on 5 month-old juvenile tench fed with artificial diet for 120 days. Our SGRs were similar with those found by Quiros et al (2003) who has a SGR of 0.65 when fish were fed with eel designed feed and 0.74 when they used sea bass feed. They found a negative SGR with the use of trout starter feed.
The same consideration on the growing performance could be made analyzing the FCR of the five groups. Best results (3.06) found in diet B with the 25% of replacement of spirulina meal, were low compared to the SGR of species traditionally cultured in intensive conditions, where the value usually range from 1.5 to 0.8 (Hardy and Barrows, 2002). Rennert et al (2003) found that the FCR ranged from 1.74 to 3.56, depending of genetic strains, since all fish were in the same farming condition and fed with commercial trout feed for a period of 450 days. Mareš (2007) tested three commercial diets with fish of 0.8 and 1.2 g and FCR ranged from 1.84 to 3.53 for bigger fish and from 2.05 to 4.15 for smaller fish. Best results were obtained by Garcia et al (2013) and Gonzalez-Rodriguez (2014), where the FCR ranged from 1.18 to 1.58 and 1.36 to 1.48 respectively. Our acceptable but not satisfying results could have been influenced by the high variability of growth that we observe in all fish groups. This phenomenon was also observed by Wolnicki et al (2003) who reported an asymmetry in body weight distribution in his tench groups, where very few large tench were present in all groups composed by small fish at the end of a feeding trial of 120 days. One possible explanation of this growing asynchrony could be found in the origin of fish used in our study. Tench spawn in ponds during a quite long period, which lasts about a month during summer. The collecting and sorting of fry at the beginning of autumn may lead to the formation of group with initially homogeneous size, but made by small individuals with different age and consequently a different potential capacity of growth. This genetic background

may influence the following growing performances. High size heterogeneity has a depressive effect on the growing of tench in intensive condition (Backiel, 1986).
Tench used in our trial came from a pond farmer which produce fish principally for restocking in open water. This farmer did not select a genetic strain that expresses a markable growing performance as habitually done by farmers who are interested in a production of marketable size fish as soon as possible. Wedekind et al (2003) reported an high variability of production in three different strains of tench tested under standard conditions. Thus our growing performances, if compared to those reach by other research group, could be linked also to the genetic strain of fish which was used, not accustomed to intensive farming conditions.
Regarding the possible substitution of fish meal with spirulina meal our results suggested that spirulina could substitute partly fish meal in tench diet. Even though the effects were not significant, probably due to the high variability in fish size at the end of experiment, best growing performance (considering SGR and FCR) were reached in diet with the 25% and 50% of fish meal substitution. Furthermore, considering the fish body composition, tench fed with diet A, where no spirulina meal was used, showed a lipid content that was significantly higher in relation to other diet, both in carcass than in whole fish. Spirulina meal has been used by several authors in fish diet formulation. Nandeesha et al (1998) find a positive effect using spirulina in carp (*Cyprinus carpio*) diet, with better results when spirulina was the sole source of protein since the algae improved the protein digestibility of the diets. In the same species Nasreen and Hawkar (2013) had the best result with the highest spirulina inclusion in their experimental diets. Olvera-Novoe et al (1998) use spirulina in the diet of tilapia (*Oreochromis mossambicus*) with no differences with control standard diet when the inclusion of spirulina was the 20 and 40%, while an higher inclusion resulted in a decrease of growing performances. Palmegiano et al (2005) and (2008) used spirulina in the diets for growing Siberian sturgeon (*Acipenser baeri*) and White sturgeon (*Acipenser transmontanus*). In both studies they found that it is possible to partially replace fish meal with Spirulina meal without any adverse effect on fish growth of sturgeons. Dernekbasi et al. (2010) used guppy (*Poecilia reticulata*) as experimental fish to study the effect of dietary supplementation of different rates of spirulina on growth and feed conversion. In their study they did not find differences between the growing performances of guppy fed with four diet with increasing quantity of spirulina, from 10 to 40 % and control diet. The FCR increased with the increase of dietary spirulina meal level. Promya and Chitmanat (2011) used of spirulina and cladophora on African sharptooth catfish (*Clarias gariepinus*). They found a positive level on growth and immunity capacity of fish fed with an inclusion of 5% of algae in the dry feed. Hernádez et al (2012)

reported that a combination of spirulina meal and soybean meal could replace totally fish meal in diet for young rainbow trout (*Oncorhynchus mykiss*). They had the best performances with a 75% of spirulina and 25% of soybean meal. The presence of spirulina in diets also improved the lysozyme activity of rainbow trout, increasing their immunity activity and reduced the phosphorus excretion of fish.

Since the spirulina meal used on this trial contained a high amount of lipids, the 11% of dry matter, the five diets have a different fatty acid composition and this diversity partially influenced the fatty acids composition of tench. Data on the fatty acid composition of wild tench are controversial. Jankowska et al (2006) and Ozgul et al (2007) found that PUFAs were the dominants fatty acids, whit 47% and 48% respectively. Cirkovic et al (2012) found that tench reared in ponds with natural feed had an amount of SFA and MUFA quite similar, 36 and 34 %. Gasco et al (2010), analyzing the PDO Italian product "Tinca gobba Dorata del Pianalto di Poirino" found that tench have MUFA ad dominant fatty acids. Results presented in chapter 3 showed that in 23 tench fished from Northern Italian lakes PUFA are the more representatives with 40 %, followed by MUFA with 32 % and SFA with 28%. Tench used in our feeding trial had MUFA already high at the beginning of experiment, and this value increased in all groups at the end of feeding period. The metabolism of some fatty acids in tench is only partially affected by dietary treatment. Fish showed the inclination of maintaining a stable level of some fatty acids. In particular DHA in tench is unaffected by the presence of this fatty acid in diets, its level at the end of the trial was similar to the beginning. To synthesize this fatty acid probably tench use shorter fatty acids, as ALA and EPA, which were found in a lower level in fish when compared with diets. Freshwater species, which evolved in an environment in which long chain *n*-3 fatty acids are not abundant as in marine environment, have the pathways of biosynthesis of *n*-3 C 20 and C 22 HUFA from C 18 *n*-3 precursor (Tocher, 2003). LA and GLA, which amount were different in all diets and in all fish group were used by tench and apparently not accumulated in body lipids. LA content of fish at the beginning of the trial was higher compared to its presence in diets but tench at the end showed a LA content similar to diet. It would seem that fish consumed the amount of LA that they had in their body to progressively reach the equilibrium with LA diet content during the 90 days of trial. GLA amount at the beginning of trial was very low (0.25%) and its presence increased following the trend of GLA content of diets, without reaching the same level of this fatty acid in diets. Tench seemed to use this fatty acid and to partially accumulate in body lipids.

The *n*-3/*n*-6 ratio was influenced by the presence of different levels of LA. *n*-3 fatty acids tend to stay in a constant level, as reported by Turchini et al (2007) who gave diet with different lipid source to tench for 84 days. At the end of their

trial they found that the *n*-3/*n*-6 ratio did not exceed a minimum level, whatever was the *n*-3/*n*-6 ratio of diet.

5.5 Conclusions

The results of the present trial shows that it is possible to partially replace fish meal with spirulina meal in the diet of juvenile tench, although the fatty acids profile of fish was affected by the presence of LA. Spirulina could be a valuable and sustainable source of protein for aqua feeding taking to account the cost of this ingredients, which could decrease in near future.

References

A.O.A.C. (1996), *Official methods of analysis of the Association of Official Analytical Chemists*, Association of Official Analytical Chemists, Arlington, U.S.A.

Backiel T. (1986) Masking effect of variability of growth on its estimation in juvenile tench Tinca tinca reared at different temperatures. *Polish Archives of Hydrobiology* 33, 69-95

Brown P.B., Kaushik S.J. and Peres H. (2008). Protein feedstuff originating from soybeans. In: *Alternative Protein Source in Aquaculture Diets* (ed. by C. Lim, C.D. Webster, & C. Lee), pp 205-223. The Haworth press, New York, USA

Ćirković M., Ljubojević D., Đorđević V., Novakov N. and Petronijević R. (2012). Chemical composition of boby including fatty acids of four cyprinid fish species cultured at the same conditions**.** *Archivia Zootechnica* 15 2 37-50

Cristie W. (1982), *Lipid analysis. Isolation, separation, identification and structural analysis of lipids*, Pergamon Press, Oxford

De Petro N., Guijarro A.I., Delgado M.J., López-Patiño M.A., Pinillos, M.L. and Alonso-Bedate M. (2001). Influence of dietary composition on growth and energy reserves in tench (*Tinca tinca*). *Journal of Applied Ichthyology* 17, 25-29

Dernekbasi S., Unal H., Karayucel I and Aral O. (2010) Effect of dietary supplementation of different rates of spirulina (*Spirulina platensis*) on growth and

feed conversion in guppy (*Poecilia rericulata* Peters, 1860). *Journal of Animal and Veterinary Advances*. 9 1395-1399

FAO Fisheries and Aquaculture Circular No. 1034 (2008). A review on culture, production and use of spirulina as food for humans and feeds for domestic animals and fish.

Folch J., Lees M. and Sloane-Stanley G. H. (1957), "A simple method for the Isolation and purification of total lipides from animal tissues". *Journal of biological chemistry*. 226, 497–509

García V., Celada J.D., Gonzalez R., Carral J.M., Sáez-Royuela M. and González Á. (2013). Response of juvenile tench (*Tinca tinca* L.) fed practical diets with different protein contents and substitution levels of fish meal by soybean meal. *Aquaculture research* 1-11

Gasco L., Gai F., Lussiana C., Lo Presti R., Malfatto V., DFaprà F and Zoccarato I (2010)**.** Morphometry, slaughtering performances, chimica and fatty acid composition of the protected designation of origin " Golden hump tench of Poirino highland" product. *Review in Fish Biology and Fisheries* 20: 357-365.

González-Rodríguez Á., Celada J.D., Carral J.M., Sáez-Royuela M. and Fuertes J.B. (2014) Evaluation of a practical diet for juvenile tench (Tinca tinca) and substitution possibilities of fish meal by feather meal. *Animal Feed Science and Technology*. 187, 61-67

Hardy R.W. and Barrows F.T. Fish Nutrition. Academic press, San Diego, Ca USA

Hemaiswarya S., Raja R., Ravi Kumar R., Ganesan V. and Anbazhagan C. (2011). Microalgae: a sustainable feed source foraquaculture. *World Journal of Microbiology Biotechnology* 27, 1737–1746

Jankowska B., Zakęś Z., Zmijewski T., Szczepkowski M. and Wunderlich K. (2006)**.** The impact of diet on the sklaughter yield, proximate composition, and fatty acids profile of fillets of tench (*Tinca tinca* L.). *Archives of Polish Fisheries*, 14: 195-211

Mareš J., Jirásek j., Baránek V., Fiala J and Kopp R. (2007) Production effect of various feeds on two size classes of juvenile tench (*Tinca tinca*) under the

conditions of intensive rearing. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* LV 111-116

Mustafa M.G. and Nakagawa H. (1995) A review: dietary benefits of algae as an additive in fish feed. *Israel Journal of Aquaculture* 47, 155-162

Nandeesha M., Gangadhar B., Varghese T.J. and Keshavanath P. (1998) Effect of feeding *Spirulina platensis* on the growt, proximate composition and organoleptic quality of common carp, *Cyprinus carpio* L. *Aquaculture research* 29 305-312

Olvera-Novoa M.A., Domínguez-Cen L.J., Olivera-Castillo L.and Martínez-Palacios C.A. (1998). Effect of the use of the microalga Spirulina maxima as fish meal replacement in diets for tilapia, Oreochromis mossambicus (Peters), fry. *Aquaculture research* 29 709-715

Ozogul Y., Ozogul F. and Alagoz S. (2007). Fatty acid profiles and fat contents of commercially importantseawater and freshwater fish species of Turkey: A comparative study. *Food chemistry* 103: 217-223

Palmegiano G.P., Agradi E., Forneris G., Gai F., Gasco L., Rigamonti E., Sicuro B and Zoccarato I. (2005) Spirulina as a noutrient source in diets for growing sturgeon (*Acipenser baeri*). *Aquaculture research* 36, 188-195

Palmegiano G.P., Gai F., Daprà F., Gasco L., Pazzaglia M. and Peiretti PG. (2008) Effects of Spirulina and plant oil on the growth and lipid traits of white sturgeon (*Acipenser transmontanus*) fingerlings. *Aquaculture research* 39 587-595

Promya J and Chitmanat C. (2011) The effects of *Spirulina platensis* and *Cladophora* algae on the growth performance, meat quality and immunity stimulating capacity of the African sharptooth catfish (*Clarias gariepinus*) *International Journal of Agriculture and Biology* 13, 77-82

Quirós M. and Alvaiño R.M. (2000). Growth and survival of tench larvae fed under different feeding strategies. *Journal of Applied Ichthyology*. 16, 32-35

Quirós M., Nicodemus N., Alonso M., Bartolomé M., Ècija J.L. and Alvaiño R.M. (2003). Survival and changes in growth of juvenile tench (*Tinca tinca*) fed defined diets commonly used to culture non-cyprinid species. *Journal of Applied Ichthyology*. 19, 149-151

Rennert B., Kolmann K. and Hack H (2003) A performance test with five different strains of tench (*Tinca tinca*) under controlled warm water condition. *Journal of Applied Ichthyology.* 19, 161- 164

Shields R.J. and Lupatsch I. (2012) Algae for Aquaculture and Animal Feeds. *Technikfolenbschätzung – Theorie und Praxis* 1, 23-37

Steffens W. (1995). The tench, a neglected pond fish species. *Polskie Archiwum Hydrobiologii* 42: 161–180.

Tocher D.R., (2003) Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in fisheries science* 11 (2) 107-184

Turchini G.M., Moretti V.M., Mentasti T., Orban E., Valfrè F. (2007) Effects of dietary lipid source on fillet chemical composition, flavor volatile compounds and sensory characteristics in the freshwater fish tench (*Tinca tinca* L.). *Food Chemistry* 102, 1144-1155

Turchini G.M., De Silva S.S., (2008). Bio-economical and ethical impacts of alien finfish culture in European inland waters. *Aquaculture International* 16:243–272

Von Lukowicz M., Tamas G. and Horvath L. (1986). Aquaculture of tench. In Aquaculture of Cyprinids. R. Billard and J. Marcel (Eds). INRA, Paris, 357-367.

Wedekin H., Rennert B. and Kohalmann K. (2003). Product quality in different strains of tench (*Tinca tinca*) tested under controlled environmental conditions. *Journal of Applied Ichthyology* 19, 174-176

Wolnicki J., Myskowski L. and Kamiński R. (2003). Effect of supplementation of dry feed with natural food on growth, condition and size distribution of juvenile tench *Tinca tinca* (L.). *Journal of Applied Ichthyology* 19, 157-160

Wolnicki J., Myskowski L., Korwin-Kossakowsky M., Kamiński R. and Stanny L.A. (2006). Effects of different diets on juvenile tench *Tinca tinca* (L.) reared under controlled conditions. *Aquaculture international* 14, 89-98.

CHAPTER 6

General Discussion

6. General discussion

Analyzing the nutritional propriety of fifteen freshwater fish species coming from lakes of Northern Italy it appears that all the species could be considered a valuable food from a nutritional perspective. Freshwater fish analyzed have a protein content which ranges from the 18.1% of burbot to the 21.37 % of whitefish. Fish are good sources of high quality proteins, due to their composition, especially rich on methionine and lysine. Fish protein provides a good combination of amino acids better than the one provided by meat, milk and eggs, therefore fish protein is highly suited to man's nutritional requirements.

Two species from the fifteen analyzed could be regarded as fatty fish. In fact landlocked shad and whitefish have an amount of lipid higher than 5%, while all others species have a lipid content that is near the 1%. Regard to lipids composition in all the species, except the Italian bleak, polyunsaturated fatty acids are dominant. Analyzing this category of fatty acids the n-3 series are the most represented, making fish one important source of this category of fatty acids that is essentials for human health.

Some of the species analyzed with the highest content of n-3 PUFA are roach and rudd, fish that found large difficulties on being appreciated by consumers. Roach is a new species for the Italian market, as this fish has been introduced in Italian water only in recent years. Rudd is an autochthonous species, but ignored by people who habitually eat fish. The muscle of these species are quite rich of bones and their preparation for consumption could be too laborious. It is necessary to promote the consumption of these species, which are very abundant in Italian waters, thus representing a potential source of income for fishermen. One possibility to valorize these species is the creation of a cooperative laboratory, where fishermen could confer their caught and where there will be the processing of fish, proposing to the market fish product such fish sauce or fish meatball, more accepted by consumers.

Similar consideration could be made considering crucian carp and wels catfish. These two species are appreciated and farmed in Eastern Europe, where people know their nutritional proprieties. The flesh of these fish could be considered an excellent food. In recent years, expecially close to the large cities, carp, crucian carp and wels catfish are sold an illegal market, where no hygienic conditions are assured, made by Eastern Europe proving that there are consumers who look

for these species. One possibility to make a profit from the fishing of these species is the creation of a processing industry, or the supply of the whole fresh fish to immigrants, according to their eating habits and culture.

Processing is a way to increase the value of fish, and to offer during all the year some products that are seasonal. Around lakes of Northern Italy there is the tradition to process fish with salt and dry it. Most famous fish product is the Missoltino. The processing technique used to obtain this fish product could be used also to process other species, since the technique provides a stable and safe product when it is correctly used. Shad, the fish used for the production of missoltino, has a risk source than other fish have not, the high amount of free histidine in its flesh. The presence of large amount of this amino acid implies the risk of histamine formation, when there are the conditions which allow bacterial growth. In our study we investigated the factors affecting histamine formation, with the cooperation of the producers. It was not possible for us to determinate which bacteria was responsible of histamine production, but we found that the drying step was the critical point during processing. It is important that the level of water activity in missoltino reaches as soon as possible a value < 0.8, that inhibits the growth of the most of microbial flora.

Northern Italy freshwater fish production consist also of farmed fish. Trout, sturgeon and eels are the species with high productions. Except sturgeon, which have a niche market with a caviar oriented production, others farmed fish production are stationary or declining, due to the competition made by sea products and fish importations from foreign country. Fish farmer are seeking new species, which could have an higher market value. Tench is one species already present on Italian market, coming from fisheries and partially from farming. Traditional farm of tench is made in extensive pond systems, with a poor yield. Intensive tench farming could be a possibility of diversifying freshwater Italian fish production.

In our study we tested the response of tench in intensive aquaculture system. Results were acceptable but not satisfying if compared with ones obtained by other research groups, due to the slow growth and to the inefficient use of the feed provided. Probably the wild tench strain used in our test was unsuitable to intensive farming conditions. More domesticated fish are necessary to improve the tench productivity in intensive farming. Domestication is a process that require several generations of fish, selecting as broodstock those fish with the best performances.

Spirulina is a high protein source that have a high potential in formulation of fish feeding, as tested by several authors. Spirulina, in contrast to other protein terrestrial plant source, such as soybean meal, does not contain antinutritional or toxic factors. Its composition include several nutrients, like antioxidants, vitamins and essential fatty acids. Many efforts have been devoted to the develops of spirulina strains that synthesize n-3 PUFA instead of the n-6 PUFA, of which is naturally rich. In our trial a partial substitution of fish meal with spirulina meal in diet of juvenile tench showed encouraging results, even if the variable growth of fish used in the experiment did not provide significant evidence.

CHAPTER 7

Summary

7. Summary

Northern Italy freshwater fish production derive from inland fisheries and mainly from aquaculture. Both of them registered some problems. The number of freshwater fishermen is in a steady reduction, caused by the modification of lakes population, that is switching from the presence of high valued fish to others cheaper species which have a limited market. Freshwater farmers suffer the competition of marine and imported products. It is necessary to find a way to valorize Northern Italian freshwater fish production. In this thesis we analyzed the chemical composition and fatty acid profile of 15 species caught in North Italy lakes, sampling species with a high value and species which are not appreciated by Italian consumers. All of them showed an high nutritional value. Species like rudd, roach or crucian carp, that now have a limited market could represent a good source of protein and lipid of great quality. Fish processing could be a way to increase the value of cheaper fish; across the lake there is the tradition of salting and drying landlocked shad, transforming them in missoltini. We analyzed this product, showing its nutritional characteristics and focusing on the formation of histamine that could occur if the processing is not performed properly. We analyzed missoltino made by seven traditional producers, finding an high histamine content in products made by three of them. We identify 99 bacterial strains, of which the majority (71) showed the ability of produce histamine and 7 were high histamine former bacteria. Despite these results we were not able to identify which bacteria strains was responsible of the histamine presence in our samples. The only parameter that we found linked to the histamine presence was the water content of products. Histamine was formed in missoltini where the drying phase of production had not be able to remove the maximum amount of water from the product.

The farming of new fish species, which presence in market is now limited, could be a way to have a higher income to fish farmers. Tench is considered one potential species for the differentiation of freshwater fish productions. We tested the response of this species in intensive aquaculture. Our results were acceptable but not satisfying, probably due to the genetic strain of tench used, which did not fit to intensive farming conditions. The use of spirulina in tench feeding showed promising results, that should be confirmed in further studies.

CHAPTER 8

Acknowledgements

My initial though goes to the memory of Professor Maria Antonietta Paleari, who collaborated in the research on the microbiology of Missoltino. Her advices were always specific and precious for me, in my approach to these aspects where my knowledge was only partial.

I am deeply grateful to the Director Prof. Valentino Bontempo and my Coordinator Prof. Giovanni Savoini for the opportunity to execute my PhD in Animal Nutrition and Food Safety.

I would like to thank the research team composed by Prof Moretti, dott. Bellagamba, Caprino and Busetto for the daily work we had together.

I would like to thank the following people who made possible the writing of this thesis:

- Dott Carlo Romanò for the sampling of the freshwater fish
-
- Dott. Piera Anna Martino and Dott Cristian Bernardi for the microbiology of Missoltino
-
- Giuseppe Beretta for the salt analysis of Missoltino