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Farming and health management: Prevention and policy measures

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Abstract. In aquaculture, more than in other animal productions, prevention is a key issue in health management. The risk of diseases increases with the intensification of the production and can be controlled mainly through the implementation of sanitary or medical prophylactic programs. Sanitary prophylaxis relates to hygienic rules, cleaning and disinfection procedures, water treatment, but also good feeding and rearing practices. Medical prophylaxis is based on the use of immunomodulators and vaccines. Proper vaccination procedures and strategies today allow for the control of the main bacterial diseases and a reduction of the consumption of antibiotics. A recent and promising area of research concerns the use of probiotics in aquaculture, especially in larviculture when fish have not raised their ontogenic maturity and cannot be immunised.

Keywords. Prevention – Hygiene – Cleaning – Disinfection – Immunostimulators – Vaccines – Probiotics.

L'élevage de poissons et la gestion sanitaire : Mesures et politiques de prévention

Résumé. *En aquaculture plus que pour d'autres productions animales, la prévention représente un facteur clé au niveau sanitaire. Le risque de maladies augmente avec l'intensification de la production et peut être contrôlé principalement par l'implémentation de programmes de prophylaxie sanitaire ou médicale. La prophylaxie sanitaire relève de l'application des règles d'hygiène, des procédures de nettoyage et désinfection, du traitement de l'eau, mais aussi de bonnes pratiques de nourrissage et d'élevage. La prophylaxie médicale est basée sur l'utilisation d'immunostimulants et de vaccins. Des procédures et stratégies de vaccination correctes permettent aujourd'hui de contrôler les principales maladies bactériennes des poissons, réduisant la consommation d'antibiotiques. Un récent et prometteur domaine de recherche concerne l'utilisation de probiotiques en aquaculture et principalement en larviculture à un stade où les poissons n'ont pas atteint leur maturité ontogénique et ne peuvent être immunisés.*

Mots-clés. *Prévention – Hygiène – Nettoyage – Désinfection – Immunostimulants – Vaccins – Probiotiques.*

I – Introduction

In recent years, the aquaculture of marine species in the Mediterranean has been one of the fastest growing industries. Currently (2003), the barrier of 100,000 tonnes should be overcome and almost 300 million fingerlings will have been stocked. The production of market size fish is mainly concentrated in Greece, with one third of the total tonnage, whereas the main markets are in Western Europe, particularly in Italy and Spain. The main production of fingerlings is spread over four different countries, which underlines the important transfer of fish in the area.

As experienced in animal husbandry and the salmon industry a few years earlier, the progress of marine fin fish production depends mainly on three dominant limiting factors: nutrition and feeding optimization, genetic improvement and pathology. In the past few years, a drop in price and the increasing demand for quality have put pressure on the producers to find solutions in order to reduce production costs and increase the quality of the final product, as well as to have a responsible attitude and an environmentally friendly industry. These last two points have been highlighted by the recent crisis faced by other sectors of activity with mad cow disease and the dioxin contamination, and the role played by the media in the development of such crisis. Correlatively, pathology has proved its importance with the production of some species, such as

Diplodus puntazzo or *Dicentrarchus labrax* being threatened by *Enteromyxum leei* or *Nodavirus,* respectively. Moreover, the list of chemicals licensed for used in aquaculture is very limited.

All these parameters have pushed the marine finfish producers to adopt a similar attitude to the salmon industry ten years earlier when they faced the same dilemma. At this time, the only EU country in which fish vaccines had a significant economical impact was Norway (Anonymous, 1994). Firstly, bacterial vaccines were tested at a commercial scale on marine species in the early 90's. Now, in most Mediterranean countries, either sanitary or medical prophylactic methods are being developed and implemented with success to reduce the impact of diseases on production, since the use of chemicals and losses are linked to pathologies.

II – Risk of diseases

The risk of diseases can be controlled, either with preventive programmes, which aim to reduce the pressure of pathogens in the environment, or with medical prophylaxis. The latter will increase the specific or non-specific resistance of the host. Contrary to salmonids, marine finfish species are reared under a wide range of environmental conditions, from cold water in northern countries to warm water in tropical areas. Depending on these environmental conditions, the structure of the local industry, the farming systems and the technology employed, these sanitary measures and medical prophylactic methods will have to be adapted to local conditions in order to be fully efficient.

1. Prevention and prophylactic programmes: Sanitary aspects

The concept of prevention and hygiene should primarily be considered in the site survey and the design of the facilities. On several occasions, production units have been set up in inappropriate areas or in a way that makes correct cleaning and disinfection almost impossible.

In hatcheries, sanitary programs represent the main way of fighting diseases. Medical prophylaxis will have a limited impact on fish that have not raised their ontogenic maturity. Hatcheries represent a closed environment in which the objective is to control the development of pathogenic and undesirable microorganisms, and not to sterilize the system.

A. Risks of contamination

The risks of contamination (Fig. 1) have to be identified and eliminated prior to any attempt at controlling the microflora in the system itself.

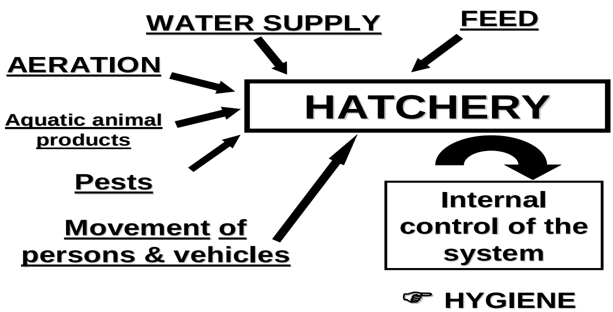

Fig 1. Risks of contamination by opportunistic or pathogenic micro-organisms in a hatchery.

Water borne contamination represents the main risk of introduction of pathogens. Unless the hatchery is supplied with borehole water, appropriate filtration and treatment of all incoming water, using ultraviolet (UV) radiation or ozonification will be required. It is clear that the system will have to be monitored through regular bacterial control of the water and the filters will need servicing on a regular basis. The efficacy of the water treatment is largely dependent of the pre-filtration of the water, the quality of the seawater pumped (Larcom *et al.*, 1981), the original bacterial load and the size of the particles in suspension. When these parameters are properly managed, ozonation and UV systems have demonstrated their efficacy for removing the main fish pathogens (Table 1) (Kimura *et al.*, 1976; Maisse *et al.*, 1981; Liltved *et al.*, 1995). Most marine hatcheries are now equipped with air filters and UV filters to avoid the risk of air borne contamination through aeration of the live food cultures or the rearing tanks. Strict control of all live stock entering the production unit can be achieved either with a set up of quarantine tanks for adult fish or the systematic disinfection of eggs, usually with iodine compounds or glutaraldehyde solutions. This will reduce the risk of vertical transmission of diseases. Most hatcheries tend to be self-sufficient and screen the introduction of live food products as much as possible, including master cultures of algae and rotifers, which, if necessary, can be supplied with the necessary warranties from reference centres. However, other risks of contamination exist and are often neglected. Movement of personnel, either visitors or workers, from an on-growing site to the hatchery needs to be controlled. Vehicles, especially lorries for fish transport or from suppliers, can introduce microbes. On some sites, footbaths for such vehicles have been installed at the entrance to the site. Pests also have to be considered as a potential risk and should be treated as such.

Table 1. Minimum UV dose rate identified for the inactivation of some of the main fish pathogens

Species	UV dosage†
Escherichia coli	4.0×10^3
Vibrio anguillarum	4.0×10^3
Aeromonas hydrophila	5.0×10^3
Pseudomonas fluorescens	5.0×10^3
IPNV (one of the most resistant virus)	1.5×10^5
VNN (Nodaviruses)	1.0×10^5††

† To destroy at least 99.9% of viable bacteria / to decrease the virus infectivity by more than 99%. Unit = $\mu W.sec^{-1}.cm^2$.
†† Inactivation of the nodavirus of striped jack is observed for an irradiation of 410 $\mu W.cm^2$ for a period of 244 sec.

III – Control of the microflora

The control of the microflora in the hatchery has three main components (Vadstein *et al.*, 1993): the non-specific reduction of microflora, the enhancement of specific microflora and the enhancement of the host resistance.

1. Non-specific reduction of microflora: Hygiene, cleaning and disinfection

The establishment of hygiene procedures, including comprehensive cleaning and sanitizing programmes (Torgersen and Hastein, 1995), has contributed to the reduction in the prevalence of bacterial flora in rearing facilities. However, basic guidelines, such as the five steps scheme (Fig. 2) and the use of adapted products at the right dosage must be respected for the success of the operation.

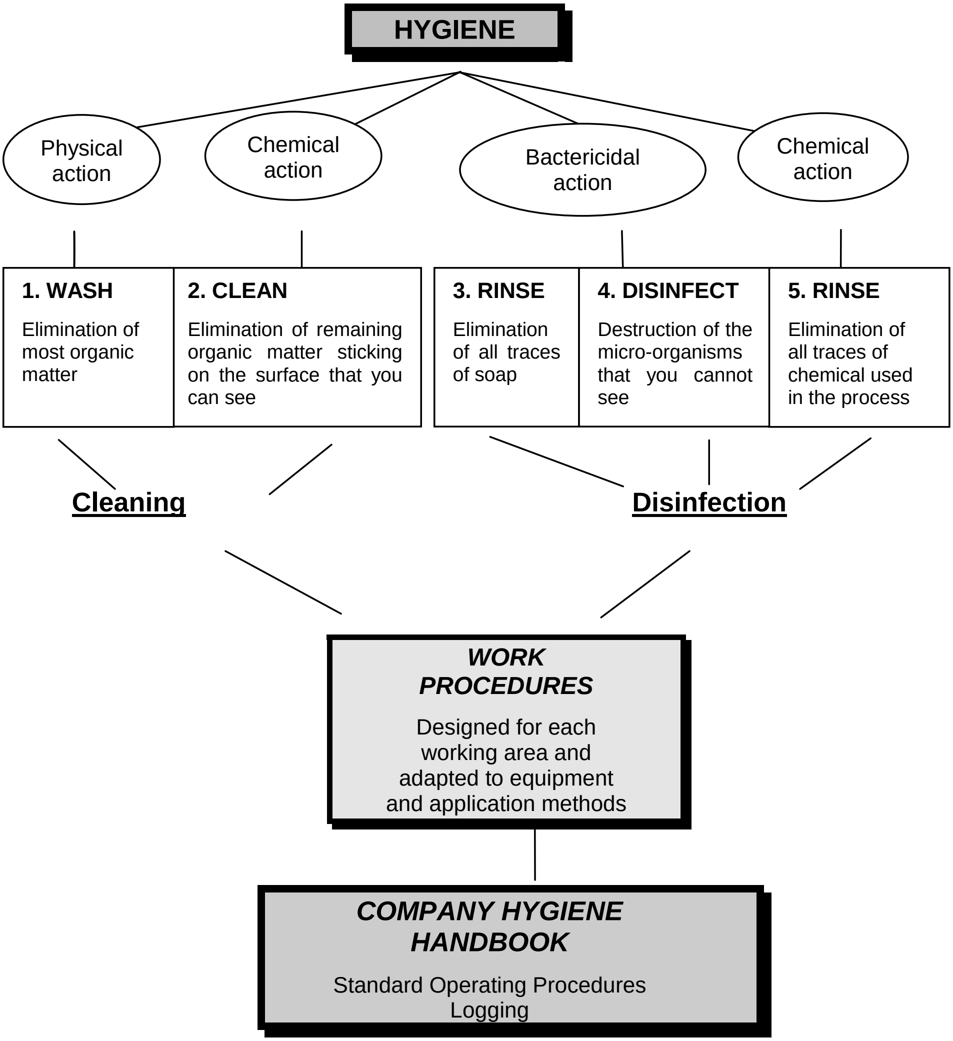

Fig. 2: Five steps scheme for the cleaning and disinfection procedures.

A. Cleaning procedures in aquaculture

The first three steps of the five-step scheme correspond to the cleaning process, which will prepare and maximize the action of disinfectants. It is recognised that effective cleaning represents more than 80 per cent of the success of a cleaning-disinfection programme. On specific surfaces, such as stainless steel or plastic, chemical cleaners have proved to be more effective than disinfectants in removing specific pathogens in biofilms. The *first step* of cleaning is due to eliminating mechanically most of the organic matter. This can be done using hot water to increase the efficiency and maximize the action of detergents (*second step*).

Generally, the function of cleaning compounds is to reduce the surface tension of water, therefore allowing the lifting and flushing away of soils. Cleaning agents are not intended to kill or inactivate micro-organisms and viruses, which is the action of disinfectants. Large numbers of micro-organisms may be removed during the cleaning operation when organic matters are flushed away. After cleaning the surface, sanitizing agents are used to destroy the remaining organisms that are exposed as a result of cleaning.

Synthetic detergents are the most common cleaning compounds, each designed to serve a specific function. Depending upon the cleaning operation planned, detergents can be chosen to achieve the objectives. The various detergents can be classified into the following types:

(i) Inorganic alkaline detergents – these are good saponifying agents, dissolving food solids. However, they are often corrosive or irritating for skin and are kept for heavy duty cleaning. The most common ones are sodium hydroxyde (caustic soda), silicates, trisodium phosphates and sodium carbonate.

(ii) Inorganic and organic acid detergents – acids are useful for removing mineral deposits formed as a result of using alkaline detergents or for use in hard water and sea water environment. Organic acids are less irritating and corrosive than inorganic acids.

(iii) Surface active detergents or wetting agents – the main action of these agents is to emulsify fat deposits. They can be classified in cationic, anionic and non-ionic agents. These wetting agents, which are a component of most detergents, have strong emulsifying, dispersion and wetting properties, as well as being non-corrosive and non-irritating.

(iv) Phosphates and enzymes – these are not commonly used in aquaculture, but phosphates are excellent emulsifying agents, while enzymes are efficient at removing protein buildups.

Finally, it should not be forgotten that one of the most important factors for an effective program is the use of suitable water. The main requirements for the water are that it should be clear, if possible soft, as well as non-corrosive and most importantly free from micro-organisms or other chemicals such as chlorine, which might inactivate the cleaning agents. A large number of detergents have antagonistic effects with disinfectant compounds, and they inactivate each other. This underlines the importance of the *third step* (washing) in the success of the whole process.

B. Specific requirements for cleaning procedures in aquaculture facilities

There is a wide range of chemicals used in the food industry as detergents, but very few performance data are available for use in the fresh water or sea water environment. Nevertheless, the specificity of aquaculture systems has to be taken into consideration while choosing the detergents to apply.

One of the main constraints of cleaning in aquaculture facilities, especially hatcheries, is to break down the biofilm formed by microorganism, as well as the accumulation of fat, greases and oil from fish food. Good saponifying and emulsifying properties will be required. Agents from the alkali group (such as sodium silicates, trisodium phosphate) can be recommended. In case of hard water and sea water, a non-ionic wetting agent will need to be chosen, as their efficacy will not be affected and they allow water to penetrate better into the organic matter remaining in the system.

The product should be as safe as possible for fish and the user. Caustic soda is an extremely powerful detergent but it is corrosive and induces skin irritation and lesions. It can be used effectively in a footbath, as its activity is not affected by soil. Some parts of aquaculture systems can be cleaned *in situ* (i.e. a CIP / Clean in Place system). This is the case of water and aeration pipe systems, or pumps. Others can be dismantled for cleaning, therefore increasing the efficacy of the process (e.g. mechanical filters, biological filters). The choice of the sanitizer to use will be wider as toxicity effects from any residues in the filter will be minimized. In this case, the cleaning and disinfection process will result in the interruption of the production facility since it includes a period of dry out. The choice of the detergent type of material used for the aquaculture facility is important. Acid compounds, including iodophor, can not be recommended on concrete structures, as they will attack the surface, removing a certain amount of cement, and lime. Their efficacy as sanitizers will be reduced. In case of mineral deposit, especially in the sea water environment, acid cleaners are the most effective in removing these deposits.

Finally, certain parameters will influence the degree of cleaning. These have to be identified and include: (i) the amount of organic matter remaining in the system after the first step of washing and the amount of time the detergent solution will be in contact with the organic matter; (ii) the temperature and concentration of the detergent solution; and (iii) the agitation at the detergent/organic matter interface. In case of the CIP (cleaning in place) system, a small "recirculating system" with a pump and a collector tank for the detergent solution can be connected to the water system for cleaning and it can be left to run for a period of 12 hours. This set-up can also be used for disinfection.

C. Disinfection in aquaculture

Disinfectant users must have clear goals and a defined protocol. The choice of the appropriate products and the technique applied must ensure the safety of fish, humans, equipment and the environment. Any organic matter remaining in systems requiring disinfection will provide protected areas for growth of micro-organisms within and under the soil, meaning that chemical sanitizers will not have the proper activity. Soil and detergents also inactivate some disinfectants partially or totally. In order for disinfectants to be applied properly, it is therefore critical to have good cleaning/washing prior to disinfection, and this constitutes the fourth step of the five-step scheme.

As for detergents, several factors will affect the activity of disinfectants:

(i) Concentration of the disinfectant solution – the effectiveness and the rapidity of action of a disinfectant will increase with the concentration of the solution employed, up to a certain limit.

(ii) Time of contact – the death of a population of microorganisms follows a logarithmic relationship. It is therefore important to reduce the initial load of bacteria or virus in the system requiring disinfection in order to shorten the time of exposure and increase the efficacy of the process. This also underlines the importance of the concentration of the disinfectant solution.

(iii) Temperature and pH – both parameters affect in a complex way the antimicrobial activity of disinfectants. An increase in pH will decrease the effectiveness of chlorine and iodophor solutions. While bacterial metabolism (growth rate and death rate) will increase correlatively with the temperature, increasing temperature will also affect the pH, the stability of sanitizer agents and their physico-chemical properties, therefore affecting their efficacy as disinfectants.

The choice of disinfectant agent will depend upon the type of material or structure to be treated. In a sea water environment, an acid solution will present different advantages, unless the treated surfaces are concrete. In earth-ponds, where complete removal of organic matter, even after cleaning, is impossible, highly alkaline solutions such as calcium oxide, calcium cyanamid or sodium hydroxide combined with a wetting agent for penetration can be recommended. Most disinfectant compounds are corrosive, and are skin irritants or caustic. The use of protection (gloves, glasses and masks) is required at all times during their application. The main disinfecting agents applied in aquaculture and their methods of use are listed in Table 2. Physical or chemical process can be applied effectively.

Table 2. Disinfectants applicable to aquaculture and methods of use (modified from Torgersen and Hastein, 1995)

Process	Indications		Method of use†	Recommendation	Warning
	Pathogens	Surface/material			
Phisical					
Desiccation, light	All pathogens	Earth ponds	Dry 3 months at a temperature >18°C / UV / temperature <0°C	Use of chemical disinfectant allows the drying period to be reduced Concrete tanks for 1 month	
Dry heat	All pathogens	Stone, iron, concrete tanks	Flame-thrower, blow-lamp		
Damp heat	All pathogens	Transport tanks	Steam at 100°C for 5 min		
UV irradiation	All pathogens	Water	(See specific table for dosage)		
Chemical					
Quaternary ammonium	Virus, bacteria	Hands, small materials, plastic Gills, fish	1 g/l >20 min (bath) 8 g/l (pulverization) 1-2 ppm (bath for 15-20 min)	Some virus (IPN) resistant	
Calcium oxide	All pathogens	Earth ponds	0.5 kg/m^2 for 1 month		Corrosive
Calcium hypochlorite	Bacteria, viruses	Water and clean surfaces	Solution with chlorometric degree of 0.01 (i.e. 30 mg Cl_2/l) left to inactivate for at least 1 day	Neutralization by thiosulphate Inactivated by organic materials	
Calcium cyanamide	All pathogens, especially spores	Earth ponds	250 g/m^2 on dry surfaces for at least 1 week		Corrosive
Formalin	Ectoparasites, bacteria, VHS virus	Treatment of fish buildings, materials, transport tanks	50-300 ppm (larvae 15 days old to large fish) Fumigation (comply with instructions)		Irritant
Iodine (iodophors)	Bacteria, viruses	Hands, smooth surfaces Eggs Gametes during fertilization	250 mg/l for a few seconds 50 mg/l for 10 min 25 mg/l for several hours	Neutralization by thiosulphate	Toxic for fish
Ozone	All pathogens	Water for sterilization	1 mg/l for 1 min	Costly. The concentration in the water must be <2µg before using it	Toxic for fish
$KMnO_4$	All pathogens	Tanks	100 g/m^3 (bath)		Corrosive

Table 2. (Cont.)

Process	Indications		Method of use[†]	Recommendation	Warning
	Pathogens	Surface/material			
Sodium hydroxide	All pathogens	On resistant surfaces (plastic, unpainted concrete), earth ponds	In mixture: - Sodium hydroxide 100 g - Teepol 10 g - Calcium hydroxide 500 g - Water 10 l Surfaces: spray 1 l/m^2, leave 48 h Earth ponds: spray 2 l/m^2, leave for 2 weeks at least	Turn water on and check the pH afterwards	Corrosive
Sodium hypochlorite	Bacteria, viruses	All clean surfaces, water hands, materials, boots	200 mg/l - 5 ml/l of bleach, leave at least for 24 h. Or neutralize after 3 h Leave for 20-30 secs, rinse with clean water or inactivate	Inactivated by thiosulphate	Irritant Toxic for fish

[†]Concentrations of the active substance.

Chlorine and iodine compounds are highly toxic for fish and must be inactivated before their release as waste or any time that one of these products might enter into contact with live fish.

A 1% solution of sodium thiosulphate can be prepared to inactivate these products. In this case, the neutralizing volumes (in ml) of solution required are:

- for chlorine: 28.5 ml of solution/100.
- for iodine: 7.8 ml of solution/100.

a) Physical process

These methods of disinfection are often underestimated, although they represent the most environmentally safe. A dry-out period is the process of choice for earth-pond disinfection. UV radiation is the easiest way to decontaminate both fresh water and sea water without toxicity risks for fish. Some forms of resistance of micro-organisms such as parasite spores can be effectively destroyed by using only dry heat, if the surface to treat allows its application.

b) Chemical process

Quaternary ammonium compounds: these are widely used to disinfect surfaces, they are also wetting agents and therefore have detergent property, which makes them good to use on porous surfaces. They are more stable than chlorine and iodine solutions in the presence of organic matters, but have a selective action on micro-organisms. They are generally less effective on Gram (-) bacteria, which are those most often involved as fish pathogens. Quaternary ammonium solutions have the advantage of being easy to measure.

Chlorine: sodium hypochlorite solutions are very active disinfectants, which are widely use for disinfection of small materials and surfaces or in footbaths. Effective against Gram (+) and Gram (-) bacteria as well as some viruses and spores under certain conditions, although chlorine is highly toxic for fish. Therefore, residues must be carefully inactivated by thiosulphate. Test kits can easily measure the concentration of active chlorine. Active chlorine is economical to use but is an irritant. Solutions of sodium hypochlorite are not stable, and are easily inactivated by organic matter and sunlight. They should be renewed on a regular basis in footbaths and tanks for disinfection of small materials. Chlorine dioxide has the advantage over chlorine of remaining active in the presence of organic matter and at high pH. It can be used when the presence of a heavy organic load is encountered. Chlorine dioxide is corrosive and a skin irritant.

Iodophors: combined with wetting agents to form iodophors, active iodine solutions are more stable than chlorine solutions, and they are active at lower concentration. Their acid nature prevents film formation but they should not be used on concrete surfaces. They are easy to measure, especially as their brown colour gives visual control, and are inactivated by thiosulfate.

Sodium hydroxide: caustic soda is a very effective virucide, being highly alkaline. Solutions are not inactivated by soil, which makes it a disinfectant of choice for earth ponds. It is usually used in a mixture with a wetting agent (Teepol) and lime, with the solution being sprayed over the earthen system. This should be followed by a dry-out period of several days. Sodium hydroxide can be employed on concrete and plastic structures, but in footbaths it is only suitable for rubber boots. Metallic structures should not be disinfected with such solutions.

Acid disinfectants: these solutions are effective against both Gram (+) and Gram (-) bacteria. They can combine the third and fourth steps of the cleaning/disinfection process as the acid will neutralise the excess alkalinity remaining from the cleaning procedure and will prevent the formation of alkaline deposits. They are very effective in sea water, removing calcium concretions. They cannot be used on concrete surfaces or at alkaline pH. Organic acid can be used for disposal of carcasses and dead fish by silage.

Aldehydes: the two compounds used for disinfection in aquaculture are formaldehyde and glutaraldehyde. Formaldehyde is wildly use as an antiparasitic compound for fish, having also a bactericidal effect. It is usually available in a 37% aqueous solution (w/w), stabilized with 15% methyl alcohol. Formaldehyde gas is a very effective tool for in situ disinfection of aquaculture systems, especially pipes (water or air systems) and rooms. Gas for fumigation is obtained from the solid polymer paraformaldehyde either by heating of the tablets or by chemical reaction with potassium permanganate. It is one of the most effective disinfection methods in case of viral contamination. Glutaraldehyde has a broad spectrum activity on viruses, bacteria and fungi, including good sporicidal properties. It has a rapid rate of kill and is not affected by the presence of organic matter. This product is not corrosive to metals or most materials and is not an irritant. It is mainly used in aquaculture for egg surface disinfection in solution (400-500 mg/l for 5 to 10 min), having a better viricide activity than iodophors. As a 2% solution, with addition of 0.4% sodium bicarbonate, it is an effective chemosteriliser that is easy to use by contact on different materials.

Calcium oxide: quicklime is the reference disinfectant for earth ponds, especially in case of infestation by *Myxosoma cerebralis*. It is applied on drained pond surfaces, and a dry-out period should be allowed before refilling. Quicklime is also recommended for disposal of carcasses and dead fish when buried.

Phenols and alcohols: due to their toxicity and the fact that phenols are inactivated by cationic and non-anionic detergents they can therefore only be used with anionic ones. Alcohols are effective only at high concentration. These products are employed in aquaculture only for small materials and hand disinfection.

Most of the systems that need disinfecting in aquaculture facilities cannot be dismantled, which leads to cleaning/disinfection in place. Therefore, a large part of the chemicals used or their residues will be released in the environment. Organic matter, grease, fat and mineral deposits are constantly present in such systems. A large range of detergents and sanitizers are available from the industry, with their own properties, specificity and limitations of use. Knowing these factors, it is possible to establish a comprehensive cleaning and sanitizing program in aquaculture facilities, which is safe for the fish, the users and the environment. Such effective programs will drastically reduce the risk of disease and will complement the action of medical prophylactic programs based on vaccination and immuno-stimulation of the fish. The introduction of new methods, such as fumigation (Hamor, 1996), has contributed to increase the efficacy of the process. Ultimately, hygiene depends on basic concepts, which, to be applied properly, require staff training and a change of mentality.

D. Non specific reduction of microflora: The particular case of hygiene in live food cultures

Improvement in live food culture through the introduction of new techniques or products has also represented a major step forward. Bacteriostatic compounds in Artemia culture enrichments represent a major help in controlling the level of hygiene and the development of bacterial flora in both live food culture and larval tanks. A bactericide treatment, such as formaldehyde or hydrogen peroxide can also be applied and eventually combined with probiotics to re-equilibrate the flora (Gatesoupe, 2002a). However, finally, the main future seems to be in new rearing methods, especially in the case of rotifer cultures, using recirculating systems and water treatment to allow high density cultures with good hygienic standards (Suantika *et al*., 2000, 2001).

2. Enhancement of specific microflora

It is obvious that there is no need for sterilization of the rearing facilities, which, in fact, will otherwise create an unstable microbial environment and induce a higher risk if new pathogens are re-introduced accidentally into the system. In order to increase the stability of the bacterial flora and avoid the development of pathogens, probiotics and mature water, which enhance specific bacterial flora, have been implemented recently mainly in larviculture (Gatesoupe, 1999; Tovar, 2002). Their application as a "biocontrol" of the microflora in the larval environment has three aims: (i) that probionts will colonize the intestine epithelium of the larvae after the opening of the mouth, avoiding colonization by primary or opportunistic pathogenic strains; (ii) most probionts tested under *in vitro* or laboratory conditions demonstrate antagonistic effects against potentially pathogenic strains (Smith and Davey, 1993) and it is expected that they should keep these properties while colonizing the intestine system of fish; and (iii) some probiotic strains are expected to increase the resistance of fish to diseases, especially by activating the non-specific immune system of their host. Recent work has underlined these properties of probionts, giving some hope in the search for new methods to control fish diseases (Nikoskelainen, 2001a,b; Chang and Liu, 2002). Other beneficial effects may be expected from probiotics, such as growth promotion induced by different mechanisms (Tovar, 2002).

Probiotics have been used for a long time in other animal production and the first probionts tested in fish production were products that already existed on the market, but they contained bacterial strains which were not selected from the marine environment. Questions concerning their efficacy in the marine environment and fish production have been raised (Gatesoupe, 2002b). Some research programs have focused on the selection of probiotic bacterial strains selected from the natural environment of the larvae, especially their intestinal heterotrophic microflora. Application of probionts in larval production has shown promising results but considerable research efforts are still required to develop applications on an industrial scale.In addition, the mesocosm system or the technique of green water for larvae can be related to the concept of microbially maturated water. In this case, bacterial flora in the environment is promoted and acts as a buffer for the culture media, avoiding a bloom of adverse microflora.

During successive production cycles, microflora will adapt to the ambient conditions, especially when antibiotics or treatments are applied, since a selective pressure often leads to the selection of opportunistic or pathogenic flora. This highlights the need for dry-out periods in production units, in order to break down these selected floras. Hence, an adaptation of production management with a physical separation of the different sectors of production and a production by cycles followed by disinfection is required.

When combined, these methods have proved their efficiency in the prevention of diseases such as viral nervous necrosis (Nakai *et al.*, 1995) or *Photobacterium damsela* subsp. *piscicida* in infected hatcheries.

On-growing operations take place in different open environments, in which a control of the ambient micro-flora is not possible. However, some hygienic principles can also be applicable. Net cleaning, disinfection and coating with anti-fouling, when allowed, help to maintain water exchange, oxygen levels and the quality of the environment for the fish. Control over the movement of boats and exchange of material from site to site should be enforced, especially in case of disease outbreaks. Separation of generations, which is a basic concept often applied in animal production, has helped to control the level of infestation by sea lice *Anilocra physodes* in sea bass aquaculture. Other measures, already applied in salmon production, such as site rotation, would also be beneficial for the production of marine species. However, in the case of open on-growing sites, medical prophylaxsis remains the most suitable solution.

IV – Enhancement of host resistance: Medical prophylaxis

1. Immunostimulators and vitamins

In recent years, the use of immunostimulants in marine species production has increased (Al-Harbi, 1992; Efthimiou, 1996; Sakai, 1999), especially for larval and juvenile stages (Vadstein, 1997). Their effects on non-specific prevention are multiple: before handling or a stressful situation, to reduce the risk of mortality after transfer, for broodstock during the maturation period and to prepare fish for vaccination, as well as to increase their immune response. The exact evaluation of their efficacy is difficult to estimate but field data generally indicate an improvement of the situation when they are applied. These products have to be considered as a treatment and not a feed additive, and the fact that their over dosage can induce immuno-suppression (Sakai, 1999) has to be borne in mind. In addition to these products, vitamins C and E can be added to the feed to obtain a similar increase of the non-specific resistance of the fish. Finally, antistress factors have been tested experimentally and can be helpful for stressful species such as sea bass.

2. Vaccines and vaccination strategies

In the early 1990s, the first vaccine commercially employed was against *Vibrio anguillarum* serotypes I and II. More recently, several *Photobacterium damsela* subsp *piscicida* vaccines have been extensively tested. These are actually the only two vaccines licensed for sea bass and sea bream. They are formalin-killed bacterins, available under different formulations for immersion, injection or oral delivery. The two first delivery presentations can be either monovalent or bivalent. In 2002, a commercial furunculosis vaccine for salmonids was successfully applied on two sites with sea bass reared in brackish water. Ongoing research has been initiated on *Flexibacter* spp. and Nodavirus (Midtlyng, 1997), and they are showing promising results.

Safety and potency tests have demonstrated, under laboratory conditions, the inequity and the efficacy of these vaccines for marine species. However, field results have not always been consistent. Nevertheless, failure in an attempt to protect fish is mostly attributable to improper application and inadequate vaccination programmes, and not to the vaccine itself. Consequently, different parameters need to be considered in drawing up appropriate vaccination procedures.

Vibrio anguillarum is a strong antigen, which induces a reasonable protection of approximately five to six months. However, the use of a booster vaccination is necessary in order to cover the whole production cycle. On the other hand, *Photobacterium damsela* subsp*. piscicida* is a weak antigen and protection of no more than two to three months can be expected. It is therefore important to identify the periods of risk during the year for the occurrence of the disease and to achieve the highest protection during these targeted periods. Fortunately, pasteurellosis is temperature dependent and occurs mainly during the summer months and early autumn, in on-growing units. Only sea bass are affected by *Vibrio anguillarum* outbreaks. However, both sea bass and sea bream are sensitive to *Photobacterium damsela* subsp*. piscicida.* In both species, the disease becomes chronic in larger fish, with low mortality unless a second pathology interferes. The planning of introduction of the fingerlings will also influence the timing of vaccination and revaccination. All this information is necessary for designing a programme of vaccination adapted to the conditions of the site considered. The sensitivity of some species such as sea bass to stress also has to be considered when choosing the method of delivery of the vaccine. The health status of the fish should be checked before any vaccination. The use of vitamins and immunomodulators are recommended for preparing the fish in order to increase the intensity of their immune response.

Two different approaches have been applied in practice. In the first option, fingerlings are vaccinated by immersion in the hatchery at 1.5 to 2 g average weight and re-vaccinated by immersion at 5 g at the on-growing unit. Generally, bivalent vaccines are employed. The fish will later need a second booster by injection at an average weight of 30 g. The second approach involves vaccinating the fry in the hatchery at 1.5 to 2 g average weight and to re-vaccinate the fish orally either once or twice, depending on the conditions faced at the site. The first method will almost certainly induce a longer immune response in the small fish during their most sensitive stage, but will not be sufficient in all cases to cover the fish up to market size. Moreover, it is a labour intensive and stressful manipulation, especially for sea bass. In addition, the cost of this vaccination programme per fish turns out to be very high. The second approach is less stressful though and appears to be more adapted to the specific conditions of marine species aquaculture.

Proper vaccination programmes against vibriosis in sea bass have proved their effectiveness, allowing control of the disease. Correlatively, the consumption of antibacterial compounds has been reduced drastically, as observed in the salmon industry a few years ago in Norway. Improvement of vaccination procedures for pasteurellosis is also expected in the near future.

V – Conclusion

Different steps can be identified in the control and prevention of diseases. The first relates to the identification of the pathogens and a proper diagnosis of the disease, which should lead to adequate treatments. The second represents the development of medical prophylactic methods to prevent these diseases. This will lead to an appropriate use of immunomodulators and the design of adapted vaccination programmes. The final step involves an understanding of the reasons for the occurrence of a particular pathology. An approach similar to the HACCP method can be followed for identifying the risk factors. More environmental studies and epidemiological research will also facilitate this. When those factors are fully understood and identified, they should lead to the development of a code of practice and design for each production site.

In Mediterranean aquaculture, the first step has been reached. Since 1991, medical prophylactic methods have been developed and applied. However, those involved in the sector should understand that vaccines do not represent the final solution in disease prevention, especially in hatcheries or when applied on farms with a low standard of hygiene. The applications of sanitary measures, in conjunction with medical prophylaxis have already proved their efficiency in marine finfish production and should be developed further. A change in mentality is definitely required and adherence to the Code of Conduct for Responsible Fisheries, as promoted by the G.F.C.M. and the FAO Committee for Aquaculture in the Mediterranean area, is strongly recommended. Furthermore, this will contribute towards securing the production and progress towards predictable quality.

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