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ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

FULL PAPER TAM MAKALE

USE OF BIOFUEL BY-PRODUCT FROM THE GREEN ALGAE *Desmochloris* sp. AND DIATOM *Nanofrustulum* sp. MEAL IN DIETS FOR NILE TILAPIA *Oreochromis niloticus*

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Abstract:

Algal by-product meals from the Hawaiian biofuels industry were evaluated as ingredients in diets for juveniles of Nile tilapia (*Oreochromis niloticus*). Four experimental diets were formulated to fulfill fish nutritional requirements. The diets were made with fish meal, soybean meal, whole diatom (*Nanofrustulum* sp.) meal, or defatted green algae (*Desmochloris* sp.) meal as the test ingredients. A feeding experiment with juvenile tilapia of 2.6 ±0.1 g initial weight was carried out in a freshwater recirculation system with each diet treatment tested in triplicate tanks. Fish were fed the experimental diets to apparent satiation twice a day for 12 weeks and fish weight was measured every three weeks. Water temperature was maintained at 22.7 ±0.8 °C, salinity at 0.1 ±0.0 ppt and dissolved oxygen at 5.6 ±0.5 mg/L. At the end of the experiment a significant effect ($P<0.05$) of diet treatments was found in fish growth and feed utilization, with specific growth rate, food conversion ratio, and retained nitrogen efficiency being highest for the green algae-based diet. Lipid content in the diets was lower than expected for the algae diets. Proximate composition analysis showed no significant difference ($P>0.05$) in the protein content of the fish bodies among the diets. Results indicate the suitability of the green algae by-product meal as an ingredient in Nile tilapia diets. Diatom meal also showed interesting potential for use as an ingredient in tilapia feeds.

Keywords: Nile tilapia, microalgae, diatom, green algae, fish feeds, Hawaii

Introduction

The Hawaiian Archipelago is one of the most isolated population centers in the world, located approximately 2,500 miles from the continental United States. Hawaii currently imports 85-90% of the food sold for human consumption (Leung and Loke, 2008), which clearly indicates sustainability and food security issues. Because no remnant processing facilities for animal products exist on the islands, there is little local protein available for use in animal feeds. The consequence of this is a high production cost for all animal production systems in the region since feeds need to be imported, including aquaculture feeds. Therefore, there is considerable interest in using locally available ingredients for fish feeds manufactured to support the growth of the aquaculture industry in Hawaii and the Pacific Basin region. Fish meal is widely used as a dietary protein source in most commercially farmed fish species, but also is the single most expensive major ingredient in aquaculture feeds (Tacon and Metian, 2008). Among the plant materials that have been tested to replace fishmeal in aquaculture feeds, soybean ingredients have performed satisfactorily for most species (Gatlin et al., 2007; Hardy, 2010). However, soybeans do not yield well as a crop under the prevalent climatic conditions in Hawaii.

The production of biofuels in Hawaii is important not only because of its renewable nature, but also because it is a potential local source of energy, which is relevant due to the fact that Hawaii is the most oil dependent state in the United States of America (Arent, 2009). A recent renewable energy model is to produce algae with high lipid contents which can be converted into biodiesel. This model also presents the opportunity to produce by-products. Thus, the algae-derived biofuels industry is a potential source of microalgae biomass for the animal feeds industry. Various strains of microalgae have been shown to possess appreciable protein content (Becker, 2007). Live microalgae are commonly used as food during the larval rearing of fish. However, few studies have been done with microalgae as a source of macronutrients in dry feeds for juvenile or adult fish. Positive effects on fish growth, protein assimilation, lipid metabolism and final product quality have been reported by the use of algae as a feed ingredient in fish diets (Mustafa and Nakagawa, 1995). Due to the high potential in Hawaii for the production of algal biomass, there is an opportunity to significantly reduce the dependence of imported ingredients for aquaculture feeds by using the by-products from the biofuel industry.

Tilapia aquaculture is widely practiced in many tropical and subtropical regions of the world. The Nile tilapia (*Oreochromis niloticus*) is the most distributed and commercially cultured species globally (El-Sayed, 2006). Nile tilapia is an omnivorous fish, with low stomach pH and a long digestive tract that allows for more effective digestion of plant material than most fish species (Rodrigues et al., 2012). The various enzymes that have been reported in tilapia fish including amylase, pepsin, trypsin, esterases and alkaline phosphatase increases their ability to utilize a wide variety of foods including aquatic larvae and insects as well as algae, weeds, and macrophytes (Tengjaroenkul et al., 2000). These attributes make the species attractive for aquaculture applications where less expensive plant derived protein can be used as the main protein source in feed. Therefore, the aim of this study was to evaluate the use of by-product green algae and diatom meals in diets for *O. niloticus*, and to compare their effect on fish growth and feed utilization to commercial fish meal and soybean-based diets.

Materials and Methods

Diets formulation and preparation

Four experimental diets were formulated to contain the maximum inclusion level of one of the following ingredients: fish meal (FM diet), soybean meal (SBM diet), whole diatom (*Nanofrustulum* sp.) meal (DIA diet), and defatted green algae (*Desmochloris* sp.) meal (ALG diet). Both algae meals were obtained from Cellana Inc., Kailua-Kona, HI, USA. All diets contained fish meal and soy protein concentrate in variable amounts depending on their formulation (Table 1). Diets were formulated to fulfill the nutritional requirements of early juveniles of Nile tilapia. Diet formulations were made with the software MIXIT WIN (V.6.14 Agricultural Software Consultants, San Diego, CA, USA). To prepare the diets, feed ingredients were thoroughly homogenized, mixed with water, and then ground in a 5.7 liter mixer (Professional 600 KitchenAid, St. Joseph, MI, USA). The resulting feed pellets were dried at 60°C for 24 hours in laboratory ovens (130D Thelco, Precision Scientific, VA, USA;

6542 Thermo Fisher Scientific, OH, USA). Feeds were then manually ground with a metal grain mill grinder and sieved to obtain particle size ranges of 0.8-1.2, 1.2-2.0 and 2.0-2.4 mm. The diets were stored in zip lock plastic bags and stored at 8°C in a refrigerator until further use.

Table 1. Formulation (g/kg dry weight) and proximate composition of the experimental diets.

	Diet			
Ingredient	**FM**	**SBM**	**DIA**	**ALG**
Fish meal[1]	584.33	50.29	244.51	143.13
Soybean meal[2]	50.00	746.58	-	-
Diatom meal[3]	-	-	401.25	-
Green algae meal[4]	-	-	-	577.62
Soy protein concentrate[5]	40.00	40.00	280.00	200.00
Dextrin[6]	191.10	-	-	-
Fish oil[7]	40.00	53.13	12.00	19.25
Canola oil[8]	44.57	60.00	12.24	10.00
Alginate[9]	20.00	20.00	20.00	20.00
Vitamin and mineral premix[10]	20.00	20.00	20.00	20.00
Di-calcium phosphate[11]	10.00	10.00	10.00	10.00
Proximate composition (% dw)				
Moisture	5.7	6.9	12.1	6.9
Protein	46.38	42.87	39.72	40.99
Lipid	13.35	12.05	8.61	5.09
Total saccharides	19.49	30.20	15.74	31.21
Ash	15.08	8.03	23.88	15.81

[1] Menhaden. Special Select, Omega Protein Inc., Houston, TX, USA. Crude protein 60% (dw).
[2] Solvent extracted. Hall Roberts' Son, Inc. Postville, IA, USA. Crude protein 46.0%.
[3] Whole cell. Cellana Kailuha-Kona, HI, USA. Crude protein 17.1%.
[4] Deffated. Cellana Kailuha-Kona, HI, USA. Crude protein 31.5%.
[5] Profine VF, Solae, St. Louis, MO, USA. Crude protein: 66.0%.
[6] Dextrin from corn D2131. Sigma-Aldrich Co., USA.
[7] Menhaden. Virginia Prime Gold, Omega Protein Inc., Houston, TX, USA.
[8] Cisco, The J.M. Smucker Co., OH, USA.
[9] Sodium alginate W201502, Sigma-Aldrich Co., USA.
[10] Rovimix, DSM Nutritional Products Mexico.
[11] Dihydrate. Squire, Neogen Corporation, KY, USA.

Experimental design

One feeding experiment was done with Nile tilapia (*O. niloticus*) juveniles produced at the PACRC freshwater fish hatchery in Panaewa, HI, USA. The experimental protocol applied in this research was approved by the Institutional Animal Care and Use Committee, University of Hawaii. The experiment was performed in a freshwater recirculation system which consisted of a biofilter coupled to a sump tank, with twelve rectangular glass aquaria of 72 L volume each. Each aquarium had a water inlet above the water surface and an outlet with a screen filter. Moderate aeration was provided to each aquarium with an air stone connected to a blower line. Water temperature, salinity and dissolved oxygen were monitored daily with a multi-meter (85D YSI, Yellow Springs, OH, USA) and were maintained at 22.7 ±0.8°C, 0.1 ±0.0 ppt, and 5.6 ±0.5 mg/L respectively. The water was maintained at ambient temperatures between 21 to 25°C. Photoperiod in the system was kept at 14L:10D.

Diet treatments were randomly assigned and each experimental diet was tested in triplicate aquaria. Ten tilapia juveniles were stocked in each aquarium with an initial individual weight for all treatments of 2.60 g ± 0.06 g. The fish were fed to apparent satiation twice daily at 09:00 and 16:00 h for 12 weeks. Every day the quantity of consumed food in each aquarium was recorded, and every three weeks the fish were group-weighed to determine growth. Fish samples for whole body proximate composition analysis were taken at the start and at the end of the experiment for each diet treatment. The fish samples were frozen at -10°C, cut into pieces with a knife and homogenized in a blender (SPB-600 Cuisinart, East Windsor, NJ, USA), then dried in an oven at 60°C for 24 h. Thereafter the samples were manually ground in a mortar and kept in zip lock bags at 8°C for further analysis.

Proximate composition analyses

Nutrient composition of experimental diets and fish bodies (three fish per tank) were confirmed through proximate composition analysis of triplicate samples. Total moisture or the inverse dry matter, was determined by the loss of mass from oven drying at 105°C until constant mass was achieved. Protein content was determined by the combustion method (AOAC Official Method 968.06, AOAC 2000). Briefly, a Costech Elemental Combustion System (ECS 4010, Valencia, CA, USA) was used to determine total nitrogen by yielding a percent nitrogen value for the sample. The nitrogen determined was converted to protein content in the sample using the protein conversion factor of 6.25. Ash was determined according to the AOAC Official Method 942.05 (AOAC 2000). Samples were dried in an oven prior to analysis to ensure that the samples were dry when analyzed. Samples were ashed at 500°C for 5.5 h and the percent ash was determined by gravimetrically measuring the difference in weight of the un-combusted and combusted samples. Lipid analysis was performed by use of an ANKOM XT10 (Macedon, NY, USA) extractor according to AOCS Official Procedure Am 5-04 (AOCS 2004). This method determines crude fat by a high temperature (90°C) extraction for 60 minutes using petroleum ether.

Amino acid analysis

All analyses were conducted by NP Analytical Labs (St. Louis, MO, USA) using standard methodologies. Specific procedures were performed to determine amino acid contents for the following groups of amino acids. Acid stable amino acids were determined by hydrolysis with hot HCl, separated on an ion-exchange column, and detected by reaction with ninhydrin. Cysteine and methionine (sulfur containing amino acids) content was determined by oxidation, and amino acid hydrolysis of the sample. The amino acids were then separated on an ion-exchange column, derivatized and quantitated upon comparison with standards that were taken through the same procedure. Tryptophan content was determined by alkaline hydrolysis of the sample, followed by HPLC using UV detection.

Fish growth and feed utilization

At the end of the experiment, for each diet treatment the specific growth rate (SGR) (% per day) of the fish was calculated as the weight gain divided by the time ((Ln final weight-Ln initial weight)/time in days * 100). The feed conversion ratio (FCR) was estimated as the feed intake divided by the fish weight gain (feed weight /weight gain) and the feed efficiency (FE) as the fish weight gain per unit of feed intake (weight gain/feed intake). The protein efficiency ratio (PER) was calculated as the fish weight gain divided by the consumed protein (weight gain/consumed protein). The retained nitrogen (RN) (g/fish) was calculated as the difference between the final and initial body nitrogen content. The retained nitrogen efficiency (RNE) (% nitrogen intake) was calculated as the retained body nitrogen multiplied by 100 and divided by the nitrogen intake (RN * 100/ N intake).

Statistical Analysis

All results were analyzed for normality and homogeneity of variance previous to a one-way ANOVA analysis to test for the effects of dietary treatments on fish growth, feed utilization and body proximate composition. If significant differences were found (P<0.05), the differences between treatments were analyzed by the Holm-Sidak method. Statistical analyses were done with SigmaStat 3.5 (Systat Software Inc., San Jose, CA, USA).

Results and Discussion

Experimental diets composition

Each of the experimental diets were formulated to contain the maximum level of one of the four test ingredients. Therefore, their proximate composition differed according to the nature of these ingredients (Table 1). At rounded values, all diets contained at least 40% protein, which fulfills the requirement of the species at this age (NRC, 2011). Lipid also varied among diets, with a lipid content lower than expected in the ALG diet, and the highest in the FM diet. Although a true lipid requirement is not specifically defined for each fish species and is influenced by diverse nutritional factors, it is generally assumed that freshwater fish as tilapia cannot tolerate high lipid levels in their diet. The lipid levels in the experimental diets in this study varied from moderate (13.4%) to low (5.1%). The ash content was high in the DIA diet (i.e. 23.9%), which is generally considered high for farmed species. The amino acid profile of the experimental diets is shown in Table 2. For all the essential amino acids, the experimental diets contained levels at or above the requirement for Nile tilapia (NRC 2011). In the case of amino acids known to be deficient in plant ingredients (i.e. methionine and lysine), their content in the diets were above requirement for the species (Furuya et al., 2001, 2012). The ash content in the DIA diet was similarly high as in other studies when diverse algae meals have been tested (Patterson and Gatlin, 2013; Vizcaíno et al., 2014).

Fish growth and feed utilization

The inclusion of algal meals in the diet had significant effects on fish growth and feed utilization. The highest final fish weight, weight gain, and specific growth rate were obtained with the ALG diet and they were significantly different (P<0.05) than that obtained with the other diet treatments (Table 3). The SBM diet produced the lowest fish growth, which was significantly different (P<0.05) than the other dietary treatments. During the first weeks of the experiment, fish growth was similar among treatments. However, it started to differentiate six weeks after the start of the experiment, and by week 12 the fish growth of the ALG diet compared to the other diet treatments was significantly higher (Figure 1). All experimental diets were well accepted by the fish and no rejection of any diet was observed. However, the feed intake was significantly different (P<0.05) among diet treatments, with the highest intake found in fish fed the ALG diet (Table 3). Similarly, the best FCR was obtained with the ALG diet (Table 3). The FE. RN and RNE were all significantly different (P<0.05) among feed treatments (Table 3). The highest protein utilization and nitrogen retention were found in fish fed the ALG diet. No significant differences (P>0.05) were found between the FM, SBM and DIA diet treatments in nutrient utilization. During the experiment no fish mortalities occurred, thus survival was 100% in all treatments.

Table 2. Essential and nonessential amino acid composition (g/100 g feed dry matter) of the experimental diets.

	Diet			
	FM	SBM	DIA	ALG
Essential amino acids				
Threonine	1.87	1.81	1.86	1.66
Valine	2.24	3.21	2.25	1.90
Methionine	1.26	0.69	0.91	0.74
Isoleucine	1.87	2.01	1.94	1.60
Leucine	3.15	3.33	3.22	2.76
Phenylalanine	1.88	2.29	2.08	1.88
Histidine	1.05	1.17	1.01	0.83
Lysine	3.30	2.67	2.80	2.07
Arginine	2.80	3.12	2.83	2.28
Tryptophan	0.40	0.52	0.45	0.48
Nonessential amino acids				
Aspartic acid	4.27	4.95	4.60	3.45
Serine	1.82	2.20	2.00	1.70
Glutamic acid	6.67	8.33	7.17	4.96
Proline	2.22	2.25	2.13	2.90
Glycine	3.31	2.04	2.53	2.03
Alanine	2.86	2.06	2.44	2.44
Tyrosine	1.35	1.54	1.41	1.13
Cysteine	0.42	0.61	0.56	0.47

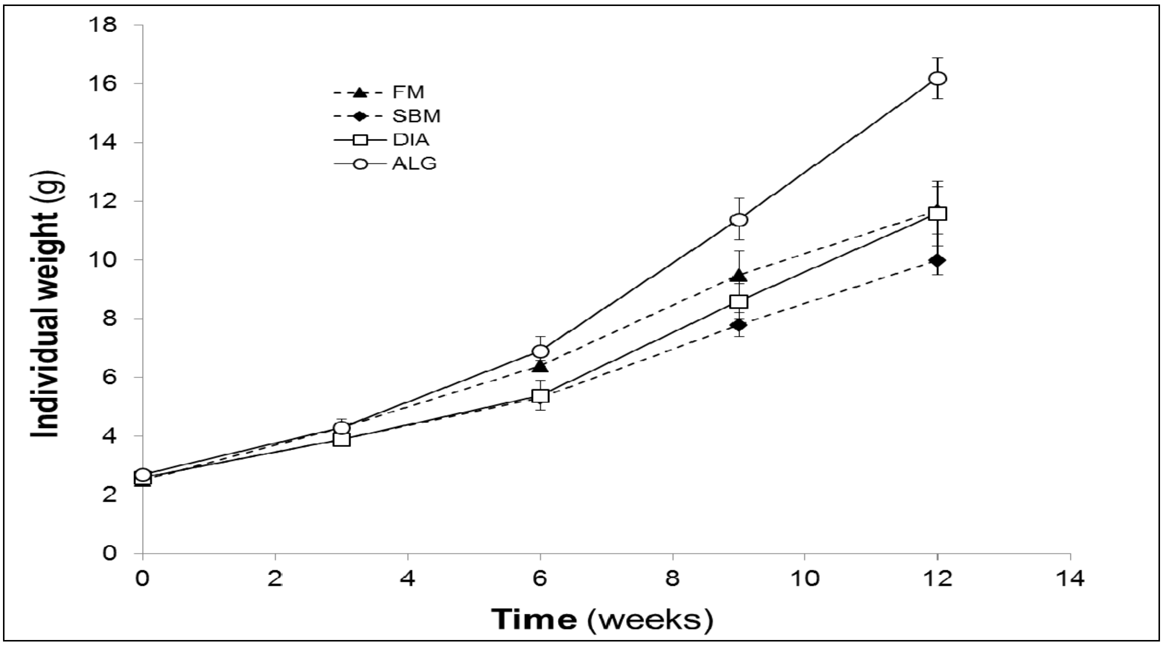

Figure 1. Mean individual weight of Nile tilapia juveniles fed experimental diets made with fish (FM), soybean (SBM), diatom (DIA) and green-algae (ALG) meals as the main protein source during a period of 12 weeks.

Table 3. Mean initial and final fish weight, weight gain, specific growth rate (SGR), feed intake, feed conversion ratio (FRC), feed efficiency (FE), protein efficiency ratio (PER), retained nitrogen (RN), retained nitrogen efficiency (RNE) and survival of Nile tilapia juveniles fed the experimental diets.

	Diet			
	FM	SBM	DIA	ALG
Initial weight (g)	2.53 ± 0.09	2.63 ± 0.03	2.56 ± 0.21	2.67 ± 0.12
Final weight (g)	11.73 ± 1.00^{b}	9.97 ± 0.57^{b}	11.60 ± 1.40^{b}	16.17 ± 0.85^{a}
Weight gain (%)	364.05 ± 41.35^{b}	278.36 ± 17.68^{c}	353.12 ± 38.30^{b}	504.77 ± 17.81^{a}
SGR (% day)	1.82 ± 0.11^{b}	1.58 ± 0.06^{c}	1.80 ± 0.10^{b}	2.14 ± 0.04^{a}
Feed intake (g/fish)	12.92 ± 0.36^{b}	12.10 ± 0.42^{b}	12.90 ± 0.54^{b}	15.57 ± 0.36^{a}
FCR	1.41 ± 0.12^{ab}	1.66 ± 0.10^{b}	1.44 ± 0.16^{ab}	1.16 ± 0.04^{a}
FE	0.71 ± 0.06^{b}	0.61 ± 0.03^{b}	0.70 ± 0.08^{b}	0.87 ± 0.03^{a}
PER	1.53 ± 0.13^{bc}	1.41 ± 0.08^{c}	1.76 ± 0.21^{ab}	2.11 ± 0.07^{a}
RN (g/fish)	0.85 ± 0.09^{b}	0.69 ± 0.05^{b}	0.82 ± 0.11^{b}	1.27 ± 0.07^{a}
RNE (% N intake)	25.97 ± 2.98^{b}	23.35 ± 1.87^{b}	29.85 ± 6.29^{b}	42.45 ± 3.27^{a}
Survival (%)*	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

Values in the same row with the same superscript are not significantly different (P>0.05).
*No significant differences were found (P>0.05).

Fish proximate composition

At the end of the experiment, significant differences were found in the fish body proximate composition among diets treatments (Table 4). The moisture content was significantly higher (P<0.05) in fish fed the DIA and ALG diets than in the fish fed the FM and SBM diets. In contrast, the lipid content was highest (P<0.05) in fish fed the FM diet followed by the fish fed the SBM treatment. No significant differences (P>0.05) were found in protein and ash content for all diet treatments (Table 4).

Suitability of microalgae meals as protein source

Tilapias have herbivorous/omnivorous feeding habits which position them at the lower levels of the aquatic food chain. This is one of the main reasons plant ingredients are successfully and extensively used in feed formulations for cultured tilapia species. Most of the plant ingredients currently used as protein sources in fish feeds are of terrestrial origin. However, due to the increasing price, future availability, and concerns on the sustainability of terrestrial crops, more research has focused on aquatic organisms as alternative sources of nutrients in aquaculture feeds. Some studies have tested duckweeds (El-Sayed 1992, 1999) and seaweeds (Amor et al., 2005) as protein sources in tilapia diets. Other studies have instead used microalgae to replace fish meal as a protein source in fish diets including tilapia diets (El-Sayed, 1994; Olvera-Novoa et al., 1998; Tartiel et al., 2008;Walker and Berlinsky, 2011; Kiron et al., 2012). The biofuel industry converts the lipid from the microalgae to fuel, but currently there is no major use for by-products left over after the extraction process (by-product meal). Thus, the by-products from this industry are a potential and valuable source of nutrients that can be used in animal feeds. Due to the expanded production of microalgae-derived biofuels, a significant source of nutrients for fish feeds may be available from biofuel production (Brennan and Owende, 2010).

Table 4. Initial and final body composition (% wet weight) of Nile tilapia juveniles fed diets with fish meal, soybean meal, diatom meal and green algae meal as major sources of protein.

	Initial	Final			
		FM	SBM	DIA	ALG
Moisture	74.2 ± 0.0	73.4 ± 0.2^{b}	73.4 ± 0.2^{b}	74.2 ± 0.1^{a}	74.1 ± 0.1^{a}
Protein*	15.0 ± 0.0	15.3 ± 1.2	15.2 ± 0.6	14.4 ± 0.4	15.0 ± 0.6
Lipid	6.1 ± 0.0	7.7 ± 0.1^{a}	6.8 ± 0.1^{b}	6.5 ± 0.1^{c}	5.5 ± 0.1^{d}
Ash*	4.3 ± 0.2	3.9 ± 0.2	3.6 ± 0.3	4.0 ± 0.1	4.0 ± 0.2

Except for the initial, values in the same row with the same superscript are not significantly different (P>0.05).
*No significant differences were found (P>0.05).

Potential causes for the significant differences found in fish growth in the present study are the protein and lipid contents of the diets, which were higher in the FM and SBM diets compared to the algae-based diets. Although it may be expected that higher protein and lipid contents in the diet produce higher fish growth, these diets yielded lower growth than the ALG diet (Table 3). Although protein and lipid requirements for Nile tilapia were met in all of the experimental diets, higher protein and lipid contents in the FM and SBM diets did not produce better growth, possibly because of the high inclusion levels of fish and soybean meals in these diets. It has been reported that high levels of soybean meal similar to the level used in the present study (SBM diet) can be detrimental to fish growth (Alam et al., 2012). Other factors may have also contributed to the observed difference in fish growth. The essential amino acid requirements for Nile tilapia were fulfilled in all diets despite the differences in amino acid content among diets (Table 2), (NRC 2011). Thus, the difference in fish growth cannot be attributed to dietary essential amino acid deficiency as the highest growth rate was found in fish fed the ALG diet, which had lower methionine and lysine content compared to the FM diet. The combination of fish meal, soy protein concentrate, and algal meal contained in the DIA and ALG diets was sufficient enough to prevent any dietary deficiency in essential amino acids. From the total protein contained in the DIA diet (39.7%), only 6.9% was derived from the diatom *Nanofrustulum* meal, with the rest of the protein derived from fish meal and soy protein concentrate. As for the ALG diet, 18.2% or close to half of the total protein (41%) in the diet was derived from the green algae meal. The contribution of the green algae *Desmochloris* sp. protein in this study was higher than previously reported for Nile tilapia of similar weight fed with diets made with a mix of either microalgae *Chlorella* spp. or *Scenedesmus* spp. (Tartiel et al., 2008). The inclusion level of the diatom *Nanofrustulum* protein in our study was higher than the maximum level tested for common carp (3.8% of total protein) which also resulted in no significant difference in fish growth when compared with a fish meal/soybean meal-based diet (Kiron et al., 2012). However, the same study demonstrated that a level of 2.1% protein from the diatom meal proved detrimental for growth of Atlantic salmon. This is a clear indication that suitability of algal meals as protein source in fish diets and their level of dietary inclusion are dependent on the type of algae and the feeding habits of the target fish.

The level of the maximum microalgae meal inclusion in the ALG diet was similar to a study with *O. mossambicus* in which the fish meal in the diet was optimally replaced by the filamentous cyanobacteria *Spirulina maxima* up to a level of 40% (Olvera-Novoa et al., 1998). However, the protein content in *Spirulina* (i.e. 66.9%) was more than double the protein content in the *Desmochloris* meal (31.5%) used in the present study indicating a different grade of utilization of the algal ingredients based on their composition. The inclusion level of microalgae meals in fish diets may be limited due to their protein content and other factors such as specific nutrient requirements in different fish species, and ingredient digestibility. For example, despite the high protein content in *Spirulina* meal, it could only replace up to 50% of fish meal in diets for silver sea bream and was suitable at even lower replacement levels for other species (El-Sayed 1994). With current processing practices of microalgae meals, it is still necessary to combine microalgae with other protein sources to partially or completely replace fish meal in diets for fish. The higher fish growth obtained with the green algae-based diet compared to the diatom-based diet may be due to the digestibility and attractiveness of the algal ingredients. The diatom meal was used in the diet as an intact cell which included the frustule or cell wall. The diatom cell wall is made of silica, and thus is difficult to digest or was possibly un-digestible for the fish. Consequently, the FE in fish fed the DIA diet was significantly lower than the one obtained in fish fed the ALG diet (Table 3). This can partially be discerned from the higher ash content in the DIA diet (Table 1) as typically, the higher the ash content in the diet the lower the feed efficiency in fish (Shearer et al., 1992). The breaking or removal of the silica cell wall of diatom material during processing may improve its value as a feed ingredient in fish feed. Nonetheless, high ash levels up to 25% in diets containing diatom meal did not produce detrimental effects on fish growth in tilapia (the present study) and common carp (Kiron et al., 2012), species that have herbivorous/omnivorous feeding habits. However, a different result is probable when these meals are tested in carnivorous fish. For example, feeding algae to rainbow trout, a carnivorous fish, is detrimental for growth (Barrows and Frost, 2014). Although feeding a diet with *Spirulina* meal as the sole protein source to common carp, an herbivorous fish, produced similar fish growth as a control diet made with fish meal (Nandeesha et al.,, 1998). Potential problems with aluminum accumulation in fish due to the algae mineral content were prevented by the dietary inclusion of di-calcium phosphate, which assists in the neutralization of aluminum in fish when fed diets containing high levels of algae meals (El-Sayed Hussein et al.,, 2014).

The feed intake was higher and the FCR was lower in tilapia fed the ALG diet compared to the values obtained with the FM diet; these values were also superior to those found in another study with Nile tilapia using fish meal, wheat bran, and sunflower cakes as protein sources (Maina et al., 2002). This may indicate one advantage (diet acceptability) of using microalgae as sources of protein over terrestrial plant proteins and fish meal by herbivorous/omnivorous fish species. Nitrogen retention in fish was not affected by algal meal inclusion in the diets compared to the fish meal-based control diet; it actually improved when the green algae *Desmochloris* was used as a protein source (Table 3). This could be the result of the type of protein present in the tested ingredients as the protein structure, size and functionality in fish meal may differ from those in the algal meals; these are factors that can affect protein utilization by fish. Omnivorous or herbivorous fish have different capacities for food digestion than carnivorous fish. Feeding tilapia with fish meal protein does not optimize the nitrogen retention as the species digestive system is adapted to utilize plant proteins. Using aquatic organisms (macrophytes, macroalgae, and microalgae) compared to angiosperms (cereals, legumes) as protein sources in fish feeds may follow the same rationale, as herbivorous and omnivorous fish have evolved ingesting and utilizing nutrients from aquatic sources.

Conclusions

As ingredients in fish feeds, microalgae meals derived from biofuel production represent an interesting alternative to fish meal and plant proteins of terrestrial origin, such as soybean products. In the present study, high inclusion levels of microalgae meal in the diets were achieved in comparison with previous research reported in tilapia. Nonetheless, the feasibility of using microalgae meals as protein and lipid sources in commercial fish feeds not only depends on their nutrient quality and utilization by fish, but also on their cost-effective production and sustained availability.

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SHORT COMMUNICATION KISA MAKALE

PRODUCTION RATE OF NITROGEN COMPOUNDS AND OTHER WATER QUALITY VARIABLES IN *Macrobrachium vollenhovenii* CULTURED IN TANKS

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Abstract:

Juvenile-size of African River prawns (*Macrobrachium vollenhovenii*) of an average length of 46.34 ±0.23mm were collected from Epe Lagoon and cultured in concrete tanks for 56 days. The production of Nitrogenous compounds was monitored and the analyses of the water quality variables were carried out weekly. The range of the water quality variables were 6.74-7.90 (pH), 25.7-30.3°C (Temperature), 6.71-8.01mg/L (Dissolved Oxygen), 0.00-0.03% (Salinity), 0.199 and 0.725 mS/cm (Conductivity), 24-48 NTU (Turbidity). The result showed that Nitrite (NO_2^-) ranged between 0.07-0.50mg/L, Nitrate (NO_3^-) was between the range of 1.32-6.63mg/L and Ammonia (NH_3^-) was between 0.32-1.65mg/L.

Keywords: Prawns, Water quality, Nitrite, Nitrate, Ammonia

Introduction

Water quality and quantity are critical to the success of prawn culture which is an important factor to consider in prawn culture medium. Freshwater is normally used for rearing freshwater prawns from post-larvae to market size. Water of 3-4 ppt salinity may be acceptable for the culture of prawns (FAO, 1982). The African River prawn, *M. vollenhovenii* belonging to the family Palaemonidae is the largest prawn species among the available prawns across the West-Africa water bodies and a good candidate for aquaculture and its omnivore. The African River prawn, *M. vollenhovenii* has good potentials for aquaculture (Bello-Olusoji *et. al.,* 2004, Bello-Olusoji *et. al.,* 1997). The species can be reared extensively in captivity like other *Macrobrachium* species such as *M. rosenbergii* in Asia, *M. idea* in India, *M. amanzonii* in America and *M. brazilensis* in Brazil, thus contributing to their economies. The oxygen requirement for prawn culture is not known in details as well as most of the water quality factors requirements for prawn culture are unknown. According to Bielsa *et. al.*, 1983; oxygen decreases with an increase in temperature. Prawns grow faster in warm water between 26-30^{0}C and water salinity of 6-33ppt (Motoh, 1981). pH also affect prawn's growth, pH of over 9 and pH lower than 5 are lethal for post-larvae. Poor water quality parameters make shrimps susceptible to diseases, a situation that caused an average reduction of 64% in prawn production and an estimated total loss of US$ 4.44 million due to the disease outbreak (Jayasinghe, 1995). Oxidation of accumulated organic wastes compounds in shrimp pond water depletes the dissolved oxygen, a reaction producing other water quality variables such as ammonia and nitrite, thus contributing greatly to shrimp mortality. High ammonia concentration causes prawn mortality. Ammonia or ammonium (NH_3 and NH_4^+) is converted into nitrite (NO_3), which, is quickly converted to non-toxic nitrate (NO_3, plant food), (Robert *et al* 1997b). Nitrites cause reddening of the fins and irritation of the gills, gasping plus excess mucus. Nitrites also bind the fish Red Blood Cells resulting in suffocation and "Brown Blood Disease". This study however, focused on the determination on the rate of production of some water quality variables in tank rearing of African river prawn (*M. vollenhovenii*).

Materials and Methods

The juvenile sizes of African river prawn *Macrobrachium vollenhovenii* were collected from Epe lagoon in Lagos State and they were transported to the Fish Farm in the Department of Fisheries and Wildlife, Federal University of Technology Akure in 4 (four) 50Litres ice-chest (in order to keep the water temperature constant during transportation), ¾ filled with oxygenated water. They were acclimatised for 7 days, after which sixty (60) healthy prawns were randomly selected between 42.24-51.03 mm and an average length value of 46.34 ±0.23mm, grouped into two concrete tanks (1.5mx1.5mx0.8m) (in order to allow a better distribution during rearing and reduction in cannibalism associated with overcrowding) with a stocking density of 30 prawns per tank and they were fed with a local formulated diet of 35% crude protein. Tanks were filled with filtered pond water and water was replaced weekly. The experiment was conducted for 56 days (8weeks). The water temperature was measured by using a standard mercury thermometer to the nearest 0.1°C. The water was analyzed at the beginning and at the end of each week and throughout the period of the experiment. Weekly measurements of concentration levels of the most important water quality parameters such as nitrate, nitrite and ammonia (NO_3^-, NO_2^-, NH_3^+) were determined using a colorimetric method for NO_3^- and NO_2, while titration method was used to determine the ammonia (NH_3^+). The tests were in triplicates. The pH was monitored daily in each tank with pH meter to the nearest 0.1 pH unit (Fisher Accumet pH meter), to obtain the mean pH value on daily basis. The dissolved oxygen "DO" concentrations was measured daily throughout the period using a portable oxygen meter (Model no: HI 9146) to the nearest 0.1mg/l. When measuring the ammonia, the pH was measured simultaneously in order to determine the relative dissociation of ammonium ions into free ammonia. The physico-chemical parameters – temperature, salinity, turbidity and conductivity were measured with Palintest comparator and Horiba U-10 water checker respectively.

Results and Discussion

The results for the water test analysed for the eight weeks are shown in Table 1 and 2. The water quality parameters were observed to change on a weekly basis, though very close to each other in comparison to the prawns' growth (Plate 1). Temperature affects the growth and survival of shrimps. Thus, the rate of growth increases with temperature. Though, higher temperature causes mortality.

Other water variables are shown in table 2. The pH ranged from 6.74-7.90, DO ranged between 6.71-8.01 mg/L, Salinity between 0.00-0.03 %, Turbidity ranged between 24-48 NTU, Conductivity between 0.199 and 0.725 mS/cm, Nitrite (NO_2) was between 0.07-0.50 mg/L, Nitrate (NO_3) ranged between 1.32-6.63 mg/L and Ammonia (NH_3) was between the range of 0.32-1.65 mg/L.

Figures 2a, b and c showed that the nitrite, nitrate and the ammonia level fluctuates respectively as the weeks increases. But the resulting mean values (Table 2) were 0.285, 3.975 and 0.985 respectively.

The growth rate of *M.vollenhovenii* is water quality variable dependent, it increases as temperature increases. Water temperature throughout the experiment ranged between 25.7-30.3°C (Table 2). Temperatures between 26-30°C are considered best in terms of maximum production. Temperature above 32°C is deleterious. Motoh (1981) reported that there exist a relationship between temperature and DO. Thus, as temperature increases, DO reduces and vice versa. These were similar to the observed result in Table 1, while figure 1 shows the temperature and DO recorded during the culture period.

However, the pH result of 6.74-7.90 supports the work of Bielsa *et. al.* (1983), which states that optimum pH for prawn, is between 7.0 and 8.5. The result was also similar to what was observed by Okoye *et. al.* (2006), he recorded a temperature range of 27.0-29.5, pH values of 6.9-7.1 and DO_2 of 6.95-7.45 in the water quality parameters during an experimental period. Wickins (1976) found that even though *Penaeus monodon* grow without suffering mortalities with water pH of 6.4 in the presence of inorganic carbon, growth was reduced to 60%. In water pH of 6.4 and less than 10-20 mg/l of inorganic carbon, *P. merguiensis* and *P. aztecus* exhibited greatly reduced growth and lower survival. When pH fell below 5.0, heavy mortalities occurred. A fall in pH have indirect effect for instance, resistance of the shrimp to pathogens might be reduced.

Its survival, growth and development in water with salinity range (0.00-0.03%) show that *M. vollenhovenii* can be cultured in any freshwater habitat. Though, it can tolerate 0-20 ppt salinity (Anetekhai, 1986). Thus *M. vollenhovenii* is a good candidate for freshwater culture (Bello-Olusoji, 2004).

Bello-Olusoji (2007) reported that the ammonia values obtained from the water samples of *M. vollenhovenii* ranged between 0.24-1.50mg/L and as the pH increases, the more ammonia is excreted by the prawns. He also reported that the species can do well at a temperature ranging between 23 and 29 °C, DO_2 ranging between 5.11 and 7.1 mg/L. These results were similar to the ones recorded in Table 2. Shrimps are quite sensitive to low oxygen levels. Growth is best at dissolved oxygen level above 3ppm. Shigueno (1975) recorded a die-off in a pond when oxygen level reached 2.7ppm during the night. The observed DO range in this study was thus safe for the prawns.

Wickins (1976) discussed the three forms of nitrogen compounds and the effects of sublethal levels on shrimp growth. Two tests with nitrate showed that the growth of *P. monodon* was not affected by a concentration of 200mg/L after five weeks of exposure. In a test with *P.indicus*, growth was reduced by nearly 50% over a period of 34 days where nitrate concentration was 6.4mg/L. For ammonia, chronic toxicity test with five species of penaeid shrimps, *P. japonicus*, *P. occidentalis*, *P. schmitti*, *P. semisulcatus* and *P. setiferus*, showed that a mean concentration of 0.45mg/L reduced growth by 50% of the control. Wickins estimated that a "maximum acceptable level" at which growth would be reduced by only 1-2% is 0.10mg/L. The concentration range of these nitrogen compounds which can be tolerated by *P. monodon* are: Un-ionized ammonia (NH_3): 0.0-1.0 mg/L, Ionized ammonia (NH_3^+): 0.0-0.5 mg/L, Nitrite (NO_2): 0.0-0.6 mg/L and Nitrate (NO_3): 0.0-2.00 mg/L (Shigueno, 1975). The pH of the water should be in the range of 7.0-8.5. Ammonia concentration in the water should not exceed 11.5 ppm of ammonia ion (NH_4^+) and 0.1 ppm of un-ionized ammonia (NH_3).

Table 1. Water parameters tested on a weekly basis (Mean ±S.D)

Week	pH	Temp. (°C)	DO (mg/L)	Salinity (%)	Conduct. (mS/cm)	Turbidity (NTU)	Nitrite (mg/L)	Nitrate (mg/L)	Ammonia (mg/L)
1	7.90±3.02	26.0±5.32	6.84±2.40	0.00±0.01	0.235±1.20	24±4.90	ND	ND	ND
2	7.25±1.12	30.3±1.03	6.71±1.08	0.01±0.01	0.257±1.08	31±3.07	0.01±1.10	1.77±1.03	0.43±0.05
3	7.16±2.23	28.0±2.65	7.37±2.43	0.00±0.01	0.212±0.12	48±4.66	0.30±1.02	1.33±2.01	0.39±1.02
4	7.07±2.12	27.7±2.22	7.37±2.21	0.00±0.01	0.199±1.09	41±6.07	0.25±0.22	3.52±1.20	0.88±1.54
5	7.02±3.24	26.2±3.30	7.93±1.09	0.00±0.01	0.234±2.01	37±6.77	0.07±0.03	1.32±1.20	0.32±0.56
6	6.74±3.01	25.8±3.01	7.90±2.01	0.03±0.10	0.725±2.03	29±6.06	0.50±1.23	6.63±1.50	1.65±0.70
7	6.98±2.05	25.7±3.22	8.01±1.22	0.01±0.01	0.305±0.07	27±6.06	0.33±2.00	4.40±1.09	1.10±1.25
8	7.26±2.60	25.7±1.22	7.99±0.90	0.00±0.01	0.244±1.02	30±4.16	0.36±1.20	3.98±2.30	1.01±1.22

Table 2. Mean water quality parameters measured during the experimental period

Measured Parameters	**Range**	**Mean values**
pH	6.74-7.90	7.32
Temperature (°C)	25.7-30.3	28
Dissolved Oxygen DO (mg/L)	6.71-8.01	7.36
Salinity (%)	0.00-0.03	0.015
Conductivity (mS/cm)	0.199-0.725	0.462
Turbidity (NTU)	24-48	36
Nitrite NO_2 (mg/L)	0.07-0.50	0.285
Nitrate NO_3 (mg/L)	1.32-6.63	3.975
Ammonia NH_3 (mg/L)	0.32-1.65	0.985

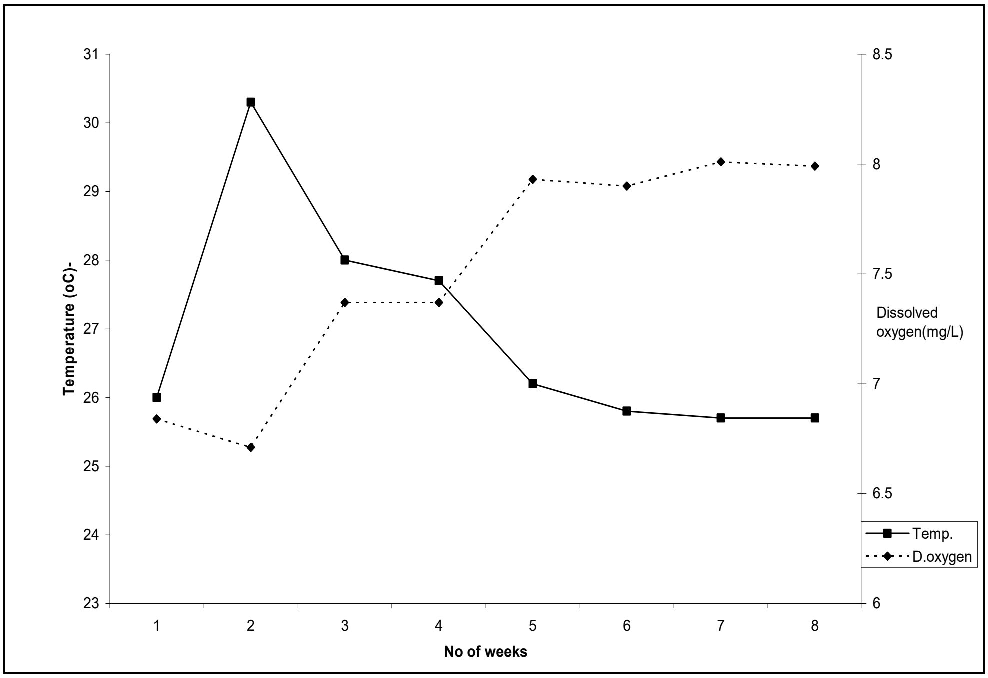

Figure 1. Water temperature (°C) and DO (mg/L) parameters in the culture tanks during the 8 weeks.

Conclusions

However, for successive prawn production in tanks, each water variables must be monitored carefully at regular intervals. Measurements must be taken at the same time in the same place using identical devices and lots of helping hands. Thus, this work seeks to address the ranges of various physico-chemical water quality for prawn culture which can as well be used as a baseline for future researchers.

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FULL PAPER TAM MAKALE

AQUACULTURE AND ITS DISTRIBUTION IN TURKEY

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Abstract:

This study discusses aquacultural practices in the world, the driving forces behind its development, its significance and present condition, aquafarming and its spatial distribution in Turkey.

Keywords: Aquaculture, Aquaculture in Turkey, Spatial Distribution of Aquaculture

Introduction

From the very old times fishing has been an important source of income for humans. It was a primitive practice once, but today it has become ramified and a more intensive undertaking.

Countries aware of the importance of balanced nutrition have focused on optimal exploitation of water resources so as to improve their animal protein sources, and to this end have developed projects. Fish are harvested in two ways: fishing and farming (aquaculture). Majority of the production is wild caught. However, the share of aquaculture in fisheries is gradually increasing. This global change has affected Turkey too, as one can understand from the data the sector has grown substantially.

Materials and Methods

Related bibliography, field studies and statistical data set the groundwork for this study. The researcher made observations on the Aegean and Mediterranean coastline and substantiate the field studies with statistical data. The study draws on the statistical data on fishing of FAO (Food and Agricultural Organization) and of Turkish Statistical Institute, of the General Directorate of Fisheries and Aquaculture (of Ministry of Food, Agriculture and Livestock), and Central Union of Fisheries Producers. ArcGIS 10.1 was used to produce the maps.

Results and Discussion

Fishing in the World

136.2 of 158 million tons of global fisheries product was consumed as food (2012). Despite national and regional variations, 16.7% of animal protein is satisfied by fishery products. 20% and 15% of the need of 2.9 billion and 4.3 billion people for animal protein respectively are met by fisheries. This share (2012) accounts for 7.1% in Germany, 3% in Austria, while 59% in Taiwan and 55% in Japan. 50% of the population in developing countries satisfy 40% of their animal protein need from fish. Across the world, people used to consume 9.9 kg per capita on average in 1960s, while it rose to 18.4 kg in 2009 and 19.2 kg in 2012. Average fish consumption amounts to 9.1 kg, 20.7 kg, 24.6 kg, 24.1 kg, and 22 kg per capita in Africa, Asia, Oceania, North America and Europe, respectively, while it is 9.9 kg in Latin America and the Caribbean. Fishing provides a great number of people not only with essential dietary needs but also with employment. Fishing is still an important source of income.

Whereas the amount of fish harvest was 28 million tons in 1955, it escalated to 89, 131 and 158 million tons in 1987, 2000, and 2012, respectively.

Table 1. Global Fish Production and Utilization in 1990-2012 (in million tons)

Production		**1990**	**1995**	**2000**	**2005**	**2012**
Inland	Aquaculture	8.17	13.86	21.3	26.8	41.9
	Capture	6.59	7.38	8.7	9.4	11.6
	Total Inland	**14.76**	**21.24**	**30.0**	**36.2**	**53.5**
Marine	Aquaculture	4.96	10.42	14.2	17.5	24.7
	Capture	79.29	85.62	86.8	82.7	79.7
	Total Marine	**84.25**	**96.04**	**101.0**	**100.2**	**104.4**
Total Aquaculture		13.13	24.28	35.5	44.3	66.6
Total Capture		85.88	93.00	95.5	92.1	91.3
Total World Fisheries		**99.01**	**117.28**	**131.0**	**136.4**	**158.0**
Utilization		70.82	86.49			
Human Consumption		-	-	96.8	107.3	136.2
Non-Food Uses		-	-	34.2	29.1	21.7
Population (billions)		-	-	6.1	6.5	7.1
Per capite food fish supply (kg)		-	-	15.9	16.5	19.2

Notes: Exluding aquatic plants.
http://www.fao.org./ Statistics for different years.

In 2012, total fish production amounts to 158.0 million tons all over the world, 66.6 million tons of which is yielded by fish farms. The figure concerning global wild catch is 91.3 million tons and the annual increase is insignificant.

Aquaculture in the World

Efforts to cultivate fisheries have increased over the last years. Fish farming in channels, creeks, pools and rice fields in Far East dates back to very old times.

Marine species farming is actually a very ancient practice. Some sources date mariculture back to 2000 BC. It is also suggested that the Japanese farmed oysters on the tidal coasts in the 4th century BC.

Fishery products are depleting due to the rapid increase in the global population, growing threat to fish reserves, various adverse environmental factors despite numerous innovations and the use of advance technology in fishing. Increasing threat to fish reserves and proportionate decrease in the catch over the past years is among the most important dynamics. Population on the Earth is over 7 billion. Aquacultural practices make considerable contributions to the satisfaction of nutrition needs of this increasing global population, healthy nourishment of people, provision of raw material for the industry and creation of employment. Its support to rural development by preserving rural population, increase in export and protection of biodiversity are its other contributions. It is clear from the statistics that Aquaculture has remarkably improved within the last years. Carp and trout were the first fish to farm. Although fresh and salt water farming has substantially developed, the substantial growth in marine aquaculture occurred in the 19th century.

Globally Aquaculture harvest increased to 90.4 million tons by 2012. The income produced amounts to 144.4 billion US dollars. 66.6 million tons of this yield is obtained from fish and 23.8 million tons from aquatic algae. These figures are 70.5 million tons of fish and 26.1 million tons of aquatic algae in 2013, respectively. China alone produces 43.5 million tons of fish and 13.5 million tons of aquatic algae. The increase in aquacultural fish harvest accounts for 6.2% from 2000 through 2012. During the same period, the increase in Africa is much higher (11.7%). It is 10% in Latin America and the Caribbean Islands and 8.2% in Asia excluding China. The growth rate accounts for 5.5% (12.7% in 1990 to 2000) in China as the biggest aquafarmer. As a matter of fact, 92.7% of aquacultural (food) products is harvested by 15 producer countries. Employment in this sector is considerably increasing. Across the world, 4.4% of economically active 1.3 billion people were employed in agriculture in 2012.

Generally speaking, the fishery harvest increased from 13.13 million tones in 1990 to 66.6 million tons in 2012. Aquaculture covers around 42.2% of global fish production and is annually growing more than 10%. Aquacultural production has turned out to be the most rapidly growing sector. Moreover, the most rapid employment increase was recorded in this sector. It employs about 55 million people, half of whom are female. 10-12% of the global population deals with fishing. The share of developing countries in global fish export is 50%, China being the forerunner. 600 aquatic species are farmed in about 190 countries.

Marine fish farming is particularly important for the developing countries. To better illustrate, 52.6% of animal meat (produced on land) is consumed by developed countries, whereas 86.4% of fishery products is consumed by developing countries.

Farmed species are fish, crustaceans, molluscs, amphibians (frogs), aquatic reptiles (excluding crocodiles) and other aquatic creatures (e.g. sea cucumbers, sea urchins and jelly fish) Aquaculture is very important for humankind. Besides its many significant features, it is a major employing sector. Lately, the number of fish farmers have substantially increased. In consideration of continents, Asia has the highest number of fish farmers and is where the highest increase has been observed. While the number of fish farmers was 3.772.000 in 1990, it was recorded to be 15.115.000 and 18.860.000 in 2005 and 2012, respectively. Remarkable increases have been reported in the number of fish farmers in all the continents.

Table 2. Distribution of Fish Farmers by Continents (in Thousand)

Fish Farmers	1995	2000	2005	2010	2012
Africa	65	91	140	231	298
Asia	7 762	12 211	14 630	17 915	18 175
Europe	56	103	91	102	103
South America and Caribbean	155	214	239	248	269
North America	6	6	10	9	9
Oceania	4	5	5	5	6
World	**8049**	**12632**	**15115**	**18512**	**18860**

(Fao, Statistics for different years.)

Aquaculture in Turkey

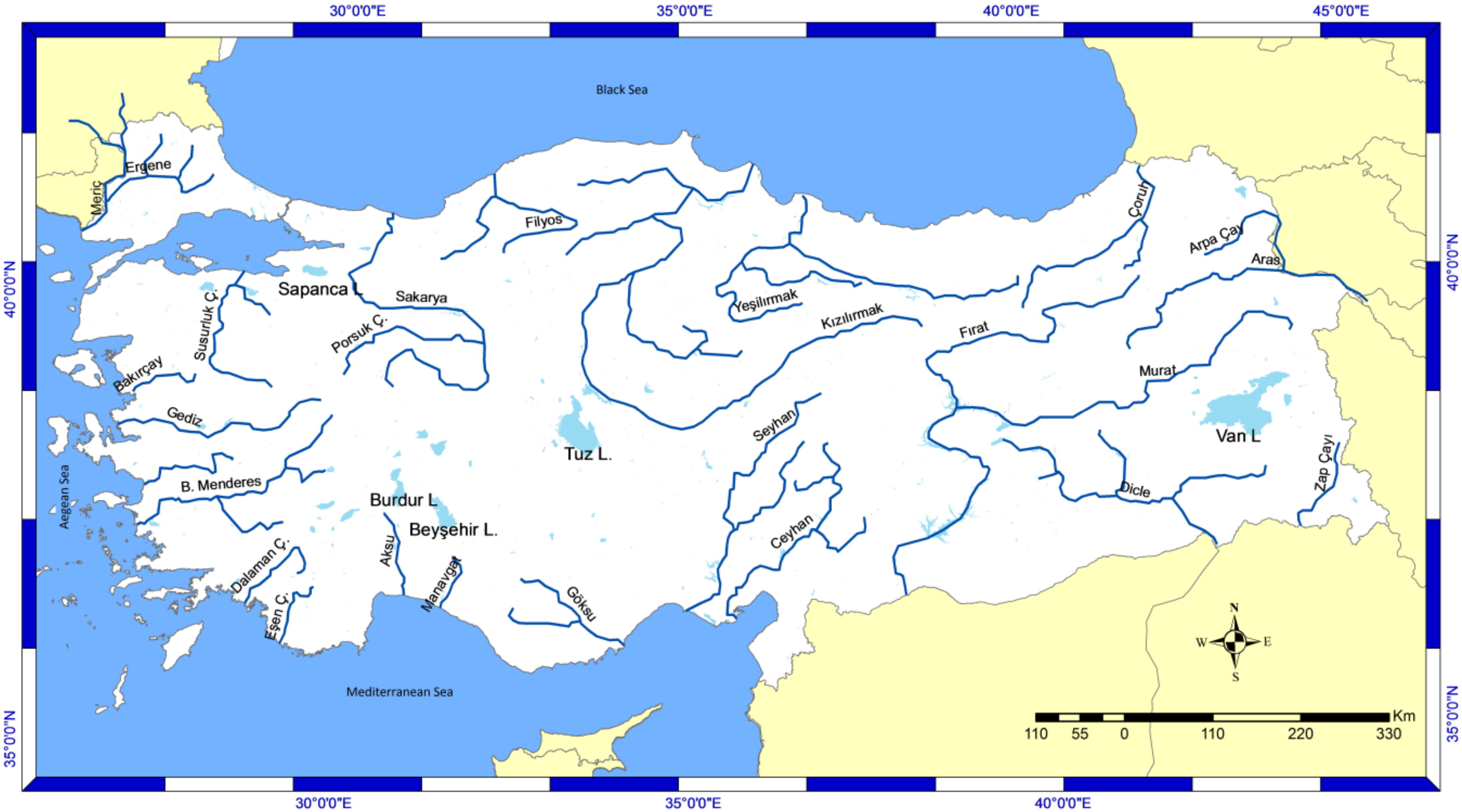
As in the entire world, technological advances in fishing have given way to increase in fishery products. However, in spite of the rapidly developing technology, production increase has come to a halt and been reversed at times due to overexploitation of fish stocks.

Fishing, underdeveloped until 1970s, has significantly developed thanks to incentives since then. The state offered low interest credit loans and customs exemptions, which in return increased the number of fishing boats, boosted fishing capabilities and supported a rapid development. However, fish flour and oil factories were founded, which were more than fish production could satisfy. Thus, fish has come to be used for the production of fish flour and oil, less economic products rather than as a primary human food. This has brought about some problems. Later (after 1988) this practice was given up and fish primarily became a human food again. Fishing in consideration of legal sizes imposed by protective and conservative efforts has contributed to fishermen, tradesmen and national economy. 1970 production of 150 thousand tons increased to 600 thousand tons by 2013. However, the amount of fish consumption is still much lower in Turkey than international figures and global average. It is clear from the statistics that the amount of consumed fish fell to 6.3 kg on average (2013). Fishery product consumption per capita in Turkey is behind the global and European average. It also varies across regions in Turkey: while it is 25 kg per capita on the coastline, it is as low as 1 kg in Central, Eastern and Southeastern regions.

In Turkey, Aquaculture is conducted with the permission of the Ministry of Food, Agriculture and Livestock. The ministry implemented new regulations so as to regulate fish farming, minimize its environmental effects, ensure a healthy and high-quality production, and published "Regulations of Fisheries (29 June 20014, No. 25507)" in the Official Gazette and put it into effect in line with the acquis on fisheries of Common Fishing Policy of the European Union.

Since 1923, Aquaculture was incentivized for the first time in 2003. In 2005 as well so as to promote the farming of new species. Incentives having been given since 2003 have made remarkable contributions to the rapid development and improvement of aquaculture sector.

A protocol was signed between General Directorate of State Hydraulic Works (DSI) and General Directorate for Agricultural Production and Development in 1994 to start fishery farming in cages in reservoirs, which are officially owned by General Directorate of State Hydraulic Works. The protocol was revised in 2004 to allow semi-intensive and extensive production. In 1995, the Ministry of Agriculture and Rural Affairs and the General Directorate of State Hydraulic Works promoted and started in natural lakes and reservoirs, respectively, which is among the causes of production increase.



Map 1. Water Potencial in Turkey

For the purpose of meeting the project needs of producers and entrepreneurs, Turkish Ministry of Agriculture and Rural Affairs and the General Directorate of Ziraat Bankası (Agricultural Bank) collaborated to create typical projects of Aquaculture in cages.

Whereas there was only one fishery facility in 1971, today there are 2912 authorized projects (Facility, Project, Prior Authorization), of which 2392 are inland and 520 are marine facilities (Suymerbir, 2014).

According to 2013 data of the Turkish Statistical Institution (TUIK, 2014), 607515 tons of fishery products were harvested in Turkey, of which 101062.8 tons were exported and 67538.21 tons were imported. Domestic consumption amounts to 479708.3 tons, processed products (fish flour and oil) 87896.2 tons and 6378.1 tons wasted. In consideration of these data, it is obvious that geographical capacities of Turkey should be realized and aquacultural practices should be promoted. Thanks to its inland and marine resources, Turkey is a suitable place for aquaculture. The surface area of Turkey's water resources for aquaculture is much larger than forests and almost equal to arable areas. Turkey is surrounded by water on three sides and its numerous streams, natural and manmade reservoirs, ponds are potential locations for aquaculture. Moreover, many dams are under way. Dams being constructed in Eastern and Southeastern Anatolia Regions to satisfy the need for energy and irrigation water would be suitable water resources for aquacultural practices. To clarify, a coastline of 8333 km, natural lakes of 178000 km^2 and reservoirs of 3442 km^2 with different ecological properties are suitable for fish farming. Realization of this potential is crucial to the development of national fishery in Turkey (Map 1).

Upon the completion of the Southeastern Anatolia Project, 201697 hectares of water area will be gained. This will create an important potential to do aquafarming in the inland waters of Southeastern Anatolia. The current production of 900 tons is expected to rise to 10000 tons thanks to efforts in the region (DPT [State Planning Organization], 2001).

Marine fish farms were removed to open and deep waters pursuant to the regulations passed by the Ministry of Environment and Urban Planning in 2009. According to these regulations, fish farms are not allow within 0.6 marine miles off the shore and in waters less than 30 meters in depth. Moreover, technological advances like the introduction of automated feeding systems, scheduled feeding and digital monitoring have substantially prevented feed-induced pollution (DPT, 2014).

Aquaculture

Aquaculture production was 3075 tons in 1986, and escalated to 167141 tons in 2010 and to 233393 tons in 2013. Fishery harvest was 607515 tons in 2013, 38.4% of which was produced by aquaculture. It can be seen that aquafarming harvest increased by 9.9% in comparison to the previous year. Aquacultural production used to account for 0.53% of total fishery harvest, whereas it increased to cover 10.42% in 1998 and 35.4% in 2013. Post-2000 increase is very eminent. Incentivization of fishery production in 2003 for the first time, increasing incentives to farm new species and incentivized juvenile fish raising are the primary drives for this development.

Fishery production decreased in 2013 in comparison with the previous year by 607515 tons (5.8%). Wild catch and farming account for 61.6% and 38.4% of the production, respectively. While production by catching amounts to 374121 tons, farms' production is 233393 tons. Fish farming in Turkey has taken a huge leap over the last years thanks to scientific, technological and economic developments. While 52.7% of the fishery products in Turkey is harvested from the inland waters, 47.3% is produced from the sea. The species widely farmed are european sea basses (*Dicentrarchus labrax*), gilt-head sea breams (*Sparus aurata*) and Rainbow trouts (*Oncorhynchus mykiss*). Rainbow Trout is the most commonly farmed species, which accounts for 56% of the entire production. The shares of bass and gilt-head sea bream farming are 29% and 15%, respectively. Rainbow trout is primarily raised in inland waters, while gilt-head sea bream and European sea bass are farmed in salt waters. Farmed species vary according to market conditions. Accordingly as a fresh water fish trout production has increased, and gilt-head sea bream and bass production went up as salt-water species. Trout farming increased three folds over 9 years (from 2004 to 2013). Further, saltwater fish production tripled over the same period. For example, bass production went up by three folds.

Juvenile Fish Farming and Their Respective Distribution Areas

The juvenile fish needed for fish farming are obtained from hatcheries or in very small amounrs abroad.

In the private hatcheries, gilt-head sea bream and sea bass fries can be successfully produced in the desired amount. Because fish population is dramatically diminishing in seas, the production of new species is of utmost importance. With new species successfully raised, the variety of species increases based on export, which facilitates the access to new markets.

Table 3. Amount of Fishery Products by Capture and Aquafarming (Selected Years between 1985 and 2013)

Years	Capture (Ton)			Aquaculture (Ton)			TOTAL	Aquaculture Percentage (%)
	Inland	Marine	Total	Inland	Marine	Total		
1985	45471	532602	**578073**	0	0	**0**	**578073**	0.0
1990	37315	342017	**379332**	4237	1545	**5782**	**385114**	1.5
1995	44983	582610	**627593**	13113	8494	**21607**	**649200**	3.3
1996	42202	474243	**516445**	17960	15241	**33201**	**549646**	6.04
1997	50460	404350	**454810**	27300	18150	**45450**	**500260**	9.09
2000	42824	460521	**503345**	43385	35646	**79031**	**582376**	13.6
2011	37097	477658	**514755**	100446	88344	**188790**	**703545**	26.8
2012	36120	396322	**432442**	111557	100 853	**212410**	**644852**	32.9
2013	**35074**	**339047**	**374121**	**123019**	**110374**	**233393**	**607515**	38.4

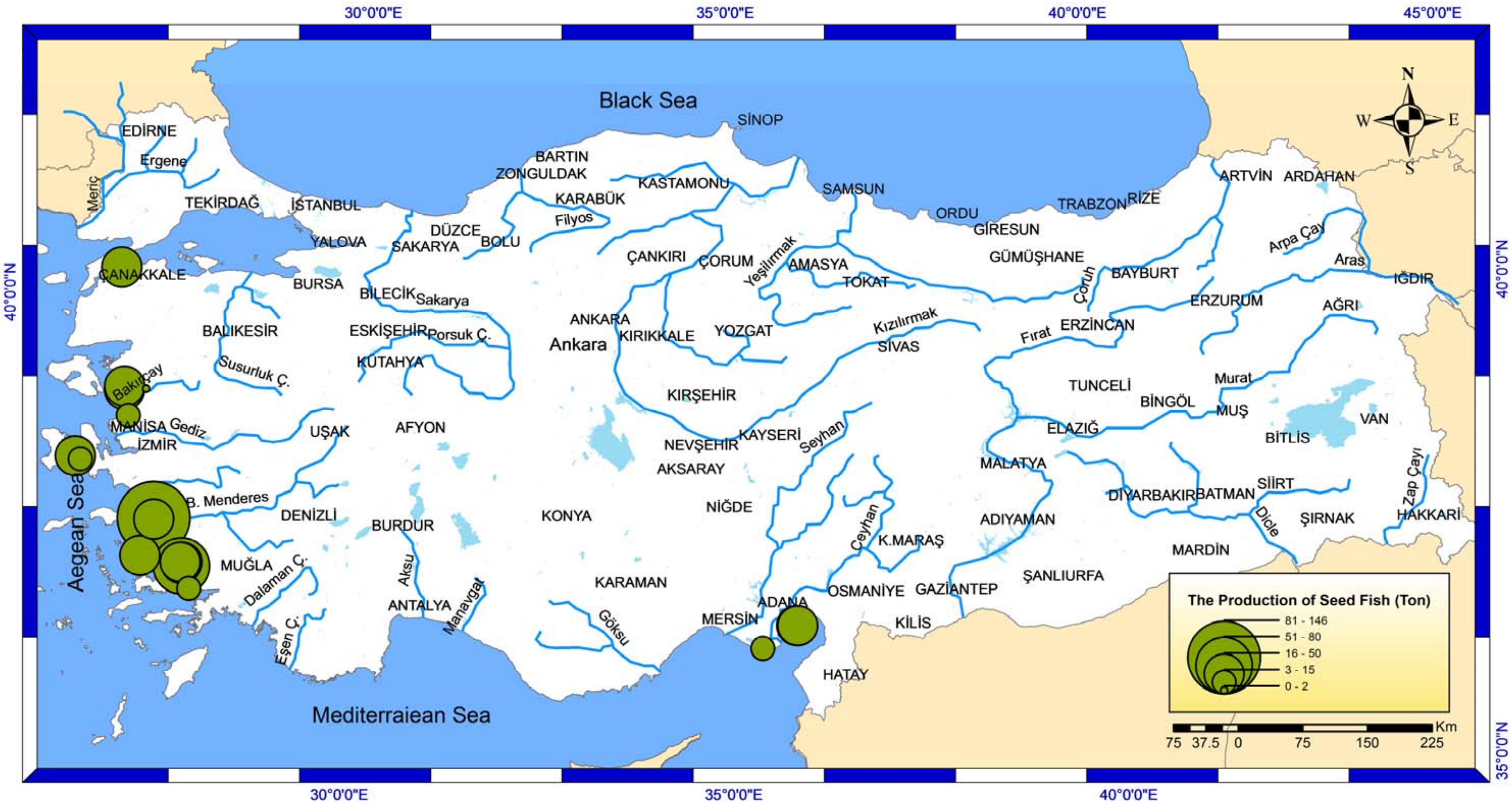
Table 4. Inland and Marine Fish Species Farmed and Their Respective Production Amounts (in Ton)

Fish Species	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Total	**94010**	**118 77**	**128943**	**139873**	**152186**	**158729**	**167141**	**188790**	**212410**	**233393.9**
Inland										
Trout	43432	48033	56026	58433	65928	75657	78165	100239	111335	122873.3
Mirror Carp	683	571	668	600	629	591	403	207	222	145.5
Marine										
Trout	1650	1249	1633	2740	2721	5229	7079	7697	3234	5186
Gilt-head sea bream	20435	27634	28463	33500	31670	28362	28157	32187	30743	35701.1
Sea Bass	26297	37290	38408	41900	49270	46554	50796	47013	65512	67912.5
Other	1513	3500	3745	2700	1968	2336	2541	1447	1364	1575.3

From the Records of the Ministry of Food, Agriculture and Livestock

Table 5. Saltwater Fish Hatcheries in Turkey - February 2013 (BSGM, 2013)

Project Title	Location	Capacity (In Ton)	Produced Species
AKUVATUR-Karataş	ADANA-Karataş	39	Gilt-head sea bream, Sea bass, Dentex, Red Seabream, Bluespotted Seabream, Meager, Red Mullet, Red Striped Seabream, Common Seabream, Axillary Seabream, Sharp Snout Seabream Sargo, Sole, Amberjack
YUNUSLAR	ADANA-Yumurtalık	15	Gilt-head sea bream, Sea bass, Shrimp
EGEMAR	AYDIN-Didim	39	Gilt-head sea bream, Sea bass, Meager, Corb, White Grouper, Dentex, Red Seabream, Common Seabream, Sharp Snout Seabream Sargo, Amberjack
KILIÇ-Bafa	AYDIN-Söke	146	Gilt-head sea bream, Sea bass, Striped Seabream, Sharp Snout Seabream Sargo, Corb, Common Seabream, White Grouper, Dentex, Red Seabream, Meager
SERÇİN	AYDIN-Söke	30	Gilt-head sea bream, Sea bass
İDA GIDA	ÇANAKKALE-Lapseki	40	Gilt-head sea bream, Sea bass
AKUA-TEK	İZMİR-Dikili	38	Gilt-head sea bream, Sea bass, Dentex, Red Seabream, Meager, Common Seabream, Corb, Clown
ÇAMLI-Alaçatı	İZMİR-Çeşme	50	Gilt-head sea bream, Sea bass
ÇAMLI-Ildırı	İZMİR-Çeşme	10	Gilt-head sea bream, Sea bass, Sharp Snout Seabream Sargo, Dentex, Axillary Seabream, Striped Seabream, Brown Meager, Oyster, Scallop, Cockle
İLKNAK	İZMİR-Dikili	20	Gilt-head sea bream, Sea bass, Sharp Snout Seabream Sargo, Dentex, Saddled Seabream, Meager
AKUVATUR -Yeni Şakran	İZMİR-Aliağa	7.5	Gilt-head sea bream, Sea bass, Dentex, Sharp Snout Seabream Sargo, Brown Meager, White Seabream, Corb, Common Seabream, Red Seabream, Meager, Red Striped Seabream, Axillary Seabream
AKUA-TEK -TURKUAZ MARİN	İZMİR-Bergama	2	Gilt-head sea bream, Sea bass
AKUVATUR-Avşar	MUĞLA-Milas	38	Gilt-head sea bream, Sea bass, Sharp Snout White Grouper, Meager, Dentex, Corb, Barbun, Red Striped Seabream, Common Seabream, Axillary Seabream, Sole, Amberjack
HATKO	MUĞLA-Ören	15	Gilt-head sea bream, Sea bass
KILIÇ-Güvercinlik	MUĞLA-Milas	39.5	Gilt-head sea bream, Sea bass, Dentex, Corb, Amberjack
KILIÇ- Akarca	MUĞLA-Milas	39	Gilt-head sea bream, Sea bass, Turbot, Sole
KILIÇ-Ören	MUĞLA-Milas	80	Gilt-head sea bream, Sea bass, Sole, Amberjack, Dentex, Corb, Sharp Snout Seabream Sargo, Red Seabream, White Grouper



Map 2. Distribution of Fish Hatcheries in Turkey

Most valued species are Red Seabream, White Grouper, Dentex and Shrimp whose population is critically low in the wild and economic value is very high. The tuna market which has rapidly grown in the world and in Turkey over the last years provides a considerable amount of foreign currency income. In Turkey, the first tuna farms were opened in 2002. Tuna farms are gradually becoming widespread, leading to economic competitions between countries. Because these farms quickly yield profit although their establishment is costly, many exporters and fishermen are attracted to this undertaking. In summer when prices are relatively lower, tunas are wild caught and fed for 3-6 months and introduced in the market when prices are higher.

As understood from the table, hatchings for aquaculture are performed along the coastline in Turkey. In consideration of total production by provinces, it is seen that 36.2% is performed in Aydın, 35.6% in Muğla, 21.5% in İzmir, 9% in Adana and 6.7% in Çanakkale. 90% of juvenile fish farming is done on the western coastline, i.e. Muğla, Aydın, İzmir and Çanakkale. Hatched fries are also released into the wild. The number of provinces where fish are released in the wild was 28 in 2002 and escalated to 39 in 2012, and the number of sources increased from 200 to 498. The area where fries are introduced covers 152 thousand hectares while the number of released fish is 3.5 million (BSGM, 2013) (Map 2).

Table 6, 7 and 8 include data on the export of farmed species. Trout is mostly exported to Romania, Russia, Germany and Poland, bass to the Netherlands, Libya, the UK, Italy, Germany and gilt-head sea bream to Lebanon, the Netherlands, Libya, Italia, Germany, the UK. Along with fish species, such species as shrimp, oyster and the like are raised in Turkey.

Locations, Characteristics and Distribution of Aquaculture

Turkey has rich water resources thanks to its location, and its climatic conditions and water resources are potentially suitable for aquaculture. These are important factor for the development of fishery sector.

Table 6. Trout Export (Patrona, 2013)

Product Type	Countries	Percentage (%)
Fresh, Cooled	Romania Russia Poland Holland	25 22 12 8
Frozen*	Germany Poland Czech Republic	48 19 7
Smoked	Germany Holland	83 11

*Inc. Fleet

Tablo 7. Bass Export (Patrona, 2013)

Product Type	Countries	Percentage (%)
Fresh, Cooled	Holland Italy Spain Russia	24 16 12 15
Fleet (Fresh, Cooled)	Holland Italy	94 3
Frozen bass	Libya Germany Russia	43 19 8
Filet (Frozen)	United Kingdom Italy France	32 27 13

Turkey has a coastline of 8333 km: Anatolian coastline covers 6480 km, while Thracian and island coastlines cover 786 km and 1067 km, respectively. The Aegean coastline is the longest in Turkey, which extends over 2805 km.dir. It is followed by the Black Sea coastline of 1695 km, the Mediterranean coastline of 1577, the Marmara coastline of 927, the Dardanelles coastline of 172 km and the Bosporus coastline of 90 km (Map 1).

As seen in the Tables, 21 natural lakes alone cover a surface area of more than 8000 km^2, and 18 researvoirs over 3000 km^2 and only 9 rivers over 4000 km.

Table 8. Gilt-head sea bream Export (Patrona, 2013)

Product Type	Countries	Percentage (%)
Fresh, Cooled	Russia Lebanon Holland Italy	14 19 16 14
Filet (Fresh, Cooled)	Holland İtaly	92 7
Frozen	Lebanon Germany Holland, Russia	67 19 4
Fillet (Frozen)	Italy Holland United Kingdom	50 21 17

Table 9. Surface Area of Major Lakes in Turkey ($35\ km^2$ and over) (TUIK, 2014)

Lake	Km^2	Lake	Km^2
Van	3713	Bafa	60
Tuz	1500	Erçek	98
Beyşehir	656	Hazar	86
Eğirdir	468	Köyceğiz	52
Akşehir	353	Işıklı	49
İznik	298	Nazik	48
Burdur	200	Sapanca	47
Kuş (Manyas)	166	Salda	45
Ulubat	134	Yay	37
Eber	126	Akyatan	35
Çıldır	115		

Table 10. Major Rivers and Their Lengths (500 km and over) (TUIK, 2014)

Rivers	Length (km)
Kızılırmak	1355
Fırat (in Turkey)	1263
Sakarya	824
Murat	562
Seyhan	560
Aras (in Turkey)	548
Dicle (in Turkey)	523
Yeşilırmak	519
Ceyhan	509

Table 11. Major Reservoir and Their Surface Areas (TUIK, 2014)

Reservoir	Surface Areas (km^2)
Atatürk	817.0
Keban	675.0
Karakaya	268.0
Hirfanlı	263.0
Manyas	167.4
Altınkaya	118.3
Alparslan-1	114.8
Yamula	88.6
Çatalan	81.9
Sarıyar	78.2
Boyabat	65.5
Kılıçkaya	64.4
Seyhan	63.0
Ermenek	58.7
Kralkızı	57.5
Birecik	56.3
Obruk	50.2
Aslantaş	49.9

Table 12. Turkey's Fishery Product Resources (State palaning Agency, 2001)

Production Area	**Amount**	**Size (Ha)**	**Length (Km)**
Natural Lakes	200	906118	-
Reservoirs	193	342377	-
Ponds	Over 750	15500	-
Rivers	33	-	177714
Seas	-	24607.200	8333
TOTAL		**25871.195**	

To mention the seas surrounding Turkey on three sides: the surface water temperature of the Mediterranean is higher than the annual average temperature. Because of light precipitation and high evaporation, salinity of the Mediterranean is very high, which is around 38-39‰. Salinity decreases towards north and become much lower where rivers discharge.

The current from the Black Sea to the Marmara via the Bosphorus forms a thin surface layer with low salinity (21-25‰). Research on the salinity of the Aegean Sea has revealed the vestiges of a current with low salinity from Marmara through the Dardanelles. For instance, the salinity of the Western Thracian Sea is 30‰ due to the salinity reducing effect of this current. However, it is 38-38.5‰ in the central and southern part of the Aegean Sea. While the salinity is 33‰ around the Biga Peninsula, it is 38.5‰ in the Edremit Gulf (Ardel, 1975). The surface water of the Marmara Sea is typically less saline, which is 22‰. Salinity increases along with the depth. There is a surface current which flows from the Black Sea to Marmara and then to the Aegean Sea and a deeper current running beneath in the opposite direction. These currents affect both salinity and temperature.

The Black Sea is under the influence of the Mediterranean climatic conditions in summer and of terrestrial climate in winter. The temperature of the surface water remains between 20°C and 26°C in summer and 7°C in the southern Black Sea in winter. Because large rivers discharge great amount of fresh water in the Black Sea and due to heavy precipitation and less evaporation, the salinity of the Black Sea is very low (18‰). Towards west and north, the salinity gets lower.

Wide sea surface is always in contact with the atmosphere and contains dissolved gases. In the Black Sea, oxygen amount rapidly decreases below 70-120 meters underwater (Ardel, 1975). Oxygen is only available in the surface water.

The Black Sea has a medium salinity and it get richer in nutrients, which creates a fertile setting. This boosts the population of the migrating pelagic fish such as sprats and anchovies. Because the northern parts of the Marmara Sea and the Aegean Sea are mixed with the warm water of the Mediterranean and the nutritious water of the Black Sea, they are also fertile areas. Besides, these are locations home to such species as mussel, sand mussel and oyster, which are economically important for exportation, be they live or processed.

Distribution of Aquaculture in Turkey

Research has revealed many locations in Turkey suitable for fish farming. For example, salmon, sea trout, turbot have been found to be the right species for the Black Sea; salmon, sea trout, turbot, sea bass, mullet, mussel and oyster for the Marmara; eel, mullet, mussel and sargo for the Aegean Sea and fish and shrimp farming for the Mediterranean Sea.

Table 13. Aquaculture by Region (2013)

REGION	**AMOUNTS (TON)**
Aegean Region	128006.7
Eastern Anatolia Region	28790.8
Mediterranean Region	22200.4
Black Sea Region	21383.8
Central Anatolia Region	19015
South Eastern Anatolia Region	10331.8
Marmara Region	3665.4
Total	**233393.9**

Aquaculture is widely performed in the Aegean Region (55%), which is followed by Eastern Anatolia Region (12%), the Mediterranean Region (10%), the Black Sea Region (9%), the Central Anatolia (8%), Southeastern Anatolia

(4%), and the Marmara Region (2%). Production in the Aegean Region is more than the total production of the remaining regions. (Map 3)

The Aegean Sea has a coastline of 2805 km and numerous bays and gulfs. Its narrow continental shelf and strategic location have increased the importance of coastal fishery.

Ecological differences are observed in the Aegean Sea. Flows from north and south intermingle here. Therefore, it is home to psychrophilic and thermophilic creatures. For aforementioned reasons, different species live in the south and north of the Aegean Sea. Thus, biodiversity is very rich: Some 400 algae, 5000 invertebrates and 300 fish species were identified. In the region fish farming makes contributions to production and market development and creates employment for many. This activity gradually increases and obtained values become indispensable for the economy.

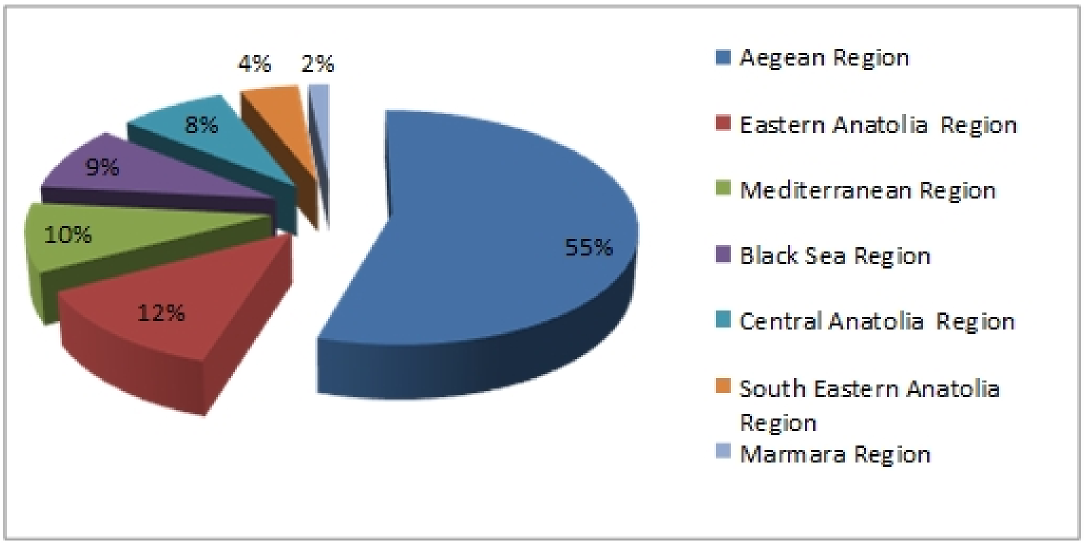
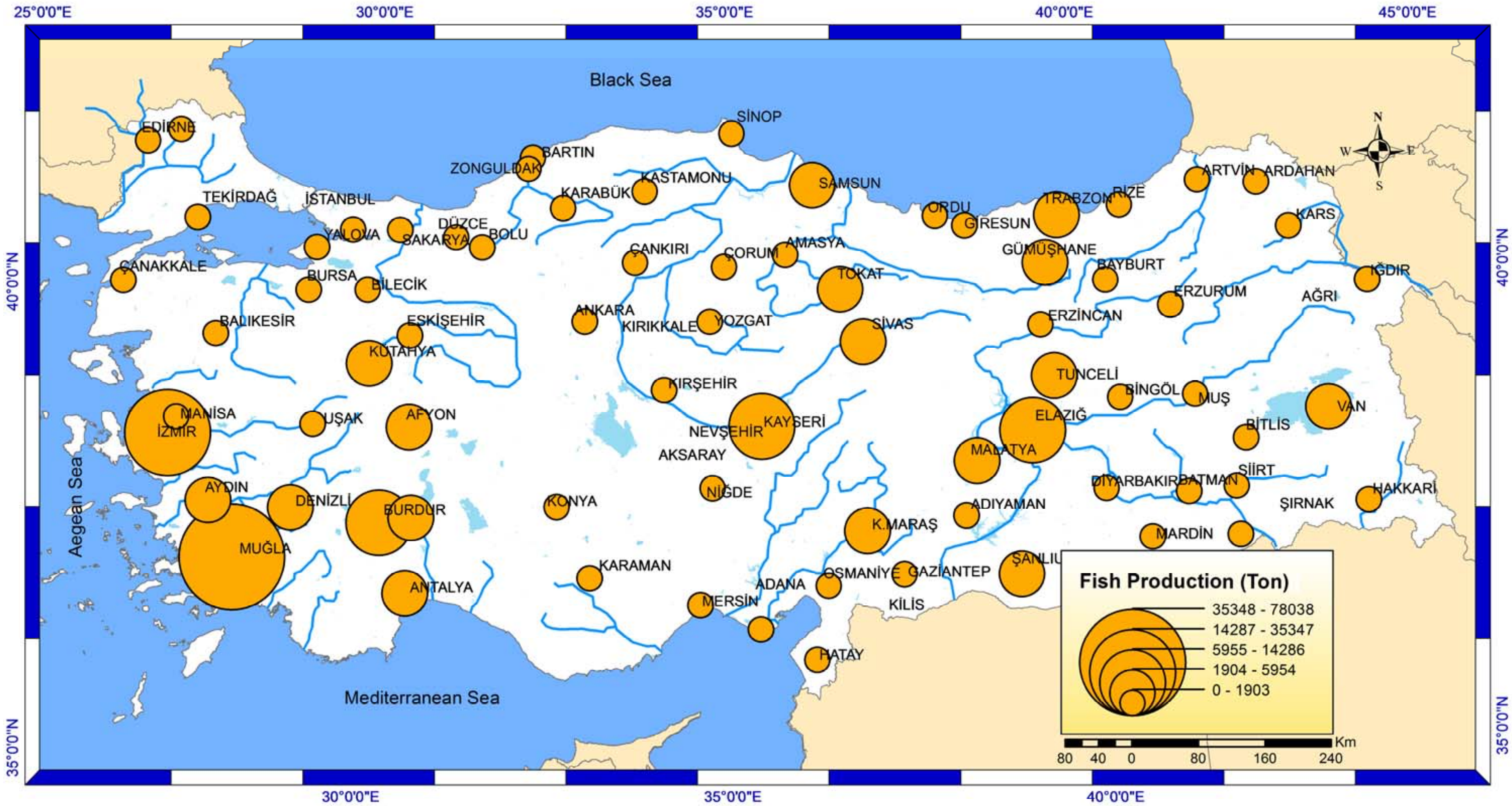


Figure 1. Distribution of Aquaculture in Turkey by Region (2013)

Table 14. Aquaculture in the Aegean Region (Species and Amounts) (2013)

PROVINCES	SPECIES	AMOUNTS (TON)
Afyonkarahisar	Trout (Inland)	2808.5
Aydın	Trout (Inland)	2895.3
	Gilt-head sea bream (Marine)	223.4
	Bass (Marine)	1265.1
Denizli	Trout (Inland)	3720.0
İzmir	Trout (Inland)	391.7
	Gilt-head sea bream (Marine)	14702.2
	Bass (Marine)	19344.7
	Other(Marine)	907.5
Kütahya	Trout (Inland)	2120.8
Manisa	Trout (Inland)	1133.3
	Mirror Carp (Inland)	44
Muğla	Trout (Inland)	13900.0
	Gilt-head sea bream (Marine)	20000.0
	Bass (Marine)	43500.0
	Other(Marine)	637.8
Uşak	Trout (Inland)	399.9
	Mirror Carp (Inland)	12.5
TOTAL		**128006.7**



Map 3. Distribution of Total Fish Production in Turkey by Provinces

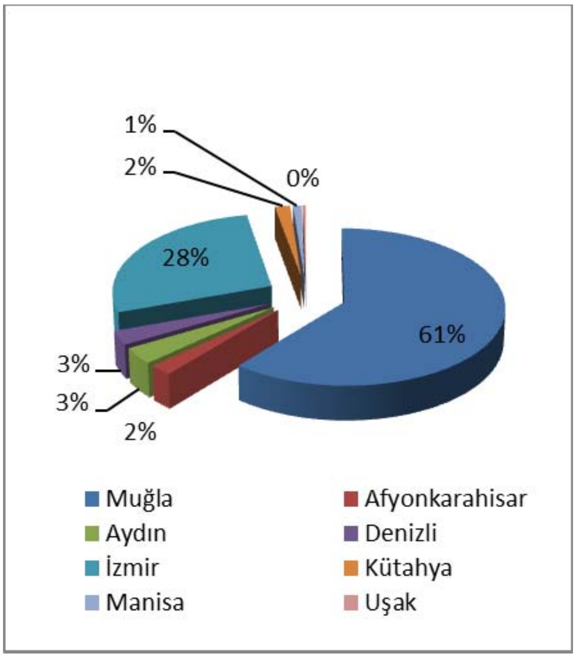


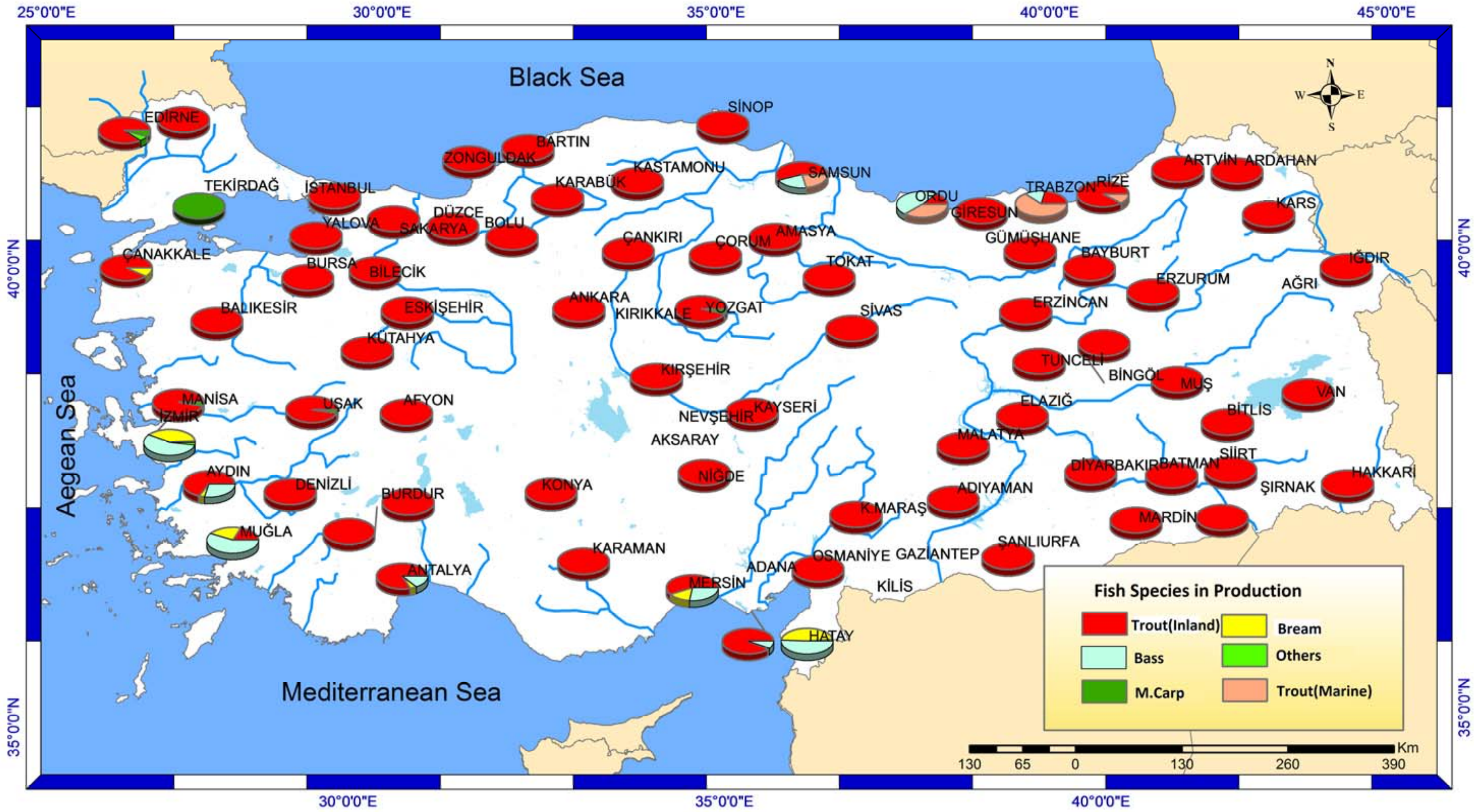
Figure 2. Distribution of Aquaculture in the Aegean Region (2013)

In consideration of regions in terms of farmed species and harvested amounts, production in the Aegean Region amounts to 128006.70 tons. Raised species are sea bass, gilt-head sea bream, trout, mirror carp and other aquatic species. (Map 4, 5, 6, 7, 9, 10)

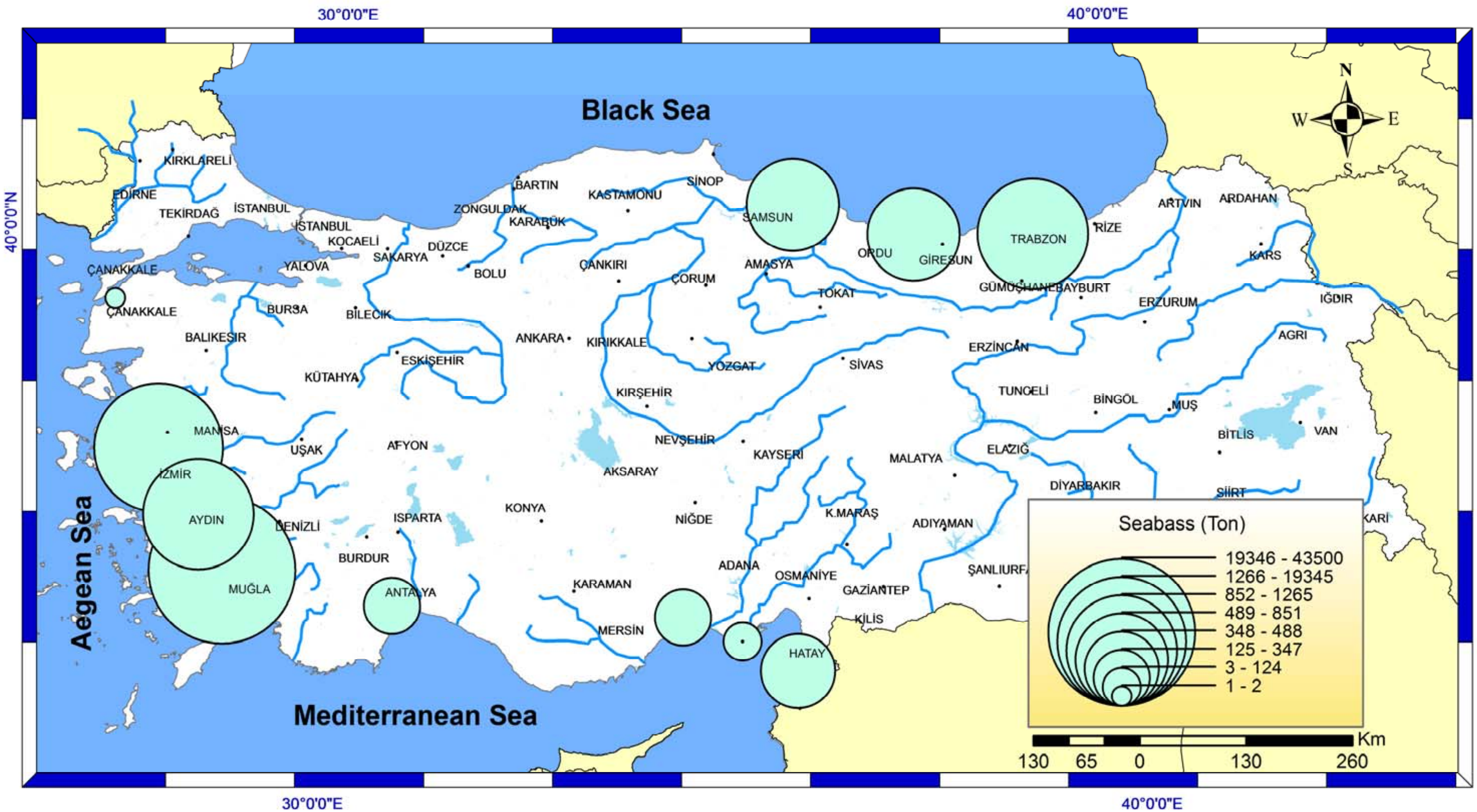
The most farmed species are sea bass and gilt-head sea bream. In addition, Muğla and İzmir stand out as top raisers. In the Aegean Region, generally trout is farmed in the inland waters and sea bass and gilt-head sea bream in the sea. Muğla produces by far the highest amount of fish (be it trout or bream and sea bass). Its production accounts for 61% of the total harvest in the Aegean Region. In consideration of other regions, Muğla's production is much higher than the total amount produced by each region. In terms of trout farming, Muğla is followed by Denizli, Aydın, Afyonkarahisar, Kütahya, Manisa, Uşak, İzmir. As for gilt-head sea bream and sea bass raising, it is followed by İzmir and Aydın. In the Aegean Region, 100580.7 tons of fish are produced, which accounts for 78.6% of the total yield of the region.

Table 15. Aquaculture in the Mediterranean Region (Species and Amounts) (2013)

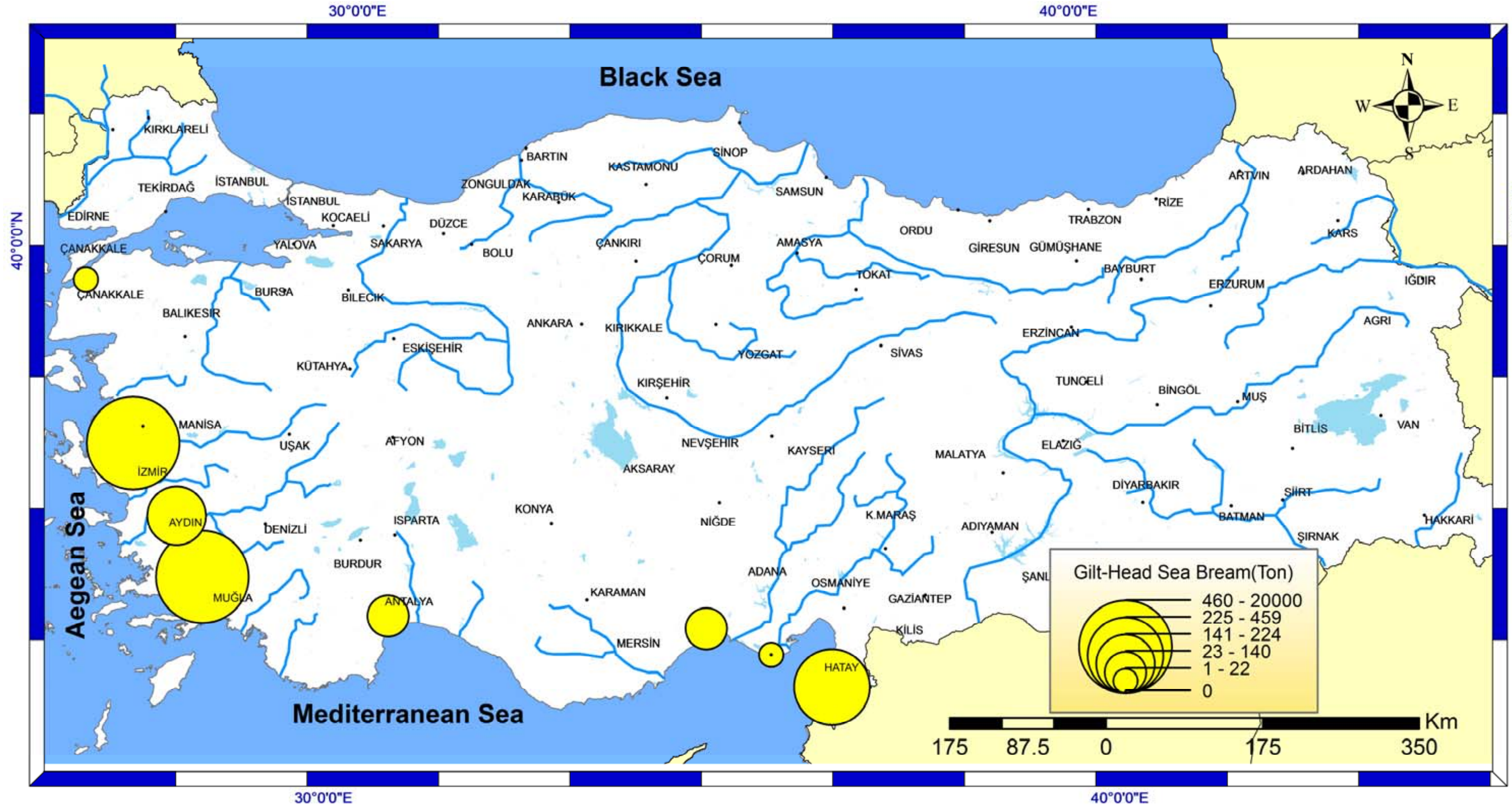
PROVINCES	SPECIES	AMOUNTS (Ton)
Adana	Trout (Inland)	1600.0
	Gilt-head sea bream (Marine)	22.1
	Bass (Marine)	123.7
	Other(Marine)	2
Antalya	Trout (Inland)	2184.1
	Gilt-head sea bream (Marine)	132.6
	Bass (Marine)	346.9
Burdur	Trout (Inland)	9724.0
Hatay	Trout (Inland)	12.9
	Mirror Carp (Inland)	9.3
	Gilt-head sea bream (Marine)	459
	Bass (Marine)	488
Isparta	Trout (Inland)	3605.0
Mersin	Trout (Inland)	499.7
	Gilt-head sea bream (Marine)	140
	Bass (Marine)	241.6
Kahramanmaraş	Trout (Inland)	2493.00
Osmaniye	Trout (Inland)	116.5
TOPLAM		**22200.4**



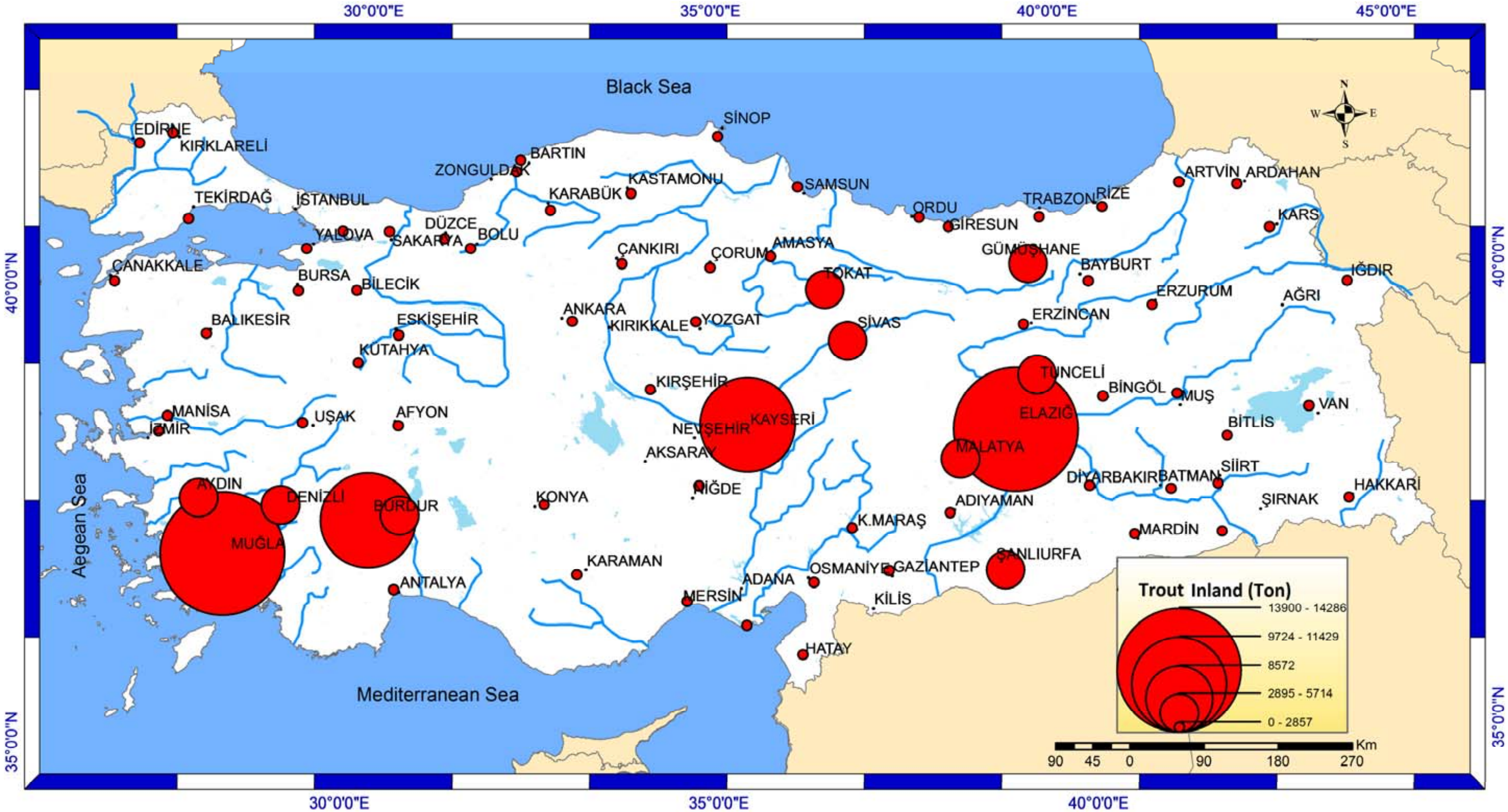
Map 4. Distribution of Fish Species in Turkey by Provinces



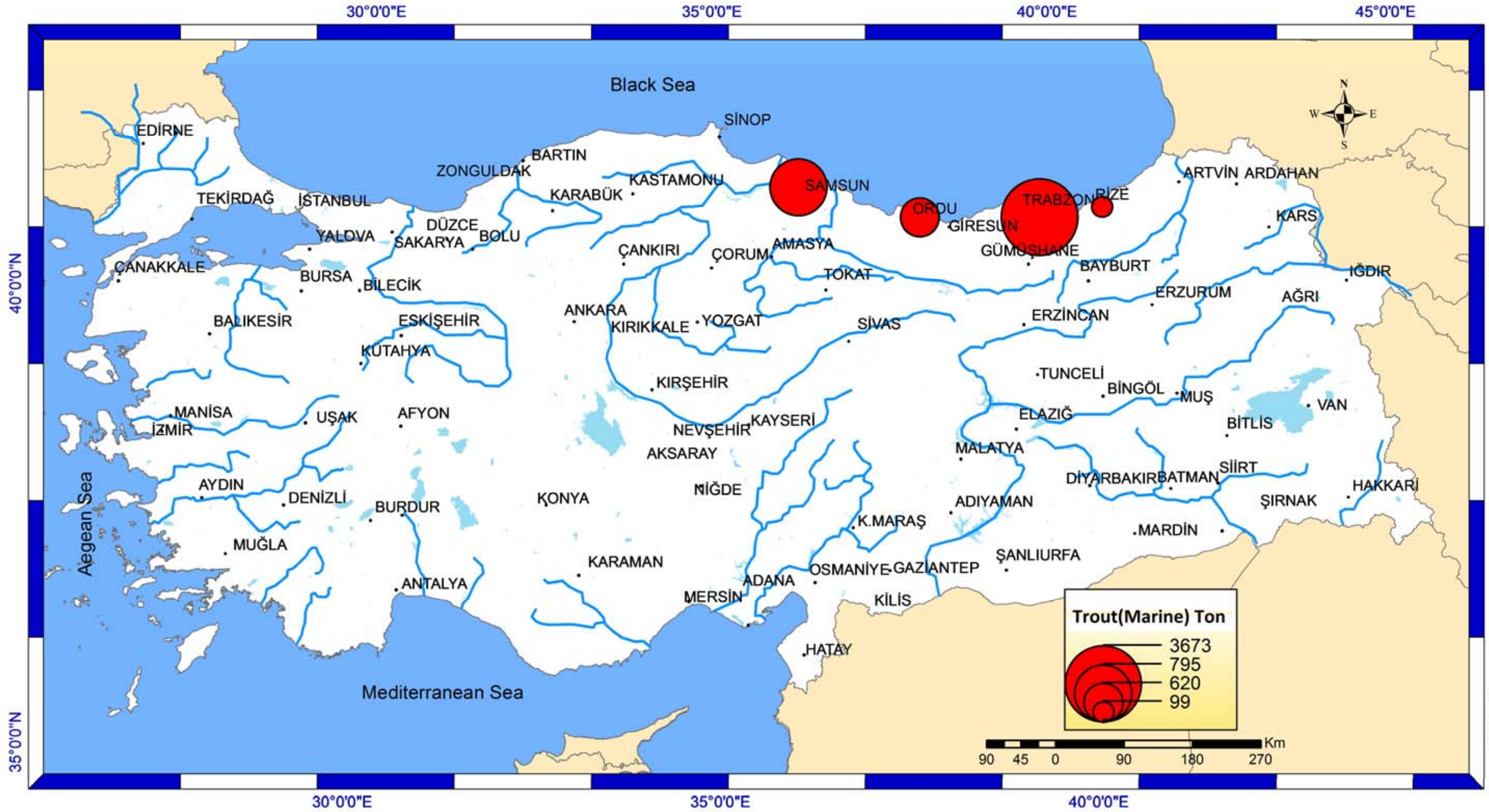
Map 5. Distribıution of Seabass Farming in Turkey by Provinces



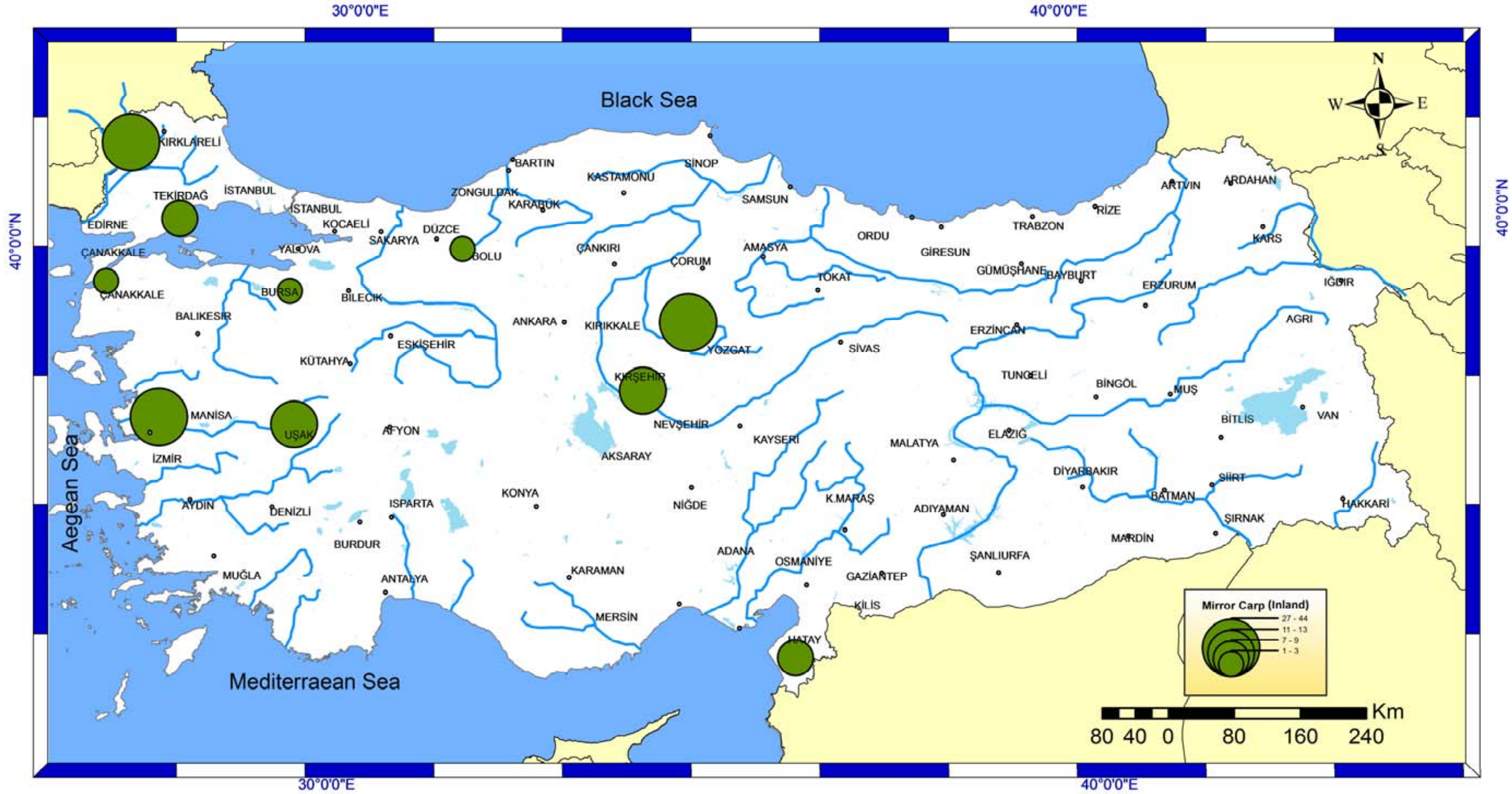
Map 6. Distribution of Gilt-head sea bream Farming in Turkey by Provinces



Map 7. Distribution of Trout Farming (Inland) in Turkey by Provinces



Map 8. Distribution of Trout Farming (Marine) in Turkey by Provinces



Map 9. Distribution of Mirror Carp (Inland Water) Farming in Turkey by Provinces

Map 10. Distribıution of Other Sea Products Farming in Turkey by Provinces

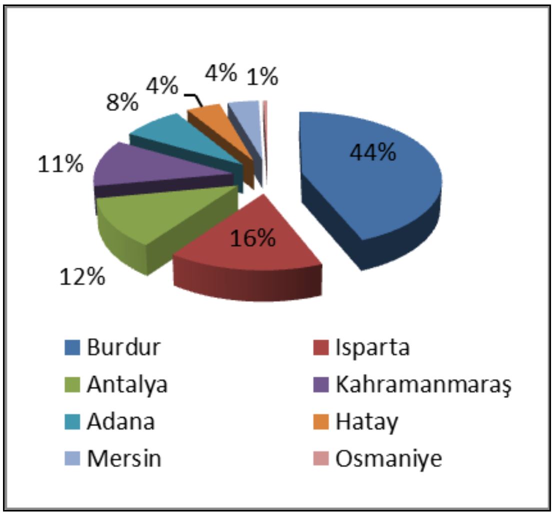


Figure 3. Distribution of Aquaculture in the Mediterranean Region (2013)

Aquaculture production amounts to 22200.40 tons in the Mediterranean Region. Raised species are predominantly trout, gilt-head sea bream, sea bass, mirror carp and other marine species. (Map 4, 5, 6, 7, 9, 10) Trout is most commonly farmed in Burdur (48%), followed by Isparta (18%), Kahramanmaraş (12%), Antalya (11%), Adana (8%), Mersin (2%), Osmaniye and Hatay (1%) and Burdur (a very small share). In terms of gilt-head sea bream production, Hatay is at the top (61%), followed by Mersin and Antalya (18%) and Adana (3%). As for sea bass production, Hatay harvests the highest amount of sea bass, followed by Antalya (29%), Mersin (20%) and Adana (10%). Mirror carp is farmed in Hatay (9.3%) and other species are raised in Adana (2 tons). In the Mediterranean Region, 1955.9 tons of fishery products are harvested in the sea, accounting for 8.8% of the total production.

Total production from aquaculture in the Black Sea Region is 21383.8 tons. As seen in the Figure, Trabzon has the biggest share in production. It is followed by Tokat, Samsun and Gümüşhane with similar shares, after which Ordu, Artvin and the others come.

The predominantly raised species in the Black Sea Region is trout along with sea bass. Moreover, mirror carp is farmed in Bolu. In the Black Sea Region, trout is farmed in both sea and inland waters. (Map 4, 5, 7, 8, 9)

Tokat has the highest amount of trout (3714 tons) raised in the inland waters. It is followed by Gümüşhane, Samsun, Trabzon, Rize, Artvin, Bolu, Bayburt, Giresun, and Ordu. Düzce, Karabük, Bartın, Zonguldak, Sinop, Kastamonu, Çorum, and Amasya are the cities where over 200 tons of trout is farmed. Trabzon is in the first place to farm trout in sea, and followed by Samsun, Ordu and Rize. In the Black Sea Region, generally trout is raised. Sea bass is farmed in Trabzon (994.3 tons), Samsun (851.2 tons) and Ordu (755.5 tons). In the region, 7787.2 tons of products have been harvested from the sea, which account for 36.4% of the total production.

The lowest amount of aquaculture products is harvested in the Marmara Region. Total amount is 3665.4 tons. Sakarya is where the most intensive aquafarming is performed. Bilecik (17%), which is followed by Balıkesir, Edirne and Bursa (13-14%), comes after Sakarya. The Figure reveals that Kocaeli, Çanakkale and Kırklareli have smaller shares. It is even smaller in Yalova and Tekirdağ.

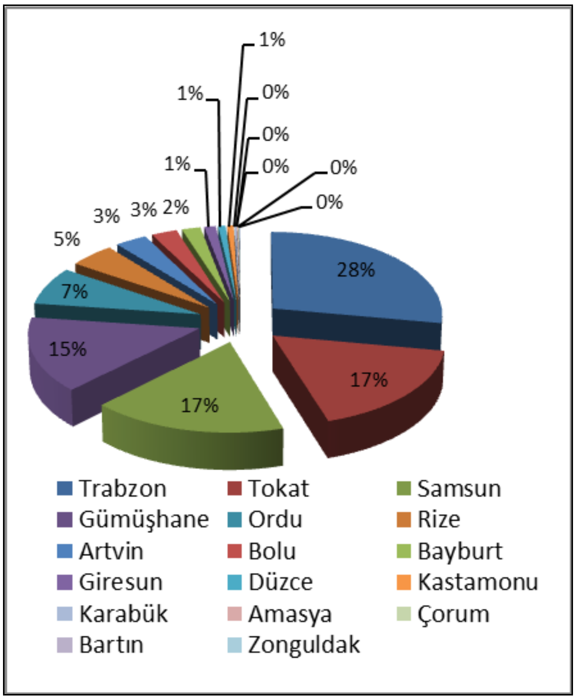


Figure 4. Distribution of Aquaculture in the Black Sea Region (2013)

Table 16. Aquaculture in the Black Sea Region (Species and Amounts) (2013)

PROVINCES	SPECIES	AMOUNTS (Ton)
Amasya	Trout (Inland)	24.6
Artvin	Trout (Inland)	697.8
Bolu	Trout (Inland)	558.8
	Mirror Carp (Inland)	0.7
Çorum	Trout (Inland)	14.8
Giresun	Trout (Inland)	250
Gümüşhane	Trout (Inland)	3121.8
Kastamonu	Trout (Inland)	134
Ordu	Trout (Inland)	203
	Trout (Inland))	620
	Bass (Marine)	755.5
Rize	Trout (Inland)	899.6
	Trout (Marine)	98.5
Samsun	Trout (Inland)	2041.0
	Trout (Marine)	795.3
	Bass (Marine)	851.2
Sinop	Trout (Inland)	17.8
Tokat	Trout (Inland)	3714.0
Trabzon	Trout (Inland)	1286.9
	Trout (Marine)	3672.4
	Bass (Marine)	994.3
Zonguldak	Trout (Inland)	11.2
Bayburt	Trout (Inland)	404
Bartın	Trout (Inland)	12.6
Karabük	Trout (Inland)	42
Düzce	Trout (Inland)	162
TOTAL		**21383.8**

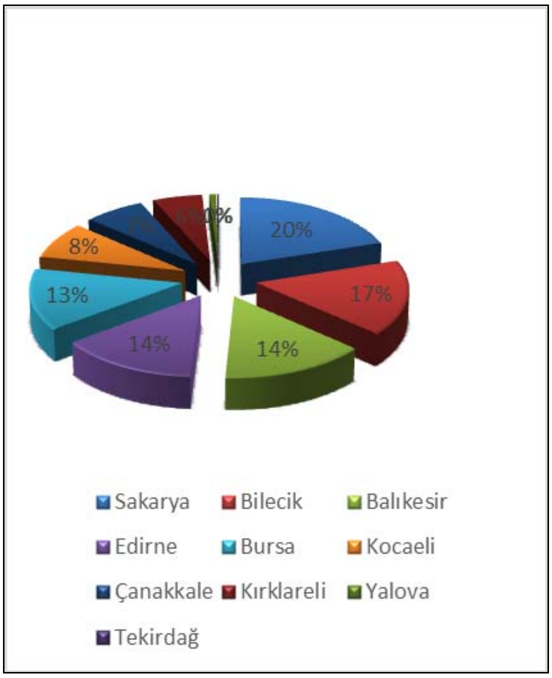


Figure 5. Distribution of Aquaculture in the Marmara Region (2013)

Table 17. Aquaculture in the Marmara Region (Species and Amounts) (2013)

PROVINCES	SPECIES	AMOUNTS (Ton)
Balıkesir	Trout (Inland)	527.6
Bilecik	Trout (Inland)	604
Bursa	Trout (Inland)	489.3
	Mirror Carp (Inland)	2.3
Çanakkale	Trout (Inland)	229.8
	Mirror Carp (Inland)	1.8
	Gilt-head sea bream (Marine)	21.8
	Bass (Marine)	1.5
Edirne	Trout (Inland)	450
	Mirror Carp (Inland)	29.5
	Other(Marine)	28
Kırklareli	Trout (Inland)	208
Kocaeli	Trout (Inland)	300.8
Sakarya	Trout (Inland)	735
Tekirdağ	Mirror Carp (Inland)	7
Yalova	Trout (Inland)	29
TOTAL		**3665.4**

The most common aquaculture species in the Marmara Region is trout. Other than trout, mirror carp, gilt-head sea bream, sea bass and other fishery species are raised in very small amounts. (Map 4, 5, 6, 7, 9 and 10)

The highest trout production is harvested in Sakarya, followed by Bilecik, Balıkesir, Bursa, Edirne, Kocaeli, Çanakkale, Kırklareli, and Yalova. Mirror carps are mostly farmed in Edirne, Bursa, Çanakkale and Tekirdağ. But production amount is not high. Total production amounts to around 50 tons, more than half of which is harvested in Edirne. Gilt-head sea bream and sea bass are farmed only in Çanakkale, while other species are raised in Edirne. 51.3 tons of the total production is obtained from the sea. This is equal to 1.4% of the total production in the Marmara Region.

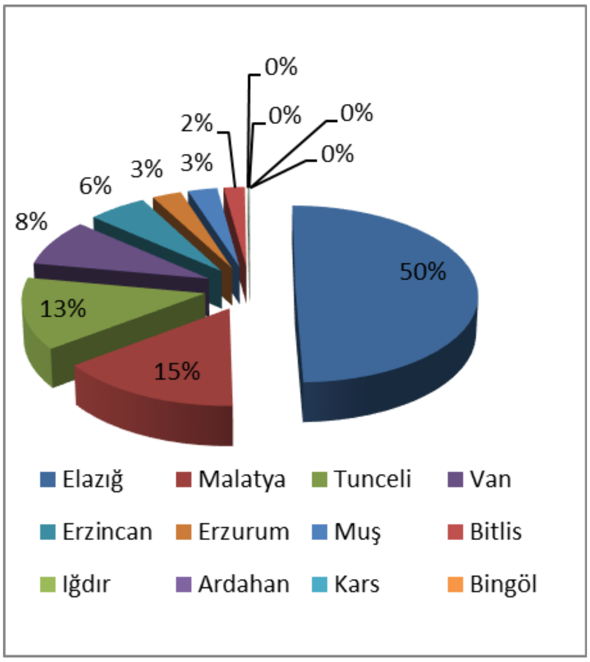


Figure 6. Distribution of Aquaculture in the Eastern Anatolia Region (2013)

Total production in the Eastern Anatolia Region is 28790.8 tons. The only farmed species is trout. (Map 7), The highest production is in Elazığ. It accounts for half of the total production in the East Anatolian Region. Elazığ is followed by Malatya, Tunceli, Van, Erzincan, Erzurum, Muş, and Bitlis, while Iğdır, Ardahan, Kars, and Bingöl have the lowest production rates.

Table 18. Aquaculture in the Eastern Anatolia Region (Species and Amounts) (2013)

PROVINCES	SPECIES	AMOUNTS (Ton)
Bingöl	Trout (Inland)	1
Bitlis	Trout (Inland)	583.8
Elazığ	Trout (Inland)	14.286.3
Erzincan	Trout (Inland)	1.643.1
Erzurum	Trout (Inland)	815.6
Kars	Trout (Inland)	10
Malatya	Trout (Inland)	4350.0
Muş	Trout (Inland)	815
Tunceli	Trout (Inland)	3779.0
Van	Trout (Inland)	2469.0
Ardahan	Trout (Inland)	15
Iğdır	Trout (Inland)	23
TOTAL		**28790.8**

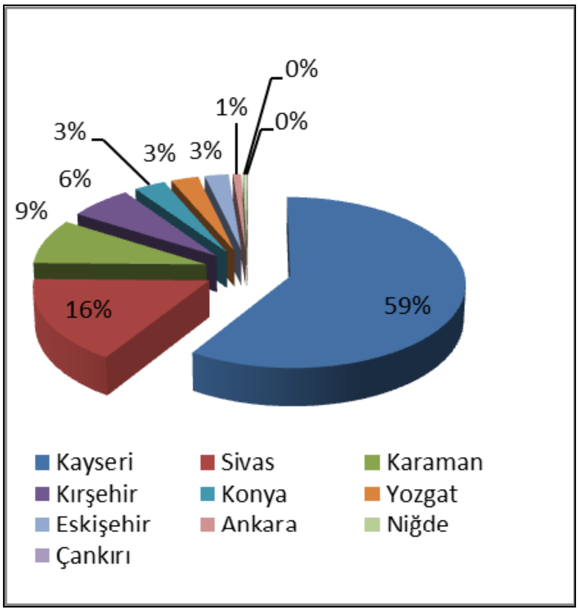


Figure 7. Distribution of Aquaculture in the Central Anatolia Region (2013)

Table 19. Aquaculture in the Central Anatolia Region (Species and Amounts) (2013)

PROVINCES	SPECIES	AMOUNTS (Ton)
Ankara	Trout (Inland)	156.4
Çankırı	Trout (Inland)	23.4
Eskişehir	Trout (Inland)	460.7
Kayseri	Trout (Inland)	11227.0
Kırşehir	Trout (Inland)	1206.3
Kırşehir	Mirror Carp (Inland)	11.2
Konya	Trout (Inland)	604.8
Niğde	Trout (Inland)	67.1
Sivas	Trout (Inland)	3084.10
Yozgat	Trout (Inland)	503.8
Yozgat	Mirror Carp (Inland)	27.2
Karaman	Trout (Inland)	1643.0
TOTAL		**19015**

Total production in the Central Anatolia Region is 19015 tons with trout being the dominant species. 27.2 tons and 11.2 tons of mirror carp is farmed only in Yozgat and Kırşehir, respectively. (Map 4, 7 and 9) Production in Kayseri constitute more than half of the total production in the Central Anatolia Region. Kayseri is followed by Sivas, Karaman, Kırşehir, Konya, Yozgat, Eskişehir, Ankara, Niğde, and Çankırı.

Total production in the Southeastern Anatolia Region is 10331.8 tons and the only species farmed is trout. (Map 7) Almost half is produced in Şanlıurfa, followed by Diyarbakır, Gaziantep, Batman, and Adıyaman. Production in Hakkari, Mardin, Siirt and Şırnak is even lower.

As in the Aegean Region, the amount of the production in Muğla is nearly half of the national production. Muğla has a special place in aquacul-ture. The indented formation of the coastline has created the suitable setting for Muğla to be able to start aquacultural activities in 1982. The number of the successful practices increased from 1986 onwards.

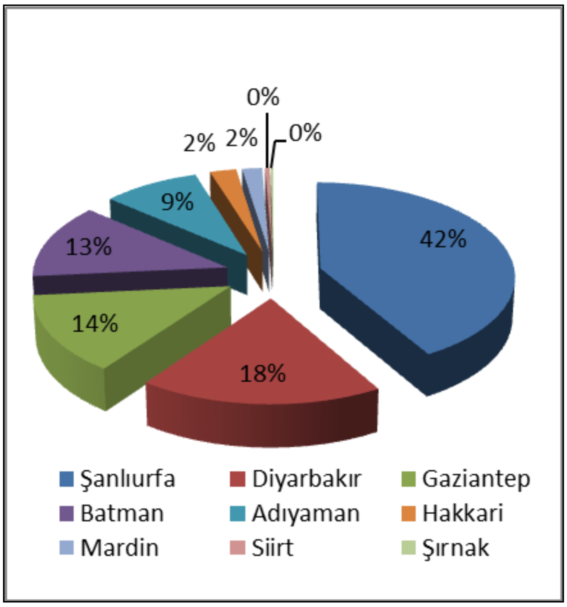


Figure 8. Distribution of Aquaculture in the Southeastern Anatolia Region (2013)

Table 20. Aquaculture in the Southeastern Anatolia Region (Species and Amounts) (2013)

PROVINCES	SPECIES	AMOUNTS (Ton)
Adıyaman	Trout (Inland)	915.5
Diyarbakır	Trout (Inland)	1902.9
Gaziantep	Trout (Inland)	1393.8
Hakkari	Trout (Inland)	247.8
Mardin	Trout (Inland)	187.7
Siirt	Trout (Inland)	40,9
Şanlıurfa	Trout (Inland)	4297.7
Batman	Trout (Inland)	1321.0
Şırnak	Trout (Inland)	24.5
TOTAL		**10331.8**

Table 21. Top Ten Provinces by Their Production Amounts (2013)

PROVINCES	AMOUNTS (Ton)
Muğla	78037.8
İzmir	35346.1
Elazığ	14286.3
Kayseri	11227.0
Burdur	9724.0
Trabzon	5953.6
Aydın	4383.8
Malatya	4350.0
Şanlıurfa	4297.7
Tunceli	3779.0
Total	**177385.3**
Other	**62008.6**
Grand Total	**233393.9**

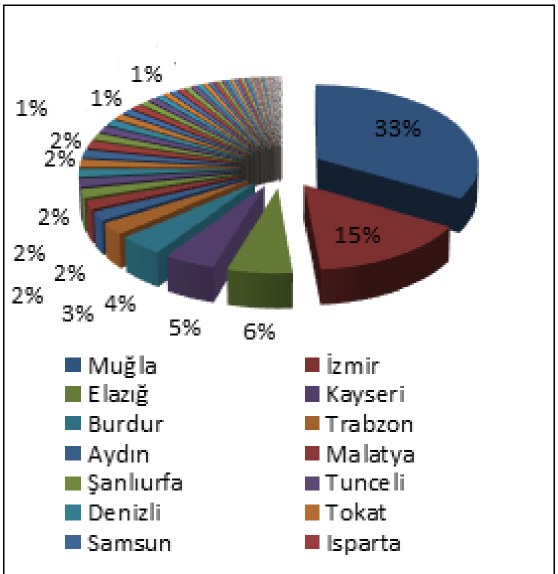


Figure 9. Distribution of Aquacultur in Turkey by Provinces (2013)

Project Capacity and Facilities by Provinces

The total capacity of 2193 aquaculture facilities (Map 11) in Turkey is 449756.5 tons. (Map 12) Turkey's potential for aquaculture is being appreciated better and therefore the number of facilities and the national capacity are increasing. For example, while there was only one aquaculture facility in 1971, the number increased to 1444 in 1999 and 2193 in 2013. In 1970, the first trout farm was established while the first sea bass and gilt-head sea bream facility was opened in 1985.

Table 22. Top Ten Provinces by Total Project Capacity (2013)

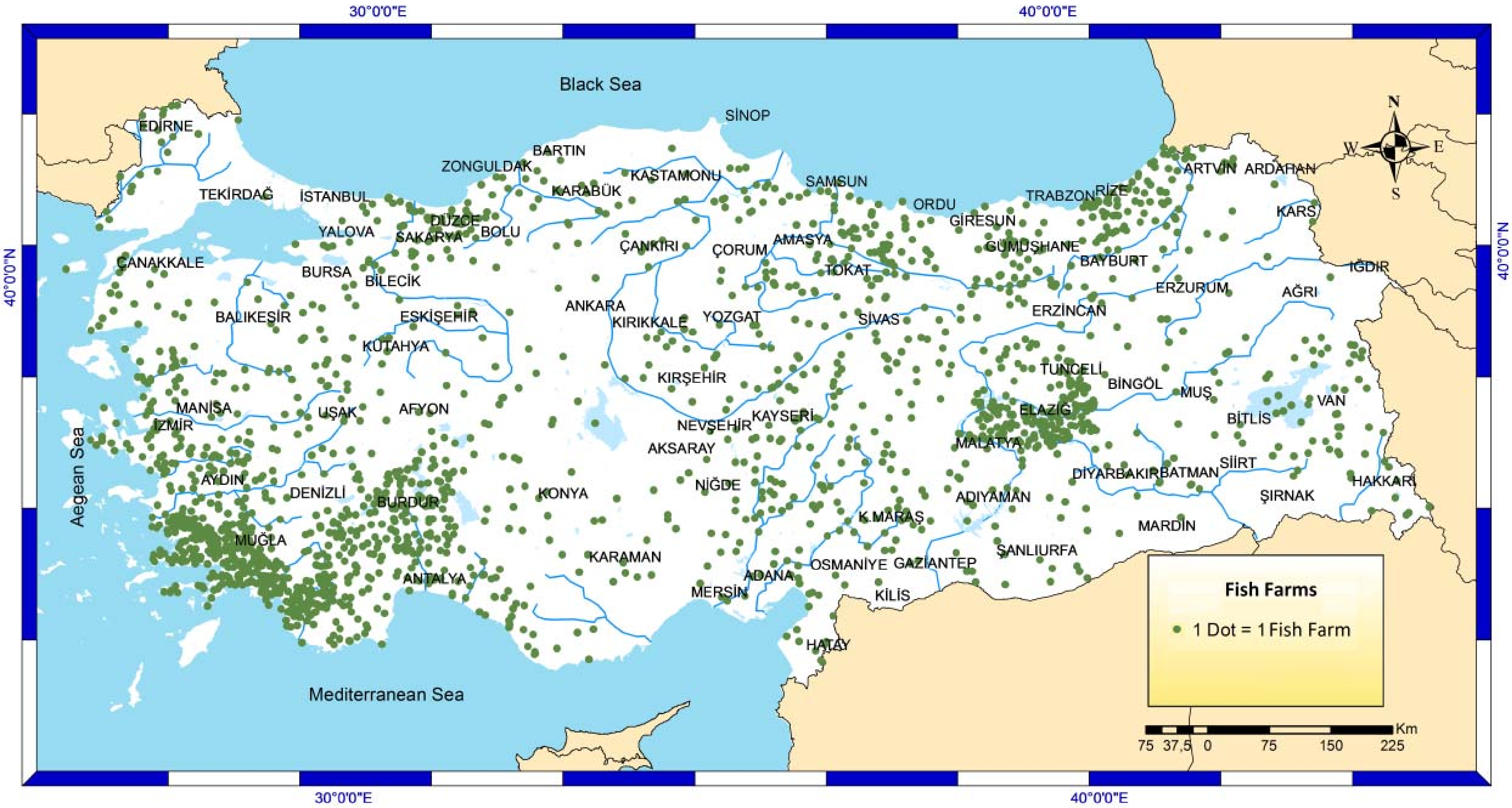
PROVINCES	PROJECT CAPACITY (Ton)
Muğla	111189
Afyonkarahisar	83388
İzmir	70264
Elazığ	31404
Kayseri	29660
Aydın	15929
Trabzon	13729.5
Malatya	10528
Şanlıurfa	10330
Burdur	8639
Total	**385060.5**
Other	**70271**
Grand Total	**455331.5**

Calculated with data from www.tarim.gov.tr

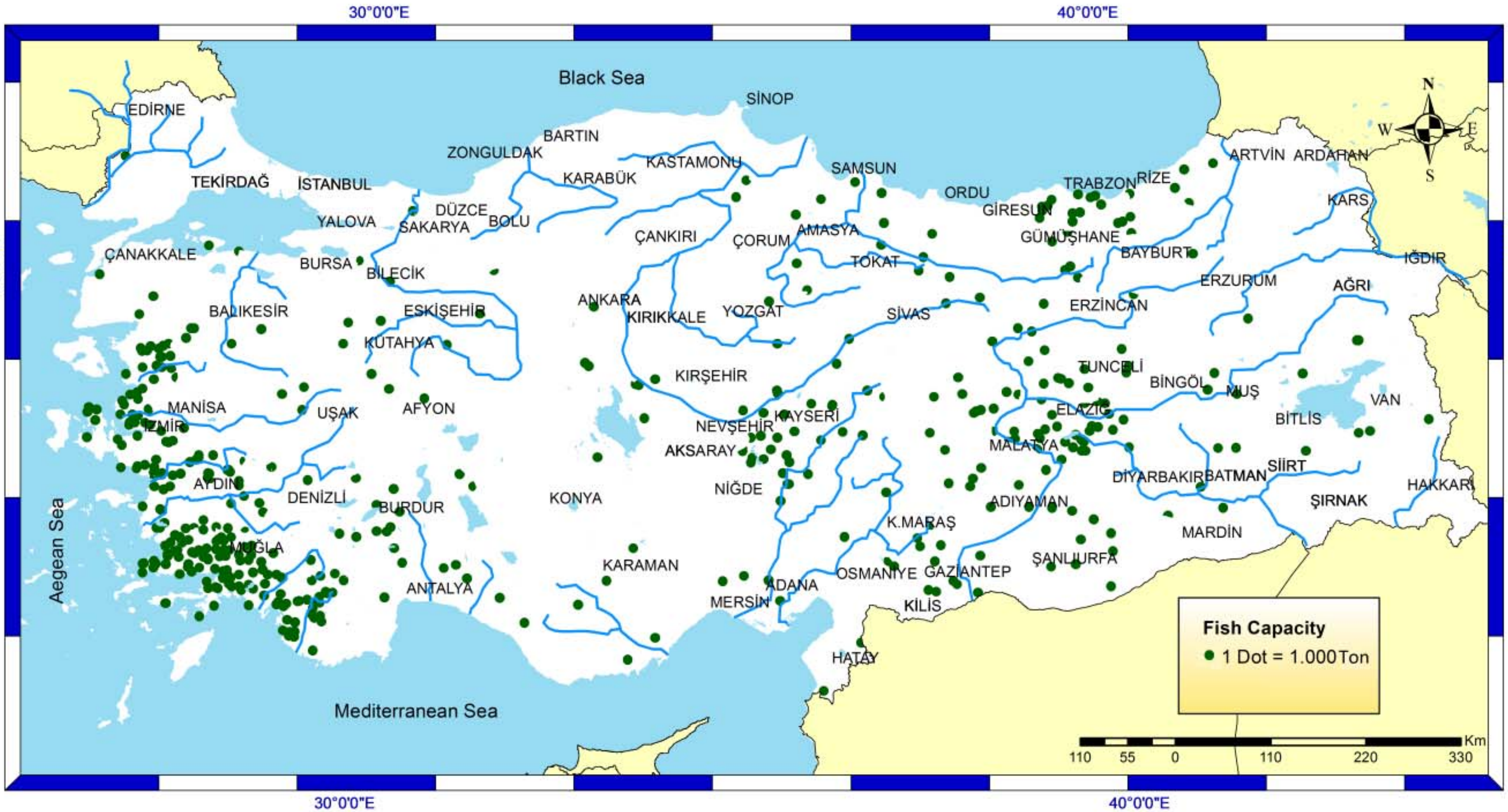
Table 23. Top Ten Provinces by the Number of Facilities (2013)

PROVINCES	NUMBER OF FACILITIES
Muğla	354
Elazığ	158
Antalya	96
İzmir	85
Isparta	82
Trabzon	82
Malatya	78
Burdur	69
Sivas	52
Mersin	51
Total	**1107**
Grand Total	**2193**

Calculated with data from www.tarim.gov.tr



Map 11. Distribution of Fish Farms in Turkey by Provinces



Map 12. Distribution of Fish Capacity in Turkey by Provinces

The capacity of the top ten provinces is 385060.5 tons, which accounts for 84.6% of the total capacity. Moreover, 1107 (50.5%) of the total facilities are owned by ten provinces at the top of the list.

Muğla is placed at the top thanks to the number and capacity of its facilities. Its capacity accounts for 25% of the total. It is followed by Afyonkarahisar with a capacity share of 18% and by İzmir with 16%. The total capacity of the first three provinces is 59%.

Figures on the number of facilities show that Muğla is at the top of the list and followed by Elazığ, Antalya and İzmir. Muğla has 354 facilities, accounting for 16.2% of the total number, while Elazığ, Antalya and İzmir's shares are 7.2%, 4.4% and 3.9%, respectively.

Bingöl has the lowest capacity, which is 25 tons and is followed by Tekirdağ, Şırnak and Iğdır. There are 6 provinces with only one aquacultural facility, which are İstanbul, Siirt, Ardahan, Iğdır, Şırnak and Tekirdağ. Of these, Iğdır, Şırnak and Tekirdağ has a capacity of 25 tons, which is higher than the others. For instance, it is 100 tons for Istanbul.

Conclusion

Rapid growth of the global population, the need for food, the demand for healthy nutrition, employment, growing export and other socio-economical factors are the most important factors for the development of aquaculture. Turkey's physical and human conditions are suitable for aquacultural practices. Preservation of water resources, highly qualified labour force and supporting these two factors by laws will promote the development of aquaculture. Aquaculture which rapidly developed on Turkish coasts in the past has come to contradict other practices and especially has been put forward as a threat to tourism activities. Today, thanks to precautions to prevent such allegations, new practices have cleared the way for aquaculture. Fish farms in the sea were moved to open and deep waters as per regulations introduced by the Ministry of Environment and Urban Planning in 2009. According to these legal regulations fish farms cannot be established within 0.6 nmi off the coast and where depth is less than 30 m. Moreover, technological advances like automated feeding mechanisms, scheduled feeding and digital monitoring have substantially prevented feed-induced pollution (DPT, 2014).

As indicated in the study, the species variety is exiguous. Sustainably farming present species, introduction of new species and diversity in farming would allow people to make better use of water resources, help expand the market and increase its share in national economy.

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FULL PAPER TAM MAKALE

SOME VARIETIES OF PATHOLOGICAL CHANGES IN EXPERIMENTAL INFECTION OF CARPS (*Cyprinus carpio*) WITH *Aeromonas hydrophila*

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Abstract:

The main clinical and pathomorphological changes in *Aeromonas hydrophila* infection in carp provoked by different field strains of this bacterium were studied. The strongest histopathological damages were seen in the functional epithelium of liver and kidneys, followed by intestine and hearth damages in addition to various haemorrhages in interstitial tissues of visceral organs and some skin haemorrhages on the ventral surface of the body and the anal region. As a whole, pathological damages consisted of degenerative changes as cloudy swelling, granular and/or hyaline droplet degeneration or vacuolation in functional epithelium in the respective internal organs in addition to some target vascular disturbances, which appeared to be good biomarkers for field assessment of that disease. Pathological damages in internal organs and haemorrhages were stronger in experimental fishes exposed to higher levels of the respective pathogens, especially those isolated from dead anaconda or the referent strain, and less pronounced damages were seen in fishes infected with of *Aeromonas hydrophila* isolated from a silver carp with marked signs of septicaemia.

Keywords: Carp, Infection, Pathology, Pathomorphological changes, *Aeromonas hydrophila*

Introduction

Aeromonas hydrophila is a common inhabitant of aquatic environments and is considered to be one of the usual constituents of the superficial and intestinal flora of cool or cold-water fishes (Lallier and Daigneault, 1984; Dooley et al., 1986), but is not necessarily restricted to fresh water environment and is often isolated from raw and processed products of marine fishes (Thampuran and Surendran, 1995). *Aeromonas hydrophila*, however, behaves frequently as a secondary invader and is responsible for the disease known as motile aeromonad septicemia (Roberts, 1978). Therefore, this bacterium is also known as one of the most important pathogens of freshwater fishes, which is commonly isolated from fish affected by ulcerative disease outbreaks accompanied by severe hemorrhages of the body surface. This disease is also known as red pest for European eel *Anguilla anguilla*, red disease for Japanese eel *A. japonica* (Hoshina, 1962), red disease for carp *Cyprinus carpio* or red sore for largemouth bass *Micropterus salmonides* (Huizinga et al., 1979). Having in mind different kind of fishes fell sick by the same disease, it is not so strange that there are some contradictions in clinical and pathomorphological signs described by some authors in different fish species (Miyazaki and Kubota, 1977; Miyazaki and Jo, 1985; Miyazaki and Kaige, 1985; Azad et al., 2001; Alagappan et al., 2009; Yardimci and Aydin, 2011). This infectious disease is also known to reduce catfish production by nearly 10% every year and is considered to be one of the common diseases accounting for the decrease of fish production (Alagappan et al., 2009). In humans, *Aeromonas hydrophila* infection has been associated with gastroenteritis and localized wound infection, especially in individuals who have an immune deficiency (Nemetz and Shotts, 1993) and therefore is of the primary concern for public health.

Having in mind the mentioned above contradictions in clinical and pathomorphological signs in different fish species as described by some authors and the reduced fish production, which can be provoked by this disease, in addition to significant health problems in humans, our objectives in this study were to clarify whether there are some differences in clinical and pathomorphological findings of this particular disease in carp as provoked by different strains of *Aeromonas hydrophila* and the possible contamination of fish produce with the same pathogen.

Materials and Methods

Experimental strains

Two of the *Aeromonas hydrophila* strains were isolated from the field, whereas the other was a referent strain. One of the field strains was isolated from a silver carp with marked signs of septicaemia, whereas the other strain was isolated from a dead anaconda. These strains were provided by the National Reference Lab on Fish, Marine Molluscs and Crustacean Diseases (Sofia, Bulgaria). The reference *A. hydrophila* (ATCC 7965) strain was purchased from the National Bank for Industrial Microorganisms and Cell Cultures (Sofia, Bulgaria). All strains exhibited β-haemolytic activity. The strains were activated 18 hours prior to the experiment by inoculation in soybean casein broth (CASO broth, Merck), incubated at 30 ºC for replication to a density of ~10^9. From initial cultures, a series of 10-fold dilutions were performed in Maximum Recovery Diluent (MRD, Merck) in order to obtain bacterial concentrations of 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} which were further used for experimental intraperitoneal infection of experimental carps.

Experimental design

An experiment was performed with 2-year-old carps, weighing from 850 to 1200 g and infected with 3 different *Aeromonas hydrophila* strains. The carps were divided in three experimental and one control group of 12 fishes each. Each carp was also marked and injected intraperitoneally with 0.5 mL of the respective bacterial culture dilution as described above. A total three fish were inoculated with each dilution. The injected carps were released into 4 separate tanks containing 800 l water each, where a constant aeration was maintained and a part of the water was also changed each day. The carps were monitored over a period of 10 days for possible changes in their behaviour, mortalities and visible pathological changes. After that period, fish were percussive stunned according to Ordinance N 15/3.02.2006 about minimum requirements for the protection and welfare of experimental animals, and then a sample was obtained from the heart via a sterile needle for bacteriological examination as per Compendium of Methods for the Microbiological Examination of Foods (Palumbo et al., 2001) by means of blood inoculation onto selective medium for *Aeromonas* spp. (GSP agar, Merck). The experimental carps were killed by destroying of cerebrum before regaining consciousness according to the mentioned above minimum requirements for the protection and welfare of experimental animals. The spleen and kidney were aseptically removed, and inoculations were made using the same selective medium as described above. Gross pathological examination was performed, and specimens for pathomorphological studies were collected from the spleen, kidney, hepatopancreas, intestine and hearth and fixed in 10% neutral buffered formalin. The fixed tissues were processed for paraffin embedding, sectioned at 6 µm and stained with haematoxylin-eosin.

The study protocol of this experiment was approved by the Trakia University Animal Care Ethic Committee as required.

Results and Discussion

Microbiological investigations revealed that the original *Aeromonas* strains were re-isolated from all investigated samples of the fishes infected with the respective strains. There were no cases of death within the 10-day period of monitoring in the infected fishes from all groups. No significant change in the fish behaviour such as anorexia or the way of swimming was noticed. Only swimming closer to water surface was seen in some fishes from all experimental groups.

Experimental fishes infected with *Aeromonas hydrophila* isolated from anaconda

The external examination of experimental fishes after the 10-day period showed a lot of haemorrhages, particularly on the ventral surface of the body and in the anal region, especially in carps injected with the highest levels of this pathogen.

In the intestine, there were degenerative changes and desquamation of epithelial cells of intestinal mucosa. Partial necrosis and desquamation was also observed in the villi which corresponded to desquamative catarrh in the intestine. The same was accompanied by bacterial invasions in the epithelium, tunica propria or submucosa and was most obvious and pronounced in fishes exposed to higher microbial density via the intraperitoneal infection. A hypersecretion of Goblet cells was also observed.

In the hearth, there was slight intermuscular edema, small hemorrhages and irregular staining of some myofibrils due to their increased acidophily. Some lytic changes, cloudy swelling, slight to moderate granular degeneration or vacuolation were also observed in part of myofibrils (Figure 1).

In the liver, there was moderate to strong granular degeneration or vacuolation of hepatocytes (Figure 2) accompanied with extensive deposition of hemosiderin in hepatocytes or macrophages.

In the kidneys, there was moderate to strong granular and/or hyaline droplet degeneration, necrosis and desquamation of tubular epithelium (Figure 3). Cellular debris and/or hyaline casts were seen in some tubules. Some nuclei of tubular epithelium showed pyknotic changes and there was deposition of hemosiderin in the epithelial cells of tubules and an increased quantity of hemosiderin-laden macrophages in the hematopoietic tissues. Sinusoids of the peritubular hematopoietic tissue were congested and some hemorrhages were also evident in the renal interstitium.

In the spleen, there was an extensive deposition of hematoidin crystals and a lot of hemosiderin-laden macrophages in the pulp.

Experimental fishes infected with *Aeromonas hydrophila* isolated from a silver carp

A few haemorrhages were detected on the ventral surface of the body and in the anal region, especially in carps injected with the highest levels of this pathogen.

In the intestine, the degenerative changes and desquamation of epithelial cells of intestinal mucosa and the partial necrotic changes in the villi were less pronounced in comparison to those described in the carps of previous group (Figure 4). A bacterial invasion in the epithelium, tunica propria or submucosa was also seen, but the same was most obvious only in fishes exposed to higher microbial density of the diluent used for intraperitoneal infection.

In the hearth, there were small hemorrhages and increased acidophily or irregular staining of some myofibrils. Slight intermuscular edema and granular degeneration or vacuolation were also observed in part of myofibrils.

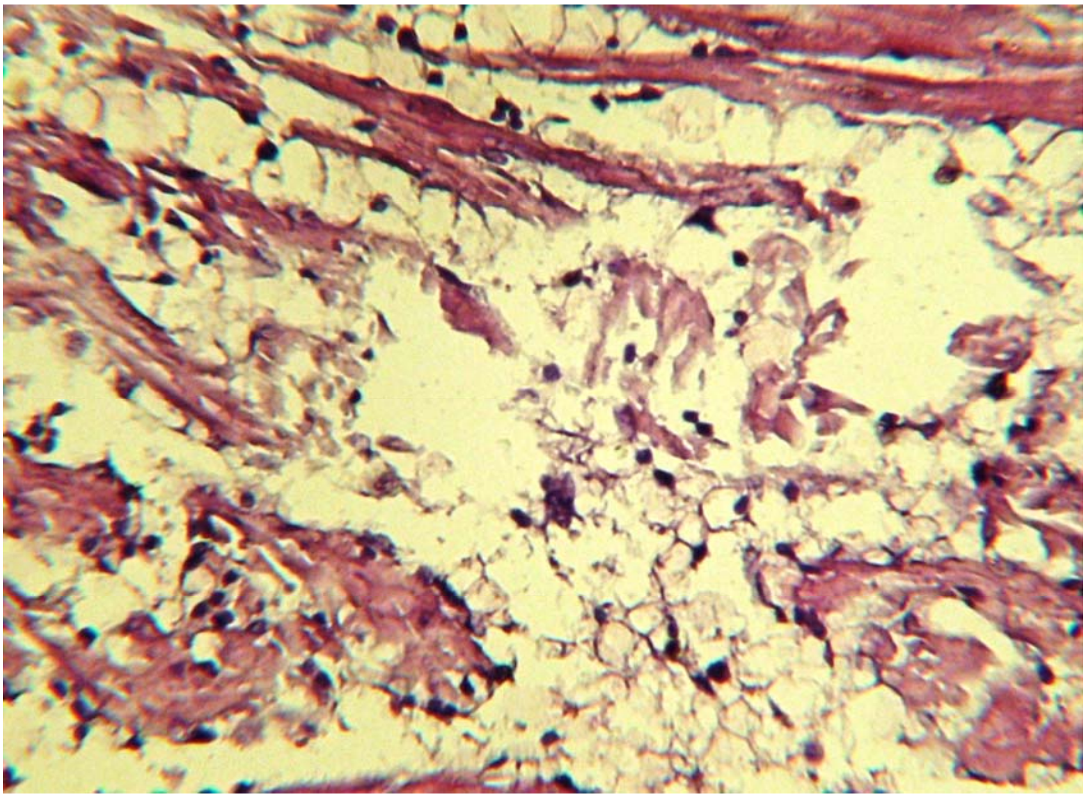

Figure 1. Lytic changes, granular degeneration or vacuolation in some myofibrils. Hearth. HE. ×260.

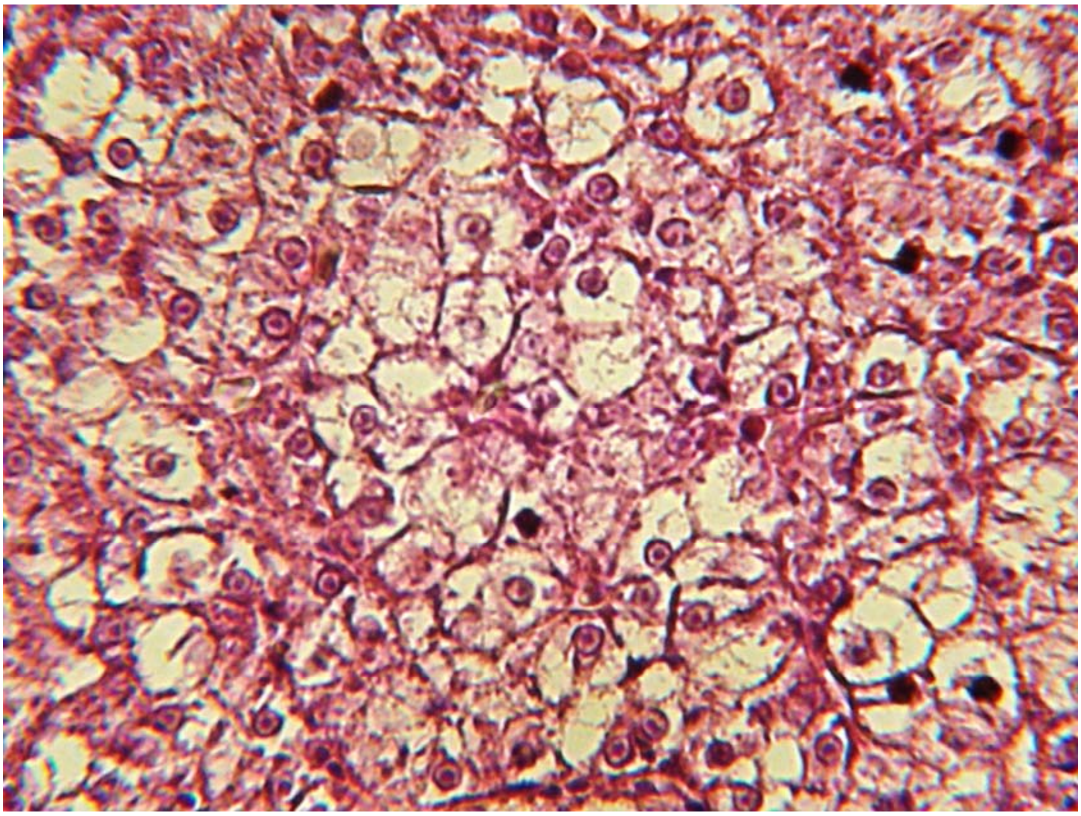

Figure 2. Granular degeneration or vacuolation in hepatocytes. Liver. HE. ×260.

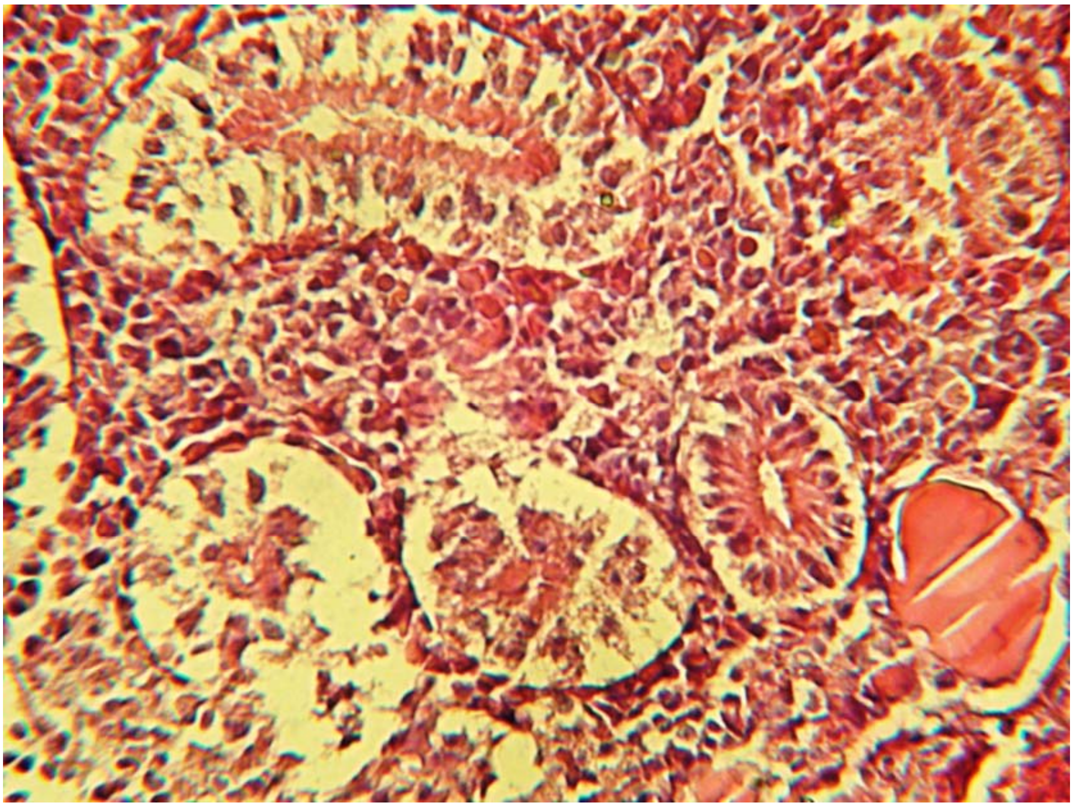

Figure 3. Granular degeneration and desquamation of tubular epithelium and hyaline casts in tubular lumens. Kidney. HE. ×260.

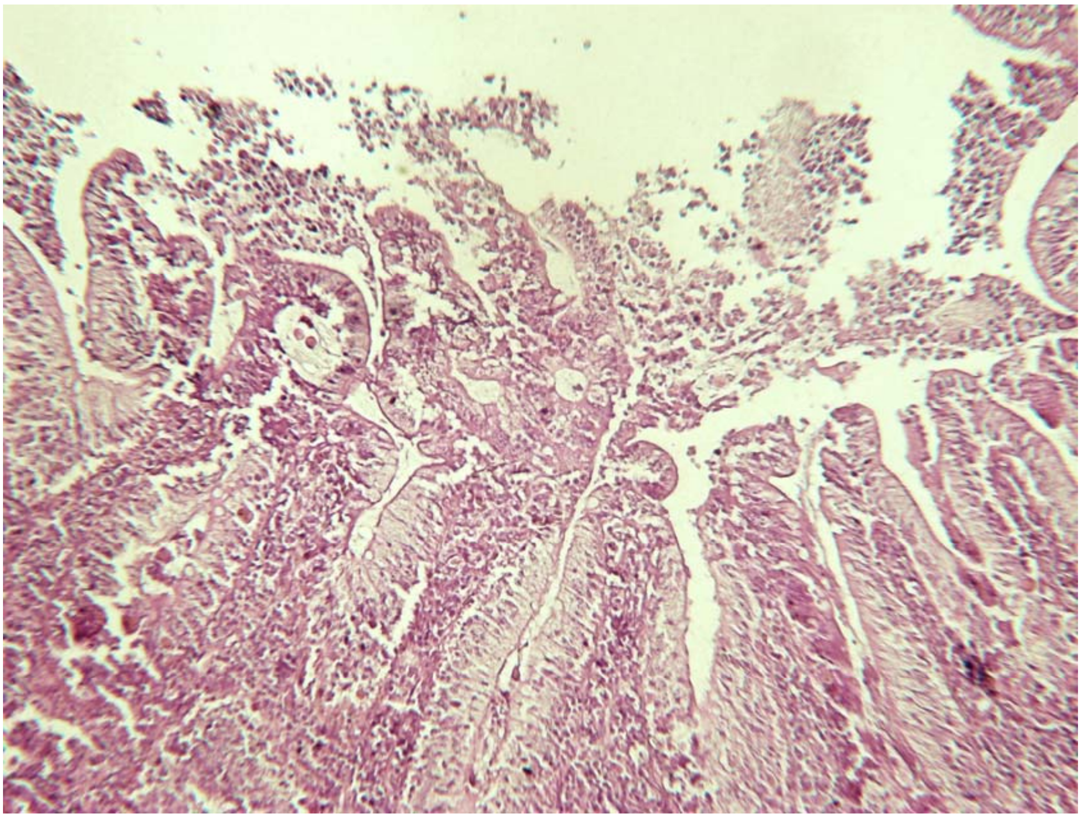

Figure 4. Desquamation of epithelial cells of intestinal mucosa and partial necrotic changes in the villi. Intestine. HE. ×100.

In the liver, there was moderate to strong granular degeneration or vacuolation of hepatocytes. A deposition of hemosiderin in hepatocytes or macrophages was also seen.

In the kidneys, there was moderate granular degeneration and desquamation of tubular epithelium. Some nuclei of tubular epithelium showed pyknotic changes and there was deposition of hemosiderin in the epithelial cells of some tubules (Figure 5). An increased quantity of hemosiderin-laden macrophages in the hematopoietic tissues was seen (Figure 5). Hyaline and/or cellular casts were also observed in the lumens of some tubules (Figure 6).

In the spleen, there was an extensive deposition of hematoidin crystals and a lot of hemosiderin-laden macrophages in the pulp.

Experimental fishes infected with referent species of *Aeromonas hydrophila*

A lot of haemorrhages on the ventral surface of the body and in the anal region were seen, mainly in carps injected with the highest levels of *Aeromonas hydrophila***.**

In the intestine, degenerative changes and desquamation of epithelial cells of intestinal mucosa (desquamative catarrh) were seen in all examined fishes. Partial necrosis and desquamation was observed in the intestinal villi. Bacterial invasions in the epithelium, tunica propria or submucosa were seen in some fishes exposed to higher microbial density of diluent. Some necrotic foci with peripheral inflammatory cell infiltration were also seen in the tunica propria or lamina muscularis of intestinal mucosa (Figure 7) probably because of bacterial invasion.

In the hearth, there were small hemorrhages (Figure 8) in addition to lytic changes, slight to moderate granular degeneration or vacuolation (Figure 8) in part of myofibrils.

In the liver, there was slight to moderate granular degeneration or vacuolation of hepatocytes accompanied with deposition of hemosiderin in hepatocytes or macrophages.

In the kidneys, there were slight to moderate degenerative changes and desquamation in the tubular epithelium in addition to some hyaline casts in tubular lumens. There was an increased quantity of hemosiderin-laden macrophages in the hematopoietic tissues. Many hemorrhages were also seen in the renal interstitium in addition to the fibrin/hyaline thrombi in some vessels.

In the spleen, there was an extensive deposition of hematoidin crystals and a lot of hemosiderin-laden macrophages in the pulp in addition to small fibrin/hyaline thrombi in some vessels.

Control fishes

There were no pathomorphological changes in internal organs and only a slight granular degeneration or karyopyknosis was sometimes seen in a few epithelial cells of kidney tubules and a single hyaline drops in their lumens. A slight granular degeneration was also seen in a few hepatocytes in the liver.

The comparison of pathomorphological findings revealed that the strongest damages were found in the liver and kidneys, followed by the hearth and the intestine and only slight damages were seen in the spleen. As a whole pathological damages consisted of degenerative changes (cloudy swelling, granular and/or hyaline droplet degeneration or vacuolation) in functional epithelium in the respective internal organs in addition to the hyperaemia and haemorrhages in their interstitial tissues.

Pathological damages in internal organs were more pronounced and stronger in experimental fishes exposed to higher levels of the respective pathogens, which was achieved by higher microbial density of diluent injected intraperitoneally (especially at levels 10^9 cfu/cm^3). LD_{50} for *Aeromonas hydrophila* usually range between 0.3 × $10^{8.66}$ cell/fish in carps (Alsaphar and Al-Faragi, 2012) and $10^{6.22}$ cell/fish in tilapia (Azad et al., 2001) after intramuscular injection up to 6.66 x 10^{11} cells/ mL in matrinxã *Brycon amazonicus* after intraperitonel injection of 0.1 mL suspension (Oliveira et al., 2011).

Similar pathological damages and increased mortality in Walking Catfish infected intraperitoneally with *Aeromonas hydrophila* were also reported by Angka (1990), where the pathomorphological changes were more pronounced in catfishes infected with higher levels of bacteria (10^7 cfu/mL) compared to those infected with lower levels of the same bacteria. According to the same author the catfishes injected intraperitoneally with such low levels of bacteria as 0.1 mL of a suspension containing 10^3 cfu/mL of *Aeromonas hydrophila* did not cause significant disease in the catfish.

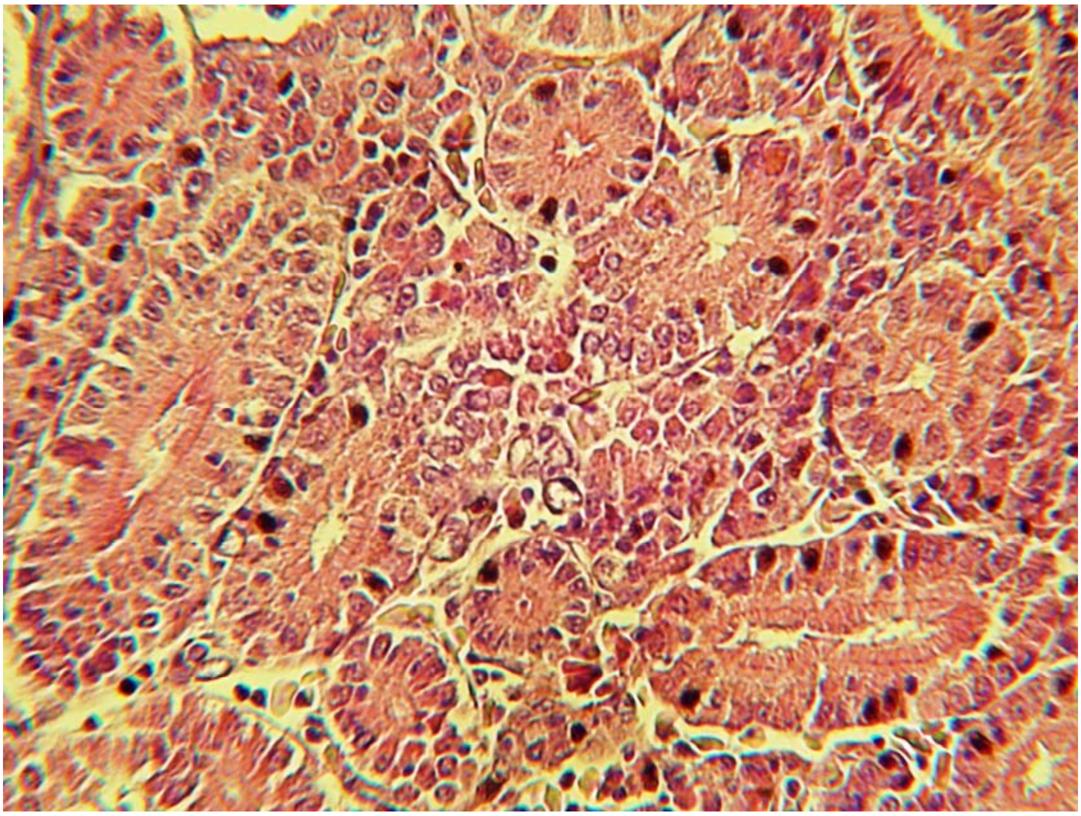

Figure 5. Pyknotic changes and/or deposition of hemosiderin in the epithelial cells of some tubules and macrophages. Kidney. HE, ×260

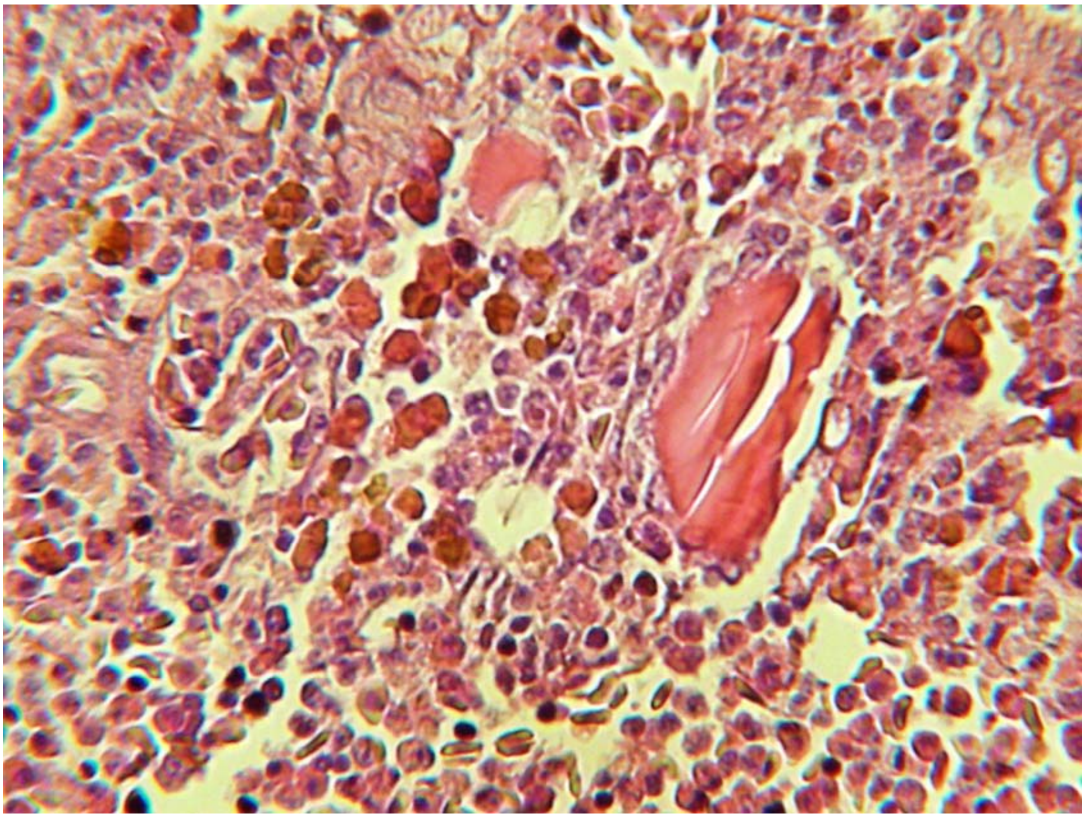

Figure 6. Hyaline casts in the lumens of some tubules and hemosiderin-laden macrophages in the hematopoietic tissues. Kidney. HE. ×260

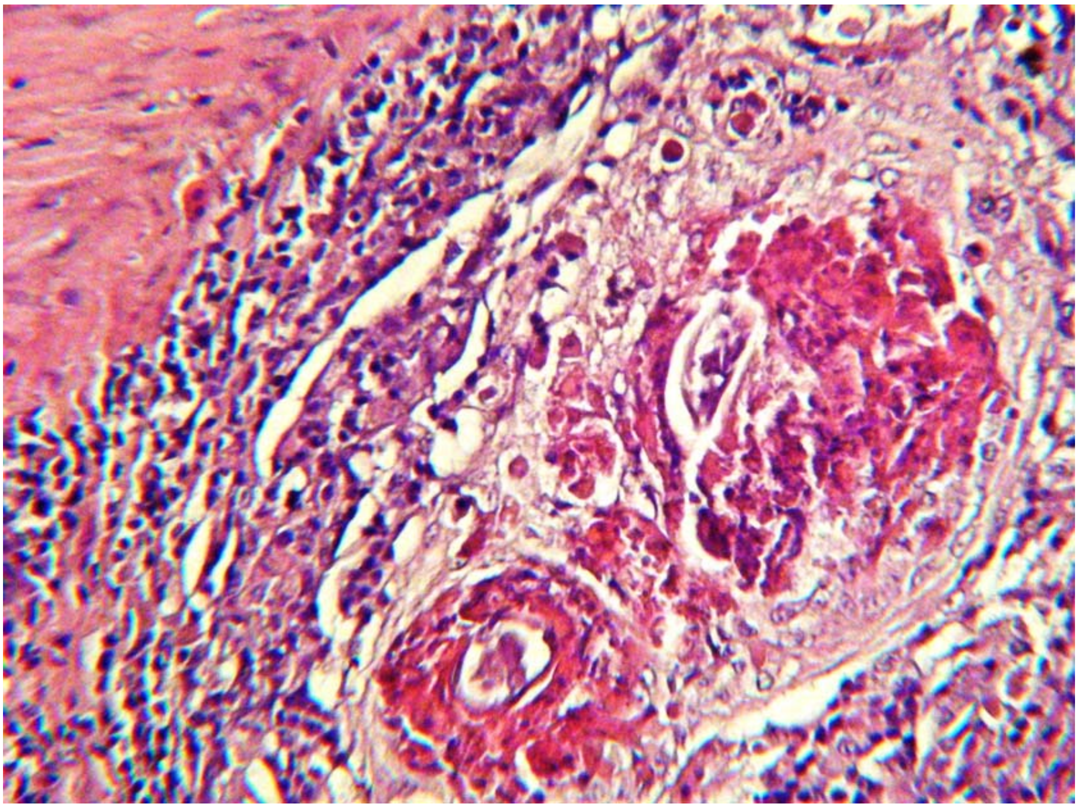

Figure 7. Necrotic foci with peripheral inflammatory cell infiltration in the tunica propria or lamina muscularis of intestinal mucosa. Intestine. HE. ×260

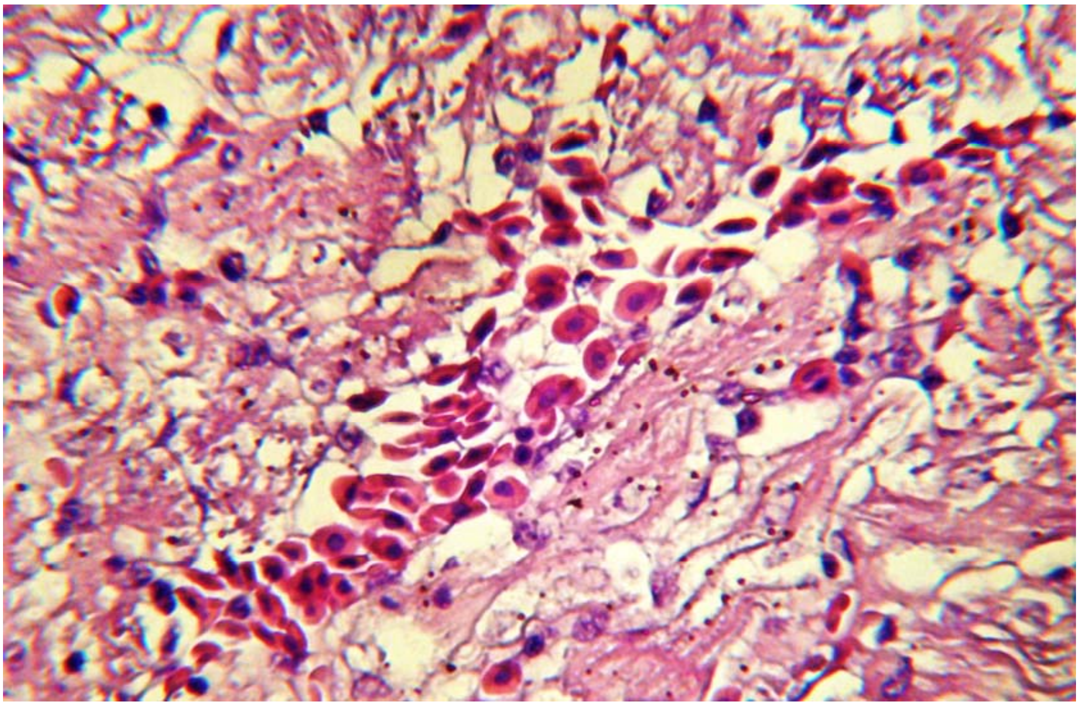

Figure 8. Small hemorrhages and slight granular degeneration or vacuolation in part of myofibrils. Hearth. HE. ×300

Similar study with Tilapia showed that experimental intramuscular infection with *Aeromonas hydrophila* at levels of 10^7 cfu/fish produced characteristic ulceration leading to open wounds, focal necrosis or vacuolation of hepatocytes, congestion of hepatic sinuses and haemorrhages in the liver, degenerative changes and necrosis of tubular epithelium of kidneys as well as degenerative changes in intestinal epithelium, erosion and sloughing of intestinal microvili (Azad et al., 2001). The infection with higher levels of the same bacteria often provoked the death of some fishes (Azad et al., 2001).

Comparing the pathogenicity of the both isolated bacteria with the referent strain of *Aeromonas hydrophila* we can conclude that the intensity of pathological damages observed in the present study was a little higher in carps infected with *Aeromonas hydrophila* isolated from anaconda, followed by carps infected with referent strain of *Aeromonas hydrophila* and a less intensity of pathological damages was seen in carps infected with *Aeromonas hydrophila* isolated from a silver carp. In the fishes infected with the strain isolated from anaconda or the referent strain there were strong damages in blood vessels as hyperaemia and haemorrhages (mainly in kidneys and hearth) in addition to degenerative changes in various internal organs.

As a whole our experimental investigations support those made by some other authors (Yardimci and Aydin, 2011; Angka, 1990; Alagappan et al., 2009; Miyazaki and Kaige, 1985) in various kinds of fishes as Tilapia, Walking Catfish, Estuarine Cattfish, Crucian Carp, etc, infected intraperitoneally with *Aeromonas hydrophila* or those of spontaneous cases of this infection (Miyazaki and Jo, 1985). In mentioned above reports there were similar or even stronger degenerative or necrotic changes and haemorrhages in internal organs (liver, kidneys, hearth, spleen, intestine) as compared to our study, in addition to some haemorrhages or ulcers/erosions on the skin surface (Azad et al., 2001; Angka, 1990; Miyazaki and Jo, 1985; Miyazaki and Kaige, 1985), haemorrhages or ulcers/erosions around pectoral fins, tail and anus (Alagappan et al., 2009; Miyazaki and Jo, 1985), accumulation of red-coloured ascitic fluid (Miyazaki and Kaige, 1985), erosive cornea, separated retina, haemorrhages and necroses in the conjunctiva, exophthalmia (Rehulka, 2002; Miyazaki and Jo, 1985). Other authors reported about increased mortality and more intensive pathological damages in Nile tilapia or Walking Catfish infected with *Aeromonas hydrophila* (Yambot and Inglis, 1994; Rodriguez et al., 1992; Angka, 1990), or in Crucian Carp (Miyazaki and Kaige, 1985) and Rainbow Trout (Rehulka, 2002) infected with the same bacterium.

Systemic infections in fishes with *Aeromonas hydrophila* were characterized by diffuse necrosis in several internal organs and the presence of melanin-containing macrophages in the blood (Ventura and Grizzle, 1988). The kidneys and liver are target organs in acute septicaemia which are apparently attacked by bacterial toxins and therefore lose their structural integrity (Afifi et al., 2000; Huizinga et al., 1979). The reason of degenerative changes and necrosis in the liver was reported to be associated with various endotoxins and extracellular products such as hemolysin, protease, elastase produced by *Aeromonas hydrophila* (Afifi et al., 2000; Kanai and Wakahayashi, 1984; Nieto et al., 1991; Angka, 1990; Rodriguez et al., 1992). The pronounced destructive changes in the skin and muscles followed by focal ulceration of the skin and vascular damages are usually attributed to hemolysin and protease activity of *Aeromonas hydrophila*, which possess also ability to adhere to cells (Azad et al., 2001; Wakayabashi et al., 1981; Kanai and Wakayabashi, 1984). The bacterial enzyme elastase could also contribute significantly to vascular damages (Wakayabashi et al., 1981) resulting in hemorrhages because blood vessels are mainly composed of elastic and collagenous fibers (Miyazaki and Kubota, 1977).

Huizinga et al. (1979) reported that focal haemorrhages and dermal lesions or ulcers were observed in chronic aeromonad infection, whereas the target organs in acute septicaemia were liver and kidneys. That could explain the mildness of the skin lesions and the pronounced damages in the liver and kidneys seen in our short duration follow up study. In addition, the intraperitoneal way of infection of fishes could contribute to intensive pathological changes in internal organs and less damages in the skin and muscles, because of ensuring a direct contact of this bacterium with visceral organs. On the other hand, stronger damages on the skin and muscles are usually reported in fishes infected via intramuscular injections (Yardimci and Aydin, 2011; Erer, 1981). However, the bacterial virulence in fishes intraperitoneally injected is always higher than in fishes intramuscularly injected with this bacterium (Erer, 1981; Miyazaki and Kaige, 1985).

For increased amounts of hemosiderin-laden macrophages or epithelial cells in the liver, kidneys and spleen in *Aeromonas hydrophila* infection in fishes reported some other authors (Ventura and Grizzle, 1988; Miyazaki and Kaige, 1985). As a rule, the infections with strong β-hemolytic strains of *Aeromonas hydrophila* usually cause stronger deposition of hematoidin crystals and increase of hemosiderin-laden cells in various internal organs (Miyazaki and Kaige, 1985).

Similar degenerative changes, including hyaline droplet degeneration in tubular epithelium of kidneys in *Aeromonas hydrophila* infection in Crucian Carp were reported by some other authors (Miyazaki and Kaige, 1985). There are also some reports for focal interstitial hemorrhages, lymphocyte infiltration, appearance of hemosiderin-laden macrophages and aggregation of melanomacrophage centres in the renal interstitium seen in other fishes infected with the same pathogen (Erer, 1981; Azad et al., 2001).

Other authors (Huizinga et al., 1979) reported that no lesions were observed in hearth and spleen in the acute cases of *Aeromonas hydrophila* infection in fishes and mainly diffuse tissue necrosis of liver and kidneys were present, which was also confirmed in the present study.

Although it is known that *Aeromonas hydrophila* has much notoriety as pathogen of fish it is important to mention that aeromonad bacteria are common inhabitant of aquatic environments and compose part of the normal intestinal or skin microflora of healthy fish (Lallier and Daigneault, 1984; Dooley et al., 1986; Yardimci and Aydin, 2011). That's why, the presence of these bacteria, by itself, in the intestines or skin of fishes, is not indicative of disease. Although this bacterium behaves as a secondary invader (Roberts, 1978) and the infection only occurs under various predisposing conditions, *Aeromonas hydrophila* is considered to be one of the most dangerous and widespread pathogens of freshwater fishes and the most important causative agent of the outbreaks of Bacterial hemorrhagic septicaemia in fishes (Austin B. and Austin D., 1993), known also as red pest or red disease (Hoshina, 1962).

According to some authors (Khalil and Mansour, 1997; Trust et al., 1974) various stressors as abrupt temperature change, handling, crowding, inadequate feed and oxygen are known to be the predisposing factors which contribute significantly to the infection of *Aeromonas hydrophila*, which is considered to be a real secondary infection in most of the fishes.

Aeromonas hydrophila is often associated with human disease (Hazen et al., 1978), which contributes further to the particular attention given to this bacterium. On the other hand, *Aeromonas hydrophila* is not generally considered to be a marine bacterium, but it could be found naturally in marine systems which interface with fresh water and therefore has a comparatively wide spreading. Having in mind the strong adherence of this bacterium to intestine and skin followed by invasion of the liver, spleen, muscle and gills of fishes (Horne and Baxendale, 1983; Kanno et al., 1989) in addition to its involving in gastrointestinal diseases and localized wound infection in humans, its recognition via some typical patomorphological damages could additionally contributes to timely diagnosis of this disease.

Conclusions

This study clearly shows that typical histopathological alterations seen in the functional epithelium of liver and kidneys, followed by intestine and hearth alterations and various haemorrhages in interstitial tissues of visceral organs, in addition to some target skin damages are good biomarkers for field assessment of that disease.

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SHORT COMMUNICATION KISA MAKALE

REOCCURRENCE OF A COMMERCIAL EURYHALINE FISH SPECIES, *Atherina boyeri* Risso, 1810 (Atherinidae) IN BÜYÜKÇEKMECE RESERVOIR (ISTANBUL, TURKEY)

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Abstract:

The big-scale sand smelt, *Atherina boyeri* Risso, 1810 was recorded for the first time from Büyükçekmece Reservoir (İstanbul, Turkey) in 1982. However, recent studies on the fish fauna of Büyükçekmece Reservoir indicated that *A. boyeri* did not exist in the reservoir. In the present study, which was originally planned for determining biological features of perch, *Perca fluviatilis* Linnaeus, 1758 in the reservoir, existence of *A. boyeri* was determined in the stomach contents of two *P. fluviatilis* individuals captured in October and November 2009. Possible reasons for reappearance of the species are discussed.

Keywords: Big-scale sand smelt, Stomach content, Temperature, Reappearance

Introduction

The big-scale sand smelt, *Atherina boyeri* Risso, 1810 is a euryhaline fish species, which migrates to sea in autumn and enters the lagoons in spring for reproduction. It has ecological and commercial relevance, and highly diffused in the most Mediterranean coastal lagoons, salt marshes and, more rarely, inland waters where it is accidentally or consciously introduced and characterized by high morphological variability among populations (Congiu et al., 2002; Andreu-Soler et al., 2003). It is also found in the northeast Atlantic, from the Azores to the northwest coast of Scotland (Bartulović et al., 2004). The natural distribution area of the fish in Turkey is Akyatan and Tuzla lakes (Adana), Bafa Lake (Aydın), Köyceğiz Lake (Muğla), Gediz Stream (İzmir), Büyükçekmece Reservoir and Küçükçekmece Lagoon (İstanbul), Peso Lake (Edirne), Sapanca Lake (Sakarya) and some estuaries in the East Black Sea Region such as Yeşilırmak (Samsun), Karadere (Kastamonu) (Altun, 1991; Kuru et al., 2001; Özuluğ et al. 2005a). *A. boyeri* is one of the most important fisheries resources in inland waters of Turkey with approximately 6500 tons production per year (Harlıoğlu, 2011).

Büyükçekmece was a lagoon located in the mouth of Karasu Stream emptying into the Sea of Marmara (Figure 1). The sea connection of the lagoon was blocked by a dam to provide drinking and usage water for İstanbul in 1985 and the lake became freshwater lake in due time. The surface of the reservoir is 28.5 km^2 with approximately 7 m water depth (Meriç, 1992; Aktan et al., 2006). The reservoir is a shallow lake, which has a dynamical structure due to climatic changes and it provides the city of İstanbul with ≈14% of its drinking water (mean = 100×10^6 m^3/year). The reservoir has progressively become mesotrophic because of industrial and domestic waste water inputs, which arrived through streams (mainly Karasu, Keşliçiftliği and Çekmece streams) emptying into the reservoir (Aktan et al., 2006; Şahin, 2006; Özuluğ, 1999).

Although several studies on the fish fauna of Turkish inland waters had not stated the presence of *A. boyeri* in Büyükçekmece Reservoir until 1950s (Ninni, 1923; Kosswig and Battalgil, 1942; Deveciyan, 1926), it was recorded for the first time from Büyükçekmece Reservoir in 1982 by Balık (1985). After then, Meriç (1986) reported this fish species in a study carried out to determine fish fauna of the reservoir. According to results of these studies, mostly marine species such as *Pomatomus saltatrix* (Linnaeus, 1766), *Mugil cephalus* Linnaeus, 1758, *Sardina pilchardus* (Walbaum, 1792), *Engraulis encrasicolus* (Linnaeus, 1758) with some freshwater fish species such as *Tinca tinca* (Linnaeus, 1758), *Rutilus rutilus* (Linnaeus, 1758), and catadromous fish species *Anguilla anguilla* (Linnaeus, 1758) existed in the reservoir. After building of the dam, freshwater species took over the marine species due to decrease in salinity down to 0.2‰ (Saç, 2010) and 23 freshwater fishes were then recorded (Özuluğ 1999). However, Meriç (1992) noted that *A. boyeri* was not present in the reservoir due to low water temperatures. In addition to this, Özuluğ (1999) also did not list *A. boyeri* in the taxonomic study of the fish fauna of the reservoir.

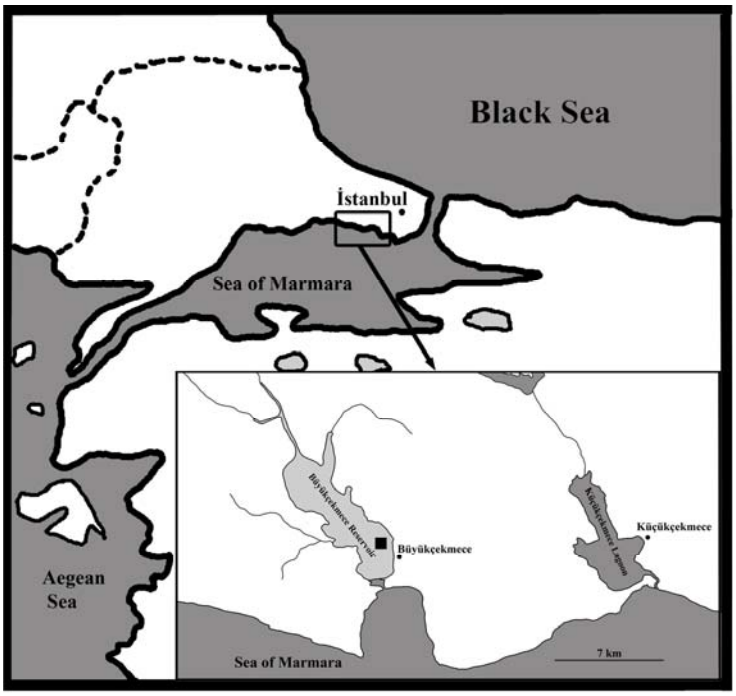

Figure 1. Map of Büyükçekmece Reservoir and Küçükçekmece Lagoon.

Materials and Methods

The present study was originally initiated with the aim of understanding food habits of perch, *Perca fluviatilis* Linnaeus, 1758 in the reservoir and fish samples were collected between March 2009 and April 2010. A total of 428 perch was captured by using gillnets having different mesh sizes (10×10 mm, 20×20 mm, 30×30 mm, 40×40 mm and 50×50 mm). The gastrointestinal tracts of 81 *P. fluviatilis* specimens were examined to determine the dietary components. Unexpectedly, two *A. boyeri* specimens were found in stomach contents of two different *P. fluviatilis* specimens captured in October and November 2009. The perch specimens were 13.8 cm total length (38.62 g) and 25.4 cm total length (268.00 g), while *A. boyeri* specimens were 8.6 cm total length (2.12 g) and 6.1 cm length (because this sample was digested to some extent, only current length was given) (1.75 g), respectively (Figure 2).

Figure 2. *A. boyeri* specimens found in stomach content of *P. fluviatilis*. Lower case indicates almost an intact *A. boyeri* specimen, probably just shortly before captured by the predator while the specimen in upper case was in a slightly advanced state of digestion.

Results and Discussion

Recent studies on the fish fauna of the reservoir showed that *A. boyeri* has disappeared in the reservoir (Meriç, 1992; Özuluğ, 1999). However, present study had undeniably proved the existence of *A. boyeri* in the reservoir. There may be three ways for the fish to present in the reservoir: (1) the fish was re-introduced to the reservoir by stocking, (2) the fish has always been in the reservoir but could not captured in the previous studies or, (3) the fish entered into the reservoir by opening of dam shutters after heavy rains. Personal communications with the authorized persons revealed that the dam shutters left open during 3 – 4 days after flood disaster in September 9, 2009. It is known that the species has been living in the Sea of Marmara and is able to swim upstream. In light of this information, the most plausible explanation was that the species might have entered to the reservoir after the dam shutters had opened. This explanation is likely, as *A. boyeri* is abundant in a lagoon-type lake, Küçükçekmece Lagoon (personal communication, Reşit Özdilek, fisherman), which has very similar character and close proximity to Büyükçekmece Reservoir (Figure 1). As sea connection is constant and maximum depth is around 20 m in Küçükçekmece Lagoon, it is expected that *A. boyeri* population has never crashed in this lagoon unlike Büyükçekmece Reservoir.

Meriç (1992) reported that connection of Büyükçekmece Lagoon with the Sea of Marmara was blocked by a barrier (11.4 m in height) in order to meet the need for fresh water in city of İstanbul and that some ecological changes have occurred making the lagoon a freshwater lake by decreasing salinity to average 0.2‰ (Saç, 2010) from ≈25‰ (Acara and Gözenalp, 1959). Meriç (1992) also concluded that disappearance of *A. boyeri* can be attributed to harsh winters in Büyükçekmece Reservoir, as the most important ecological condition might be water temperature because feeding of *A. boyeri* ceases when water temperature is lower than 8°C and, water temperature lower than 4°C is fatal for this species. According to Henderson et al. (1988), the reason of migration of *A. boyeri* from coast (spawning area) to sea was protecting itself from low winter temperature of shallow waters in England. Büyükçekmece is a shallow reservoir and has average 2–3 m depth. Meriç (1992) recorded 3.2°C at 6.5 m depth and 3.0°C at surface water in a station having 7.15 m maximum depth in Büyükçekmece Reservoir. According to Aktan et al. (2006), surface temperature was 3.8°C in February, 2004 while Şahin (2006) was reported 2°C at 2.5 m depth in February, 2005 in the reservoir. At the present study, minimum water surface temperature was measured as 3.5 °C on February, 2010 (Figure 3). It is highly remarkable for *A. boyeri* to attain Büyükçekmece Reservoir despite fatal water temperatures for the species in the winter. However, as only two specimens encountered in the stomach contents of a predator species for almost three decades (since 1986), it is obvious that this species has low abundance and a restricted distribution in this reservoir. This fact would support the idea that it can enter the reservoir through sea connection when it opens periodically. Notwithstanding a couple of general faunal studies did not report *A. boyeri* in Büyükçekmece Reservoir before 1980s, these studies were superficial (i.e. not in detail) and high abundance of *A. boyeri* was confirmed by personal communication with a researcher (Nurettin Meriç) who worked in the lake in 1970s. He has also admitted that *A. boyeri* was not commercially caught however it was one of the dominant fish species in Büyükçekmece Lagoon. Given this species was present in the coastal areas of the Sea of Marmara in 1940s (Erazi, 1942) and migrates to lagoons in spawning time, it should be one of the abundant natural fish species in Büyükçekmece Lake before the dam construction.

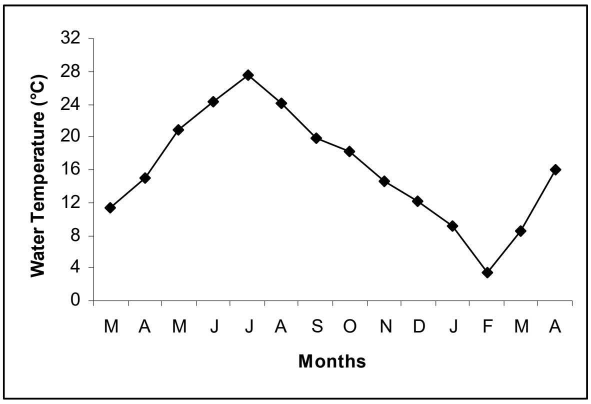

Figure 3. Surface water temperature values of Büyükçekmece Reservoir during study period.

Conclusion

A. boyeri is a commercialized fish species for Turkish inland waters. For this purpose, it has been translocated to some fresh water bodies in Turkey such as İznik, Eğirdir lakes and Karacaören-I, Kesikköprü and Ömerli reservoirs, adapted successfully and became the dominant fish species of these ecosystems (Özuluğ et al., 2005b; İnnal and Erk'akan, 2006; Altındağ and Ahıska, 2006; Gülle et al., 2008; Özvarol and Karabacak, 2011).

These water bodies are deeper than Büyükçekmece Reservoir and *A. boyeri* can migrate from coastal or surface areas to deep for protecting itself from cold fatal water temperatures. This difference between Büyükçekmece Reservoir and other water bodies may explain higher colonization success of *A. boyeri* in other water bodies. Reappearance of *A. boyeri* in Büyükçekmece Reservoir is most likely because of opening the sea connection of the reservoir. Unfavorable environmental conditions for *A. boyeri* in the reservoir seem to continue and this might prevent increase the abundance of the species but sea connection of the reservoir may still provide its regular entrance to the reservoir. Reoccurrence of *A. boyeri* in the reservoir can be considered as a very important phenomenon in terms of ecological and commercial perspectives especially when the population size reaches considerably high levels. However, Büyükçekmece Reservoir should continuously be monitored and more detailed comparable field works should be conducted to get more solid results on this hypothesis.

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