

Effect of Temperature and Seasonality Principal Epizootiological Risk Factor on Vibriosis and Photobacteriosis Outbreaks for European Sea Bass in Greece (1998-2013)

Georgios Bellos^{1*}, Panagiotis Angelidis² and Helen Miliou¹

¹Department of Applied Hydrobiology, Faculty of Animal Science and Aquaculture, Agricultural University of Athens, GR-11855 Athens, Greece

²Laboratory of Ichthyology, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece

Abstract

Our epizootiological survey was focused on bacterial diseases of European sea bass, *Dicentrarchus labrax* L., an important Mediterranean species for aquaculture, in Greece. Vibriosis and Photobacteriosis were the most severe bacterial diseases, located in the majority of Greek mariculture areas (Argolikos gulf, North Evoic gulf Maliakos gulf and Thesprotia Sagiada coast, Amvrakikos gulf, Aitolokarmania Mitikas coast, Ionian island coasts) during the period 1998-2013. A database of 152 cases was formulated, from which 134 cases concerned vibriosis and photobacteriosis, while the rest outbreaks were motile aeromonas septicemia and tenacibaculosis. PCA pointed out three principal components with the following ranking order: a) temperature and seasonality, b) group of mariculture areas and average body weight, and c) case year. The results of logistic analysis showed the temperature - seasonality as the first, in ranking, and the only statistically significant epizootiological risk factor. Contrast Test (low vs. high temperature values) also proved the significant effect of temperature ($p < 0.05$). Vibriosis pathogen *Listonella* (*Vibrio*) *anguillarum* was recorded in a wide temperature range (12-26°C) in the most of Greek rearing locations. In contrast, Vibriosis from rest vibria was found to a narrow temperature range, especially in Argolikos gulf, North Evoic gulf and Ionian Island coasts. Specifically, *V. harveyi* cases emerged at high temperatures (19-22°C), while those of *V. alginolyticus* and *V. splendidus* II at low temperatures (15-17°C). Photobacterium damsela subspecies piscicida in most of Greek rearing areas and Photobacterium damsela subspecies damsela in Argolikos gulf, North Evoic gulf and Ionian Island coasts appeared in a relatively wide range (19-25°C). However, they showed higher frequencies in warm period. The results will support an evolutionary epizootiological survey and will reinforce a preventive biosecurity program in Greek mariculture taking into consideration the temperature - seasonality factor along with the classical sanitary approach.

Keywords: Epizootiological survey; Risk factors; Vibriosis; Sea bass; Aquaculture; Greece; Preventive biosecurity

Short Communication

European sea bass [1] commonly known as “sea bass” in Mediterranean and European Union countries, is one of the most commercially important, mainly brackish and seawater fish species. It is characterized by the second higher global production amount of 48,000 tons in Greece in comparison with the Turkish highest production amount of 51,600 tons, in 2013 [2]. In Greece, as in other Mediterranean countries, mariculture is a dynamic aquaculture sector with an also intensive development of hatchery tanks and cage culture in Spain, Italy and France [3]. The hatchery production establish Greece as the most significant provider with a production amount of 192,000 thousand sea bass juveniles, not only nationally, but also for other Mediterranean countries, in 2013 [2]. European sea bass was the target fish species of our research with emphasis on causal pathogen agents, geographic distribution of their incidences through this epizootiological survey, for a long period 1998-2013. It has focused on clear case results, for the assessment of the epizootiological risk factors in most important coastline rearing areas in Greece. Similarly, epizootiological surveys have been performed in other Mediterranean or European countries, especially in France and Spain [4,5]. The Greek coastline most important mariculture locations of this survey consist of four Mediterranean fish culture area groups with approval from Greek Government Authorities have as follow: a) Thesprotia Sagiada and Mitikas gulf – Ionian Island coasts (Ionian Sea), b) West – Central – East Amvrakikos gulf (Ionian Sea), c) Maliacosgulf – Biotia – Evoia (North Evoic gulf) (Central Aegean Sea) d) Argolikos gulf – (South Aegean Sea). A database of 152 cases of severe bacterial diseases, for

European sea bass, was formulated. The 134 cases concerned vibriosis and photobacteriosis outbreaks caused from the pathogen bacteria shown (Figure 1). The rest 18 cases concerned motile aeromonas septicemia and tenacibaculosis (former marine flexibacteriosis) caused from *Aeromonas hydrophila*, *A. sobria*, *Aeromonas* spp. and *Tenacibaculum maritimum* (former *Flexibacter maritimus*). The 152 cases were classified into groups according to: a) temperature as low (11-19°C) and high (20- 28°C) values, b) seasonality as winter (December – February), spring (March – May), summer (June – August) and autumn (September – November), c) the above area groups of Greek coastline, d) average body weight as 0.1-5 g (immature innate non-specific immunity and almost absent specific acquired immunity class of larvae and juveniles), 6-80 g (mature innate and immature acquired immunity class of elder juveniles), 81-150 g (mature innate non-specific and mature acquired specific immunity class of young fish), 151-400 g (mature innate non-specific and mature acquired specific immunity of adult fish), and e) case-year from the

***Corresponding author:** Georgios Bellos, Department of Applied Hydrobiology, Faculty of Animal Science and Aquaculture, Agricultural University of Athens, GR-11855 Athens, Greece, Tel: 923326-022624; E-mail: elenmi@aua.gr

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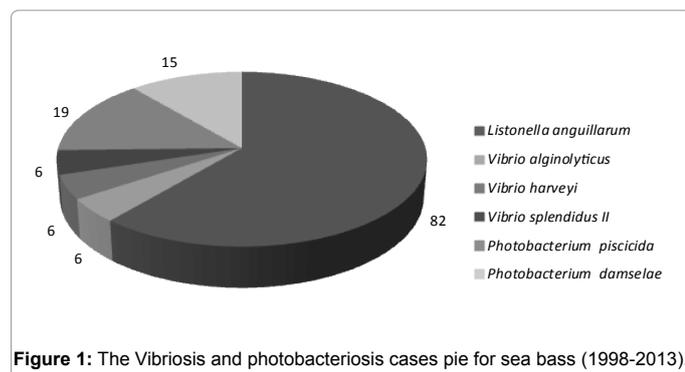


Figure 1: The Vibriosis and photobacteriosis cases pie for sea bass (1998-2013).

period (1998-2013). PCA pointed out three principal components with the following ranking order: a) temperature and seasonality, b) group area and average body weight, and c) case year. In a following Logistic Procedure, the two first principal components were considered. The results of logistic analysis showed the temperature - seasonality as the first, in ranking, and the only statistically significant epizootiological risk factor ($p < 0.05$). Contrast Test [6] with temperature as the only variable (low vs. high temperature values), also proved the significant effect of this risk factor ($p < 0.05$). Statistical analysis was performed with SAS [7]. In this survey, the sea bass classical vibriosis outbreaks from *Listonella anguillarum* appeared in a wide temperature value range (12-26°C). However, the acute classical vibriosis affected larvae and juveniles mainly at high temperatures, while the chronic or asymptomatic form appeared in young and adult fish at low temperatures. The chronic form skin ulcerative and visceral or intestinal of classical vibriosis has been reported only for adult fish at low temperatures in Greek coastline areas [8,9]. Larsen [10] reported that *L. anguillarum* can survive, not only in winter but also in summer, reaching a maximum population number. It has been suggested that vibriosis appears when the water temperature exceeds 10-12°C and occurs more often in areas where overcrowding exists and environmental equilibrium is fragile [11]. Outbreaks from *Vibrio harveyi* were recorded for the acute vibriosis form in juveniles at 19°C in May with high losses and for the sub-acute intestinal and visceral vibriosis in adult fish, at 22°C in August. Incidences from *V. harveyi* have also been reported for sea bass, at similar temperature values, in North Ionian Sea (South Italy) [12]. Contrary to the above, vibriosis from *Vibrio alginolyticus* and *V. splendidus* sero-type II outbreaks were recorded at low temperatures 15-17°C (November - February) in both juveniles and adults, causing higher mortalities in the former. Similarly, cases from *V. alginolyticus* have been reported for sea bass at low temperature values in Adriatic Sea (North Italy) and North Ionian Sea (South Italy) [12]. The photobacteriosis outbreaks from *Photobacterium damsela* subspecies *piscicida* (former *Pasteurella piscicida*) emerged at temperature values 19-25°C (April - September). At this temperature range, cases from sea bass juveniles and young fish with acute septicemia epizootics in Amvrakikos and Argolikos gulf have been reported [9,13-15]. In addition, chronic visceral or asymptomatic photobacteriosis cases have been referred to adult fish in Mediterranean coasts of Turkey [16,17] Egypt [18] Italy [19] and Spain [2]. Other researchers [3,20] have identified photobacteriosis epizootics mainly in summer, after heavy rain falls in brackish water coast areas, with high losses. The motile *Photobacterium damsela* subspecies *damsela*, in our survey, was found in sea bass at a temperature value range 19-24, 5°C (May - August). Specifically, chronic or asymptomatic photobacteriosis cases appeared in adults at 19°C, while acute septicemic photobacteriosis outbreaks with high losses in juvenile and young fish at 21-24.5°C,

particularly under stress conditions caused by cages transportation. This newer motile subspecies has been detected in Italy and Spain coasts from sea bass at high temperature values [21-24]. All the above data for the principal epizootiological risk factor, temperature and seasonality, effect on vibriosis and photobacteriosis, for European sea bass, may be utilized to an innovative marine biosecurity program. It can be applied especially for the stenothermal vibria like *V. harveyi* and *V. alginolyticus* and *V. splendidus* serotype II. For the eurythermal vibrio *L. anguillarum* a preventive medicine procedure has recently been applied in Greece for sea bass, including probiotics and immunostimulants administration to larvae (0.1 g), bath vaccinations of *L. anguillarum* serotypes O1 and O2 to 2/5 g and intraperitoneal vaccination to 25g juveniles [10,25,26]. A bivalent bacterin vaccine for the protection from both classical vibriosis and photobacteriosis has also been applied, particularly in areas with higher water temperatures [27]. A further research is needed for vaccination procedures concerning vibriosis from the rest vibria. In these vaccination programs, the role of temperature seasonality should be taken into consideration. In addition, the rearing temperature adjustment at adverse levels for each stenothermal *Vibrio* sp. can be applied where this is feasible, such as in recirculating aquaculture systems. Specifically, the up-to-date data point out optimum temperatures low (e.g. <19°C) for *V. harveyi* and high (e.g. >17°C) for *V. alginolyticus* and *V. splendidus* serotype II. It seems that rearing of sea bass at a temperature about 18°C prevents main health problems related to *Vibrio* pathogens. Therefore, under such preventive veterinary medicine biosecurity conditions, we can reinforce the sustainability and profitability through continual epizootiological survey and strict surveillance.

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