# Chemotherapeutics in Shellfish: Potential Uses and Limitations

Carolyn Friedman

*California Department of Fish and Game,*
*Fish Disease Laboratory,*
*2111 Nimbus Rd., Rancho Cordova, California, 95670 and*
*Department of Medicine,*
*School of Veterinary Medicine,*
*University of California,*
*Davis,*
*California,*
*95616,*
*USA*

## INTRODUCTION

Few chemotherapeutants are approved for use in shellfish aquaculture. This may be due, in part, to difficulties in the application of therapeutic agents on many aquatic animals as a result of the biology and/or methods of culture of a particular species. In addition, relative to the commercial culture of major agricultural species, the marine aquaculture industry is quite small. The cost of Food and Drug Administration (FDA) approval of a drug is substantial. In many cases, it may not be cost-effective for pharmaceutical companies or other interested parties to fund studies required for FDA approval of a drug. Despite these difficulties, efforts to approve selected therapeutants are increasing.

The National Aquaculture Act (NAA) was enacted in 1980 with a goal of advancing and encouraging aquaculture. The Joint Subcommittee on Aquaculture (JSA) formed a Working Group on Quality Assurance in Aquaculture Production (QAA) in November 1990. The goal of this group, which is made up of representatives of federal agencies with interests in aquaculture production, state aquaculture coordinators, and industry, is to help direct efforts of industry, researchers and private companies in the use of drugs and chemicals in aquaculture production.

As of last June, the QAA has initiated development of (1) a document that clarifies federal regulatory oversight of drugs and chemicals used in aquaculture production, (2) species specific producer guidelines on chemical and drug use, and (3) a central databank of information on drugs and chemicals approved for use by aquaculturists that will be incorporated into the USDA Extension Service's Food Animal Residue Avoidance Databank (FARAD). The Interregional Project No. 4 or IR-4 of the USDA also promotes testing of chemotherapeutants in minor species via funding and sponsorship: with aquaculture animals

as one of its focuses. Certain pharmaceutical companies have also become interested in or have sponsored efficacy, residues and other studies for FDA approval of specific drugs.

At present, only oxytetracycline is approved for treatment of gaffkemia in lobsters. Oxytetracycline was approved for use in feed (1 g/lb feed for 5 d as the sole ration with a 30 d withdrawal time) on January 3, 1986 (PMF 5028). Formalin is in the last stages of approval for the treatment of external parasites of penaeid shrimp; the data for this PMF (No. 3543) was accepted May 6, 1991. Formalin should be approved in 1992.

I would like to briefly discuss some general aspects of the biology and culture methods of several species of invertebrates that are most frequently cultured in North America. Then I will discuss the etiology, histological pathology and management or treatment of principle diseases of selected species of oysters, abalone, shrimp and lobsters. Before I continue, I would like to stress that treatment with chemotherapeutic agents should be a last resort, or should be either metaphylactic or therapeutic in conjunction with close pathological monitoring. Attempts should be made to improve management practices before initiating a drug treatment. The best method to control diseases is by avoidance of a particular pathogen via good husbandry techniques, use of certified disease-free animals as broodstock or grow-out animals and careful monitoring of water quality and of the health status of the cultured animals.

Bivalve shellfish (such as oysters, mussels and clams) filter phytoplankton to attain food and are generally cultured in large embayments. Therefore, treatment of these animals in field situations is not feasible. However, broodstock and seed, which are often held in confined areas, may be treated. Treatment may be used to save important broodstock from a parasite or disease or as a prophylactic treatment of broodstock or seed prior to shipment to another geographic location. Of course, this approach must be regulated by a pathologist to assure that the therapeutant is effective.

Cultured abalone are amenable to the application of therapeutants. In this new and growing field, abalone are reared in tanks and/or barrels and raceways during the entire culture period and may be easily manipulated. Adult abalone grown in tanks or raceways may be given bath treatments or orally administered drugs. Post larval abalone may also be given therapeutants incorporated into a pelleted diet that is available on a limited basis (K. Norman-Boudreau, Sebastopol, CA). Like abalone, many crustaceans appear to be amenable to drug treatments, as they are reared in relatively confined areas and may be fed a prepared diet. In addition, bath or dip treatments could be applied to pond, tank or raceway culture of shrimp, crayfish and to holding tanks of lobsters to treat external parasites.

## OYSTERS

### Principle Protozoan Diseases and Treatments

Two haplosporidian parasites, *Haplosporidium nelsoni* and *H. costale* plague the culture of the American oyster, *Crassostrea virginica*, along the east coast of the United States. *Haplosporidium nelsoni*, the etiological agent of Delaware Bay disease or MSX, is a more problematic pathogen than *H. costale*, the agent of Seaside disease (SSO). The geographic distribution, seasonality and degree of mortality are broader and more intense

for MSX than for SSO. In addition, the prepatent period for MSX is only about 6 weeks, while SSO has a 9 to 10 month incubation period. *Haplosporidium costale* is limited to waters with salinities of >25 ppt, while *H. nelsoni* causes overt disease in oysters reared at salinities between 15-30 ppt.

The European flat oyster, *Ostrea edulis*, and the Pacific oyster, *Crassostrea gigas*, are also infected with haplosporidan parasites. *Bonamia ostreae*, the etiological agent of bonamiasis or hemocytic parasitosis, devastated the French oyster industry upon introduction from California in 1979 and causes yearly mortality in 1-2 year old flat oysters grown in California and Washington state. The disease was recently observed in flat oysters from Maine during a routine health examination. Infections with *B. ostreae* render oyster hemocytes incapable of normal cellular defenses against invading organisms and eventually leads to oyster death. Mortalities range between 30-100 %.

Unlike *B. ostreae*, the etiological agent of Denman Island disease, *Mikrocytos mackini*, infects the hosts' connective tissue cells. Pacific oysters grown along Denman Island and Vancouver Island in British Columbia, Canada, historically become infected between April and July. Mortality is observed in 2-4 year old oysters during the late spring and early summer and generally does not exceed 25-30 %. No infected individuals have been observed beyond July or when water temperatures approach 16° C.

An apicomplexan protozoan, *Perkinsus marinus*, infects American oysters grown along the east coast of the United States from Long Island Sound south to the Gulf of Mexico. The disease caused by *P. marinus*, frequently referred to as "Dermo," also occurs in waters off Mexico south to Brazil where it also infects *Ostrea frons* and *O. equestris*. The prepatent period for Dermo in warm waters (20-25° C) is 4 to 5 weeks and mortality may reach 100% within three infection cycles.

No treatments are available for the aforementioned protozoan diseases. Control of MSX, SSO, bonamiasis, Denman Island disease and Dermo have been through management of culture practices and/or genetic selection of resistant populations. Management practices include specific seasons for outplanting of seed to avoid infective stages or to allow a longer prepatent period, harvest of oysters earlier than normal in a particular season, culture of resistant species or strains of oysters and so on.

Due to the increased desire for intra and interstate transport of oysters (and shellfish in general), an antiprotozoal therapeutant may be useful to treat either broodstock and/or seed prior to shipment to another location. The therapeutant could be administered as an additive to algal cultures or to preserved algae paste. Whether or not such a treatment is feasible is questionable at present.

**Principle Bacterial Diseases and Treatments**

Several species of *Vibrio* have been reported to infect larval oysters, clams, scallops, abalone, shrimp and other shellfish species. Vibriosis occurs seasonally under high water temperature conditions and is generally accompanied by a rapid onset of high mortality. Up to 100% mortality within 18 h post infection has been reported for oyster larvae.

Vibriosis may be treated by bath exposure to antibiotics, such as streptomycin or erythromycin. Oxytetracycline reacts with calcium in seawater and would not be very effective. Due to the rapid onset and heavy mortality often associated with larval vibriosis,

treatment may be limited to very early infections or be of a prophylactic nature. Development of drug-resistant strains of *Vibrio* spp. may also limit the efficacy of antibacterial therapeutants. In addition the potential for drug-resistant human-pathogenic *Vibrio* spp. is also a major concern in the application of antibiotics in aquaculture production.

Pacific oyster nocardiosis (PON) affects *Crassostrea gigas* > 2 year in age that are grown in warm embayments (18-30° C) in the Pacific northwest of the United States, southern British Columbia, Canada, and Japan. The disease is generally associated with the phenomenon termed 'summer mortality'. Oysters with PON may have raised nodules or focal areas of yellow, green or brown discoloration. Pacific oyster nocardiosis is associated with up to 30 % mortality between the late summer and early fall. No therapeutants are available for PON. Treatment is via management and includes early harvest of oysters.

## ABALONE

### Principle Diseases and Treatments

In general, North American abalone are not known to suffer from many infectious agents. Mortality of juvenile pinto abalone, *Haliotis kamschatkana*, grown in British Columbia, Canada, has recently been reported in association with muscle and nerve infections with an eucaryotic achlorophyllous protist, *Labyrinthuloides haliotidis*. Red abalone, *H. rufescens*, are reported to be susceptible to this pathogen in laboratory experiments. *Labyrinthuloides haliotidis* causes 100% mortality in abalone <5 mm in maximum dimension within 2 weeks after infection. A combination of equipment and tank disinfection with chlorine (25 mg/L HOCl in seawater for 20 min) and treatment of infected abalone with cycloheximide (1-2 mg/L for 23 h/d for 5 consecutive days) only temporarily controlled active infection. Zoospores were not affected by the drug and reinfection recurred. In addition, the parasites showed an increased resistance to cycloheximide after only 3 treatments. Cycloheximide does not appear to be an effective therapeutant to control *L. haliotidis* due to its short-lived efficacy and that it has recently been suspected to be carcinogenic.

The principle concern regarding infectious agents in abalone is a newly discovered renal coccidian parasite of abalone from California. The unidentified coccidian does not appear to be very pathogenic in the six abalone species examined. Under normal culture conditions, seed abalone < 10 mm in size lack coccidian infections. However juvenile and adult abalone >17 mm in maximum dimensions are generally infected. The concern regarding this parasite is due to the uncertainty of the timing of juvenile abalone infections and the degree of pathogenicity of this coccidian for naive species including abalone, other invertebrates and fishes outside of California. The red abalone is a very desirable culture organism and many states and countries are interested in importing abalone seed from California. However, these potential customers do not want to risk native species that may be susceptible to infection with this coccidian.

Despite the seemingly low virulence of the coccidian parasite, there is great potential for the application of anticoccidial drugs in abalone culture. Coccidiocidal or coccidiostatic therapeutants could be administered to seed abalone in an artificial diet prior to shipment

out of California to preclude introduction of the parasite into new waters. Wild or cultured broodstock could also be treated for coccidian infections prior to spawning. Application of such therapeutants in conjunction with pathological examination of seed abalone would greatly enhance and encourage growth of the abalone culture industry.

Many anticoccidian therapeutants, such as lasalocid, sulphamethazine, sulphaquinoxaline and Romet (sulphamerazine + ormetoprim, 5 : 3) are approved for use in sheep, goats and poultry. A slightly different formulation of Romet-B (5 : 1) is an approved antibacterial drug for use in salmonids and catfish. Unfortunately, in vivo trials using both formulations of sulphadimethoxine and ormetoprim via oral administration have not been effective against the renal coccidia of abalone in California. Other potential coccidiostats include monensin, amprolium + ethopabate, erythromycin and Robenidine.

## CRUSTACEANS

### Principle Diseases of Shrimp

Several infectious agents cause disease in cultured shrimp including bacteria, fungi, protozoans and viruses. At present, no known anti-viral therapeutants exist; viral diseases will not be discussed. Bacterial pathogens, such as *Vibrio* and *Aeromonas*, are generally problematic in stressed shrimp populations and during early growth stages that include larval, post-larval and juvenile stages. Candidate antibacterial therapeutants based on the therapeutic index (NOEC/highest MIC to sensitive bacteria) formulated by Williams et al. (in press) and Mohney et al. (in press) via in vitro MIC and larval toxicity data include enrofloxacin, oxytetracycline, Romet-30 and sarafloxacin.

*Leucothrix mucor*, a filamentous bacterium, causes filamentous gill disease in all life-stages of penaeid shrimp. Death due to hypoxia may result from severe infections. Aquatrine is approved by the EPA for use in shrimp culture and is effective in controlling infestations with *L. mucor* as a bath treatment (0.25-0.5 mg Cu/L for 4-8 h or 0.1 mg Cu/L for 24 h).

High densities of fouling epicommensal organisms (such as algae and protozoans) on the epipodites and gills of shrimp can become problematic in shrimp culture systems. Formalin-F is an effective parasiticide and is in the last stages of approval for treatment of external parasites of shrimp including *Epistylis* sp., *Zoothamnium* sp. and *Vorticella* sp. Information regarding this drug is available in the Federal Register (5-6-91) Vol. 56(87):20618. The report on studies of the efficacy, residues, toxicity and environmental safety of formalin was accepted by the FDA in January of 1992. Companies interested in selling formalin have submitted Supplemental New Animal Drug Applications with the FDA in January 1992.

The major problem in shrimp and lobster culture results from fungal infections. Larval mycosis, caused by *Lagenidium callinectes* and *Sirolpidium* sp. is associated with up to 100% mortality in early shrimp, lobster and crab larval stages (protozoea and mysis) within 48-72 h post infection. The infection may originate from adult animals during spawning or from infected shrimp within a shared rearing tank, raceway or pond. Successful treatment against these phycomycetous fungal infections in shrimp and crab larvae has been reported for trifluralin (Treflan). Trifluralin is effective against zoospores of *Lagenidium* and

*Sirolpidium* at very low concentrations (5-15 ppb) and toxicity to larvae occurs at concentrations >100 times the effective dose. Treflan is ineffective against fungal hyphae and, hence, not efficacious once a zoospore has germinated and penetrated a host. This compound is considered a water quality additive and is being considered for approval in shrimp culture by the EPA.

Fusarium disease affects juvenile and adult shrimp and lobsters during grow-out and maturation phases. Opportunistic infections with *Fusarium solani* usually occur under stressed conditions such as overcrowding, heavy metal exposure or primary disease. Infections frequently occur in cuticular wounds. Mortality associated with *F. solani* infections range between 10-90 %. No approved chemotherapeutants are available for fusarium disease. Only the compound "BG-101", 5-chloro-2-methyl-4-isothiazolin-3-one mixed with 2-methyl-4-isothiazolin-3-one, appears to be effective in reducing fusarium disease. However, "BG-101" is expensive to produce and may be highly toxic to marine organisms other than shrimp.

**Principle Diseases of Lobsters**

Lobsters are affected by many of the bacterial and fungal pathogens described in the previous section. Lobsters maintained in holding tanks or tidal ponds prior to sale often become infected with *Aerococcus viridans var. homari*, the etiological agent of gaffkemia. Bacterial infection generally occurs through a break in the exoskeleton. Mortality of infected lobsters is directly temperature-dependent. Death may result within 48 h after infection with the bacterium at 20° C. Lobsters may live for 5 mo after *A. viridans* infection when held in waters maintained at 2° C. Oral administration of oxytetracycline was recently approved for use in lobster culture to combat gaffkemia. A 21 day withdrawal time is required prior to sale for human consumption.

## CONCLUSIONS

Due to culture practices or lack of approved therapeutants, many diseases of shellfish, such as MSX, SSO, Dermo, bonamiasis and Denman Island disease of oysters, coccidian and *L. haliotidis* infections in abalone, viral diseases and fusarium disease of shrimp may not be treated under field or laboratory conditions. Research is needed to determine effective and safe therapeutants to control these diseases.

Oxytetracycline is approved to treat gaffkemia of lobsters and Formalin-F is close to being approved to treat fouling protozoans of shrimp.

Enrofloxacin, oxytetracycline, Romet-30 and sarafloxacin (Sarafin) are currently being tested for FDA approval for use in shrimp culture to treat larval bacterial infections.

Trifluralin (Treflan) is currently being tested for EPA approval as a water quality additive against zoospores of *Lagenidium* and *Sirolpidium*.

Several anticoccidial drugs (lasalocid, sulphamethazine, sulphaquinoxaline, monensin, amprolium + ethopabate, erythromycin and Robenidine) should be tested for use in abalone culture.

Application of certain therapeutants may be limited to seed and/or broodstock as a prophylaxis against disease agents prior to shipment to another location. Due to the long

duration between treatment of seed and human consumption, the problem of determining withdrawal times would be reduced and may expedite approval of such treatments.

Research in shellfish pathology and animal husbandry will strengthen our understanding of mechanisms of disease in cultured organisms. Application of this knowledge with the development of therapeutants and continued advancement by the FDA and EPA in the approval process for minor-use drugs will help enhance and encourage the potential for shellfish aquaculture.