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Antibacterial agents in Mediterranean finfish farming: A synopsis of drug pharmacokinetics in important euryhaline fish species and possible environmental implications

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Abbreviations: $t_{1/2\alpha}$ (h) – distribution half-life; $t_{1/2\beta}$ (h) – elimination half-life; V_d (l kg^{-1}) – volume of distribution; V_{dss} (l kg^{-1}) – apparent volume of distribution at a steady state; $F(\%)$ – bioavailability – fraction of administered dose available systemically, determined as follows: 100 × [non-intra-vascular dose/intra-vascular dose]; MRL (ng g^{-1}) – maximum residue level; AUC (μg h mL^{-1}) – area under the curve – total area under tissue drug concentration versus time curve; *WTs* (h) – withdrawal times; MIC (μg mL^{-1}) – minimum inhibitory concentration; C_{max} (μg g^{-1} or mL^{-1}) – maximum drug concentration in tissue or plasma; K_{oc} – organic carbon coefficient; $T1/2$ – biodegradation half life; EC_{50} – effect concentration; 50% effect on group of organisms; NOEC – no observable effect concentration; the highest test concentration with no adverse effects

Abstract

The literature pertaining to the use of registered antibacterial agents in Mediterranean finfish farming is reviewed, with an emphasis on the Greek fish-farming industry. This review provides a scientific resource dedicated to the design of future antibacterial dosing regimes in Mediterranean fish farming, where insufficient supporting information is currently available. This paper addresses the paucity in knowledge concerning pharmacokinetics and the efficacy and environmental impact of commonly used antibacterials needed to direct future research and promote good practices in the euryhaline fish farming industry. Several

registered antibacterials are currently available for combating bacterial infections, including tetracyclines, (fluoro) quinolones, potentiated sulfa, penicillin and chloramphenicol derivatives. Based on the available data, oxytetracycline (OTC) and quinolone drugs (oxolinic acid – OA and flumequine – FLU) are the most widely used in Mediterranean aquaculture. As a result these drugs have received the most extensive studies, whereas, there is considerable paucity of reliable data on pharmacokinetic and the depletion characteristics of other drugs used, particularly potentiated sulfa, penicillin derivatives and florfenicol. We find there is incomplete data on drug efficacy and minimum inhibitory concentrations (MIC) for common antibacterials used against the major bacterial pathogens of Mediterranean fish species. Furthermore, a considerable lack of data on environmental drug concentrations around Mediterranean fish farms was also identified, highlighting the need for more extensive environmental studies to monitor contamination in environmental components i.e., water and sediment, and in non-target species (flora and fauna). Prudent selection and use of antibacterials can encourage lower dosage applications, enhance treatment efficacy, and help to minimize contamination of the environment. Selection of readily bioavailable drugs which have low environmental persistence, low aquatic toxicity and high antibacterial efficacy is advised, to reduce potential losses to the environment and associated toxic effects on target species and the development of bacterial resistance. Lack of present data made it impossible to provide thorough and accurate guidance on selection and use of antibacterials and approaches for minimizing environmental impacts for the treatment of major euryhaline aquaculture species.

Introduction

The term aquaculture encompasses all activities associated with rearing aquatic organisms including fish, oysters, crustaceans and algae. Fish farming in the Mediterranean region is an activity that began many centuries ago although modern marine Mediterranean fish farming has only been practised effectively over the last two decades. As in several parts of the world, finfish production in the Mediterranean area has grown rapidly (Table 1). Mediterranean as well as Greek euryhaline fish production is dominanated by two species, European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*), representing about 97% of the total euryhaline fish production (FGM, 2000; Table 1). As a result, research has mainly focussed on these two species over the last two decades. The remaining aquaculture production is comprised of species new to farming, such as sharpsnout sea bream (*Diplodus puntazzo*), common dentex (*Dentex dentex*) and other sparids. New candidates are likely to play a vital role in the survival and growth of the euryhaline aquaculture industry over the next century, particularly due to a saturation of markets for the two main species, resulting in high competition and reduced profit margins (Theodorou, 2002).

The rapid development of fish farming has led to increased levels of disease. As aquaculture often involves cage farming where large numbers of animals are kept together in a confined space, disease outbreaks are common regardless of the quality of the hygiene practised. Pathogens such as bacteria, parasites, viruses and fungi may cause infection in caged fishes. However, bacteria remain the major cause of fish diseases in fish farming in Europe and are therefore a potential source of significant financial loss. A range of bacterial pathogens encountered in Mediterranean euryha-

Table 1. Euryhaline fish (sea bass, gilthead sea bream) production (metric tonnes in thousands) in the Mediterranean over the last decade

Year	1994	1995	1996	1997	1998	1999	2000	2001	2002
Greece	13	17	21	26	27	48	59	61	70
Total	36	47	51	62	91	113	127	128	140

Source: FEAP (2003).

line fish farming have been reported in the literature. The most devastating are *Vibrio anguillarum* serotype 1*b* and *Photobacterium damsela* subsp.*piscicida* (formely *Pausterella*), although several other species have also been described (Christophilogiannis et al., 1997a; Babelona et al., 1998; Doukas et al., 1998; Rigos et al., 1998; Athanassopoulou et al., 1999; Company et al., 1999; Le-Breton, 1999; Zorrilla et al., 2003; Table 2). High standards of hygiene, in theory, are the most effective preventative control measure against infectious bacterial agents. Vaccination is also a very effective approach, however, developed vaccines are only available for a limited range of pathogens (mainly *V. anguillarum* serotype 1b and *P. damsela* subsp.*piscicida*; Le-Breton, 1999). In contrast, a range of antibacterial drugs are available for combating bacterial diseases, for which vaccines have not yet been developed.

The aim of this review is to summarize and discuss the pharmacokinetics and efficacy of registered antibacterial agents used in Mediterranean euryhaline fish farming. It is anticipated that this work will (a) provide a scientific foundation to aid the design of future antibacterial dosing regimes in euryhaline fish farming, (b) highlight the paucity of knowledge of many pharmacokinetics, and (c) direct future research in support of good practice in the industry.

Chemotherapy and legislation

Disease outbreaks in aquaculture are normally confronted with mass therapy, usually orally administered via incorporation of drugs into the feed. Other methods of application, for example direct injection and bathing treatments, are less frequently employed (Table 3). The best approach in conventional veterinary medicine is to use a therapeutic treatment whereby the target pathogen and its drug sensitivities have been identified. Other criteria must be also considered, such as the fraction of a drug that is bioavailable and its ability to reach infected sites in the species of interest.

Unfortunately, antibacterial drugs have not always been used in a responsible manner in the aquaculture industry. The urgency of the farmer's response to an outbreak, often results in ill-informed decision-making based on a rushed diagnosis and possible use of inappropriate drugs. Chemotherapy in the aquatic environment is not free from practical problems (Table 4), including

Table 2. Summary of important bacterial diseases affecting Mediterranean finfish farming

Bacterial pathogen	Sensitive fish species
Vibrio anguillarum serotype 1b	*D. labrax, S. aurata*
V. anguillarum serotype 3	*D. labrax*
Photobacterium damsela subsp. *piscicida* (formely *Pausterella*)	*S. aurata*, *D. labrax*, *D. puntazzo*, *D. dentex*
V. alginolyticus	*D. labrax*, *S. aurata*, *D. puntazzo*, *D. dentex*
V. damsela	*D. labrax*, *P. puntazzo*, *S. aurata*, *D. dentex*
V. fluvialis	*D. labrax*
V. ordalii	*D. labrax*
V. splendidus	*S. aurata*, *D. puntazzo*
V. vulnificus	*D. puntazzo*
V. harveyi	*D. labrax*, *S. aurata*, *D. dentex*
Aeromonas hydrophila	*D. labrax*, *D. puntazzo*
Aeromonas spp.	*S. aurata*
Staphylococccus epidermatitis	*D. puntazzo*
Pseudomonas spp.	*S. aurata*, *D. labrax*
Flexibacter maritimus	*D. labrax*
Cytophaga-like bacteria	*S. aurata*, *D. labrax*

Sources: Christophilogiannis et al. (1997a), Babelona et al. (1998), Doukas et al. (1998), Rigos et al. (1998), Athanassopoulou et al. (1999), Company et al. (1999), Le-Breton (1999), Zorrilla et al. (2003).

Table 3. Methods of drug administration against bacterial fish diseases

Administration route	Concerns	Type of farming
Via the feed	Palatability	Cages
	Leaching	Raceways
	Fish appetite	Ponds
	Environmental risk	Tanks
Bath treatment	Water solubility (lipophilicity)	Cages (small)
	Environmental pollution	Raceways
	High cost	Tanks
	Unpractical in large cages	Hatcheries
Injection	Required anaesthesia	Brood stock
	Unpractical – labour intensive	Valuable pet fish

the risk of environmental pollution and potential hazards for human consumers or for farm workers. Chemicals have been used in fish farming for more than 100 years, however, intensive efforts to register fish toxicants only commenced in the 1950's to late 1970's (Schnick, 1999). Public concern for chemical pollution in the environment began almost 40 years ago, coinciding with the first use of drugs against furunculosis (Carson, 1962). Several organisations including the *Food & Agriculture Organization* (FAO), the *World Health Organization* (WHO), the *International Office of Epizootics* (OIE) and a number of national governments have all raised the issues associated with irresponsible use of antibiotics in all production sectors, with particular concern for potential risks to public health. Not until the early 1980s, was the concept of environmental risk assessment (ERA) of drugs in fish farming proposed (Jorgensen and Halling-Sorensen, 2000). At that time, chemotherapeutants were already widely used in marine salmon farming.

The aquaculture industry is currently subjected to international, European and national regulatory constraints. Discussions on the control of trade, development and legal employment of veterinary medicines among European Union (EU) member countries has been ongoing for almost 20 years. The establishment of the open market within the EU in 1993 further increased the importance of regulating the use of medicines throughout the EU (Alderman, 1999). Consequently, legislation of EU countries has had to bear a common regulatory environment across all member countries. Directive 81/851/EEC, relating to veterinary medicinal products, was the initial stage of this process in 1983. The availability and authorization of drugs for European veterinary medicine, including aquaculture, was regulated by the EU in 1990 via Council Regulation 2377/90

Table 4. Practical problems associated with chemotherapy in aquaculture

Problems	Exposure
Residues and persistence in the aquatic environment	Un-absorbed and un-ingested medicated feed or direct drug loss from bath treatments
Antibiotic resistance	Un-metabolised drug in fish excretions (faeces, urine, gills)
Uptake by aquatic flora and fauna (possible toxicity)	Metabolites and possible back-conversion to parent compound
Effects on workers	Contact with a possibly carcinogenic substance
Effects on human health	Fish with drug levels above MRLs possibly leading to adverse health effects
	Direct contact and consumption of improperly cooked farmed items or cross-contaminated food items; direct transfer of resistant bacteria

which was superceded by Council Regulation 2309/93 in 1993. Several EU Directives are applicable to the use of drugs for farming purposes. Veterinary medicine is currently regulated by the *Committee for Veterinary Medicinal Products* (CVMP) of the *European Medicines Evaluation Agency* (EMEA).

Several antibacterial drugs regulated by EU legislation are currently used in Mediterranean finfish farming (Table 5). Circumstances and conditions in fish farms vary considerably according to the species farmed, type of farming, water quality and water temperature. In many instances of disease outbreak in farmed fish, there are limited or no guidelines for selecting the appropriate medication and a treatment regime. In these cases, exiting knowledge and research for other similar drugs and/or other closely-related fish species are used to select a treatment. Although this approach is far from ideal, it is an attempt to overcome the paucity of data, and is unfortunately usually done without any formal guidance leading to unreliable extrapolation. European Union Directive 90/676/EEC provides a ''prescribing cascade'' to support the use of drugs authorised for other farmed animals, where no suitable registered compound has been recommended to treat diseases in fish. In such cases, a standard withdrawal period is imposed, corresponding to 500-degree days in fish (Alderman, 1999). This is to ensure consumer safety is enforced by an established maximum residue level (MRL), which is derived from toxicity testing data. The MRL is the maximum concentration of residue tested to be without toxicological risk (e.g., hypersensitivity reactions) to human health (Table 5). To ensure that no residues above the MRL exist in the edible tissues of farmed products, a withdrawal period is determined for each drug in the target fish species, at different temperature conditions.

Efficacy and optimisation of drug treatments

The prudent use of antibacterial agents must be practiced in all types of intensive farming (terrestrial or aquatic) to minimize potential hazards. The effective design of a dosage regimen in veterinary medicine is a rather complex procedure requiring consideration of several factors. These include:

Table 5. Maximum residue limits (MRLs) for commonly-used registered antibacterial drugs used in fish farming in the European Union

Antibacterial drugs	Maximum residue level, MRL (ng g^{-1})	Sources
Tetracyclines		
Oxytetracycline*	100	EMEA (1995a)
Chlortetracycline	100	
(Fluoro)Quinolones		
Oxolinic acid*	100	EMEA (2003)
Flumequine*	600	EMEA (1999)
Sarafloxacin	30	EMEA (1998a)
Potentiated sulfa		
Sulfonamides (sulfadiazine)*	100**	EMEA (1995b)
Trimethoprim*	50	EMEA (1997)
nameend = ''c3''		
Penicillin derivatives		
Amoxycillin	50**	EMEA (undated)
Ampicillin	50**	EMEA (undated)
Chloramphenicol derivatives		
Florfenicol	1000	EMEA (2000)
Thiamphenicol	50	EMEA (1998b)

*Licensed for use in Greece.
**MRLs established for terrestrial animals.

(a) *Physico-chemical properties* of the candidate selected drug. These dictate the ability of the drug to penetrate biological barriers (membranes). For example, lipophilicity (which increases its ability to penetrate membranes), number and type of functional groups with an affinity for ionic binding (which influences potential for formation of complexes with cations which alter molecular charge and thus reduce membrane permeability), molecular weight and size (e.g., micronisation, which increases permeability by increasing solubility) and p*K*a value which is the extent to which a drug is ionised at a certain pH (most drugs are absorbed by passive diffusion in the unionised form).

(b) *Antibacterial mechanism* of the candidate drug. This is the type of action it has against the bacterial pathogen, for example, bacteriostatic action (able to inhibit bacterial growth) and bactericidal action (able to kill bacteria with time- or concentration-dependent action).

(c) *Nature of the disease* (e.g., infections with slow progression, asymptomatic infections which may persist for long periods and reappear under optimum conditions for the pathogen).

(d) *Sites of infection* (e.g., poorly vascularized areas versus highly blood-diffused areas).

(e) *Kinetic profile of the candidate drug* in the species of interest. This is dictated by pharmacokinetic parameters (distribution and elimination half-lives, $t_{1/2\alpha,\ \beta}$, volume of distribution, V_d; maximum drug tissue concentrations, C_{max}; area under the curve analyses, AUC).

(f) *Pharmacodynamics* of the candidate drug expressed in the form of minimum inhibitory concentrations (MIC) against the causative bacterial pathogen.

(g) *Health status of the infected fish*. This is to address (in addition to reduced fish appetite) the possibility of reduced capacity in infected animals to absorb and metabolize the drug, which must be taken into consideration when treatment regimens are designed. For example, Uno (1996) demonstrated significant differences in absorption of oxytetracycline (OTC) after oral administration between *vibrio*-infected and healthy ayu (*Plecoglossus altivelis*). Bioavailability of OTC was reduced by 60% in infected fish with both plasma and tissue levels considerably lower, compared to healthy fish. Coyne et al. (2004a) reported a dramatic difference in the oxolinic acid (OA) concentrations between healthy and moribund rainbow trout (*Oncorhynchus mykiss*) (<0.005 versus >0.015 μg mL^{-1}). The metabolic capacity of common carp (*Cyprinus carpio*) was also found to be altered during bacterial infection since bacterial endotoxin caused basal hepatic cytochrome P450 activity levels to be significantly lower in infected liver (Marionnet et al., 1998).

The efficacy of a treatment is commonly evaluated by the integration of MIC values with maximum drug concentrations in plasma. There is some evidence to support the use of MIC data for *in vivo* application, to predict the treatment efficacy of OTC (Bruun et al., 2003). However, such assessments must be carefully performed, since MICs reflect a quantitative measure of bacterial sensitivity to drugs and are determined *in vitro* which does not represent the biological activity of the drug in the target animal *in vivo*, so their validity comes into question (Smith et al., 1994; Branson, 2001). These are based on theoretical assessment of a drug's efficacy against a bacterial pathogen based on the requirement that the drug's C_{max} plasma (maximum plasma concentration) following administration in the target species, exceeds a factor of 4:1 (C_{max}:MIC) (Stamm, 1989) or even 8:1 (Blaser et al., 1987) to ensure effective antibacterial action. However, there is concern regarding the generalised over-simplicity of these guidelines, therefore they should be treated with caution (Smith et al., 1994). Coyne et al. (2004a,b) failed to determine any formula for estimating MIC breakpoint values including the 4:1 ratio and suggest that the application of such ratio is not valid at least for farmed fish populations, although it may be beneficial in terrestrial large animal farming. Other criteria suggest that C_{max} plasma should be at least 2×MIC and the plasma concentration must be at least 1×MIC for half the dosing interval (Shojaee AliAbadi and Lees, 2000). However these factors have received some criticism since the authors stress that the time for which concentration exceeds MIC ($T >$ MIC) is an important determinant of the outcome of the therapy, as during periods when drug levels drop below MIC, bacterial re-growth and thus re-infection is possible. Moreover, no account is

taken in these criteria of the concentration of free unbound drug at an infection site, since this may differ from the drug plasma concentration, especially in tissues that are not highly vascularized (Smith, 2001; Liu et al., 2002). Another argument related to the prediction of the outcome of the therapy lies with the fact that although estimation of bacterial susceptibility (MICs) can be obtained in advance of the treatment, the plasma-tissue C_{max} values can be based on predictions in pretreatment status and only when they are consistent with the actual *in vivo* concentrations can they contribute to a valid application of any ratio (Coyne et al., 2004b).

Shojaee AliAbadi and Lees (2000) in an attempt to particularise drug treatments, proposed that an optimum dosage schedule should achieve drug concentrations at sites of infection in excess of MIC in the case of bacteriostatic drugs (e.g., tetracyclines and chloramphenicol derivatives) and bactericidal drugs with time-dependent action (e.g., penicillins) and high AUC or C_{max}:MIC ratios, and bactericidal drugs with concentration-dependent effect (e.g. fluoro-quinolones). In conclusion, the integration of different guidelines from the literature to attempt to optimise and design dosage regimens, is a rather complicated procedure not least due to the generalized over-simplistic nature of existing guidelines and exceedance factors. It is recommended that existing approaches be used with considerable caution.

Registered antibacterial agents in Mediterranean finfish farming and their pharmacokinetics

Tetracyclines

Tetracyclines are broad-spectrum bacteriostatic antibiotics produced by *Streptomyces* spp. fungi. Their mode of action is via interference with bacterial protein synthesis (mRNA translation) by binding to bacterial 30S ribosomal subunit of microbial 70S ribosomes. Oxytetracycline (OTC) is the most common tetracycline used worldwide for the treatment of bacterial fish diseases. The drug is orally administered by incorporation into the feed, usually at a dosage of 75 mg kg^{-1} fish for 10 days (Scott, 1993). There are cases of chlortetracycline (another tetracycline) being used as an antibacterial in fish in Spain (Costello et al., 2001), but no information with regard its kinetics in euryhaline fish species is currently available. However, the chemical behavior and kinetic profiles of these two drugs in other food-producing animal species, has been shown to be very similar (EMEA, 1995a). The pharmacokinetics of OTC have been extensively investigated in experimental studies of euryhaline farmed fish species including European sea bass, gilthead sea bream and sharpsnout sea bream (Malvisi et al., 1996; Rigos et al., 2002a, 2003a, 2004a,b) (Table 6). Incomplete absorption of the drug was observed in all euryhaline fish species tested (Rigos et al., 2003a2004a, b). The bioavailability (F%) of OTC has been found to be higher in European sea bass (22%) (Rigos et al., 2004a) compared to gilthead sea bream (9%) (Rigos et al., 2003a), while F was almost negligible in sharpsnout sea bream (Rigos et al., 2004b). Therefore, a significant fraction of the administered OTC is not found in the circulation of euryhaline fish. Consequently, the use of OTC should be discouraged, at least when the drug is administered orally, as in sharpsnout sea bream.

The major barrier to OTC absorption in euryhaline fish species results from functional groups with an affinity for ionic binding. These cause complex formations with cations (Mg^{2+} and Ca^{2+}) in the feed and the intestinal environment of the fish, which in turn reduces OTC membrane permeability (Clive, 1968). This is evidenced by the considerable amount of orally administered OTC (40–73%) recovered unmetabolised in faeces of euryhaline fish (Rigos et al., 1999, 2002d). It has been widely reported that this is due to considerable first pass elimination of OTC, supported by the observation that a high proportion of the drug is detected in liver and bile soon after oral administration (Plakas et al., 1988; Rigos et al., 2004b).

The large volume and distribution observed in these species indicates the drug is adequately distributed throughout the body through transfer outside of the blood, which is a favorable property for treating poorly vascularized infected areas, such as the skin or muscle. Maximum plasma OTC concentration in gilthead sea bream and European sea bass following a single oral administration has been reported to be $\sim$2.6 μg mL^{-1} (Rigos et al., 2003a, 2004a). Penetration of OTC in the tissue compartment of euryhaline fish is significant since the *apparent volume of distribution at steady-state*

Table 6. Pharmacokinetic studies of registered (EU) antibacterial agents in euryhaline fish farming

Reference	Drug	Administration	Dose (mg kg^{-1})	Duration (days)	Fish species	Weight (g)	Temperature (C°)	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ or γ (h)	V_{dss} L kg^{-1}	F%	MIC μg mL^{-1} or g^{-1}	*WTs* (h)
Malvisi et al. (1996)	OTC	OR-M	75	14	GSB	50–70	19–28					14.7 (liver)	480
Malvisi et al. (1996)	OTC	OR-M	75	14	SB	80–100	19–28						
Rigos et al. (2002a)	OTC	IV	40		SB	110	13.5	1.0	69	5.6			
Rigos et al. (2002a)	OTC	IV	40		SB	110	22	0.2	10	2.6			
Rigos et al. (2003a)	OTC	IV	40		GSB	100	20	2.0	53	2.9			
Rigos et al. (2003a)	OTC	OR-S	75		GSB	100	20				9	2.6 (plasma)	
Rigos et al. (2004b)	OTC	IV	40		SSB	90	19	1.4	35	4.0			
Rigos et al. (2004b)	OTC	OR-S	75		SSB	90–200	17–19				0	6.2 (liver)	
Rigos et al. (2004a)	OTC	OR-S	50		SB	120	22				22	2.6 (plasma)	
Christophilogiannis et al. (1997b)	OA	OR-M	25	10	SB	350	21					6 (liver)	
Christophilogiannis et al. (1997b)	OA	OR-M	30	10	GSB	65	26					6.4 (liver)	
Poher et al. (1997)	OA	IV	10		SB	100	15.4	0.7	87	2.6			
Rigos et al. (2002b)	OA	IV	15		SB	110	14	2.8	315	14.8			
Rigos et al. (2002b)	OA	IV	15		SB	110	22	1.2	55	5.0			
Rigos et al. (2002c)	OA	IV	20		GSB	100	20	0.5	12	2.1			
Rigos et al. (2002c)	OA	OR-S	30		GSB	100	20				14	1.0 (plasma)	
Rigos et al. (2003b)	OA	OR-M	30	10	GSB-SSB	120–170	19		13–19			0.9 (plasma)	0
Rigos et al. (2004d)	OA	IV	20		SSB	90	19	0.4	10	2.1			
Rigos et al. (2004d)	OA	OR-S	40		SSB	90	19				15	0.9 (plasma)	
Malvisi et al. (1997)	FLU	OR-M	12	5	GSB	60–80	25–28					0.4 (vertebrae)	
Rigos et al. (2002e)	FLU	IV	10		SB	120	18	1.0	11	1.5			
Rigos et al. 2003c	FLU	IV	10		GSB	170	19	0.2	30	0.6			
Rigos et al. 2003c	FLU	OR-S	20		GSB	170					29	1.7 (plasma)	
Tyrpenou et al. (2003b)	FLU	OR-M	35	5	GSB	237–307	18–24		22.1–21.4			2.4 (muscle + skin)	107–76
Tyrpenou et al. (2003a)	SARA	OR-M	10	5	GSB	163–237	18–25		32.5–17.8			0.3 (liver)	42
della Rocca et al. (2004)	AMO	OR-S	80		GSB	120–160	22				0.3	0.1 (skin)	
della Rocca et al. (2004)	AMO	OR-M	80	10	GSB	50–180	22–26						
Castells et al. (2000)	THI	OR-S	15–30		SB	250–300						5.6–9.4 (plasma)	
Intorre et al. (2002)	THI	OR-M	15–30	5	SB	250–350	18–20					0.9–1.3 (plasma)	120–144
Malvisi et al. (2002)	THI	OR-M	40	5	SB-SSB	110–150	20–28					6.4–8.5 (liver)	80–89

OTC: oxytetracycline; OA: oxolinic acid; FLU: flumequine; SARA: sarafloxacin; AMO: amoxycillin; THI: thiamphenicol

$t_{1/2\alpha}$: absorption half life (plasma or tissue); $t_{1/2\beta}$: elimination half time (plasma or tissue); V_{dss}: apparent volume of distribution of the drug at a steady state; F: bioavailability = 100*(non-intra-vascular dose/ intra-vascular dose); MC: maximum concentration (tissue when no plasma was sampled) after oral dosing (single or multiple); *WTs*: withdrawal times

IV: intravascular; OR-S: oral single dose; OR-M: oral multiple dose; SB: European sea bass; GSB: gilthead sea bream; SSB: sharpsnout sea bream

($V_{d(ss)}$), after single injection has been found to be 2.6–4.0 L kg^{-1} (Rigos et al., 2002a2003a2004b). Similarly, high concentrations of OTC have also been reported in gilthead sea bream skin and vertebrae (7.7 and 6.0 $\mu g\ g^{-1}$), following multiple oral dosing (Malvisi et al., 1996).

The elimination of OTC is strongly affected by water temperature in euryhaline fish (Rigos et al., 2002a), with longer elimination half-lives observed in sparid plasma ($t_{1/2\beta}$ of 35–53 h at 19–20 °C) (Rigos et al., 2003a, 2004b) than in European sea bass (10 h at 22 °C) following single oral dosing (Rigos et al., 2002a). The differences in the metabolism of OTC between sparids and European sea bass can be attributed to differences in their physiology (e.g., differences in hepatic microsomal cytochrome P450 activity). As a result, long withdrawal times (*WTs*) (20 day period) are necessary for OTC-treated gilthead sea bream at 19–28 °C (Malvisi et al., 1996) to achieve a MRL of 100 ng g^{-1} (EMEA, 1995a). Due to the relatively slow elimination of OTC in sparids, a sequential dosing schedule of OTC in these species might be a more prudent and cost-effective alternative if adequate tissue levels are maintained in the treated fish (drug concentrations at sites of infection remain in excess of MIC). Currently, there is no published information available on efficacy of OTC and MICs (for marine conditions) against important bacterial diseases of euryhaline fish.

(Fluoro)quinolones

Quinolones, such as including oxolinic acid (OA) (first generation quinolone) and fluoroquinolones (first generation derivatives; including flumequine; FLU and sarafloxacin; SARA), are synthetic modern antibacterials effective against broad spectrum systemic infections of gram negative bacteria. Their mode of antibacterial action is by interfering with bacterial DNA gyrase (converts relaxed closed-circular, duplex DNA to a negatively superhelical form) preventing completion of the super-coiling of bacterial chromosomes and possess post-antibacterial action in a dose-dependent manner (Shojaee AliAbadi and Lees, 2000). The recommended dosages of OA, FLU and SARA are 10–30, 12 and 10 mg kg^{-1} fish for 5–7 days, respectively (EMEA, 1998a, 1999, 2003). Quinolones are used extensively to combat bacterial fish pathogens in several European Mediterranean countries (Costello et al., 2001).

The kinetic profiles and residue depletion of quinolones have been widely investigated in several euryhaline fish species (Table 6). Due to the low p*K*a values of quinolones (6–6.9), their absorption is influenced by the alkaline environment of the gut in marine fish species. However, OA absorption studies in European sea bass and sparids have revealed relatively high apparent digestibility (absorption) values (64–92%) (Rigos et al., 1999, 2002d). In contrast, *F* for OA in sparids is surprisingly low (14–15%) (Rigos et al., 2002c, 2004d), indicating that complexing of OA with cations reduces its membrane permeability and is indicative of significant first pass elimination following absorption. On the contrary, FLU has been shown to be more bioavailable than OA in gilthead sea bream (29%) (Rigos et al., 2003c). This might indicate that FLU is preferable to OA in diseased gilthead sea bream, assuming no differences in bacterial sensitivity to these two quinolones.

Maximal plasma OA levels following single or multiple oral dosing in both gilthead and sharpsnout sea bream, were found to be $\sim$1 $\mu g\ mL^{-1}$ (Rigos et al., 2002c, 2003b, 2004d), while respective values for FLU after single dosing in gilthead sea bream was higher at 1.7 $\mu g\ mL^{-1}$ (Rigos et al., 2003c). This difference may be attributable to the greater *F* of FLU in gilthead sea bream, and is therefore indicative of the greater therapeutic efficacy of FLU as previously mentioned.

Although the penetration of intravascularly-injected OA in the tissue compartment of euryhaline fish is moderate ($V_{d(ss)}$ = 2.1–2.6 L kg^{-1}) (Poher et al., 1997; Rigos et al., 2002c, 2004d) it is higher than that of FLU (0.6–1.5 L kg^{-1}) (Rigos et al., 2002e, 2003c). A study of the tissue distribution of OA following multiple dosing, revealed that liver, bile and skin act as reservoirs of the drug treatment in both gilthead and sharpsnout sea bream with rapid depletion of the drug from all tissues to "consumer safe levels" (MRL = 100 ng g^{-1}; EMEA, 2003) 24 h after completion of treatment at 19 °C (Rigos et al., 2003b). The rapid clearance of OA in euryhaline fish species is a beneficial feature, allowing fish are to enter the market more rapidly.

Malvisi et al. (1997) reported that skin and vertebrae of gilthead sea bream act as reservoirs

for FLU for prolonged periods even after cessation of treatment, but accumulation is at levels below the MRL (MRL = 600 ng g^{-1}; EMEA, 1999). Very low FLU levels have been measured in muscle of European sea bass (21 ng g^{-1}) dosed at 12 mg kg^{-1} for 5 days, at 21–25 °C (Luzzana et al., 1996). Flumequine depletion from edible tissues of gilthead sea bream has been investigated in two studies with contradicting results. In one study, separate muscle and skin samples showed "safe consumer FLU levels" 24 h post-treatment at 25–28 °C (16 and 85 ng g^{-1} for muscle and skin, respectively) (Malvisi et al., 1997), while in another study much higher FLU levels were observed in muscle and skin samples (1225–2394 ng g^{-1} at 18–24 °C) 24 h post-treatment, requiring *WTs* of 106 and 76 h, for low and high temperatures, respectively (Tyrpenou et al., 2003b). The considerable differences between the two studies were attributed to the lower in-feed administration of the drug in the first study (12 versus 35 mg kg^{-1}) and differences in experimental design (Tyrpenou et al., 2003b). Therefore, no final conclusions can be made with respect to FLU depletion in sparid edible tissues and *WTs* to ensure safe levels for human consumption.

Tissue distribution studies of SARA in gilthead sea bream, have revealed that the liver accumulates highest levels of the drug during treatment, but the vertebra are also a reservoir for the drug, since levels persist in vertebra even once treatment has ceased (Tyrpenou et al., 2003a). These findings have led to suggested *WTs* from muscle and skin, of 42 h at 25 °C for a MRL of 30 ng g^{-1} (EMEA, 1998a). As with OA, rapid depletion of SARA from fish tissues is a favorable characteristic allowing shorter *WTs* for treated fish to be sold to market. Despite this, absorption-bioavailability studies on SARA in euryhaline fish is still needed to evaluate its efficacy (coupled with MIC studies), as its *F* has been found to be low (2%) in Atlantic salmon (*Salmo salar*) (Martinsen and Horsberg, 1995) and low levels of the drug have been measured in tissues of gilthead sea bream (highest concentration in liver; 300 ng g^{-1}) (Tyrpenou et al., 2003a).

In the case of OA, $t_{1/2\beta}$ values following multiple dosing of sharpsnout and gilthead sea bream, have been estimated to be 12 and 11–14 h in muscle and skin, respectively, at 19° C (Rigos et al., 2003b). Corresponding values for FLU (18–24 °C) and SARA (18–25 °C) for muscle and skin of the same species were found to be 22 and 18–33 h (Tyrpenou et al., 2003a,b). Overall, the elimination of quinolones appears to be rapid in euryhaline fish species and highly temperature-dependent (Rigos et al., 2002b; Tyrpenou et al., 2003a,b). Consequently, daily dosing of quinolone drugs should be mandatory, especially at higher water temperatures, to maintain maximum tissue drug concentrations in euryhaline fish. Since quinolones are bactericidal drugs with concentration-dependent action, high AUC or C_{max}:MIC ratios are desirable in infected fish. Thus, dosages should be maximised wherever possible and the duration of treatment kept to a minimum, e.g., 5 days, to compensate for drug loss (and subsequent loss to the environmental) and minimize costs.

As is the case with OTC, studies on the efficacy and MIC (under marine conditions) of quinolone drugs against important bacterial diseases of euryhaline fish in the literature, are scarce. Ledo et al. (1987) reported OA MIC values of OA against several *Vibrio* spp. and *P. damsela* subsp.*piscicida* of 0.075–0.3 μg mL^{-1}. Only limited MIC information for FLU is available for a few bacterial fish pathogens. A very low MIC of FLU of 0.15 μg mL^{-1} against *V. damsela* under marine conditions has been reported (Rigos et al., 2003c). On the contrary, the last study revealed higher MIC values (ranging between 4.78 and 38.25 μg mL^{-1}) for *V. anguillarum*, *P. damsela* subsp. *piscicida*, *V. alginolyticus* and *V. flubialis*, indicating that these species are rather resistant to this quinolone according to the guidelines proposed by Tsoumas et al. (1989). Determination of MICs of quinolone drugs and challenge tests against other bacterial strains are needed to confirm the efficacy of these drugs for use in euryhaline finfish treatments.

Potentiated sulfa

Potentiated sulfa drugs are a combination of sulfonamides and pyrimidine potentiators, such as trimethoprim (TRM) and ormetoprim (OMP), in a concentration ratio of 5:1. Sulfonamides are a large range of structurally-related synthetic compounds (e.g., sulfadiazine (SDZ), sulfamethoxazole (SMX) and sulfadimethoxine (SDM)) that are derivatives of sulfanilamide. Potentiated sulfa antibiotics have a broad spectrum of bactericidal

activity against bacterial pathogens and their combined efficacy is greater than the sum of the potencies of any two separate drugs. This is because they interfere with the nucleid acid metabolism of bacteria, by acting as competitive inhibitors of folic acid metabolism. Sulfadiazine plus TRM is the most commonly used combination in veterinary medicine. The recommended dosage in fish treatments is 25 and 5 mg kg^{-1} fish (for 5–10 days) for SDZ and TRM, respectively (Scott, 1993; EMEA, 1995b; 1997).

Unfortunately, the kinetic profile of SDZ and TRM and their efficacy against bacterial pathogens, have not been studied in euryhaline fish species, although they have been used extensively in several Mediterranean countries (Costello et al., 2001). On the other hand, considerable information is available on kinetic profiles of SDZ and TRM for cold and/or fresh water fish species (Stoffregen et al., 1996; Horsberg et al., 1997; Samuelsen et al., 1997). These have demonstrated high bioavailability of SDZ (46%) and TRM (100%) in Atlantic salmon, but high MIC values against important bacterial pathogens (MIC_{50} = 1.6–32 $\mu g\ mL^{-1}$) (Horsberg et al., 1997).

Attempts to extrapolate kinetic information from cold and/or fresh water species to design/or correct dosage regimens and predict withdrawal times for euryhaline fish species is suspect. The physiological differences and thus capacity to process drugs, between fresh and/or cold water and euryhaline fish species are significant, which can lead to ineffective treatment and potential cross-infection of human consumers. Clearly, there is an urgent need for studies of pharmacokinetics and drug efficacy of potentiated sulfa, at least in the most commonly farmed euryhaline fish species.

Penicillin derivatives

Penicillin derivatives (β-lactams), including amoxycillin (AMO) and ampicillin (AMP), are broad spectrum antibacterial agents widely used in veterinary medicine. These drugs possess bactericidal action (time-dependent action) by inhibiting bacterial cell wall synthesis in a time-dependent manner (Shojaee AliAbadi and Lees, 2000). The recommended dosage of penicillin derivatives in fish treatments is 80 mg kg^{-1} fish for 5–10 days (Scott, 1993).

These compounds have not been widely employed in euryhaline fish farming, probably due to the lack of kinetic studies in fish and incomplete standards – for example, MRLs are only available for terrestrial farmed animals (Lalumera et al., 2004). AMP and AMO are used in Spain (Costello et al., 2001) and more recently AMO has been introduced in Italy to combat bacterial fish pathogens (della Rocca et al., 2004). The kinetic profile and efficacy of AMP have not yet been investigated in euryhaline fish species. Published MIC values for AMO indicate this drug is a promising antibacterial for combating *V. anguillarum* and *P. damsela* subsp.*piscicida* in gilthead sea bream with values of 0.04 and 0.08 $\mu g\ mL^{-1}$ (Mazzolini et al., 1997). Plasma C_{max} following oral dosing, is recommended to exceed MIC by a factor of 5–12 at 1–12 h from initial dosing (della Rocca et al., 2004). However, AMO has negligible bioavailability in gilthead sea bream (0.33%; della Rocca et al., 2004), questioning its use in this species, at least using oral administration. However, kinetic studies in other euryhaline fish species, may demonstrate more acceptable levels of AMO absorption, where it may be more appropriately used for antibacterial therapy. Since penicillin drugs possess bactericidal effect with time-dependent action it is recommended that dosing should aim at achieving drug concentrations at sites of infection that exceed the MIC (lowest possible dosage with longest possible duration; e.g. sequential dosing if drug tissue levels and MICs are acceptable).

Chloramphenicol derivatives

Chloramphenicol (CAP) derivatives including florfenicol (FLO) and thiamphenicol (THI), are primary bacteriostatic broad-spectrum compounds that inhibit bacterial protein synthesis by binding to the 50s subunit of the bacterial ribosome. Both antibacterial agents have been used in veterinary medicine without serious adverse effects, such as aplastic anaemia which has been seen with CAP use leading to a ban on its use in food-producing animals (EC 1430/94). The recommended dosage of FLO and THI against bacterial fish diseases is 10 and 40 mg kg^{-1} fish for 10 and 5 days, respectively (EMEA, 1998b, 2000).

The kinetic profile and efficacy of FLO have not yet been determined for euryhaline fish species.

In contrast, FLO has been found to have excellent bioavailability (97%) in Atlantic salmon (Martisen et al., 1993) and high efficacy against *Aeromonas salmonicida* (Nordmo et al., 1998). However, as previously mentioned, although useful, there are serious limitations in extrapolating this data to treat warm water euryhaline fish with this drug.

Fortunately, there are studies of the kinetics of THI in euryhaline fish, particularly in European sea bass (Intorre et al., 1997, 2002; Castells et al., 2000; Malvisi et al., 2002) and to a lesser extent in gilthead sea bream, where THI was found to be well-distributed in tissue compartments of both species, following a 5-day dosing at 40 mg kg^{-1} at 20–28 °C (Malvisi et al., 2002). Maximum tissue concentrations of THI during treatment reached 6.4 and 3 $\mu g\ g^{-1}$ in European sea bass liver and skin, respectively. Respective values for gilthead sea bream were 8.5 and 2 $\mu g\ g^{-1}$ in liver and skin (Malvisi et al., 2002). Following single oral THI administration (oral gavage in aqueous solution), peak plasma THI concentration was as high as 5.6 and 9.4 $\mu g\ mL^{-1}$ in European sea bass, following 15 and 30 mg kg^{-1} dosing, respectively (Castells et al., 2000). However, maximal plasma THI levels following 5-day treatment administered in-feed, were found to be considerably lower for both dosing levels at 0.8 and 1.3 $\mu g\ mL^{-1}$, respectively (Intorre et al., 2002). These differences may be due to the different routes of administration employed, and the influence of feed constitutes on THI absorption (e.g. drug complexing with cations in feed). The lower drug levels attained in treated fish in the latter study are more representative to "at site" treatments where drugs are delivered via the feed indicating that absorption of THI is inhibited in the gut environment.

MIC_{50} values for THI against *V. anguillarum* and *P. damsela* subsp.*piscicida* as high as 2.5 and 5 $\mu g\ mL^{-1}$, respectively, have been reported (Mazzolini et al., 1997). Additional MIC studies (where a wider range of species and isolates are tested) is needed to achieve a comprehensive understanding of the efficacy of this drug, since it is likely that the above isolates may be resistant to THI as reflected by the high MIC values. It is important to note, that MIC values for CAP against these two pathogens were also high (4.8 $\mu g\ mL^{-1}$) (Tyrpenou et al., 2003c). Thus, it is difficult to speculate that the administration of THI even under a high dosage regimen as used by Intorre et al. (2002) would be effective at least, against the aforementioned bacteria. This indicates a relative inefficacy of THI against bacterial infections of euryhaline fish species, however further research is needed to confirm this. On the contrary, respective MIC values for FLO against the two aforementioned pathogens were considerably lower (0.2–0.8 and 0.2–0.6 $\mu g\ mL^{-1}$ for *V. anguillarum* and *P. damsela* subsp. *piscicida*, respectively) (Fukui et al., 1987; Zhao et al., 1992; Kim and Aoki, 1993), indicating a higher efficacy of FLO compared to THI for combating bacterial diseases.

Following multiple oral dosing, the elimination of THI in European sea bass is relatively fast, as indicated by its short half-life (20–23 h; Intorre et al., 1997). Based on an MRL of 50 ng g^{-1} (EMEA, 1998b), *WT*s of 120 and 144 h are necessary for 5-day dosing at 15 and 30 mg kg^{-1}, respectively, at 18–20°C to ensure treated sea bass are suitable for human consumption (Intorre et al., 2002). Malvisi et al. (2002), suggested *WT*s of 80 and 89 h for THI in sea bass and gilthead sea bream, respectively, which is in accordance with that recommended by Intorre et al., (2002) for higher temperature conditions (20–28 °C). Long *WT*s are not beneficial due to the increased time needed before fish can be sold to market. Chloramphenicol derivatives are bacteriostatic compounds, thus dosing should be aimed at achieving drug concentrations at sites of infection that exceed the MIC. Consequently, use of a lower dosage (e.g., 10 mg kg^{-1}) over a longer treatment period (e.g., 10 days) would be a more economical, rational and effective approach to reducing *WT*s. However, use of a longer-term lower-level dosing approach requires experimentation to ensure the resulting tissue drug concentrations are indeed adequate to combat the infection at hand.

Environmental implications of antibacterial drug use in Mediterranean fish farming

The incidence of bacterial infections in euryhaline farming sites requires the treatment of a considerable proportion of the caged fish population. Despite optimization of antibacterial drug therapy for bacterial fish infections, significant quantities of drugs are nevertheless released in to the vicinity of the fish farms from different routes (see Figure 1). This is via non-ingested medicated pellets (due to

reduced fish appetite or low feed palatability), unabsorbed drug in the feaces or unprocessed parent drug from renal and gill excretion and in the form of metabolites following renal and faecal excretion of the treated fish (Rigos et al., 1999, 2002d, 2004c). For example, using information from absorption studies of OTC, one of the most commonly used drugs, 40%, 60% and 74% of total orally administered drug to European sea bass, sharpsnout and gilthead sea bream in Greek fish farms, is passed unabsorbed from fish in to the environment in faeces, due to its low absorption (Rigos et al., 1999, 2002d; Rigos, 2003). To offset these characteristics, high dosing levels of OTC are used which in turn leads to greater drug loss to the environment.

A drug's potential for degradation and its lipophilicity, combined with ambient environmental conditions of receiving waters and sediments, are critical factors in determining its environmental impact. Antibacterial drugs used in aquaculture have been shown to persist in water and sediments in the vicinity of euryhaline fish farms, sometimes long after their use has ceased (Jacobsen and Berglind, 1988; Samuelsen et al., 1992; Capone et al., 1996). The persistent of drugs released from fish farms prolongs their life in the water column and sediment and lipophilicity allows them to enter the aquatic food chain leading to contamination of non-target organisms. Upon release into water, abiotic degradation (mainly by photolysis) of antibiotics can take several days or weeks (Oka

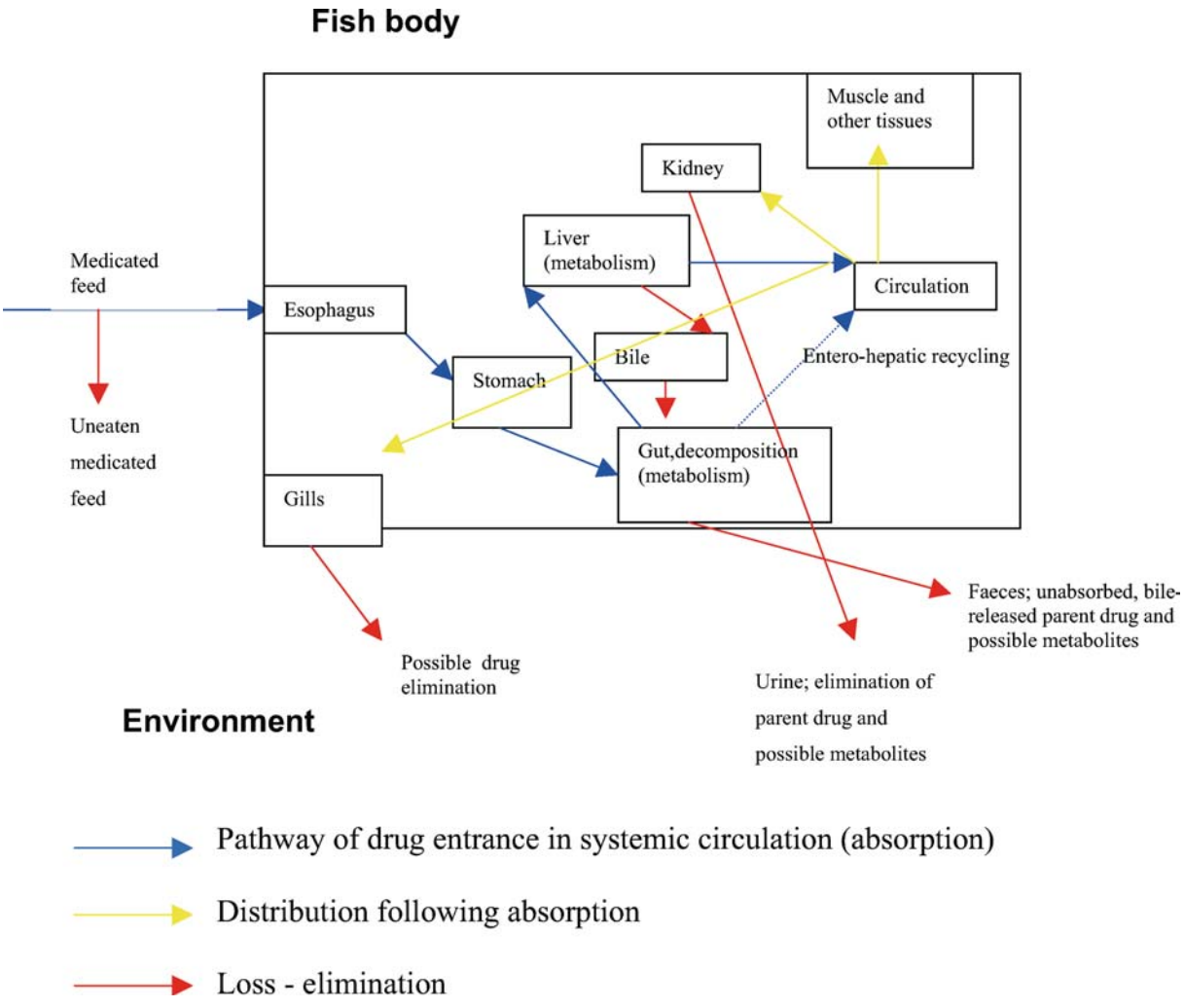

Figure 1. Pathway of orally administered antibacterials in fish; a hypothetical model (adapted from Rigos, 2003).

et al., 1989; Samuelsen, 1989). Furthermore, with the exception of bath treatments, only a small proportion of a drug released during fish therapy (oral administration) to the environment actually ever reaches the surface waters, where light penetration is adequate for effective photolysis (Lunestad et al., 1995).

Antibacterial drugs, particularly OTC, are easily partitioned into organic matter of aquatic sediments and suspended particulates (organic carbon coefficient (K_{oc}) of OTC can be as high as 93,000; Rabolle and Spliid, 2000). Many of the drugs reviewed here, such as OTC, OA and SARA, are relatively resistant to biodegradation, as indicated by long half-life ($T_{1/2}$ = 151 days) in cold water euryhaline surface sediments (Hektoen et al., 1995). Fortunately, degradation of drugs tends to be more extensive in Mediterranean euryhaline environments where temperature, salinity and light intensity are greater than in cold water mariculture and thus degradation is higher (Oka et al., 1989; Samuelsen, 1989; Samuelsen et al., 1994; Doi and Stoskopf, 2000). However, determination of abiotic and biological degradation rates for antibiotic drugs, specifically under warm-water euryhaline conditions, is necessary to fully appreciate their overall persistence in sediments and water around Mediterranean fish farms.

The environmental fate and behavior of antibacterials in the marine environment are illustrated in Figure 2, using OTC orally administered to euryhaline fish, as an example (adapted from

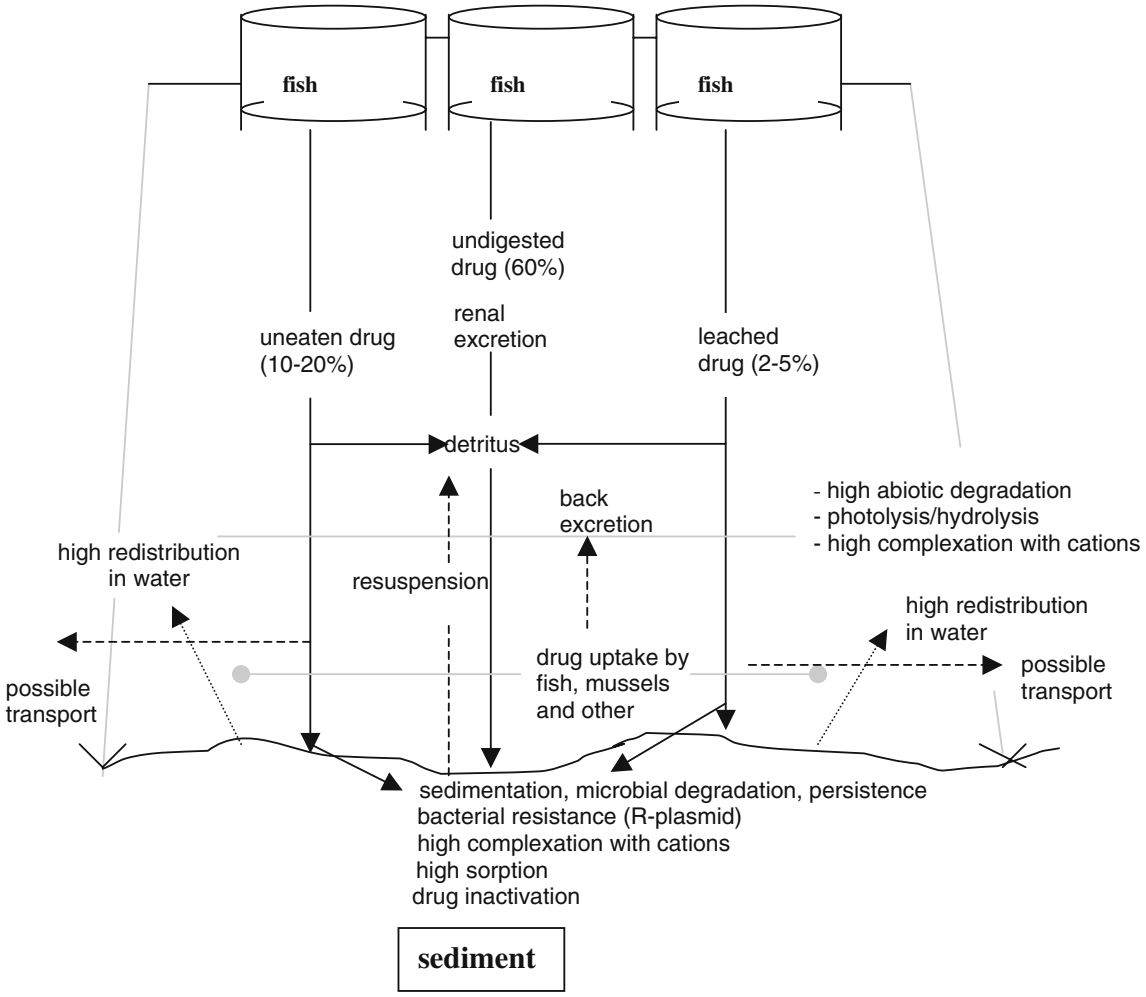

Figure 2. Environmental fate (hypothetical model) of orally administered OTC in euryhaline fish farming sites (adapted from Rigos, 2003).

Rigos, 2003). The figure shows, that during treatments a fraction of the administered drug is lost to the environment due to known depressed absorption and poor bioavailability of this drug. Additional loss of the administered dosage may occur due to poor palatability and/or depressed appetite of treated fish (10–20%) (Rigos et al., 1999). Leaching of the medicated pellets (2–5%) is also possible, especially in cases where the drug is incorporated in the diet by oil-coating, coupled with delayed consumption by anorectic diseased fish (Rigos et al., 1999). Upon release of OTC to the environment, the figure shows that the drug can be subjected to abiotic degradation (e.g., photolysis) and complexing in the water column, following which it may it be taken up by non-target species and accumulate in marine sediments via high drug sorption; abiotic drug processing; development of resistance; drug complexing with marine cations (Samuelsen, 1989; Capone et al., 1996; Pouliquen and Le Bris, 1996; Coyne et al., 2001). OTC may also be flushed back from the sediment to the water column and transported away from the site by water currents (Figure 2).

One of the major consequences of drug pollution from aquatic farms is the development of antibiotic resistance in human and fish pathogens which reduces their therapeutic value for fish therapy and human medicine (transfer of drug resistance) (Angulo, 1999; Schmidt et al., 2000). The occurrence of resistant bacterial populations in sediments beneath and around cold-water marine fish farms is well documented (Kerry et al., 1994, 1996; Capone et al., 1996; Angulo, 1999). There has been only one study of bacterial resistance in water and sediments around Mediterranean fish farms. Chelossi et al. (2003) reported high incidences of quinolone, tetracycline and penicillin–resistant bacterial populations in sediments from the vicinity of Ligurian Sea coastal fish farms. OTC-resistant bacteria have even been found in sediments around salmon farms off the coast of Chile which had no history of OTC use, demonstrating antibacterial resistance is transferable between bacterial populations in warm water environments regardless of drug availability in the local environment (Miranda and Zemelman, 2002).

Antimicrobial resistant bacterial strains have been found in invertebrates, farmed and wild fish destined for human consumption. High incidences of multi-drug resistant bacteria (e.g.,mm *Vibrio* spp., *Pseudomonas* spp. and *Aeromonas* spp.) have been detected in a range of pelagic and demersal wild fish species caught near warm water fish farms in Chile (Miranda and Zemelman, 2001; Castro-Escarpulli et al., 2003) and Japan and Korea (Kim et al., 2004). In the Mediterranean, similar findings have also been made for gilthead sea bream collected from waters around the fish farms of southwest Spain (Zorilla et al., 2003). A high incidence of OA-resistant bacteria was reported in mussels collected from areas close to a Norwegian fish farm (Samulesen et al., 1992). Clearly, there are opportunities for horizontal transmission of resistant bacterial strains to human populations from the consumption of farmed and wild-caught fish and shellfish (Sorum and L'Abbe-Lund, 2002). Indeed there is evidence of this in Italy (Ottaviani et al., 2001) and Taiwan (Wong et al., 2000). Transmission to Greek consumers has not yet been studied and may indeed be an unquantified public health issue. Considering that Greece is currently the largest producer of euryhaline farmed fish in Europe, this issue requires further study.

Another consequence of drug pollution from aquatic farms is the potential accumulation of drug residues in the aquatic food chain leading to exposure of biota around farm sites with possible toxic effects. Shellfish and wild-caught fish destined for human consumption harvested from areas around farm sites, have been shown to be contaminated with antibiotic drugs to levels beyond the respective MRLs (Table 5). OTC residues have been detected in mussels (*Mytilus edulis*) from Galway, Ireland (max. 10.2 $\mu g\ g^{-1}$) (Coyne et al., 1997), oysters (*Crassostrea gigas*; max. 0.1 $\mu g\ g^{-1}$), Dungerness crabs (*Cancer magister*; max 0.1 $\mu g\ g^{-1}$) and red rock crabs (*Cancer productus*; 0.8–3.8 $\mu g\ g^{-1}$) from Puget Sound, U.S. (Capone et al., 1996). Up to 4.4 $\mu g\ g^{-1}$ of OA have been detected in muscle and liver of coalfish (*Pollachius virens*) and mackerel (*Scomber scombrus*) caught near Norwegian fish farms (Samuelsen et al., 1992). There have been no studies to date investigating drug levels in non-target species (e.g. wild-caught fish, shellfish and sea weeds) from the vicinity of Mediterranean fish farms. Routine drug testing of these potential human foods is not required/enforced in the Mediterranean as is the case with farmed fish. Currently, any incidences of elevated drug residues in biota may go undetected,

which may lead to deleterious consequences for human consumers and have adverse ecological effects.

Data on the toxic doses of antibiotics for aquatic wildlife are available for a limited number of species and limited to short term test data which do not reflect toxicity resulting from long term exposure to antibiotics. Furthermore, the influence of higher water temperature, salinity and light availability found around coastal Mediterranean fish farms on ecological effects of antibacterials has not been studied to date. In the case of antibacterial phytotoxicity, cyanobacteria (e.g., *Microcystis aeruginosa*; effective concentration for 50% toxicity (EC_{50}) FLU = 0.16, OA = 0.18, SARA = 0.015, OTC = 0.2 mg L^{-1}) are an order of magnitude more sensitive to toxic effects of these drugs than eukaryotic green algae (e.g. *Selenastrum capricornutum*; EC_{50} FLU = 5, OA = 16, SARA = 16, OTC = 4.5 mg L^{-1}) (Holten-Lutzhoft et al., 1999; Halling-Sorensen, 2000). In the aquatic weed *Lythrum salicaria*, FLU concentrations over 100 mg L^{-1} are necessary for adverse effects on post-germinative development (Migliore et al., 2000) indicating that higher plants may be less sensitive to the toxicity of antibiotics than prokaryotic algae. There is little monitoring of antibiotic concentrations in under-cage seawater at fish farms. OTC concentrations of <0.3 mg L^{-1} were observed at a salmon farm in Galway, Ireland, following a 12 day OTC fish therapy (125 mg kg^{-1} b. wt. d^{-1}) (Coyne et al., 2001). This indicates there may indeed be incidences of seawater antibacterial concentrations adequate to cause some toxicity to cyanobacteria. To date there are no studies of under-cage seawater drug concentrations in Mediterranean fish farms, however considering drug use in countries with high farmed fish production volumes, such as Greece, monitoring programs clearly need to be put in place.

In the case of the toxicity of antibiotics to aquatic animals, only limited data are available and only for cold water freshwater/estuarine crustaceans. FLU has been shown to cause adverse effects on cyst hatching and nauplii larval development in brine shrimp (*Artemia salina*; EC_{50} (72 h) = 96 mg L^{-1}; Migliore et al., 1997). Acute and no observable effects concentration (reproductive NOEC) values for water fleas (*Daphnia magna*), indicate that quinolone antibiotics (e.g., OA; EC_{50} (48 h) = 4.6 mg L^{-1}, NOEC = 0.38 mg L^{-1}) are more toxic to crustaceans than most other antibiotics, such as potentiated sulpha and tetracyclines (Wollenberger et al., 2000). On the basis of the limited available toxicity data, it would appear that water and sediment drug levels beneath and around fish farms reported to date, are unlikely to be adequate to cause toxic effects in wildlife. For example, even the maximum reported concentration of FLU (0.58 mg kg^{-1}) in sediments from the vicinity of Italian sea bass farms reported by Lalumera et al. (2004) is well below the EC_{50} value for FLU determined for brine shrimp (Miglione et al., 1997). However, the possible non-lethal effects of antibacterial agents with low exposure over time on aquatic biota can not be neglected (especially in the case of potential DNA damage on long lived species).

To minimize local contamination of water, sediments and non-target species, use of antibacterials with a high potential for absorption combined with a high bioavailability in the treated fish species would improve practice. This would enable use of lower therapeutic doses with proportionately lower drug losses to the environment. Thereby the likelihood of the development of ecotoxic effects on non-target species and resistant bacterial populations is reduced. Furthermore, this would improve cost-effectiveness of the therapy.

Conclusions and recommendations

Vaccination has effectively minimized the use of antibacterials against a limited number of bacterial pathogens in the euryhaline environment, however there is still a range of antibacterial drugs, which remain the last resort to combat many bacterial fish diseases. It is apparent from this review, that although the euryhaline fish farming industry is well established, basic pharmacokinetic and residue depletion studies are incomplete (e.g. penicillin derivatives) or totally lacking (e.g. potentiated sulfa and florfenicol) for some commonly used drugs.

The pertinent literature (Costello et al., 2001; Lalumera et al., 2004) reveal that OTC and quinolone drugs (FLU and OA) are the most commonly used in euryhaline fish farming across the Mediterranean. Naturally, most pharmacological research has been directed towards these two

important drug groups (see Table 6). Still however, there is a lack of knowledge, mainly on the efficacy and potential environmental impacts of these drugs in warm water euryhaline conditions. No evaluation can be made for the candidacy of potentiated sulfa since there is a significant lack of knowledge (pharmacokinetics, efficacy and environmental effects) of this drug in the euryhaline fish industry. Additional work is also needed for penicillins. The single published pharmacokinetic study of penicillins (AMO) for only one species (gilthead sea bream) indicates poor bioavailability (della Rocca et al., 2004). Although extensive research exists on THI in euryhaline fish (Castells et al., 2000; Intorre et al., 2002; Malvisi et al., 2002), no data is available for FLO despite the fact that this drug has been shown to be an excellent candidate for cold water fish farming (Nordmo et al., 1998).

Due to the fact that fundamental data is still lacking, dosage regimens currently employed for these drugs have usually been designed by extrapolation from salmonid studies, which is wholly inappropriate due to the inter-specific differences in drug kinetics among fish species, already clearly demonstrated in the literature. Extrapolation errors can considerably influence drug efficacy, safety and cost of treatment. Until new effective and practical vaccines are available to combat major bacterial diseases, it is paramount that good practice is employed during the use of antibacterial drugs to minimize environmental pollution which can lead to adverse ecological effects and the development of drug resistance in bacterial populations. Management and hygiene practices must be optimized addressing the whole product lifecycle and therapies must be carefully designed taking into account physico-chemical and pharmacokinetic properties and aquatic toxicity of the candidate antibacterial agent to be used, within the context of the nature of the disease. Considering our knowledge of the environmental fate, behavior and toxicity and ecological impacts of antibacterials used in fish farming, when selecting oral therapy, preference should be given to easily absorbed bioavailable drugs with low persistence, low aquatic toxicity and high antibacterial efficacy to encourage use of lower doses. This will help to minimize loss to the environment since the drug will be well retained and eventually metabolized by the treated fish and any portion lost to the environment (e.g., due to lost medicated feed) will not persist sufficiently to bioaccumulate in food chains or encourage development of resistant bacterial populations. For example, on this basis, OTC and AMO are not recommended for treating sharpsnout and gilthead sea bream respectively, due to their negligible bioavailability in these species. Unfortunately, due to data paucity it was not possible to make more reliable and accurate recommendations on selection and use of antibacterials and approaches for minimizing environmental impacts for the treatment of major euryhaline aquaculture species. The selection of administration methods with least drug wastage are advised to minimize environmental contamination (loss from bath treatments > in feed > *in vivo*).

Substantial research is needed to construct a database of environmental fate and ecotoxicological data (for indigenous species from different phyla and trophic levels) for commonly used antibacterials, specific to the Mediterranean aquatic environment, to facilitate proper assessment of ecological risks of drug pollution from Mediterranean fish farms. Considering the long term and large volume usage of antibacterials in Mediterranean fish farming, respective governments should encourage sustainable drug use and regulate monitoring of antibacterials in fish farm sediments to ensure levels do not accumulate in excess of thresholds for ecological effects or encourage the development of bacterial resistance. Clearly, the availability of reliable data on drug use and properties from the aquaculture industry and drug manufacturers, combined with guidance and regulation from government and environmental scientists, are critical to improving practice and achieving sustainable fish production.

References

Alderman, D.J. (1999) Requirements for the approval of veterinary therapeutics or growth enhancers in fish production. *In*: Smith, D.J., Gingerich, W.H., Beconi-Barker, M.G. (eds.), *Xenobiotics in Fish*. Kluwer/Plenum Press, NY, pp. 25–38.

Angulo, F. (1999) Antibiotic Use in Aquaculture: Centre for Disease Control Memo to the Record. Use of antimicrobial agents in aquaculture: potential for public health impact. Public Health Service, Department of Health & Human Services, Centres for Disease Control and Prevention (CDC), 5 pp.

Athanassopoulou, F., Prapas, T. and Rodger, H. (1999) Diseases of *Puntazzo puntazzo* Cuvier in marine aquaculture systems in Greece. *J. Fish Dis.* **22**, 215–218.

Babelona, M.C., Zoriilla, I., Morinigo, M.A. and Borrego, J.J. (1998) Survey of bacterial pathologies affecting farmed gilthead sea bream (*Sparus aurata* L.) in southwestern Spain from 1990 to 1996. *J. Fish Dis.* **166**, 19–35.

Blaser, J., Stone, B.B., Growner, M.C. and Zinner, S.H. (1987) Comparative study with enoxacin and netilmicin in a pharmacodynamic model to determine importance of ratio of antibiotic peak concentration to MIC for bactericidal activity and emergence of resistance. *Antimicrob. Agents Chemother.* **31**, 1054–1060.

Branson, E. (2001) Clinical relevance of Minimum Inhibitory Concentrations (MICs). *Aquaculture* **196**, 289–298.

Bruun, M.S., Madsen, L. and Dalsgaard, I. (2003) Efficiency of oxytetracycline treatment in rainbow trout experimentally infected with *Flavobacterium psychrophilum* strains having different *in vitro* anti-biotic susceptibilities. *Aquaculture* **215**, 11–20.

Capone, D.G., Weston, D.P., Miller, V. and Shoemaker, C. (1996) Antibacterial residues in marine sediments and invertebrates following chemotherapy in aquaculture. *Aquaculture* **145**, 55–75.

Carson, R.L. (1962) *Silent Spring*. Houghton Mifflin, Boston, MA, 368 pp.

Castells, G., Intorre, L., Bertini, S., Cristofol, C., Soldani, G. and Arboix, M. (2000) Oral single-dose pharmacokinetics of thiamphenicol in the sea–bass (*Dicentrarchus labrax*). *J. Vet. Pharmacol. Therapeut.* **23**, 53–54.

Castro-Escarpulli, G., Figueras, M., Aguilera-Arreola, G., Soler, L., Fernandez-Rendon, E., Aparicio, G., Guarro, J. and Chacon, M. (2003) Characterisation of *Aeromonas spp.* isolated from frozen fish intended for human consumption in Mexico. *Int. J. Food Microbiol.* **84**, 41–49.

Chelossi, E., Vezzulli, L., Milano, A., Branzoni, M., Fabiano, M., Riccardi, G. and Banat, I.M. (2003) Antibiotic resistance of benthic bacteria in fish-farm and control sediments of the Western Mediterranean. *Aquaculture* **219**, 83–97.

Christophilogiannis, P., Rigos, G., Yiagnisi, M., Andriopoulou, A., Koutsodimou, M., Anastasopoulou, G., Alexis, M. and Richards, R.H. (1997a) Histopathological diagnostic findings of fish diseases reported in Greek maricultures during the period 1995–1996. *Proceedings of the 5th Hellenic Symposium on Oceanography and Fisheries*, April 15–18, 1995, Kavala, Greece, pp. 231–233.

Christophilogiannis, P., Alexis, M. and Richards, R.H. (1997b) Antibiotics use in Greek maricultures: study of the oxolinic acid retention time in gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*), at high temperatures after oral treatment. *Proceedings of the 5th Hellenic Symposium on Oceanography and Fisheries*, April 15–18, 1995, Kavala, Greece, pp. 181–184.

Clive, D.L.G. (1968) Chemistry of tetracyclines. *Quar. Rev. Chem. Soc.* **22**, 435–456.

Company, R., Sitja-Bobadilla, A., Pujalte, M.J., Garay, E., Alvarez-Pellitero, P. and Perez-Sanchez, J. (1999) Bacterial and parasitic pathogens in cultured common dentex, Dentex dentex L. *J. Fish Dis.* **22**, 299–309.

Costello, M.J., Grant, A., Davies, I.M., Cecchini, S., Papoutsoglou, S., Quingley, D. and Saroglia, M. (2001) The control of chemicals used in aquaculture in Europe. *J. Appl. Ichthyol.* **17**, 173–180.

Coyne, R., Hiney, M. and Smith, P. (1997) Transient presence of oxytetracycline in mussels (*Mytilus edulis*) following its therapeutic use at a marine Atlantic salmon farm. *Aquaculture* **149**, 175–181.

Coyne R., Smith P. and Moriary C. (2001) The fate of oxytetracycline in the marine environment of a salmon cage farm Mar. Environ. Healt. Ser. No 3, 24 pp.

Coyne, R., Samuelsen, O., Kongshaug, H., Andersen, K., Dalsgaard, I., Smith, P. and Bergh, O. (2004a) A comparison of oxolinic acid concentrations in farmed and laboratory held rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **239**, 1–13.

Coyne, R., Samuelsen, O., Bergh, O., Andersen, K., Pursell, L., Dalsgaard, I. and Smith, P. (2004b) A comparison of oxolinic acid concentrations in farmed and laboratory held rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **239**, 23–35.

della Rocca, G., Zaghini, A., Zanoni, R., Sanguinetti, V., Zanchetta, S., DiSalvo, A. and Malvisi, J. (2004) Seabream (*Sparus aurata* L.): disposition of amoxicillin after single intravenous or oral administration and multiple dose depletion studies. *Aquaculture* **232**, 1–10.

Doi, A.M. and Stoskopf, M.K. (2000) The kinetics of oxytetracyline degradation in deionised water under varying temperature, pH, light, substrate and organic matter. *J. Aqua. Anim. Health* **12**, 246–253.

Doukas, B., Athanassopoulou, F., Karagouni, E. and Dotsika, E. (1998) *Aeromonas hydrophila* infection in cultured sea bass, *Dicentrarchus labrax* L., and *Puntazzo puntazzo* Cuvier from the Aegean Sea. *J. Fish Dis.* **21**, 317–320.

EMEA (1995a) Oxytetracycline, Tetracycline, Chlortetracycline. Summary report (3). Committee for Veterinary Medicinal Products / The European Agency for the Evaluation of Medicinal Products. Committee for Veterinary Medicinal Products. EMEA/MRL/023/95.

EMEA (1995b) Sulphonamides. Summary report (2). Committee for Veterinary Medicinal Products / The European Agency for the Evaluation of Medicinal Products. Committee for Veterinary Medicinal Products. EMEA/MRL/026/95.

EMEA (1997) Trimethoprim. Summary report (2). Committee for Veterinary Medicinal Products / The European Agency for the Evaluation of Medicinal Products. Committee for Veterinary Medicinal Products. EMEA/MRL/255/97FINAL.

EMEA (1998a) Sarafloxacin (Salmonidae). Summary report (2). Committee for Veterinary Medicinal Products / The European Agency for the Evaluation of Medicinal Products. Committee for Veterinary Medicinal Products. EMEA/MRL/349/98-FINAL.

EMEA (1998b) Thiamphenicol (extension to pigs, sheep and fin fish). Summary report (3). Committee for Veterinary Medicinal Products / The European Agency for the Evaluation of Medicinal Products. Committee for Veterinary Medicinal Products. EMEA/MRL/505/98-FINAL.

EMEA (1999) Flumequine. Summary report (2). Committee for Veterinary Medicinal Products / The European Agency for

the Evaluation of Medicinal Products. Committee for Veterinary Medicinal Products. EMEA/MRL/624/99.

EMEA (2000) Florfenicol. Summary report (5). Committee for Veterinary Medicinal Products / The European Agency for the Evaluation of Medicinal Products. Committee for Veterinary Medicinal Products EMEA/MRL/760/00-FINAL.

EMEA (2003) Oxolinic acid. Summary report (4). Committee for Veterinary Medicinal Products / The European Agency for the Evaluation of Medicinal Products. Committee for Veterinary Medicinal Products EMEA/MRL/892/03-FINAL.

EMEA (undated) Penicillins. Summary report. Committee for Veterinary MedicinalProducts / The European Agency for the Evaluation of Medicinal Products. Committee for Veterinary Medicinal Products .

FEAP (2003) Federation of European Aquaculture Producers. Aquamedia, Production. http://www.feap.info/feap/aquaculturedata/.

FGM (2000) Federation of Greek Mariculture. 2000 Annual Report (In Greek). http://www.imbc.gr/fgm/.

Fukui, H., Fujihara, Y. and Kano, T. (1987) *In vitro* and *in vivo* antibacterial activities of florfenicol, a new fluorinated analog of thiamphenicol, against fish pathogens. *Fish Path*. **22**, 201–207.

Halling-Sorensen, B. (2000) Algal toxicity of antibacterial agents used in intensive farming. *Chemosphere* **40**, 731–739.

Hektoen, H., Berge, J.A., Hormazambal, V. and Yndestad, M. (1995) Persistence of antibacterials in marine sediments. *Aquaculture* **133**, 175–184.

Holten-Lutzhoft, H.C., Halling-Sorensen, B. and Jorgensen, S.E. (1999) Algal toxicity of anti-bacterial agents applied in Danish fish farming. *Arch. Environ. Contam. Toxicol.* **36**, 1–6.

Horsberg, E., Martinsen, B., Sandersen, K. and Zernichow, L. (1997) Potentiated sulfonamides: *in vitro* inhibitory effect and pharmacokinetic properties in Atlantic salmon in seawater. *J. Aqua. Anim. Health* **9**, 203–210.

Intorre, L., Castells, G., Perez, B., Bertini, S., Mengozzi, G., Soldani, G. and Arboix, M. (1997) The oral disposition of thiamphenicol in the sea–bass J. *Vet. Pharmacol. Therap.* **20**(Suppl. 1): 184–185.

Intorre, L., Castells, G., Cristofol, C., Bertini, S., Soldani, G. and Arboix, M. (2002) Residue depletion of thiamphenicol in the sea–bass. *J. Vet. Pharmacol. Therap.* **25**, 59–63.

Jacobsen, P. and Berglind, L. (1988) Persistence of oxytetracycline in sediments from fish farms. *Aquaculture* **70**, 365–370.

Jorgensen, S.E. and Halling-Sorensen, B. (2000) Drugs in the environment. *Chemosphere* **40**, 691–699.

Kerry, J., Hiney, M., Coyne, R., Cazabon, D., NicGabhainn, S. and Smith, P. (1994) Frequency and distribution of resistance to oxytetracycline in microorganisms isolated from marine fish farm sediments following therapeutic use of oxytetracycline. *Aquaculture* **123**, 43–54.

Kerry, J., Hiney, M., Coyne, R., Cazabon, D., NicGabhainn, S. and Smith, P. (1996) Spatial distribution of oxytetracycline and elevated frequencies of oxytetracycline resistance in sediments beneath a marine salmon farm following oxytetracycline therapy. *Aquaculture* **145**, 31–39.

Kim, E. and Aoki, T. (1993) Drug resistance and broad geographical distribution of identical R plasmids of *Pausterella piscicida* isolated from culture yellowtail in Japan. *Microbiol. Immunol.* **37**, 103–109.

Kim, S., Nonaka, L. and Suzuki, S. (2004) Occurrence of tetracycline resistance genes tet(M) and tet(S) in bacteria from marine aquaculture sites. *FEMS Microbiol. Lett.* **237**, 147–156.

Lalumera, G.M., Calamari, D., Galli, P., Castiglioni, S., Crosa, G. and Fanelli, R. (2004) Preliminary investigation on the environmental occurrence and effects of antibiotics used in aquaculture in Italy. *Chemoshpere* **54**, 661–668.

Le-Breton, A. (1999) Mediterranean finfish pathologies: present status and new developments in prophylactic methods. *Bull. Eur. Ass. Fish Pathol.* **19**, 250–253.

Ledo, A., Dopazo, C.P., Romalde, J.L., Toranzo, A.E and Barja, J.L. (1987) Effectiveness of different chemotherapeutic agents for controlling bacterial fish diseases. *Bull. Eur. Ass. Fish Pathol.* **7**, 20–22.

Liu, P., Muller, M. and Derendorf, H. (2002) Rational dosing of antibiotics: the use of plasma concentrations versus tissue concentrations. *Inter. J. Antimicr. Agents* **19**, 285–290.

Lunestad, B.T., Samuelsen, O.B., Fjeld, S. and Ervik, A. (1995) Photostability of eight antibacterials in seawater. *Aquaculture* **134**, 217–225.

Luzzana, U., Moretti, V.M., Scolari, M., Mentasti, T. and Valfre, F. (1996) Residue depletion of orally administered flumequine from muscle tissue of European sea bass (*Dicentrarchus labrax*) fed diets differing in fat content. *In*: Haagsma N. and Ruiter (eds.), Euroresidue III. Proceedings of the Euroresidue III Conference, 6–8 May 1996, Veldhoven, The Netherlands, pp. 659–663.

Malvisi, J., della Rocca, G., Anfossi, P. and Giorgetti, G. (1996) Tissue distribution and residue depletion of oxytetracycline in sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) after oral administration. *Aquaculture* **147**, 159–168.

Malvisi, J., della Rocca, G., Anfossi, P. and Giorgetti, G. (1997) Tissue distribution and depletion of flumequine in sea bream (*Sparus aurata*). *Aquaculture* **157**, 197–204.

Malvisi, J., della Rocca, G., Anfossi, P., Tomasi, L., Di Salvo, A., Zanchetta, S., Magni, A., Sello, M. and Giorgetti, G. (2002) Tissue distribution and residue depletion of thiamphenicol after multiple oral dosing in and seabass (*Dicentrarchus labrax L.*) and seabream (*Sparus aurata* L.). *J. Appl. Ichthyol.* **18**, 35–39.

Marionnet, D., Chambras, C., Taysse, L., Bosgireaud, C. and Deschaux, P. (1998) Modulation of drug-metabolizing systems by bacterial endotoxin in carp liver and immune organs. *Ecotoxicol. Environ. Saf.* **41**, 189–194.

Martinsen, B. and Horsberg, T.E. (1995) Comparative single-dose pharmacokinetics of four quinolones, oxolinic acid, flumequine, sarafloxacin and enrofloxacin in Atlantic salmon (*Salmo salar*) held in seawater at 10 °C. *Antimicr. Agents Chemoth.* **39**, 1059–1064.

Martisen, B., Horsberg, T.E., Varma, K.J. and Sams, R. (1993) Single dose pharmacokinetic study of florfenicol in Atlantic salmon (*Salmo salar*) in seawater at 11 °C. *Aquaculture* **112**, 1–11.

Mazzolini, E., Vismara, D., Geschia, G., Malvisi, J., Fabris, A., Passera, A., Danielis, L. and Giorgetti, G. (1997) *In vitro* activity of several antibiotics against clinical isolated of

marine fish pathogens. Int. Workshop: "Aquaculture Application of Controlled Grud and Vaccine Delivery", 21–23 May, 1997, Udine, Italy.

Migliore, L., Civitareale, C., Brambilla, G. and Di Delupis, G. (1997) Toxicity of several important agricultural antibiotics to Artemia. *Water Res.* **31**, 1081–1806.

Migliore, L., Cozzolino, S. and Fiori, M. (2000) Phytotoxicity to and uptake of flumequine used in intensive aquaculture on the aquatic weed *Lythrum salicaria*. *Chemosphere* **40**, 741–750.

Miranda, C.D. and Zemelman, R. (2001) Antibiotic resistant bacteria in fish from the Conception Bay, Chile. *Mar. Pollut. Bull.* **42** (11): 1096–1102.

Miranda, C.D. and Zemelman, R. (2002) Bacterial resistance to oxytetracycline in Chilean salmon farming. *Aquaculture* **212**, 31–47.

Nordmo, R., Holth Rishet, J.M., Varma, K.J., Sutherland, I.H. and Brokken, E.S. (1998) Evaluation of florfenicol in Atlantic salmon, *Salmo salar* L.: efficacy against furunculosis due to *Aeromonas salmonicida* and cold water vibriosis due to Vibrio salmonicida. *J. Fish Dis.* **21**, 289–297.

Oka, H., Ikai, Y., Kawamura, N., Yamada, M., Harada, K., Ito, S. and Suzuki, M. (1989) Photodecomposition products of tetracycline in aqueous solution. *J. Agricult. Food Chem.* **37**, 226–231.

Ottaviani, D., Bacchiocchi, I., Masini, L., Leoni, F., Carraturo, A., Giammarioli, M. and Sbaraglia, G. (2001) Antimicrobial susceptibility of potentially pathogenic halophilic vibrios isolated from seafood. *Int. J. Antimicrob. Agents* **18**, 135–140.

Plakas, S.M., McPearson, R.M. and Guarino, A.M. (1988) Disposition and bioavailability of ^{3}H-tetracycline in the channel catfish (*Ictalurus punctatus*). *Xenobiotica* **18**, 83–93.

Poher, I., Blanc, G. and Loussouarn, S. (1997) Pharmacokinetics of oxolinic acid in sea-bass *Dicentrarchus labrax* (L., 1758), after a single rapid intravascular injection. *J. Vet. Pharmacol. Therapeut.* **20**, 267–275.

Pouliquen, H. and Le Bris, H. (1996) Sorption of oxolinic acid and oxytetracycline in marine sediments. *Chemosphere* **33**, 801–815.

Rabolle, M. and Spliid, N.H. (2000) Sorption and mobility of metronidazole, olaquindox, oxytetracycline and tylosin in soil. *Chemosphere* **40**, 715–722.

Rigos G. (2003) The kinetic behaviour of oxytetracycline and oxolinic acid in euryhaline farmed fish species (Sparidae). PhD dissertation, University of Kingston, London, 166 pp.

Rigos, G., Grigorakis, K., Nengas, I., Christophilogiannis, P., Yiagnisi, M., Koutsodimou, M., Andriopoulou, K. and Alexis, M. (1998) Stress related pathology seems a significant obstacle for the intensive farming of common dentex, *Dentex dentex* (Linnaeus 1758). *Bull. Eur. Ass. Fish Pathol.* **18**, 15–19.

Rigos, G., Alexis, M. and Nengas, I. (1999) Leaching, palatability and digestibility of oxolinic acid and oxytetracycline in sea bass (*Dicentrarchus labrax* L.). *Aquacult. Res.* **30**, 1–7.

Rigos, G., Alexis, M., Andriopoulou, A. and Nengas, I. (2002a) Pharmacokinetics and tissue distribution of oxytetracycline in sea bass (*Dicentrarchus labrax*) at two water temperatures. *Aquaculture* **210**, 59–67.

Rigos, G., Alexis, M., Andriopoulou, A. and Nengas, I. (2002b) Temperature-dependent pharmacokinetics and tissue distribution of oxolinic acid in sea bass, *Dicentrarchus labrax* L. after a single intravascular injection. *Aquacult. Res.* **33**, 1–7.

Rigos, G., Alexis, M., Tyrpenou, A.E., Nengas, I., Piper, I. and Troisi, G. (2002c) Pharmacokinetics of oxolinic acid in gilthead sea bream, *Sparus aurata* L. *J. Fish Dis.* **25**, 401–408.

Rigos, G., Nengas, I, Alexis, M., Tyrpenou, A.E., Arbanitis, P. and Troisi, G. (2002d) Digestibility of oxytetracycline and oxolinic acid in Sparidae farmed fish. 10th International Symposium on Nutrition & Feeding in Fish, Feeding for Quality, June 2–7, Rhodes, Greece, 113 pp.

Rigos, G., Tyrpenou, A., Nengas, I. and Alexis, M. (2002e) A pharmacokinetic study of flumequine in sea bass, *Dicentrarchus labrax* L. *J. Fish Dis.* **25**, 101–105.

Rigos, G., Nengas, I, Tyrpenou, A.E., Alexis, M. and Troisi, G.M (2003a) Pharmacokinetics and bioavailability of oxytetracycline in gilthead sea bream (*Sparus aurata*) after a single dose. *Aquaculture* **221**, 75–83.

Rigos, G., Nengas, I., Alexis, M., Tyrpenou, A.E. and Troisi, G. (2003b) Tissue distribution and residue depletion of oxolinic acid in gilthead sea bream (*Sparus aurata*) and sharpsnout sea bream (*Diplodus puntazzo*) following multiple in-feed dosing. *Aquaculture* **224**, 245–256.

Rigos, G., Tyrpenou, A.E., Nengas, I., Yiagnisi, M., Koutsodimou, M., Alexis, M. and Troisi, G. (2003c) Pharmacokinetics of flumequine in gilthead sea bream, Sparus aurata and *in vitro* activity against important bacterial fish pathogens. *Dis. Aqua. Org.* **54**, 35–41.

Rigos, G., Nengas, I., Athanasopoulou, E. and Alexis, M. (2004a) Bioavailability of oxytetracycline in sea bass, *Dicentrarchus labrax* (L.). *J. Fish Dis.* **27**, 119–122.

Rigos, G., Tyrpenou, A.E., Nengas, I., Alexis, M., Athanasopoulou, E. and Troisi, G.M (2004b) Poor bioavailability of oxytetracycline in sharpsnout sea bream (*Diplodus puntazzo*). *Aquaculture* **235**, 489–497.

Rigos, G., Nengas, I., Alexis, M. and Troisi, G. (2004c) Potential drug (oxytetracycline & oxolinic acid) pollution from Mediterranean sparid farm fish farms. *Aquat. Toxicol.* **69**, 281–288.

Rigos, G., Nengas, I., Tyrpenou, A.E., Alexis, M. and Troisi, G. (2004d) The kinetic profile of oxolinic acid in sharpsnout sea bream *Diplodus puntazzo* (Cetti, 1777). *Aquacult. Res.* **35**, 1299–1304.

Samuelsen, O.B. (1989) Degradation of oxytetracycline at two different temperatures and light intensities, and the persistence of oxytetracycline in the sediment from a fish farm. *Aquaculture* **83**, 7–16.

Samuelsen, O.B., Lunestad, B.T., Husevag, B., Holleland, T. and Ervik, A. (1992) Residues of oxolinic acid in wild fauna following medication in fish farms. *Dis. Aqua. Org.* **12**, 111–119.

Samuelsen, O.B., Lunestad, B.T., Ervik, A. and Fjelde, S. (1994) Stability of antibacterial agents in an artificial marine aquaculture sediment studied under laboratory conditions. *Aquaculture* **126**, 283–290.

Samuelsen, O.B., Pursell, L., Smith, P. and Ervik, A. (1997) Multiple-dose pharmacokinetic study of Romet^{30} in Atlantic salmon (*Salmo salar*) and *in vitro* antibacterial activity against *Aeromonas salmonicida*. *Aquaculture* **152**, 13–24.

Schmidt, A.S., Bruun, M.S., Dalsgaard, I., Pedersen, K. and Larsen, J.L (2000) Occurrence of antimicrobial resistance in

fish-pathogenic and environmental bacteria associated with four Danish Rainbow Trout Farms. *Appl. Environ.. Microbiol.* **66**, 4908–4915.

Schnick, R.A. (1999) Use of chemicals in fish management and fish culture. *In*: Smith, D.J., Gingerich, W.H., Beconi-Barker, M.G. (eds.), *Xenobiotics in Fish*. Kluwer/Plenum Press, NY, pp. 1–14.

Scott, P. (1993) Therapy in aquaculture. *In*: Brown, L. (ed.), *Aquaculture for Veterinarians*. Pergamon Press, Oxford, pp. 131–153.

Shojaee AliAbadi, F. and Lees, P. (2000) Antibiotic treatment for animals: effect on bacterial population and dosage regimen optimization. *Inter. J. Antimicr. Agents* **14**, 307–313.

Smith, P. (2001) Accuracy, precision and meaning of antimicrobial agent susceptibility testing of bacteria associated with fish diseases. *Aquaculture* **196**, 253–266.

Smith, P., Hiney, M. and Samuelsen, O.B. (1994) Bacterial resistance to antimicrobial agents used in fish farming; a critical evaluation on method and meaning. *Ann. Rev. Fish Dis.* **4**, 273–313.

Sorum, H. and L'Abbe-Lund, T.M. (2002) Antibiotic resistance in food-related bacteria-a result of interfering with global web of bacterial genetics. *Inter. J. Food Microbiol.* **78**, 43–56.

Stamm, J.M. (1989) *In vitro* resistance by fish pathogens to aquacultural antibacterials, including the quinolones difloxacin (A-56619) and sarafloxacin (A-56620). *J. Aquat. Anim. Health* **1**, 135–141.

Stoffregen, D.A., Bowser, P.R. and Babish, J.G. (1996) Antibacterial chemotherapeutants for finfish aquaculture: A synopsis of laboratory and field efficacy and safety studies. *J. Aqua. Anim. Health* **8**, 181–207.

Theodorou, J.A. (2002) Current and future technological trends of European seabass-seabream culture. *Rev. Fish. Sci.* **10**, 529–543.

Tsoumas, A., Alderman, D.J. and Rodgers, C.J. (1989) *Aeromonas Salmonicida*: Development of resistance to 4-quinolone antibacterials. *J. Fish Dis.* **12**, 493–507.

Tyrpenou, A.E., Iossifidou, E.G., Psomas, I.E. and Fotis, G.D. (2003a) Tissue distribution and depletion of sarafloxacin hydrochloride after in feed administration in gilthead sea bream (*Sparus aurata* L.). *Aquaculture* **215**, 291–300.

Tyrpenou, A.E., Kotzamanis, Y.P. and Alexis, M.N. (2003b) Flumequine depletion from muscle plus skin tissue of gilthead sea bream (*Sparus aurata* L.) fed flumequine medicated feed in seawater at 18 and 24 °C. *Aquaculture* **220**, 633–642.

Tyrpenou, A.E., Rigos, G., Yiagnisi, M., Nengas, I. and Alexis, M. (2003c) Pharmacokinetics of chloramphenicol (CAP) in gilthead sea bream (*Sparus aurata*) and its *in vitro* activity against important bacterial fish pathogens. *J. Hellen. Vet. Med. Soc.* **54**, 311–320.

Uno, K. (1996) Pharmacokinetic study of oxytetracycline in healthy and vibriosis-infected ayu (*Plecoglossus altivelis*). *Aquaculture* **143**, 33–42.

Wollenberger, L., Halling-Sorensen, B. and Kusk, K.O. (2000) Acute and chronic toxicity of veterinary antibiotics to *Daphnia magna*. *Chemosphere* **40**, 723–730.

Wong, H.C., Liu, S., Ku, L., Lee, I., Wang, T., Lee, Y., Lee, C., Kuo, L. and Shih, D. (2000) Characterisation of Vibrio parahaemolyticus isolates obtained from food borne illness outbreak during 1992 through 1995 in Taiwan. *J. Food Prot.* **63**, 900–906.

Zhao, J., Kim, E., Kobayashi, T. and Aoki, T. (1992) Drug resistance of *Vibrio anguillarum* isolated from ayu between 1989 and 1991. *Nippon Suisan Gakkaishi* **58**, 1523–1527.

Zorilla, I., Chabrillon, M., Arijo, S., Diaz-Rosales, P., Martinez-Manzanares, E., Babelona, M. and Morinigo, M. (2003) Bacteria recovered from diseased cultured gilthead seabream (*Sparus aurata*) in southwestern Spain. *Aquaculture* **218**, 11–20.