Pathological Findings in farmed *Sparus aurata* with Special Attention to Parasites of the Gills, Fins and Skin.

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1. INTRODUCTION

The consumption of seafood is a human practice that dates back to ancient times, when communities lived as hunter-gatherers, travelling from one region to another. Out of necessity, humans had to adapt in order to survive and hence the skill of fishing was developed and fish became a major source of sustenance. The practice kept evolving throughout the ages and has climaxed into the modern aquaculture methods, trying to reduce the detrimental effects of overfishing which is a direct result of the global increase in seafood demand. The vast majority of fish are of the class osteichthyes, which is an extremely diverse and abundant group consisting of over 30,000 species. Taxonomically, it encompasses all fish that have bone skeletons, therefore excluding all fish that have a cartilaginous skeleton. It is also the largest class of vertebrates in existence today (Kotpal 2009).

Sparus aurata (Linnaeus 1758), also known as gilthead sea bream in English and ‘awrat’ in Maltese, has earned its scientific name *Sparus* (which has given the whole family of Sparidae its name) due to their dorsal fin having strong spines resembling barbed spears. The second part of the scientific name is *aurata*, derived from the presence of a gold band marking between its eyes (Clare 2012). The species is widely regarded as one of the most important fish farm species in intensive rearing systems. Initially, farming mainly involved capturing juveniles from open waters but now most of the production comes from juveniles produced in technologically sophisticated hatcheries requiring skilled staff, consequently, it is one of the most important staple broodstocks in aquaculture since most of the world’s sea bream is directly supplied from aquaculture centres to the open water fish farms.

Mediterranean countries are renowned for mastering the art of seafood cuisine and sea bream is one of the main dishes in any reputable seafood restaurant. Sea bream is an incredibly versatile foodstuff, with the gilthead sea bream being generally considered the best tasting of the breams. They have a coarse, succulent and quality flesh that is ideal for grilling, baking and frying. Sea bream is sold whole mainly from local fish markets or as fillets from chain supermarkets.

Gilthead sea bream is not the only marketed sea bream species in the world. The Sparidae family comprises of only one *Sparus* genus sea bream but the common tongue has adopted the term sea bream to be Sparidae’s moniker. Other species that are therefore regarded as ‘sea bream’ include common dentex (*Dentex dentex*), common Pandora

(*Pagellus erythrinus*), red porgy (*Pagrus pagrus*) and salema porgy (*Sarpa salpa*), the latter being known to cause hallucinations when eaten (Daily Telegraph 2009). Differentiation between the different genera within the family is not a difficult task due to clear phenotypical differences. A clear example would be that *Sparus aurata* has a gold bar marking between its eyes while *Sarpa salpa* has thin gold stripes longitudinally along its body. This makes it easy for a knowledgeable restauranteur to distinguish between the delicious gilthead and the hallucinogenic salema porgy, even if the restaurant's patrons will see no real difference and simply call it a fish.

2. AIM AND GOALS

In this Diploma thesis, the author's main objective is to review the literature available on *Sparus aurata* and the most common diseases observed in the species together with their pathological findings. Special attention will be given to pathological findings on the skin, gills and fins, including whether any parasites where visible both macroscopically and microscopically. The samples are all obtained from Malta and with this fact taken into consideration, the author hopes that this thesis may help shed some light on the parasites and diseases present in Maltese *Sparus aurata* and that it may point the right direction for future research on the matter. As a result, the author aims to identify flaws and suggest improvements, if any, in the husbandry methods used in the rearing of sea bream. This thesis will focus on only one instance of sampling and the author recommends that a future study explores the possibility of setting the goal of identifying the presence of parasites and diseases found in different seasons of the year due to a significant number of the pathogens exhibiting seasonality and sensitivity to temperature.

3. LITERATURE REVIEW

Gilthead sea bream is the only species from the Sparidae family that has dominated the modern fish farming industry. It is common throughout the Mediterranean and is also found along the Eastern Atlantic coasts, from the United Kingdom to the Canary Islands (European Commission 2012). The total aquaculture production of gilthead sea bream was 173,062 tonnes in 2013 (FAO 2015).

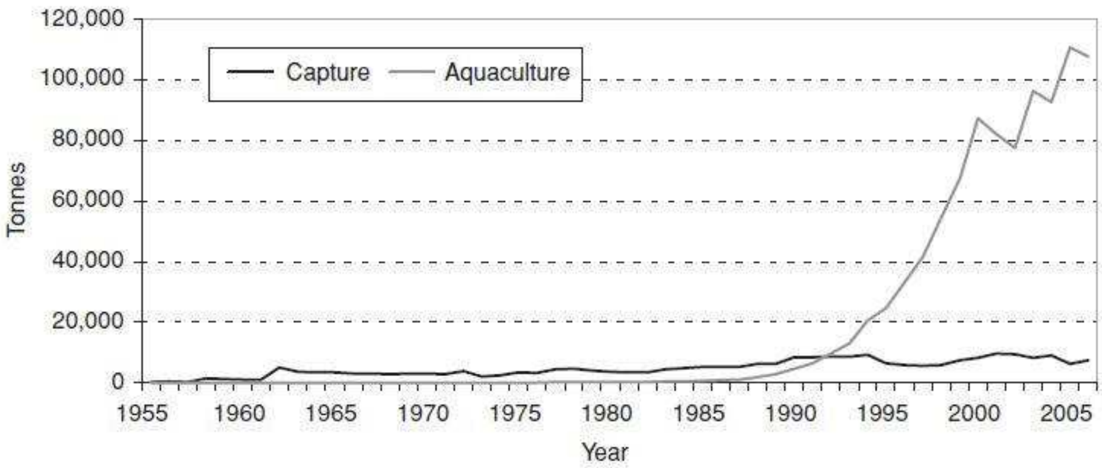

Figure 1: Evolution of World Capture and Aquaculture Production of Gilthead Seabream (FAO-FishStat 2008)

3.1 Taxonomy

Sparidae are fish in the Perciformes order (the largest order of vertebrates, containing over 40% of all Osteichthyes). Taxonomy of this particular order is an arduous and controversial task. This is due to the order being paraphyletic, resulting in various suborders that are not included into the Perciformes order. A paraphyletic taxon group is a group composed of a collection of organisms, including the most recent common ancestor of all those organisms. Unlike a monophyletic group, a paraphyletic taxon does not include all the descendants and all the monophyletic descendant groups of the most recent common ancestor (Hennig 1966).

Kingdom:	Animalia
Phylum:	Chordata
SuperClass:	Actinopterygii
Class:	Actinopteri
SubClass:	Neopterygii
InfraClass:	Teleostei
SuperClass:	Acanthopterygii
Order:	Perciformes
SubOrder:	Percoidei
Family:	Sparidae
Genus:	*Sparus*
Species:	*Sparus aurata*

This taxonomic hierarchy has been retrieved October 13th 2015 from the Integrated http://www.itis.gov.

3.2 Sparidae Biology

Sparidae are mostly coastal dwellers and are classified as tropical to temperate marine species. This means that their presence in colder waters is rarely observed, being one of the main reasons why sea bream is not widely commercialized in the northern hemisphere. Larvae are born in the open sea during October-December and juveniles typically migrate in early spring towards protected coastal waters, where they can find abundant nutrients for their optimal growth and milder temperatures. These young sparids, or smaller species of the family, usually aggregate into schools and reside in shallow waters compared to adult sparids, or larger species of the family, which to prefer solitary lives and deeper stretches of water. Sea breams are found in all forms of coastal waters, be it soft-bottomed, rocky or entire meadows of *Posidonia oceanica*. In comparison, this does not mean that all sparid species do not show any substrate preferences as adults, confining certain species to very specific habitats. A clear example of this is shown in early studies (García-Rubies & Macpherson 1995; Harmelin-Vivien et al. 1995) in which strict shallow water species like *Diplodus* spp. and *Sarpa salpa* all shared a similar preference for a substrate consisting primarily of pebbles, but also recruited to substrates of sand or gravel and small or medium blocks and "all juveniles recruited along the shore in very shallow water, less than 2 meters in depth, for 5 of the 6 sparid species studied. One species only, *Diplodus annularis*, recruited in deeper water, between 5 and 8 meters" (Harmelin-Vivien *et al.* 1995). *Sparus aurata* is usually found on rocky and seaweed bottoms, but it is also frequently observed on sandy grounds. The following schematic (Figure 2) exhibits how juvenile sparids extend their habitat and eventually migrate into deeper waters.

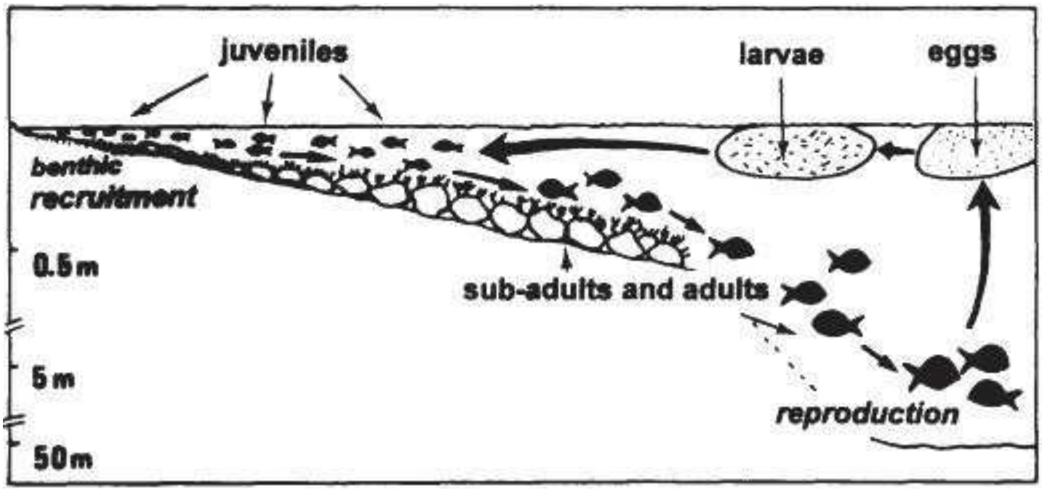

Figure 2: Schematic view of the various habitats occupied by Diplodus spp. on Mediterranean rocky shore during their entire life-cycle (Harmelin-Vivien et al (1995))

Sparids are considered as one of the most diverse families concerning reproductive capabilities and sexuality. It is a unique case in which despite the relatively moderate size of the family, single species differ considerably in their reproductive strategy and exhibit all known reproductive systems known for fish. Buxton & Garratt (Buxton and Garratt 1990) describe how some of the species exhibit protogynous hermaphroditism (changing sex from fertile females to fertile males), others in the family display protandrous hermaphroditism (the exact opposite of protogynous hermaphroditism) and how many of the members of the family exhibit classical gonochorism (separate sexes) in which hermaphroditic tissue is absent in any of the developmental stages. The same study refers to another reproductive group as "late gonochorists". These so-called late gonochorists develop an immature gonad of no discernible sex prior to distinct sex determination with no evidence of sex reversal and may be regarded as rudimentary hermaphroditism. In sea breams, like in fish in general, sequential hermaphroditism is believed to be the most common expression of hermaphroditism and is practiced by the individual reproducing as one sex at a certain stage of life and then as the other sex at a later stage (Hanel & Tsigenopoulos 2011). Adults reproduce in deeper waters and after spawning, the eggs are pelagic and so are the larvae after hatching. The fingerlings migrate closer to shore in search for food and milder sea temperatures. Later on in their lives, the fish begin to sexually mature and benthic recruitment occurs in the shallow waters.

3.3. Gilthead Sea Bream Anatomy and Characteristics

The anatomy of a fish is genetically optimized to reflect its needs and its habitat and one of the constants throughout all fish habitats is the physical presence of water as its medium. Fish are ectothermic creatures, meaning that in their habitat, their body temperature

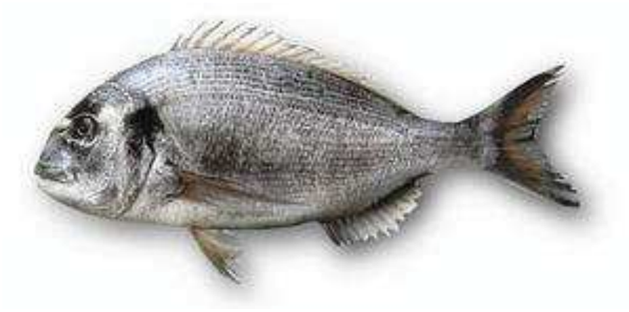

Figure 3: Phenotypical anatomy of Sparus aurata (Cultured Aquatic Species Information Programme. Sparus aurata. (FAO 2005)) *http://www.fao.org/fishery/culturedspecies/Sparus_aurata/en*

As previously mentioned, sparids have various forms of sexuality and gilthead sea bream is no exception. In fact it is one of the most well studied hermaphroditic fish. It is a protoandric hermaphrodite with its breeding season ranging from October to December. During the species' first 2 years of life, the gilthead is a functional male. However, when the fish reaches sizes over 30cm at the usual age of 3 years, the gilthead sea bream loses its functionality as a male and becomes a functional female, spawning transparent spherical eggs that have a diameter of less than 1mm and have an oil drop. Females are batch-spawners that can lay 20,000-80,000 eggs every day for a period up to 4 months. In captivity, sex reversal is conditioned by social and hormonal factors.

3.4. Production Systems of *Sparus aurata*

Before the modern methods of aquaculture came into force, marine fish rearing in the Mediterranean was greatly based on the collection of wild juveniles from the sea either naturally or by trapping. In fact, Cataudella et al explain that gilthead sea bream has been traditionally cultured in Italy in natural geological formations known as "valli" in the northern Adriatic regions in extensive farming systems in which the juveniles are captured and stocked in these lagoons, spurring locals into naming this method of culturing as "vallicoltura". These extensive farming systems acted like natural fish traps to which juveniles migrated from the sea in search for better resources. This method allowed the fish to spend two to three seasons in the valli before reaching sizes that are favourable to the market (Cataudella *et al*. 1995). However, this system was no longer a viable market option after the 1960s since pollution and overfishing began to play a major antagonizing role in the availability of gilthead sea bream fry. This encouraged the industrialization of sea bream, establishing by the end of the 1980s an intensive production scheme based on a reliable and programmed quantity of juveniles and its success has been regarded as the first step towards intensive aquaculture systems in the Mediterranean basin (Basurco *et al* 2011).

The core definition of extensive farming systems is that growth and intensity is limited by the available food supply by natural resources and in the case described above, the availability of captured wild juvenile sea bream. The system is based on the natural migration of sparids. This method is still a viable option in modern days and if these systems are utilised, gilthead seabream reach the first commercial size (350g) in approximately 20 months (FAO 2005). However, it is not regarded as having a significant impact on the market and is unable to provide the supply required to meet the consumers' demand. The best way to run a modern extensive farming operation is to rely on both wild-caught and hatchery-reared juveniles that are introduced into the lagoon in April-May weighing at 2-3g each since the natural system only provides an unreliable and unpredictable source of natural fry (FAO 2005).

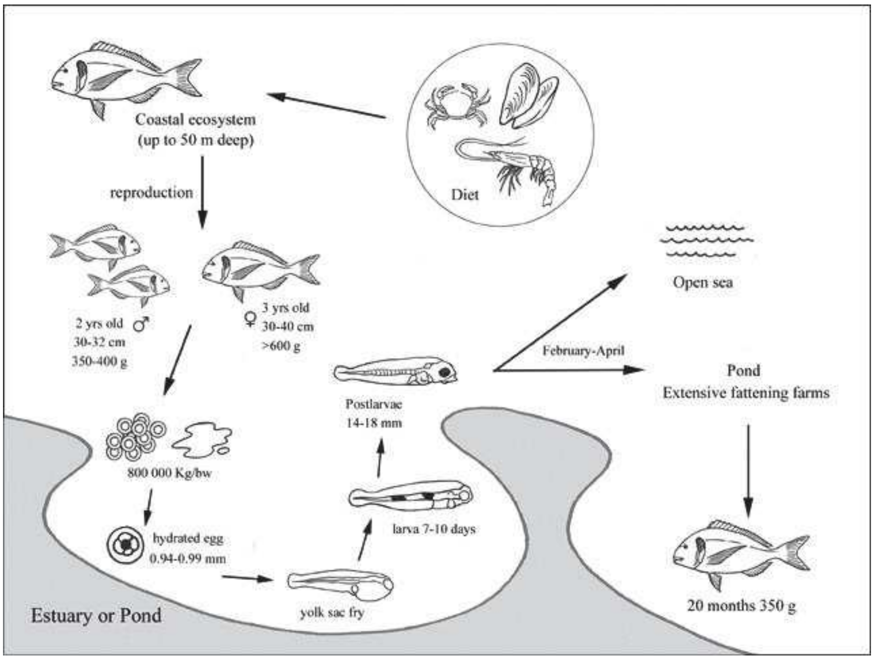

Figure 4: Production Cycle of Sparus aurata - Extensive System (Cultured Aquatic Species Information Programme. Sparus aurata. (FAO 2005))
http://www.fao.org/fishery/culturedspecies/Sparus_aurata/en

On the other hand, the core definition of intensive farming systems is that it allows fish production to be increased almost indefinitely as long as all contributing factors are respected, these being sufficient oxygen, fresh water and food. The only factor that can be disregarded in intensive sea bream farming systems is that cages are placed in the sea, supplementing the fish crop with sufficiently aerated water. This farming system obviously requires more labour and attention especially when one considers that the fish cannot naturally search for their food due to the population density and closed space, making the cost per fish higher that in extensive farming systems. Large production units usually have their own hatcheries but this does not exclude the possibility of stocking up the cage systems with fish from third party establishments. If the gilthead seabream is being reared in tanks, a large quantity of oxygen injection is required to ensure fish survival in such densely populated spaces.

The culture period varies with location and water temperature, but usually it takes between 18 and 24 months for a specimen to reach 400 g from hatched larvae. Commercial size can vary from 250 g to more than 1.5 kg. Tank-rearing pre-fattened 5g gilthead seabream under excellent conditions (18-26^{o}C) will allow the fish to reach first commercial size (350g) in approximately a year. Utilizing sea cages is more economical due to significantly less energy costs as previously pointed out and it is the main method of rearing in the Mediterranean basin. A disadvantage that is hard to control is that temperature cannot be efficiently regulated in open waters, resulting in a longer rearing period to market size. On average, larger pre-fattened gilthead seabream (10 g) reach first commercial size (350-400 g) in about one year, while smaller juveniles (5 g) reach the same size in about 16 months (see Figure 5). Malta's waters are temperate and close to the gilthead's ideal conditions all year round, resulting in larger market weights after 18 months of cage rearing (See Table 2 in 5.1. Results).

When the farmed seabream is scheduled for harvesting, a few days of starvation are required, the length of which being modulated by the water temperature and the feeding rate used. 24 hours of starving is enough when temperatures are over 25^{o}C. Lower temperatures however require a greater number of hours usually ranging between 48-72 hours. After the correct starving procedure has been applied, harvesting can be carried out and any dead or dying fish need to be examined. Fish must be corralled into small areas so that they can be gathered with the use of dipnets, or more effectively, with vacuum pumps. Sea cages should be harvested when weather conditions are permitting so that the safety of the workers is safeguarded.

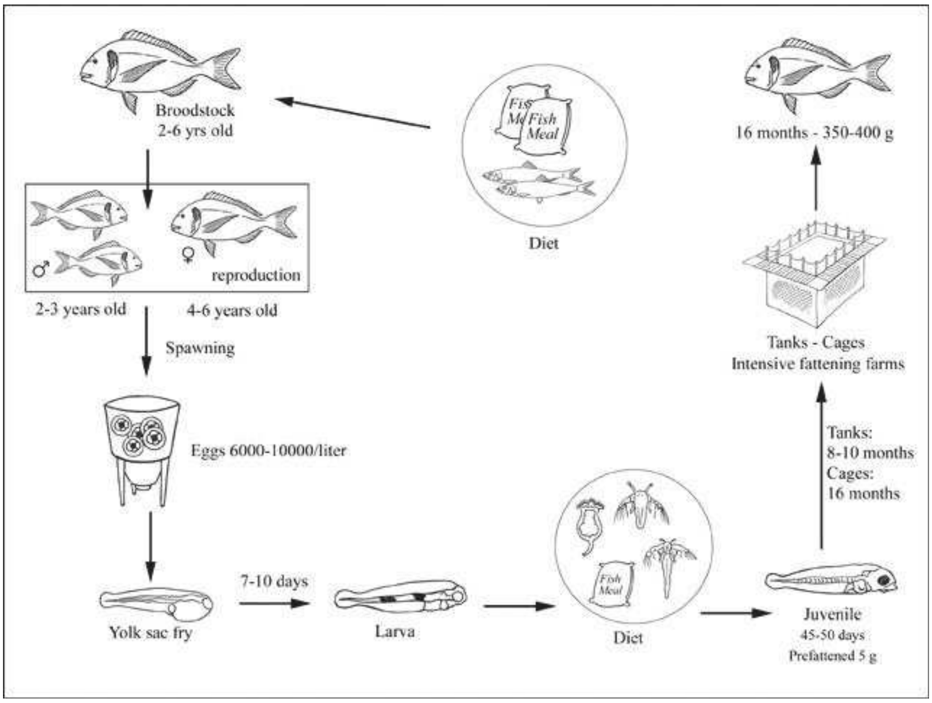

Figure 5: Production Cycle of Sparus aurata - Intensive System (Cultured Aquatic Species Information Programme. Sparus aurata. (FAO 2005))
http://www.fao.org/fishery/culturedspecies/Sparus_aurata/en

The sea bream eggs required to replenish fattening crops are produced by land-based hatcheries, which usually have their own broodstock units of various age groups ranging from 1 year old males to possibly 10 year old females. At the beginning of the spawning season, breeders are transferred to spawning tanks with great care at controlling the sex ratio, normally housed at a 3:1 male to female ratio to ensure a good fertilization rate. It has been found that young males at the end of the spawning period increase the number of older fish that become female. In contrast, the presence of older females diminishes the occurrence of sex reversal in the younger fish. The normal fertilization ratio is 90-95%, a significant amount when one considers a single female can produce more than 1 million eggs in a reproductive season. In turn, the spawning period can be further extended by environmental manipulation, primarily through the use of artificial photoperiods in the hatchery (Sola et al. 2007). 3 to 4 days after hatching, the gilthead larvae generally would have depleted their yolk sac and begin to practice exogenous feeding. The most commonly used live starter feed are the rotifers, *Branchionus plicatilis*. After 10 days, rotifers are integrated with *Artemia salina* until the larvae accomplish metamorphosis. It is common practice that both the rotifers and the *Artemia* are routinely enhanced with lipid preparates to enrich the levels of essential fatty acids and vitamins that promote growth and survivability. Weaning of the larvae is carried out when the fish reach a weight of 5-10mg. Juveniles approximately 45 days old are moved to larger tanks in which they are presented feed at 2 hour intervals from 08.00hrs to 20.00hrs.

3.5. The Effect of Stress, Biotic and Abiotic Factors on Farmed *Sparus aurata*

Among the sparids, gilthead sea bream may show physiological alterations when subjected to environmental stressors, in particular when subjected to low temperatures and may be affected by what is known as the winter syndrome, a multifactorial syndrome prompted by a decrease in temperature below 13°C (Tort *et al.* 1998). Therefore it may be assumed that temperatures below 13°C may be stressful for *Sparus aurata*. When these temperatures coincide with other potential stressors and opportunistic pathogens that may also induce the syndrome, the total synergistic effect may sometimes cause mortality. Sparids are able of coping with large ranges of oxygen concentrations, however, relative oxygen consumption increases with temperature, food intake, activity and stress levels, while it decreases with increasing body size (Tort *et al.* 2010). Water quality is another important parameter that needs to be respected in order to avoid stress. Ammonia is one of the most significant limiting factors for survival and growth and in farming conditions, the main source of ammonia in the water is through fish metabolism while a minor source is through decomposing uneaten feed (MacIntyre *et al.* 2008). However, ammonia in seawater is not a real issue in net pen sea cage systems because it is generally diluted at non-limiting levels by natural sea water currents (EFSA 2008).

High stocking density has been shown to produce chronic stress, poor growth, poor feed utilization and other adverse effects, including possible mortality (Montero *et al*. 1999; Sangiao-Alvarellos *et al*. 2003). Behavioural problems, aggressiveness in particular, can negatively affect survival, growth and welfare of the farmed fish caused by competition for food and space.

3.6. Diseases in *Sparus aurata* and their Pathological Findings

High population densities in relatively enclosed space increases the risk of infections such as lice, fungi, bacteria, protozoa and other parasitic species. *Sparus aurata*, although being a relatively advanced model in aquaculture and is regarded as one of the more resistant farmed species, has its fair share of diseases even though most are rather uncommon.

3.6.1. Viral Infections in *Sparus aurata*

Lymphocystis Disease (LCD) is caused by a virus in the Iridoviridae family. Affected fish develop macroscopic, white, wart-like pseudotumours of extremely hypertrophic dermal fibroblasts however, invasion of visceral organs, such as spleen and heart, can also occur in heavy infections (Schäperclaus 1992; Colorni & Diamant 1995). The disease has been frequently reported in gilthead sea bream aquaculture nurseries around the Mediterranean basin, where the virus is probably endemic, including Italy (Masoero *et al.* 1986) which is geographically close to Malta. The disease occurs mainly at temperatures ranging between 22-27^{o}C, which is followed by a benign and self-limiting course with skin lesions that eventually heal in 30 days and leave no scarification. Mortalities usually occur in young individuals whose breathing, swimming or feeding are severely impaired by excess growths of infected tissue.

Viral Encephalopathy and Retinopathy (VER) is characterized by nervous symptoms ranging from whirling swimming to blindness. In larvae and juveniles, mortality may reach 100% within 1 week from the onset of nervous signs. The virus can persist in marine environments for months (Frerichs *et al.* 2000) and survivors of the disease acquire some degree of immunity, but can also become carriers. A treatment has yet to be devised but vaccinations in experimental studies have shown high levels of protection. Unfortunately, no commercial vaccines are available in the market. VER seems to have a somewhat lesser impact on members of the Sparidae family but it was demonstrated that Gilthead can act as asymptomatic carriers and infect European seabass (Castric *et al.* 2001), which is one of the reasons why they are raised is separate cages and separated with a good mass of water between them.

3.6.2. Bacterial Infections in *Sparus aurata*

Vibriosis is caused by several species of the Vibrionaceae family mainly associated with fish mortalities. Many are facultative pathogens, requiring predisposing factors in order to express their pathogenicity. Vibriosis is characterised by systemic haemorrhagic septicaemia, with anaemic gills, lethargy and erythema at the base of the fins being the typical external signs. In advanced cases, liquefaction of the liver, kidney and spleen are present. Treatment with medicated feeds may be effective if administered in the early stages. *Vibrio spp.* have been isolated frequently from various sparid farms around the Mediterranean and should be suspected when clinical signs suggest its presence. *Vibrio parahaemolyticus* and some biotypes of *Vibrio vulnificus* in particular, hold the potential of being zoonotic, infecting humans through the eating of contaminated or raw fish. These bacteria damage the inner wall of the human intestine, causing diarrhoea and in immunocompromised individuals, wound infections by the latter may lead to fatal septicaemia.

Photobacteriosis, or as previously termed, pasteurellosis due to originally being placed in the *Pasteurella* genus, is caused by *Photobacterium damselae ssp. Piscicida*. This disease rapidly develops into an acute septicaemic condition characterised by a splenomegaly and is also regarded as a pseudotuberculosis due to the granulomatous-like lesions in the spleen and kidney in advanced stages. Temperatures above 21^{o}C increases the morbidity and mortality of the outbreaks, with young fish being the most susceptible. At lower temperatures, mortality decreases but the fish remain carriers (Magariños *et al.* 2001; Toranzo *et al.* 2005). It has caused significant losses in gilthead sea bream and European seabass in the Mediterranean region, making it one of the more important diseases (Kvitt *et al.* 2002; Toranzo *et al.* 2005). Transmission can be through ovarian and seminal fluids from apparently healthy broodstocks (Romalde *et al.* 1999) as well as via horizontal route, with the bacteria being able to infect its host through the gills, the gastrointestinal tract and possibly the skin. Vaccines against photobacteriosis have been developed, some of which being specifically targeted for gilthead sea bream, even though the level of protection is of short duration and not of the highest calibre.

Pseudomonas anguilliseptica is the bacterial agent thought to be behind the haemorrhagic septicaemia present in what is known as the Winter Syndrome due to the recurrent isolation of *Pseudomonas anguilliseptica* from this multifactorial disease, indicating that the bacterium plays a significant role in the syndrome's pathogenesis (Berthe *et al*. 1995, Doménech *et al*. 1997, 1999). External lesions are not very common, possibly finding petechial haemorrhages on the skin and the liver. Other than that, not much else can be observed except for keratitis and abdominal distension, which do not offer much information for disease differentiation.

Tenacibaculum maritimum is a bacterium commonly found in sea water and it is the opportunistic bacteria responsible for flexibacteriosis, also known as "gliding bacterial disease". Fish of any size and any raising environment can be affected, although the disease is more severe in juveniles. The mouth appears as having haemorrhagic erosions, lesions may open in the skin, fins and tail appear frayed and gill rot may develop. The disease can become systemic and the skin lesions can act as ports of entry for secondary invading organisms (Colorni & Padrós 2010). *Tenacibaculum maritimum* has been described in gilthead sea bream, nevertheless when compared to other cultured species such as *Dicentrarchus labrax*, infections in *Sparus aurata* seem to be generally less severe. Coinfections with monogenean gill parasites (*Sparicotyle* and *Furnestinia*) are often observed in gilthead sea bream (Colorni & Padrós 2010).

Hundreds of fish species have been reported to being susceptible to various *Mycobacterium spp.*, suggesting that virtually any fish species may be infected and develop piscine mycobacteriosis. Infection likely occurs by invasion through skin wounds or through the gill tissue, or by ingesting *Mycobacterium*-contaminated materials (Frerichs 1993). External lesions and symptoms are not severe and they include skin ulcers, cachexia and exophthalmia. On the otherhand, internal lesions are of a more serious nature, with the internal organs appearing granulomatous and enlarged. In advanced cases, characteristic whitish nodules appear in the liver, mesenteries and heart. No vaccines are yet available against fish mycobacteriosis. Antibiotics such as rifampicin and clarythromycin are used to treat the condition in valuable ornamental fish but they should be avoided in farmed fish that are to be used as human food. Regardless, if antibiotics are used, the treatment takes months to eliminate the mycobacterium and results are still uncertain. Diseased fish are unmarketable and should be disposed of safely since cases of human infections by aquatic mycobacteria have been reported with increasing frequency. The zoonotic potential of certain *Mycobacterium marinum* strains is considerably high, with Mediterranean countries included in confirmed cases of human infections.

Epitheliocystis is caused by obligate intracellular prokaryotes related to *Chlamydia* and they are strongly host specific with similar pathologies in different hosts. Infections are characterized by the formation of ellipsoid cysts in the host gills and skin, which are actually hypertrophic epithelial cells filled with Gram-negative coccoid microorganisms. Heavy infections and mortalities occur in juveniles and transmission by the natural way is still unclear but contaminated nets and farming equipment may be responsible for the rapid infection spread in culturing facilities.

3.6.3. Fungal Infections in *Sparus aurata*

Ichthyophonus lies on the borderline taxonomical class, Mesomycetozoea, and it is known to cause cutaneous ulcers and granulomatous lesions in highly vascularized organs such as the gills, spleen, liver, kidney and heart. The infection follows a chronic course and susceptibility seems to increase with age, causing the fish to appear emaciated and may lead to a fatal outcome. The pathogen is an obligate pathogen and its spores can survive in seawater for a maximum of two years (McVicar 1999). *Ichthyophonus* is uncommon among cultured fish, however, the potential infection from wild fish species should not be underestimated since no effective treatment has been devised so far.

3.6.4. Protozoan Parasitic Infections of *Sparus aurata*

Velvet disease, which is caused by *Amyloodinium ocellatum*, is one of the most devastating parasitic diseases that has the potential to affect virtually any species of teleost fish in the Mediterranean basin (Colorna & Pedrós 2010). It primarily resides on the gills, with gill damage and osmoregulatory impairment being the likely causes of death, but it can also occasionally attack the skin. Being a dinoflagellate, its life cycle is composed of three stages: the parasitic feeding stage (trophont), in which it firmly anchors to the fish and feeds on its epithelia; the encysted reproductive stage (tomont) in which it the trophonts loosen their attachment and encyst in the substratum; and the free-swimming infective stage (dinospore), in which the highly motile dinospore remains infective for only one week. Due to the nature of the agent's rapid propagation, fish confined in ponds or tanks are quickly overwhelmed by the infection, whereas *Amyloodinium ocellatum* is virtually absent in open water cage systems when applying logic to the same knowledge. Dinospores are susceptible to medicinal treatment but the other stages are not, so time must be given to ensure that all trophonts and tomonts reach the dinospore stage. In light of this, 0.75ppm copper sulphate treatment for a minimum of 14 days proves to be effective (Paperna 1984).

Trichodina spp. are bell-shaped ciliates commonly occurring on the skin and gills of fish worldwide. Trichodinosis is spread via direct transmission and its host specificity is variable, depending on the *Trichodina* species in question, but generally low. They are of no significance on healthy fish, but stressed or debilitated fish are ideal hosts for massive proliferation. Their repeated or numerous adherence and suction eventually damages and erodes the gills. The recommended treatment is the use of formalin since marine trichodinids are sensitive to the chemical. (Colorni & Pedrós 2010)

Cryptocaryon irritans is the parasitic ciliate that causes cryptocaryonosis, also known as Marine White Spot Disease. The clinical signs of cryptocaryonosis include pinhead-sized whitish vesicles, mucus hyperproduction, epithelial hyperplasia, corneal cloudiness, skin discoloration and, with the disruption of the gill structure, severe respiratory distress since osmoregulatory balance becomes increasingly difficult to maintain and gas exchange in severely damaged gills gradually fails. This parasite can virtually infect any teleost fish due to its very low host specificity.

Entoromyxum leei, a myxozoan, is one of the more important endoparasites causing disease problems in marine aquaculture. A chronic infection develops and affected fish become anorexic, develops a foul-smelling extensive necrosis of the intestinal lining and eventually a fatal outcome is reached, in which a typical knife-edge body shape and a bloated abdomen is observed. The infection shows variable severity and incidence, possibly indicating a genetically based susceptibility to the disease (Palanzuela 2006).

Polysporoplasma sparis is also one of the more important endoparasites observed in gilthead sea bream and its spores are found mainly in the glomerular capillaries of the kidney, but the inflammatory response and disruption of the renal physiology seems to be limited. The mxyozoan has been frequently observed and has been on occasion been associated with poor growth and chronic mortality. Other than that, its pathogenicity seems to generally follow a mild course (Colorni & Pedrós 2010).

The presence of a new microsporidium, *Enterospora nucleophila*, is believed to be responsible for an emaciative syndrome observed in farmed gilthead sea bream (Sparus aurata). The microsporidium is mainly found in the intestinal mucosa with clinical signs including anorexia, cachexia and pale internal organs. Severe histopathological damage occurs in intense infections and this microsporidian is considered a serious emerging threat in sea bream production (Panazuela *et al.* 2014). In recent times, quite worryingly, it has been causing problems in Malta's sea bream that is still in its juvenile stages (Abela *et al.* 1996).

3.6.5. Platyhelminth Parasitic Infections of *Sparus aurata*

Class Monogenea is comprised of mostly ectoparasitic flukes, the most frequently encountered worm in mariculture, all hermaphroditic and no intermediate hosts are required to complete their life cycle. Free-swimming ciliated larvae hatch from the eggs and if they fail to locate a suitable host within a few hours, they die. Flukes either draw blood or feed off the tissues of the host, causing irritation, hyperplasia, haemorrhage and anaemia. A 1-hour formalin treatment 150-200ppm repeated a few days later is effective in ridding the fish of most of these worms. *Furnestinia echeneis* is frequently observed on gilthead sea bream in the Mediterranean and they are usually encountered at the distal extremities of the gill lamellae. Recent studies show that rates of infestation are very low and the fluke was never found in abundance. All fish appeared to be in good health and no evident symptoms were exhibited. The investigation done on Corsican gilthead farms demonstrated that the rate of infestation showed a correlation to seasonal variations, with the increasing presence of *Furnestinia echeneis* directly related to temperature (Antonelli L, Quilichini Y, and Marchand B 2010). *Sparicotyle chrysophrii* is another common monogenean pathogen for cultured sea bream in the Mediterranean, with its haematophagous activity producing severe anaemic conditions in the winter seasons. Secondary infections by *Tenibaculum* and Vibrionaceae are commonly observed (Colorni & Pedrós 2010).

Class Digenea are endoparasitic platyhelminths that require at least one intermediate host to complete their life cycle. All major groups of vertebrates serve as hosts for the adult stage and in fish they can be found as encysted larval or juvenile stages and as free adults. Small free-swimming miracidia hatch from the eggs of the fluke, they survive for several hours, during which it must locate an adequate first intermediate host that is always a bivalve or a mollusc. Free-swimming cercariae then leave the first host and a have a 24 hour time window to find a second host in which they develop into cysted or uncysted metacercariae. When the intermediate host is predated by the definitive host, the metacercaria develops into an adult but these are not considered particularly harmful for the definitive host. Acute infections by the cercariae have occasionally been observed due to severe damage in the host tissues during penetration and migration but once encysted, metacercariae do not produce further tissue damage. However, the intense melanisation reaction around the cysts renders the affected fish unmarketable (Colorni & Pedrós 2010). The general picture in case of Sparids is that they are intermediate or definitive hosts. In Malta's scenario, the sanguinicolid trematode *Cardicola aurata* is relatively widespread among the local farm crops (Brinch-Iversen J. personal observation).

3.6.6. Parasitic Crustacea Infections of *Sparus aurata*

A large number of copepods and isopods parasitize the integument of fish but other species that move more freely can cause irritation and macrophage infiltration. Gill filaments can be severely damaged and skin haemorrhages typically occurring in heavy infestations, however, copepod presence on fish has only rarely been associated with mortalities. The morphology of the Copepod adult stages can be highly adapted for parasitic functions with caligalids having the ability to spread to epizootic proportions. Mucus and epidermal tissue seems to be the Copepod's main diet. These "sea-lice" can be controlled mainly with formaldehyde, organophosphate insecticides, hydrogen peroxide, ivermectin and others; however, some degrees of chemical resistance is being exhibited, while the therapeutic dose and the host's safety margin are often dangerously close. (Colorni & Pedrós 2010).

Parasitic isopods are grossly visible on skin, in the mouth or gill chamber. Because of their very large size, isopods can cause considerable damage with their biting, sucking mouthparts and hooked appendages. Suborder Flabellifera consists of isopods that are up to 6 cm in length, and include the family Cymothoidae which constitutes the great majority of isopod parasites in fish. Fish are vulnerable to the larvae of the Gnathiidae family, of which most are highly specialized to specific hosts but some like *Gnathia piscivora* are indiscriminate in the host they attack and are regarded as potentially dangerous pests. Injured or stressed fish are particularly susceptible and even more so if they are in cage reared facilities, where high fish densities provide optimal transmission conditions, and as of yet, no effective antiparasitic treatment has been discovered. The most common isopod affecting gilthead sea bream in the Mediterranean cage-reared gilthead sea bream is *Ceratothoa parallela* (Papapanagiotou & Trilles 2001) as well as *Ceratothoa oestroides*, which has become a major pest primarily for sea bass but also for sea bream.

4. Materials and Methods

4.1 Sampling

A total of 30 specimens of *Sparus aurata* were collected and examined for this thesis. A random sample of 10 specimens was obtained and examined in July 2015 from the Malta Aquaculture Research Centre, San Luċjan. The research centre raises gilthead larvae from the broodstock and sells the fry to fish farming companies when they reach 2g of body weight. This sample was taken to act as a control since the Research Centre takes excellent care in controlling the environmental variables, virtually asserting the production of healthy, non-infected fish. The batch from which this sample was taken is P15/BRFL03, which was due to leave the Centre's hatchery and start its life in the open water cage systems in the southern region of Malta.

Figure 6: Sparus aurata weighing 2g housed in an indoor tank. (Robert Schembri 2015)

Three months later, another random sample of 10 specimens was captured from the same batch that was residing in a pen in the Delimara/Marsaxlokk area, in October 2015. This was done to investigate whether any parasites or diseases had established themselves in the farming crop since leaving the Research Centre's controlled environment.

Since batch P15/BRFL03 was still growing and had months ahead of it before reaching market weight, a second sampling was done from another batch that had reached market weight. This batch was being harvested in October 2015 and the fish farming company provided me with another random sample of 10 sea breams from their crop before being placed onto the processing line. The market weight batch will them be introduced to the local market with a large portion of the produce exported to Italy.

In all instances, the freshly caught fish were immediately transported to the laboratory, where they were inspected, dissected and examined. The samples were transported by using polyethylene chill boxes since the laboratory was only 10 minutes away by car, meaning that no freezing processes were used in the transporting of these samples.

Figure 7: The Area where the Open Water Cage Systems are Located. Visible on Close Inspection. (Google Maps 2015)

4.2. Visual Inspection

The first step in visual inspection is assigning an ID number to each specimen and then measuring the length and weighing the body weight of each specimen. The skin was inspected with the naked eye for any macroparasites or suspicious lesions. If any macroparasites were found, they were collected using a forceps for possible further examination. The fins were also inspected with the naked eye for the presence of any macroparasites or suspicious lesions. If any macroparasites were found, they were collected using forceps for possible further examination. The mouth was opened and visually inspected to see if there were any isopod parasites present. If any were found, they were collected using forceps for possible further examination. Lastly, the operculum of either side was lifted in order to expose the gills, allowing the author to examine the gills with the naked eye for macroparasites and suspicious lesions. If any macroparasites were found, they were collected using forceps for possible further examination.

4.3. Dissection

An incision along the belly starting from the anus all the way to the opercular aperture was done to expose the internal organs. Most of internal organs were inspected macroscopically without any intention of further investigation. The swim bladder was punctured so that the kidney located above it could be macroscopically investigated. A sample was excised from both gills and prepared on a glass slide and pressed down with a glass cover slip for microscopic inspection using a Zeiss Axioskop 2 plus Microscope. This procedure was carried out on all specimens.

Figure 8: Method of Dissection. (Robert Schembri 2015)

5. Results and Discussion

5.1. Results

The control sample taken from the Malta Aquaculture Research Centre resulted in very predictable results. Their length ranged from 6.0cm to 7.7cm and their weights were all above the required 2g before housing in the open water cage systems. Absolutely no macroscopic abnormalities were observed and it can be said that virtually all deaths that occur in the rearing tanks are not linked with parasitic infections.

The random sample obtained from batch PF15/BRFL03 three months later had results that could have been more promising when considering the title of this thesis. Their length ranged from 10cm to 13.5cm and their weight ranged from 14g to 46g (See Table 1). All of the specimens resulted negative to macroparasites except for one, which had a possibly parasitic copepod resting on the skin. Upon microscopic examination of the gills, 2 specimens from the sample seemed to have the presence of trematodes in the gills being either *Furnestinia echeneis* or *Sparicotyle chrysophrii*, monogeans that are known to usually host on *Sparus aurata,* the latter being the more unlikely one since its haematophagous activity usually causes anaemic conditions as previously explained.

Table 1: Results of Visual Inspection of Batch PF15/BRFL03 Conducted in October 2015

Batch PF15/BRFL03 Gilthead Sea Bream Visual Inspection (October)								
Specimen Number	**Length**	**Weight**	**Gills Macroparasite / Lesion Count**		**Fins Macroparasite / Lesion Count**		**Skin Macroparasite / Lesion Count**	
PF15-1	13.0cm	37g	0	0	0	0	0	0
PF15-2	12.5cm	29g	0	0	0	0	0	0
PF15-3	10.5cm	18g	0	0	0	0	0	0
PF15-4	13.0cm	29g	0	0	0	0	0	0
PF15-5	11.5cm	21g	0	0	0	0	1	0
PF15-6	14.5cm	46g	0	0	0	0	0	0
PF15-7	11.0cm	21g	0	0	0	0	0	0
PF15-8	11.5cm	22g	0	0	0	0	0	0
PF15-9	10.0cm	14g	0	0	0	0	0	0
PF15-10	12.5cm	24g	0	0	0	0	0	0

The sample composed of market weight fish from another batch had a more evident presence of parasites. Sea lice were more abundant than in the younger generation but pathological findings were virtually invisible and the fish themselves, healthy. Their length ranged from 27.7cm to 31.5cm and their weight ranged from 412g to 628g (See Table 2). The majority of sea lice were found on the skin of the specimens but some could be found on the fins as well. The author of this thesis did not investigate what species the sea lice were but it can be safely said that they were copepods and definitely not isopods. Once again, no macroparasites or evident lesions were visible on the gills. However, upon microscopic examination of the gills, 2 fish from the sample had monogeans present, being either *Furnestinia echeneis* or *Sparicotyle chrysophrii,* the latter being the more unlikely one since its haematophagous activity usually causes anaemic conditions as previously explained. Another specimen (MW-4) may also have exhibited the presence of monogeans, however, the microscopic examination was not confirmatory. Specimen MW-1 had some abrasions on the skin of the flanks, although there is doubt on whether it was of any pathological significance. Specimen MW-6 had erythema on its ventral side on the belly region while specimen MW-7 had what looked like small petechiae on the left pectoral fin that was more prominent than the others present as natural post mortem findings on other specimens. The only fish among all the samples to have an evident lesion in an internal organ was MW-2: the spleen weighed 2g and measured 22mm with 5+ round, fluid-filled nodules (See Figure 9).

Table 2: Results of Visual Inspection of Market Weight Batch Conducted in October 2015

Market Weight (MW) Gilthead Sea Bream Visual Inspection								
Specimen Number	**Length**	**Weight**	**Gills Macroparasite / Lesion Count**		**Fins Macroparasite / Lesion Count**		**Skin Macroparasite / Lesion Count**	
MW-1	31.0cm	596g	0	0	0	0	0	1
MW-2	31.5cm	557g	0	0	0	0	0	0
MW-3	31.0cm	532g	0	0	0	0	0	0
MW-4	30.5cm	607g	0	0	1	0	1	0
MW-5	31.5cm	628g	0	0	0	0	0	0
MW-6	27.7cm	412g	0	0	0	0	3	1
MW-7	31.0cm	560g	0	0	2	1	0	0
MW-8	30.0cm	566g	0	0	0	0	0	0
MW-9	31.5cm	583g	0	0	1	0	0	0
MW-10	30.5cm	585g	0	0	0	0	3	0

Figure 9: Cystic Spleen Found in Specimen MW-2 (Robert Schembri 2015)

5.2. Discussion

The results seem to indicate that the general population of the farm crops in Maltese waters seem to be healthy without parasites of the gills, fins and skin causing pathological findings that slowly destroy the farmed species. The results, however, must not be taken as representative of all fish farming regions in Malta throughout the entire year. In fact, this experiment tackles the parasitic and pathological findings in October, which is a particular month of the year in which temperatures and climate begin to change from summer conditions to autumn conditions. Most parasites are seasonal creatures and this could be a factor in the lack of parasites on the samples obtained. Further studies are encouraged on a seasonal basis so that a complete picture may eventually be described when it comes to intensive *Sparus aurata* culturing in open cage systems. Since the samples obtained were living members of the sparid population being reared, the infestations may have not developed enough and in retrospect, when comparing to other studies, the pathological findings are less marked and abundant than the studies that had access to freshly naturally dead fish in which the disease had fulfilled its course. There are currently no studies the author can compare to when it comes to sea bream rearing in Malta so comparisons will be made with studies that were conducted in waters of other countries of the Mediterranean basin.

The copepods present on the samples did not seem to have much pathological effect on the specimens. Sea lice feed on the mucus of the host as well as blood and tissue, and their feeding and attachment are responsible for any primary pathological lesions and diseases that develop. The severity and the number of the copepods seem to correlate with each other with the size and age of the fish, the health status of the fish and the stage in which the particular copepod species is in. Although the author of this thesis has not specified the species of the copepods observed, members of the family Caligidae and Ergasilidae are the most commonly reported species throughout the world, the latter having the greater disease potential of the two (Johnson *et al*. 2004). Johnson *et al.* state that parasitic copepods have a major impact on marine aquaculture, there are limited reports of instances in which disease has manifested as a direct consequence and even less reports on methods of disease treatments used. Their pathological impact on intensive farming is even further diminished when one considers that there is no evidence to suggest that sea lice can act as vectors for fish diseases. As with any other infective disease, stress management and welfare practices that maintain optimal fish health are very likely to reduce the effect of parasitic copepods since reduction in these parameters are regarded as predisposing factors.

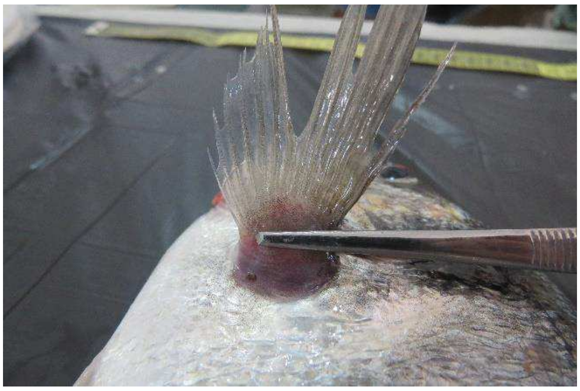

Figure 10: A Copepod was nested underneath the fin of a specimen in the Market Weight batch. (Robert Schembri 2015)

The gills are predilection sites for infestation of the other most common crustacean causing parasitic infections in fish, the isopoda, and negligible incidents in which it attaches to the skin. In Egypt, it has been found that in summer, incidence of isopod infestation may affect 2 sea bream out of every 3 (Khalil *et al.* 2014), which is contrasting with the data observed in Malta so far. It is virtually impossible to miss an attached isopod due to their large bodies protruding from under the host's operculum. The increase in isopod prevalence is another serious problem which is affecting a number of fish-farms (Athanassopoulou *et al.* 2009) and should not be underestimated when it reaches Maltese waters in more numbers.

Trichodina spp. and *Ichthyobodo necatrix* seem to be common recurring parasites in sea bream and their presence in Maltese *Sparus aurata* in common knowledge among fish farms and pathologists, even though there are no scientific papers confirming this. This is because they are considered to be of little bother since they are normally easily controlled with formalin baths and not really of significant concern. In contrast with local claims, heavy infections with *Trichodina spp.*, *Furnestinia echeneis* and epitheliocystis have caused mortalities among *Sparus aurata* cultures in previous instance such as in the Red Sea and France (Paperna & Baudin Laurencin 1979). Years have passed since Paperna and Baudin Laurencin made these findings and the parasites' pathological significance may have been well reduced with the use of modern aquaculture technology but the potential risk is always there if the agents and their pathologies are allowed to fester.

This thesis with regard to monogeans, seems to agree with Papoutsoglou *et al.* (1996), with the most frequent parasite observed being a monogean. However, unlike Papoutsoglou *et al.*'s observation in which *Furnestinia echeneis* levels decreased with bream size, no clear relationships between parasite levels and fish size were observed. Their study can be safely relied on since two years of data are analysed in which water quality and ectoparasites infesting gilthead sea-cages are recorded in comparison to the total 30 specimen investigated in this thesis. Although digeneans were not explored in this thesis, these intestinal endoparasites have been previously recorded on wild sea bream, such as *Monorchis monorchis* and the acanthocephalan *Telosentis exiquus*. The cestode *Callibothrium sp.* was also recorded by Papoutsoglou (1976). The digeneans and cestodes, however, are unlikely to be found in intensively farmed fish since the availability of live prey, some of which act as intermediate hosts, is limited to naturally occurring zooplankton and cage fouling. Once again, this thesis is in agreement with Papoutsoglou *et al.* since at the observed levels of parasitism, pathogenic effects and lesions were not physically evident on the gilthead sea bream.

It has been brought to my attention by a local fish pathologist (Brinch-Iversen J. personal observation) that the major problems in Malta's sea bream population comes from endoparasites, mostly Myxosporea but also Microspora. Of the former it is mainly *Enteromyxum leei* and *Polysporoplasma sp.*, and of the latter it is most recently and quite worryingly *Enterospora nucleophila* in juvenile sea bream. Since this thesis did not have the opportunity to tackle these endoparasites, this information would best be investigated further in a future scientific study so that their effect on sea bream can be quantified and backed with actual published data.

Figure 11: Microscopic Inspection of Gill Samples (Robert Schembri 2015)

Parasite control in aquaculture requires a keen awareness of parameters and if the data produced from this thesis had to be representative of the fish farming efficiency of the managerial company, then it is following an efficient protocol dealing with parasite prevention and elimination. There is still more that could be done but it is subject to the constraints of economics and environmental protection and sustainability. As global aquaculture continues to expand, the impact of parasitic disease is also likely to grow, meaning that anti-parasitic treatments should be further explored. Before embarking on a course of treatment, it is important to have a proper identification of the causative disease agent. An inaccurate diagnosis can lead either to the true problem going undiagnosed and becoming worse or to an inappropriate course of treatment being used, which is why the author of this diploma thesis decided not to attempt to guess the parasitic agent observed without sufficient data and materials and methods.

6. Conclusion

Although the samples of this thesis represent only a small portion of *Sparus aurata*'s life in the sea cages, the initial parasitic picture of intensively raised sea bream in Malta seems promising, with only 50% of the marketable fish having parasites of minor importance with virtually no zoonotic potential. Further studies are recommended so that a more complete conclusion can be reached on the parasites present, their pathological potential and economic importance since literature on Maltese-reared sea bream is virtually non-existent. Even though some parasites were found on some of the samples, the presence of a parasite does not necessarily mean that it requires treatment. When present in low numbers, most of today's commonly encountered sea bream parasites may cause negligible damage to their hosts unless the host is stressed and immunocompromised, highlighting the importance of good governance of fish farms and hatching facilities.

Strong cooperation and trust is needed between the local Aquaculture Research Centre and the fish farms so that the best possible produce is released into the market while aiming for better economic production for the companies in the same instance. The Research Centre should pay particular attention to the parasites that are encountered on a regular basis and neglected on the basis of funding and passiveness since some of these underestimated pathogens have caused disease in other regions of the Mediterranean, even potentiating more severe secondary infections. Based on the author's personal observation, a very limited range of chemotherapeutants are used in local fish farming. It is suggested that Rogers' and Basurco's textbook on veterinary drugs and vaccines (2009) used in marine aquaculture is explored for other possible preventive and therapeutic treatments (such as ivermectin per os against Isopoda and Copepoda, and ivermectin/levamisole bath against Monogeneans and other helminths) other than simply formalin baths, especially since there is the possibility that this carcinogenic chemical will be prohibited in the European Union in the near future. Having fry raised in precisely controlled environments and having excellent weather and sea conditions in Malta's climate is advantageous, but should not be overly relied on without effective treatments reinforcing the industry.

7. SUMMARY (ABSTRACT)

Although *Sparus aurata* is regarded as being a healthy and resistant fish compared to other farmed fish species, intensively cultured sea bream still have a significant amount of problems with technological mistakes and primary and secondary infections. This thesis explores literature for the different pathologies that can be found in cage-reared gilthead sea bream in the Mediterranean. Special attention is dedicated to the pathological findings and occurrences on the skin, fins and gills found in cage-reared sea bream when they reach market weight and are ready to be harvested, as well as in cage-reared sea bream 3 months after introduction into the pens from the aquaculture centre. All samples have been obtained from the island of Malta and all presented data and conclusions should not define Malta's aquaculture status. This is due to this thesis having a low representative sample, obtained from only one fish farming area, and only one fish farming company participated in the data sampling. Further studies are suggested based on the author's findings. Options on how to tackle the parasites present in the current spectrum of pathogens are given in the conclusion

8. REFERENCES

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