Research Article

Effect of Cold Chain on *Vibrio spp.* Isolation from Frozen Shrimp and Fish of the Kribi Coastal Waters, Cameroon

Rhoda Nsen Bughe[1,2*], Charles Awe [2,4], Akindeh Mbuh Nji[2,3], Calvino Fomboh Tah[2], Marius Dongmo[2], Florent Mougou Essama[2], André Pagnah Zoli [5], Wilfried Fon Mbacham[2,3].

[1.]Department of Biological Sciences, Higher Teacher Training College, University of Yaoundé 1, BP. 47, Yaoundé, Cameroon,

[2.]Laboratory for Public Health Research Biotechnologies, and Food and Drug Safety Unit (FODRUS), Biotechnology Center, Nkolbisson University of Yaoundé 1, P.O. box: Box 8094 Yaoundé 8, Cameroon

[3.]Department of Biochemistry, University of Yaoundé 1, P. O. box: 812 Yaoundé, Cameroon

[4.]Laboratoire National véterinaire, Garoua, BP: 503 Garoua, Cameroun

[5.]Department of Physiology and Biotechnology of reproduction, University of Ngaoundere, BP: 454, Ngaoundere, Cameroon

*** Correspondences:** Dr. Rhoda Nsen Bughe, rhodabughe@yahoo.com, & Wilfried Fon Mbacham, wfmbacham@yahoo.com

Abstract

Vibrio species that inhabit seafood are often implicated in seafood disease outbreaks. Recently, *Vibrio harveyi* has been reported as an emerging human pathogen from seafood. However, the cold chain is a major control method against pathogens from seafood. This study investigates the effectiveness of the cold chain in isolating *Vibrio spp.* and *Vibrio harveyi* from shrimp and fish collected in Kribi. A total of 122 samples consisting of 60 shrimp (*Penaeus monodon* and *Penaeus kerathurus*) and 62 fish (*Galeoides decadactylus* and *Dicentrarchus labrax*) were collected from fishermen at the Boamanga beach in Kribi in this longitudinal study. Samples were frozen at -20°C in the laboratory and subjected to microbiological and molecular analyses. Of the 122 samples analyzed, 94(77.1%) were contaminated with 121 bacterial isolates. Isolates were identified as *Vibrio* phenotypically and genotypically recording 96(79.3%) and 83(68.6%) respectively. No *Vibrio harveyi* was detected. This therefore, suggests *Vibrio harveyi* sensitivity to cold chain. Notwithstanding, *Vibrio cholerae*, *V. parahaemolyticus*, *V. vulnificus*, *V. fluvialis; and Aeromonas hydrophila and Pasteurella pnemotropica* were identified. The high prevalence of *Vibrio* species presents a public health risk, emphasizing the essence for stringent hygienic practices during seafood processing to minimize risks of infection for consumers or fishermen.

Key words: Phenotypically and genotypically identification, *Vibrio species*, *Vibrio harveyi*, frozen fish and shrimp, Kribi Boamanga Beach.

Received: 06/02/2025
Accepted: 01/04/2025
DOI: https://dx.doi.org/10.4314/jcas.v21i3.1

Résumé

Les Vibrions sont impliqués dans les épidémies liées aux produits de la mer. Récemment, *Vibrio harveyi* a été signalé comme un pathogène humain émergent. Cependant, la chaîne du froid est une méthode majeure de contrôle des agents pathogènes dans les fruits de mer. Cette étude examine l'efficacité de la chaîne du froid pour isoler *Vibrio harveyi* dans les fruits de mer collectés à Kribi. Un total de 122

échantillons (60 crevettes : *Penaeus monodon* et *Penaeus kerathurus)* et 62 poissons : Galeoides decadactylus et Dicentrarchus labrax) ont été collectés auprès de pêcheurs de la plage de Boamanga à Kribi. Les échantillons ont été congelés à -20°C au laboratoire puis soumis aux analyses microbiologiques et moléculaires. Sur les 122 échantillons analysés, 96(79.3%) et 83(68.6%) étaient contaminés avec *Vibrio spp.* sur plan phénotypique et génotypique respectivement. Aucun *Vibrio harveyi* n'a été détecté, suggérant donc une sensibilité de *Vibrio harveyi* à la chaîne du froid. Cependant un risque de contamination aux autres espèces de *Vibrio: Vibrio cholerae*, *V. parahaemolyticus*, *V. vulnificus*, and *V. fluvialis* subsiste, ce qui constitue un problème pour la santé publique, soulignant l'importance de pratiques hygiéniques lors de la transformation de ces produits de mer afin de réduire le risque d'infection des pêches ou consommateurs.
Mots clés : identification phénotypiquement et génotypiquement, *Vibrio spp.*, *Vibrio harveyi*, crevettes et poissons congelés, débarcadère de Boamanga, Kribi.

1. INTRODUCTION

The fishery sector, through its products remains a resource of primary importance to global nutrition (FAO, 2018, 2020). Fish and shrimp are sources of proteins and essential trace elements that are valuable for nutritional balance and health (rich in long-chain omega 3 and low in cholesterol) (FAO, 2020; MINEPIA, 2020). In many developing countries and Cameroon inclusive, fish are the primary source of animal protein consumed because of their relative proximity and availability to all segments of the population. Shrimp are considered luxury products unlike fish (MINEPIA, 2020). However, these fishery products have been identified as sources of pathogenic bacteria, particularly Vibrio species that pose a threat to public health (CDC, 2010; Paudyal et al., 2017). In Cameroon, several species of *Vibrio* (*V. parahaemolyticus*, *V. cholera*e, *V. vulnificus* and *Vibrio alginolyticus*) have been reported in shrimp, fresh fish, and marine waters (Ndip et al., 2002; Koji et al., 2015; Bughe et al., 2016; 2017).

V. harveyi has been reported as the aetiology of diseases in fish and shrimp in marine and aquaculture environments (especially in fish and shrimp larvae) (Cano-Gómez, 2012; Hashem et El-Barbary, 2013; Kumaran and Citarasu, 2016; Torky et al., 2016; Zhang et al., 2020; Pavlinec et al., 2022; Triga et al., 2023). Additionally, few rare cases of less severe infections (digestive, skin, and septicaemia) in (immunocompromised) humans have been reported (Stalin and Srinivasan, 2016; Del Gigia-Aguirre et al., 2017; Brehm et al., 2020). Besides health interest, a high prevalence of pathogenic Vibrios in seafood products can hinder the exportation of these products to the international market. This may impede Cameroon, which intends to focus its economic plan on the development of the fishery sector (a booming fertile heritage).

Albeit, *Vibrio harveyi* inhabits coastal and marine water, attached on the surface and gut of marine organism and shrimp pond water (Khamesipour et al., 2014), the depletion of organic particles or plankton in seawater can lead to competition between *Vibrio harveyi* and other *Vibrio spp.* or with other marine bacteria (Long et al., 2005). This competition is responsible for the inhibition of growth or even death of certain *Vibrio* species on the surface of organic particles or plankton in suspension, making their detection impossible (non-cultivable) (Wong et al., 2024).

Climatic and environmental factors (Salinity and temperature) (Toni et al**.,** 2009; Zarei et al**.,** 2012; Liang et al. 2019; Mirbakhsh et al**.,** 2013) can influence the survival of Vibrio species. Salinity is especially important for the survival of obligate halophilic Vibrio species such as *V. harveyi* (Mirbakhsh et al**.,** 2013). Temperature tolerance varies between species, but the highest densities of Vibrios are found in water temperatures between 20°C and 30°C (Tantillo et al**.,** 2004).

So these conditions can be used to control Vibrio in seafood.

Thus, cold chain is usually used to maintain the nutritional value of these products to avoid depreciation immediately after catch (Amos, 2007) and also has a positive effect of inhibiting the growth of or killing microbes present in fish or shrimp (Bate and Bendall, 2010; De Silvia et al., 2018, Di Salvo et al., 2023). It is of paramount importance to assess the effectiveness of the cold chain practice given that there is paucity of information on *Vibrio harveyi* and *Vibrio spp*. from frozen fish and shrimp available in Cameroon despite its potential hazards. Therefore the objective of this study was to assess the effectiveness of cold chain in isolating *V. harveyi* and *Vibrio spp*. in fish and shrimp from the coastal waters of Kribi.

2. MATERIALS AND METHODS

2.1. Study area and sampling

This study was carried out in Boamanga beach, Kribi, capital of the Ocean Division, South Region (Cameroon); located on the edge of the Gulf of Guinea between 2° and 3° north latitude and 9° and 10° east longitude (Tiafack, 2014).
A longitudinal study was conducted on One hundred and twenty-two (122) samples consisting of 60 shrimp (30 Penaeus monodon and 30 Penaeus kerathurus) and 62 fish (31 Galeoides decadactylus and 31 Dicentrarchus labrax) collected at the Boamanga beach from the month of June to September, 2021. Sampling was performed weekly and five fish and five shrimp were bought, placed in sterile bags, and transported in an isothermal cooler containing frozen icepacks to the Food and Drug Safety Research Laboratory, Biotechnology Center, University of Yaoundé1. Sample processing was performed after two days of freezing at -20°C.

2.2. Isolation and phenotypic identification of *Vibrio* spp.

The hepatopancreas and intestines of frozen shrimp and fish were analysed; five grams each of aseptically extracted hepatopancreas and intestines was placed in separate sterile plastic bags containing 45 ml of 3% NaCl-supplemented alkaline peptone water (Biotec lab, UK). The samples were homogenised in plastic bags using a 400 W Bag-Mixer (Interscience, St. Nom, France) and incubated at 28°C for 24 h. After incubation, a tenfold serial dilution of up to 10^{-4} was performed and 0.1 ml was pipetted, spread onto Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar plate (Huankai Microbial (HKM) LTD, Guangzhou, China), and incubated at 28°C for 24 h. Suspected discrete colonies of *Vibrio* species were identified based on cultural characteristics: yellow or green colonies with smooth surfaces and circular or regular outlines with a diameter of ± 2 mm. These colonies were streaked on 3% NaCl-supplemented nutrient agar plate (Liofilchem, Italy) and incubated for 24 h at 28°C. Pure isolates were phenotypically identified using; motility, Gram staining, oxidase and catalase tests (Cheesbrough, 2006). This was followed by the Analytical Profile Index (API) 20 E strip kit test (BioMerieux, France). The results were analyzed using catalogue version 4 and online microbial identification software (API web software, BioMerieux).

2.3. Molecular identification of *Vibrio spp.* and *Vibrio harveyi*

2.3.1. DNA extraction

DNA extraction was performed by boiling method as previously described by Kim et al. (2007). Briefly, two or three 24-hour pure colonies of phenotypically identified Vibrio isolates were obtained from a 3% NaCl-supplemented Tryptic Soy agar plate (Liofilchem, Italy) and transferred into a 1.5 ml sterile Eppendorf tube containing 500 µl of nuclease-free water. Next, 0.5 µl triton

X 100 (Sigma, Germany) was added to the mixture and vortexed for 5 seconds. The solution was then heated in a hot block at 100°C for 20 min and cold shocked at -20°C for 20 minutes. This was followed by centrifugation at 10000 rpm for 10 min. Finally, 200 µl of the supernatant (DNA) was collected and transferred into a new sterile Eppendorf tube. The DNA concentration was measured using a Nanodrop spectrophotometer and stored at -20°C.

2.3.2. **Amplifications and Electrophoresis**

Amplifications of the 16S rDNA gene and the 16S rRNA gene for the genus *Vibrio* and *V. harveyi* identification were performed respectively. The 16S rDNA gene was amplified for then identification the genus *Vibrio,* following the protocol described by Bughe (2017). Amplification was conducted using a T3 Thermal cycler (Biometra, UK).

Each PCR run had a total volume of 20µl which included 7µl of nuclease-free water (QIAGEN), 10µl of 'One Hot Start Taq Master Mix' (BIOLABS.), 0.5µl of 0.1M of each primer (Universal Primers; FD2: 5'-AGAGTTTGATCATGGCTCAG-3'; RP1: 5'-ACGGTTACCTTGTTACGACTT-3') and 2 µl of DNA extract. DNA amplification was performed under the following conditions: initial denaturation at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 1 minute, annealing at 65°C for 1.5 minutes, extension at 72°C for 2 minutes, and final extension at 72°C for 5 minutes. The 16 rRNA gene for the identification of *V. harveyi* was amplified as described by Fukui and Sawabe (2007) with slight modification. Only genotypically identified *Vibrio spp.* samples were retained. Each PCR run had a total volume of 20µl containing 7µl of nuclease-free water (QIAGEN), 10µl of 'One Hot Start Taq Mix' (BIOLABS), 0.5µl of 0.2M of each primer (specific primers; VHARF: 5'-CCGCATAATACCTACGGGTC-3'; VHARR: 5'- ACCCGAAGTGCTGGCAAACA-3') and 2 µl of chromosomal DNA extract from genotypically identified *Vibrio* genus. DNA amplification was performed under the following conditions: initial denaturation at 94°C for 3 minutes, followed by 20 cycles of denaturation at 94°C for 30 seconds, annealing at 65°C for 30 seconds, extension at 72°C for 30 seconds and final extension at 72°C for 10 minutes. The amplicons were visualised using a UV reader after electrophoresis of the PCR product in a 1% agarose gel electrophoresis system at 150 volts for 30 minutes.

2.4 Statistical analysis

The data were input in Microsoft excel sheet version 2010 from which the percentage of occurrence were recorded and the tables were constructed.

3. RESULTS

3.1. **Phenotypic and genotypic Identification of *Vibrio* species.**

The prevalence of bacteria in the samples was 77.1% (94), with shrimp and fish accounting for 36.9% and 40.2% respectively. A total of 121 bacterial isolates were obtained from the fish (49) and shrimp (45). Phenotypic identification recorded ninety-six (96) *Vibrio spp.* out of the 121 isolates, with 45 contaminated shrimp and 49 contaminated fish. The results obtained were based on the morphological analysis of colonies, which were yellow or green in color, demonstrated flagella evidence when motility tested, and presented as short, straight, curved, or slightly comma-shaped Gram-negative rods (Figure 1 and 2).

The biochemical analyses included catalase and oxidase tests, as well as the Analytical Profile Index (API) 20E test, interpreted using catalogue version 4 and online microbial identification software (API web software, BioMérieux).

All Vibrio species tested positive for catalase and oxidase. The kit used for identifying Vibrio

primarily described it as "good" for the genus and "indiscriminate" at the species level, as some substrate reactions were similar to those of *Aeromonas* and *Pasteurella*. However, satisfactory identification of *Vibrio parahaemolyticus* was achieved. All *Vibrio parahaemolyticus* isolates possessed the following enzymes: lysine deaminase and gelatinase, fermented glucose, produced indole, and reduced nitrate, while all *Vibrio cholerae* produced indole and reduced nitrate. The other Vibrio species varied in their reactions to the different substrates used for their identification (Table 1). There was no identification code that matched the identity of *Vibrio harveyi* from the API web software.

Among the 25 isolates that could not be classified as Vibrios, six were identified as *Pasteurella pneumotropica* and five as *Aeromonas hydrophila*; the remainder could not be identified at the genus level using the Analytical Profile Index (API) 20E. Overall, this resulted in a prevalence of 79.3% for Vibrio species in frozen fish and shrimp (Table 2).

Vibrio cholerae was the most predominant isolate from both shrimp and fish samples, with a prevalence of 35.8%, followed by *Vibrio parahaemolyticus* (15.7%), *Vibrio spp.* (17.4.8%), *Vibrio vulnificus* (9.1%), and *Vibrio fluvialis* (1.7%) (Table 2). Of the 96 bacterial isolates analysed by conventional PCR, 83 were identified as belonging to the genus *Vibrio,* resulting in a prevalence of 68.6% (37 shrimps and 42 fish). Fish samples were more contaminated (45.5%) than shrimp (33.9%) (Table 3, Figure 3 and 4). Despite the high contamination rate with the genus *Vibrio*, no *Vibrio harveyi* was found among the 83 genotypically identified *Vibrio* isolates. Additionally, 31.4% of the bacterial isolates did not belong to the genus *Vibrio.*

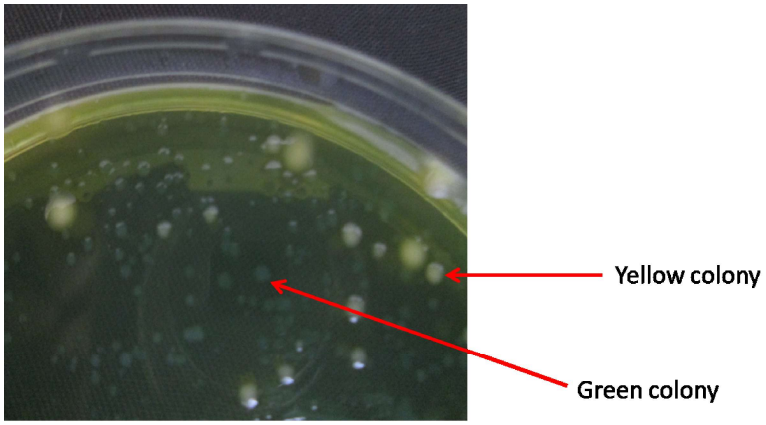


Figure 1 Legend: This is a Thiosulphate citrate bile sucrose agar plate on which two types of colonies have grown appearing yellow and green. Each of the colony depict a different species of a bacterium

Figure 1: Yellow and Green colonies of bacteria on Thiosulphate citrate bile salt sucrose agar plate

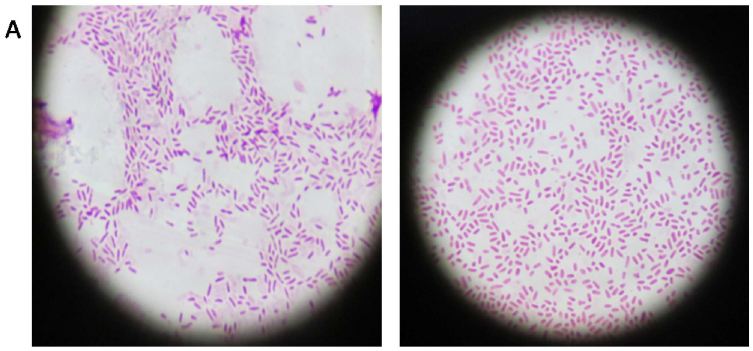


Figure 2 Legend: In A: the pink comma-shaped rods are Gram negative bacteria of the genus *Vibrio* and in B: the pink short straight rods still show Gram negative bacteria of the same genus *Vibrio* viewed under X 100 objective oil immersion of an optical Microscope. These are the different types of rods shaped bacteria that were isolated both in fish and Shrimp.

Figure 2: Gram negative comma-shaped and short straight rods of Vibrio species from fish and shrimp

Table 1: Biochemical identification of Vibrio isolates from frozen Shrimp and Fish of the Kribi coastal water

BIOCHEMICAL TEST	Shrimps					Fish			
	Vibrio parahaemolyticus N=9	*V. cholerae* N=19	*V. vulnificus* N=3	*V. fluvialis* N=2	Vibrio spp. N=8	*V. parahaemolyticus* N=10	*V. cholerae* N=24	*V. vulnificus* N=8	Vibrio spp. N=13
Cytochrome-Oxidase (OX)	100	100	100	100	100	100	100	100	100
Catalase	100	100	100	100	100	100	100	100	100
Ortho nitrophenyl-βD- galactopyranosidase (ONPG)	0	78.9	66.7	100	37.5	0	50	75	53.8
Arginine Dihydrolase(ADH)	0	52.6	66.7	50	62.5	0	30	25	69.2
Lysine Decarboxylase (LDC)	100	100	0	100	75	100	83.3	37.5	76.9
Ornithine Decarboxylase(ODC)	77.8	52.6	0	0	25	50	87.5	25	0
Citrate Utilization (CIT)	55.5	26.3	0	0	75	50	68.0	0	15.4
Hydrogen Sulphide Production (H_2s)	0	0	0	0	0	0	0	0	0
Urease (URE)	11.1	5.3	0	0	25	40	0	0	0
Tryptophane Deaminase (TDA)	0	21.1	0	0	0	0	83.3	37.5	53.8
Indole production (IND)	100	100	100	0	75	100	100	100	76.9
Acetoin production (VP)	55.5	57.9	0	0	37.5	60	41.6	0	15.4
Gelatinase (GEL)	100	84.2	66.7	0	50	100	83.7	0	53.8
Glucose (Fermentation-Oxidation) (GLU)	100	42.1	100	0	50	100	94.2	0	69.2
Fermentation-Oxidation (Mannitol, MAN)	88.8	47.4	100	0	100	100	94.2	25	76.9
Fermentation-Oxidation (Inositol, INO)	11.1	0	0	50	25	20	0	0	0
Fermentation-Oxidation(Sorbitol, SOR)	0	42.1	0	0	37.5	0	0	0	15.4
Fermentation-Oxidation (Rhamnose, RHA)	0	0	0	0	37.5	0	5.3	37.5	23.1
Fermentation-Oxidation (Saccharose, SAC)	0	5.2	100	0	37.5	0	68.0	87.5	53.8
Fermentation-Oxidation (Melibiose, MEL)	0	0	0	100	0	0	15.7	0	0
Fermentation-Oxidation (Amygdaline, AMY)	77.8	89.5	33.3	0	62.5	100	88.9	87.5	92.3
Fermentation-Oxidation(Arabinose, ARA)	77.8	84.2	100	50	0	100	68.0	87.5	0
Nitrate (NO_3)	100	100	100	50	100	100	100	100	53.8
Nitrite (N2)	0	0	0	50	0	0	0	0	46.2

Legend: The value with the tables against the different biochemical test indicates the percentages of the isolates that were positive for the different biochemical tests contain in the API 20E kit.

N: number of isolates of the different species

Table 2: Prevalence of Vibrio species and other bacteria isolated from frozen Fish and shrimp of Kribi coastal water from phenotypic identification.

Isolates	Fish	shrimp	Total	
Vibrio parahaemolyticus	10(8.3%)	9(7.4%)	19(15.7%)	
Vibrio cholerae	24(19.8%	19(15.7%)	43(35.5%)	
Vibrio vulnificus	8(6.7%)	3(2.4%)	11(9.1%)	
Vibrio fluvialis	0	2(1.7%)	2(1.7%)	
Vibrio species	13(10.7%)	8(6.7%)	21(17.4%)	
Total Vibrio isolates	**55(45.5%)**	**41(33.9%)**	**96(79.3%)**	
Aeromonas hydrophila	3(2.4%)	2(1.7%)	5(4.1%)	
Pasteurella pneumotropica	5(4.1%)	1(0.8%)	6(4.9%)	
Unclassified bacteria	8(6.7%)	6(4.9%)	14(11.6%)	
Total_non –Vibrio_isolates	**16(13.2%)**	**9(7.5%)**	**25(20.7%**	
Total	71(58.7%)	50(41.3%)	121(100%)	

Table 3: Prevalence of Vibrio species isolated from frozen Fish and shrimp of Kribi coastal water from PCR.

Isolates	Fish	shrimp	Total
Vibrio parahaemolyticus	10(8.3%)	9(7.4%)	19(15.7%)
Vibrio cholerae	19(15.7%	16(13.2%)	35(28.9%)
Vibrio vulnificus	7(5.8%)	3(2.4%)	10(8.3%)
Vibrio fluvialis	0	2(1.7%)	2(1.7%)
Vibrio species	11(9.1%)	6(4.9%)	17(14.0%)
Total identified	**47 (38.9%)**	**36(29.7%)**	**83(68.6%)**
Non-Vibrio isolates	**24 (19.8%)**	**14((11.6%)**	**38(31.4%)**
Total	71(58.7%)	50(41.3%)	121(100%)

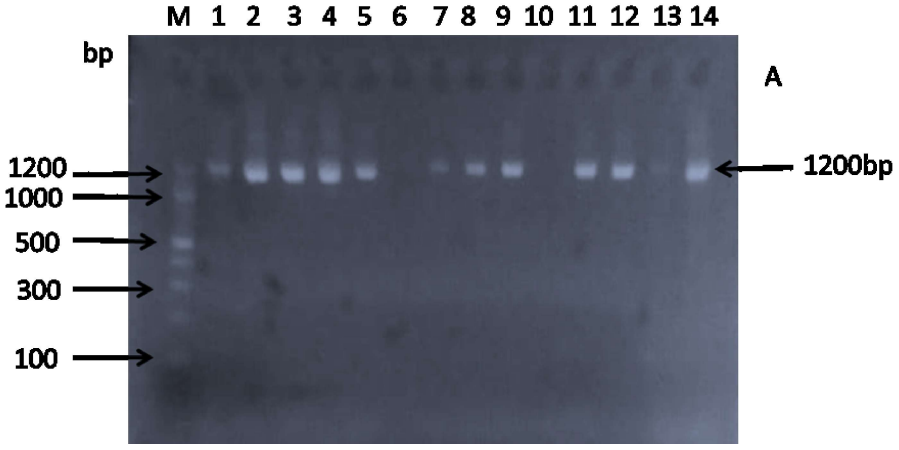
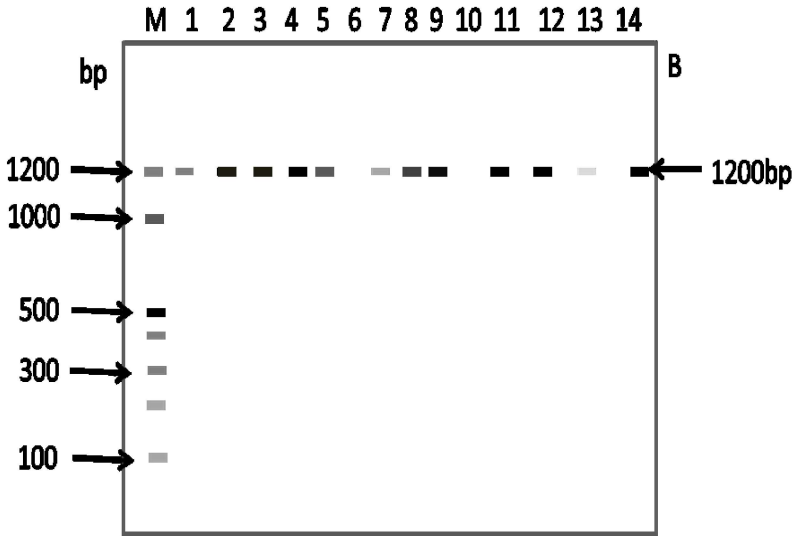


Figure 3: A; Polymerase Chain reaction of the 16S rDNA gene Agarose gel electrophoresis for identification of *Vibrio spp*. for Fish

Legend: lane M; molecular weight marker (100bp), lane 1; positive control,

Lanes 2, 3, 4, 5, 7, 8, 9, 11, 12, 13, and 14; represent isolates that were positive for *Vibrio* genus, Lane 6, and 10; represent isolates that were negative for *Vibrio* genus;

bp: base pair



3B: Pictogram of the above gel

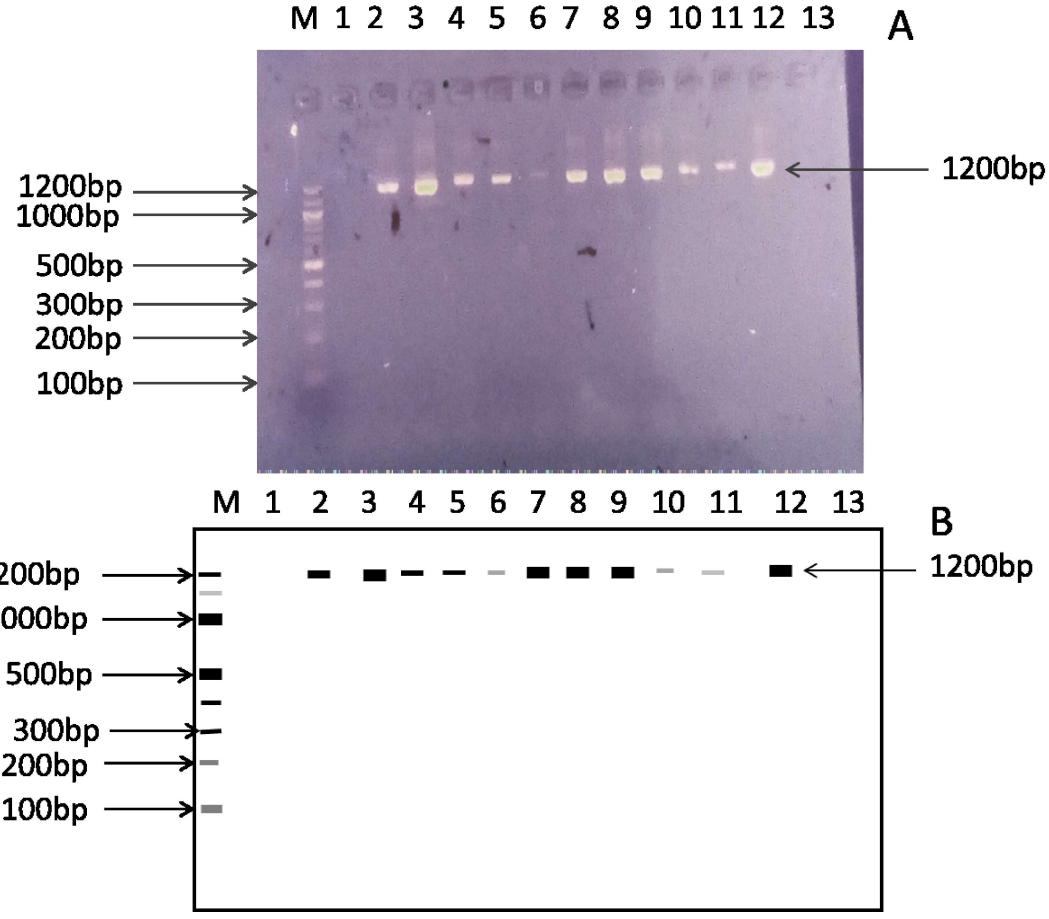


Figure 4 A: Polymerase Chain reaction of the 16S rDNA gene Agarose gel electrophoresis for identification of *Vibrio spp*. from Shrimp

4B: Pictogram of the above gel

Legend: lane M; molecular weight marker (100bp),
Lanes 1 and 13; isolates that were negative for *Vibrio* genus,
Lanes 2, 3, 4, 5, 6, 7, 8, 9, 11, 12; isolates that were positive for *Vibrio* genus,
bp: base pair.

4. DISCUSSION

This study highlighted that, though there was a high contamination of laboratory-frozen shrimp and fish samples of the Kribi Coastal water, Cameroon with the genus *Vibrio*, *Vibrio harveyi* was absent. Phenotypic identification detected other species of Vibrio: *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio fluvialis as well as Pasteurella pneumotropica* and *Aeromonas hydrophila*. This means, the cold chain killed the viable -culturable *Vibrio harveyi* while other *Vibrio species* and other bacteria were unaffected and remained viable. Other studies report that when Vibrio species enter the viable-but- non-culturable state, they can survive for long periods in frozen seafood (Di Salvo et al., 2023). The existence of *Vibrio* cells in the VBNC state in fish and shellfish pose a public health risk as the VBNC bacterium resuscitates under favourable conditions and proliferate resulting to disease outbreak, when in contact with open wounds, or ingested. However, if proper hygienic processing conditions are respected, there will be probably less public health risk.

The absence of *Vibrio harveyi* in this study ties with the findings of De Silva et al. (2018) who could not detect *Vibrio harveyi* in frozen shrimps but detected in live ones. This implies that some Vibrio species for example *Vibrio harveyi* cannot survive freezing. The cold chain does not only affect the growth of *Vibrio harveyi*, but other Vibrio like *Vibrio parahaemolyticus* whose load decreases when cold chain is practiced (Di Salvo et al., 2023; Love et al., 2020). This statement justifies the presence of *Vibrio parahaemolyticus* reported in this study and other species like *Vibrio cholerae, Vibrio vulnificus*, and *Vibrio fluvialis*. This study agrees with findings of Bughe et al. (2020) who transported shrimp in ice for the investigation of Vibrio species in freshly caught shrimps of the same study site and reported the absence of *Vibrio harveyi*, but *V. alginolyticus* and *V. parahaemolyticus* were identified in the samples. This implies that, *Vibrio harveyi* is very sensitive to low temperature. The absence of *Vibrio harveyi* from the laboratory -frozen fish and shrimp in this study, attest to the effect of cold chain on the detection of *V. harveyi*.

Even with optimal culturing conditions, such as suitable salinity levels in the culture medium and appropriate incubation temperatures, *Vibrio harveyi* was not detected in the samples. This indicates that the cold chain negatively impacts the survival of *Vibrio harveyi* cells in frozen fish and shrimp. This finding corroborates with the report of Tantillo et al. (2004), who noted variations in temperature tolerance among different Vibrio species and highlighted that high densities of Vibrios are typically found in water temperatures ranging from 20°C to 30°C.

Vibrio harveyi is commonly found in low numbers in marine environments. Although appropriate isolation techniques have been employed for isolating *V. harveyi* from shrimp, the isolation rate is often low among marine shrimp but is high and easily detected in aquaculture ponds (Torky et al., 2016; Pavlinec et al., 2022). The presence of *V. harveyi* in farmed-shrimp and fish can be attributed to the favourable conditions for their growth and survival, such as temperature, salinity, pH, organic particles, and plankton (Copin et al., 2019). Several studies have been documented showing the presence of *Vibrio harveyi* in farmed-shrimp and fish across different regions; Asia (China, Vietnam, Malaysia, and India) (Zhu et al., 2020; Sony et al., 2021; Yang et al., 2021) and Europe (Spain) (Pujalte et al., 2003).

Some species of Vibrio have been reported as both pathogens and opportunistic pathogens to immune-compromised humans and animals leading to conditions such as septicaemia, and digestive tract and skin infections. *Vibrio harveyi*

is reported in diseased-farmed shrimp and fish (Torky et al. (2016); Amatul-Samahah et al. 2022; Gan et al., 2022). Additionally, *Vibrio harveyi* has been linked to wound infections with the first report case study in Spain (Del Gigia-Aguirre et al. (2017) and another case following traumatic leg amputation (Brehm et al. 2020). These reports highlight the risk associated with handling fish and shrimp with open wounds, which may expose individuals to infection by *Vibrio harveyi* and other *Vibrios*.

A imitation of this study is that DNA for molecular analysis was extracted from colonies of bacteria isolated from frozen shrimps and fish; meanwhile if it was extracted from the frozen shrimp and fish directly, the DNA of viable-but-non-culturable state of Vibrio harveyi could have been detected, if present. Further research should be performed on similar frozen shrimps and fish samples without necessarily isolating the bacteria. To confirm *Vibrio harveyi* on culture media, live fish and shrimp samples of marine water should be used.

5. Conclusion

Freezing effectively kills bacteria in seafood, as this study demonstrate the absence of *Vibrio harveyi* in laboratory-frozen shrimp and fish collected from Kribi coastal waters. However, the cold chain could not eliminate completely all *Vibrio* species in the samples as other species including *Vibrio cholerae*, *V. parahaemolyticus*, *V. vulnificus* and *V. fluvialis* were presence; therefore, there is a public health risk. Thus, careful processing of these products is essential to prevent self-contamination.

Acknowledgements

We would like to thank the staff of the Divisional Delegation of Livestock, Fisheries and Animal Industries of the Ocean division in Kribi for their collaboration and availability, and the entire team of Food Safety and Public Health Research Biotechnology laboratories at the Biotechnology Centre of the University of Yaoundé 1, who provided us with the material support necessary for the success of this work.

Conflict of Interest

There is no conflict of interest to declare

Author Contribution

Rhoda Nsen Bughe: conceptualization(lead); writing-original(lead); formal analysis (lead), writing-review and editing(equal); Wilfred Fon Mbacham: writing-original draft (supporting); writing-review and editing (equal); André Pagnah Zoli: Conceptualization (supporting); writing-original draft (supporting); writing-review and editing (equal); Awe Charles: writing – original draft(supporting); formal analysis (supporting); writing-review and editing (equal); Akindeh Nji: Conceptualization(supporting); writing-review and editing (equal); Calvino Fomboh Tah; Marius Dongmo, Essama Mougou; were all involved in formal analysis (supporting); writing -review and editing.

REFERENCES

Amatul-Samahaha, Md. A., Mohamad, A., Al-saari, N., Zamri-Saade, M., Azmaif, M. N. A., Yusof, M. T., Ina-Salwanya, Md.Y., Tanaka, M., Mino, S., Sawabe, T. (2022). Draft genome sequence data of Vibrio harveyi VH1 isolated from a diseased tiger grouper, Epinephelus fuscoguttatus, cultured in Malaysia. Data in Brief 44, 108533 https://doi.org/10.1016/j.dib.2022.108533

Amos, B (2007). Analysis of quality deterioration at critical steps/points in fish handling in Uganda and Iceland and suggestions for improvement. United Nations University, Uganda. P. 45.

Bate, E. C., and Bendall, J. R. (2010). Changes in fish muscle after death. British Medical Bulletin (12), 2305.

Brehm, T. T., Berneking, L., Rohde, H., Chistner, M., Schlickewei, C., Martins, M. S., and Schmiedel, S. (2020). Wound infection with Vibrio harveyi following a traumatic leg amputation after a motorboat propeller injury in Mallorca, Spain: a case report and review of literature. BMC Infectious Diseases 20(1), 104-120 Doi: 10.1186/s12879-020-4789-2.

Bughe, R. N., Oben, M. P., Oben, B. O., Nji, M. A., Ali, M. I., Netongo, M. P. and Mbacham, F. W. (2016). Prevalence of *Vibrio parahaemolyticus* in *Penaeus Monodon* (Fabricius, 1798) from the Douala Coastal Waters of Cameroon: Implication for Food Safety. International Journal of Research Studies in Biosciences 4, 10-20.

Bughe, R. N. (2017). Characterisation of Penaeid shrimps, pathogenic Vibrios and the antagonistic potential of *Bacillus* spp. from shrimps of the south and littoral coastal waters, Cameroon. PhD thesis, University of Buea, Cameroon, pp 1-196

Bughe, R. N., Oben, B. O., Nji, A. M., Chedjou, J. P. K. , Mbange, A. E., Ali, I. M., Oben, P. M., and Mbacham, W. F., **(2020)**. Occurrence and antibiotics susceptibility of *Vibrio* species from Penaeid shrimps from Kribi Coastal Water, Cameroon. *International Journal of Advanced Research in Biological Sciences* 7(7): 96-111 http://dx.org/10.22192/ijarbs.2020.07.012

Cano-Gómez (2012). Molecular identification of *vibrio harveyi*-related bactaria and *Vibrio owensii* sp.nov. pathogenic to larve of the ornate spiny lobster Panulirus ornatus. PhD thèses James Cook Université

CDC (2010). Preliminary Food Net Data on the Incidence of Infection with Pathogens Transmitted Commonly Through Food – 10 States, 2009. Morbidity and Mortality Weekly Report 59 (14), 418-22.

Cheesbrough M. (2006). Biochemical tests to identify Bacteria. In: District Laboratory Practice in Tropical Countries Part II. Second Edition. Cambridge University Press, New York pp. 64.

Copin, S., Robert-Pillot, A., Gay, M. et Quilici, M-L. (2019). *Vibrio* impliqués en pathologie humaine : une étude de leur répartition dans des produits de la mer consommés en France. *Bulletin épidémiologique, santé animale et alimentation* no 68/ Spécial Vigilance sur la chaîne alimentaire.

De Silva, B. C. J., Hossain, S., Dahanayake, P. S., De Zoysa, M., Heo, G-J. (2018). Comparative prevalence and characterization of Vibrio spp. isolated from live and frozen white-leg shrimp (Litopenaeus vannamei) in Korean market. Journal of Food safety 2018; e12487 https://doi.org/10.1111/jfs.12487

Del Gigia-Aguirre, L., Sánchez-Yebra-Romera, W., Garcia-Munoz, S., and Rodríguez, M. (2017). First description of wound infection with *Vibrio harveyi* in Spain. New Microbe and New Infections 19, 15–16

Di Salvo, E., Panebianco, F., Panebianco, A., Ziino, G. (2023). Quantitative Detection of Viable but Nonculturable *Vibrio parahaemolyticus* in Frozen Bivalve Molluscs. Foods 12, 2373. https://doi.org/ 10.3390/foods12122373

FAO. (2018). La situation mondiale des pêches et d'aquaculture. Atteindre les objectifs de développement durable. Rome. 224 p. Licence : CC BY-NC-SA 3.0 IGO (consultable à l'adresse suivante : http://www.fao.org/3/i9540fr/i9540fr.pdf).

FAO. (2020). La situation mondiale des pêches et d'aquaculture. La durabilité en action. Rome. 247p.

Gan, L., Zheng, J., Xu, W. H. *et al*. (2022). Deciphering the virulent *Vibrio harveyi* causing spoilage in muscle of aquatic crustacean *Litopenaeus vannamei*. Scientific Report **12**, 16296. https://doi.org/10.1038/s41598-022-20565-1

Fukui, Y. and Sawabe, T. (2007). Improved One-step Colony PCR Detection of *Vibrio harveyi*. Microbes and Environment 2(1), 1-10.

Hashem M. and El-Barbary, M. (2013). *Vibrio harveyi* infection in Arabian Surgeon fish (Acanthurus sohal) of Red Sea at Hurghada, Egypt. Egyptian Journal of Aquatic Research 39, 199-203

Khamesipour, F, Noshadi, E., Moradi, M., Raissy, M. (2014). Detection of Vibrio spp. in shrimp from aquaculture sites in Iran using polymerase chain reaction (PCR). Aquaculture, Aquarium, Conservation & Legislation (AACL) BIOFLUX 7(1), 1-8

Kim, J. S., Lee, G. G. G., Park, J. S., Jung, Y. H., Kwak, H. S., Kim, S. B., Nam, Y. S. and Kwon, S. (2007). A Novel Multiplex PCR Assay for Rapid and Simultaneous. Detection of Five Pathogenic Bacteria: *Escherichia coli* O157:H7, *Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Vibrio parahaemolyticus*. Journal of Food Protection 70(7), 1656–1662.

Koji, E., Djimeli, C. L., Arfao, A. T., Ewoti, V. N., Tchakonte, S., Bricheux, G. *et al.* (2015) Abundance dynamic of *Vibrio* cells associated with freshwater shrimps Atyidae (Crustacea-decapoda) in the coastal surface waters of Cameroon (Central Africa): Assessment of the role of some environmental factors. International Journal of Current Microbiology Applied Sciences 4(6), 358-378

Kumaran, T. and Citarasu, T. (2016). Isolation and Characterization of Vibrio Species from Shrimp and Artemia Culture and Evaluation of the Potential Virulence Factor. Intel Prop Rights 4, 153. doi:10.4172/2375-4516.1000153

Long, R. A., Rowley, D. C., Zamora, E., Liu, J., Bartlett, D. H. and Azam, F. (2005). Antagonistic interactions among marine bacteria impede the proliferation of *Vibrio cholerae*. Applied and Environmental Microbiology 71, 8531-6.

Liang, J., Liu J, Wang X, Lin H, Liu J, Zhou S, Sun H, Zhang XH (2019) Spatiotemporal dynamics of free-living and particle associated *Vibrio* communities in the northern Chinese marginal seas. Applied and Environmental Microbiology 85(9), e00217-e219

Love, D., C., Kuehl, L. M., Lane, R. M., Fry, J. P., Harding J., Benjamin J.K., Clanc Kate, Hudson B. (2020). Performance of cold chains and modeled growth of *Vibrio parahaemolyticus* for farmed oysters distributed in the United States and internationally. International Journal of Food Microbiology 313, 1083 https://doi.org/10.1016/j.ijfoodmicro.2019.108378 Received 27

MINEPIA. (2020). Le Cameroun veut développer la filière aquacole. En ligne sur le site du MINAPIA. http://minepia.gov.cm.

Mirbakhsh, M., Akhavan, S.A., Afsharnasab, M., Khanafari, A. and Razavi, M.R. (2013). Molecular Identification of *Vibrio harveyi* from Larval Stage of Pacific White Shrimp *(Litopenaeus vannamei)* Boone *(Crustacea: Decapoda)* By Polymerase Chain Reaction and 16S rDNA Sequencing. Iranian Journal of Fisheries Sciences, 13(2), 384-393

Ndip, N. R., Akochere, K. J-F., Mokosso, K. D., Ndip, M. L. and Anyangwe, A. N. I., (2002). Carriage of Vibrio spp. by shrimps harvested from the coastal waters of south west Cameroon. East Africa Medical Journal 79(3), 146-149.

Paudyal, N., Anihouvi, V., Hounhouigan, J., Matsheka, M., Sekwati-Monang, B., Amoa-Awua, W., Atter, A., Ackah, N., Mbugua, S. and Asagbra, A. (2017). Prevalence of food borne pathogens in food from selected African countries–A meta-analysis. International Journal of Food Microbiology 249, 35-43.

Pavlinec, Ž., Zupi
i, I. G., Orai, D. , Lojki, I., Fouz, B. & Zrn
i S. (2022). Biochemical and molecular characterization of three serologically different *Vibrio harveyi* strains isolated from farmed Dicentrarchus labrax from the Adriatic Sea. Scientifc Reports 12:7309 https://doi.org/10.1038/s41598-022-10720-z

Pujalte, J.M., Sitjà-Bobadilla, A., Macián, C.M., Belloch, C., Álvarez-Pellitero, P, P., PérezSánchez, P., Uruburu, F, F. & Garay, E. (2003). Virulence and Molecular Typing of *Vibrio harveyi* Strains Isolated from Cultured Dentex, Gilthead Sea Bream and European Sea Bass. Systematic and Applied Microbiology. Doi: 10.1078/072320203322346146

Song, M., Sumithra, T. G., Anusree, V. N., Amala, P. V., Reshma, K. J, Alex, S. *et al.* (2021). Antimicrobial resistance and virulence characteristics of *Vibrio vulnificus*, *Vibrio parahaemolyticus* and *Vibrio harveyi* from natural disease outbreaks of marine/estuarine fishes. Aquaculture. Doi.org/10.1016/j.aquaculture.2021.736608

Stalin, N. and Srinivasan, P. (2016). Molecular characterization of antibiotic resistant *Vibrio harveyi* isolated from shrimp aquaculture environment in the south east coast of India. Microbial Pathogenesis 97, 110-118.

Tantillo, G.M., Fontanarosa, M., Di Pinto, A. and Musti, M. (2004). Updated perspectives on emerging vibrios associated with human infections. Letters in AppliedMicrobiology 39, 117-26

Tiaf ack, O., Chrétien, N., and Emmanuel, N. N. (2014). Development Polarisation in Limbe and Kribi (Littoral Cameroon): Growth Challenges, Lessons from Douala and Options. Current Urban Studies 2(04), 361.

Toni, D. A., Touron-bodilis, A. et Wallet, F. (2009). Effet du changement climatique sur les micro-organismes aquatiques pathogènes : quelques exemples. Environnement, Risques & Santé 8 (4).

Torky, A. H., Gaber, S., Abdellrazeq, M. M. H. and Nourhan, H. G. (2016). Molecular Characterization of *Vibrio harveyi* in Diseased Shrimp. Alexandria Journal of Veterinary Sciences 51(2), 358-366.

Wong Yi You, Weng Choon, Bong Chui Wei, Lim Joon Hai, Ng Ching Ching, Narayanan Kumaran, Sim Edmund Ui Hang and Wang Ai jun (2024). Environmental factors that regulate Vibrio spp. abundance and community structure in tropical waters. Anthropocene Coasts 7, 21 https://doi.org/10.1007/s44218-024-00054-w

Triga, A., Smyrli M. and Katharios P. (2023). Pathogenic and Opportunistic Vibrio spp. Associated with Vibriosis Incidences in the Greek Aquaculture: The Role of Vibrio harveyi as the Principal Cause of Vibriosis. Microorganisms 11, 1197. https://doi.org/10.3390/microorganisms11051197

Yang, A., Li, W., Tao, Z., Ye, H., Xu, Z., Li, Y. Gao, Y. and Yan, X. (2021). *Vibrio harveyi* isolated

from marine aquaculture species in eastern China and virulence to the large yellow croaker (*Larimichthys crocea*). Journal of Applied Microbiology ISSN 1364-5072

Zarei, M., Borujeni, P. M., Jamnejad, A. and Khezrzadeh, M. (2012). Seasonal prevalence of *Vibrio* species in retail shrimps with an emphasis on *Vibrio parahaemolyticus*. Food Control 25, 107-109

Zhang, X-H., He, X. and Austin, B. (2020). *Vibrio harveyi:* a serious pathogen of fish and invertebrates in mariculture. Marine Life Science Technology. Doi.org/10.1007/s42995-020-00037-z

Zhu, Z., Duan, C., Dong, C., Weng, S. and He, J. (2020). Epidemiological situation and phylogenetic relationship of *Vibrio harveyi* in marine-cultured fishes in China and Southeast Asia. Journal Preproof. Doi.org/10.1016/j.aquaculture.2020.735652.