

Listonella has been determined Chemically in Natural Fish from the Ocean

Dr. Faiz Alam *

Fisheries Department, Southwest Area
Integrated Water Resources Planning
and Management Project, India

Corresponding author: Dr. Faiz Alam

✉ alamfaij77@edu.co

Fisheries Department, Southwest Area
Integrated Water Resources Planning and
Management Project, India

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Abstract

Infectious diseases caused by marine bacterial pathogens are increasingly causing economic losses to fisheries and aquaculture, while posing increasing risks to public health and the protection of affected species. Four wild marine fish species were collected from five fishing grounds and analyzed for listnera in two regions: northern (Acre, Haifa and Shefaim) and central and southern (Tel Aviv and Ashdod). Initial screening was performed using PCR analysis with specially designed primers on DNA extracted from liver and kidney. For *P. damselae*-positive specimens, 16S rRNA amplicons were sequenced. An attempt was then made to determine related subspecies using a three-step genetic screen auto, ureC and toxR 20 of which were classified as *P. damselae* subsp. piscicida (Phdp), two as *P. Damsel* subspecies. (Phdd) and 25 could not be assigned to any subspecies. Our results reinforce the idea that fish living in contaminated environments are susceptible to pathogenic microbes. To assess how pathogen presence affects population management and conservation, this study should be followed by studies aimed at: Establish a standardized contamination index as a basis for (i) quantifying the levels of contaminants that can affect pathogen outbreaks and (ii) establishing criteria upon which authorities will take preventive action.

Keywords: Wild fish pathogens; Listonella; Diseases; Mediterranean sea

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Introduction

Fish are carriers of many pathogens, including viruses, bacteria, parasites and fungi, some of which are potentially zoonotic. The studies done have focused mainly on farmed fish and to a lesser extent on wild populations. This also applies to the Levant Basin in the Mediterranean Sea. The survey is part of a long-term monitoring program in the Mediterranean conducted by the University of Haifa, Israel. The program aims to advance ecological research, assess the health of marine ecosystems, and create a database to identify key changes indicative of degradation and degradation of biological systems, their diversity and functions. was set as Samples were collected from two geographical regions in Israel [1].

North (Acre, Haifa, Shefaim) and Central South (Tel Aviv, Ashdod). From each region he sampled four different fish species [2].

Round sardine (*Sardinella aurita*), brushtooth lizardfish (*Saurida undosquamis*), striped mullet (*Mullus surmuletus*) and gold-

banded mullet (*Upeneus moluccensis*). These were selected for their ecological importance and the fact that they are widely available and consumed by humans. It is part of a broader effort to create a database of the prevalence of marine fish pathogens along the Mediterranean coast. Other research conducted under this program has focused on Nervous Necrosis Virus (NNV) and *Streptococcus*. - especially *p. iniae* *Vibrio* spp. and *Mycobacterium* spp. and *S. iniae*, *V. harveyi* and *P. Maiden*. All three studies included kidney and liver sampling [3].

This study focuses on her two potentially pathogenic marine bacteria.

listella and rickettsia-like organisms (RLO). These putative pathogens have been identified in fish in other studies around the world and are known to be a significant and widespread cause of disease and death in marine animals and humans [4].

P. Damsel subspecies. *Damselae* (Phdd) and *P. damselae* subsp.

piscicida (Phdp). Phdd is a common pathogen in a wide variety of marine animals, including many species of fish, crustaceans, mollusks, marine reptiles, elasmobranchs, and cetaceans [5]. In humans, it can cause opportunistic infections; contribute to necrotizing fasciitis and, in extreme cases, death. Strains of this pathogen have been isolated from seawater, algae, marine organisms without clinical signs, and seafood, and are thought to be abundant in the gut of certain shark species. Marine animal infections can be transmitted through water and are affected by temperature and salinity. Symptoms are hemorrhages and sores around the skin, gills, pelvic fins, and caudal peduncle in certain fish species. Other species of fish show bleeding in the eyes, mouth and jaws. Human infections have been reported mainly from open wounds during work with fish or bodies of water containing fish, although rare reports of infection after eating raw fish and urinary infections after swimming in contaminated water have been reported, fish pseudotuberculosis. This is because when fish are chronically infected, there are nodules/whitish granulomas in some internal organs that contain bacterial build-up. The most important surrogate animal for this disease is saltwater fish. It has been isolated in numerous outbreaks in large areas around the world, including the United States, Japan, northern Europe, and coasts throughout the Mediterranean. However, reports in wild fish are relatively rare and recent, and the majority of reports over the years have been of disease outbreaks in edible fish species. Today, pasteurellosis is a major limiting factor in marine aquaculture, affecting flounder, trout, eels, sea bass, and more, with widespread economic impact worldwide due to the losses it causes [6]. The route of infection is still unknown, but water temperature (23°C or higher), salinity, and water quality seem to affect the epidemic in summer. Studies on the viability of this bacterium in seawater and marine sediments demonstrate both its short-term viability in culture far from fish and its ability to switch to vigor-maintaining survival modes. The aquatic environment can serve as a reservoir and source of potential infectious diseases. Several studies have shown high homology of the 16S rRNA gene between the two subspecies, but there is disagreement about the percentage of homology. Some have reported 100% homology, while others have found one or two nucleotide differences [7]. In one study, by combining 16S rRNA with primers specific for the ureC gene, he was able to distinguish between the two subspecies using a PCR amplification technique. The ureC gene, which enables Phdd to hydrolyze urea, is not present in her Phdp DNA, making it a marker that distinguishes her two subspecies of *P. damselae*. Another way to distinguish between the two subtypes involves virulence factors. The gene involved in its production, toxR, is considered a valuable phylogenetic marker in the study of *P. damselae* species. This is because the high divergence helps him distinguish between the Phdd and Phdp subspecies. There are Phdd-specific phenotypic features such as mobility on sheep blood agar, nitrate reduction, and hemolysis. Additionally, most Phdd strains can grow at 37 °C (the inhibition temperature of Phdp) [8].

Materials and Methods

Fish collection

Between August 2016 and December 2017, local fishermen used

nets or trawlers to catch the fish. I caught a fish in the following 5 fishing grounds.

Acre, Kishon estuary, Shefaim, Tel Aviv and Ashdod were purchased by traders in the associated fish markets. The fishing grounds were divided into northern (Acre, Kishon, Shefaim) and central and southern (Tel Aviv and Ashdod). All fish were inspected externally and appeared healthy. Fish were placed on ice immediately after purchase, transported to the Morris Kahn Marine Research Laboratory (MKMRS) laboratory, and frozen at -80 °C until preparation [9].

Sample collection

Fish were thawed and examined externally for fins, skin and gills, weighed and measured from nose to tip of tail, and then examined internally. All fish appeared generally healthy with no obvious internal or external signs of serious illness. Kidneys (n = 166) and livers (n = 168) were sampled during necropsy. I was. A total of 92 brushtooth lizards, *Saurida undosquamis* (liver = 47, kidney = 45), Shimabora, *Mullus surmuletus* (liver = 41, kidney = 40) 81, snapdragon, *Upeneus* 81 samples were collected. *Moluccensis* (liver = 39, kidney = 42) and 80 round sardines, *Sardinella aurita* (liver = 41, kidney = 39). Aiming for a similar number of samples per species from each region, we collected a total of 179 samples from the northern sites and 155 samples from the central south [10].

Results

No RLO positive results were found in any of the samples tested. *P. damselae* was found in 47 samples (overall prevalence of 14%). Further grouping of the positive samples into subspecies of *P.*

Discussion

The overall aim of this study was to investigate the prevalence of *P. damselae* along the Israeli coast and determine whether it correlated with different levels of contamination. Prevalence of *P. damselae* was found in all four wild fish species tested, albeit in varying proportions. Out of 334 samples, 47 positive samples of *P.* were observed. with an overall prevalence of 14%, results are comparable to estimates based on studies conducted in the Mediterranean and around the world. Of these 47 *P. damselae*-positive specimens, 29/47 (62%) became positive in summer and 18/47 (38%) in winter. This is somewhat consistent with data from other studies. Round sardines had the highest prevalence, at 16/80 (20%). This may be because, unlike other studied fish species, which live at depths of 30-300 m, this species is thermophilic and also lives close to shore. Possibly, the difference could be due to the species' high food plasticity or preference for conditions that are ideal physiological temperatures for diffusion of clear, saltwater *P. spp.*, It can also result from phenotypic differences between fish species in terms of susceptibility to pathogens. We also hypothesized that the high prevalence of this species may be related to the level of environmental contamination in that environment, as polluted environments are known to weaken the immune system of fish. If the source of the pollution is on land, it would be expected that fish living closer to the shore would be more affected, as the data in this study seem to suggest.

In this case, sardines can be considered bioindicators (sentinels) of coastal pollution. If confirmed, the occurrence of *P. damselae* in round Sardinella would allow the creation of a spatial map of contaminants, whether they were from land, deeper in the sea, and associated with fish. , may provide a tool for future research to help identify the origin of contaminants population and public health. Due to the importance of identifying subspecies of *P. damselae*, we used PCR primers from previous studies for tentative identification. We then used other primers to identify subspecies of samples that turned out to be *P. Damsel* positive. Apparently, an efficient PCR-based assay to discriminate between the two subspecies has not yet been developed (only 47% could discriminate), as shown in other studies. Perhaps the solution lies in more advanced molecular techniques such as amplified fragment length polymorphism (AFLP) and PCR described in

several studies, or in a combination of phenotypic traits and other classification methods based on bacterial culture. *Damselae* specimens assigned to subspecies were classified as *P. damselae*. *Damsel* subspecies *piscicida* is prominent.

Conclusions

The prevalence of two important fish pathogens in marine fish from the eastern Mediterranean. We showed that organisms like *Rickettsia* are unlikely to pose a risk to the health of fish in our region, but *Photobacterium* species were indeed present in the marine fish tested. Further research is needed to understand the impact of potential pathogens of common infectious diseases on local aquaculture and the magnitude of the threat to public health.

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