Molecular identification and characterization of *Vibrio* and *Mycobacterium* in wild and cultured marine finfish in Israel

Yael Regev

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTER'S DEGREE

University of Haifa

Faculty of Natural Sciences

Leon H. Charney School of Marine Science

The Department of Marine Biology

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(Chairperson of Master's studies committee)

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Table of Contents

Molecular identification and characterization of *Vibrio* and *Mycobacterium* in wild and cultured marine finfish in Israel

Yael Regev

Abstract

Wild and cultured marine fish may contain several species of pathogens, and the prevalence of different pathogens varies widely among different species and life history stages. Although aquaculture in the Mediterranean is a relatively young industry, finfish diseases have been reported to cause considerable problems and mortalities among the farmed stocks. In general, the farming activity and the open design of Mediterranean aquaculture systems allow the transmission of infectious pathogens inter- and intra-farm facilities. A wide range of marine pathogens from aquaculture is well documented, but there is a lack of baseline data and information regarding pathogenic agents' prevalence in wild fish population.

This study focuses on *Mycobacterium* and *Vibrio*, both of which are known to be major causes of fish loss, occasionally to the extent of being the limiting factor. Mycobacteriosis, caused by Non-Tuberculous Mycobacteria (NTM), is among the most chronic diseases within aquatic animals. In addition, fish mycobacteriosis has substantial economic consequences in the aquaculture and fisheries industry, as infections may significantly decrease production and trade. Some fish NTM pathogens are highly virulent and zoonotic. Another pathogen to be examined is *Vibrio*, a gram-negative curved rod which occurs naturally in marine, estuarine, and freshwater systems worldwide. They occupy habitats ranging from deep sea to shallow aquatic environments. Some species include human and animal pathogens capable of causing gastroenteritis, wound infections, cholera, and fatal septicemia. The disease is known to have increased death rates (> 50%) in fish farms soon after an outbreak took place.

From the Eastern Mediterranean Sea, a total of 210 wild marine indigenous and Lessepsian fish from four different species were sampled and tested for *Vibrio* and *Mycobacterium* for two years (2016-2017), using PCR with 16S rRNA primers. Based on the sequencing results, the total prevalence of positive results for *Vibrio* in wild fish in 2016 was significant higher compared to 2017 ($F= 5.91$, $P= 0.031$). In addition, 72 gilthead sea bream (*Sparus aurata*) from an Israeli offshore marine farm were also examined for two years (2017-2018) in order to assess the possibility of horizontal pathogen transmission from wild to maricultured fish and *vice*

versa. The results exhibited that *Mycobacterium* prevalence was significantly higher in 2018 (F= 9.943, P= 0.002), while in the 2017 study there was no positive results for *Mycobacterium*.

Following this survey, an additional study was conducted in order to characterize *Vibrio* and *Mycobacterium* strains and their prevalence within the five fish species. The Lessepsian species Randall's Threadfin Bream (*Nemipterus randalli*) and the local species striped red mullet (*Mullus surmuletus*) showed high prevalence of both pathogens, suggesting that those species may serve as a carrier and might horizontally infect other susceptible species, which live in proximity. The phylogenetic analysis revealed that all the twenty detected *Vibrio* strains belonged to four different groups, and there was an overlap in one group between the wild and the cultures species; this may suggest spontaneous transmission between wild and farm fish. All eleven detected *Mycobacterium* strains belonged to three different groups, without overlapping between the wild and the cultured species.

The results of this study highlight the necessity of continuous molecular monitoring in order to characterize the prevalence of pathogenic agents in both wild and cultured fish populations, to assess the possibility for horizontal transmission of pathogens between wild fish and mariculture fish, and to improve our understanding of the potential for zoonotic infections.

List of Tables

List of Figures

1. Introduction

Over the past few decades, there has been a worldwide increase in reports of diseases affecting marine organisms of different taxa (Harvell et al. 1999). Climate change is additional pressure on marine ecosystems which are already subject to many anthropogenic disturbances, such as overfishing, pollution and habitat destruction. Environmental conditions play a crucial role not only in pathogen transfer but also as risk factors for clinical disease occurrence. Unlike mammals that regulate their internal environments, most fish are poikilotherms, meaning that they have little ability to regulate their core body temperature. In this situation, both the microbe and the host are physiologically tied to the environment and have an optimal temperature range for survival; above and below this range they become stressed and immunosuppressed. Extended periods outside the optimal range usually results in death.

This is especially true in the Mediterranean Sea, which is one of the biggest reservoirs of biodiversity in the world (Coll et al. 2010). Diverse cold and tropical marine fauna combine and mix due to the basin's oceanographic and biogeographical properties and, therefore, might serve as "miniature model" for the world's oceans and provide insights into global patterns of marine ecosystems (Lejeusne et al. 2010). Climate warming can increase pathogen development and their survival rates, disease transmission and host susceptibility (Harvell et al. 2002).

Wild fish have an important ecological role in the ecosystem and economic role as a major protein source for humans (Holmlund and Hammer 1999). Although aquaculture production has increased dramatically, fish consumption still largely depends on fisheries (FAO 2016). A wide range of marine pathogens from aquaculture is well documented, but there remains a lack of baseline data and information regarding pathogenic agents' prevalence in the wild fish population (Ward and Lafferty 2004).

1.1. Marine aquaculture

Aquaculture is a fast-growing industry for the production of high protein-sourced foods, including fish production as well (FAO 2016). This growth is accompanied by concerns from both the public and private sectors (FAO 2016), as fish production is commonly associated with serious environmental impacts (e.g. water pollution, pathogen transmission, temperature changes, *etc*.). For this reason, strict regulations have been imposed regarding the quality of aquaculture effluent discharge (Partridge et al. 2008), which leads to improvements in environmental performance, technologies, and sustainability of this sector (FAO 2016). Well

established method for fish production is in isolated floating cages in the open sea, which can be either singular with a circumferential walkway for operation purposes or grouped with a walkway that forms a raft. Cage farms are anchored with cables to anchorage facilities on the sea or lake floor. Offshore cages are submerged when there is a risk of damage by high waves in stormy seas and are refloated when the storm passes. Production management in cages and pens is based on the removal of waste matter from the culture unit by the water flow (Fernandez-Gonzalez and Sanchez-Jerez 2017). Although this maintains high water quality in the cages, it also may become an ecological nuisance. Production is usually very dense, similar to the density in intensive production ponds. Fish are routinely treated and harvested from the walkway or raft around the cages, and boats are used for transportation of feed and for harvest (Fernandez-Gonzalez and Sanchez-Jerez 2017). In Israel, there are several marine cage farms along the Israeli Mediterranean coast. According to the FAO report from 2018, the annual production of marine fish from all the cages in Israel is over 2,100 tons. Over 85% of this production consists of the gilthead seabream, *Sparus aurata*.

1.2. The concern of infectious disease in mariculture and aquaculture

Aquaculture production has constantly increased during the last five decades (FAO, 2018). Although aquaculture in the Mediterranean is considered a relatively young industry, finfish diseases have been reported to cause substantial problems and mortalities among farmed stocks (Arechavala-Lopez et al. 2013). Two apparent reasons that play a central role in the transmission of infectious pathogens are the farming activity and the open design of Mediterranean aquaculture systems. As such, the transport of infected farmed fish from hatcheries, infected equipment, staff and vessels, as well as through water currents have been the

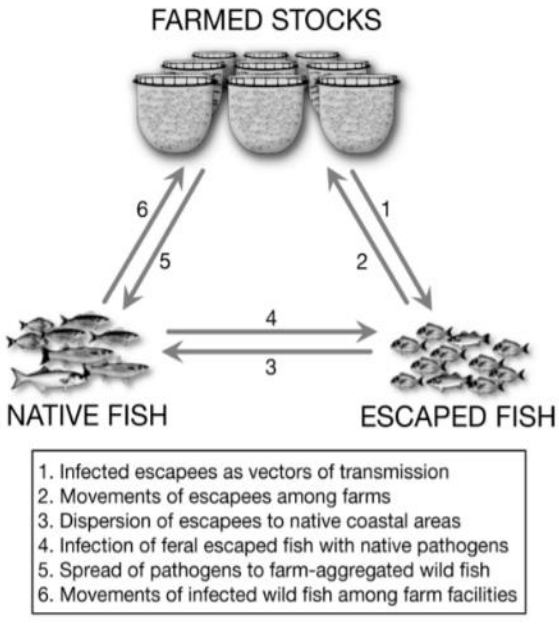

Figure 1: Generalized flow chart of potential pathogen transmission through movements of wild fish and escapees. (Arechavala-Lopez et al. 2013)

main focus of fish health and biosecurity programs (Arechavala-Lopez et al. 2013).

Infectious diseases are also common in marine waters. Bacteria and viruses can be transmitted both horizontally and vertically, due to oceanographic conditions in nearshore environments that are strongly influenced by local conditions (Johansen et al. 2011), and they can reduce commercial species' growth and survivorship or decrease seafood quality. These impacts seem most problematic in the stressful and crowded conditions of aquaculture, which increasingly dominates seafood production as wild fishery production plateaus (Lafferty et al. 2014). The fish's skin condition also plays an important role in the onset of some bacterial diseases, since skin lesions act as sites of pathogen entry (Colorni 1992). For instance, *Vibrio algynolyticus* and *Mycobacterium marinum* are associated with stress conditions and disruption of fish skin integrity after handling (Colorni 1992; Toranzo et al. 2005).

It is often difficult to accurately estimate impacts of diseases on wild populations, especially those of pelagic and subtidal species. However, there are few quantitative data demonstrating that wild species near farms suffer more from infectious diseases than those in other areas. The movement of exotic infectious agents to new areas continues to be a great concern.

1.3. Relationship between infection and disease

It is important to note that exposure of a host to a pathogen does not always result in infection which, in turn, does not always lead to disease (Wade 2014). Moreover, infection may occur far from the immediate vicinity where infection is either detected or disease first becomes evident (Figure 2). These variables make a diagnosis and/or pathogen evasion highly problematic. For example, in the case of a clinical disease observed in a wild fish population, the exposure was likely to have occurred several days to weeks beforehand. Further complications in diagnosis may occur when migratory fish are the hosts, as their migratory path may not have been monitored. In addition, the pathogen may have also moved and no longer be present at the location of exposure/infection due to host migration or current (Wade 2014).

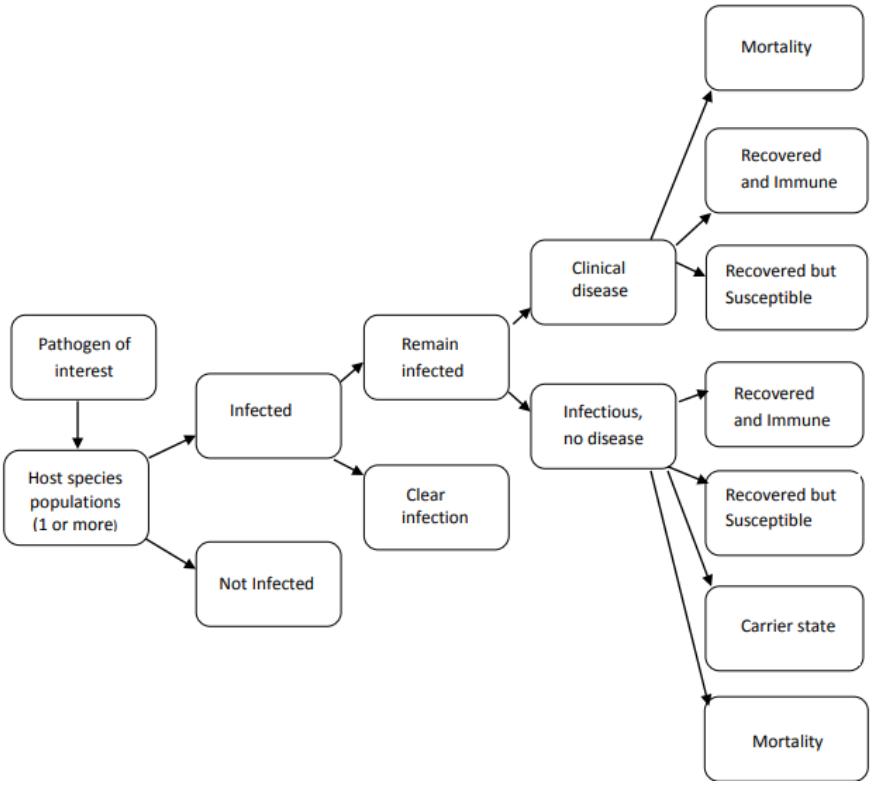

Figure 2: Generalized flow chart of possible outcomes of pathogen exposure among wild and farmed fish. The state of being infected includes latent infections (Wade 2014).

1.4. Marine zoonotic diseases

Zoonotic pathogens, diseases that can be transmitted between animals and humans, presents a global public health concern. Those pathogens are known to cause protracted illness, especially in immuno-compromised individuals (Gauthier and Rhodes 2009). As aquaculture production and the consumption of aquaculture products increases, the possibility of contracting zoonotic infections from either handling or ingesting these products also increases. Aquatic pathogens (e.g. *Mycobacterium marinum*, *Streptococcus iniae*, *Vibrio vulnificus* etc.) have also been associated with disease outbreaks in food fish. Outbreaks are often related to management factors, such as the quality and quantity of nutrients in the water and high stocking density, which can increase bacterial loading on the external surface of the fish. As a result, diseased fish are more likely to transmit infection to humans (Haenen et al. 2013).

This research focuses on two types of bacterial pathogens; *Vibrio* and *Mycobacterium* which are known as zoonotic pathogens (Gauthier and Rhodes 2009) and are major causes of fish mortality (García-Rosado et al. 2007).

1.5. *Vibrio* in the marine environment

Vibrio species is a gram-negative curved rod that occurs naturally in marine, estuarine, and freshwater systems worldwide. They occupy habitats ranging from the deep sea to shallow aquatic environments (Reen et al. 2006). More than 70 different *Vibrio* species are known, and 12 of which are recognized as human pathogens (Kokashvili et al. 2015). Some species include human and animal pathogens capable of causing gastroenteritis, wound infections, cholera, and fatal septicemia (Ceoccarelli and Colwell 2014). *Vibrio* infections have long since been observed and documented in marine- and estuarine-type fishes. The disease is known to have increased death rates (> 50%) in fish farms soon after an outbreak (Haldar et al. 2010). Symptoms of Vibriosis are akin to other signs of bacterial diseases of fish, and usually start with sluggishness and a loss of appetite. The disease may cause discoloration and eventual decay of the skin. Swollen sores may appear on the body, and in many cases protrude through the skin surface. Another symptom is redness around the fins and mouth. When the disease becomes systemic, it can cause exophthalmia ("pop-eye"), and the gut and rectum may be bloody and filled with fluid (Haldar et al. 2010).

Figure 3: Vibriosis in fish. Visible swollen sores on the body, exophthalmia and redness around the fins and mouth (Mancuso et al. 2018).

1.6. *Mycobacterium* in the marine environment

Mycobacteria (family Mycobacteriaceae), are pleomorphic, gram-positive, acid-fast, aerobic, non-motile rods (Gauthier and Rhodes 2009). Mycobacteriosis is caused by non-tuberculous mycobacteria (NTM), and considered among the most chronic diseases occurring in aquatic animals (Novotny et al. 2010). The NTM group comprises more than 150 different species with distinct virulence features (Tortoli 2014). In addition, fish mycobacteriosis has substantial economic consequences, especially in the aquaculture and fisheries industry as infections may significantly decrease production and trade. Some fish NTM pathogens are highly virulent and

zoonotic (Gauthier and Rhodes 2009). As such, infection of aquaria with these pathogens is a public health concern. Typical signs of *Mycobacterium* in fish are weight loss or emaciation, scale loss, ulcerations or hemorrhage along the body wall, granulomas, infection at necropsy, poor appetite and attitude, and often a history of reproductive problems (Francis-Floyd 2011).

Both external and internal clinical signs caused by each pathogen are dependent on the host species, age of the fish, and stage of the disease (acute, chronic, sub-clinic carrier); the signs are not always correlated or present at all (Toranzo et al. 2005).

Figure 4: Mycobacteriosis of *Sparus aurata*: (A) Granulomas visible within the spleen during necropsy. (B) Ulcerative skin lesion on the gill. (Photographed by O. Ashoulin)

1.7. Hypothesis

Vibrio and *Mycobacterium* are widespread pathogens that may become a threat to farmed fish and may impact the wild fish populations in the Eastern Mediterranean Sea.

1.8. Objectives

a. To compare pathogen prevalence between different years in farmed and wild fish.
b. To compare pathogen prevalence in different internal organs and maturity age.
c. To identify different strain variations of *Mycobacterium* and *Vibrio spp.* in wild and cultured fish.
d. To assess transmission of the pathogens from wild to cultured fish and *vice versa*.

1.9. Significance of the study

This research is part of the long-term marine ecological monitoring program of the Morris Kahn Marine Research Station (University of Haifa). The aim of this study is to establish a scientific baseline of the marine pathogens in the eastern Mediterranean Sea. The fish species that were sampled and analyzed for *Mycobacterium* and *Vibrio* are fish with both ecological and economical importance in the region.

2. Materials and Methods

2.1. Fish and tissue sampling

Four wild fish species (*Mullus surmuletus*, *Nemipterus randalli*, *Saurida lessepsianus* and *Sardinella aurita)* were caught by trawlers and fishermen and collected at four ports along the Israeli Mediterranean shoreline: Akko, Kishon, Jaffa and Ashdod (Figure 5). In addition, *Sparus aurata* specimens were sampled during their growth period from a fish farm located 12 km offshore in the southern area of the Israeli Mediterranean Sea. All wild specimens sampled during 2016 and 2017, and the cultured *S. aurata* sampled during 2017 and 2018, are detailed in Tables 1a and 1b.

Fish were placed on ice immediately on the boat and transferred on ice to the laboratory where weight, total length and visual inspections were carried out. It should be mentioned that the fish were obtained at the ports and nearby fish markets, but the exact fish capture sites are not recorded in this study.

All specimens were aseptically dissected for tissue sample collection according to fish necropsy protocol of Yanong (2003). All samples were kept frozen at - 80 °C until further analysis. From each fish specimen, liver and kidney tissues were isolated and kept frozen at -80 °C until use.

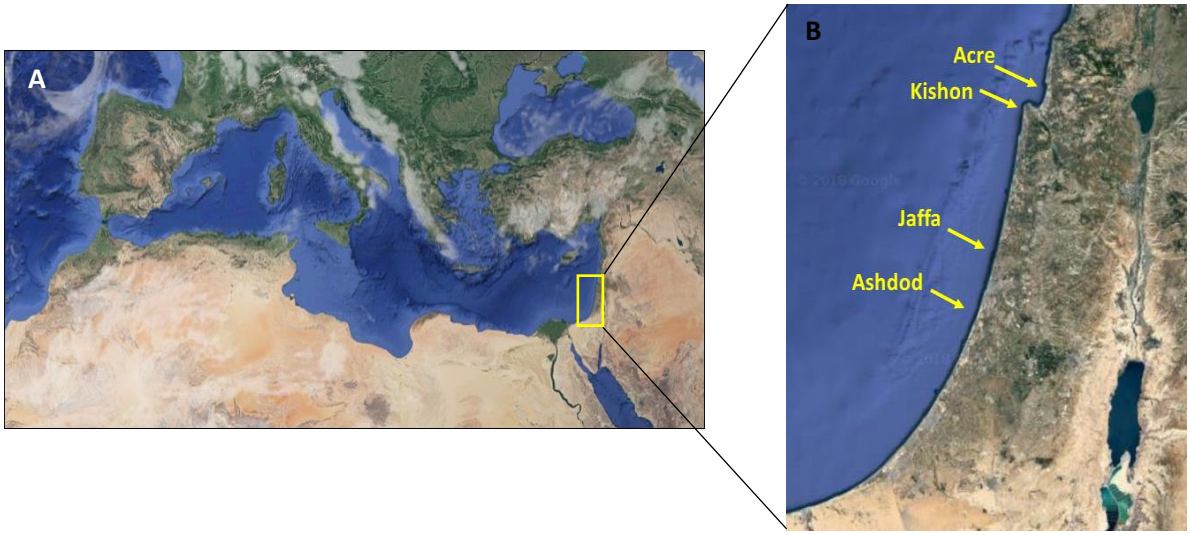

Figure 5: Geographical distribution of the sampling locations described in this study. A –Sampling area is in the most eastern basin of the Mediterranean Sea, B – fish were sampled from four fishing ports along the Israeli coast – two in the North: Akko, Kishon; and two in the South: Jaffa, Ashdod. Template map source: Google Earth.

Table 1a: Wild fish organisms sampled and analyzed in this study. Total number of specimens tested and number of wild fish sampled from each sampling site in 2016 and 2017.
*M- Mediterranean natives, L- Lessepsian migrants

Year	Family, Species	Common name	Origin*	Akko	Kishon	Jaffa	Ashdod	Total
2016	***Mullidae***							
	Mullus surmuletus	Striped red mullet	M	3	13	3	3	22
	Nemipteridae							
	Nemipterus randalli	Randall's Threadfin Bream	L	3	13	10	3	29
	Synodonitidae							
	Saurida lessepsianus	Brushtooth lizardfish	L	9	13	13	3	38
	Clupeidae							
	Sardinella aurita	Round sardinella	M	3	11	10		24
2017	***Mullidae***							
	Mullus surmuletus	Striped red mullet	M		15			15
	Nemipteridae							
	Nemipterus randalli	Randall's Threadfin Bream	L		15	15	12	42
	Synodonitidae							
	Saurida lessepsianus	Brushtooth lizardfish	L			15		15
	Clupeidae							
	Sardinella aurita	Round sardinella	M			20	5	25

Table 1b: Cultured *Sparus aurata* sampled and analyzed in this study. Total number of specimens tested, and number of cultured fish farm sampled in 2017 and 2018.
*M- Mediterranean natives, F- Fish farm

Family, Species	Common name	Origin*	2017	2018	Total
Sparidae					
Sparus aurata	Gilthead sea bream	M, F	45	27	72

2.2. DNA extraction

Extraction of total DNA from the tissues of each specimens were performed using the Wizard SV Genomic DNA Purification System kit (Promega Corporation, USA) and in according to the manufacturer's specifications. Up to 20 mg of tissue sample were placed in 275 µl of manufacturer's Digestion Solution Master Mix, followed by an overnight incubation in 55 °C (16-18 hours), then a 250 µl of Lysis buffer was added. For purification of DNA, the lysates were transferred to the manufacturer's minicolumn assembly, centrifuged for 3 minutes at 13,000Xg, followed by 4 subsequent centrifuges at 13,000Xg for 1 minute with 650 µl Wash

Solution. DNA was eluted using a 2 minute incubation with 250 µl nuclease-free water and 13,000Xg centrifuge for 2 minutes. The DNA concentration and quality were determined at 280 nm with a NanoDrop One (NanoDrop Ins., Thermo Scientific), and the extracted DNA were stored at -20 °C until further analysis.

2.3. PCR amplification

PCR reactions for both *Vibrio* and *Mycobacterium* were performed in reaction tubes preloaded with 3 µl of DNA, 0.2 µl of each primer, 9.1 µl of ultra-pure PCR water and 12.5 µl GoTaq Green Master mix (Promega, WI, USA) on a SimpliAmp Thermal Cycler (Applied Biosystems, CA, USA).

The amplification reaction for *Mycobacterium* 16s rRNA was subjected to 40 cycles (4 min at 95°C, 30 sec at 62°C, 30 sec at 72°C), followed by 10 min of extension at 72 °C. Primers T39 5´-GCGAACGGGTGAGTAACACG-3' and T13 5´-TGCACACAGGCCACAAGGGA-3' amplified a 924 bp segment of the published gene sequence (Talaat, Reimschuessel, and Trucksis 1997).

The amplification reaction for *Vibrio* 16s rRNA was subjected to 40 cycles (4 min at 95°C, 30 sec at 61°C, 30 sec at 72°C), followed by 10 min of extension at 72 °C.

Primers 63f 5'-CAGGCCTAACACATGCAAGTC-3' and 763r 5'-GCATCTGAGTGTCAGTATCTGTCC-3' amplified a 700 bp segment of the published gene sequence (Montiert et al. 2010).

Six µl of each amplicon were checked by 1.5% Agarose gel electrophoresis along with a 1kb BenchTop ladder (Promega, WI, USA).

2.4. Comparison of kidney and liver tissues

A total of 251 specimens were taken from the kidneys and livers of each fish species. These were analyzed and compared to determine *Vibrio* and *Mycobacterium* prevalence from all the five fish species. Only specimens tested for both tissues were included in the statistical analysis.

2.5. Comparison of juvenile and adult fish

A total of 150 mature fish and 95 juveniles, from four fish species (only mature *M. surmuletus* were tested, and therefore weren't tested for statistical significance), were analyzed and compared for *Vibrio* and *Mycobacterium* prevalence. The separation of mature and juvenile fish was determined by the total length: *N. randalli* reach sexual maturity at a total length of ~ 110 mm (Demirci et al. 2018); *S. aurita* at a total length of ~ 155mm (Tsikliras and Antonopoulou

2006); *S. lessepsianus* at a total length of ~180 mm (El- Halfawy et al. 2007); and *S. aurata* at a total length of ~ 260 mm (Emre et al. 2009).

2.6. Sequencing and phylogenetic analysis

Vibrio PCR (primer '63f') and *Mycobacterium* PCR (primer 'T39') amplicons were purified by ExoSAP-IT (Affymetrix, Santa Clara, CA) and sequenced by Sanger sequencing method (HyLabs, Rehovot, IL). Sequences were aligned and compared to representative sequences available in Arb-Silva website and in GenBank by BLAST using the BioEdit Sequence Alignment Editor and MEGA10 software.

Phylogenetic trees visualized with the MEGA10 software. Robustness of nodes on the phylogeny was assessed by 1000 bootstrap replicates using Maximum Parsimony analysis. All sequences from positive samples were deposited in GenBank and accession numbers are provided in the Appendices (Tables S1 and S2).

2.7. Statistical analyses

All statistical analyses were conducted using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, USA). Multiple logistic regression analysis was chosen to analyze the various effects on *Vibrio* and *Mycobacterium* prevalence. For all tests, a P-value of < 0.05 was considered significant.

3. Results

3.1. *Vibrio* results

3.1.1. *Vibrio* prevalence in wild and cultured fish

A total of 113 wild fish in 2016, and 97 in 2017, were tested for *Vibrio* by PCR amplification (Table 2a). In addition, a total of 45 cultured fish (*Sparus aurata*) in 2017, and 27 in 2018 were also tested (Table 2b). Based on the sequencing results, in the 2016 study the total prevalence of positive results for *Vibrio* in wild fish was significantly higher compared to 2017 ($F= 5.91$, $P= 0.031$). Figure 6 shows the significant effect of interaction between fish species and years ($F = 2.68$, $P = 0.048$). *N. randalli*, *S. aurita* and *S. lessepsianus* exhibited a decay in *Vibrio* prevalence in 2017. However, *M. surmuletus* showed an increase in *Vibrio* prevalence in 2017. As for the cultured fish farm, no change in prevalence was found between the years ($P > 0.05$). In the 2017 study the total prevalence of positive results for *Vibrio* was 8.89%, compared to 2018 when the prevalence for *Vibrio* was 3.7%.

Table 2a: *Vibrio* prevalence in wild fish species from the Mediterranean Sea. Results based on the PCR targeting 16s segments. Positive results refer to one or more isolated tissue.

Fish species	**2016**			**2017**			**Total**		
	N	**Positive**	**% Positive**	**N**	**Positive**	**% Positive**	**N**	**Positive**	**% Positive**
Mullus surmuletus	22	2	9.09%	15	2	13.33%	37	4	10.81%
Sardinella aurita	24	4	16.67%	25	0	0.00%	49	4	8.16%
Saurida lessepsianus	38	1	2.63%	15	0	0.00%	53	1	1.89%
Nemipterus randalli	29	6	20.69%	42	0	0.00%	71	6	8.45%
Total wild	113	13	11.50%	97	2	2.06%	210	15	7.14%

Table 2b. *Vibrio* prevalence in the cultured fish *S. aurata* from the Mediterranean Sea fish farm. Results based on PCR targeting 16s segments. Positive result refers to one or more isolated tissue.

Fish species	**2017**			**2018**			**Total**		
	N	**Positive**	**% Positive**	**N**	**Positive**	**% Positive**	**N**	**Positive**	**% Positive**
Sparus aurata	45	4	8.89%	27	1	3.70%	72	5	6.94%

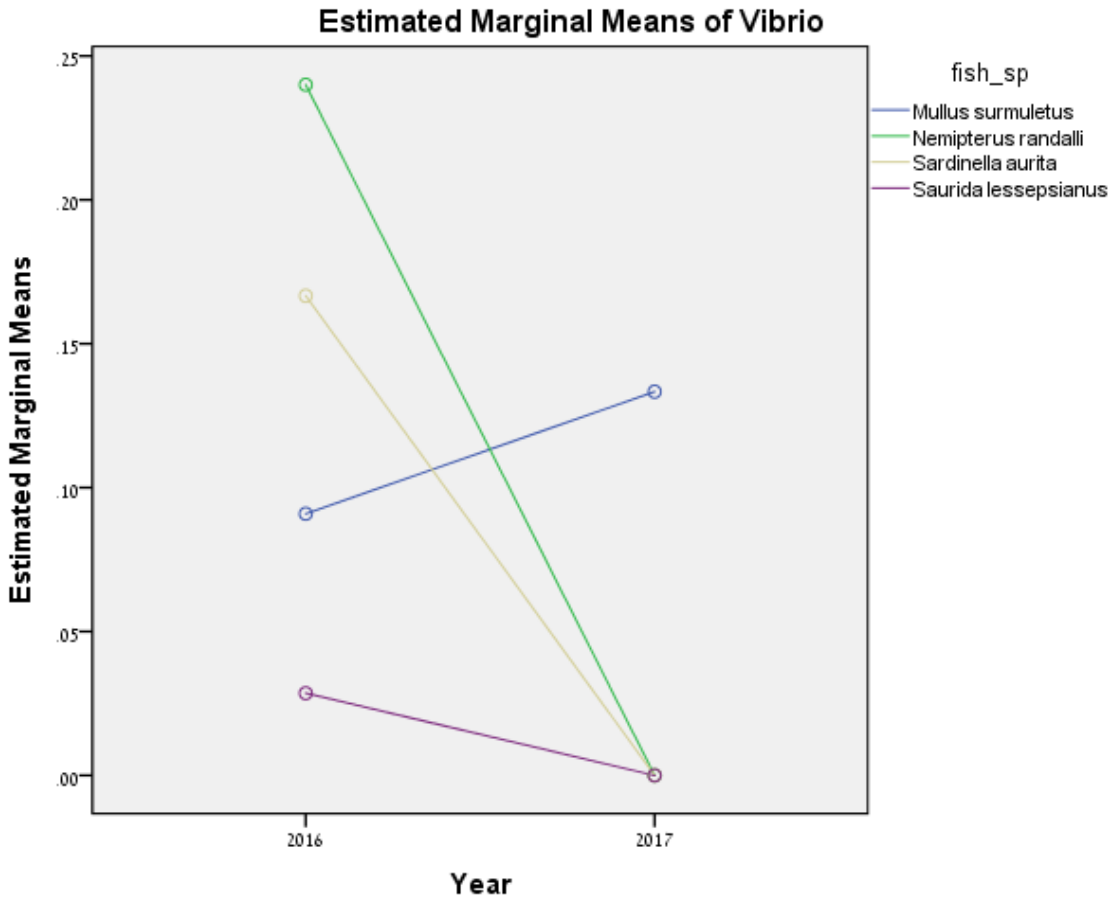

Figure 6: *Vibrio* prevalence interaction between fish species and between years.

3.1.2. Relative distribution of *Vibrio* in kidney and liver tissues

A total of 251 samples were taken from kidney and livers from all the five species, and were tested for *Vibrio* (Table 3). No significantly higher prevalence was found in the kidney samples when compared to liver tissues ($P > 0.05$). From kidney samples, positive results were obtained in two samples of *M. surmuletus* (5.4%), six *N. randalli* (9.7%), two *S. aurita* (4.5%), one *S. lessepsianus* (2.1%) and two *S. aurata* (5.2%). From the liver samples, positive results were obtained in two samples of *M. surmuletus* (5.4%), two *S. aurita* (4.5%) and three *S. aurata* (4.9%). No positive results were obtained in *N. randalli* and *S. lessepsianus* liver tissues. Two specimens of *M. surmuletus* exhibited presence of *Vibrio* in both the liver and kidney tissues.

Table 3: Relative distribution and percentage of *Vibrio* in kidney and liver tissues from wild and cultured fish. Only specimens tested for both tissues were included in the statistical analyses.

	Positive for *Vibrio*			
	Kidney tissue		**Liver tissue**	
Fish species	**N**	**%**	**N**	**%**
Mullus surmuletus	2/37	5.4%	2/37	5.4%
Nemipterus randalli	6/62	9.7%	0/62	0.0%
Sardinella aurita	2/44	4.5%	2/44	4.5%
Saurida lessepsianus	1/47	2.1%	0/47	0.0%
Sparus aurata	2/61	3.3%	3/61	4.9%
Total	13/251	5.2%	7/251	2.8%

3.1.3. Comparison of juvenile and adult fish

A total of 150 mature fish and 95 juveniles from four fish species (only mature *M. surmuletus* were tested, and therefore this species was not included in the statistical analysis), were analyzed and compared for *Vibrio* prevalence (Table 4). No positive results were obtained in *N. randalli* and *S. aurita* juveniles, yet infected mature fish of the same species showed a prevalence of 12.5% and 6.1%, respectively. A positive result as obtained for one juvenile *S. lessepsianus* (25%) and higher prevalence was found in juvenile *S. aurata* (7.7% juvenile compared to 5% of mature specimens).

Table 4: Relative distribution and percentage of *Vibrio* in juvenile and adult wild and cultured fish.

	Positive for *Vibrio*			
	Mature		**Juvenile**	
Fish species	**N**	**%**	**N**	**%**
Nemipterus randalli	6/48	12.5%	0/23	0.0%
Sardinella aurita	2/33	6.1%	0/16	0.0%
Saurida lessepsianus	0/49	0.0%	1/4	25.0%
Sparus aurata	1/20	5.0%	4/52	7.7%
Total	9/150	6%	5/95	5.3%

3.1.4. Phylogenetic analysis of *Vibrio*

The phylogenetic tree constructed from the 16S rRNA gene partial sequences (Figure 7) revealed a similarity within four different groups of *Vibrio*: Six *N. randalli* and two *M. surmuletus* segments from wild Mediterranean Sea fish shared an identical nucleotide sequence

and displayed similarity to *V. alginolyticus* and *V. parahaemolyticus* (see Orange group). All these segments were detected from kidney tissues in 2016. The second group (Green) showed similarity to *V. harveyi* and contained two samples of *S. aurita* from 2016, which in both of them segments from kidney and liver tissues, two 2017 *M. surmuletus* (liver tissue) and two cultured Mediterranean Sea fish *S. aurata* (one from 2017 and one from 2018). The third group contain one strain from 2016 *S. lessepsianus* which didn't showed any similarity to specific *Vibrio* specie (Yellow group). Three 2017 *S. aurata* isolates from cultured Mediterranean Sea fish, also didn't showed any similarity to specific *Vibrio* specie (Blue group). specific *Vibrio*

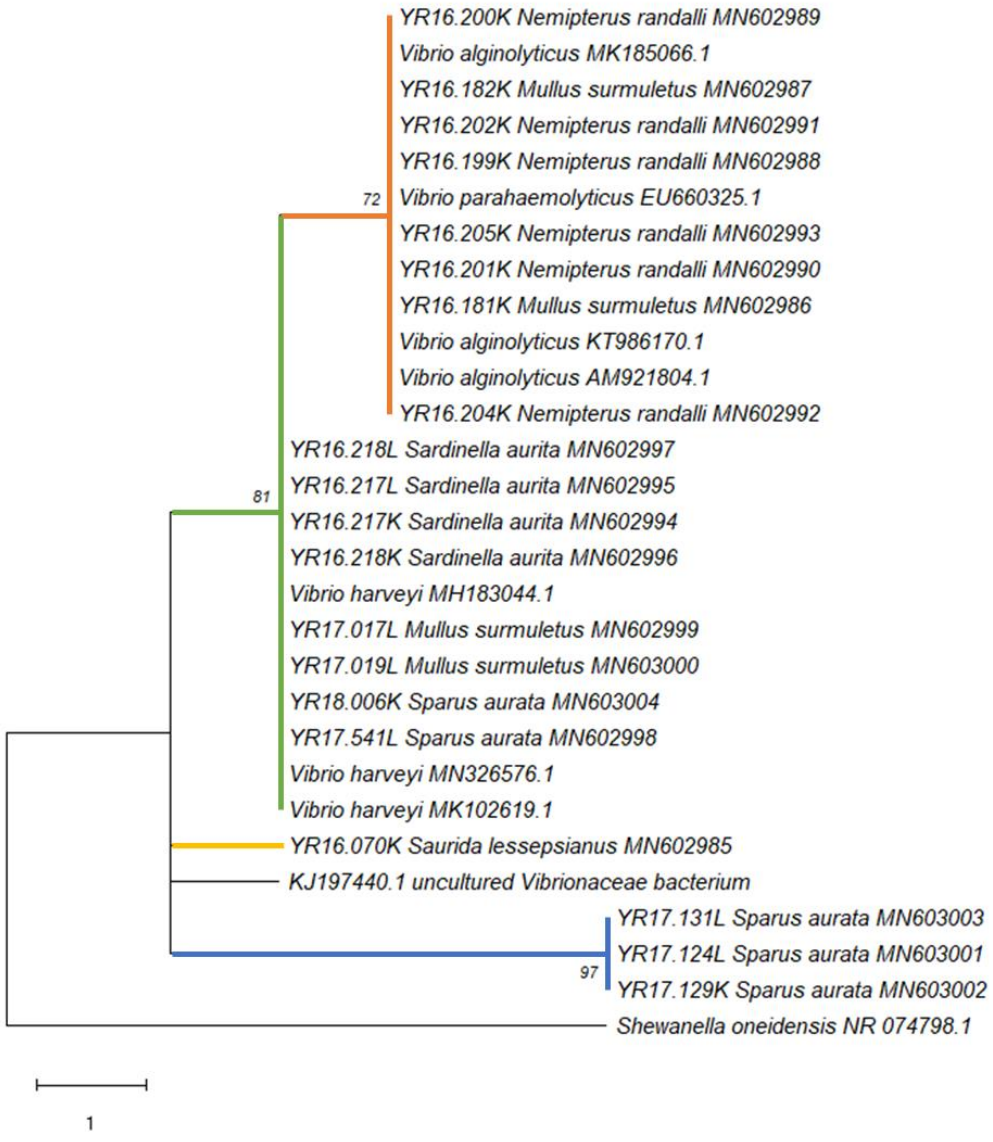

Figure 7: *Vibrio* phylogenetic tree. Maximum Parsimony analysis phylogenetic tree of the *Vibrio spp.* derived from 16s rRNA gene partial sequences. The sequence name for positive samples of this study begins with YR and includes the capture year, identification number, tissue (L-liver, K- kidney), host species and GenBank accession number. The tree was rooted by using *S. oneidensis* as the outgroup. Numbers on the branches indicate bootstrap proportions (1000 replicates, only values ≥ 70% are reported). Available Gen-Bank and Arb-Silva accession numbers are shown. The scale bar represents 1 nucleotide substitution per site

3.2. *Mycobacterium* results

3.2.1. *Mycobacterium* prevalence in wild and cultured fish

A total of 113 wild fish in 2016, and 97 in 2017, were tested for *Mycobacterium* by PCR amplification (Table 5a). In addition, a total of 45 cultured fish (*Sparus aurata*) in 2017, and 27 in 2018 were tested (Table 5b). Based on the sequencing results, in the 2016 study no positive results were recorded in wild fish, compared to 2017 when the overall prevalence for *Mycobacterium* was 5.15%. The only species that showed the presence of this pathogen were *M. surmuletus* (6.67%) and *N. randalli* (9.52%). According to the multiple logistic regression, no significantly higher prevalence was found over the total prevalence of *Mycobacterium* between the years. As for the fish farm, a significantly higher prevalence (F= 9.943, P= 0.002) was found in 2018 (18.52%) compared to 2017 study, in which there was no positive results for *Mycobacterium*.

Table 5a: *Mycobacterium* prevalence in wild fish species from the Mediterranean Sea. Results based on PCR targeting 16s segments.

Fish species	2016			2017			Total		
	N	Positive	% Positive	N	Positive	% Positive	N	Positive	% Positive
Mullus surmuletus	22	0	0.00%	15	1	6.67%	37	1	2.70%
Sardinella aurita	24	0	0.00%	25	0	0.00%	49	0	0.00%
Saurida lessepsianus	38	0	0.00%	15	0	0.00%	53	0	0.00%
Nemipterus randalli	29	0	0.00%	42	4	9.52%	71	4	5.63%
Total wild	113	0	0.00%	97	5	5.15%	210	5	2.38%

Table 5b: *Mycobacterium* prevalence in the cultured fish *S. aurata* from the Mediterranean Sea fish farm. Results based on PCR targeting 16s segments.

Fish species	2017			2018			Total		
	N	Positive	% Positive	N	Positive	% Positive	N	Positive	% Positive
Sparus aurata	45	0	0.00%	27	5	18.52%	72	5	6.94%

3.2.2. Relative distribution of *Mycobacterium* in kidney and liver tissues

A total of 251 samples were taken from kidney and livers of each specimen, and were tested for *Mycobacterium* from all the five fish species (Table 6). A significantly higher prevalence was found in kidney samples compared to liver tissues (F= 2.518, P= 0.041). From the kidney samples, positive results were obtained in one specimen of *M. surmuletus* (2.7%), four *N. randalli* (6.5%), and two *S. aurata* (3.3%). From liver samples, positive results were only

obtained in two *N. randalli* (3.2%). No positive results were obtained in *S. aurita* and *S. lessepsianus* tissues. One specimen of *N. randalli* exhibited presence of *Vibrio* in both the liver and kidney tissues.

Table 6: Relative distribution and percentage of *Mycobacterium* in kidney and liver tissues from wild and cultured fish. Only specimens tested for both tissues were included in the statistical analysis.

	Positive for *Mycobacterium*			
	Kidney tissue		**Liver tissue**	
Fish species	**N**	**%**	**N**	**%**
Mullus surmuletus	1/37	2.7%	0/37	0.0%
Nemipterus randalli	4/62	6.5%	2/62	3.2%
Sardinella aurita	0/44	0.0%	0/44	0.0%
Saurida lessepsianus	0/47	0.0%	0/47	0.0%
Sparus aurata	2/61	3.3%	0/61	0.0%
Total	7/251	2.8%	2/251	0.8%

3.2.3. Comparison of juvenile and adult fish

A total of 150 mature fish and 95 juveniles from four fish species (only mature *M. surmuletus* were tested and therefore weren't included in the statistical analyses), were analyzed and compared to ascertain *Mycobacterium* prevalence (Figure. 11). No positive results were obtained in both mature and juvenile *S. aurita* and *S. lessepsianus*. Higher prevalence was observed in mature *N. randalli* and *S. aurata* compared to their juvenile counterparts. There was no significant difference ($p > 0.05$) in total prevalence of *Mycobacterium* between the mature and the juvenile (Table 7).

Table 7: Relative distribution and percentage of *Mycobacterium* in juvenile and adult wild and cultured fish.

	Positive for *Mycobacterium*			
	Mature		**Juvenile**	
Fish species	**N**	**%**	**N**	**%**
Nemipterus randalli	5/48	10.4%	0/23	0.0%
Sardinella aurita	0/33	0.0%	0/16	0.0%
Saurida lessepsianus	0/49	0.0%	0/4	0.0%
Sparus aurata	2/20	10.0%	3/52	5.8%
Total	7/150	4.7%	3/95	3.2%

3.2.4. Phylogenetic analysis of *Mycobacterium*

The phylogenetic analysis of *Mycobacterium* segments revealed similarity across three different groups of *Mycobacterium* (Figure. 8): Three 2017 *N. randalli* and one *M. surmuletus* segments from wild Mediterranean Sea fish shared an identical nucleotide sequence (YR17.015K differed by one nucleotide) and showed similarity to *M. peregrinum* (Orange group). *N. randalli* YR17.001L (Green group) showed similarity to *M. neoaurum*, only differing from the orange group by 9 nucleotides. All five segments from the 2018 fish farmed *S. aurata* were identical to each other, were similar to *M. marinum* (Blue group), and differed from the orange group by 21 nucleotides.

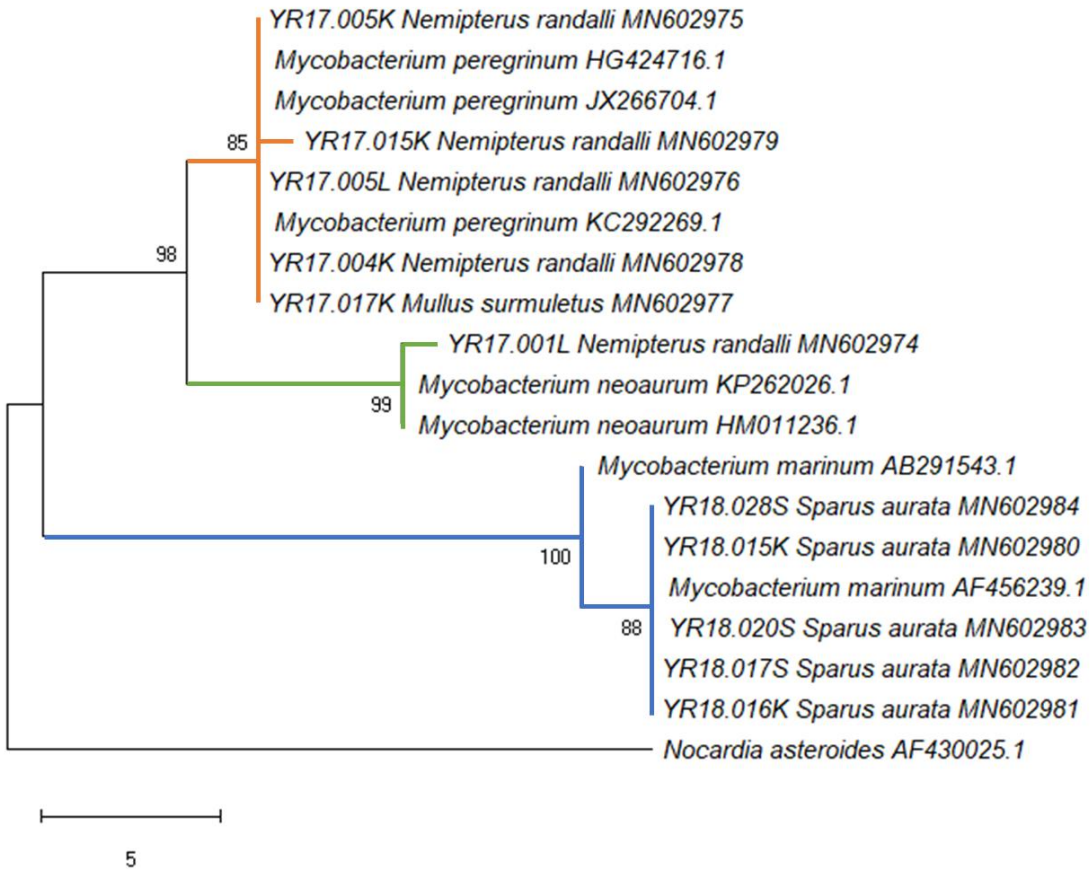

Figure 8: *Mycobacterium* phylogenetic tree. Phylogenetic tree output from the Maximum Parsimony analysis of the *Mycobacterium spp.* derived from 16s rRNA gene partial sequences. The sequence name for positive samples of this study begins with YR and includes the capture year, identification number, tissue (L-liver, K- kidney, S- spleen), host species and GenBank accession number. The tree was rooted by using *N. asteroides* as the outgroup. Numbers on the branches indicate bootstrap proportions (1000 replicates, only values ≥ 70% are reported). Available Gen-Bank and Arb-Silva accession numbers are shown. The scale bar represents 5 nucleotide substitution per site.

4. Discussion

Vibrio and *Mycobacterium* are widespread in nature, especially in a marine environment. Fish infected by these bacteria could be a source of zoonotic risk for human health (Francis-Floyd 2011) and are known to cause infections in humans with different degrees of severity, especially in immunocompromised individuals (Bercovier and Vincent 2001; Ceoccarelli and Colwell 2014). Although *Vibrio* and *Mycobacterium* have been studied and detected in many fish species around the world, including various wild marine fish species (Diamant et al. 2000; Ucko et al. 2002; Sevim et al. 2015; Kokashvili et al. 2015; Senderovich et al. 2010; Bluford et al. 2017), at the time of writing this is the first study to identify *Vibrio* in the Lessepsian migrant *Saurida lessepsianus* and the endemic Mediterranean fish *Sardinella aurita.*

In this study, the presence of two important aquatic bacteria were investigated in four marine wild fish and one cultured farm fish in the eastern Mediterranean Sea. Both pathogens were detected in indigenous and Lessepsian species, and their prevalence varied greatly between fish species. In both pathogens, there were no statistically significant differences between the different wild species. However, the overall prevalence of *Vibrio* was significantly higher in 2016 compared to 2017.

Vibrio species are omnipresent and widely distributed in aquatic environments all over the world (Urakawa and Rivera 2006). The occurrence of *Vibrio spp.* is commonly associated with temperature, especially in temperate climes. Generally, *Vibrio* species are detected in summer but less common in winter, whereas the *Vibrio* population variation is lower in tropical and subtropical waters (Urakawa and Rivera 2006). Reports have shown a significant association between rising seawater temperature and an increase in the number of *Vibrio* infections, suggesting that global warming could be a factor in the emergence of *Vibrio* diseases in temperate areas, due to its influence on resident bacterial communities (Huehn et al. 2014). In 2016, during a continuous survey, most of the positive samples were detected in the summer period, which can explain the higher prevalence of *Vibrio* in 2016. However, most of the surveys in 2017 were between October to December, and the prevalence was lower.

Within the cultured fish species, in 2018 the prevalence for *Mycobacterium* in *S. aurata* was significantly higher compared to 2017, where no infection was detected at all. The total prevalence for both pathogens was higher in the Lessepsian fish *N. randalli* and in the indigenous *M. surmuletus* with no reported clinical signs. The relatively high prevalence in those species can be a result of low susceptibility to those pathogens. High prevalence in an asymptomatic fish may indicate they can serve as carriers and horizontally infect other susceptible species living in proximity (Castric et al. 2001). *N. randalli* is an invasive species,

first reported in the Mediterranean in 2005 (Golani and Sonin 2006), and has become a dominant fish species in the Israeli ichthyofauna in the past within 5 years (Stern et al. 2014); the reasons for its successful establishment are unknown (Edelist 2013).
Although *Vibrio* and *Mycobacterium* can be isolated from different organs (e.g. spleen, liver, gut), it has been suggested that these pathogens have an affinity for, or are better detected, in kidney tissue (Skorecki et al. 2015; Hammarén 2017). This is in consensus with my results, where I observed that the prevalence for both pathogens in kidney was higher than in liver tissues in most of the examined fish species.

In addition, the comparison of *Vibrio* and *Mycobacterium* prevalence in juvenile and adult fish was examined in four out of the five fish species (only mature *M. surmuletus* were detected in this study and therefore were not included in the statistical analysis). In the wild fish, most of the prevalence detected were adult-stage specimens. However, as for the cultured fish *S. aurata*, positive results were obtained in both adult and juvenile specimens. It seems that these pathogens do not display a "preference" for specific life stage of the fish, and all species, especially cultured fish, of all ages are susceptible (Colorni et al. 2014).

Phylogenetic analyses, based on the 16S rRNA gene sequences, revealed that all detected *Vibrio* strains were divided to four different genogroups of *Vibrio spp.*, with overlap in one group between the wild and the cultured species. This suggests spontaneous transmission between the wild and the farm fish. The first group showed high similarity to *V. parahaemolyticus* and *V. alginolyticus*. According to Montieri et al. (2010), these two *Vibrio* species share nearly identical sequences in 16S rRNA gene (99.8% identity), and therefore this gene is not a good inadequate for the separation between the two species. The second group showed high similarity to *V. harveyi*. These species are known to be dangerous to of human, marine fish and invertebrates and they can cause various diseases including vasculitis, gastroenteritis, septicemia, skin infection etc. (Bej et al. 1999; Austin and Zhang 2006; Osunla and Okoh 2017). The third group contained only one strain that belongs to the Lessepsian fish *S. lessepsianus*, which showed similarity to uncultured Vibrionaceae bacterium isolated from *Lagodon rhomboides* pinfish (Givens et al. 2015), with a 95% homology. To my knowledge, there were no records in the literature about the presence of *Vibrio* in this fish species. A possible explanation is that there is species-specific adaptation of some *Vibrio* species (Nishiguchi and Nair 2003) and, therefore, this specific genotype might be a new *Vibrio* genotype that is specific for *S. lessepsianus* and possibly other marine fish. Further isolation and characterization of *Vibrio* species is needed from this fish to understand its full genetic properties. In addition, the

fourth group contained three identical strains all belonging to the cultured *S. aurata* from 2017 without any similar references. It seems that this group have a unique insertion of 11 nucleotides which is common in 16S rRNA gene sequences, when insertion–deletion events are frequent and result in length differences among homologous sequences (Urbanczyk et al. 2007).

The phylogenetic analysis of *Mycobacterium* reveals a clear separation between the wild and the cultured species: the wild species were positive across two main groups of *Mycobacterium spp*. (with high similarity to *M. peregrinum* and *M. neoaurum*), while positive samples of the cultured fish *S. aurata* were similar to *M. marinum*. All these *Mycobacterium spp.* are known as pathogenic species. Both *M. peregrinum* and *M. neoaurum* are rapidly growing mycobacteria that can cause bloodstream infections in immunocompromised hosts, and unlike other NTM species, it rarely causes pulmonary infections (Kim et al. 2014). *M. marinum* is a recognized fish pathogen that can also infect endothermic organisms including humans. In human infections, *M. marinum* gains access to the body through skin abrasions and generally produces superficial and self-limiting lesions which involve the cooler parts of the body such as hands, forearms, elbows, and knees (Ucko and Colorni 2005; Todorova et al. 2015). Although *M. marinum* is a well-recognized pathogen of fish, *M. peregrinum* has only recently been associated with diseases in fish (Kent et al. 2004). Even though Mycobacteriosis cases have been reported previously in wild and cultured fish species (Sevim et al. 2015; Colorni 1992), in this study, the identify of Mycobacteria in *N. randalli* is documented for the first time.

Over the last few decades, the 16S rRNA gene has emerged as a good standard for determining phylogenetic relationships of bacteria (Woese 1987) . By using PCR amplification and direct sequencing of 16S rRNA products, Knibb et al. (1993) identified *M. marinum* directly from infected fish. This has allowed both proper taxonomic assignment and has opened the way to molecular epidemiologic analysis at the same time. Yet, even though this gene is still considered a key standard for bacterial identification (Gillman et al. 2001; Turenne et al. 2001), as more sequence information has accumulated over time it has become evident that the resolution power of 16S rRNA sequences alone is often insufficient when closely related organisms are compared (Palys et al. 1997). Furthermore, Palys et al. (1997) suggested that protein-encoding genes may be more discriminative than those encoding rRNA, while the analysis of two or more unlinked loci would prevent bacterial misclassification due to possible homologous recombination with other taxa. In this study, I tried to use highly conserved genes, such as *rpoB* and *hsp65*, in order to obtain a better identification of *Vibrio* and *Mycobacterium* species. Unfortunately, I did not succeed. Therefore, further molecular analyses are needed to

understand the epidemiology and pathogenicity of the *Vibrio* and *Mycobacterium spp.* identified in this study, both on fish and humans.

In the summer of 2018, there was an outbreak of *M. marinum* in three out of the seven fish cages examined. The death rates ranged between 5.7% - 13.5%, and some of the fish who survived showed clinical signs for the pathogen or no signs at all. *M. marinum* was isolated from the kidney and the spleen of both juvenile and adult fish. There was no evidence of the disease in 2017. A possible explanation is that in 2017 each cage was populated with ~ 350 K fish, in contrast to 2018 where each cage was populated with ~ 470 K fish. These conditions might have led to the disease outbreaks. As farmed fish are monitored regularly, they can be used as sentinels to evaluate pathogen exposure in the aquatic environment. However, this approach has utility only if farmed fish are susceptible to the pathogen and enter the marine environment free of the pathogen of interest. In addition, occurrence of disease in farmed fish populations does not necessarily imply occurrence of the same disease in wild populations.

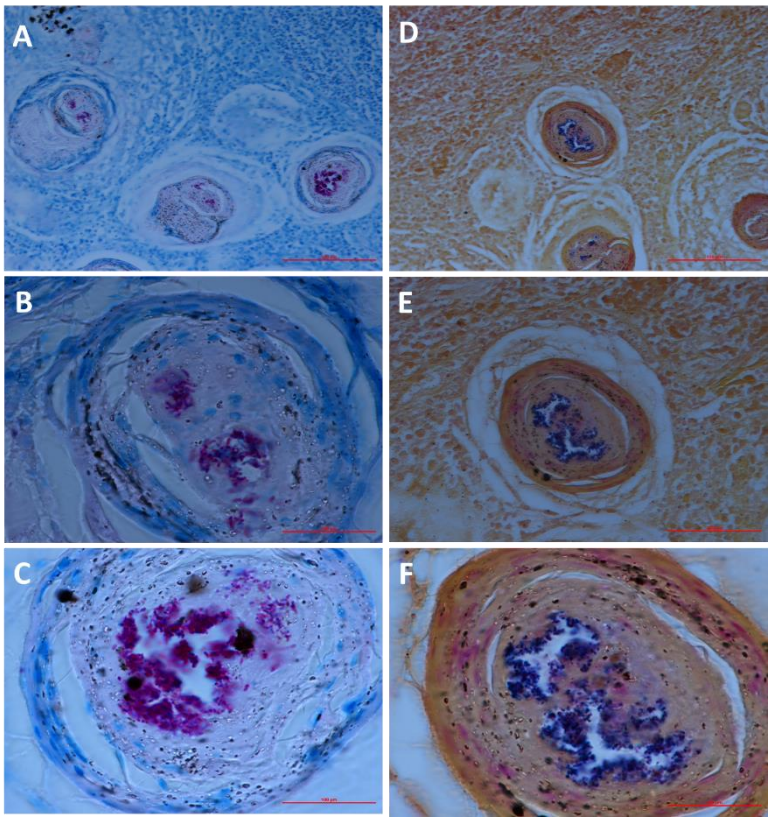

Figure 9: Histopathology of a granulomatous lesion in the spleen of *Sparus aurata* infected by *M. marinum*. (A-C) gram stain, (D-F) 'ziehl neelsen' staining. Inset bar = 100 µm. (Histopathology by A. bouznach**)**

It is difficult to evaluate the health effect of escapees on the ecosystem without taking into consideration the qualitative aspects of wild fish assemblages around farms. Cross-contagion between farmed and wild fish species with shared pathogens may occur (Diamant et al. 2007) either through movements of individual fish or through species-specific migrations (Butler 2002; Krkošek 2010). Connection among farms and other marine areas of interest through wild fish movements have been demonstrated both in Norway (Uglem et al. 2009) and in Mediterranean fish farms (Diamant et al. 2007).

Unlike parasitic pathogens, bacteria seem to exhibit higher potential to spread between wild and farmed fish. This is likely because the ecological barriers that exist for parasite transfer do not represent a great obstacle for bacteria (Arechavala-Lopez et al. 2013). Firstly, bacteria are almost always present on the skin surface of fish. Secondly, bacterial diseases are usually treated by non-professional staff at the farms, and consequently involve increased risk of developing resistance and more pathogenic strains. Finally, bacteria are often generalists and do not need wild conspecifics to spread from farmed fish (Arechavala-Lopez et al. 2013).

There is a potential risk of pathogen transmission through movements of escaped and wild fish in Mediterranean fish farming areas, but actual transmission has been documented only in a handful of cases (Raynard et al. 2007). Due to technical or operational malfunctions, infected farmed fish may escape and, in theory, spread pathogens to other cages/farms and wild fish as well. Furthermore, infected wild fish might also transfer pathogens to the farmed fish (Arechavala-Lopez et al. 2012). This co-infection process leads to a large variety of shared pathogens among wild and farmed fish, while the various pathways of pathogen transmission increase the potential for infection and render epidemiological risk management difficult (Raynard et al. 2007).

Further research on molecular mechanisms of disease transmission in aquaculture and marine environments, as well more holistic analyses of pathogenic events in the Mediterranean Sea, are needed to clarify the potential of transmission of pathogens from aquaculture to the marine ecosystems.

5. Conclusions

As Mediterranean aquaculture and mariculture are expected to increase in the near future, pathogens like Vibriosis and Mycobacteriosis remain important infectious diseases of wild and cultured finfish, and should be extensively studied and investigated. *Vibrio* and *Mycobacterium* prevalence in asymptomatic fish may indicate that they can serve as carriers and infect other susceptible species. As many *Vibrio* and *Mycobacterium* species infect fish and also humans, the potential for zoonotic infection presents an additional challenge. Although these diseases have been studied in fish for over a century, basic questions about their pathobiology, including transmission and host defense mechanisms remain unknown. Additionally, effective prophylaxis, control measures, and non-lethal diagnostics require research and development. However, with the advent of modern molecular detection methods, epidemiological techniques, and vaccinology, considerable potential exists for the improvement of our understanding and control of those diseases in the future.

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7. Supplementary

Table S1: List of *Mycobacterium* positive results with GenBank accession numbers.

Host species	Tissue	Year	Accession number
Nemipterus randalli	Liver	2017	MN602974
Nemipterus randalli	Kidney	2017	MN602975
Nemipterus randalli	Liver	2017	MN602976
Mullus surmuletus	Kidney	2017	MN602977
Nemipterus randalli	Kidney	2017	MN602978
Nemipterus randalli	Kidney	2017	MN602979
Sparus aurata	Kidney	2018	MN602980
Sparus aurata	Kidney	2018	MN602981
Sparus aurata	Spleen	2018	MN602982
Sparus aurata	Spleen	2018	MN602983
Sparus aurata	Spleen	2018	MN602984

Table S2: List of *Vibrio* positive results with GenBank accession numbers.

Host species	Tissue	Year	Accession number
Saurida lessepsianus	Kidney	2016	MN602985
Mullus surmuletus	Kidney	2016	MN602986
Mullus surmuletus	Kidney	2016	MN602987
Nemipterus randalli	Kidney	2016	MN602988
Nemipterus randalli	Kidney	2016	MN602989
Nemipterus randalli	Kidney	2016	MN602990
Nemipterus randalli	Kidney	2016	MN602991
Nemipterus randalli	Kidney	2016	MN602992
Nemipterus randalli	Kidney	2016	MN602993
Sardinella aurita	Kidney	2016	MN602994
Sardinella aurita	Liver	2016	MN602995
Sardinella aurita	Kidney	2016	MN602996
Sardinella aurita	Liver	2016	MN602997
Sparus aurata	Liver	2017	MN602998
Mullus surmuletus	Liver	2017	MN602999
Mullus surmuletus	Liver	2017	MN603000
Sparus aurata	Liver	2017	MN603001
Sparus aurata	Kidney	2017	MN603002
Sparus aurata	Liver	2017	MN603003
Sparus aurata	Kidney	2018	MN603004

זיהוי ואפיון מולקולארי של ויבריו ומיקובקטריום בדגי בר ודגי כלובים בישראל

יעל רגב

תקציר

דגי בר ודגי כלובים יכולים להכיל מינים רבים של פתוגנים (גורמי תחלואה), ושכיחותם של פתוגנים שונים משתנה מאוד בין מינים ושלבי חיים שונים. על אף שהחקלאות הימית בים התיכון הוא ענף צעיר יחסית, דווח כי מחלות דגים גורמות לבעיות ותמותות ניכרות בקרב החוות. באופן כללי, פעילות החוות והתכנון הגלוי של מערכות חקלאיות ים-תיכוניות מאפשרות העברת פתוגנים זיהומיים בתוך ובין המתקנים. מגוון רחב של פתוגנים ימיים מחקלאות ימית מתועד היטב, אך חסרים נתונים בסיסיים ומידע לגבי שכיחותם של פתוגנים באוכלוסיית דגי הבר.

מחקר זה מתמקד במיקובקטריום ובויבריו, שניהם חיידקים הידועים כגורמים מרכזיים לתמותת דגים, לעיתים עד כדי היותם גורם מגביל. מיקובקטריוזיס, הנגרמת על ידי Non-Tuberculous Mycobacteria (NTM), היא בין המחלות הכרוניות ביותר של בעלי חיים ימיים, וחלק מהמינים אף עלולים להיות מאוד ויראליים וזואונוטיים (מועברים באופן טבעי בין בעלי חיים בעלי חוליות לבני אדם). בנוסף, למיקובקטריוזיס בדגים ישנן השלכות כלכליות משמעותיות במיוחד בענף החקלאות והדייג מכיוון ומחלות זיהומיות עלולות להקטין משמעותית את הייצור והסחר. הפתוגן הנוסף שנבדק הוא ויבריו, חיידק גרם שלילי בעל צורת מתג מעוקל המופיע באופן טבעי במערכות ימיות, אסטואריים ומים מתוקים ברחבי העולם. הם תופסים בתי גידול שנעים בין הים העמוק לסביבות מימיות רדודות. מינים מסוימים כוללים פתוגנים של בני אדם ובעלי חיים המסוגלים לגרום לדלקות במערכת העיכול, זיהומים עוריים, כולרה ואלח דם. זמן קצר לאחר התפרצות של המחלה בחוות הדגים, ישנה עלייה חדה בשיעורי התמותה.

במחקר זה, נדגמו 210 דגי בר במהלך שנתיים (2016-2017) לצורך זיהוי ואיפיון רמת נגיעות החיידקים ויבריו ומיקובקטריום באמצעות שיטות מולקולריות. הדגים שנלקחו היו ממינים מהגרים, שמקורם בים סוף (מינים לספסיים) ומינים מקומיים לים תיכון, כולם בעלי חשיבות מסחרית וסביבתית. התוצאות הראו שכיחות כוללת גבוהה יותר של ויבריו בדגי הבר שנדגמו בשנת 2016 לעומת השכיחות הכוללת ב- 2017 (F=5.91, P=0.031) .

בנוסף, נבדקו 72 דגי דניס (*Sparus aurata*) במשך שנתיים (2017-2018) מחוות דגים ישראלית הממוקמת בים הפתוח במטרה להעריך אפשרות הדבקה בין אוכלוסיות דגי בר לדגי חקלאות ולהפך. התוצאות הראו שכיחות גבוהה יותר של מיקובקטריום בשנת 2018 (F = 9,943, P = 0.002), בעוד שב- 2017 לא נראתה נוכחות של פתוגן זה כלל.

מחקר נוסף נערך במטרה לאפיין מיני ויבריו ומיקובקטריום ושכיחותם בחמשת מיני הדגים. *Nemipterus randalli* ו- *Mullus surmuletus* הראו שכיחות גבוהה של שני הפתוגנים ביחס לשאר מיני הדגים שנבדקו. תוצאות אלו עלולות לרמז כי המינים הללו יכולים לשמש כנשאים ועלולים להדביק בצורה אופקית מינים רגישים אחרים, החיים בסמיכותם. הניתוח הפילוגנטי של מקטעי הויבריו שנמצאו בדגים הראה חלוקה לארבע קבוצות שונות, כאשר באחת

הקבוצות היו דגים הן מהבר והן מהכלובים, מה שעשוי לרמז על העברה ספונטנית של הפתוגן ביניהם. כל קטעי המיקובקטריום שזוהו התחלקו לשלוש קבוצות שונות, כאשר דגי הכלובים היו בקבוצה נפרדת משאר דגי הבר.

מחקר זה מדגיש את החשיבות של המשך סקרי ניטור על מנת לענות על המחסור הקיים במידע על שכיחות פתוגנים באוכלוסיות דגי הבר. מידע זה יאפשר להבין טוב יותר את הדינמיקה של העברת פתוגנים שונים בין אוכלוסיות שונות בבר ובינם לבין מינים של חקלאות ימית.

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הפקולטה למדעי הטבע

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