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Main sanitary problems and needs in Turkish fish farming

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Abstract. Fish diseases are often introduced by the inlet water, purchased eggs and fish, as well as by birds, visitors, transport vehicles and farm equipment or by farm employees themselves. Eggs should be purchased only from disease-free farms and they must be disinfected routinely on arrival. Employees also represent a disease hazard, mainly after making deliveries to other farms. Hands, boots, and vehicle tyres should be disinfected. Equipment should be kept separate for each pond and be disinfected after each use. Duration of disinfection should be sufficient to kill pathogens. Dead fish should be removed and losses must be recorded daily. There can be serious problems with fish purchased from other farms and sea bass/sea bream broodstock caught from the marine environment. Such fish can enter the farm without any health control. Farmers should follow quarantine measures strictly. Good husbandry and vaccination programmes should be applied. Disease maps, certification programmes and regulations must be established.

Keywords. Fish – Sanitation – Disinfection – Quarantine – Aquaculture.

Principaux problèmes sanitaires et besoins pour la pisciculture en Turquie

Résumé. Les maladies des poissons sont souvent introduites par l'eau des bras de mer, par les œufs et les poissons achetés, ainsi que par les oiseaux, les visiteurs, les véhicules de transport et l'équipement des fermes piscicoles ou les employés eux-mêmes. Les œufs doivent être achetés uniquement à partir de fermes indemnes de maladies, et il faut les désinfecter de façon routinière à la réception. Les employés représentent également un danger de maladies, surtout après avoir fait une livraison aux autres fermes. Les mains, les bottes et les pneumatiques des véhicules doivent être désinfectés. L'équipement doit être gardé séparément pour chaque bassin et doit être désinfecté après chaque utilisation. La durée de la désinfection doit être suffisante pour éliminer les pathogènes. Les poissons morts doivent être retirés et les pertes sont à enregistrer quotidiennement. Il peut survenir de sérieux problèmes avec les poissons achetés à d'autres fermes et avec les géniteurs de bar ou de daurade capturés dans le milieu marin. Ces poissons risquent d'entrer dans la ferme sans aucun type de contrôle sanitaire. Les aquaculteurs doivent suivre de manière stricte des mesures de quarantaine. Une bonne gestion et des programmes de vaccination doivent être appliqués. Des cartes de maladies, des programmes de certification et des réglementations restent à établir.

Mots-clés. Poissons – Protection sanitaire – Désinfection – Quarantaine – Aquaculture.

I – Introduction

Infectious diseases have always been a limiting factor for aquaculture. High population densities, adverse environmental conditions and poorly designed culture systems frequently cause stress, which consequently reduces the immunity of fish to pathogens. Bacteria, viruses, parasites and fungi can all cause disease outbreaks and high level mortality. All viral, many parasitic and some of the bacterial diseases can not be treated successfully with the chemicals available. It is a reality that treatment of disease is expensive and more difficult than prevention. Avoidance of contact between the host and the pathogen may be the only available control measure. Supplying pathogen-free good quality water, pathogen-free diets, hygiene and sanitation, good husbandry, improved diet formulation, modification of the environment, contact prevention with other animals, active immunisation and quarantine of new animals are major topics for prevention of infectious diseases in aquaculture (Ahne and Winton, 1988). Sanitation

practices should include disinfection between groups of fish, cleanliness while fish are growing and prevention of disease transmission by equipment, personnel or water (Francis, 1996). In this paper, sanitation applications and problems for fish culture in Turkey will be presented.

II – Water sanitation

It is not possible to find completely sterile water in aquaculture. Effective treatment of sea water, especially for sea bass and sea bream larval rearing units, can be achieved using pre-filtration by sand, filter cartridge and ultraviolet (UV) treatment. Aquaculturists in Turkey prefer to use 20-50 micron sand filters, and 10, 5 and 1 micron cartridge filters to remove particles, as well as UV for disinfection of sea water. In rainbow trout farms, huge concrete gravel plus sand containing ponds are used to remove and filter coarse particles. Trout hatcheries always use good quality spring, artesian or surface waters. However, saprolegniasis is a serious problem for hatching rainbow trout eggs if the temperature is high. Trout hatcheries have been using hydrogen peroxide and/or formaldehyde to overcome saprolegniasis even though it is not as effective as malachite green.

The installation of parallel 55 watt UV lamps emitting light at 254 nm is used to disinfect sea bass and sea bream hatchery water in Turkey. The number of lamps depends on the required volume of water. The lamp must be installed in such a manner that there is a maximum layer of 6 mm of water passing around the lamp. However, in the new type of UV lamps, water can be disinfected at 4000 l per hour and can be used for 8700 hours without loss of disinfection ability, and there is no need for a maximum layer of 6 mm. The effectiveness of a UV system is related to water volume and colour, the presence of humic acids, dissolved iron and suspended solids (Bruno, 1995). A dose of 50,000 $\mu\text{W}/\text{sec}.\text{cm}^2$ can inactivate bacteria and viruses, including zoospores of *Saprolegnia*. However, 350,000 $\mu\text{W}/\text{sec}.\text{cm}^2$ UV can kill all viruses, except IPNV, as well as bacteria and microsporidian spores (De Kinkelin *et al.*, 1985).

Another disinfection method in aquaculture is the use of ozone. A dose of 1-8 ppm ozone (O_3) for 2-6 minutes is quite effective for killing bacteria and viruses, and also removes odour, but above 0.002 ppm it is toxic to fish and may be harmful for human health (Bruno, 1995). Ozone is even more effective than UV, and a few sea bass and sea bream hatcheries are using the techniques.

UV disinfection is used in every hatchery but most of them have no facility to assess UV efficiency. Additionally, if a lamp breaks and/or there are any fluctuations in the electricity supply, undisinfected water with high microorganism loads can enter larval tanks and trigger bacterial diseases.

III – Cleaning and disinfection of ponds and tanks

Fish housed in tanks are increasingly subject to disease as the intensity of rearing increases. The increased organic load associated with high feeding and stocking rates causes an environment where opportunistic bacteria, fungi and parasites can flourish. To minimise this, the water exchange should be adequate for the stocking densities and feeding rates used. Particulate matter (e.g. faeces, uneaten food, etc.) and dead fish should be removed as soon as possible because they can transmit infection to other fish. Cleaning, with removal of debris by siphoning and removal of most excess particulate matter, should be undertaken at least once a week. It is important that equipment used for cleaning must be disinfected between uses (Francis, 1996).

An appropriate disinfectant technique must be used, which considers fish, human and environmental safety, as well as cost. However, there is no disinfectant that can fulfil all of these conditions. Additionally, certain limitations are established in the EU for control of water discharge from farms using such treatments.

Good cleaning before disinfection is very important. For instance, mud and algae prevent the penetration of the disinfectant and must be removed by brushing first.

Drying of concrete or earth ponds under direct sunlight is an effective form of self disinfection. Unfortunately, this method may not be effective in all cases because complete drying can not be guaranteed. Disinfection of earth ponds can be achieved by using quick lime (calcium oxide), which is commonly used. For routine disinfection of sandy soil and swampy soil, 75 g/m² and 400 g/m² can be used, respectively. If used at 1 kg/m², pathogens down to a soil depth of 3 cm can be eradicated. Chloride of lime at 100-250 g/m² is better than quick lime, although it is expensive and its use is not allowed, for instance, in Germany (Bruno, 1995).

Concrete ponds are disinfected by painting with lime in Turkey. Disinfection of concrete and earth ponds can be enhanced by the addition of sodium hydroxide (that increases the pH) and teepol (reduces surface tension) to calcium hydroxide. The mixture indicated below can be used at 1 l/m² for spraying on concrete surfaces and at 2 l/m² for earth ponds. If used for concrete surfaces it has to be left for 48 hour. However, if used for earth ponds it should be left for at least 2 weeks (De Kinkelin *et al.*, 1985). The formulation of an appropriate surface disinfectant is:

Sodium hydroxide	100 g
Teepol	10 ml
Calcium hydroxide	500 g
Water	10 l

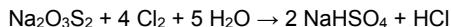
Fish can be restocked after refilling with flowing water and the pH falls to 8.0-8.5.

A solution of 5% formalin (from 35-40% formaldehyde) can be used for concrete surfaces when liming is impossible. Formaldehyde should not be used without wearing protective cloths, gloves and respirators. Formaldehyde is cheap but aggressive to some metals and mucous membranes, and it should not be used in enclosed spaces. Formalin can be used to inactivate bacteria, the VHS virus and some parasites (Le Breton, 1996). The disinfectant properties of formaldehyde are related to reduction and protein precipitation. The duration of disinfection for a metal item is 30 minutes, whereas plastic and wooden surfaces should be left for 2 hours (Schaperclaus *et al.*, 1992). Formaldehyde should be kept in the dark and cool, but at not less than a storage temperature of 5-8°C. If formaldehyde is kept in unfavourable conditions a white paraformaldehyde precipitate develops, which is very toxic to fish (Schaperclaus *et al.*, 1992).

Tanks and concrete ponds must be cleaned of mud, films of organic materials and algae by brushing. To remove them, the marine aquaculturist prefers to use technical grade hydrochloric acid. However, this causes a high acid effluent and may be harmful to the environment. Instead of using hydrochloric acid, a high pressure water nozzle device such as used for car washing can be employed. Similar devices are being used successfully for *in situ* cleaning of cage nets from algae and fouling materials.

Active chlorine containing sodium hypochlorite, calcium hypochlorite, and granular chlorine can be used for disinfection of tanks, equipment, boots, cage nets, etc.

Tanks can be disinfected with 200 ppm active chlorine for 30-60 minutes or 100 ppm for several hours (Francis, 1996). Better results can be obtained if the chlorine is mixed with detergent (e.g. 0.1% teepol or byprox). Chlorine can be purchased as a dairy industry disinfectant or as liquid bleach from known suppliers. The latter contains 4.5% active chlorine. Hypochlorite is not suitable for long term storage and should be bought only in sufficient quantities for a few weeks (Bruno, 1995). However, chlorine can cause harm to nets if used at a high concentration. Calcium hypochlorite, which is a solution with a chlorometric level of 0.01 (i.e. 30 mg/l) should be left to inactivate bacteria and viruses for at least one day. However, it should be neutralised afterwards because it is toxic to fish and the environment. Chlorine can be neutralised by sodium thiosulphate and the reaction is:



1 g sodium thiosulphate neutralises 354 mg chlorine or 1,275 mg iodine (De Kinkelin *et al.*, 1985).

Granular chlorine does not volatilize as readily as liquid bleach. In a poorly ventilated room, fumes from liquid bleach may cause fish kills in adjacent tanks. A concentration of 10 ppm granular chlorine (e.g. chloramine T) for 24 hours is effective for disinfection of tanks and submersed equipment. In addition, it degrades more easily and is less harmful than bleach but it is more expensive. Fish can be disinfected with chloramine T for 1 hour, and doses are dependent on water hardness and pH. The quantities required are given in Table 1.

Table 1. Dose of chloramine T (ppm) for a one hour bath of fish depending on pH and calcium carbonate hardness (De Kinkelin *et al.*, 1985)

pH	Calcium carbonate hardness (mg/l)	
	<40	100
6	2.5	7
6.5	5	10
7	10	15
7.5	18	18
8	20	20

PVP-iodine can also be used against bacteria and viruses. For smooth surfaces, 250 ppm is suggested.

Potassium permanganate is cheap and can be found in tablet form. A concentration of 100 ppm is quite effective against all pathogens (Le Breton, 1996). The action of potassium permanganate is related to the production of atomic oxygen, hydrogen peroxide and the formation of manganese dioxide (MnO_2), although the latter causes an unwanted brownish coloration on treated materials.

The main disinfectants and their usage are listed in Table 2.

IV – Pathogen free diet

Pelleted or granulated feed, which is assumed to be safer, is used for grow-up fry and fattening for marine and fresh water fish. However, aflatoxicosis may occur in fish if the feed ingredients are contaminated.

Live feeds (e.g. Artemia, rotifer and algae) are used to feed marine fish larvae. Trash fish, bivalve molluscs, octopus, and krill are used for feeding marine fish broodstock and sometimes for trout. It has been reported that IPN virus has been isolated from rotifers (Comps *et al.*, 1990) and some bacteria have been isolated from Artemia. Ichthyophoniasis has been reported in rainbow trout through the use of contaminated trash fish as feed (Holliman, 1993), such as cod, herring and sea bass (Sitjà-Bobadilla and Alvarez Pellitero, 1990). Vibriosis caused by *V. ordalii* was observed in sea bass juveniles fed with trash fish (Çağırğan, 1993). *L. anguillarum* and some marine fish nematodes were observed from trash fish fed to rainbow trout (Çağırğan, unpublished data). Consequently, trash fish should be pasteurised in order to prevent outbreaks by feeding. Some aquaculturists treat cut or minced trash fish with boiling water for a few minutes.

Recently, blue fin tuna (*Thunnus thynnus*) culture has begun in Turkey. For feeding, frozen fish imported from Norway is used, without the use of any heat treatment. These animals are

therefore at risk of exposure to infectious agents through the food. Additional care is needed in cases such as these to avoid the importation of exotic diseases with the purchase of diets from foreign countries (Ahne and Winton, 1988). However, rotifers, *Artemia nauplii* and algae are also a potential contamination source for infections, and they should be disinfected to at least partly prevent problems. Antibiotics and/or fresh water treatments are used to control bacterial contamination of rotifers. Rotifers are kept in 10 ppm chlorine-containing water for 6 hours which is then neutralised with sodium thiosulphate. Then, in hatcheries, a 5 ppm flumequine bath is used, whereas other establishments only use clean fresh water.

Table 2. Main disinfectants and usage in aquaculture (modified from Le Breton, 1996)

Chemical	Pathogen	Indication	Method of use	Recommendation
Calcium cyanamide	All pathogens, especially spores	Earth ponds	250 g/m ² on dry surfaces for at least 1 week	
Calcium hypochlorite	Bacteria, viruses	Water and surfaces	Chlorometric degree 0.01 (i.e. 30 mg Cl ₂ /l left to inactivate for at least 1 day)	Neutralisation by sodium thiosulphate, inactivated by organic material
Calcium oxide	All pathogens	Earth ponds	500 g/m ² for 1 month	
Formalin	Bacteria, virus (VHSV) and some ectoparasites	Treatment of fish Concrete ponds	50-300 ppm Fumigation with 3-5% formaldehyde	Do not use in closed spaces
Hexachlorophene soap	Virus, bacteria	Hands	Hand disinfection	
Iodine	Bacteria, viruses	Hands and smooth surfaces Eggs Gametes during fertilisation	250 ppm - few seconds 50-100 ppm for 10 min 25 ppm for several hours	Neutralisation by sodium thiosulphate
Ozone	All pathogens	Water disinfection	1-8 ppm	More than 0.002 ppm toxic for fish
Potassium permanganate	All pathogens	Tanks	100 ppm	
Quaternary ammonium	Virus, bacteria	Hands, small plastic surfaces Fish gills	1/1000, 20 min bath 8/1000 pulverisation 1-2 ppm, 15-20 min bath	Some viruses (IPNV) are resistant
Sodium hydroxide	All pathogens	Concrete ponds, resistant surfaces	0.2%	Cheap but corrosive
Sodium hypochlorite	Bacteria, viruses	All cleaned surfaces, water, hands, materials, boots	200 ppm for 1 hour	Neutralisation by sodium thiosulphate

V – Prevention of contact with animals and predatory mammals

Sea gulls, herons and other aquatic birds are common in the aquatic environment and related sustained losses can be very significant. Besides losses from fish-eating animals, they can also spread diseases, and birds may be intermediate hosts of certain parasites. For example, ligulosis can be spread by aquatic birds. Musk rats, crayfish, and geese may harbour large numbers of *Yersinia ruckeri* (Busch, 1978; Stevenson and Airdrie, 1984). It has been observed that cats can catch sluggish fish from concrete ponds, and this may also facilitate the spread of pathogens. Contamination of the pathogens between ponds by cats can be prevented by

lowering the water level in the ponds. Cages can be covered with nets for prevention of attacks by birds (University of Stirling, 1988). Some farms prefer to use bird scarers or deterrents using noise to discourage birds. The Mediterranean seal, which is a marine mammal, can suddenly attack sea cages, which not only tears the nets and destroys the cages but can also spread disease from one farm to another. Protective nets can be used for prevention of marine mammal attacks (University of Stirling, 1988).

VI – Disinfection of hands, feet and equipment

Footbaths and areas for employees to wash their hands with disinfectant soap should be placed at the entrance to buildings and between rooms within a building. These steps not only directly decrease the potential for spread of infectious disease, but also encourage employees to think about cleanliness (Francis, 1996). Hands can be disinfected and cleaned with commercially available hexachlorophene-containing soap. No-one should be allowed to enter a farm wearing shoes, and they should change to boots. This is very important, especially for disease inspectors who could carry pathogens from farm to farm. However, to date, this is not standard practice on many farms.

Disinfectant baths for boots, as well as car and lorry tyres are used on all large farms. Unfortunately, most of the baths are not long enough for large lorry tyres. The disinfectant should be chosen carefully because the presence of mud is unavoidable.

VII – Transplantation of new species

The advantages of transplants and transfers in aquaculture mostly concern fish and are foremost for commercial gain. Provision of consumer choice in the human diet is important and other advantages are related to sport fisheries and ornamental use. The disadvantages are disease threats to other aquaculture enterprises from the introduction of either new disease agents or new strains of existing agents and the potential of transplanted or transfer of species that cause damage to the environment or native habitats through escapees. Such damage includes habitat destruction, predation and competition with native species, exotic disease and pest introductions and, especially in the case of transfers, possible loss of fitness in native strains due to interbreeding or competition (Munro, 1988). One example is the irradiated sterile striped bass hybrids imported from Israel for trial purposes that were put in cages in Bozdoğan dammed lake. The results have shown that they adapted perfectly and the company concerned wants to improve this business. However, research carried out by the Ege University Fisheries Faculty has shown that the striped bass are not sterile, larvae have been obtained and the nature of the striped bass is too aggressive. This may cause the unwanted conditions already mentioned, as outlined by Munro (1988). Additionally, such fish may harbour new viruses, bacteria or parasites.

VIII – Disinfection of eggs

Eggs can act as an important vehicle for transmission of diseases from parent to offspring and between hatcheries because opportunistic pathogens may be present in the epiflora of fish eggs. The surface disinfection of eggs reduces the probability of pathogen development (Planas and Cunha, 1999). Disinfection of eyed rainbow trout eggs is important to reduce egg and fry mortalities due to RTFS caused by *Flavobacterium psychrophilum* (Rangdale *et al.*, 1997). Eggs can be disinfected by polyvinyl pyrolidone iodine (PVP-iodine) (Betadine, Batticone, Vanodine) or hydrogen peroxide. Both can be used as a bath treatment at a concentration of 100 ppm for 10 minutes. However, if iodine is used it should be neutralised with sodium thiosulphate (Bruno, 1995). For the prevention of RTFS, disinfection of eggs with a bath of 100 ppm hydrogen peroxide for 10 minutes is very effective.

For eyed sea bass and sea bream eggs, 50 ppm PVP-iodine for 10 minutes is effective. It can

also be used at 25 ppm for several hours during fertilisation (Le Breton, 1996). A 5-10 min treatment of 400-800 ppm glutaraldehyde has been used successfully for surface disinfection of cold water marine fish eggs (e.g. plaice, halibut, and cod). Egg disinfection is not used for trout eggs in Turkey. However, PVP-iodine has rare use for sea bass and sea bream eggs.

IX – Prevention of fish movements

It is necessary to minimise transfer of diseased or disease-suspected fish from farm to farm. Many of the potential diseases are spread by fish and eyed egg movements. ERM, pasteurellosis, lactococcosis, *Lernanthropus kroyeri* beginning on one farm or in an area has spread quickly all over the country because of the absence of regulations and effective quarantine measures.

Minimising the transport of dangerous pathogens can be performed only by the use of applicable regulations. EC regulations can be helpful for the preparation of national regulations. However, the reality is that the regulations which have to be established should be modified for each individual country. Nevertheless, before establishing regulations, fish disease maps/geographical information systems should be prepared in order to establish approved and unapproved zones.

If the purchase of fish is unavoidable they should be kept in quarantine for at least two weeks, separated from the rest of the farm stock, using separate equipment and following strict quarantine measures (Bruno, 1995) There can be serious problems with fish purchased from another farm and sea bass/sea bream broodstock caught directly from the marine environment. The fish enter a farm without any health control and farmers put them in separate tanks or ponds but without following any quarantine rules. The farmers need to know that quarantine and restriction of fish movement helps to protect farm populations from disease spread. However, it is not easy to persuade either the farmers or the authorities to consider this approach seriously enough.

The quarantine rules vital for aquatic diseases and the principles of quarantine are outlined below (Rosenthal, 1988):

1. Aquatic organisms which have to be placed in quarantine are by definition a potential health risk. The aim of quarantine is to establish that they are either free of prescribed pathogens and pests or, if they are not, it may be acceptable to use progeny from them which are proven pathogen and pest free.
2. In case of exotic species, imports must in most cases be held in quarantine for life and be subjected to repeated tests to establish their pathogen free status. If they are pathogen free only the F1 generation may be released.
3. Introductions, whether as gametes or fertilised eggs for fish, or as other stages for molluscs, crustaceans or marine plants, should be disinfected upon arrival at the quarantine unit.
4. Acclimation of introduced species to the environmental conditions of the quarantine unit should be undertaken in a manner which prevents contact between transport and the final holding medium.
5. The effluent waters from the quarantine unit should either be sterilised or treated by a disinfection technique sufficient to kill all prescribed pathogens and pests. The effectiveness of sterilisation/disinfection techniques should be monitored by the regulatory authority. Intake waters should be sterilised if their origin is from surface waters.
6. The quality of water used in quarantine should be monitored at regular intervals to ensure that any mortality in the quarantine population is not due to environmental conditions rather than disease agents.

7. Disposal of wastes and dead organisms, and of materials which have been in contact with them, must be conducted by an approved manner.
8. If more than one stock or species are kept in the quarantine unit, each must be kept in self-contained compartments in such a way that does not cause transmission of pathogens between different stocks.
9. When recirculation systems are employed, both assessment and control of water quality must be carried out.
10. No equipment should enter or leave the quarantine unit without disinfection.
11. Personnel operating the quarantine unit should enter and leave through a disinfection station, which should be regularly serviced to guarantee continued effectiveness.
12. Personnel operating the quarantine unit should not visit other aquaculture establishment on the same day.
13. The design of a quarantine unit should minimise any risk that operator error could cause any escape of animals.

X – Facility design

Recent design techniques for aquaculture facilities have resulted in improvements to aquatic animal health. Factors such as effective cleaning, less stressful handling and demand feeders have been used to produce healthier animals. These newer facilities, while more expensive, are able to rear animals more efficiently with lower losses due to diseases (Ahne and Winton, 1988). However, choosing sites for aquaculture can be problematic. There are many trout farms that undergo fluctuations in water temperature and changing water quality caused by, for instance, flooding, which cause stress and where disease outbreaks are observed frequently.

XI – Nutrition

Improved diet formulations have resulted in more rapid growth and better conversion ratios, as well as less disease outbreaks for fish. For instance, between 0 to 33 mg/kg of vitamin C was found in commercial pelleted fish feed produced by four different feed factories (Çağırhan and Firat, 1996).

XII – Vaccination

Immunisation is the most effective method for controlling endemic diseases. There are several effective commercial vaccines available against ERM, furunculosis, lactococcosis, streptococcosis, vibriosis, pasteurellosis and IPN. However, the cost of vaccines for Turkish farmers is high compared to the market price of fish. Another problem is obtaining protection until the fish are sold. Injection vaccination provides good protection in sea bass but, needless to say, it is very difficult to vaccinate billions of fish. Oral vaccines are used as a booster but they add extra cost. Primary vaccination can be undertaken when rainbow trout reach 1 g (preferably 3-5 g) and the same rules are applied for trout, sea bass and sea bream in Turkey. However, research on sea bass has shown that they can be vaccinated and protected against pasteurellosis when they reach 50 mg (Magariños *et al.*, 1999). For sea bass, a weight of 0.2 g is enough for vaccination but there is a gap before primary vaccination can be carried out.

There are no commercial vaccines against parasites (e.g. *Caligus* spp., *Meinertia oestroides*, or *Ichthyophthirius multifiliis*).

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