10

Bacteria Isolated from Diseased Wild and Farmed Marine Fish in Greece

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1. Introduction

World production of sea bream and sea bass farming has been rising over time. The total production of gilthead sea bream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax*, L.) in Europe has been increased. These marine fish species have high economic value in the aquaculture industry. More specifically, the total production of 92,310 tonnes in 1999 amounted to 175,196 tonnes in 2006, representing an average annual growth rate of 9.6%. Greece is the country with the largest production of euryhaline fish (sea bream and sea bass) between Mediterranean countries. In 2008, Greece held its largest production (130,000 tonnes of which 95,000 tonnes sea bass and sea bream). Climatic and geomorphologic conditions of Greece promoting the cultivation of euryhaline fish, grants and projects given by the European Union, the decline of fish stocks and the restrictions have been imposed last yeas in fishing, contributed significantly in the development of the industry of fish farming. Today the industry fully covers the needs of the Greek market and most of the quantity is exported to foreign markets, the main destination countries Italy, Spain, France, England and Portugal. Currently, additional species have entered in the farming, belonging mainly to the family Sparidae, such as *Puntazzo pntazzo*, *Diplodus sargus*, *Lithognathus mormyrus, Pagrus pagrus, Pagellus erythrinus* and *Dentex dentex*.

Bacterial diseases of fish origin have become one of the major agents of economical losses since the beginning of marine farming (Kubota & Takakuwa, 1963; Anderson & Conroy, 1970). The development of intensive marine fish farming in the form of the concentration of large quantities of biomass in a relatively small volume water leads-under certain conditions (combination of factors) – to the emergence of diseases which lead to losses in the population. The occurrence of a disease can lead to death or symptoms both refer to deviation from the normal structure or function of the host (Hedrick, 1998). Most diseases of farmed fish originate from wild populations. The close contact between farmed and wild fish results in exchange of pathogens. The clinical symptoms caused by any pathogen depend on the type of host, age of the fish and stage of disease (acute, chronic, subclinical form). Moreover, in some cases, there is no correlation between internal and external injuries. In fact, systemic diseases (eg. pasteurellosis) with high mortality rates, causing internal damage to infected fish, but often have a healthy appearance. Conversely, other diseases with relatively low mortality cause significant physical damage, including ulcers, necrosis, exophthalmos, making the fish unfit for the market.The diseases, the number and

types of bacterial pathogens have been well documented in several farmed fish species, such as global production of sea bream and sea bass farming has been rising over time. Also the incidence of diseases and the number and types of bacterial pathogens have been well documented in farmed fish species, such as salmonids and turbot. However, for farmed and wild fish species in Greece the number and type of bacteria associated with pathology have been described sporadically (Athanassopoulou et al., 1999; Bakopoulos et al., 1995; Varvarigos 1997; Yiagnisis et al., 1999, 2007).
The purpose of this chapter is to give an accurate, as possible, description of the main bacterial pathogens isolated from farmed and wild fish in Greece and to give information of their occurrence in relation to age and season. This is the first integrated study from Greece including a lot of farmed and wild fish species.

1.1 Clinical signs

Signs of diseases include anorexia, lethargies, pale gills, disorientation, darkening in colour, abdominal retention, exophthalmos, abdominal swelling, and external haemorrhages in the head, eyes, skin, gills and at the bases of the fins as well as skin ulcers.

1.2 Post-mortem findings

Visceral petechiation, pale kidneys, enlarged spleen and kidney, liquefactive renal necrosis, lesions in the kidney, tubercles in the spleen, are some of the post-mortem findings.

1.3 Laboratory testing

Diseased fish collected from Greek fish farms. Dead (recently) or moribund fish, showing any symptoms, were transported to the laboratory under refrigeration (2 ºC -8 ºC) and opened aseptically. Sampling was carried out mainly from headkidney, but sometimes from the spleen, liver and the brain (especially in small fry). Tryptic Soy agar (Oxoid), Tryptic Soy Broth (TSB Oxoid) enriched with 2% NaCl (TSAS, TSBS), Marine agar (MA, Difco) and Thiosulfate-Citrate-Bile salt-Sucrose agar (TCBS, Oxoid) were used as culture media for the bacteria. All inoculated culture media incubated at 22 º C for 2-5 days. A representative number of different types of colonies detected on culture media was collected from plates and streaked on TSAS plates for purity and to carry out identification. Pure cultures of the isolates, obtained by repeated plating on TSAS, were identified by biochemical characterization. Bacteria, identified as *Listonella anguillarum* and *Photobacterium damselae* subsp.*piscicida,* were confirmed serologically by agglutination test (system BIONOR).

2. Identification of bacteria

When identifying bacteria, certain characteristics are selected and used for this purpose. Pure cultures of the isolates, obtained by repeated plating on TSAS are used for identification. Primary identification usually involves simple tests such as morphology, motility, growth on various types of culture media, catalase and oxidase tests. Gram staining reveals the morphology and divides bacteria in two categories - the Gram-positive and the Gram-negative bacteria. For morphological appearance it is preferable to examine young cultures from non-selective media. Bacterial colonies of a single species, when grown on specific media under controlled conditions are described by their characteristic size, shape and pigment. The fermentation of glucose may also be used to distinguish between groups of bacteria. Using these few simple tests it is usually possible to place bacteria, provisionally, in one of the main groups. Then the isolated bacterium is subjected to a battery of tests. Some of them are found in commercial identification systems.

2.1 Growth of bacteria at different temperatures

The growth medium used, for these tests, was the Tryptic Soy Broth with 2% NaCl. The temperatures chosen were 4, 35 and 40 º C for 7, 2 and 1-2 days. The growth of microorganisms at different temperatures is used as an identification key. For example *Vibrio aestuarinus* does not grow at 40 º C but *Vibrio alginolyticus* and *V.parahaemolyticus* can grow.

2.2 Growth of bacteria in different concentration of sodium chloride

The concentrations of sodium chloride were selected 0,3,6,8 and 10%. The growth medium used for these tests was the Tryptic Soy Broth with 2% NaCl. This culture medium is a differential medium because it allows the investigator to distinguish between different types of bacteria based on the trait of NaCl tolerance. Thus a selective, differential medium for the isolation of *Vibrio alginolyticus,* contains a high concentration of salt (10%) that inhibits the growth of *Vibrio harveyi* (it grows until 8% NaCl).

2.3 Using the system API 20E (Biomerieux)

Many commercially available diagnostic kits have been introduced into routine laboratory diagnostics of fish pathogens, such as API 20E, API ZYM, API 20NE, API 50 CH, API Rapid ID 32 (bioMerieux,Marcy-l'Etoile, France), Biolog MicroPlatesGN2, GP2, AN (Biolog, Inc., Hayward, CA, USA), Enterotubes, BBL Crystal E/NF (Becton-Dickinson& Company, Franklin Lakes, NJ, USA), and some others. Of these, API 20E rapid identification system has been the most widely used for identification of fish pathogenic bacteria (Popovic et al., 2007).
The API-20E test kit, a very specific means of bacterial identification (for Enterobacteriaceae and other non-fastidious Gram-negative rods), is a collection of mini test tubes, each with a reagent that test for a different aspect of bacterial metabolism. It provides an easy way to inoculate and read tests. After incubation with an unknown Gram-negative bacterium, the interpretation of positive and negative tests allows for identification to the species level. The preparation of inoculum for the API 20E, was the addition of pure-developed colonies in 5 ml of 2% NaCl. The solutions used for inoculation of cells as indicated respectively by the manufacturer. The inoculation of cells of the tiles and the addition of paraffin oil was made with the help of sterile pipettes volume of 1ml, under sterile conditions. Incubation of plates was at 22-25 º C for 24-48 h. Reading the results was made according to the instructions. It is known that the information from the database of microorganisms API20E identification system is based on results from the study of human clinical strains. For this reason, the identification through the database API 20E system was not used in mind completely. The results of the reactions, however, were considered for identification of bacteria.

2.4 Using the system API Staph (Biomerieux)

For identification of Gram-positive cocci, that give a positive reaction of catalase and negative oxidase reaction, the Api Staph identification system was used (Biomerieux) according to manufacturer's instructions. *Staphylococci* are gram-positive cocci occurring most often in irregular "grape-like" clusters. API-Staph consists of a strip containing dehydrated test substrates in individual microtubes. The tests are reconstituted by adding an aliquot of the API-Staph medium to each tube inoculated with the strain to be studied. The tests included acid production from d-glucose, d-trehalose, d-mannitol, d-mannose, xylose, maltose, lactose, sucrose, *N*-acetylglucosamine, raffinose, d-fructose, d-melibiose, xylitol, and α-methyl-glucosamine; nitrate reduction; and alkaline phosphatase, arginine dihydrolase, urease, and acetoin production. Coagulase production is a significant additional key. It is the ability to clot plasma. A rapid slide coagulase test may be performed. Most *Staphylococcus* species isolated from fish are coagulase negative. The data have been processed using the software program ApiLab Plus. API Staph (Biomerieux) is an established identification system for *Staphylococci*, used for many years.

2.5 Identification of isolated bacteria: Present work

Pure cultures of the isolates, obtained by repeated plating on TSAS, were identified by biochemical characterization following the criteria described by Austin et al., 1995. Biochemical keys, described by Alsina & Blanch, 1994a, 1994b, were used for identification of *Vibrio* species. These biochemical keys are designed for environmental and clinical isolates and can be used for strains that are Gram negative, giving a positive oxidase reaction, grow in TCBS medium. Some trials were included in the system identification Api 20E. The following biochemical and morphological tests were made: Gram stain, cell morphology, oxidase reaction, catalase reaction, motility, test glucose O-F, growth in TCBS agar, swarming ability of colonies in TSAS, growth in 0, 3,6,8 and 10% NaCl, growth at 4 º C, 35 º C, 40 º C, resistance at 10 and 150 mg O/129, resistance to 10μg ampicillin and tests of API 20E system as the ortho-nitro-phenyl-BD-galaktopyranozidium, hydrolysis of arginine, the decarboxylation of lysine and ornithine, assimilation of citrate, production H_2S, production of urease, deamination of tryptophan, indole production and aketoinis, hydrolysis of gelatin, fermentation of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, meliviozis, amygdalin and arabinose as carbon sources.

Two thousands one hundred twenty four bacterial isolates obtained from diseased farmed and wild fish species in Greece were identified. During the nine years (1997-2005) a total of 2124 strains of bacteria were isolated from 430 cases, as shown in Table 2.5.1.

Fish species	Number of bacterial isolates
Dicentrarchus labrax	1017
Sparus aurata	887
Puntazzo puntazzo	99
Pagellus erythrinus	21
Pagrus pagrus	19
Diplodus sargus	12
Dentex dentex	12
Mugil cephalus	6
Epinephelus marginatus	4
Lithognathus mormyrus	4
Wild fish species	43

Table 2.5.1 Origin and number of 2124 identified bacterial isolates from diseased farmed and wild fish in Greece (Yiagnisis and Athanassopoulou, 2011).

Bacteria were identified initially at the genus level and then at the species level.

2.5.1 Bacteria isolated from European sea bass (*Dicentrarchus labrax*)

Figure 2.5.1.1 shows the isolation rates of bacterial groups from diseased farmed European sea bass in Greece from 1997-2005 (Yiagnisis and Athanassopoulou, 2011).

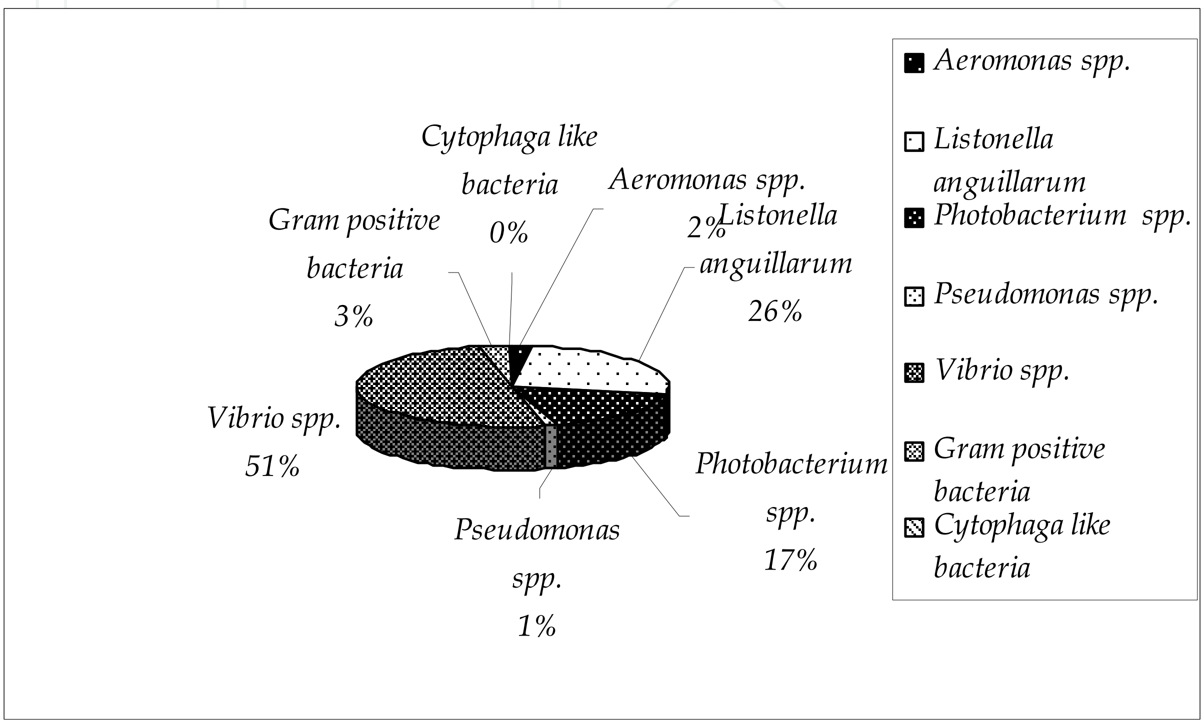

Fig. 2.5.1.1. Bacterial groups isolated from diseased farmed European sea bass in Greece (Yiagnisis and Athanassopoulou, 2011).

Different species of *Vibrios* together with *Listonella (Vibrio) anguillarum* constitute 77% of total bacteria isolated from diseased European sea bass. The percentage of isolated *Photobacterium spp.* is 17% while *Photobacterium damselae* subsp. *piscicida* is the main species. The remaining groups of bacteria were isolated at low rates. Table 2.5.1.1 shows the bacteria isolated from sea bass divided into age groups.

Bacterial species most frequently isolated from sea bass are *Listonella anguillarum*, *Vibrio alginolyticus*, *Photobacterium damselae* subsp. *piscicida*, *Vibrio splendidus* II and *Vibrio parahaemolyticus. L. anguillarum* is the most frequent bacterial isolated species from European sea bass. *L. anguillarum* and *Photobacterium damselae* subsp. *piscicida* were isolated mainly from larger fish. *Vibrio splendidus* II has the highest incidence in fry. The majority of bacteria were isolated during spring, as season but on September, as month (Figure 2.5.1.2). As shown in Figure 2.5.1.3, the greater isolation frequency of *Listonella anguillarum* occurred in April while the greater isolation frequency of *Vibrio splendidus* II occurred in May and *Photobacterium damselae* subsp. *piscicida* and *Vibrio alginolyticu*s increased frequencies of isolation were observed in September. For *Vibrio parahaemolyticus* the most observed incidence was in October. It is observed that the bacterium *Vibrio alginolyticus* was isolated simultaneously with *Photobacterium damselae* subsp. *piscicidae.*

Bacterial species	sea bass fry	sea bass	no data	Total
Aeromonas sobria	4			4
Aeromonas spp.	11	1	4	16
Bacillus circulans		2		2
Bacillus pumilus	3			3
Bacillus spp.	4			4
Cytophaga like bacteria	4			4
Flavobacterium spp.		3		3
Listonella anguillarum	58	203		261
Photobacterium damselae subsp.damselae		12		12
Photobacterium damselae subsp.piscicida	61	102		163
Photobacterium phosphoreum	1			1
Pseudomonas fluorescens/putida		8		8
Pseudomonas spp.	6	1		7
Staphylococcus auricularis	2			2
Staph.cohnii	4			4
Staph.epidermidis		6		6
Staph.hominis		3		3
Staph.schleiferi		3		3
Staph.warneri		1		1
Staph.xylosus		4		4
Stenotrofomonas maltophilia		1		1
Vibrio alginolyticus	82	105	4	191
Vibrio anguillarum like	2	3	4	9
Vibrio harveyi	13	8		21
Vibrio costicola	8	4		12
Vibrio fisheri		1		1
Vibrio fluvialis	25			25
Vibrio logei	4			4
Vibrio mediterranei	3			3
Vibrio nereis	4			4
Vibrio nigrapulchritudo	4			4
Vibrio ordalii	3			3
Vibrio parahaemolyticus	32	42		74
Vibrio splendidus II	105	16		121
Vibrio tubiashii	28	1		29
Vibrio vulnificus	4			4
Total	475	530	12	1017

Table 2.5.1.1. Number of bacterial isolates from diseased farmed European sea bass in correlation with the age of fish (Yiagnisis and Athanassopoulou, 2011).

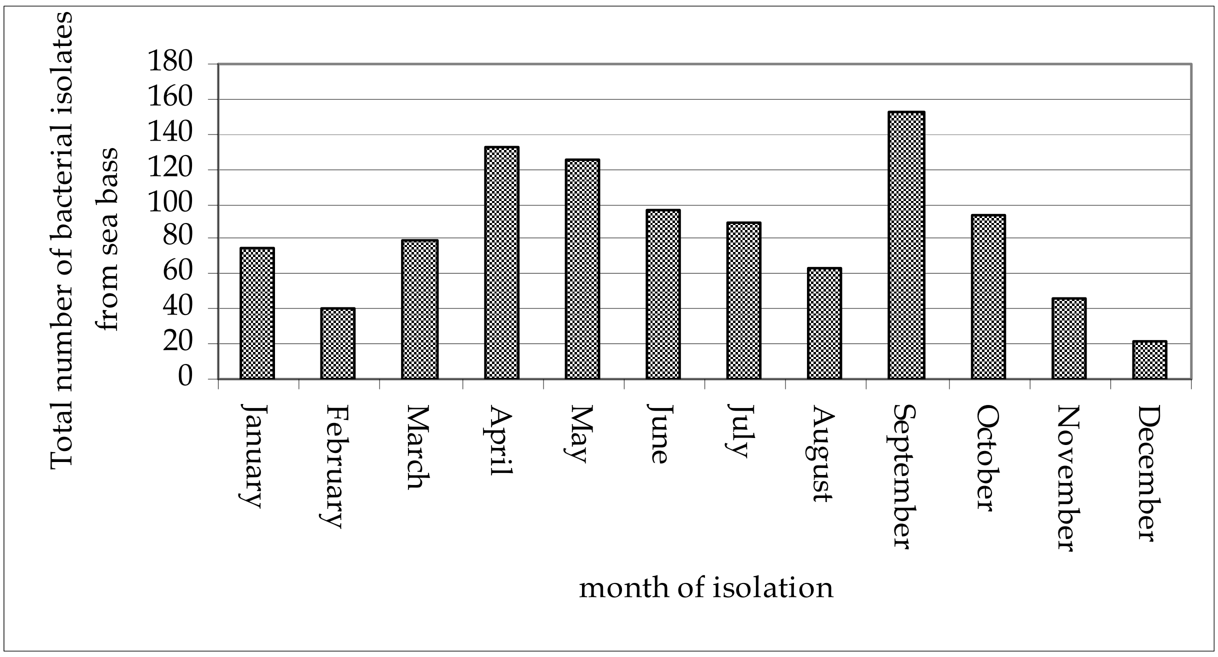

Fig. 2.5.1.2. Total number of bacterial isolates from diseased farmed European sea bass in relation to the month of isolation (Yiagnisis and Athanassopoulou, 2011).

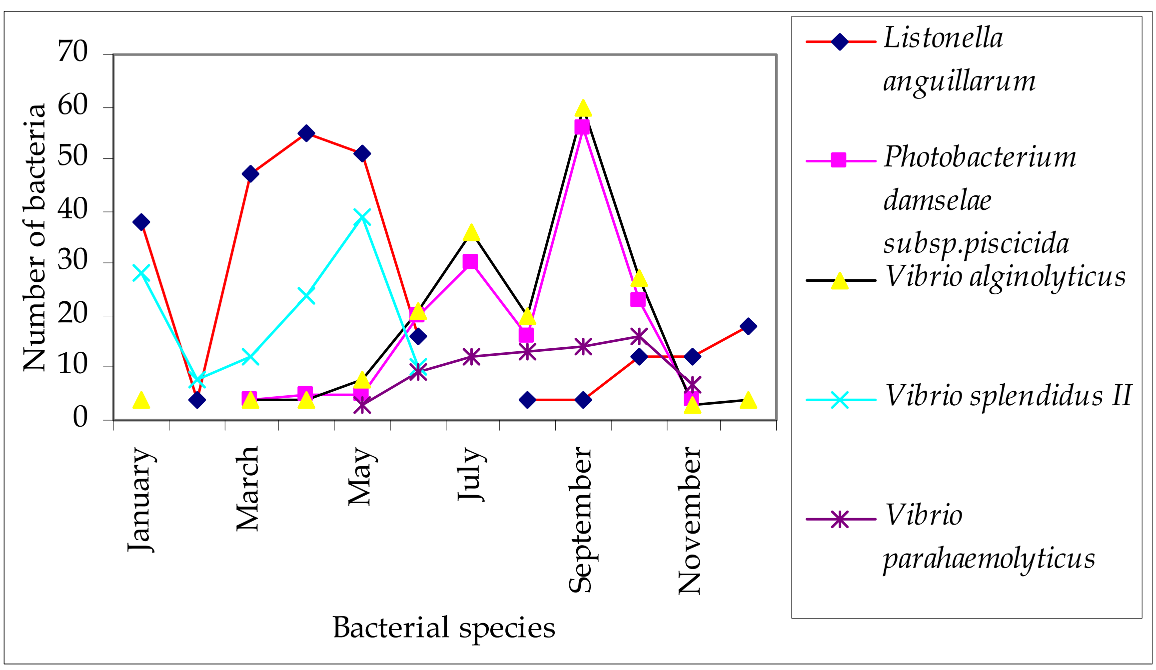

Fig. 2.5.1.3. Number of bacterial species, most frequently isolated from diseased farmed European sea bass in relation to the month of isolation (Yiagnisis and Athanassopoulou, 2011).

Listonella anguillarum was the most frequent species isolated from farmed sea bass during the 9-year sample period.

2.5.2 Bacteria isolated from gilthead sea bream (*Sparus aurata*)

Figure 2.5.2.1 shows the isolation rates of bacterial groups from diseased farmed gilthead sea bream in Greece from 1997-2005 (Yiagnisis and Athanassopoulou, 2011).

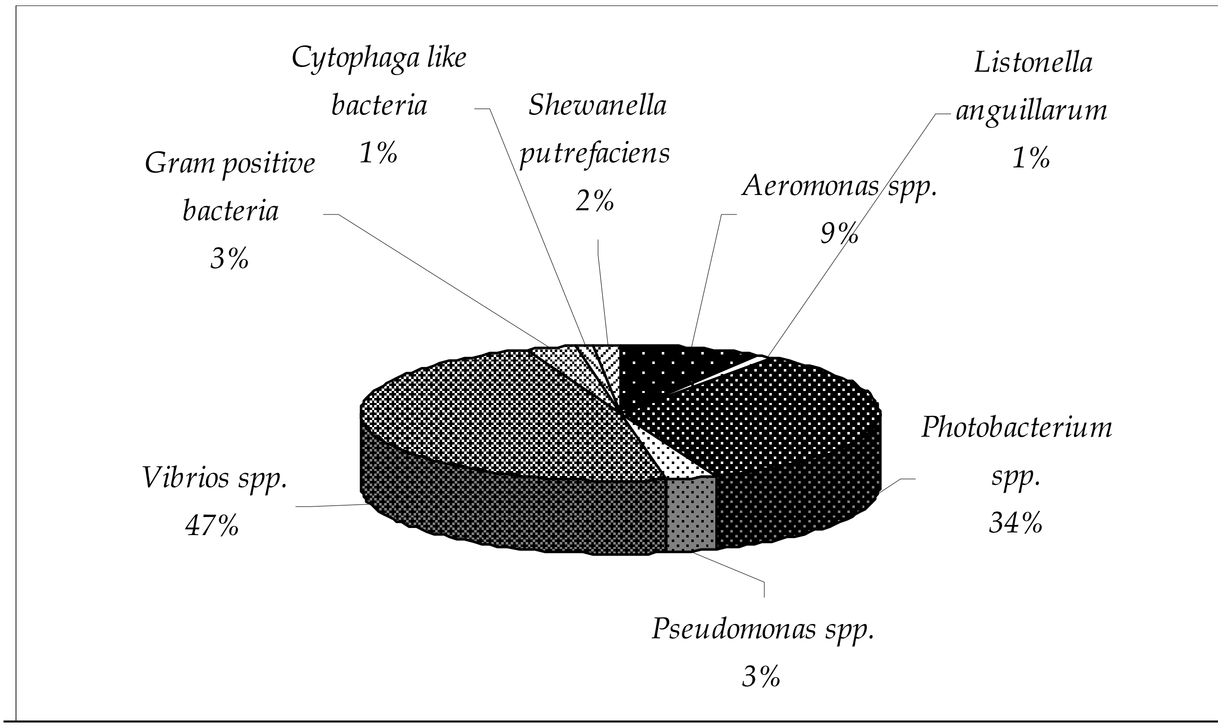

Fig. 2.5.2.1. Rates of isolation of different bacterial groups from diseased farmed gilthead sea bream reared in Greece from 1997 to 2005 (Yiagnisis and Athanassopoulou, 2011).

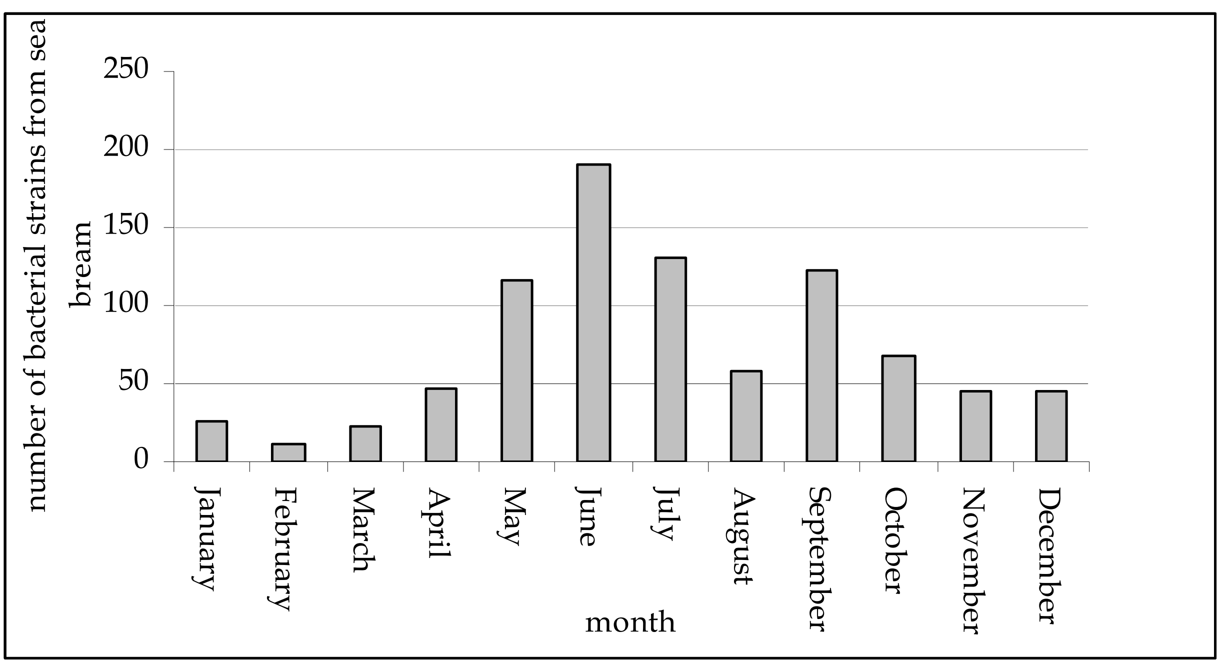

Fig. 2.5.2.2. Number of bacterial strains isolated from diseased farmed gilthead sea bream in correlation to the month of isolation (Yiagnisis and Athanassopoulou, 2011).

Bacterial species	sea bream	fry sea bream	no data	Total
Aeromonas hydro/caviae	7			7
Aeromonas sobria	22			22
Aeromonas spp.	27	8		35
Bacillus spp.		4		4
Cytophaga like bacteria	4	4		8
Listonella anguillarum	8			8
Photobacterium damselae subsp.*damselae*	56	24		80
Photobacterium damselae subsp.*piscicida*	139	18		157
Photobacterium spp.		4		4
Pseudomonas cepacia		2		2
Pseudomonas fluorescens/putida	6			6
Pseudomonas spp.	15	2		17
Shewanella putrefaciens	8	4		12
Staph. cobuii	3			3
Staph.lentus		3		3
Staph.schleifer		3		3
Staph.warneri	5			5
Vibrio alginolyticus	117	42	4	163
Vibrio anguillarum like		2	3	5
Vibrio harveyi	20	7		27
Vibrio costicola	60	12		72
Vibrio fisheri	4	12		16
Vibrio fluvialis	15	5		20
Vibrio harveyi	11	4		15
Vibrio mediterranei	4	4		8
Vibrio nereis	4			4
Vibrio nigrapulchritudo	4			4
Vibrio ordalii	2			2
Vibrio parahaemolyticus	50	6		56
Vibrio pelagius		6		6
Vibrio pelagius II	3			3
Vibrio splendidus II	56	8		64
Vibrio tubiashii	8			8
Vibrio vulnificus	31	4		35
Bacillus megaterium	3			3
Grand Total	692	188	7	887

Table 2.5.2.1. Number of bacterial isolates from diseased farmed gilthead sea bream in correlation with the age of fish (Yiagnisis and Athanassopoulou, 2011).

In Figure 2.5.2.1 it is observed that different *Vibrio* species along with *Listonella anguillarum* constitute 48% of total bacteria isolated from diseased sea bream. The percentage of isolated *Photobacterium spp.* is 34% while *Photobacterium damselae* subsp. *piscicida* is the main species. The incidence of *Aeromonas* spp. was 9%, of *Pseudomonas* spp. 3%, of *Shewanella putrefaciens* 2%, of Gram positive cocci 3% and of *Cytophaga* like bacteria 1%. As it is shown in the table 2.5.2.1, the majority (692) of total isolated bacteria (887) belongs to sea bream fry. Bacteria with the highest incidence in the sea bream is *Photobacterium damselae* subsp.*piscicida*, *Vibrio alginolyticus, Photobacterium damselae* subsp. *damselae, Vibrio costicola, Vibrio splendidus* II, *Vibrio parahaemolyticus* and *Vibrio vulnificus*. *Listonella anguillarum* was isolated from fry only, with very low frequency. The majority of bacteria were isolated during summer, especially June (Figure 2.5.2.2).
Figure 2.5.2.3 shows the number and species of bacterial species, most frequently isolated from sea bream in relation to month of isolation.

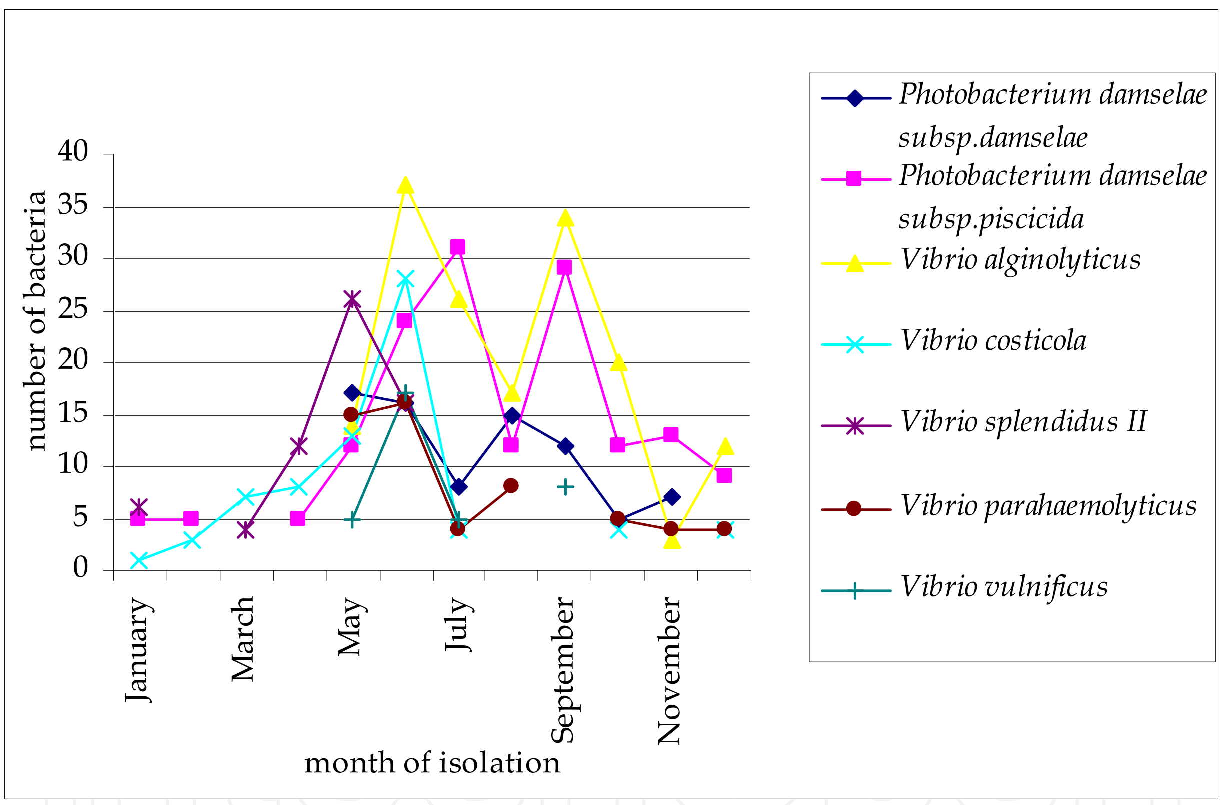

Fig. 2.5.2.3. Number and species of bacterial species, most frequently isolated from diseased farmed gilthead sea bream in relation to month of isolation (Yiagnisis and Athanassopoulou, 2011).

Vibrio alginolyticus, Photobacterium damselae subsp. *piscicidae, Photobacerium damselae* subsp. *damselae, Vibrio vulnificus, Vibrio parahaemolyticus* and *Vibio costicola* are most frequently isolated from diseased farmed sea bream in the summer and *Vibrio splendidus* II in May. It is observed that the bacterium *Vibrio alginolyticus* was isolated almost simultaneously with *Photobacterium damselae* subsp. *piscicida,* as in sea bass.
The lower numbers of bacteria from diseased farmed fish were isolated during the winter. These results are in agreement with those of other researchers (Company et al., 1999) where

in a bacteriological and parasitological study of farmed dentex conducted in the Mediterranean region, it was reported a relationship between high mortality and high temperature water. These results however are not in agreement with the results of Zorilla et al, 2003, who in a bacteriological study of farmed sea bream (*Sparus aurara*, L.) held in southwest Spain reported the lower numbers of bacterial isolates during summer.

2.5.3 Bacteria isolated from sharpsnout sea bream (*Diplodus puntazzo*)

Figure 2.5.3.1 shows the isolation rates of bacterial groups from diseased farmed sharpsnout sea bream in Greece from 1997-2005. It is observed that different *Vibrio* species along with *Listonella anguillarum* constitute 73% of total bacteria (99) isolated from diseased sharpsnout sea bream. The percentage of isolated *Photobacterium damselae* subsp. *damselae* is 12% while *Vibrio alginolyticus* is the main isolated species. The incidence of *Aeromonas* spp. was 4%, of *Pseudomonas fluorescens/putida* 4% and of *Staphylococcus* spp. 7%. The table 2.5.3.1 shows the bacteria isolated from sharpsnout sea bream divided into age groups. Most of bacterial strains have been isolated from larger fish than fry.

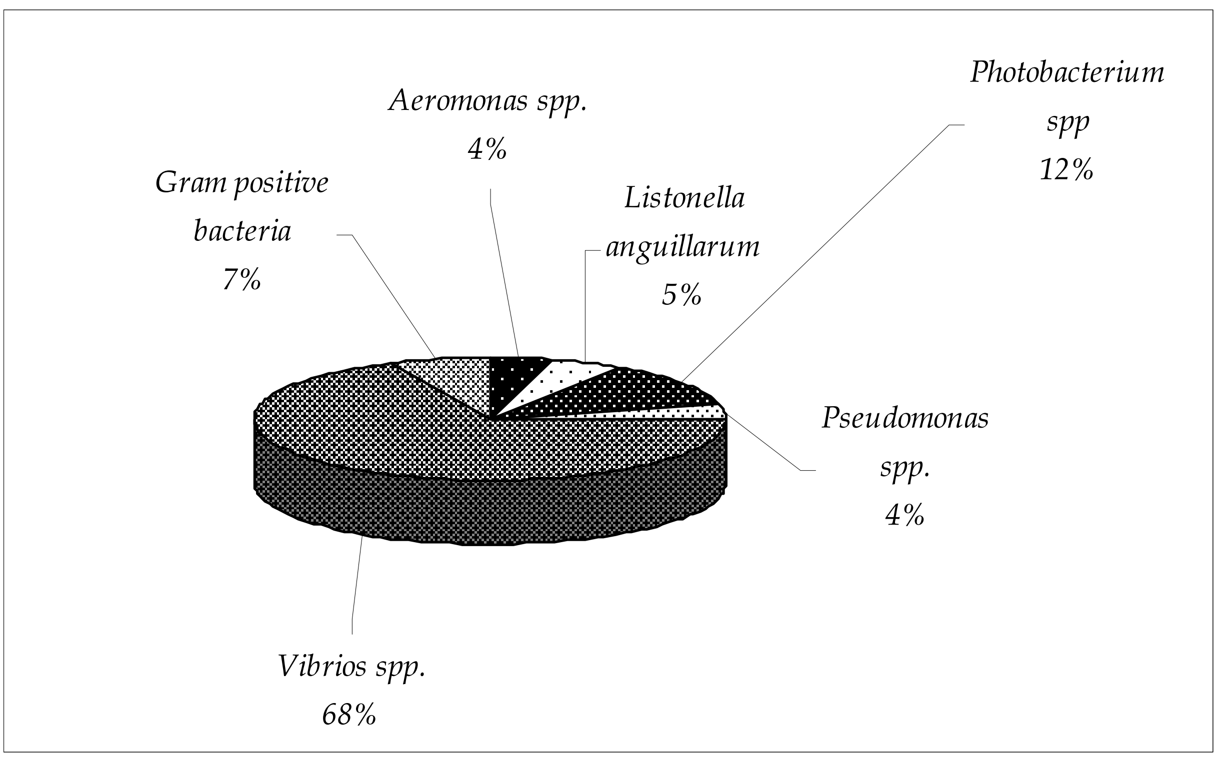

Fig. 2.5.3.1. Bacterial groups isolated from diseased farmed sharpsnout sea bream in Greece (Yiagnisis and Athanassopoulou, 2011).

Bacteria most frequently isolated from farmed diseased sharpsnout sea bream is *Vibrio alginolyticus*, *Vibrio costicola* and *Photobacterium damselae* subsp. *damselae. Vibrio alginolyticus* is the most often isolated bacterium during the summer and autumn but it is not responsible for great losses. Most of the bacteria from sharpsnout sea bream have been isolated in June, October and November (Fig 2.5.3.2).

Bacterial species	sh. sea bream fry	sh. sea bream	Total
Aeromonas caviae		4	4
Listonella anguillarum	5		5
Photobacterium damselae subsp.damselae		12	12
Pseudomonas fluo/putida	4		4
Staphylococcus capitis		4	4
Staphylococcus epidermidis	3		3
Vibrio alginolyticus	14	19	33
Vibrio harveyi		8	8
Vibrio costicola		14	14
Vibrio fisheri		4	4
Vibrio parahaemolyticus	4		4
Vibrio.splendidus II		4	4
Total	30	69	99

Table 2.5.3.1. Number of bacterial isolates from diseased farmed sharpsnout sea bream in correlation with the age of fish (Yiagnisis and Athanassopoulou, 2011).

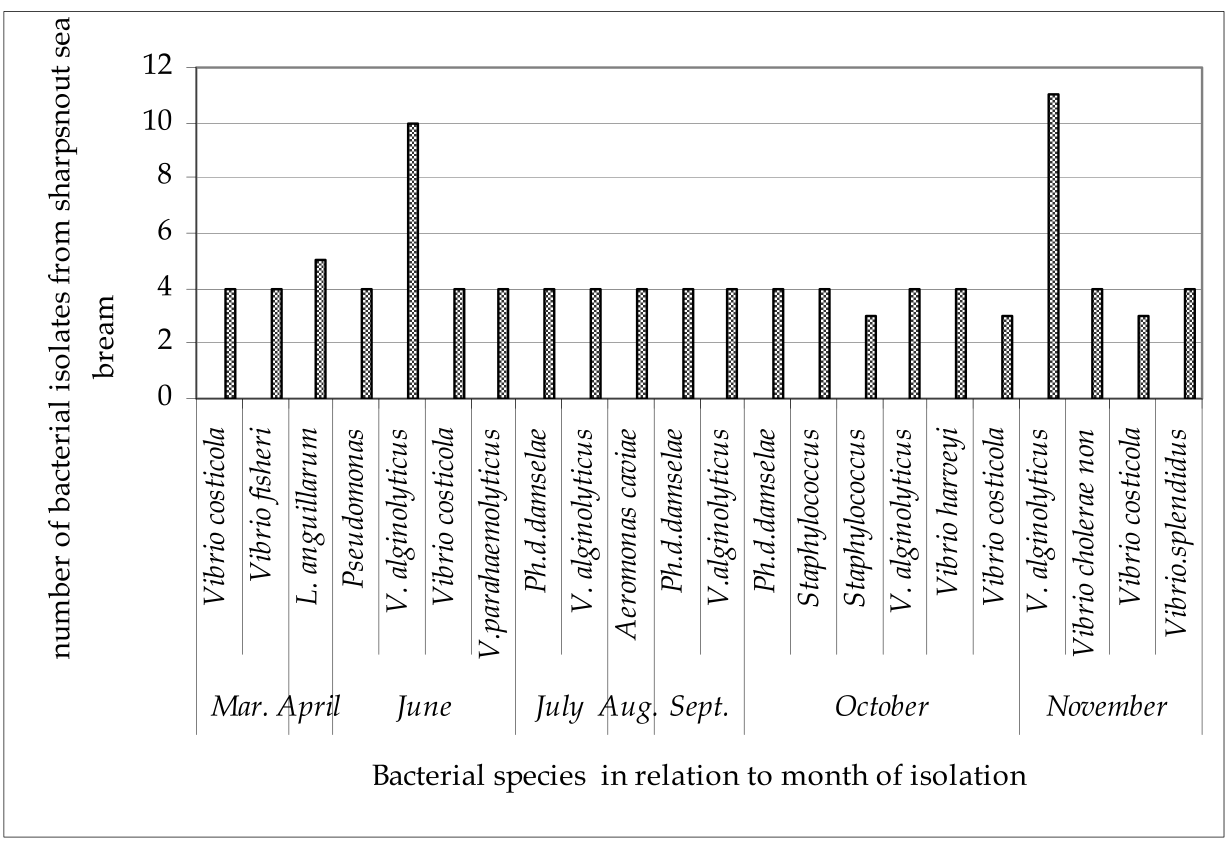

Fig. 2.5.3.2. Number of bacterial species isolated from diseased farmed sharpsnout sea bream in correlation to the month of isolation (Yiagnisis and Athanassopoulou, 2011).

2.5.4 Bacteria isolated from new species of farmed fish

In table 2.5.4.1 it is shown the number of bacterial strains, been isolated, from new species of farmed fish in relation to month of isolation. *Vibrio alginolyticus* is the most commonly isolated bacterial species. ***Listonella anguillarum*** was isolated from *Epinephelus* sp., *Photobacterium damselae* subsp. *piscicida* was isolated from *Mugil cephalus and Pagellus erythrinus. Photobacterium damselae* subsp. *damselae* was isolated from *Pagellus erythrinus*. The species isolated from *Diplodus sargus* are *Vibrio fisheri, Vibrio nereis,* and *Vibrio harveyi*. The majority of bacterial strains have been isolated during fall. No bacteria were isolated, from these new species of diseased farmed fish, during the winter.

New species of farmed fish	Month of isolation	Number of bacterial strains	Bacterial species	Total bacterial number
Pagrus pagrus	September	15	*Vibrio alginolyticus*	19
		4	*Vibrio splendidus II*	
Mugil cephalus	September and October	3	*Vibrio alginolyticus*	6
		3	*Photobacterium damselae* subsp. *piscicida*	
Epinephelus sp.	March	2	***Listonella anguillarum***	4
	February	2	*Vibrio anguillarum like*	
Diplodus sargus	April	4	*Vibrio fisheri*	12
	March	4	*Vibrio nereis*	
	April	4	*Vibrio harveyi*	
Pagellus erythrinus	October	4	*Photobacterium damselae* subsp. *damselae*	21
		4	*Aeromonas hydrophila*	
	November	5	*Photobacterium damselae* subsp. *piscicida*	
		4	*Vibrio harveyi*	
		4	*Vibrio costicola*	
Lithognathus mormyrus	August	4	*Vibrio alginolyticus*	4
Dentex dentes	August	4	*Aeromonas spp.*	12
		4	*Vibrio nereis*	
	February	4	*Vibrio costicola*	

Table 2.5.4.1. Number of bacterial strains, isolated, from new species of diseased farmed fish in relation to month of isolation (Yiagnisis and Athanassopoulou, 2011).

2.5.5 Bacteria isolated from wild fish

In table 2.5.5.1 it is shown the number and the species of bacterial strains, been isolated, from wild fish species in relation to month of isolation. *Listonella anguillarum* (Yiagnisis et al., 2007) and *Photobacterium damselae* subsp.*damselae* are the most frequently isolated species.

Fish species	Month of isolation	Number of bacterial strains	Bacterial species	Total bacterial number
Mugil cephalus	September	1	*Photobacterium damselae* subsp.*damselae*	3
		1	*Vibrio harveyi*	
		1	*Vibrio alginolyticus*	
Atherina boyeri	December	28 (1 case)	*Listonella anguillarum*	28
Gobius niger	April	1	***Vibrio splendidus*** II	1
Labrus spp	April	1	***Vibrio splendidus*** II	1
Sciaena umbra	October	1	*Pseudomonas* spp.	1
Boops boops	July	9 (1 case)	*Photobacterium damselae* subsp.*damselae*	9
Total number of bacterial strains	43			

Table 2.5.5.1. Number of bacterial strains, isolated, from diseased wild fish species in relation to month of isolation (Yiagnisis and Athanassopoulou, 2011).

Most of the bacteria, isolated from both farmed and wild fish, were Gram-negative. Specifically 77% (for farmed sea bass), 73% (for farmed sharpsnout sea bream) and 48% (for farmed sea bream) of the isolated bacteria were identified as *Vibrio* species (including *Listonella anguillarum*). For farmed species *Sparus aurata* (sea bream) and *Diplodus puntazzo* (sharpsnout sea bream) the most frequent type was *Vibrio alginolyticus*. This high incidence of *Vibrio* spp. have was found in previous studies done in Spain by Balebona et al, 1998b and Zorilla et al, 2003. *Vibrio alginolyticus* isolated frequently with other *Vibrio* species and *Photobacterium damselae* subsp. *piscicida*. In fact, from our results it appears that the incidence of *Vibrio alginolyticus* in *sea* bream and sea bass is similar to that of *Photobacterium damselae* subsp. *piscicida. V. alginolyticus* was isolated from water of fish farms and live food. Other authors have reported the isolation of this species from water aquaculture (Angulo et al., 1993, Blanch et al., 1997).

3. Conclusion

European sea bass (*Dicentrarchus labrax*) is the fish species with the majority of isolated bacterial strains in this study. Bacterial species most frequently isolated from sea bass are *Listonella anguillarum*, *Vibrio alginolyticus*, *Photobacterium damselae* subsp. *piscicida*, *Vibrio splendidus* II and *Vibrio parahaemolyticus. Listonella anguillarum* is the main bacterial species isolated. *L. anguillarum* constitute 26% of total bacteria isolated from diseased sea bass, with the greater isolation frequency occured in April. *Photobacterium damselae* subsp. *piscicida* is an obligate fish pathogen, isolated also from sea bass fry. *Vibrio alginolyticus* is an opportunistc vibrio, isolated from sea bass. *Photobacterium damselae* subsp. *piscicida* and *Vibrio alginolyticu*s increased frequencies of isolation were observed in September. Another opportunistic vibrio, *Vibrio splendidus* II is isolated mainly from sea bass fry and its greater isolation frequency occurred in May. The majority of bacteria from sea bass were isolated during spring and fall. The majority of total isolated bacteria from sea bream comes from fry. Bacteria with the highest incidence in the sea bream is *Photobacterium damselae* subsp.*piscicida*, *Vibrio alginolyticus, Photobacterium damselae* subsp. *damselae*, *Vibrio costicola*, *Vibrio splendidus* II, *Vibrio parahaemolyticus* and *Vibrio vulnificus*. *Listonella anguillarum* was isolated only from fry with 1% frequency. The majority of bacteria were isolated from sea bream during summer, especially June. *Photobacterium damselae* subsp. *piscicida* had its greater isolation frequency occurred in July. *Vibrio alginolyticus* was isolated almost simultaneously with *Photobacterium damselae* subsp. *piscicidae,* as in sea bass. The percentage of isolated *Photobacterium damselae* subsp. *damselae* is 12% from sharpsnout sea bream *Vibrio alginolyticus* is the main isolated species from sharpsnout sea bream and other new species of farmed fish. The majority of bacterial strains from all these species have been isolated during fall. Main pathogens as *Listonella anguillarum and Photobacterium damselae* subsp. *piscicida* can be isolated from wild fish species.

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5. References

Alsina, M. & Blanch, A. R. (1994a). A set of keys for biochemical identification of environmental *Vibrio* species. *Journal of Applied Bacteriology* Vol.76, pp. 79-85.

Alsina, M. & Blanch, A. R. (1994b). Improvement and update of a set of keys for biochemical identification of environmental *Vibrio* species. *Journal of Applied Bacteriology* Vol.77, pp. 719-721.

Anderson, J. & Conroy, D. (1970). *Vibrio* Disease in Marine Fishes. In: *A Symposium in Marine Fishes and Shellfishes*. Snieszko, F.F. (Ed.), Special Publication No. 5, American Fisheries Society, USA.

Angulo, L., López, J.E &. Vicente, J.A. (1993). Distribución del género *Vibrio* en el agua de entrada de dos piscifactorias de rodaballo (*Scophthalmus maximus*). In: A. Cerviño, A. Landín, A. de Coo, A. Guerra and M. Torre, Editors, *Proceedings of the IV National Congress of Aquaculture*, Xunta de Galicia, Vilanova de Arousa (Spain) (1993), pp. 611–616.

Athanassopoulou, F., Prapas, A. & Rodger, H. (1999). Diseases of *Puntazzo puntazzo* Cuvier in marine aquaculture systems in Greece. *Journal of Fish Diseases* Vol.22, pp. 215-218.

Austin, B., Alsina, D.A. Austin, A.R. Blanch, F.Grimont, et al. (1995) Identification and typing of *Vibrio anguillarum:* a comparison of different methods. *Systematic and Applied Microbiology,* Vol.18, pp. 285-302.

Bakopoulos, V., Adams, A. &. Richards, R.H. (1995). Some biochemical properties and antibiotic sensitivities of *Pasteurella piscicida* isolated in Greece and comparison with strains from Japan, France and Italy. *Journal of Fish Diseases* Vol.18, pp. 1-7.

Balebona, M.C., Zorrilla, I., Morin˜igo, M.A., Borrego, J.J., 1998b. Survey of bacterial pathologies affecting farmed gilt-head sea bream (*Sparus aurata* L.) in southwestern Spain from 1990 to 1996. *Aquaculture* Vol. 166, pp.19–35.

Blanch A. R., Alsina M., Simón M. & Jofre J. (1997) Determination of bacteria associated with reared turbot (*Scophthalmus maximus*) larvae. *Journal of Applied Microbiology* Vol.82, pp. 729–734.

Company R., Sitjà-Bobadilla A., Pujalte M.J., Garay E., Alvarez-Pellitero P. & Pérez-Sánchez J. (1999) Bacterial and parasitic pathogens in cultured common dentex, *Dentex dentex* L. *Journal of Fish Diseases* Vol.22, pp. 299–309.

Hedrick, R. P. (1998). Relationships of the host, pathogen, and environment: Implications for diseases of cultured and wild populations. *Journal of Aquatic Animal Health* Vol.10, No.2, pp. 107-111.

Kubota, S. & Takakuwa, M. (1963). Studies on the diseases of marine cultured fishes, I. General description and preliminary discussion of fish diseases in the Mie Prefecture, *Journal of the Faculty of Fisheries, Prefectural University of Mie-Tsu* 6 (1963), pp. 107–124 (In Japanese) (Translation of the Fisheries Research Board of Canada, Biological Station, Nanaimo, BC, 1966).

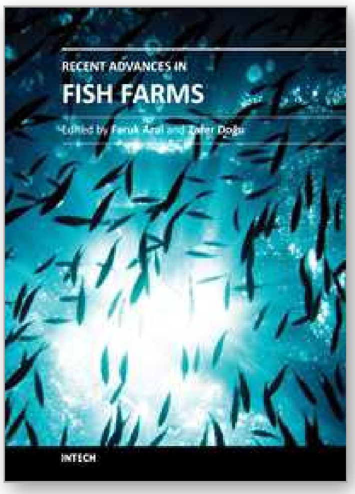
Popovic, N.T. Coz-Rakovac, R. & Strunjak-Perovic, I., 2007. Commercial phenotypic tests (API 20E) in diagnosis of fish bacteria: a review, *Veterinarni Medicina* 52, pp. 49–53.

Varvarigos, P. (1997): Marine fish diseases in Greece Fish Farmer November /December, pp. 33-34.

Yiagnisis, M., Christofilogiannis, P., Rigos, G., Koutsodimou, M., Andriopoulou, A., Anastasopoulou, G., Nengas, I. & Alexis, M. (1999). Review of bacterial isolates in Greek mariculture during the period of 1995-1998. *Abstract book p-060. 9th International Conference EAFP "Diseases of Fish and Shellfish"*, 19-24 September 1999, Rhodos, Greece.

Yiagnisis, M., Vatsos, I.N., Kyriakou, C. & Alexis, M. (2007). First report of *Listonella anguillarum* isolation from diseased big scale sand smelt, *Atherina boyeri* Risso 1810, in Limnos, Greece. *Bulletin of the European Association of Fish Pathologist* Vol.27, No.2, pp. 61-69.

Zorrilla, M., Chabrillon, A. S., Rosales, P. D. Manzanares, E. M., Balebona, M. C. & Morinigo, M. A. (2003): Bacteria recovered from diseased cultured gilthead sea bream, *Sparus aurata* L. in southwestern Spain.Aquaculture, Vol.218, No.1-4, 27 March 2003, pp. 11–20.



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The world keeps changing. There are always risks associated with change. To make careful risk assessment it is always needed to re-evaluate the information according to new findings in research. Scientific knowledge is essential in determining the strategy for fish farming. This information should be updated and brought into line with the required conditions of the farm. Therefore, books are one of the indispensable tools for following the results in research and sources to draw information from. The chapters in this book include photos and figures based on scientific literature. Each section is labeled with references for readers to understand, figures, tables and text. Another advantage of the book is the "systematic writing" style of each chapter. There are several existing scientific volumes that focus specially on fish farms. The book consists of twelve distinct chapters. A wide variety of scientists, researchers and other will benefit from this book.