FAS http://e-fas.org
Fisheries and Aquatic Sciences

Low-value Fish used as Feed is a Source of Disease in Farmed Fish

Do-Hyung Kim*

Department of Aquatic Life Medicine Pukyong National University, Busan 608-737, Korea

Abstract

Low-value fish is the most commonly used feed in Asian fish farms despite the fact that its application is controversial in regard to the sustainability and biosecurity of aquaculture. In this study, the causal agent of a disease outbreak at a Korean rockfish *Sebastes schlegeli* farm was investigated to determine whether the low-value fish used at the farm was the source. Infected Korean rockfish and Pacific sand eel used as feed were sampled from the farm, and bacterial cultures recovered from the internal organs of all sampled rockfish were isolated as pure cultures and later identified as *Vibrio harveyi*. The causal agent of the disease was also isolated from the kidneys of some of the sampled Pacific sand eels. This study provides additional evidence that the low-value fish used as feed at fish farms can be a key source of infectious diseases.

Key words: Low-value fish, Korean rockfish, *Ammodytes personatus*, *Vibrio harveyi*, Biosecurity

Introduction

Low-value, or trash fish (i.e., as raw ingredients not reduced to fishmeal), is a term used to describe fish species with various characteristics, generally including fish that are small in size and have little or no direct commercial value (Funge-Smith et al., 2005). The FAO (2012) estimates that the total worldwide volume of low-value fish used as feed in aquaculture is approximately 9 million tons. The use of low-value fish as feed is a common husbandry practice in marine fish farms in several countries within the Asia-Pacific region (Funge-Smith et al., 2005). Approximately 90% of the marine fish (e.g., Korean rockfish) cultured in Korea are fed with farm-made aquafeed, whose formulation generally consists of low-value fish mixed with 10–20% (w/w) industrially manufactured compound fish meal feed (Kim et al., 2013). Although accurate information about the exact species of low-value fish used as feed in Korea is lacking, Pacific sand eel *Ammodytes personatus* and big head croaker *Collichthys niveatus* are generally recognized as the most common species. In this study,

the causal agent of a disease that occurred at a Korean rockfish farm was investigated, including examination for the presence of pathogens in the low-value fish used as feed on the farm. Additionally, a brief literature review was conducted, which indicated that low-value fish and baitfish are disease vectors.

Materials and Methods

Bacterial isolation and identification

Infected Korean rockfish *Sebastes schlegeli* ($n = 10$; weight = ~ 600 g) were sampled in October 2012 from a sea-cage fish farm located in Namhae on the southern coast of Korea. Pacific sand eel ($n = 10$), which were typically used at the farm as low-value fish for feed, were also obtained. Moribund or freshly dead rockfish exhibiting skin ulcers and hemorrhages in the operculum and on the body surface (Fig. 1) were asepti-

Fig. 1. Clinical signs of Korean rockfish *Sebastes schlegeli* infected with *Vibrio harveyi*. Skin ulcers and hemorrhages at the operculum, around the base of the pectoral and pelvic fins and caudal peduncle were commonly observed in most sampled fish.

cally dissected, and swabbed materials derived from the kidney and spleen were transferred to brain heart infusion agar (BHIA; Difco, Detroit, MI, USA) containing 1% NaCl to isolate the causal agent. The plates were incubated at 25°C for 7 d, following a thorough examination for signs of clinical disease.

Frozen low-value fish samples were thawed quickly in a microwave oven, and the kidney and liver were aseptically removed and used to isolate bacteria. Several colonies with different morphologies appeared on BHIA after a 7-d incubation at 25°C. A colony polymerase chain reaction was then performed on each well-isolated colony using 16S rRNA gene primers (fD1: 5′-AGAGTTTGATCCTGGCTCAG-3′; rP2: 5′-ACGGCTACCTTGTTACGACTT-3′) (Weisburg et al., 1991), following the procedure described in Kim and Kim (2013). Sequencing reactions were conducted using a BigDye Terminator v.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA), and the amplified DNA fragments were sequenced on an automatic sequencer (Applied Biosystems 3730xl DNA Analyzer). Specific primers (toxRF1 5′-GAAGCAGCACT-CACCGAT-3′; toxRR1 5′-GGTGAAGACTCATCAGCA-3′) amplifying a part of the *toxR* gene (Pang et al., 2006) were used for further identification of *Vibrio harveyi*. Isolates closely related to *V. harveyi* were tested for growth at 40°C in media devoid of the NaCl supplement.

Analysis of the 16S rRNA gene sequences

All sequences were subjected to similarity searches using BLAST, and the closest type strain was determined using RDP SEQMATCH (www.rdp.cme.msu.edu) (Cole et al., 2009). The sequences of all isolates were aligned using the ClustalX software for phylogenetic analysis, and positions 63–779 (*Escherichia coli* 16S rRNA numbering) were chosen. The neighbor-joining tree was constructed based on Kimura two-parameter distances, and bootstrap analysis of 1,000 re-samplings was performed using MEGA 5 (Saitou and Nei, 1987; Tamura et al., 2011). The accession numbers of representative sequences are presented in Table 1.

Antimicrobial susceptibility testing

A disc diffusion assay was performed for all isolates and reference strains according to the protocols specified in the CLSI guideline M42-A (CLSI, 2006). All tests were conducted on Mueller–Hinton agar supplemented with 1% NaCl, with incubation at 22°C for 24–28 h. Eight antimicrobials, including discs containing gentamicin (10 μg), erythromycin (15 μg), florfenicol (30 μg), nalidixic acid (30 μg), oxolinic acid (2 μg), enrofloxacin (5 μg), oxytetracycline (30 μg), and trimethoprim/sulfamethoxazole (SXT) (1.25/23.75 μg) (Oxoid, Cambridge, UK) were used. These are all antibiotics that have

been approved for use in aquaculture. *Aeromonas salmonicida* subsp. *salmonicida* ATCC 33658 was used as the quality control strain (CLSI, 2006).

Results and discussion

The closest type strains to the 32 isolates retrieved in this study and their accession numbers are listed in Table 1. The phylogenetic relationships are presented in (Fig. 2). *Psychrobacter* spp., including *P. faecalis* (90%; 9/10), *P. maritimus* (20%; 2/10), *P. celer* (10; 1/10) and *P. luti* (10%; 1/10), were predominantly isolated from the kidney or liver of the frozen Pacific sand eels. In general, *Psychrobacter* species are primar-

ily isolated from cold to warm saline ecosystems, in samples ranging from chilled meat and fish to clinical samples (Bowman, 2006). Furthermore, a few *Psychrobacter* species, such as *P. maritimus*, have been isolated from the intestinal tract of fish (Ringø et al., 2008) and freshwater fish (Gonzalez et al., 2000). However, *P. faecalis*, which was initially obtained from a bioaerosol originating from pigeon feces (Kämpfer et al., 2002), has not previously been isolated from or associated with disease in fish. Hisar et al. (2002) found that *Psychrobacter immobilis* caused infection in rainbow trout (*Oncorhynchus mykiss*), but hypothesized that the species may act as a secondary, not primary, invader, as no mortality was observed for 35 days after challenge. Based on the present and previous studies (Gonzalez et al., 2000; Gennari et al., 1999), it was

Table 1. Representative sequences (16S rRNA gene) of isolates obtained from the internal organs of pacific sand eel *Ammodytes personatus* and infected Korean rockfish *Sebastes schlegeli*

| Isolation source	Isolate code	Closest type strain (determined by RDP SEQMATCH)	Identity (%)	Length (bp)	Accession no.
Farmed fish- Korean rockfish					
Fish 1	RFHW1ka	*Vibrio harveyi*; NCIMB1280T; AY750575	99.0	810	KR051262
	RFHW1kb	*Vibrio harveyi*; NCIMB1280T; AY750575	99.9	810	KR051263
Fish 2	RFHW2k	*Vibrio harveyi*; NCIMB1280T; AY750575	99.8	810	RFHW1kb*
Fish 3	RFHW3ka	*Vibrio harveyi*; NCIMB1280T; AY750575	99.0	811	RFHW1ka*
	RFHW3kb	*Vibrio harveyi*; NCIMB1280T; AY750575	100	810	RFHW1kb*
Low-value fish- Pacific sand eel					
Fish 1	AP1La	*Psychrobacter faecalis*; Iso-46; AJ421528	99.0	906	KR051241
	AP1Kb	*Aerococcus urinaeequi*; IFO12173; D87677	99.5	843	KR051240
	AP1Ka	*Vagococcus fluvialis*; CCUG 32704; Y18098	96.8	845	KR051239
	AP1Lb	*Vibrio harveyi*; NCIMB1280T; AY750575	99.9	809	RFHW1kb*
Fish 2	AP2La	*Psychrobacter faecalis*; Iso-46; AJ421528	98.8	886	KR051245
	AP2Ka	*Pseudomonas caeni*; HY-14; EU620679	99.3	844	KR051242
	AP2Kb	*Trichococcus collinsii*; 37AN3; AJ306612	95.2	915	KR051243
	AP2Kc	*Vagococcus fluvialis*; CCUG 32704; Y18098	99.3	913	KR051244
Fish 3	AP3Ka	*Psychrobacter faecalis*; Iso-46; AJ421528	98.6	900	KR051246
	AP3O	*Psychrobacter celer*; SW-238; AY842259	98.8	916	KR051247
Fish 4	AP4Ob	*Psychrobacter faecalis*; Iso-46; AJ421528	99.5	930	KR051250
	AP4La	*Aerococcus urinaeequi*; IFO12173; D87677	98.1	880	KR051249
	AP4Kc	*Psychrobacter maritimus*; KMM 3646; AJ609272	95.1	910	KR051248
Fish 5	AP5Lb	*Psychrobacter faecalis*; Iso-46; AJ421528	99.1	880	KR051252
	AP5Lc	*Psychrobacter faecalis*; Iso-46; AJ421528	97.6	880	KR051253
	AP5La	*Aerococcus urinaeequi*; IFO12173; D87677	99.0	880	KR051251
Fish 6	AP6K	*Psychrobacter faecalis*; Iso-46; AJ421528	99.8	890	AP4Ob*
	AP6L	*Vibrio harveyi*; NCIMB1280T; AY750575	98.5	809	KR051254
Fish 7	AP7La	*Psychrobacter faecalis*; Iso-46; AJ421528	98.8	880	KR051255
Fish 8	AP8Lc	*Aerococcus urinaeequi*; IFO12173; D87677	97.2	887	KR051257
	AP8Ob	*Psychrobacter faecalis*; Iso-46; AJ421528	98.0	864	KR051258
	AP8Lb	*Psychrobacter maritimus*; KMM 3646; AJ609272	98.8	882	KR051256
	AP8Oc	*Vagococcus fluvialis*; CCUG 32704; Y18098	96.2	820	KR051259
Fish 9	AP9Kb	*Psychrobacter faecalis*; Iso-46; AJ421528	97.2	724	KR051261
	AP9L	*Vibrio harveyi*; NCIMB1280T; AY750575	99.0	810	RFHW1ka*
	AP9Ka	*Psychrobacter luti*; NF11; AJ430828	97.3	660	KR051260
Fish 10	AP10L	*Psychrobacter faecalis*; Iso-46; AJ421528	99.0	906	AP1La*

Asterisks indicate that the isolates (in "Isolate code" and "Accession no." column) had identical 16S rRNA gene sequences.
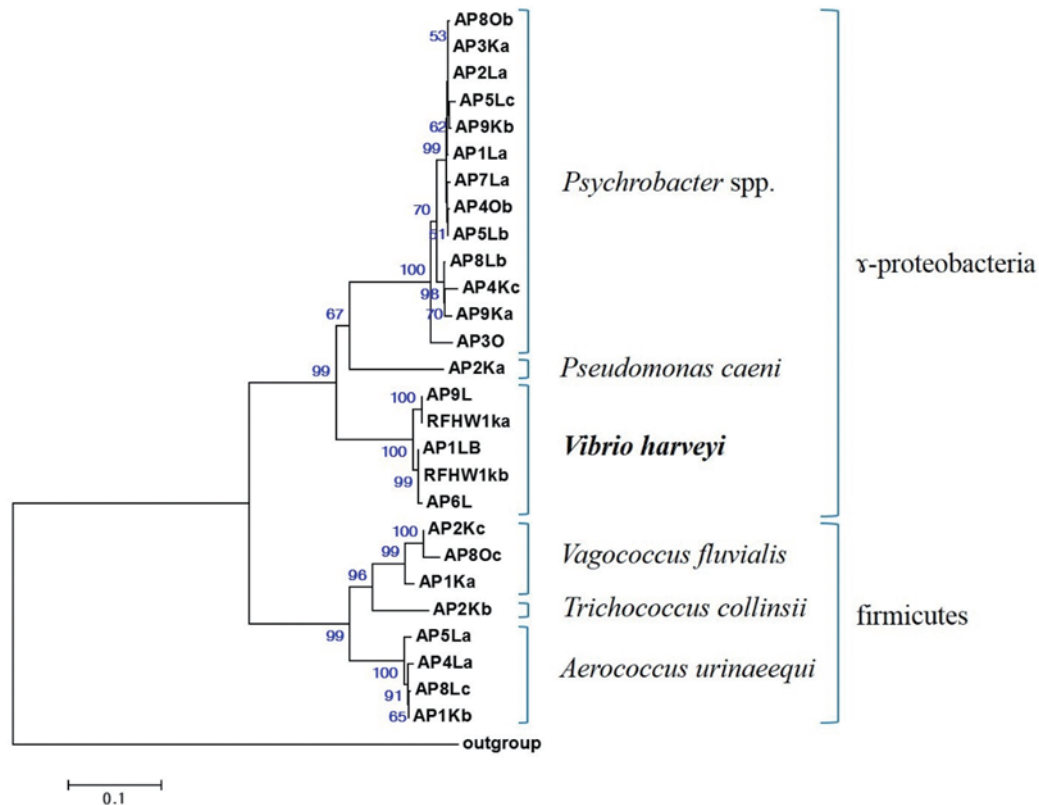
Fig. 2. Neighbor-joining phylogenetic tree showing the relationships among 27 16S rRNA gene sequences of isolates retrieved from Pacific sand eel *Ammodytes personatus* and infected Korean rockfish *Sebastes schlegeli*. Approximately 717 nucleotides *Escherichia coli* 16S rRNA gene positions 63-779) were analyzed to conduct the tree. Bootstrap values based on 1,000 re-samplings display the significance of the interior nodes, and are shown at the branch points; only values displaying >50% are given. Scale bar represents a 10% estimated difference in nucleotide sequences.

speculated that *Psychrobacter* are rarely associated with disease and that this is a common bacterial group that may often be isolated after *post mortem* storage. *Aerococcus urinaeequi* (40%; 4/10), *Vagococcus fluvialis* (30%; 3/10), *Pseudomonas caeni* (10%; 1/10) and *Trichococcus collinsii* (10%; 1/10) were also isolated from the kidney or liver of Pacific sand eels at different frequencies. AP2Kb, AP4Kc, and AP8Oc, which were most closely related to *T. collinsii*, *P. maritimus*, and *V. fluvialis* type strains, respectively, are highly likely to be affiliated with different or new phylogenetic groups, as the similarities of their 16S rRNA gene sequences were significantly low (<97%). Although it is difficult to determine why these bacterial species existed in the kidney or liver, we hypothesize that they may not be primary pathogens, but instead are secondary invaders of already-dead tissues and organisms.

Most of the Korean rockfish sampled in this study displayed skin ulcers and hemorrhaging in the operculum and around the base of the pectoral and pelvic fins and the caudal peduncle (Fig. 1). Most of the bacteria isolated from the fish showed similar colony morphology; thus, five isolates were selected for further examination. The 16S rRNA gene sequences of the five selected rockfish isolates (RFHW1ka, RFHW1kb,
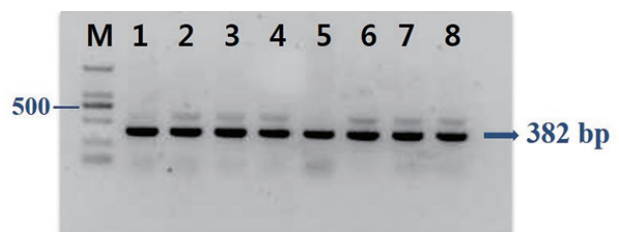
Fig. 3. Agarose gel showing polymerase chain reaction products (band size = 382 bp) of the *toxR* gene in isolates obtained from Korean rockfish *Sebastes schlegeli* and Pacific sand eel. M, DNA marker; lane 1, RFHW1ka; lane 2, RFHW1kb; 3, RFHW2k; 4, RFHW3ka; 5, RFHW3kb; 6, AP1Lb; 7, AP6L; 8, AP9L.

RFHW2k, RFHW3ka, and RFHW3kb) and three Pacific sand eel isolates (AP1Lb, AP6L, and AP9L) were found to be most closely related to the *Vibrio harveyi* type strain, a well-known opportunistic fish pathogen. It was further confirmed that the *V. harveyi toxR* gene could be specifically amplified from all eight isolates (Fig. 3). Additionally, all isolates that grew well on thiosulfate citrate bile salt sucrose agar (TCBS agar) did not grow at 40°C or on culture media devoid of the NaCl sup-

plement, which is in agreement with the typical characteristics of *V. harveyi* (e.g., Lee and Yii, 1996; Won et al., 2007).

The inhibitory zones obtained for the reference strains were within the acceptable ranges specified in the CLSI guideline M42-A (CLSI, 2006). The zone sizes obtained from the *V. harveyi* isolates are shown in Table 2. The inhibitory zone diameters for gentamicin, tetracycline, and the quinolones of all eight isolates were similar. According to the epidemiological cut-off values (CO_{WT}) using the data for 52 *V. harveyi* strains isolated in Korea, including the eight isolates, and a normalized resistance interpretation analysis (data not shown), RFHW1ka, RFHW3ka, and AP9L were classified as non-wild type for SXT, erythromycin, and florfenicol (indicated in bold type in Table 2). Additionally, RFHW2k was treated as non-wild type for both SXT and erythromycin. RFHW1kb, RFHW3kb, AP1Lb, and AP6L showed almost the same antibiotic susceptibility patterns as those of RFHW1ka, RFHW3ka, AP9L, and RFHW2k.

Vibrio harveyi is a Gram-negative marine bacterium that

causes serious penaeid shrimp and fish disease (Austin and Zhang, 2006). Many studies have demonstrated that *V. harveyi* causes disease in various fish species, such as sea bream (*Sparus aurata*) (Haldar et al., 2010), wedge sole (*Dicologoglossa cuneate*) (López et al. 2009), olive flounder (*Paralichthys olivaceus*), and Korean rockfish (Won and Park, 2008). *Vibro harveyi* was also observed to be more virulent in Korean rockfish than in other fish species (e.g., olive flounder) (Won and Park, 2008). Although the portal of entry of *V. harveyi* is not yet fully understood, the gastrointestinal tract may be a key route of invasion, as this pathogen has been isolated from several species with gastroenteritis (Yii et al., 1997; Soffientino et al., 1999; Lee et al, 2002). Notably, in this study, *V. harveyi* was isolated from three Pacific sand eels (frequency, 30%; 3/10) used as fish farm feed. It is presumed that, unlike the other bacterial species isolated in this study, natural infection by *V. harveyi* can occur in Pacific sand eel. Hence, use of the eel as a feed in aquaculture could be a source of disease. Although there is no direct evidence for the pathogenicity of

Table 2. Inhibitory zone diameters (mm) of *Vibrio harveyi* isolates obtained from pacific sand eel *Ammodytes personatus* and infected Korean rockfish *Sebastes schlegeli*

Isolate codes	Aminoglycoside	Antifolate	Macrolide	Penicols	Quinolones			Tetracycline
	CN	SXT	E	FFC	OA	NAL	ENR	OT
RFHW1ka	14	**6**	**6**	**13**	24	28	22	28
RFHW1kb	14	26	14	28	25	31	24	29
RFHW2k	15	**6**	**6**	31	26	30	23	30
RFHW3ka	14	**6**	**6**	**13**	24	29	24	29
RFHW3kb	10	28	14	33	24	30	26	31
AP1Lb	13	32	16	31	22	30	25	29
AP6L	15	28	11	32	23	27	22	30
AP9L	14	**6**	**6**	**15**	21	27	21	24

Abbreviations (also see Table 2): CN, gentamicin; SXT, trimethoprim/sulfamethoxazole; E, erythromycin; FFC, florfenicol; OA, oxolinic acid; NAL, nalidixic acid; ENR, enrofloxacin; OT, oxytetracycline.
Numbers in bold type indicate non-wild type.

Table 3. Aquatic pathogens found in baitfish or low-value fish used as aquaculture feed

Species of low-value fish or baitfish	Storage condition	Pathogens found	References
Common shiners (*Notropis cornutus*)	Live	*Aeromonas salmonicida*	Ostland et al., 1987
White suckers (*Catostomus commersoni*)			
Creek chub (*Semotilus atromaculatus*)			
Golden shiners (*Notermogonus crysoleucas*)			
Shrimp (8 different kinds)[1]	Frozen	*Vibrio* spp.[2] and *Listeria monocytogenes*	Rashid et al., 1992
Trash fish and shrimp[1]	Frozen	*Streptococcus iniae*, *S. parauberis* and iridovirus	Kim et al., 2007
Japanese jack mackerel (*Trachurus japonicas*)	Frozen	Betanodavirus (viral nervous necrosis virus (VNNV))	Gomez et al., 2010
Japanese anchovy (*Engraulis japonicius*)			
Japanese common squid (*Todarodes pacificus*)			
Big head croaker (*Collichthys niveatus*)	Frozen	*A. salmonocida*	Kim et al., 2013
Japanese anchovy (*Engraulis japonicas*)			
Konoshiro gizzard shad (*Konosirus punctatus*)			

[1]There was no specified species.
[2]Vibrio spp. include *V. parahaemolyticus*, *V. mimicus*, *V. alginolyticus*, *V. vulnificus*, and *V. fluvialis*

the three isolates (AP1Lb, AP6L, and AP9L) to rockfish, they were phenotypically very similar to the rockfish isolates. Nevertheless, these results may not strongly support the supposition that low-value fish was the direct source of vibriosis in this study. However, the use of low-value fish contaminated with or infected by pathogens as feed in fish farms should be avoided due to biosecurity concerns.

In general, the use of baitfish and low-value fish for feed raises the possibility of disease transmission (Gill, 2000; Hardy, 2004; FAO, 2011). Several studies have shown that baitfish or low-value fish pose significant risks for the spread of diseases, as summarized in Table 3 (e.g., Ostland et al., 1987; Rashid et al., 1992; Kim et al., 2007; Gomez et al., 2010; Kim et al., 2013). Low-value fish are often used in close proximity to their source; however, larger numbers of low-value fish captured from the wild are moved and released into new regions far from their origins (Goodwin et al. 2004). Indeed, frozen low-value fish are of particular concern, given evidence that freezing is insufficient to inactivate all of the viruses and bacteria present in infected fish (Arkush et al. 1998; Hervé-Claude et al. 2008; Kim et al., 2013). Thus, as described previously by Hyatt et al. (1997) and Ward et al. (2001), imported frozen whole feed fish could play an important role as a vector for the introduction and spread of exotic diseases. In light of this, many countries have restricted the use of low-value fish in fish farming activities. For example, the use of low-value fish is prohibited in Denmark, and fish farms have been forced to switch to formulated feeds. Re-feeding of salmon to the same species (intra-species recycling) is also banned by law in Norway and Chile (FAO, 2006). Finfish aquaculture activities in Korea still depend too much on low-value fish feed, so serious biosecurity concerns continue to exist in the country. Therefore, the introduction of new regulations to ban the use of whole low-value fish (containing internal organs) on Korean fish farms may be needed in the near future to ensure the sustainability of aquaculture, as well as protect the natural environment. Furthermore, as low-value fish or baitfish imported into Korea are not a designated quarantine item under current Korean law, legislation to require inspection of the imported fish to be used as aquaculture feed by a competent authority should be urgently introduced to minimize the occurrence of exotic diseases.

Acknowledgments

References

Arkush KD, Frasca S and Hedrick RP. 1998. Pathology associated with the Rosette Agent, a systemic protest infecting salmonid fishes. J Aquat Anim Health 10, 1-11.

Austin B and Zhang XH. 2006. *Vibrio harveyi*: a significant pathogen of marine vertebrates and invertebrates. Lett Appl Microbiol 43, 119-124.

Bowman J. 2006. The genus *Psychrobacter*. Prokaryotes 6, 920-930.

CLSI. 2006. Methods for antimicrobial disk susceptibility testing of bacteria isolated from aquatic animals. Approved guideline M42-A.

Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Kulam-Syed-Mohideen AS, McGarrell DM, Marsh T, Garrity GM and Tiedje JM. 2009. The ribosomal database project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res 37, D141-D145.

FAO. 2006. Use of fishery resources as feed inputs to aquaculture development: trends and policy implications. FAO Fisheries Circular, no. 1018, 114.

FAO. 2011. Aquaculture development. 5. Use of wild fish as feed in aquaculture. FAO technical guidelines for responsible fisheries no. 5, supplement 5, 94.

FAO. 2012. The state of world fisheries and aquaculture 2012. FAO, 209.

Funge-Smith S, Lindebo E and Staples D. 2005. Asian fisheries today: The production and use of low value/trash fish from marine fisheries in the Asia-Pacific region. Asia-pacific fishery commission RAP PUBLICATION 2005/16 (Bangkok, 1pp) (ftp://ftp.fao.org/docrep/fao/008/ae934e/ae934e00.pdf).

Gennari M, Tomaselli S and Cotrona V. 1999. The microflora of fresh and spoiled sardines, *Sardina pilchardus*, caught in Adriatic (Mediterranean) Sea and stored in ice. Food Microbiol 16, 15-28.

Gill TA. 2000.Waste from processing aquatic animals and animal products: implications on aquatic pathogen transfer. FAO Fisheries Circular, no. 956, 26.

Gomez DK, Mori KI, Okinaka Y, Nakai T and Park SC. 2010. Trash fish can be a source of betanodaviruses for cultured marine fish. Aquaculture 302, 158-163.

Gonzalez CJ, Santos JA, Garcia-Lopez ML and Otero A. 2000. Psychrobacters and related bacteria in freshwater fish. J Food Protect 63, 315-321.

Goodwin AE, Peterson JE, Meyers TR and Money DJ. 2004. Transmission of exotic fish viruses: The relative risks of wild and cultured bait. Fisheries 29, 19–23.

Haldar S, Maharajan A, Chatterjee S, Hunter SA and Chowdhury N. 2010. Identification of Vibrio harveyi as a causative bacterium for a tail rot disease of sea bream *Sparus aurata* from research hatchery in Malta. Microbiol Res 165, 639-648.

Hardy RW. 2004. Problems and opportunities in fish feeds – fisheries processing byproducts. International Aquafeeds 7, 33-34.

Hervé-Claude LP, Carpenter TE and Hedrick RP. 2008. Risk of introducing viral hemorrhagic septicemia virus (VHSV) to the Chilean South Pacific via sardine imports from Europe. Dis Aquat Org 78, 199-207.

Hisar O, Yanik T and Hisar SA. 2002. Clinical and pathological investigation of *Psychrobacter immobilis* infection in rainbow trout (*Oncrohynchus mykiss*, Walbaum). Isr J Aquacult-Bamid 54, 189-196.

Hyatt AD, Hine PM, Jones JB, Whittington RJ, Kearns C, Wise TG,

Crane MS and Williams LM. 1997. Epizootic mortality in the pilchard *Sardinops sagax neopilchardus* in Australia and New Zealand in 1995. II. Identification of a herpesvirus within the gill epithelium. Dis Aquat Organ 28, 17-29.

Kämpfer P, Albercht A, Buczolits S, Busse HJ. 2002. *Psychrobacter faecalis* sp. nov., a new species from a bioaerosol originating from pigeon faeces. Syst Appl Microbiol 25, 31-36.

Kim DH, Choi SY, Kim CS, Oh MJ and Jeong HD. 2013. Low-value fish used as feed in aquaculture were a source of furunculosis caused by atypical *Aeromonas salmonicida*. Aquaculture 408-409, 113-117.

Kim DH and Kim DY. 2013. Microbial diversity in the intestine of olive flounder (*Paralichthys olivaceus*). Aquaculture 414-415, 103-108

Kim JH, Gomez DK, Choresca CH and Park SC. 2007. Detection of major bacterial and viral pathogens in trash fish used to feed cultured flounder in Korea. Aquaculture 272, 105-110.

Lee KK and Yii KC. 1996. A comparison of three methods for assaying hydrophobicity of pathogenic Vibrios. Lett Appl Microbiol 23, 343-346.

Lee KK, Liu PC and Chuang WH. 2002. Pathogenesis of gastroenteritis caused by *Vibrio carchariae* in cultured marine fish. Mar Biotechnol 4, 267-277.

López JR, Roca E de la, Núñez S, Herran R de la, Navas JI and Manchado M. 2009. Identification of Vibrio harveyi isolated from diseased cultured wedge sole Dicologoglossa cuneate. Dis Aquat Organ 84, 209-217

Ostland AE, Hicks BD and Daly FG. 1987. Furunculosis in baitfish and its transmission to salmonids. Dis Aquat Organ 2, 163-166.

Pang L, Zhang XH, Zhong Y, Chen J, Li Y and Austin B. 2006. Identification of *Vibrio harveyi* using PCR amplification of the toxR gene. Lett Appl Microbiol 43, 249-255.

Rashid HO, Ito H and Ishigaki I. 1992. Distribution of pathogenic vibrios and other bacteria in imported frozen shrimps and their decontamination by gamma-irradiation. World J Microb Biot 8, 494-499.

Ringø E, Sperstad S, Kraugerud OF and Krogdahl A. 2008. Use of 16S rRNA gene sequencing analysis to characterize culturable intestinal bacteria in Atlantic salmon (*Salmo salar*) fed diets with cellulose or non-starch polysaccharides from soy. Aquaculture Res 39, 1087-1100.

Saitou N and Nei M. 1987. The neighbor-joining method — a new method for reconstructing phylogenetic trees. Mol Biol Evol 4, 406-425.

Soffientino B, Gwaltney T, Nelson DR, Specker JL, Mauel M and Gomez-Chiarri M. 1999. Infectious necrotizing enteritis and mortality caused by *Vibrio carchariae* in summer flounder Paralichthys dentatus during intensive culture. Dis Aquat Organ 38, 201-210.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M and Kumar S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28, 2731-2739.

Ward TM, Hoedt F, McLeay L, Dimmlich WF, Kinloch M, Jackson G, McGarvey R, Rogers PJ and Jones K. 2001. Effects of the 1995 and 1998 mass mortality events on the spawning biomass of sardine, *Sardinops sagax*, in South Australian waters. ICES J Mar Sci 58, 865-875.

Weisburg WG, Barns SM, Pelletier DA and Lane DJ. 1991. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173, 697-703.

Won KM and Park SI. 2008. Pathogenicity of *Vibrio harveyi* to cultured marine fishes in Korea. Aquaculture 285, 8-13.

Won KM, Choi JH, Kim YC and Park SI. 2007. Microbiological characteristics of *Vibrio harveyi*. J Fish Pathol 20, 237-247.

Yii KC, Yang TI and Lee KK. 1997. Isolation and characterization of *Vibrio carchariae*, a causative agent of gastroenteritis in the groupers, *Epinephelus coioides*. Curr Microbiol 35, 109-115.