Fish as Hosts of *Vibrio cholerae*

Malka Halpern¹,²* and Ido Izhaki²

¹ Department of Biology and Environment, Faculty of Natural Sciences, University of Haifa, Tivon, Israel, ² Department of Evolutionary and Environmental Biology, Faculty of Natural Sciences, University of Haifa, Haifa, Israel

*Vibrio cholerae*, the causative agent of pandemic cholera, is abundant in marine and freshwater environments. Copepods and chironomids are natural reservoirs of this species. However, the ways *V. cholerae* is globally disseminated are as yet unknown. Here we review the scientific literature that provides evidence for the possibility that some fish species may be reservoirs and vectors of *V. cholerae*. So far, *V. cholerae* has been isolated from 30 fish species (22 freshwater; 9 marine). *V. cholerae* O1 was reported in a few cases. In most cases *V. cholerae* was isolated from fish intestines, but it has also been detected in gills, skin, kidney, liver and brain tissue. In most cases the fish were healthy but in some, they were diseased. Nevertheless, Koch postulates were not applied to prove that *V. cholerae* and not another agent was the cause of the disease in the fish. Evidence from the literature correlates raw fish consumption or fish handling to a few cholera cases or cholera epidemics. Thus, we can conclude that *V. cholerae* inhabits some marine and freshwater fish species. It is possible that fish may protect the bacteria in unfavorable habitats while the bacteria may assist the fish to digest its food. Also, fish may disseminate the bacteria in the aquatic environment and may transfer it to waterbirds that consume them. Thus, fish are reservoirs of *V. cholerae* and may play a role in its global dissemination.

**Keywords: fish, *Vibrio cholerae*, waterbird, bacteria–fish interactions, reservoir, vector**

**INTRODUCTION**

The devastating disease, cholera, is known to occur globally causing epidemics and pandemics. However, the way this disease is worldwide disseminated is still unknown. *Vibrio cholerae*, the causing agent of cholera is ubiquitous in marine and freshwater aquatic environments. Copepods (*Crustaceaen*) (Colwell and Huq, 2001) and chironomids (*Diptera; Chironomidae*) (Broza and Halpern, 2001; Halpern et al., 2004, 2006, 2007; Senderovich et al., 2008; Halpern and Senderovich, 2015) were described as natural reservoirs of *V. cholerae*. Copepods and chironomids are abundant in fresh and marine water ecosystems and are consumed by different fish species. Halpern et al. (2008) raised the hypothesis that fish that feed on copepods and chironomids, and waterbirds that also may feed on these invertebrates and consume fish as well, may be reservoirs and vectors of *V. cholerae*. Here we review the scientific literature that indicates that fish are indeed significant reservoirs of *V. cholerae* in water ecosystems.

**Vibrio cholerae**

*V. cholerae*, a Gram-negative motile rod causes massive cholera outbreaks such as the one following the 2010 earthquake in Haiti (Sack et al., 2004; Chin et al., 2011; Katz et al., 2013). Cholera is a
global threat to public health and it was estimated that between 2008 and 2012 cholera caused an annual average of 2.9 million cases, and 95,000 deaths, worldwide (Ali et al., 2015). Particular serogroups (O1 and O139) of this bacterium are responsible for cholera epidemics and pandemics. Human infection with \(V. \text{cholerae}\) begins with ingestion of contaminated food or water containing the bacterium. \(V. \text{cholerae}\) colonizes the small intestine and secretes cholera enterotoxin (CT) into the host cells resulting in rapid efflux of chloride ions and water into the lumen of the intestine, leading to profuse diarrhea and severe dehydration (Kaper et al., 1995).

Non-O1/non-O139 \(V. \text{cholerae}\) serogroups are also linked to \(V. \text{cholerae}\) gastroenteritis as well as to wound infections and bacteremia (Deshayes et al., 2015). \(V. \text{cholerae}\) O1, O139 and non-O1/O139 comprise a single taxonomic species and their habitats attributes are similar (Lewin, 1996), however, recently it has been suggested that not all strains of \(V. \text{cholerae}\) species share the same niche (Kirchberger et al., 2016). The role of CT in the environment is not understood.

\(V. \text{cholerae}\) is commonly associated with chitin-containing zooplankton, particularly copepods (Huq et al., 1983) and chironomids (Broza and Halpern, 2001; Halpern et al., 2004). Recent evidence supports the hypothesis that fish and waterbirds may also be intermediate reservoirs and vectors of \(V. \text{cholerae}\) (Halpern et al., 2008; Halpern and Izhaki, 2010).

Fish as Possible Reservoirs of \(V. \text{cholerae}\)

**V. cholerae** O1 and O139 Serogroups in Fish

In a laboratory experiment that was conducted more than 50 years ago, Felsenfeld (1963), infected sardines (Stolephorus) and mullets (Liza) with pathogenic \(V. \text{cholerae}\) O1 strains (Ogawa and Inaba). *Vibrio* concentration in the water was 10^2 cells/ml. The strains were detected in the fish intestine after the fish were exposed to the bacteria (Table 1). In another laboratory experiment, Runft et al. (2014) used *V. cholerae* O1 strains to colonize zebrafish gut. They found that the bacteria attached to the fish intestinal epithelium and formed micro-colonies. They suggested that zebrafish can act as a host model for pathogenic *V. cholerae* strains (Rowe et al., 2014; Runft et al., 2014) (Table 1). Evidence for the presence of pathogenic serogroups of *V. cholerae* in fish was published by du Preez et al. (2010) who detected large numbers of *V. cholerae* O1 and O139 in fish scale samples collected in Mozambique. These researchers obtained their evidence by a direct fluorescent antibody technique. *V. cholerae* O1, positive for cholera toxin gene, was isolated from Tilapia gills in Tanzania (Hounmanou, 2015). *V. cholerae* O1 isolates, positive to ctxA and tcpA genes were detected from two marine fish in Cochin, India (no details were given as to the fish species) (Kumar and Lalitha, 2013). In the same study, Kumar and Lalitha (2013) also identified 141 non-O1/O139 isolates from unidentified marine fish species (Table 1).

**V. cholerae** Non-O1/O139 in Fish

Carvajal et al. (1988) identified *V. cholerae* non-O1/O139 serogroups in healthy Lorna fish (Sciaena deliciosa) sampled from inshore marine sites during a Peruvian cholera epidemic (Table 1). Senderovich et al. (2010) examined freshwater and marine fish species. Ten freshwater (71%) and one marine (2.3%) fish species tested positive for the presence of *V. cholerae* non-O1/O139 in their intestine (Table 1). *V. cholerae* non-O1/O139 was also detected in four fish species collected from the Fowl River in the Gulf of Mexico (Jones et al., 2013) (Table 1). The prevalence of *V. cholerae* isolates in Tilapia (Oreochromis niloticus) intestines, sampled from a water reservoir in Ouagadougou, Burkina Faso in Africa, was 6.3% (Traoré et al., 2014) (Table 1). In Qingdao in China, *V. cholerae* was detected by means of metagenomic tools in the gastrointestinal tract of a farmed adult turbot fish (Scophthalmus maximus) (Xing et al., 2013). In India, *V. cholerae* was isolated from two fish species (Bulls eye, Priacanthus hamrur and Hard tail scad, Megalaspis cordyla) caught off Royapuram coast (Sujatha et al., 2011) (Table 1). When the microbial quality and safety of Pangasius fish processed for export in Vietnam was evaluated, *V. cholerae* was isolated from tra fish (Pangasius hypophthalmus) fillets and from the water used to rinse them (Thi et al., 2014) (Table 1).

*V. cholerae* Isolated from Diseased Fish

A few studies have reported the isolation of *V. cholerae* non-O1/O139 from diseased fish. *V. cholerae* was isolated from internal organs of diseased ayu (Plecoglossus altivelis) and guppy fish (Poecilia reticulate) in Japan and Iran, respectively (Yamanoi et al., 1980; Kiyukia et al., 1992; Kiani et al., 2016) and from Nile tilapia (Oreochromis niloticus) that were cultured in floating cages in Thailand (Dong et al., 2015) (Table 1). Rehulka et al. (2015) demonstrated that an intraperitoneal injection of *V. cholerae* into common carp, rainbow trout and common nase caused the death of the injected fish (Table 1).

Indirect Evidence on the Possible Presence of *V. cholerae* in Fish

According to the Hong Kong Food and Environmental Hygiene Department, *V. cholerae* serotype Ogawa biotype El Tor was found in a supermarket fish tank water in Pok Fu Lam in September 2003 (Press Release, 2003). They were not able to explain the source of the bacteria. *V. cholerae* O1 was detected from aquarium water and fish imported from Thailand and Sri Lanka to Czech Republic (Plesník and Procházková, 2006). Using molecular methods, Smith et al. (2012), identified *V. cholerae* from aquarium water containing common goldfish (Carassius auratus) purchased from aquarium shops in Rhode Island (Table 1). When the bacterial community of zebrafish intestinal tracts was studied using cloning of the 16S rRNA gene, *V. cholerae* was found as the dominant OUT in the Gammaproteobacteria class (Lan and Love, 2012).

Epidemiological Evidence of Fish Consumption as the Cause of Cholera

Evidence from the literature correlated fish with a few cholera outbreaks. The first records date back over more than 50 years. It was postulated that cholera endemicity in India was due to hilsa fish (Pandit and Hora, 1951) (Table 2). Morgan et al. (1960), suggested that the origin of El Tor vibrios outbreaks in Thailand might have been fish that are often eaten raw in...
Table 1: Isolation of *V. cholerae* strains from healthy fish species that were sampled from different habitats and regions around the world.

| Fish species | Habitat | Site of isolation | Isolated from fish organ/comments | References |
|--------------|---------|-------------------|----------------------------------|------------|
| *V. cholerae* O1 | | | | |
| Sardines (Stolephorus sp.) | Intestine colonization lab experiment | Medical Research Laboratory, Bangkok, Thailand | Intestine, survival in the intestine lasted only 5 days | Felsenfeld, 1963 |
| Mullet (Liza sp.) | Intestine colonization lab experiment | Medical Research Laboratory, Bangkok, Thailand | Intestine, O1 survival in the intestine lasted only 5 days | Felsenfeld, 1963 |
| Unidentified sea fish, Beira beach | | The Pungwe estuary at Beira, Mozambique | *V. cholerae* O1/O139 were detected on fish scale using direct fluorescent antibody | du Preez et al., 2010 |
| Unidentified sea fish, Cochin | Marin environment | Cochin, India | *V. cholerae* O1, and non O1 | Kumar and Laitila, 2013 |
| Zebrafish (Danio rerio) | Intestine colonization lab experiment (demonstrating a host model) | Wayne State University IACUC, Michigan, USA | O1, Intestine, micro-colonies were observed on the intestinal epithelium | Runft et al., 2014 |
| Tilapia sp. Mzumbe sewage stabilization ponds | Morogoro, Tanzania | | *V. cholerae* O1, and non-O1 from gills | Hounmanou, 2015 |

| *V. cholerae* NON O1/O139 | | | | |
| Iorna fish (Sciaena delicosa) | Marine inshore waters | Peru | Information not available | Carvajal et al., 1988 |
| Tilapia sp. | Fish pond | Haifa and Nir David, Israel | Intestine | Halpern et al., 2008 |
| Common St. Peter's fish (Tilapia sp. and Tilapia zillii) | Fish pond | Nahalat, Israel | Intestine | Senderovich et al., 2010 |
| Josephus chichlid (Astatotilapia flavijosephi) | Fish pond | Nr David, Israel | Intestine | Senderovich et al., 2010 |
| Grass carp, white-amur (Ctenopharyngodon idella) | Fish pond | Atlit, Israel | Intestine | Senderovich et al., 2010 |
| Common carp (Cyprinus carpio) | Fish pond | Atlit, Israel | Intestine | Senderovich et al., 2010 |
| Flathead gray mullet (Mugil cephalus) | Fish pond | Nahalat, Israel | Intestine | Senderovich et al., 2010 |
| Gaielle St. Peter's fish (Gasterosteus gauvella) | Fish pond | Kfar Rupin, Israel | Intestine | Senderovich et al., 2010 |
| Jordan St. Peter's fish (Oreochromis aureus) | River | Nr David, Israel | Intestine | Senderovich et al., 2010 |
| Carasobarbus canis | Lake | The Sea of Gaielle, Israel | Intestine | Senderovich et al., 2010 |
| Longhead barbel (Barbus longiceps) | Lake | The Sea of Gaielle, Israel | Intestine | Senderovich et al., 2010 |
| Flathead gray mullet (Mugil cephalus) | Lake | The Sea of Gaielle, Israel | Intestine | Senderovich et al., 2010 |
| Biocichyay soldierfish (Myripristis murdjan) | Mediterranean Sea (Marine water) | Aiko, Israel | Intestine | Senderovich et al., 2010 |
| Bulls eye (Priacanthus hamrur) | Royapuran coast (Marine water) | Chennai, Tamil Nadu, India | Intestine and the muscles | Sujatha et al., 2011 |
| Hard tail scad (Megalaasps cordyla) | Royapuran coast (Marine water) | Chennai, Tamil Nadu, India | Gills, intestine, muscles and skin | Sujatha et al., 2011 |
| Zebrafish (Danio rerio) | Adult zebrafish cultured in tanks | Auckland, New Zealand | *V. cholerae* was detected in intestine samples using cloning of 16S rRNA gene | Lan and Love, 2012 |
| Turbot fish (Scophthalmus maximus) | Marine aquaculture | Qingdao, China | *V. cholerae* was detected by metagenomic tools | Xing et al., 2013 |
| Sheephead (Archosargus probatocephalus) | Fowl River (estuarine) | Gulf of Mexico | Intestine | Jones et al., 2013 |
| Sea catfish (Arius fels) | Fowl River (estuarine) | Gulf of Mexico | Intestine | Jones et al., 2013 |
| Pin fish (Lagodon rhomboides) | Fowl River (estuarine) | Gulf of Mexico | Intestine | Jones et al., 2013 |
| Crevale jack (Caranx hippos) | Fowl River (estuarine) | Gulf of Mexico | Intestine | Jones et al., 2013 |
| Frozen tra fish (Pangasius hypophthalmus) fillet | Food industry | Vietnam | Final packaged products (fillets) | Thi et al., 2014 |
| Tilapia (Oreochromis niloticus) | Tanghin freshwater reservoir | Ouagadougou, Burkina Faso (Africa) | 6.3% (15 out of 238) | Traoré et al., 2014 |

(Continued)
Table 1 | Continued

| Fish species | Habitat | Site of isolation | Isolated from fish organ/comments | References |
|--------------|---------|------------------|-----------------------------------|------------|
| Unknown tropical fish | Water from Fish tank | UK | Reported to be the cause of a wound | Booth et al., 1990 |
| Common goldfish (Carassius auratus) | Aquarium water | Rhode Island | Indication using molecular methods | Smith et al., 2012 |

V. cholerae ISOLATED FROM DISEASED FISH

| Fish species (order) | Habitat | Site of isolation | Isolated from fish organ/comments | References |
|----------------------|---------|------------------|-----------------------------------|------------|
| Ayu fish (Plecoglossus altivelis) and Guppy Fish (Poecilia reticulata) | River | Japan | Livers, spleens, or kidneys of diseased fish | Yamanoi et al., 1980; Kiiyukia et al., 1992 |
| Goldfish (Carassius auratus) | No data available | No data available | No data available | Reddacliff et al., 1993 |
| Nile tilapia (Oreochromis niloticus) | Floating cage cultured Nile tilapia farms | Mekong River, Thailand | V. cholerae from internal organs of diseased fish | Dong et al., 2015 |
| Guppy Fish (Poecilia reticulata) | Aquaculture ponds | Kasha, Iran | Skin, gill, kidney and brain tissue from diseased fish | Kiani et al., 2016 |
| Cardinal tetra (Paracheirodon axelrodi) | Fish aquarium | Czech Republic | Diseased fish | Rehulka et al., 2015 |
| Raphael catfish (Platydoras costatus) | Fish aquarium | Czech Republic | Diseased fish | Rehulka et al., 2015 |
| Common nase (Chondrostoma nasus) | Fish aquarium | Czech Republic | Diseased fish | Rehulka et al., 2015 |

the Pacific area. Cholera was associated with eating salted fish, sardines and other fish from an atoll lagoon in the Pacific Ocean (Merson et al., 1977; Kuberski et al., 1979; McIntyre et al., 1979). A cholera outbreak in Tanzania (67 patients, including 11 deaths) was correlated with handling and eating fish at social gatherings (Killewo et al., 1989). Out of 12 cholera cases caused by V. cholerae O1, serotype Ogawa, biotype El Tor, in the southern Italian region of Puglia, in 1994, three patients reported consumption of raw fish (Maggi et al., 1997) (Table 2). Consumption of dried fish correlated significantly with cholera risk in Tanzania (Acosta et al., 2001). In July 2001, a case of cholera, caused by V. cholerae O1, serovar Inaba, biovar El Tor, was reported in Berlin. Interestingly, the patient had most likely been infected while handling and preparing fish imported from Nigeria (Schürmann et al., 2002). A food traceback investigation following three cases of cholera in Sydney, Australia, found that the only exposure common to all cases was consumption of raw whitebait imported from Indonesia (Forsman et al., 2007). V. cholerae O1 serovar Ogawa was identified as the causative agent in all three cases. V. cholerae non-O1 was isolated from stools of a fisherman who had fished, cooked and eaten a lake fish in Italy (Piantieri et al., 1982). The source of Vibrio cholerae non-O1 that was found in a wound, was linked with a tropical fish tank (Booth et al., 1990) (Table 2).

V. cholerae and Fish—Mutualistic Interactions?

A few publications (mentioned above and in Table 1), correlated the presence of V. cholerae with a disease in fish (Yamanoi et al., 1980; Kiiyukia et al., 1992; Dong et al., 2015; Rehulka et al., 2015; Kiani et al., 2016). Kiiyukia et al. (1992), who isolated V. cholerae non-O1/O139 from diseased ayu fish in Japan, emphasized that healthy ayu fish caught in Lake Biwa, Japan in the rivers running into this lake, also harbored V. cholerae but without showing any signs of a disease. Kiani et al. (2016) isolated V. cholerae along with other pathogens from diseased Nile tilapia that were cultured in floating cages in Thailand. However, it was not proven that V. cholerae was indeed the causative agent of the disease (Table 1). All the above studies simply assumed that because they isolated V. cholerae from the diseased fish, this species was responsible for the disease. Rehulka et al. (2015) injected a fish with relatively large dose of bacteria (e.g., 2 × 10⁸ cells) to obtain fish mortality but without following all Koch postulates rules. Hence we argue that at least for some cases other bacterial species or viruses and not V. cholerae were probably responsible for the fish disease (Table 1).

Senderovich et al. (2010) isolated V. cholerae from 15 different healthy fish species. They found 5 × 10³ and 1.4 × 10⁴ colony forming units (cfu) of V. cholerae per gr intestine content in Sarotherodon galilaeus (Galilee St. Peter's fish) and in Mugil cephalus (Flathead gray mullet), respectively. None of these fish showed any signs of disease. Nevertheless, there is a scarcity of quantitative studies of V. cholerae in fish. Many other studies reported the presence of V. cholerae in different healthy fish species that were sampled from both marine and freshwater habitats (listed in Table 1) but these studies did not quantify the numbers of V. cholerae in the fish.

Not all the fish species are inhabited by V. cholerae. For example, Jones et al. (2013) detected V. cholerae only in 4 out of 10 fish species sampled in the Gulf of Mexico (estuarine habitat). Similarly, Senderovich et al. (2010) did not detect V. cholerae in 4 out of 14 freshwater and in 43 out of 44 marine fish species. Scrutiny of the list of the fish species found to host V. cholerae revealed that all belonged to Actinopterygii class (Table S1). V. cholerae was identified from 30 species belonging to 9 different orders within this class.
Fish may actually benefit from *V. cholerae* that inhabit their intestine. Strains of *V. cholerae* secrete extracellular enzymes such as proteases (Halpern et al., 2003) and chitinases (Pruzzo et al., 2008; Senderovich et al., 2010). These enzymes may have a role in the digestion of macromolecules like proteins and chitin in the fish gut. Chitin, a polymer of β-1,4-N-acetylglucosamine, is the main component of crustaceans' (copepods) and insects' (chironomids) exoskeletons. This insoluble polymer is a source of carbon and nitrogen (Cohen-Kupiec and Chet, 1998; Laviad et al., 2016). Senderovich et al. (2010) found that all *V. cholerae* strains isolated from 15 different fish species were able to degrade chitin. Thus, it is possible that the fish intestine serves as hosts for *V. cholerae* while the bacteria may play a role in helping the fish digest its chitinous zooplankton prey. As the fish that carry the bacteria swim from one location to another (some fish species move from rivers to lakes or sea and the reverse), they serve as vectors for *V. cholerae*. Nevertheless, fish are consumed by waterfowls, which disseminate the bacteria on a global scale (Halpern et al., 2008; Halpern and Izhaki, 2010).

From an epidemiological point of view, the fish carry the cholera bacteria from one place to another. So eventually, if waterbirds feed on the fish, *V. cholerae* may be transferred in some waterbird species' digestive tracts and thus be globally spread.

**UNRESOLVED QUESTIONS AND FUTURE RESEARCH**

1. Copepods and chironomids are natural reservoirs of *V. cholerae*. Do fish that feed on these zooplankton species get infected with *V. cholerae*?
2. Is *V. cholerae* transferred vertically or horizontally among fishes? Does an infected female transfer *V. cholerae* to her offspring?
3. Can the bacteria be transferred from one fish species' droppings to another fish species that lives in the same habitat?
4. When the fish intestine becomes infected with *V. cholerae*, does the bacteria become part of its normal microbiota?
5. What are the differences between fish species that carry *V. cholerae* in fresh and marine waters?
6. Does *V. cholerae* prevalence in fish vary by season? Or by different fish age and gender?
7. Can we determine a model fish species that carries *V. cholerae* as against those fish species that do not?

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