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Similarities and differences between vibriosis in European sea bass caused by *Vibrio anguillarum* and *Vibrio harveyi*

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ABSTRACT

Vibrioses caused by different members of the genus *Vibrio* are common diseases in aquaculture characterised by systemic infections, high mortalities, and economic losses of fish and shellfish. Farming of European sea bass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) has rapidly grown over the last decade in Croatia but its economic efficacy is significantly jeopardized by losses due to *Vibrio anguillarum* and *Vibrio harveyi* infection. Therefore, we studied and compared the most prominent similarities and differences in the environmental conditions, clinical signs and conventional and molecular diagnostic methods of vibriosis caused by both bacterial pathogens. Outbreaks in sea bass and sea bream caused by *V. anguillarum* mostly occur during the spring and autumn, following a fast increase or decrease in the sea temperature, whereas infections with *V. harveyi* occur during the summer months at a temperature above 20°C. They have a similar clinical appearance in the acute form but in subacute forms they differ. *V. anguillarum* infection is characterized by massive haemorrhages in the abdominal wall, while during infection with *V. harveyi* keratitis, corneal opacity and uncoordinated swimming behaviour are noticed. Necropsy disclosed haemorrhages in the liver, intestine and stomach during infection with *V. anguillarum,* and sero-catarrhal enteritis with distension of the intestine and ascites in the case of *V. harveyi*. Although molecular tools enable correct identification, in this study we defined the main differences capable of distinguishing *V. anguillarum* from *V. harveyi* based on traditional bacteriological identification such as sensitivity to O/129 (10 μg) and novobiocin, and the enzymatic activity of β-galactosidase, arginine dihydrolase and amygdalin fermentation.

Key words: European sea bass; *Vibrio anguillarum*; *Vibrio harveyi;* environmental conditions; diagnostics; differentiation

Introduction

Vibrioses caused by different members of the genus *Vibrio* are common diseases in aquaculture, characterized by systemic infections, high mortalities and economic losses in fish and shellfish (FRANS et al., 2011). Bacteria of the genus *Vibrio* are Gram-negative, fermentative bacteria with polar flagella, they are oxidase-positive, and most of them require sodium chloride for growth. Various *Vibrio*

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species are environmental bacteria, inhabitants in brackish and marine waters. It is suggested that *V. anguillarum*, *V. ordalii*, *V. salmonicida*, *V. harveyi* and *V. vulnificus* are pathogens that cause the greatest losses in aquaculture worldwide (TORANZO et al., 2005; SANDLUND et al., 2010; SITJÀ- BOBADILLA et al., 2014).

Some commercially important marine fish species, including European sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), sole (*Solea senegalensis*), turbot (*Scophthalmus maximus*) and Atlantic salmon (*Salmo salar*), are seriously affected by vibriosis in many countries (KORUN and TIMUR, 2008). Generally, the term 'vibriosis' has been used to define *V. anguillarum* infections (GHITTINO et al., 2003) and *V. anguillarum* has become widely spread in various cultured and wild fish, bivalves and crustaceans in salt or brackish water, causing a fatal haemorrhagic septicaemic disease (AGUIRRE-GUZMÁN et al., 2004; PAILLARD et al., 2004; TORANZO et al., 2005).

V. harveyi is commonly found in the marine environment and the physiological intestinal microflora of free-living marine organisms (RUBY and MORIN, 1979; HERNANDEZ et al., 2004). Nevertheless, numerous isolates have been described as causative agents of disease in marine fish (ALVAREZ et al., 1998; ZHANG et al., 2000; PUJALTE et al., 2003), crustaceans (KARUNASAGAR et al., 1994; LAVILLA-PITOGO et al., 1998) and shellfish (NISHIMORI et al., 1998).

Vibriosis is generally characterized by dark skin coloration, pale gills, and haemorrhages near the mouth and on the fin base, exophthalmia, corneal opacity and ulcers on the skin surface. Internally, moribund fish show severe anaemia and have haemorrhages in the abdominal fat, kidney and liver (TORANZO et al., 2005; ZORRILLA et al., 2003a).

European sea bass is one of the economically most important fish species in the Mediterranean region (VARSAMOS et al., 2006; AFONSO et al., 2005), and vibrioses are reported as highly detrimental bacterial diseases affecting sea bass farming (TORANZO et al., 2005; PUJALTE et al., 2003; ZORRILLA et al., 2003b). Farming of European sea bass and gilthead sea bream in Croatia has been growing rapidly over the last ten years (KATAVIĆ and GAVRILOVIĆ, 2017) and significant economic loss is caused by infection with bacteria *V. anguillarum* and *V. harveyi* (ČOLAK and ZRNČIĆ, 2015).

This paper aims to present and compare the most prominent similarities and differences in the environmental conditions, clinical signs and diagnostics of vibriosis caused by both *V. anguillarum* and *V. harveyi* in the farming of European sea bass in Croatia, based on our findings between 2013 and 2019.

Materials and methods

Environmental conditions and sampling. The environmental conditions and clinical signs of the disease were monitored in a marine fish farm in the central Eastern Adriatic. The fish are farmed in net pens with a diameter ranging from 8 to 12 m and with the depth of the nets of 6 to 8 meters. The average density was up to 10 kilos per m^3. The depth under the cages is from 15 to 30 meters, and the distance to the shore is from 100 to 500 meters. Mostly specimens weighing from 40 to 150 grams were monitored. Temperatures and mortalities in the growing cages were noted daily. Mortalities were calculated as a percentage of dead fish during the outbreak in a cage with approximately 40,000 individuals. Changes in the behaviour and appearance of the fish were checked. Fish showing an unusual appearance and behaviour were sampled for necropsy and further laboratory analysis. We euthanized fish with an overdose of MS-222 (EFSA, 2009).

Additionally, samples from other farms on different sites along the Adriatic coast submitted for diagnostics were included in the study (Fig. 1).

External examination and the necropsy. Changes in the external appearance of the fish were determined with the naked eye. We examined body cavities, gills and fins. Skin observation included any discolouration, swelling, lesions, or haemorrhages. We then removed the operculum and observed the appearance and colour of the gills and the possible presence of macroscopically visible

ectoparasites. We prepared native preparations of gills using the standard technique, and examined them microscopically at low magnification. After opening the body cavity, the viscera were visually inspected for abnormalities such as discoloration or mottled appearance, enlargement, haemorrhages, abscesses or cyst, fluid in the abdominal cavity, and any foreign bodies, such as metazoan parasites or tissue growths etc.

Fig. 1. Map of Croatian Adriatic coast showing sampling sites

Isolation and identification of bacterial isolates. After the aseptic opening of the abdominal cavity of the fish, material from the heart, spleen and kidney was sampled using a sterile inoculating loop, and streaked onto solid bacteriological media. The plates were incubated at 22 ± 2°C for 24-48 hours. For isolation of the *V. anguillarum* and *V. harveyi,* the non-selective media Marine Agar (MA, Difco, USA) and Trypticase Soy agar (TSA), supplemented with 1.5% NaCl (Difco, USA) were used. Colonies grown on solid media were transferred onto selective medium Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS, Difco, USA). The isolated bacterial colonies morphologically corresponding to the colonies of the species *V. anguillarum* and *V. harveyi* (AUSTIN and

AUSTIN, 2012), were kept at +4 ± 2ºC on slant MA. The isolates were identified on the basis of the morphology of the bacterial colonies, and tinctorial and biochemical properties. In the first step, the bacterial colonies were checked for purity under a reverse microscope, followed by the preparation of Gram-stained smears to differentiate Gram-positive and Gram-negative bacteria (HABRUN, 2014). Motility was determined using an API M Medium (BioMerieux, France), according to the manufacturer's instructions. A glucose oxidation-fermentation test was conducted using an OF Medium (BioMerieux, France), according to the manufacturer's instructions. The production of the catalase was tested by the detection of foaming on a glass slide after the addition of 3% solution hydrogen peroxide to the bacterial colony (HABRUN, 2014). Oxidase was demonstrated by detecting the colour change after wetting the filter paper with Oxidase Reagent Droppers (Becton, Dickinson and Company, USA), transferring the colonies from agar, and rubbing them onto filter paper. Bacteria of the *Vibrio* genus were differentiated from others by the vibriostat O/129 susceptibility test using discs impregnated with 150 µg and 10 µg of 2,4-diamino-6,7-diisopropylpterydine (Oxoid, USA), respectively, and 5 µg of novobiocin (BD, USA) placed on Mueller-Hinton agar (Difco, US) supplemented with 1.5% NaCl, and inoculated with 24 hours bacterial culture. The type of haemolysis was determined visually in bacterial colonies grown on blood agar supplemented with 1.5% NaCl incubated 24-48 hours at 22 ± 2°C. The oxidative or fermentative metabolism of carbohydrates was determined, using a semi-solid substrate, containing glucose and bromothymol blue as pH indicators. Each isolate was inoculated into two tubes, one was sealed with sterile paraffin oil, and both were incubated at 30 ± 2°C. Salt tolerance was determined in peptone water with the addition of 0.5%, 3%, 6% and 10% salt. To determine the bacterial species, multiple biochemical properties were tested using API 20E (BioMerieux S.A., Marcy-l'Etoile, France) (use of β-galactosidase (ONPG), arginine, lysine, ornithine, citrate, production of hydrogen sulphides, indole, the Voges Proskauer test, hydrolyzing urea, degrading gelatine, fermentation of glucose, mannose, inositol, sorbitol, melibiose, amygdalin, arabinose and reduction of nitrates to nitrites). The tests were carried out after incubation for 24 to 48 hours at 28 ± 2°C, and in some of the wells the colour changed due to pH differences. We read results according to the manufacturer's instructions. Identification of all strains was confirmed using different polymerase chain reaction (PCR) protocols.

Molecular identification of isolated bacterial isolates. DNA from bacterial colonies was extracted using the NucleoSpin Microbial DNA kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. To confirm the genus identity of all used isolates, we amplified the 16s rRNA gene sequence using primers 27FYM (5'-AGAGTTTGATYMTGGCTCAG-3') (FRANK et al., 2008) and 1492YR (5'-TACGGYTACCTTGTTACGACTT-3') (NEWBY et al., 2004). PCR was set up as follows: with 10 µl of GoTaq G2 Hot Start Master Mix (Promega, USA), 2 µl DNA, 0.5 µM of each primer and nuclease-free water to a final volume of 20 µl. The amplification was performed using a ProFlex PCR System (Applied Biosystems, USA) with an initial denaturation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 60 seconds, primer annealing at 50°C for 30 seconds, elongation at 72ºC for 90 seconds, ending with the final elongation step at 72ºC for 7 minutes. The results of PCR were analysed by electrophoresis on a QIAxcel system (Qiagen, Germany), using the QIAxcel DNA Screening Kit. PCR products were sequenced by Macrogene (Netherlands). The obtained sequences were compared against The Nucleotide database (2020) using BLAST (JOHNSON et al., 2008), and identified taxonomically on the basis of similarities greater than 99%. *V. anguillarum* was additionally identified by amplifying the amiB gene sequence using the PCR method developed by GYEONG-EUN et al. (2007). PCR was performed as above, but using the primers van-ami8 (5'-ACATCATCCATTTGTTAC-3') and van-ami417 (5'-CCTTATCACTATCCAAATTG-3'), with the following changes to the temperature

protocol: the primer annealing temperature was set to 54°C, and the elongation time in each cycle to 30 seconds. Identification of *V. harveyi* was performed by specific amplification of the toxR gene (PANG et al., 2006). PCR was performed in the same way as for the amiB gene, but using the primers toxRF1 (5'-GAAGCAGCACTCACCGAT-3') and toxRR1 (5'-GGTGAAGACTCATCAGCA-3'), with an annealing temperature of 55°C.

Susceptibility testing. The susceptibility of isolated bacteria to antimicrobials was determined by disc diffusion and microdilution methods. The disc diffusion test (BAUER et al., 1966) was performed on Mueller-Hinton agar (Difco, USA) supplemented with 1% NaCl, using the following discs: ampicillin 25 µg (Oxoid, UK), trimethoprim/sulfadiazine 1.25/23.75 µg (Becton Dickinson and Company, USA), florfenicol 30 µg (Oxoid, UK), oxytetracycline 30 µg (Becton, Dickinson and Company, USA), flumequine 30 µg (Becton, Dickinson and Company, USA), nalidixic acid 30 µg (Becton, Dickinson and Company, USA), gentamicin 30 µg (Oxoid, UK) and neomycin 30 µg (Oxoid, UK). The plates were incubated at 28±2°C and read after 24 to 28 hours by measuring the inhibition zone around each different antibiotic disc in mm (SMITH and EGAN, 2018). The Minimum Inhibitory Concentrations (MICs) of the antimicrobial substances (ampicillin, erythromycin, florfenicol, oxytetracycline, oxolinic acid, and trimethoprim-sulfamethoxazole) for *V. anguillarum* and *V. harveyi* isolates were determined using VetMIC Aquatic (SVA, Sweden). CAMHB (Muller-Hinton Broth Adapted Cation) (Becton, Dickinson and Company, USA) was prepared according to the manufacturer's instructions. The bacterial suspension was according to the manufacturer's instructions inoculated into each well of the VetMIC panel, and incubated for 48 hours at 22°C. All substances in the commercial panel were diluted in concentrations ranging from 16 to 0.002 mgL^{-1} except trimethoprim-sulfamethoxazole, which was diluted from 4/76 to 0.03/0.06 mgL^{-1} (Table 5). The conditions for these assays were based on those provided in the CLSI guideline VET04-A2 for microdilution susceptibility testing of non-fastidious organisms. The plates were incubated at 28±2°C and read after 24 to 28 hours (CLSI, 2014; SMITH et al., 2013). The highest dilution of antibiotic at which no growth was visually determined was considered to be the MIC (ZRNČIĆ and PAVLINEC, 2020).

Results

Environmental conditions and mortalities associated with V. anguillarum and V. harveyi outbreaks. Temperature and mortalities were noted daily as the production results indices. The regimes of temperatures associated with *V. anguillarum* outbreaks in October 2014 and 2016 were decreases from 20.0 °C to 16.5 °C, and from 20.0 °C to 17.0 °C, respectively, while in May 2013 there was an increase from 14.0 °C to 19.0 °C; in May 2014 from 17.0 °C to 19.0 °C and in May 2016 from 17.5 °C to 20.0 °C (Fig. 2a). Mortalities caused by *V. anguillarum* were about 1.5 % in October 2014, 3.12 % in October 2016, 1.7 % in May 2013, 1.6% in May 2014 and 2.7 % in May 2016 (Fig. 2c). In all cases, antibacterial treatment was undertaken soon after the increase in the number of dead individuals. Temperatures during the outbreak of *V. harveyi* during August 2017 fluctuated between 21.0 and 26.0 °C; in July 2018 and August 2018 between 23.0 and 26.0 °C; in August 2019 between 21.0 and 26.0 °C and in September 2019 between 22.0 and 24.7 °C (Fig. 2b). Mortalities were up to 4.6 % in August 2017, 1.6 % in July 2018, 4.5 % in August 2018, 5.3 % in August 2019 and 4.6 % in September 2019 (Fig. 2d). Just as in the case of the outbreaks of *V. anguillarum,* antibacterial treatments were commenced soon after the increase in the number of dead individuals.

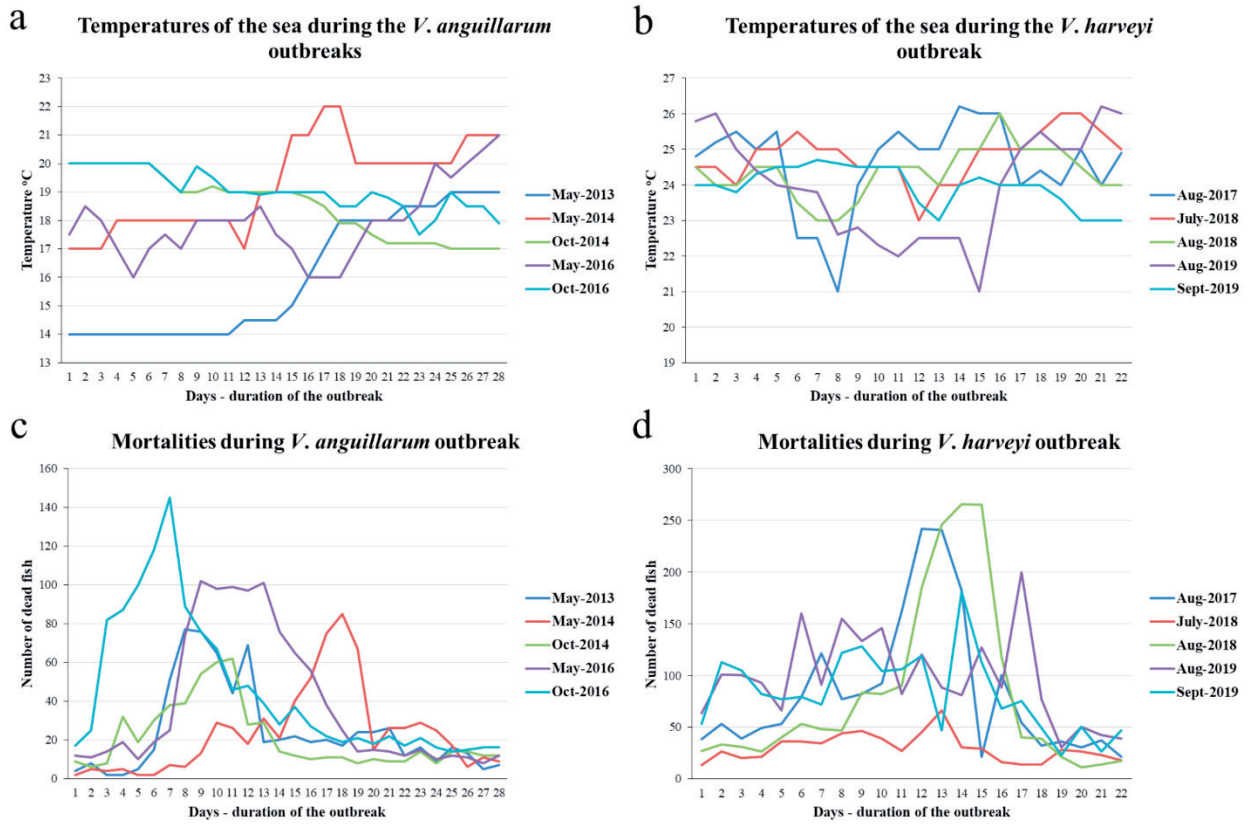

Fig. 2**.** Regimes of temperatures and mortality rates associated with *V. anguillarum* and *V. harveyi* Temperatures of the sea during the *V. anguillarum* outbreaks (1a). Temperatures of the sea during the *V. harveyi* outbreak (1b). Mortalities during *V. anguillarum* outbreaks (1c). Mortalities during the *V. harveyi* outbreak (1d). Mortalities are expressed as the number of dead specimens daily in a cage with approximately 40,000 individuals.

External examination and necropsy. The results of the external examination and necropsy are summarized in Table 1. In seabass infected with *V. anguillarum* loss of scales, erythema around the mouth and vent, haemorrhages on the head, operculum, the fin bases, oedematous lesions in the skin, and ulceration and pale gills with haemorrhages were observed. More pronounced subacute cases were characterized by massive haemorrhages on the abdominal wall, head and operculum, while in the chronic course large granulating lesions in musculature and even ulceration, and severe anaemia of the gills, with grey corneal opacity were observed (Fig. 3a and 3b). Specimens infected with *V. harveyi* showed depigmentation, erosions, haemorrhages at the base of the fins, kidney necrosis, skin ulceration, keratitis, corneal opacity, exophthalmia and pale gills, with haemorrhages (Fig. 3c and 3d). In specimens infected with *V. anguillarum,* autopsy showed haemorrhages on the liver and posterior part of the intestines, rarely in the stomach (Fig. 3e). Those infected with *V. harveyi* showed sero-catarrhal enteritis, with distension of the intestines, which were filled with white to yellowish exudate (Fig. 3f).

Results of bacteriological examination. During the study some other bacterial pathogens were isolated and stored. Since comparison of the bacterial species *V. anguillarum* and *V. harveyi* was the topic of this study, we will only consider these two species. A total of 30 *V. anguillarum* isolates and the same number of *V. harveyi* isolates were isolated from sea bass from different farms along

Table 1. Differences in macroscopic changes between fish infected with *V. anguillarum* and *V. harveyi*

Macroscopic changes	Infection with V. anguillarum	Infection with V. harveyi
Loss of scales	+	+
Haemorrhages on fin basis, operculum, the head	+	+
Erythema around the mouth and vent	+	+/-
Depigmentation	-	+
Edematous lesions in the skin,	+	+
Massive haemorrhages on the abdominal wall	+	-
Keratitis, corneal opacity	-	+
Uncoordinated swimming	-	+
Haemorrhages on the liver, intestine, stomach	+	-
Sero-catarrhal enteritis with distension of the intestine	-	+
Exophthalmia	+	+
Ulceration	+	+
Pale gills	+	+
Granulating lesions in musculature	+	-

the Croatian Adriatic coast (Fig. 1) and identified and compared. All isolates grew on TCBS medium as yellow colonies due to glucose fermentation, and they were Gram-negative and showed motility. All *V. anguillarum* isolates grew in peptone water with the addition of 0.5 to 6% NaCl, while none grew in peptone water with the addition of 10% NaCl. All *V. harveyi* isolates grew in peptone water with the addition of 0.5 to 3% NaCl, five did not grow at 6% NaCl and none grew in 10% NaCl. All *V. anguillarum* isolates caused β-haemolysis just like all the *V. harveyi* isolates. All isolates showed enzyme oxidase and catalase. All *V. anguillarum* and *V. harveyi* isolates showed fermentative metabolism of carbohydrates. All isolates of both species were sensitive to vibriostat O/129 (150 mg), while there were some differences in sensitivity to O/129 (10 mg) and novobiocin, which are shown in Table 2. Among the 26 biochemical properties tested of the isolated *V. anguillarum*, 21 were determined using the API 20 E system (Table 3). All isolates had the enzyme β-galactosidase (ONPG) and most tested (25 out of 30) isolates possessed the arginine dihydrolase enzyme (ADH). None of them had ornithine decarboxylase (ODC) or lysine decarboxylase (LDC). All isolates produced acetoin in the Voges Proskauer test. None of the tested *V. anguillarum* was capable of utilizing citrate, and they could not release hydrogen sulphide from the amino acid. All tested isolates were capable of conversion of the amino acid tryptophan into indole, and could produce nitrate. Due to enzymatic activity, all isolates had gelatinase but tryptophan deaminase was not produced. The enzyme urease was present in only 2 out of 30 isolates. All isolates fermented glucose, sorbitol, rhamnose and sucrose, mannose was fermented by half the isolates, while 12 out of 30 isolates fermented arabinose, but none fermented inositol, melibiose and amygdalin. The same scheme of determination of biochemical properties was applied for *V. harveyi* and it was found that none of them had β-galactosidase (ONPG), arginine dihydrolase (ADH), ornithine decarboxylase (ODC) or enzyme urease. All of them were capable of conversion of the amino acid tryptophan into indole, and they degraded gelatine, but none of them had the tryptophan deaminase. Two out of 30 isolates had the enzyme lysine decarboxylase (LDC). All isolates fermented glucose, rhamnose, sucrose and amygdalin, while mannose was fermented by 27 out of 30 isolates. No isolated bacteria fermented inositol, melibiose and arabinose. None of the isolates tested positive for the Voges Proskauer test, five isolates out of thirty were capable of utilizing citrate, and none released hydrogen sulphide from the amino acid, but all produced nitrate (Table 3).

Fig. 3. External changes and necropsy of seabass infected with *V. anguillarum* and *V. harveyi*
Seabass infected with *V. anguillarum* showed massive haemorrhages on the abdominal wall, head and opercula (a and b). Specimens infected with *V. harveyi* showed depigmentation, erosions, haemorrhages at the base of fins, necrosis, ulceration, keratitis, corneal opacity, exophthalmia and pale gills with haemorrhages (c and d). Haemorrhages on the liver and posterior part of the intestine, rarely in the stomach, in a specimen infected with *V. anguillarum* (e). Sero-catarrhal enteritis with distension of the intestines, filled with white to yellowish exudate in those infected with *V. harveyi* (f).

Table 2. Physiological and biochemical properties of isolated *V. anguillarum* and *V. harveyi* isolates

Property / Isolates	NaCl 0.5%	NaCl 3.0%	NaCl 6.0%	NaCl 10.0%	Oxidase	Catalase	O/129 150µg	O/129 10µg	Novo biocin	OF	Type of hemolysis
V. anguillarum (n=30)	30/30	30/30	30/30	0/30	30/30	30/30	30/30 S	25/30 S	28/30 S	30/30	β (30/30)
V. anguillarum (Manfrin 2020)	+	+	+	-	+	+	S	S	n/a	+	n/a
V. harveyi (n=30)	30/30	30/30	25/30	0/30	30/30	30/30	30/30 S	30/30 R	30/30 R	30/30	β (30/30)
V. harveyi (Pretto 2020)	+	+	+	-	+	+	S	R	n/a	+	α & β ≈50%

Legend: S = sensitive, R = resistant, OF = glucose oxidation-fermentation test

Table 3. Biochemical properties of isolated *V. anguillarum* and *V. harveyi* isolates determined by API 20E system

Isolates / Property	*V. anguillarum* (n=30)	*V. anguillarum* (Manfrin, 2020)	*V. harveyi* (n=30)	*V. harveyi* (Pretto 2020)
ONPG	30/30	+	0/30	-
ADH	25/30	+	0/30	-
LDC	0/30	-	2/30	+
ODC	0/30	-	0/30	+
CIT	0/30	v	5/30	+
H_2S	0/30	-	0/30	-
URE	2/30	-	0/30	-
TDA	0/30	n/a	0/30	-
IND	30/30	+	30/30	+
VP	30/30	+	0/30	-
GEL	30/30	+	30/30	+
GLU	30/30	+	30/30	+
MAN	15/30	+	27/30	+
INO	0/30	-	0/30	-
SOR	30/30	+	22/30	+
RHA	30/30	-	30/30	-
SUC	30/30	+	30/30	+
MEL	0/30	-	0/30	-
AMY	0/30	+	30/30	+
ARA	12/30	+	0/30	-
NO_2	30/30	+	30/30	+

Legend: ONPG = β-galactosidase, ADH = arginine dihydrolase, LCD = lysine decarboxylase, ODC = ornithine decarboxylase, CIT = citrate, H2S = hydrogen sulphide production, URE = urease, TDA = tryptophan deaminase, IND = indole, VP = Voges-Proskauer, GEL = gelatinase, GLU = glucose, MAN = mannitol, INO = inositol, SOR = sorbitol, RHA = rhamnose, SAC = sucrose, MEL = melibiose, AMY = amygdalin, ARA = arabinose, NO2 = reduction of nitrates to nitrites; n/a = not available; v = variable

The sensitivity of isolated bacterial isolates of V. anguillarum and V. harveyi to antibacterial agents. The disc diffusion test showed that all *V. anguillarum* isolates were from sensitive to moderately sensitive to trimethoprim-sulfamethoxazole, flumequine and florfenicol by disc diffusion. Only five *V. anguillarum* isolates were susceptible to ampicillin, while the others were resistant. Twenty-nine isolates showed sensitivity or moderate sensitivity to oxytetracycline, while one isolate was completely resistant to this antibacterial agent (Table 4). Isolates of *V. harveyi* tested by disc diffusion methods were found to be resistant to gentamicin and neomycin. They were from sensitive to moderately sensitive to trimethoprim-sulfamethoxazole and florfenicol, and moderately sensitive to nalidixic acid. Twenty-five isolates were sensitive and moderately sensitive to oxytetracycline, five isolates were completely resistant to this antibacterial agent (Table 4).

Table 4. Susceptibility of isolated *V. anguillarum* and *V. harveyi* isolates to antibacterial substances (disc diffusion method according to PRETTO, 2018)

Antimicrobial substance	Disc content µg	Results for *V. anguillarum* (n=30)	Results for *V. harveyi* (n=30)
Ampicillin	25	5/30 I 25/30 R	n/d
Trimethoprim-sulfamethoxazole	1.25/23.75	27/30 S 3/30 I	27/30 S 3/30 I
Florfenicol	30	28/30 S 2/30 I	28/30 S 2/30 I
Oxytetracycline	30	24/30 S 5/30 I 1/30 R	23/30 S 2/30 I 5/30 R
Flumequine	30	30/30 S	30/30 S
Nalidixic acid	30	n/d	30/30 I
Gentamicin	30	n/d	30/30 R
Neomycin	30	n/d	30/30 R

Legend: S = sensitive, I = intermediate, R = resistant

The sensitivity of V. anguillarum and V. harveyi isolates to antibacterial agents by the microdilution method. All the tested *V. anguillarum* isolates were inhibited by low concentrations of florfenicol, oxytetracycline, oxolinic acid and trimethoprim-sulfamethoxazole, but were resistant to ampicillin and erythromycin (Table 5). All tested *V. harveyi* isolates were inhibited by low concentrations of florfenicol, oxytetracycline, trimethoprim-sulfamethoxazole and oxolinic acid, and resistant to ampicillin and erythromycin (Table 6).

Table 5. Minimum inhibitory concentration (MIC) values (mg L^{-1}) determined for 30 *V. anguillarum* isolates

Antimicrobial substance mgL^{-1}	No of isolates	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16
Ampicillin	30														
Erythromycin	30														
Florfenicol	30									15	2	13			
Oxytetracycline	30								10	20					
Oxolinic acid	30						15	10	2	3					
Trimethoprim-Sulfamethoxazole*	30							30							

*dilution of the trimethoprim-sulfamethoxazole was from 4/76 to 0.03/0.06

Table 6. Minimum inhibitory concentration (MIC) values (mg L^{-1}) determined for 30 *V. harveyi* isolates

Antimicrobial substance mgL^{-1}	No of isolates	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16
Ampicillin	30														
Erythromycin	30														
Florfenicol	30										20	10			
Oxytetracycline	30								10	20					
Oxolinic acid	30								5	25					
Trimethoprim-Sulfamethoxazole*	30								25	5					

*dilution of the trimethoprim-sulfamethoxazole was from 4/76 to 0.03/0.06

Results of molecular identification of isolated bacterial isolates of V. anguillarum and V. harveyi. Analysis of partial 16S rRNA gene sequences using BLAST showed that all isolates belonged to the *Vibrio* genus. Isolates that were suspected to be *V. anguillarum* showed over 99% sequence similarity with most *V. anguillarum* sequences available in the database. Likewise, suspected *V. harveyi* isolates showed over 99% sequence similarity with *V. harveyi* sequences. All the suspected *V. anguillarum* isolates were confirmed as *V. anguillarum* by amplification of the amiB gene. The amplified product was 429 bp long, as expected. All the suspected *V. harveyi* isolates were confirmed as *V. harveyi* by amplification of the toxR gene. The amplified product was 382 bp long, as expected.

Discussion

The aquaculture sector plays an important role in sustaining food security and contributing to economic growth (MOHD-ARIS et al., 2019). Vibriosis is nowadays a limiting factor in sea bass aquaculture throughout the Mediterranean region (HAENEN et al., 2014) and in recent years, increasingly frequent outbreaks caused by the emerging pathogen *V. harveyi*, have occurred. The results of our study show that vibriosis caused by either *V. anguillarum* or *V. harveyi* has a very similar clinical appearance in its acute forms, such as dark pigmentation or haemorrhages on the fin bases and operculum or loss of scale. The subacute forms are different, with massive haemorrhages in the abdominal wall in fish infected with *V. anguillarum* and keratitis, corneal opacity and uncoordinated swimming behaviour in fish infected with *V. harveyi*. However, it should be highlighted that samples submitted during the summer months exhibiting nerve symptoms were usually tested for the presence of viral nervous necrosis (VNN).

Necropsy usually disclosed some additional differences in the subacute course of the disease, similar to the signs provoked by the challenge of seabass with a pure culture of *V. harveyi,* such as external haemorrhages in the mouth, operculum and fins, and inflammation of the vent (FIRMINO et al., 2019). It should be highlighted that outbreaks in Croatia caused by *V. anguillarum* mostly occur during the spring and autumn, following a rapid increase or decrease in the sea temperature, which supports the findings of HAENEN et al. (2014). In contrast, infections with *V. harveyi* occur during the summer months at temperatures of 20°C and over, and most often independently to variations in the water temperature, but often due to changes in the weather or farming conditions. Our study showed that sometimes during September it is possible to diagnose outbreaks caused by both *Vibrio* pathogens.

Determination of the haemolysis type showed that β haemolysis dominated in our *V. anguillarum* isolates, which matches the data provided by AUSTIN and AUSTIN (2012). Similarly, all *V. harveyi* isolates showed β haemolysis, which is in line with findings of RATTANAMA et al. (2012).

Sodium chloride tolerance studies showed that our *V. anguillarum* isolates grew in peptone water

supplemented with 0.5% to 6% NaCl, while they did not grow in peptone water supplemented with 10% NaCl, which is in line with the data reported by AUSTIN and AUSTIN (2012). Likewise, most of our *V. harveyi* isolates grew in peptone water supplemented with 0.5 to 6%, while 5/30 isolates could not grow at 6% of NaCl, which is in contrast to the findings of LOPEZ et al. (2009) who observed growth of their *V. harveyi* isolates in peptone water supplemented with 3 to 8% of NaCl. However, TENDENCIA (2002) observed a similar growth pattern regarding the salt tolerance in isolates from cage-cultured Asian seabass, and this points to the conclusion of a diversities in salt tolerance among geographically different isolates.

Identification of isolates using the API 20 E system revealed that *V. harveyi*, unlike *V. anguillarum*, could not utilize β-galactosidase to produce acetoin in the Voges Proskauer test, but could produce nitrate. Both isolates fermented glucose and sucrose, but did not ferment melibiose. Another difference was susceptibility to O/129 10mg and novobiocin:; almost all our *V. anguillarum* isolates were sensitive to both substances, while all *V. harveyi* isolates were resistant to them. Interestingly, this finding is opposite to the findings of KORUN and TIMUR (2008), who reported that all their isolates of *V. harveyi* were sensitive to both O/129 150mg and O/129 10mg but in line with findings of AUSTIN and AUSTIN (2012).

Susceptibility testing of isolated bacteria to antimicrobial substances is an indispensable part of diagnostic procedures. Hence, we tested the susceptibility of *V. anguillarum* and *V. harveyi* isolates to antibacterial agents by both disc diffusion and microdilution methods. Both bacterial species showed sensitivity to trimethoprim-sulfamethoxazole and florfenicol with the disc diffusion method in the majority of isolates of both species. *V. anguillarum* and *V. harveyi* were sensitive to moderately sensitive to oxytetracycline in a similar percentage. The results of susceptibility testing using the microdilution method were in line with those obtained by disc diffusion, and showed that *V. anguillarum* was resistant to erythromycin. *V. anguillarum* and *V. harveyi* isolates were inhibited by similar concentrations of florfenicol and oxytetracycline. *V. anguillarum* was inhibited by lower concentrations of oxolinic acid and trimethoprim-sulfamethoxazole compared to those needed to inhibit the growth of *V. harveyi* isolates. This susceptibility pattern might be correlated to the frequent use of antibiotics for the treatment of *V. harveyi* infections on marine farms (DENG et al., 2020; SCARANO et al., 2014). It is known that vaccination against *V. anguillarum* has become a usual practice in the farming of sea bass (SARROPOULOU et al., 2012). Therefore, antibacterial treatments against *V. anguillarum* infections are applied less often to mitigate production losses, compared to the control of the disease outbreaks caused by *V. harveyi*. In the last twenty years, there have been numerous examples of justification for fish vaccination in the light of the sustainable growth, development and cost-effectiveness of aquaculture (THORARINSSON and POWELL, 2006). All our *V. anguillarum* isolates were obtained before vaccination against this pathogen became a part of the farming procedures, while *V. harveyi* isolates were collected from farms where sea bass had been vaccinated and from those where vaccination against *V. anguillarum* has been accepted. Unfortunately, there are no commercially available vaccines against *V. harveyi* and the most efficient practice of disease mitigation is still the use of antimicrobials. Therefore, it is necessary to make additional efforts to develop a vaccine against *V. harveyi,* and introduce it into the farming cycle. Moreover, it is necessary to highlight the insufficient availability of harmonized protocols to evaluate the results of susceptibility testing, and the adoption of "cut off" values for both *Vibrio* species important for Mediterranean marine aquaculture, as proposed by SMITH and EGAN (2018).

Since *V. anguillarum* is mostly successfully controlled, in the last decade *V. harveyi* has become an increasingly important pathogen, and it could be considered to be an emerging pathogen in the Mediterranean marine aquaculture. AMARO et al., (2020) related *V. harveyi* outbreaks to the climate changes in the Mediterranean basin, as previously described, mainly as a fish pathogen in subtropical areas (ZHANG and AUSTIN., 2020). Therefore, more effort should be invested in the study of all

aspects of this pathogenic bacteria and its influence on marine aquaculture.

Our study attempted to contribute to easier diagnostic differentiation of these two *Vibrio* species, based on epidemiological conditions, morphological changes and by the implementation of conventional bacteriological techniques, to enable faster diagnosis in farm laboratories and to mitigate the losses caused by this pathogen more efficiently.

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References

AFONSO, A., S. GAMES, J. DA SILVA, F. MARQUES, M. HENRIQUE (2005): Side effects in sea bass (*Dicentrarchus labrax*, L.) due to intraperitoneal vaccination against vibriosis and pasteurellosis. Fish. Shellfish. Immunol. 19, 1-16.
DOI: 10.1016/j.fsi.2004.09.001

AGUIRRE-GUZMÁN, G., H. M. RUÍZ, F. ASCENCIO (2004): A review of extracellular virulence product of *Vibrio* species important in diseases of cultivated shrimp. Aquac. Res. 35, 1395–1404.
DOI: 10.1111/j.1365-2109.2004.01165.x

ALVAREZ, J. D., B. AUSTIN, A. M. ALVAREZ, H. REYES (1998): *Vibrio harveyi*: a pathogen of penaeid shrimps and fish in Venezuela. J. Fish. Dis. 21, 313-316.
DOI: 10.1046/j.1365-2761.1998.00101.x

AMARO, C., B. FOUZ, E. SANJUAN, J. C. ROMALDE (2020): Vibriosis. In: Climate Change and Infectious Diseases. (Woo, P. T. K., J. A. Leong, K. Buchmann, Eds), CAB International, Wallingford, pp. 182-210.

AUSTIN, B., D. A. AUSTIN (2012): Bacterial Fish Pathogens. Diseases of Farmed and Wild Fish. Springer-Praxis Publishing, Chichester, pp. 171-172, 141-142.
DOI: 10.1007/978-1-4020-6069-4

BAUER, A. W., W. M. KIRBY, J. C. SHERRIS, M. TURCK (1966): Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45(4), 493-6.
DOI: 10.1093/ajcp/45.4_ts.493

CLSI (2014): M100-S24 Performance standards for antimicrobial susceptibility testing. CLSI Supplement. 24th ed., Wayne, PA.

ČOLAK, S., S. ZRNČIĆ (2015): Health situation in Croatian mariculture. In: Atti del XXI Convegno Nazionale S.I.P.I. Società Italiana Patologia Ittica. (Della Pozza, M., A. Manfrin, Eds.), Chioggia, Italy, pp. 68–68.

DENG, Y., L. XU, S. LIU, Q. WANG, Z. GUO, C. CHEN, J. FENG (2020): What drives changes in the virulence and antibiotic resistance of *Vibrio harveyi* in the South China Sea? J. Fish. Dis. 43, 853–862.
DOI: 10.1111/jfd.13197

EFSA (2009): Scientific opinion "Species-specific welfare aspects of the main systems of stunning and killing of farmed seabass and seabream". Scientific Opinion of the Panel on Animal Health and Welfare. EFSA J. DOI: 10.2903/j.efsa.2009.10101010, 1-52.

FIRMINO, J., M. D. FURONES, K. ANDREE, C. SARASQUETE, J. B. ORTIZ-DELGADO, G. ASENCIO-ALCUDIA, E. GISBERT (2019): Contrasting outcomes of *Vibrio harveyi* pathogenicity in gilthead seabream, *Sparus aurata* and European seabass, *Dicentrachus labrax*. Aquaculture. 511, 734210.
DOI: 10.1016/j.aquaculture.2019.734210

FRANK, J. A., C. I. REICH, S. SHARMA, J. S. WEISBAUM, B. A. WILSON, G. J. OLSEN (2008): Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. Appl. Environ. Microb. 74, 2461–2470.
DOI: 10.1128/AEM.02272-07

FRANS, I., C. W. MICHIELS, P. BOSSIER, K. WILLEMS, B. LIEVENS, H. REDIERS (2011): *Vibrio anguillarum* as a fish pathogen: virulence factors, diagnosis and prevention. J. Fish. Dis. 34, 643–661.
DOI: 10.1111/j.1365-2761.2011.01279.x

GHITTINO, C., M. LATINI, F. AGNETTI, C. PANZIERI, L. LAURO, R. CIAPPELLONI, G. PETRACCA (2003): Emerging pathologies in aquaculture: effects on production and food safety. Vet. Res. Commun. 27(1), 471-479.
DOI: 10.1023/b:verc.0000014204.37722.b6

GYEONG-EUN, H., K. DONG-GYUN, B. JU-YOON, A. SUN-HEE, C. B. SUNGHUL, K. IN-SOO (2007): Species-specific PCR detection of the fish pathogen, *Vibrio anguillarum*, using amiB gene, which encodes N-acetymuramoyl-L-alanine amidase. FEMS Microbiol. Lett. 269, 201-206.
DOI:10.1111/J.1574-6968.2006.00618.X

HABRUN, B. (2014): Clinical Veterinary Bacteriology. Medicinska naklada, Zagreb, Croatia.

HAENEN, O. L. M., B. FOUZ, C. AMARO, M. M. ISERN, H. MIKKELSEN, S. ZRNČIĆ, M. A. TRAVERS, T. RENAULT, R. WARDLE, A. HELLSTÖRM, I. DALSGAARD (2014): Vibriosis in aquaculture. B. Eur. Assoc. Fish. Pat. 34, 138-148.

HERNÁNDEZ, C. R., C. H. L. MARTÍNEZ, A. DÍAZ, O. A. ROMERO, R. C. GODÍNEZ, G. A. ZAVALA, R. A. VERDUGO (2004): Aerobic bacterial flora of the nasal cavity in Gulf of California sea lion (*Zalophus californianus*). Vet. J. 170, 359-63.
DOI: 10.1016/j.tvjl.2004.08.008

JOHNSON, M., I. ZARETSKAYA, Y. RAYTSELIS, Y. MEREZHUK, S. MCGINNIS, T. L. MADDEN (2008): NCBI BLAST: a better web interface. Nucleic. Acids. Res. 36, W5–W9.

DOI: 10.1093/nar/gkn201

KARUNASAGAR, I., R. PAI, G. R. MALATHI, I. KARUNASAGAR (1994): Mass mortality of *Penaeus monodon* larvae due to antibiotic-resistant *Vibrio harveyi* infection. Aquaculture. 128, 203-209.

KATAVIĆ, I., A. GAVRILOVIĆ (2017): Focus on Croatia: Progress towards sustainable aquaculture. Aquac. Eur. 42, 5-11.

DOI: 10.1016/0044-8486(94)90309-3

KORUN, J., G. TIMUR (2008): Marine Vibriosis associated with diseased sea bass (*Dicentrarchus labrax*) in Turkey. J. Fish. Sci. 2(1), 66-76.

DOI: 10.3153/jfscom.2008007

LAVILLA-PITOGO, C. R., E. M. LEAÑO, M. G. PANER (1998): Mortalities of pond-cultured juvenile shrimp, *Penaeus monodon*, associated with dominance of luminescent vibrios in the rearing environment. Aquaculture. 164, 337–349.

DOI: 10.1016/S0044-8486(98)00198-7

LOPEZ, R. J., E. DE LA ROCA, S. NUNEZ, R. DE LA HERRRAN, J. I. NAVAS, M. MANCHADO, M. HERRERA, A. E. TORANZO (2009): Identification of *Vibrio harveyi* isolated from diseased cultured wedge sole *Dicologoglossa cuneate*. Dis. Aquat. Organ. 84, 209-217.

DOI: 10.3354/dao02045

NEWBY, D. T., D. W. REED, L. M. PETZKE, A. L. IGOE, M. E. DELWICHE, F. F. ROBERTO, J. P. MCKINLEY, M. J. WHITICAR, F. S. COLWELL (2004): Diversity of methanotroph communities in a basalt aquifer. FEMS Microbiol. Ecol. 48, 333–344.

DOI: 10.1016/j.femsec.2004.02.001

NISHIMORI, E., O. HASEGAWA, T. NUMATA, H. WAKABAYASHI (1998): *Vibrio carchariae* causes mass mortalities in Japanese abalone, *Sulculus diversicolor supratexta*. Fish. Pathol. 33, 495-502.

NUCLEOTIDE [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004 – [cited 2020 06 03]. Available from: https://www.ncbi.nlm.nih.gov/nucleotide/

MANFRIN, A. (2020): *Vibrio anguillarum*. In: Diagnostic manual for the main pathogens in European seabass and Gilthead seabream aquaculture. (Zrnčić, S., Ed.), Ciheam, Zaragoza, pp. 67-73.

MOHD-ARIS, A., M. Z. SAAD, H. M. DAUD, T. M. YUSOFN, M. Y. INA-SALWANY (2019): *Vibrio harveyi* protease deletion mutant as a live attenuated vaccine candidate against vibriosis and transcriptome profiling following vaccination for *Epinephelus fuscoguttatus*. Aquacult. Int. 27, 125–140.

PAILLARD, C., F. LE ROUX, J. J. BORREGO (2004): Bacterial disease in marine bivalves: review of recent studies: trends and evolution. Aquat. Living. Resour. 17, 477–498.

DOI: 10.1051/alr:2004054

PANG, L., X. H. ZHANG, Y. ZHONG, J. CHEN, Y. LI, B. AUSTIN (2006): Identification of *Vibrio harveyi* using PCR amplification of the toxR gene. Lett. Appl. Microbiol. 43(3), 249-55.

DOI: 10.1111/j.1472-765X.2006.01962.x

PRETTO, T. (2020): *Vibrio harveyi* group. In: Diagnostic manual for the main pathogens in European seabass and Gilthead seabream aquaculture. (Zrnčić, S., Ed.), Ciheam, Zaragoza, pp. 75-82.

PUJALTE, M. J., A. SITJA-BOBADILLA, M. C. MACLAN, C. BELLOCH, P. ALVAREZ-PELLITERO, J. PEREZ-SANCHEZ, F. URUBURU, E. GARAY (2003): Virulence and molecular typing of *Vibrio harveyi* strains isolated from cultured dentex, gilthead sea bream and European sea bass. Syst. Appl. Microbiol. 26, 284-292.

DOI: 10.1078/072320203322346146

RATTANAMA, P., J. R. THOMPSON, N. KONGKERD, K. SIRINITIWARAWONGN, V. VUDDHAKUL (2012): Sigma E Regulators Control Hemolytic Activity and Virulence in a Shrimp Pathogenic *Vibrio harveyi*. PLOS ONE. 7(2), e32523.

DOI: 10.1371/journal.pone.0032523

RUBY, E. G., J. G. MORIN (1979): Luminous enteric bacteria of the marine fishes: a study of their distribution, densities, and dispersion. Appl. Environ. Microbiol. 38, 406-411.

DOI: 10.1128/aem.38.3.406-411.1979

SANDLUND, N., O. M. RODSETH, D. H. KNAPPSKONG, I. U. FIKSDAL, O. BERG (2010): Comparative susceptibility of turbot, halibut, and cod yolk-sac larvae to challenge with *Vibrio spp*. Dis. Aquat. Organ. 89, 29-37.

DOI: 10.3354/dao02176

SARROPOULOU, E., J. GALINDO-VILLEGAS, A. GARCÍA-ALCÁZAR, P. KASAPIDIS, V. MULERO (2012): Characterization of European *Sea Bass* Transcripts by RNA SEQ After Oral Vaccine Against *V. anguillarum*. Mar. Biotechnol. 14, 634–642.

DOI: 10.1007/s10126-012-9466-z

SCARANO, C., C. SPANU, G. ZIINO, F. PEDONESE, A. DALMASSO, V. SPANU, S. VIRDIS, E. P. DE SANTIS (2014): Antibiotic resistance of *Vibrio* species isolated from *Sparus aurata* reared in Italian mariculture. New. Microbiol. 37(3), 329-37.

SITJÀ-BOBADILLA, A., C. ZARZA, B. FOUZ (2014): Pathology. In: The biology of Sea bass. (Sánchez-Vázquez J. A., F. J. Muñoz-Cueto, Eds.), CRC Press, Boca Ratón, USA, pp. 287-341.

DOI: 10.1201/b16043-13

SMITH, P., V. ALDAY-SANZ, J. MATYSCZAK, G. MOULIN, C. R. LAVILLA-PITOGO, D. PRATER (2013): Monitoring and surveillance of antimicrobial resistance in microorganisms associated with aquatic animals. Rev. Sci. Tech. 32(2), 583-93.
DOI: 10.20506/rst.32.2.2237

SMITH, P., S. EGAN (2018): Standard protocols for antimicrobial susceptibility testing of *Vibrionaceae* isolated from aquatic animals. Bull. Eur. Ass. Fish. Pathol. 38(4), 160-164.

TENDENCIA, E. A. (2002): *Vibrio harveyi* isolated from cage-cultured seabass *Lates calcarifer* Bloch in the Philippines. Aquac. Res. 22, 455-458.
DOI: 10.1046/j.1365-2109.2002.00688.x

THORARINSSON, R., D. B. POWELL (2006): Effects of disease risk, vaccine efficacy, and market price on the economics of fish vaccination. Aquaculture. 259, 42-49.
DOI:10.1016/J.AQUACULTURE.2006.01.033

TORANZO, A. E., B. MARGARINOS, J. L. ROMALDE (2005): A review of the main bacterial diseases in mariculture systems. Aquaculture. 246, 37-61.
DOI: 10.1016/j.aquaculture.2005.01.002

VARSAMOS, S., G. FLIK, J. F. PEPIN, S. E. WENDELAAR BANGA, G. BREVIL (2006): Husbandry stress during early life stages affects the stress response and health status of juvenile sea bass, *Dicentrarchus labrax*. Fish. Shellfish. Immun. 20, 82-96.
DOI: 10.1016/j.fsi.2005.04.005

ZHANG, X. H., B. AUSTIN (2020): *Vibrio harveyi*: a serious pathogen of fish and invertebrates in mariculture. Mar. Sci. Tech. 2, 231–245.
DOI: 10.1007/s42995-020-00037-z

ZHANG, X. H., X. HE, B. AUSTIN (2000): Pathogenicity of *Vibrio harveyi* to salmonids. J. Fish. Dis. 23, 93-102.
DOI: 10.1046/j.1365-2761.2000.00214.x

ZORRILLA, I., S. ARIJO, M. CHABRILLON, P. DIAZ, E. MARTINEZ-MANZANARES, M. BALEBONA, M. A. MORIÑIGO (2003a): *Vibrio* species isolated from diseased farmed sole, *Solea senegalensis* (Kaup) and evaluation of the potential virulence role of their extracellular products. J. Fish. Dis. 26, 103-108.
DOI: 10.1046/j.1365-2761.2003.00437.x

ZORRILLA, I., M. A. MORIÑIGO, D. CASTRO, M. C. BALEBONA, J. J. BORREGO (2003b): Intraspecific characterization of *Vibrio alginolyticus* isolates recovered from cultured fish in Spain. J. Appl. Microbiol. 95, 1106-1116.
DOI: 10.1046/j.1365-2672.2003.02078.x

ZRNČIĆ, S., Ž. PAVLINEC (2020): Introduction to bacterial diseases. In: Diagnostic manual for the main pathogens in European seabass and Gilthead seabream aquaculture. (Zrnčić, S. Ed.), Ciheam, Zaragoza, pp. 63-66.

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ZUPIČIĆ, I. G., Ž. PAVLINEC, T. VEIĆ, D. ORAIĆ, S. ZRNČIĆ: Sličnosti i razlike vibrioze kod lubina uzrokovane bakterijama *Vibrio anguillarum* i *Vibrio harveyi*. Vet. arhiv, 92, 509-524, 2022.

SAŽETAK

Vibrioze uzrokovane bakterijama roda *Vibrio* su česte bolesti u akvakulturi, a karakteriziraju ih sustavne infekcije, visoka smrtnost i ekonomski gubici riba i školjkaša. Uzgoj lubina (*Dicentrarchus labrax*) i komarče (*Sparus aurata*) u Hrvatskoj je u posljednjem desetljeću naglo porastao, a ekonomska učinkovitost značajno je ugrožena gubicima zbog infekcije bakterijama *Vibrio anguillarum* i *Vibrio harveyi*. Stoga smo proučavali i usporedili okolišne uvjete, kliničke znakove te konvencionalne i molekularne dijagnostičke metode vibrioze uzrokovane s oba bakterijska patogena. Vibrioze uzrokovane bakterijom *V. anguillarum* kod lubina i komarče uglavnom se javljaju tijekom proljeća i jeseni, nakon brzog porasta ili sniženja temperature mora, dok se infekcije *V. harveyi* javljaju tijekom ljetnih mjeseci na temperaturama mora iznad 20 °C. Imaju sličnu kliničku sliku u akutnom obliku, ali se u subakutnom obliku razlikuju. Infekciju *V. anguillarum* karakteriziraju masivna krvarenja po trbušnoj stijenci, dok se kod infekcije *V. harveyi* uočava keratitis, zamućenje rožnice i nekoordinirano plivanje. Tijekom infekcije bakterijom *V. anguillarum,* razudbom su vidljiva krvarenja na jetri, crijevima i želucu. U slučaju infekcije s *V. harveyi*, vidljivi su sero-kataralni enteritis s distenzijom crijeva i ascitesom. Iako molekularna dijagnostika omogućava ispravnu identifikaciju, u ovom istraživanju smo definirali glavne čimbenike koje mogu razlikovati *V. anguillarum* od *V. harveyi* na temelju tradicionalne bakteriološke identifikacije kao što su osjetljivost na O/129 (10 μg) i novobiocin, te enzimska aktivnost β -galaktozidaze, arginin dihidrolaze i fermentacija amigdalina.

Ključne riječi: lubin; *Vibrio anguillarum*; *Vibrio harveyi*; okolišni uvjeti; dijagnostika; diferencijacija