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Athanassopoulou F., Pappas I.S., Bitchava K.

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An overview of the treatments for parasitic disease in Mediterranean aquaculture

F. Athanassopoulou*, I.S. Pappas and K. Bitchava***

*Laboratory of Ichthyology and Fish Pathology, Faculty of Veterinary Medicine,
University of Thessaly, 224 Trikalon str., Karditsa 43100 (Greece)
**Laboratory of Pharmacology, Faculty of Veterinary Medicine,
University of Thessaly, 224 Trikalon str., Karditsa 43100 (Greece)

Abstract. The purpose of this paper is to provide a general overview of important manifested parasitic diseases of Mediterranean fish and their control methods. Currently available treatments practised in Mediterranean mariculture are explained briefly under two main groups: parasites located on the skin and gills, and those located in internal organs. Available pharmaceutical and biological products for the treatment of Monogenea, Isopoda, Copepoda, Cestoda and Protozoa are described, and problems related to their therapy are discussed. The mode of action of these drugs is also explained and new research developments in this area are presented.

Keywords. Aquaculture – Drugs – Parasites – Mediterranean fish.

Passage en revue des traitements des maladies parasitaires en aquaculture méditerranéenne

Résumé. *La finalité de cet article est de présenter un rappel général des maladies parasitaires d'importance qui se manifestent chez les poissons méditerranéens ainsi que leurs méthodes de contrôle. Les traitements actuellement disponibles qui sont appliqués en mariculture méditerranéenne sont expliqués brièvement, en fonction de leur division en deux groupes principaux : les parasites situés dans la peau et les branchies, et ceux qui se trouvent dans les organes internes. Les produits pharmaceutiques et biologiques disponibles pour le traitement de Monogenea, Isopoda, Copepoda, Cestoda et Protozoa sont décrits, avec discussion des problèmes liés à leur thérapie. Le mode d'action de ces produits est également expliqué, et les nouveaux progrès de la recherche dans ce domaine sont présentés.*

Most-clés. *Aquaculture – Produits pharmaceutiques – Parasites – Poissons méditerranéens.*

I – Introduction

Fisheries and intensive fish farming represent very important financial sources in Mediterranean countries. The sudden increase of marine production of fish, in all countries where intensive aquaculture is practiced, has resulted in serious pathological problems. More than five marine species are commercially reared in Mediterranean regions and a wide variety of parasites are recognized as serious pathogens of these fish. These are mostly due to parasites that either retard the normal growth of fish or cause sudden mortalities.

The most common parasites affecting Mediterranean fish are the myxosporeans (especially *Myxidium leei*) that have been implicated in serious losses in cultured sharp snout sea bream and sea bream (Athanassopoulou *et al*., 1999). The increase in prevalence of Isopoda is another serious problem which nowadays affects a number of fish farms (Athanassopoulou *et al*., 2001a). Parasitic metazoa also, under particular conditions, especially in young fish, can cause serious pathological problems and increased mortality.

In contrast to mammalian therapeutics, the use of pharmaceutical substances and particularly antiparasitic is rather limited in fish. Although recently adequate research has been carried out on antibiotic treatments, sensitivity and tissue residues, as well as work on anthelminthic treatment of warm water fish, such as sea bream and bass, is virtually non existent. There are

no licensed antiparasitic compounds for Mediterranean species or official MRL's currently available and all information is extrapolated from coldwater species and especially salmonids. This can cause problems as treatment conditions are very different in these species in terms of environmental (temperature, pH, stability, toxicity to other aquatic animals) and individual fish factors (safety, metabolism, stress, residues, etc.). Therefore, the treatment of parasites in commercial situations is very difficult and should be targeted through a "holistic approach".

II – Brief overview of the main parasitic diseases

1. Ectoparasites

A. Protozoa: Amyloodinium / Oodinium *spp. and* Trichodina *sp.*

These parasites can affect all marine species both in cages and in tanks. They have a direct life cycle that is difficult to treat. They can both cause high mortality, especially in areas with deterioration of the water quality and high temperatures (Bruno *et al*. 1997).

B. Monogenea

These parasites affect mainly fish in cages and have a direct life cycle; eggs are produced and deposited on the net meshing resulting in an accumulation of eggs within the culture system. The most common parasites of sea bass are *Diplectanum* sp., whereas in sea bream both *Furnestinia* sp. and *Microcotyle* sp. are found. (see EAFP Publication: A practical guide for the marine farmer).

C. Isopoda/Copepoda

The copepod *Lernanthropus kroyeri* mainly causes losses in small sea bass in both semi-intensive (lagoons) and intensive cage farming systems as a result of asphyxia and anaemia. The losses in farmed fish can be higher after heavy rainfall and there is evidence that the parasite is associated with degradation of environmental conditions (Theohari *et al*., 1997; Athanassopoulou *et al*., 2001a). Its life cycle is direct (Woo and Poynton, 1995).

a] ***Ceratothoa oestroides***

This is an important isopod pathogen of sea bass and bream in cage culture in the Aegean and Adriatic Sea (Sarusic, 1999; Athanassopoulou *et al*., 2001a). The parasite is found in the mouth, the skin and branchial cavity. The adult parasites are seen in pairs in the buccal cavity and after copulation pullus larvae are hatched in the marsipium of the female parasites and then released to the environment where they undergo several metamorphoses. It is the 2nd stage (pulli II) that is believed to attack cultured fish and this is the stage that causes most problems (Lindsay and Moran, 1976; Mladineo, I., 2003; Vagianou *et al.*, 2006). These pulli II show little specificity in terms of attachment location on the host in contrast to the adult stages that normally attach to the buccal epithelium and cause less damage. Lesions are caused mainly by the copulation activity or are due to the size of the adult parasites.

2. Endoparasites

A. Microsporida

These protozoan parasites form cysts affecting many fish. Sea bream have been reported to be affected by *Pleistophora* and *Glugea* spp. (Mathieu-Daude *et al*., 1992; Athanassopoulou, 1998). They are often located in the muscles where they cause hypertrophy and degeneration, thus affecting the quality of the flesh. According to Lom and Dykova (1992), their life cycle is direct. Artificial infection of freshwater fish through oral administration of spores, injection or

exposure to spore suspension has been successful in *Glugea* spp. (Takahashi and Egusa, 1977). However, this does not mean that the life cycle of these parasites is always direct in fish culture. Trials attempting to infect yellowtail with *Microsporidium seriolae* through the above methods were unsuccessful (Ogawa and Yokoyama, 1998). Thus, the life cycles of marine microsporidians have not yet been elucidated.

B. Myxosporida

These are important pathogens of fish that affect internal organs (histozoic species such as *Myxidium leei*, *Polysporoplasma sparis*, etc.) or cavities (coelozoic species, such as *Ceratomyxa* spp.). *P. puntazzo* (sharp snout sea bream) is particularly susceptible to *Myxidium leei*. In recent years, the life cycles of many myxosporidians infecting freshwater fish have been studied and elucidated (El-Matbouli and Hoffma, 1995), with the oligochaetes being shown to act as alternate hosts. However, the life cycles of marine species are almost unknown. Diamant (1997) experimentally demonstrated direct fish to fish transmission of *Myxidium leei*, the most pathogenic myxosporean of sea bream and sharp snout sea bream.

C. Amoeba-like organisms

These are very common parasites of marine fish, especially sea bream, that affect gills but also internal organs (Kent *et al*., 1988; Dykova *et al*., 1995; Athanassopoulou *et al*., 2002a).

III – Pharmaceuticals used in Mediterranean aquaculture and existing therapy

1. Treatments for Ectoparasites

A. Protozoan infections

The most common substances used to treat protozoa and monogenean parasites in fish are shown in Table 1. Most of these drugs concern freshwater fish and only a limited number of these drugs have been tested in Mediterranean fish but mainly in experimental trials (Athanassopoulou *et al*., 2003). Although some of these have proven effective, the commercial use of drugs requiring a bath application is very limited to marine cages due to the labour involved. Therefore, drugs applied per os are preferred.

Marine protozoa and especially *Oodinium* and *Trichodina* spp. are usually treated with disinfectants. *Oodinium* is one of the most difficult parasites to treat and such treatment only concerns land-based systems (tanks). Copper sulphate as a constant drip is the only drug of choice. Trichodinids can be treated successfully with a combination of malachite green and formalin but, again, only in tanks (hatcheries). Formalin (saturated 37% aqueous solution of formaldehyde gas) is also widely used.

Mebendazole and levamisole have been used to treat monogeneans in marine fish at doses similar to those applied in freshwater fish but with no great success. Levamisole in bath treatments was used also to treat amoebic gill disease in Atlantic salmon (Zilberg *et al*., 2000). No successful treatment was noticed when levamisole was given in the food.

Ivermectin is very effective against monogeneans and isopod/copepod infections (Athanassopoulou *et al*., 2002b). However, this drug has a lot of disadvantages as it produces residues in tissues and the environment, it is very toxic in low temperature (Roth *et al*., 1993) and inhibits respiration in gills (Toovey *et al*., 1999).

a] Formalin

Formalin (= a saturated 37% aqueous solution of formaldehyde gas) is a widely used chemical for parasite treatments or disinfections in both freshwater farms and marine hatcheries. It is also

used as an anti-bacterial and anti-fungal agent and has become popular because of its recent licensing as an approved therapeutic for farmed finfish in the USA and Canada (Speare *et al*., 1997). Intermittent use in salmon at prophylactic treatment doses is effective and safe in this species (Powell and Speare, 1996; Speare and McNair, 1996).

Table 1. Summary of the chemotherapeutants used in aquaculture

Parasites/use	Compound	Administration	Dosage
Isopoda and Copepoda	Ivermectin	Per os	0.05 mg/kg/2 times/week OR 0.2 mg/kg once
	Emamectin (Slice)	Per os	50 µg/kg x 7 days
	Chitin synthesis inhibitors (teflubenzuron)	Per os	10 mg/kg x 7 days
	Organophosphates	Per os	0.15 ppm x 20 min
	Dichlorvos	Bath>17C	0.5 ppm x 30min
	Azamethiphos	Bath	0.01 ppm x 1 h
	Pyrethrin and pyrethroids	Bath	0.1 ppm x 1 h
	Hydrogen peroxide	Bath	1,500 ppm x 30 min
	Chloramine-T	Bath	2.5-10 ppm X 1 h
	Deltamethrin-*C. oestroides*	Bath (Alphamax)	0.01 ppm x 30 min
Monogeneans and other helminths	Levamisole	Bath	2 ppm x 24 h
	Ivermectin	Bath	0.05 mg/kg/2 times/week OR 0.2 mg/kg once
Protozoa-Internal	Formalin	Per os	200 ppm x 1 h
	Organophosphates	Bath, per os	0.15 ppm x 20 min
	Amprolium	PER OS	190 g/t biomass
	Oregano essential oils	Per os	8-12 ml/5 kg biomass
	Quinine	Bath	30 ppm x 1h daily for 3 weeks
	Salinomycin	Per os	5 mg/feed/14days
	Sulphonamides	Per os	30 mg/kg
		bath	5-10 ppm x 4 hours
	Toltrazuril	Bath	25 mg/kg x 10 days
Protozoa-external *Cryptocaryon* sp.	Fumagillin	Per os	3 mg/kg x 8 weeks
	TNP-470	Per os	0.1-1 mg/kg x 4weeks
Oodinium sp.	Formalin Reduced salinity	Bath	200 ppm x 1 h
	Copper sulphate	Bath	0.75 ppm x 2 week
Disinfectants	Benzalkonium chloride	Bath Bath	10 ppm x 10 min OR 5 ppm x 30 min OR 2 ppm x 1 h OR 1 ppm several hours
	Chloramine-T	Bath	2.5-10 ppm x 1 h
	Copper sulphate	Bath - Prolonged immersion	0.1 mg/l x 10 days
	Formalin	Prolonged immersion –12hrs bath	2 ml/100 l 200 ppm x 1 h
	Hydrogen peroxide	Bath	1,500 ppm x 30 min
	Iodophores	Bath Dip	500 mg/100 l x 1 h 100 g/100 l x 10 sec

The literature on the safety of formalin is conflicting (Rucker, 1962; Smith and Piper, 1972). The doses, however, have never been tested in Mediterranean fish or even seawater and if not used

at an appropriate level it can cause necrosis and serious gill damage (Wedemeyer, 1971; Hammell, unpubl. data). The toxic effects of formalin increase with increasing temperatures (Alderman and Michael, 1992). Formalin solutions will degrade with precipitation of paraformaldehyde, which is toxic to salmonids and must be removed prior to use. Formaldehyde is a potential carcinogen and proper safety precautions should be taken by staff administrating this agent (Burka *et al*., 1997).

b] Hydrogen peroxide

Hydrogen peroxide has been used as a bactericidal agent (Derksen *et al*., 1999; Itou *et al*., 1997) and as a fungicidal for eggs (Burka *et al*., 1997), but its main use has been against sea lice in the treatment of chalimus and mobile stages. Treasurer and Grant (1997) found that under commercial farming conditions lice treated with hydrogen peroxide at 1500 ppm for 20 minutes at 10ºC did not reattach to salmon. Treated egg strings did not produce viable copepodites (McAndrew *et al*., 1998). Hydrogen is a strong oxidizing agent and causes the parasite to separate from its host, possibly by generating lethal oxygen emboli inside the sea lice (Thomassen, 1993). Unfortunately, the chemical has a very narrow safety margin and becomes narrower with increasing temperature (Bruno and Raynard, 1994). Also, a tremendous volume of the hydrogen peroxide is required to treat sea pens, and this is costly. Hydrogen peroxide can damage the gills of fish in experimental treatments and some mortalities have been reported when farmed fish are treated (Burka *et al*., 1997). For all these disadvantages, the chemical is not likely to be used for Mediterranean fish.

c] Chloramine T

Chloramine-T is an organic chlorocompound, which slowly breaks down to release chlorine. It is most commonly used as an antibacterial disinfectant and as a mucous stripping agent. It is also used for treatment of protozoa and for skin and gill flukes in a variety of fish species (Meinertz *et al*., 1999). Mixed infections can simultaneously be treated with this chemical (Ostland *et al*., 1995). Treatment using low doses (2.5-10 ppm) for up to one hour does not normally lead to problems and is not stressful for either the freshwater or the marine environment (Powell *et al*., 1994; Athanassopoulou, unpublished data).

d] Potassium permanganate ($KMnO_3$)

Potassium permanganate is very effective against cichlidogyriasis of tilapia fish (*Oreochromis hornorum*) (Flores-Crespo *et al*., 1995) and an ectoparasitic ciliate (*Ambiphrya ameiuri*) of channel catfish (*Ictalurus punctatus*) (Goncharenko *et al*., 1985).

e] Copper sulphate ($CuSO_4$)

It is used as a source of copper ion (Cu++) for the treatment of marine fish diseases caused by the protozoan parasites *Amyloodinium (Oodinium) ocellatum* and *Cryptocaryon irritans* (Cardeilhac and Whitaker, 1988). It is also used for controlling fresh water fish diseases caused by Costia, *Chilodonella*, *Epistylis* and *Trichodina* (Plumb, 1991), and ichthyophthiriasis (Schlenk *et al*., 1998). Total suspended solids are normally correlated to the efficacy of the chemical and it is usually toxic to fish, especially in soft water (Schlenk *et al*., 1998). Its use therefore in marine farms is very limited.

f] Iodophores

Iodine is an effective disinfectant for most egg pathogens and has been widely adopted by the aquaculture industry (Evelyn *et al*., 1986). Iodophor treatment may prevent *Loma salmonae* infections of the endothelial cells of Chinook salmon (*O. tshawytscha* Walbaum) by reduction of viability of spores. However, some spores survive even when high concentrations of iodophor are used (Shaw *et al*., 1999). Iodophor toxicity varies with species, parental stock, pH, egg condition and development stage (Alderman, 1984; Fowler and Banks, 1990).

g] Malachite green

Malachite green is used as an ectoparasiticide and external fungicide on fish and fish eggs (Culp and Beland, 1996) in freshwater. The dye seems to act as an irreversible respiratory enzyme poison (Alderman, 1982). The tissue levels of malachite green especially when co-administrated with formalin accumulate in exposed fish to levels greater than in the initial exposure concentration (Clifton-Hadley and Alderman, 1987). It is effective against proliferative kidney disease (PKD) of rainbow trout, *Oncorhynchus mykiss*, in established clinical or sub-clinical infection caused by the ciliate *Ichthyophthirius multifiliis* exposed to malachite green under laboratory conditions (Clifton-Hadley and Alderman, 1987). Since it has long term persistence in tissues (Alderman and Clifton Hadley, 1993) and it has possible teratogenic and carcinogenic effects, it is now banned from most countries (Meyer and Jorgenson, 1983).

B. Monogenean infections

a] Benzimidazoles

Benzimidazoles act either by disruption of energy metabolism in helminths (inhibition of fumarate reductase), or disruption of the polymerization of tubulin in cellular microtubules (Manger, 1991). Albendazole, mebendazole and fenbendazole have significant deleterious effects on uni- and multinucleate merots, sporogonial plasmodia, sporoblasts, and later sporogonic stages of microsporidian species *Glugea anomala*, Moniez, 1887 (Schmahl and Benini, 1998). Fenbendazole and triclabendazole were effective without signs of toxicity against *Gyrodactylus* sp. infecting rainbow trout. In contrast, oxibendazole and albendazole were effective but were toxic as well. Other benzimidazoles, such as thiabendazole, oxfendazole and flubendazole were totally ineffective (Tojo *et al*., 1992). Mebendazole has been used to treat monogeneans in marine fish at doses similar to those applied in freshwater fish but with no great success.

b] Levamisole

Levamisole has been demonstrated, in some fish species, to be a good enhancer of non-specific or specific immune responses when given alone or with a vaccine (acting as an adjuvant), respectively (Anderson, 1992). *In vitro* treatment of fish leukocytes with levamisole enhanced phagocytic cell activities (chemotactic activity, phagocytosis, respiratory burst and myeloperoxidase activity) or natural cytotoxic activity in carp (Siwicki, 1987, 1989; Baba *et al*., 1993), rainbow trout (Kajita *et al*., 1990), coho salmon (Olivier *et al*., 1985) and gilthead seabream (Meseguer *et al*., 1997; Mulero *et al*., 1998a, 1998b; Cuesta *et al*., 2002). Levamisole acts on nicotinic receptors of *Ascaris suum* and other large nematodes (Martin, 1993). Levamisole is found to be effective in a freshwater bath against the nematode *Anguillicola crassus*, pathogenic in eels under *in vivo* conditions (Taraschewski *et al*., 1988). Levamisole was effective against the monogenean *Gyrodactylus aculeate* parasitizing the skin of sticklebacks (*Gasterosteus aculeatus*), causing damage of the parasite tegument, and *Diplozoon paradoxum* parasitizing the gills of chub (*Squalius cephalus*) and bream (*Abramis brama*) causing severe damage along the mid-body (Schmahl and Taraschewski, 1987).

c] Niclosamide

Niclosamide is a chlorinated salicylamide that has been for many years the drug of choice for the treatment of most human and animal tapeworm and trematode infections. It interferes with the energy metabolism of helminths, possibly by inhibiting adenosine triphosphate (ATP) production as well as by uncoupling oxidative phosphorylation in the mitochondria of the parasite during electron transport from NADH to flavoprotein (James and Gilles, 1985). Niclosamide is found to be effective against the histophagous ciliate *Philasterides dicentrarchi* that causes fatal scuticociliatosis in farmed turbot (*Scophthalmus maximus*) and sea bass (*Dicentrarchus labrax*) (Iglesias *et al*., 2002). Niclosamide was also effective against the monogenean *Gyrodactylus aculeate* parasitizing the skin of sticklebacks (*Gasterosteus*

aculeatus) and *Diplozoon paradoxum* parasitizing the gills of chub (*Squalius cephalus*) and bream (*Abramis brama*) causing damage of the parasite tegument and severely affecting the mid-body, respectively (Schmahl and Taraschewski, 1987).

d] Bithionol

Bithionol is a halogenated diphenylsulphide and it was the drug of choice for the treatment of fascioliasis and paragonimiasis until praziquantel became available. Its mechanism of action is related to interference in ATP production in parasites. The succinic dehydrogenase system of liver flukes is very vulnerable and it is selectively inhibited by bithionol and niclosamide. Bithionol has an uncoupling effect on oxidative phosphorylation, thus prohibiting the formation of ATP in parasites (James and Gilles, 1985). Bithionol sulphoxide is found to be effective against the histophagous ciliate *Philasterides dicentrarchi* that causes fatal scuticociliatosis in farmed turbot (*Scophthalmus maximus*) and sea bass (*Dicentrarchus labrax*) (Iglesias *et al*., 2002).

C. Isopoda and Copepoda

The most common substances used to treat these parasites in marine fish are shown in Table 1. The most frequently used treatments against salmon lice *Lepeophtheirus salmonis* on commercial farms are various bath treatments including hydrogen peroxide, dichlorvos and cypermethrin (Burka *et al*., 1997; Pike and Wadsworth, 1999; Toovey and Lyndon, 2000). All of these treatments are effective in removing the adult or pre-adult stages, but only cypermethrin has been shown to reduce larval stages. Recently, hydrogen peroxide has also been found to affect eggs (McAndrew *et al*., 1998). Among the systemic treatments for lice, Ektoban (teflubenzuron) and Slice (emamectin benzoate) have shown good results (Armstrong *et al*., 2000; Stone *et al*., 2000; Ramstad *et al*., 2002). However, all these drugs are tested against the copepod *Lepeophtheirus salmonis* (salmon sea lice). Mediterranean fish are mainly infected by the isopod *C. oestroides* and *Caligus* sp., but it is mainly the isopod that causes problems and for which treatments are needed. Therefore, data related to the efficacy of the above drugs cannot be used in the case of the isopod parasite. *In vitro* treatments with deltamethrin against *C. oestroides* have proven effective (Athanassopoulou *et al*., 2001a), but preliminary trials with emamectin have been disappointing (Athanassopoulou, unpubl. data). Experimental treatment of sea bass with diflubenzuron has also shown good results (Athanassopoulou *et al*., 2004).

a] Organophosphates

Organophosphates inhibit many enzymes, especially acetylcholinesterase by phosphorylating its esterification site. They cause a blockage of cholinergic nerve transmission in the parasite, resulting in spastic paralysis. Organophosphates are used to treat crustacean parasites, such as sea lice, *Ceratothoa gaudichaudii* in salmon and *Argulus* sp. in freshwater salmonids, as well as gill and skin flukes, although the use of these compounds as anti-trematodes has decreased because of the development of resistance (Burka *et al*., 1997). Organophosphates (dichlorvos, trichlorphon) have been used as standard treatments for mobile stages of sea lice (*Lepeophtheirus* sp. and *Caligus* sp.) by the immersion bath method in salmon. Their therapeutic index is low and they affect cholinesterase both in the host and the parasite, as well as other organisms in the aquatic environment. They represent a safety risk for chemical handlers when they administer treatments, and parasite resistance is well documented (Roth *et al*., 1993; Burka *et al*., 1997). Azamethiphos, a newer organophosphate, has recently been used in Atlantic salmon because it is shown to be more effective against chalimus, pre-adult and adult stage of the sea lice (O'Halloran *et al*., 1992). The clinical application is 0.1 ppm for 30-60 min but this is very difficult to achieve in cages. Effects of azamethiphos on the environment have not been published (Roth *et al*., 1993).

b] Pyrethrins and pyrethroids

The main mechanism of action of the synthetic pyrethroids, such as deltamethrin and cypermethrin, involves the slowing down of the sodium channels of the nerve cells (Blagburn

and Lindsay, 1995) and interaction with the GABA receptors of flukes. They have a broader spectrum of activity than the organophosphate compounds since they also decrease the numbers of the chalimus, in addition to the pre-adult and adult stages of sea lice. Pyrethroids are synthetic analogues of pyrethrins with similar pharmacological properties. Pyrethroids can also inhibit brain AChE in both juvenile and adult fish (Reddy *et al*., 1991). Pyrethrins and pyrethroids are very safe to use for arthropod infections in mammals, but, in fish, the safety margin is considerably reduced, and fish toxicity has been reported (Roth *et al*., 1993).

Deltamethrin under laboratory conditions has high potential toxicity to fish. It has been reported that deltamethrin influences the ontogenesis of fish causing changes in hatching rate, abnormalities in development, and a decrease in body length (Roth *et al*., 1993).

c] Chitin synthesis inhibitors

Chitin synthesis inhibitors, lufenzuron, diflubenzuron and triflubenzuron, are effective against larval stages of sea lice but less effective against adult sea lice. Teflubenzuron was used for treatment of farmed Atlantic salmon *Salmo salar* L. infected with sea lice *Lepeophtheirus salmonis*, Kroyer, 1838. Maximum efficacy was observed toward chalimus and pre-adult stages of *L. salmonis* at approximately 26 d post-medication without any adverse drug reactions or palatability problems (Ritchie *et al*., 2002). Since they bind to marine sediments and remain in the environment for prolonged periods of time, they currently have limited, temporary approval in Norway. Diflubenzuron showed good results against the isopod *C. oestroides* of sea bass (Athanassopoulou, unpubl. data) and it is a promising drug because it is administered in feed.

d] Avermectins and related drugs

Avermectins have been used in aquaculture mainly to control sea lice infestation. Ivermectin is used extensively in livestock and companion animals and is a safe drug to use in mammals but, in fish, the blood brain barrier is not as impervious as it is in mammals and CNS depression and deaths have been reported in salmon at therapeutic doses (Burka *et al*., 1997). It also has a prolonged residence time and the drug is not metabolized (Hoey *et al*., 1992). Sea lice, including chalimus stages, can be killed by ivermectin but the therapeutic index is rather narrow (Johnson and Margolis, 1993). In contrast to ivermectin, emamectin benzoate has recently been registered for use in European countries and has proved to be very effective in salmon against sea lice (Stone *et al*., 1999, 2000; Armstrong *et al*., 2000; Ramstad *et al*., 2002). It is found to interfere with the GABA receptors of the nervous system, leading to flaccid paralysis. Ivermectin is also very effective against monogeneans and isopod/copepod/nematode infections (Heckman, 1985; Hyland and Adams, 1987; Athanassopoulou *et al*., 2002b). However, this drug has a lot of disadvantages as it produces residues in tissues and the environment, it is very toxic at low temperature (Roth *et al*., 1993) and it inhibits respiration in gills (Toovey *et al*., 1999).

2. Treatments for endoparasites

A. Anticoccidial drugs

Anticoccidials are generally given to poultry in the feed to prevent acute disease and the economic loss often associated with subacute infection. Prophylactic use is preferred because most of the dosage occurs before signs become apparent, and delayed treatment may not benefit the entire flock. There are a lot of drugs developed, including amprolium, clopidol, halofuginone, ionophores, nicarbazin, tetracyclines and sulphonamides (Croft, 1997) but only a few are used in fish for infections of parasites producing similar spores (i.e. Myxosporea and Microspores) and these are described below.

a] Fumagillin

Fumagillin, an antibiotic isolated from *Aspergillus fumigatus*, was used primarily for treating *Nosema apis* (Microsporea) infections in honey bees *Apis mellifera* (Ketznelson and Jamieson, 1952), and for treating patients with amoebiasis (Killough *et al*., 1952). The molecular mechanism of fumagillin action on microsporidial replication is poorly understood. In *in vitro* ultrastructural studies, *Encephalitozoon cuniculi* organisms treated with fumagillin were irregularly shaped. Proliferation stage organisms were typically swollen and contained irregularly shaped cytoplasmic vesicles (Shadduck, 1980).

In microsporidian *Octosporea muscaedomesticae*, fumagillin treatment caused a decrease in total RNA, suggesting that fumagillin inhibited RNA synthesis (Jaronski, 1972). In fish, fumagillin was effective against the microsporean *Pleistophora anguillarum* in eels *Anguilla japonica* (Kano *et al*., 1982) and against *Myxidium giardii* infections by blocking the development and preventing the formation of new spores in European eels *Anguilla anguilla* (Székely *et al*., 1988). It is used to control *Enterocytozoon salmonis* (Hedrick *et al*., 1991) and *Loma salmonae* (Kent and Dawe, 1994; Speare *et al*., 1999) infection in Chinook salmon (*Oncorhynchus tshawytsha*). It is effective against haemorrhagic thelohanellosis caused by *Thelohanellus hovorkai* and *Sphaerospora renicola* in common carp (*Cyprinus carpio*, L.) when administrated during the infective period (Yokoyama *et al*., 1990, 1999; Molnár *et al*., 1987), early intracellular trophozoites and more developed plasmodia of *Hoferellus carassi* (Yokoyama *et al*., 1990), *Myxobolus cerebralis* in rainbow trout (El-Matbouli and Hoffman, 1991) and against the myxosporean *Sphaerospora testicularis* in sea bass (Sitja-Bobadilla and Alvarez-Pellitero, 1992).

TNP-470 is a semi-synthetic analogue of fumagillin that possibly acts in the same way as fumagillin by inhibition of RNA synthesis (Jaronski, 1972), as well as by affecting host cell function or growth by reduction of levels of mRNA encoding for cyclin D1, which plays a role in regulating cell division at the mid-G1 phase in human umbilical endothelial cells (Hori *et al*., 1994). TNP-470 is effective in vitro against the Microsporidia *Encephalitozoon intestinalis* and *Vittaforma corneae* (Didier, 1997).

Fumagillin is known to reduce growth in rainbow trout and sea bass (*Dicentrarchus labrax*) when feeding 1% fumagillin for one month, and is accompanied by mortality and a decrease in haematopoietic tissues (Wishkovsky *et al*., 1990; Sitja-Bobadilla and Alvarez-Pellitero, 1992), as well as depletion of the renal interstitium in Chinook salmon (Hedrick *et al*., 1988).

b] Toltrazuril

Toltrazuril displays both anthelminthic and antiprotozoal activity. It is a symmetric triazine derivative, and is capable of entering the host cell as well as the intracellular or tissue-inhibiting parasitic stages in chicken-parasitizing coccidia *Eimeria* sp. (Mehlhorn *et al*., 1984). Incubation of *Glugea anomala*, which parasitizes the connective tissue of sticklebacks (*Gasterosteus aculeatus*), led to complete destruction of multinucleate meronts and to fragmentation of sporogonial plasmodia, suggesting an inhibitory effect of the drug on nuclear division (Schmahl and Mehlhorn, 1989; Schmahl *et al*., 1990) possibly via inhibition of enzymes involved in pyrimidine synthesis and nuclear division (Harder and Haberkorn, 1989).

It is active against pre-spore stages of *Myxobolus* sp. in the gills of bream (*Abramis brama*), as well as against developmental stages of *Henneguya* sp. parasitic on the gills of the tapir fish (*Gnathonemus petersi*) (Schmahl *et al*., 1989b, 1991). It is also effective against parasitic ciliates, such as *Ichthyophthirius multifillis*, *Trichodina* spp. and *Apiosoma* spp. (Mehlhorn *et al*., 1988; Schmahl *et al*., 1989a; From *et al*., 1992) and fish-infecting members of the Coccidia, Microsporidia and Myxozoa (Mehlhorn *et al*., 1988). It has been suggested that in Mediterranean fish, it is effective for treatment of Myxosporean infections including *Myxidium leei* (Lytra, pers. comm.). It is also known to be effective against the monogenean *Gyrodactylus aculeatus* (Schmahl *et al*., 1988).

Similar results for fish and crustacean parasites have been shown with the asymmetric triazinone derivative HOE 092 V when dissolved in medicinal baths against the gill-parasitic *Henneguya* sp. in tapir fish (*Gnathonenus petersii*) and against the skin-parasitic *H. laterocapsulata* in hybrid clariid catfish (*Clarias gariepinus* x *Heterobranchus bidorsalis*) (Schmahl *et al*., 1992, 1993).

c] Amprolium

Amprolium is a structural analogue of thiamine (vitamin B1) causing competitive inhibition of thiamine utilization by the parasite. It acts upon the first generation in the cells of the intestinal wall, preventing differentiation of the metrozoites. It may also suppress the sexual stages and sporulation of the oocysts. *Amprolium* has been shown to be ineffective against *Hexamita salmonis*, *Gyrodactylus* sp. and *Ichthyobodo necator* in rainbow trout (Tojo and Santamaria, 1998a, 1998b, 1998c).

d] Quinine

Quinine acts on skin-inhabiting trophozoite stages of *Ichthyophthirius multifiliis* in ornamental fish and causes severe alterations in the parasite's structure, mainly consisting of an enlargement of the alveolar sacs, a partial destruction of the nephroplasm, and a sloughing off of the membranes bordering the contractile vacuoles (Schmahl *et al*., 1996). It has also been shown to be effective against the plasmodial developmental stages of *Henneguya* sp. in tapir fish (Zegula, 1997). Quinine administrated orally led to malformation of the presporogonic and pansporoblastic stages of *Henneguya* sp. parasitizing the gills of tapir fish (Dohle *et al*., 2002).

e] Metronidazole

Metronidazole is a nitroimidazole primarily used in the treatment of infections caused by anaerobic bacteria and protozoa such as *Trichomonas vaginitis* and *Giardia lamblia* (Tally and Sullivan, 1981; Lau *et al*., 1992). Metronidazole has a low molecular weight, resulting in easy penetration of the cell membrane of both aerobe and anaerobe microorganisms. Once inside the cell metronidazole has been shown to reduce, via a ferri-doxin system that exists in anaerobic microorganisms, to reactive intermediates that damage the DNA of microorganisms (Tally and Sullivan, 1981). Its effect on fish parasites is not proven. Experimental use against *Loma salmonae* did not show good results (Speare *et al*., 1999).

f] Salinomycin

Salinomycin is an antibiotic belonging to ionophorous polyethers and acts as a chelator with monovalent cations, especially for potassium, disturbing the intracellular concentration balance for monovalent ions (Kinashi *et al*., 1973). It has a strong activity against many microorganisms including the coccidia. It causes shrinking of the pellicula in *Eimeria* sp., vacuolisation of the cytoplasm and destruction of mitochondria (Raether *et al*., 1991). Salinomycin administrated orally causes deleterious effects on the trophozoite cytoplasm and on the presporogonic and pansporoblastic stages of *Henneguya* sp. parasitizing the gills of tapir fish. Severe shrinking of the plasmodia was also observed and an enlargement of the sutural ridges in the pansporoplasts and malformation of the polar capsules (Dohle *et al*., 2002).

g] Benzimidazoles

In monosporidia, benzimidazoles cause a decrease in the number of ribosomes, gross enlargement of the SER, vacuolization of the cytoplasm and complete disintegration of the nuclear structures (Schmahl and Benini, 1998). Albendazole acts on *Encephalitozoon cuniculi* by inhibition of the formation of the intranuclear spindle (Colbourn *et al*., 1994) and on *Nosema bombysis*, a silkworm parasite, by clumping of chromatin in the nuclei, inhibition of spindle formation and spore malformation (Haque *et al*., 1993). Experimental use against *Loma salmonae* showed good results (Speare *et al*., 1999).

h] Sulphonamides

Sulphonamides have been used as chemotherapeutic agents against bacterial diseases in mammals and fish for decades (Alderman, 1988). They compete with para-aminobenzoic acid (PABA) in the biosynthesis of tetrahydrofolic acid in the pathway to form folic acid. Some drugs of this group can have anticoccidial action especially when combined with other compounds such as toltrazuril (Laczay *et al*., 1995; Haberkorn, 1996).

i] Oregano oils

In oregano (*Oregano vulgare*) essential oil the most abundant compounds were also γ-terpinene, p-cymene, carvacrol and thymol (Daferera *et al*., 2000). These components are found to have inhibitory effects on microorganisms (Athanassopoulou *et al*., 2000) especially in spore forming organisms (Sivropoulou *et al*., 1996; Mejiholm and Dalgaard, 2002).

IV – Recent research, developments and constraints

The main parasitic diseases of Mediterranean fish causing problems (mortalities, delay in growth rates and/or loss of fecundity) and requiring treatments are the Myxosporeans *Myxidium leei*, *Polysporoplasma sparis* and *Ceratomyxa* sp., as well as isopods and copepods. In some areas there are also heavy mortalities due to monogenean infections. Some preventive methods are currently available for Isopoda infections but there is an urgent need for research into anthelminthic treatments, as well as background information on life cycles and early diagnostic procedures, as, in many cases, this is non-existent for Mediterranean parasites.

In view of the EEC policies concerning reduction of chemical use in the water environment, the strategic and effective use of chemotherapeutants becomes essential. As in the case of salmonid aquaculture, the combination of the correct treatments with other health management and disease prevention strategies, such as vaccination, water quality improvement, production of tolerant fish, alternative treatments will help ensure the successful development of the sector in the future.

In recent years, a few major research projects have been undertaken in Greece, funded by the Greek Ministry of Research and Technology, concerning the efficacy of different antiparasitic drugs in Mediterranean fish. The results of these experiments are under publication and are discussed briefly below. In addition, a joint EEC project involving most of Mediterranean countries dealing with early diagnosis, epidemiology and prevention methods for the pathogen *Myxidium leei* in *P. puntazzo* and *S. aurata* has just started (2003).

1. Research on Monogenea

There has been limited funded work carried out in recent years concerning the treatment of Monogenea in Mediterranean cultured fish. Preliminary experimental treatments with ivermectin at the levels used for sea lice in salmon but for sea bass have been promising (Athanassopoulou, unpubl. data).

2. Research on Isopoda/Copepoda

Due to local funding a little more information exists on the drugs used for these infections.

A. Ivermectin

The toxicity and histopathology of ivermectin was studied in 3 and 35 g healthy sea bass, *Dicentrarchus labrax* L., following in-feed, oral intubation and injection administration at dose rates ranging from 0.5 to 3.5 mg/kg. Estimated LD50 values for 3 g fish were 0.335 and 0.106 mg/kg following oral intubation and injection administration, respectively, for fish reared at 11ºC and 0.839 and 1.023 mg/kg following oral intubation and injection administration, respectively,

for fish reared at 20ºC. For 35 g fish reared at 11ºC, the estimated LD50 was 0.523 and 0.361 mg/kg following oral intubation and injection administration, respectively. No signs of toxicity were observed when the compound was administered via the feed at 0.5 and 0.7 mg/kg.

However, toxicity (>10%) was observed at dose rates of 0.2 mg/kg and higher when the compound was administered via oral intubation and at 0.5 mg/kg when administered via injection. The compound was found to be significantly more toxic to fish reared at 11ºC than 20ºC. Furthermore, ivermectin was found to be more toxic to 3 g than 35 g sea bass when administered via injection. Histopathological examination of the major organs revealed pathology was largely restricted to gills and intestinal tissue. In 3 g sea bass, lesions were also found in the kidneys (Athanassopoulou *et al*., 2002b). The therapeutic effect of the drug was very satisfactory in copepod (*Lernanthropus kroyeri*) infections of sea bass (Athanassopoulou *et al*., 2001b). However, due to the problems associated with this drug, research is required to find other alternative treatments that will be both economical and easy to administer. Emamectin benzoate could be an alternative drug if adequate research is undertaken to assess toxicity, dose ranges and tissue residue levels in Mediterranean fish.

B. Deltamethrin

Experimental treatments of 30 min duration were undertaken for both sea lice (*Ceratothoa oestroides*) *in vitro* and for sea bass of average weight 5.73 and 20.06 g infected with *C. oestroides*, kept in experimental tanks at a temperature of 20ºC. In both experiments different concentrations were used and evaluated. For the sea bass tests, the following concentrations of the drug were tested: 10 µg/l, 5 µg/l, 3 µg/l, 0.15 µg/l, 0.1 µg/l, and 0.05 µg/l. The results were assessed after one hour and at 24 hours and 48 hours. The best results were achieved at a dose of 10 µg/l (0.01 mg/l) where the prevalence was reduced from 100% to 0% over 24 h in both large and small fish. The parasites were dead also at 48 hours. The dose of 5 µg/l reduced the prevalence from 100% to 11.74% and from 85.7% to 0% for large and small fish, respectively. Finally, at a dose of 3 µg/l the results were respectively from 88.2% to 37.5% (large fish) and from 87.5 to 13.3% (small fish). Smaller doses did not have any effect on the parasites at 24 or 48 hours.

C. Diflubenzuron

Experimental treatments were undertaken with sea bass of average weight 5.73 and 20.06 g infected with *C. oestroides*, kept in experimental tanks at a temperature of 20ºC. The results were very promising and the toxicity of the drug very limited (Athanassopoulou *et al*., unpublished data).

3. Research on Myxosporea/Microsporea

Extensive research was undertaken in the period 1997-2001 concerning the immunology and treatment of different myxosporeans of cultured *Sparus aurata*, *P. puntazzo* and sea bass and *Sargus* sp.

Three sets of experiments were undertaken, two in land-based experimental tanks (at 22ºC) and one in small experimental cages (25ºC). The drugs and doses used in each experiment are shown in Table 2. As no *M. leei* were present at the time of these research projects, the myxosporan *P. sparis* in sea bream and *Myxobolus* sp. in *P. puntazzo* were considered. In all three experiments, and in both species, the treatment with amprolium and sanilomycin at 12% was the most effective in terms of both therapy and toxicity to fish (Athanassopoulou *et al*., 2003).

Oregano oils have also been found to be effective in treating bacterial (*Vibrio anguillarim* and *V. alginolyticus*) *in vitro*, as well as in treating myxosporean infections in Sparidae (Athanassopoulou *et al*., 2000, 2003).

Table 2. Compounds used in experimental treatments for Myxosporea in Mediterranean fish (*S. aurata* and *P. puntazzo*)

Compound	Dose	Scheme	Comments
Fumidil-1	2 - up to 25 mg/kg	3 and 6 weeks	2 land based experiments and 1 exp. field trial
Toltrazuril-2	600 ml/t biomass	2 days on, 3 days off, 2 days on, repeat after 15 days	2 land based experiments
Toltrazuril-2	600 ml/t biomass	2 days on, repeat after 15 days	2 land based experiments
Amprolium	190 g/t biomass	30 days	2 land based experiments
EsB-3	200 g/t biomass	3 days on, repeat after 15 days	2 land based experiments
Ampr+EsB-3	100 + 100 g/t biomass	30 days	2 land based experiments
Ampr+Salilomycin 12%	100 + 70 g/t biomass	30 days	22 land based experiments and 1 exp. field trial
Oregano oils (Ecodiar)	8-12 ml/5 kg biomass	30 days	2 land based experiments and 1 exp. field trial

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