

Field Studies on Gas Bubble Disease Affecting Red Sea Cultured Asian Seabass, *Lates calcarifer*, *Oreochromis spilurus* and Hamour *Epinephelus coioides* in Jeddah, Saudi Arabia with Special References to Control

¹Hussien A.M. Osman, ²Ahmed M.E. El-Refaey, ³Abdul Rahman Q. Al-Zahrani and ³Mohamed S. Hazzaa

¹Department of Hydrobiology, Veterinary Research Division, National Research Center, Dokki, Egypt

²Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abou Hammad, Sharkia, Egypt

³Fisheries Research Center, Jeddah, Saudi Arabia

Abstract: The present study was carried out in Fisheries Research Center, Jeddah, Saudi Arabia on a total number of 794 fish, the study aimed to investigate all circumstances and causes of gas bubble disease in certain Red Sea cultured fish species; 533 Asian seabass *Lates calcarifer* 235 *Oreochromis spilurus* and 26 Hamour, *Epinephelus coioides*. The total prevalence of disease was 25.55%, different prevalence and susceptibility for the disease. *O. spilurus* was the highest prevalence 31.91% followed by *L. calcarifer* 25.52% then Hamour, *E. coioides* 19.23%. Asian Sea bass and *O. spilurus*, affected fish was less than 5 kg body weight on contrast affected Hamour was more than 5 kg. The most characteristic clinical signs were large and small external gas filled bubbles easily visible to the naked eye. The bubbles were located and distributed on the external body surface especially on the fin margins and body skin (emphysema in dermis), microscopically there was large gas emboli in the blood vessels of gills. It was noticed that all water parameters; approximately normal except gas saturation, temperature and salinity of water during the problem. Easy and simple procedures were applied to control the disease. The most pronounced histopathological alterations were odema, hyperplasia and gas emboli in gills, vacular degeneration of hepatocytes in liver, hyalinization and odema of some musculature bundles with sloughing of some intestinal villi.

Key words: Saudi Arabia • Gas Bubble Disease (GBD) • Red Sea Cultured Fish • *Oreochromis spilurus* • Asian Sea Bass • Hamour • *Epinephelus coioides*

INTRODUCTION

One of the most important of all diseases of cultured fish is the condition generally known as gas-bubble disease. First described in aquarium fishes in 1898 by Gorham. Gas bubble disease refers to the development of gases in a fish's bloodstream. This can occur when its aquarium or pond water is supersaturated with gases [1]. It has now been observed clinically in a wide variety of farmed species and under a number of different circumstances [2-5].

Fishes are cold-blooded creatures (poikilothermic animals), meaning their body temperature depends on the temperature of their environment. The water they live in and their bloodstreams can become supersaturated with

gases when there is a sudden rise in water temperature or a sudden rise in pressure [6]. When the cold water in the aquarium is suddenly heated, it can release and trap gases within the water causing gas bubble disease in the aquarium fish. Similarly, pond or tank water can become supersaturated with gases when they are filled with well water through a submerged hose. These gases can also lead to gas bubble disease [7].

Gas bubble disease is caused by super saturation of water with dissolved gases. It is more common in coldwater aquaculture, in which cold, inflowing water, already saturated with gas, may be heated without adequate time or aeration for volatilization of excess gas [8]. It also commonly results when water from deep wells, often high in nitrogen gas and carbon dioxide, is brought

into an aquaculture facility without proper aeration. Gas bubble disease is still incompletely understood, despite its significance in many types of intensive fish culture. It is in fish-farming systems, however, that the greatest potential for sudden serious outbreaks arises [9]. As production levels have increased with the advent of water recirculation and concomitant use of air and oxygen injection, it has been shown that even injection of oxygen alone can induce gas-bubble disease due to oxygen super saturation [1,10].

So the present study was aimed to investigate all circumstances, causes and trials for control of gas bubble disease in certain Red Sea cultured fish species; Asian sea bass *Lates calcarifer*, *Oreochromis spilurus* and Hamour, *Epinephelus coioides* in Fisheries Research Center (FRC) Jeddah, Saudi Arabia.

MATERIALS AND METHODS

Fishes: The present study was carried out on a total number 794 Red Sea cultured marine fishes, (533 Asian seabass *Lates calcarifer* 235 *Oreochromis spilurus* and 26 Hamour, *Epinephelus coioides*). January, 2013 in Fisheries Research Center Jeddah, Saudi Arabia. Large numbers of fish was suddenly died without clear clinical signs for some Red Sea cultured species; *O. spilurus*, Asian sea bass *L. calcarifer* and Hamour *E. coioides*, all investigated fish were sorted and divided according to species and weight into less or more than 5 kg for each species.

Clinical Signs and Postmortem: Clinical examination of scarified or freshly dead fishes of the investigated three species was adopted using the methods described by Lucky [11] for determining any lesions or abnormalities on the external body surface, also determining any postmortem lesions by examining gills and all the internal organs including liver, spleen, kidney, intestine, testis, ovaries and gas bladder.

Determination of Water Parameters: Water temperature and dissolved oxygen of water (DO) were recorded in all ponds in the farm two times daily; 6 AM and 10 PM. While the rest of parameters were measured once daily; saturation of water was measured using Senso Direct Oxi 200, Germany and recorded daily morning. Ammonia, nitrite and nitrate were measured in the water samples using (Hach model DR/2000) according the operator manual. Water temperature and dissolved oxygen, salinity and pH were measured using (JENWAY 9200).

Histopathological Studies: Autopsy samples were taken from the skin, fins and gills of naturally infected investigated fish fixed in 10% formal saline for twenty four hours. Washing was done in tap water then serial dilutions of alcohol (ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slide microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin and examination was carried out using light electric microscope [12].

RESULTS

Clinical Signs: Fish deaths occurred suddenly with large and small external gas filled bubbles easily visible to the naked eye. The bubbles were located and distributed on the external body surface in the fin margins and body skin (emphysema in dermis), the bubbles may be so large that small fish may lose their balance and float uncontrollably in the water due to the large amount of gas contained within the bubbles. Eyes were bulged (exophthalmia) contained bubbles and fins be damaged quite severely (Fig. 2), respiratory distress and opened mouth (plate 1, A&B).

Postmortem Lesions: Signs of asphyxia, open mouth and Gas emboli in blood vessels of virtually any organ, including skin, gills, eyes, viscera and peritoneal cavity. Gills were greatly congested and damaged and may bleed all internal organs; liver, spleen, ovaries and intestine were greatly pale, internal gaseous accumulation in the gas bladder with hyperinflation (plate 1 A &B). The clinical signs were characteristic and diagnostic; fish may die suddenly without apparent cause. The presence of gas emboli is characteristic. Bubbles may appear in fins margins or in gills blood capillaries. Postmortem findings include internal gaseous accumulation in the swim bladder and visceral peritoneum.

Prevalence, Species and Weight of Fish: The present study was carried out on the total number 794 Red Sea cultured marine fishes, (533 Asian seabass *Lates calcarifer*, 235 *Oreochromis spilurus* and 26 Hamour, *Epinephelus coioides*). Present study displayed that the total prevalence of gas bubble disease in investigated fishes was 25.55%; Asian Seabass (*L. calcarifer*), Hamour *E. coioides* and *O. spilurus* showed different prevalence

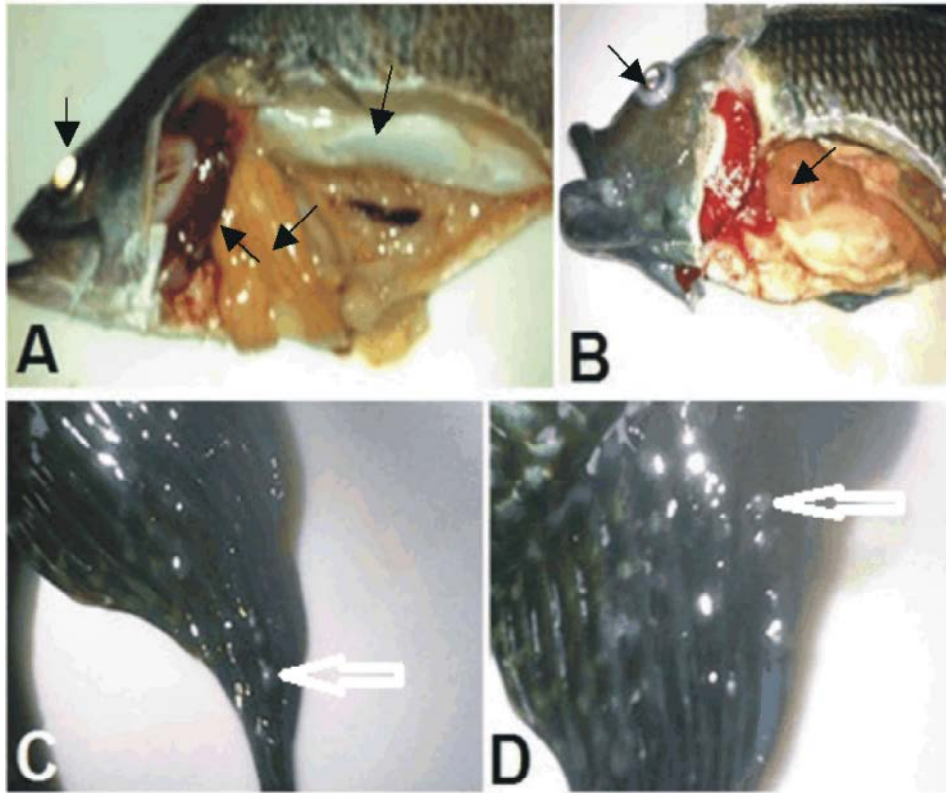


Plate 1: (A) & (B) Open mouth, exophthalmia, severe congestion of gills with bleedings accompanied with severe paleness of all internal organs liver, spleen with distended gas bladder of Asian sea bass and *Oreochromis spilurus* (arrows) (C) & (D) Gas bubbles on the skin at the margins of fins of *O. spilurus* (arrows)



Fig. 2: Showing destructed eyes with gas bubbles of Hamour, *Epinephelus coioides* (arrow).

Table 1: Showing numbers of fish species affected with GBD in relation to body weight and prevalence

species	No of fish	Weight	Dead fish No	Survival fish	Prevalence %
<i>Lates calcarifer</i>	533	<5 kg	136	397	25.52
<i>Epinephelus coioides</i>	26	>5 kg	5 21	19.23	
<i>Oreochromis spilurus</i>	235	<5kg	75 160	31.91	
Total	794	-----	216	578	25.55

Table 2: Water parameters for one week during the prevalence of disease

Parameter								
day	Gas sat. mg/l	Water temp. °C	Salinity (g/l)	Ammonia (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	DO. (mg/l)	pH
D1	----	35.6	42	0.05	5.40	0.07	5.0	8.03
D2	2.3	38.5	39	0.06	7.22	0.08	5.5	8.05
D3	6.1	35.3	37	0.07	6.23	0.08	5.0	8.10
D4	8.2	33.3	37	0.09	4.45	0.09	4.3	8.12
D5	11.2	24.5	35	0.07	6.56	0.06	5.2	8.16
D6	6.3	29.5	38	0.05	5.12	0.05	4.5	8.01
D7	----	36.7	41	0.05	5.89	0.06	4.9	8.03

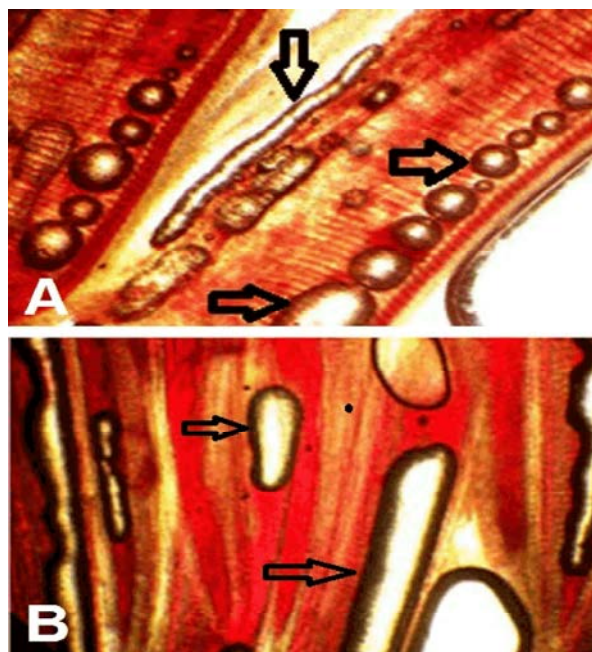


Plate 3: Showing (A&B) wet mount showing large and small gas bubbles emboli embedded in branchial blood vessels between primary gill filaments of Asian sea bass, *Lates calcarifer* (arrows).

and susceptibility for the disease. *O. spilurus* was the highest 31.91% followed by Asian sea bass (*L. calcarifer*), 25.52% then Hamour (*E. coioides*) 19.23%. Asian sea bass and *O. spilurus* infected fish was less than 5 kg body weight on contrast infected Hamour was more than 5 kg Table 1.

Water Parameters: Most water parameters were recorded daily for all ponds, it was noticed that all water parameters; Ammonia, Nitrate, Nitrite, dissolved oxygen and pH. Approximately normal except gas saturation, temperature and salinity of water during the week at the time of disease appearance GBD in investigated fishes Table 2.

Histopathological Studies: Wet mount and histopathological studies revealed that several large and small gas emboli embedded in the branchial vessels of gills (plate 3 A & B). Odema is showed in the secondary lamellae of the gills and occlusion of the large branchial vessels. Hyperplasia and swollen gill lamellae with congestion of blood capillaries (plate 4, A). Hyalinized musculature bundles with edema and scant infiltration of inflammatory cells (plate 4, B) liver suffered from necrosis, hydropic and vascular degeneration of hepatocytes (plate 4, C). Intestine suffered from sloughing and detachment of villi with infiltrations of inflammatory cells (plate 4, D).

Trials of Control: Application of simple and easy procedures; introducing sea water to the pond through the pipes directed water up and release down in plastic container for releasing of excesses gases with slow worming by atmospheric air for the introduced water (plate 5,A). It is noticed that there is more excesses gas bubbles comes from water around the container in the pond, Other treatment introducing sea water to the pond through perforated pipes for releasing injected gasses (plate 5 B&C).

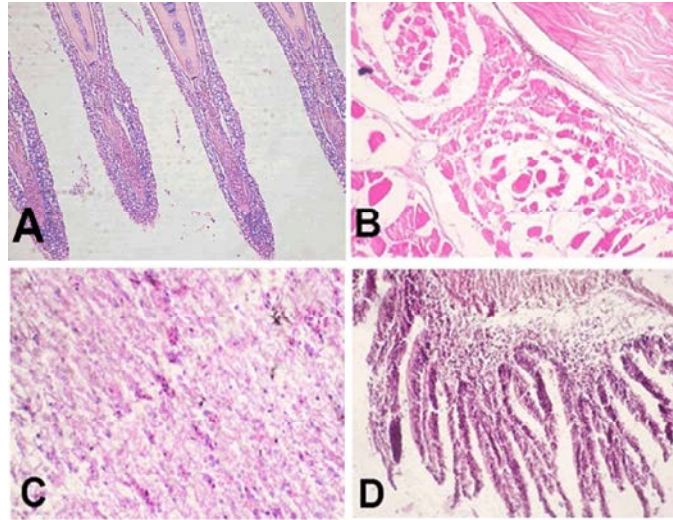


Plate 4: (A) distal hyperplasia and swollen gill lamellae with congestion of blood capillaries (B) hyalinized muscle bundles with minimal infiltration of inflammatory cells (C) liver suffered from necrosis, vascular degeneration of hepatocytes (D) intestine suffered from detachment and sloughing with odema of villi accompanied with infiltration of inflammatory cells)X 100, H&E).

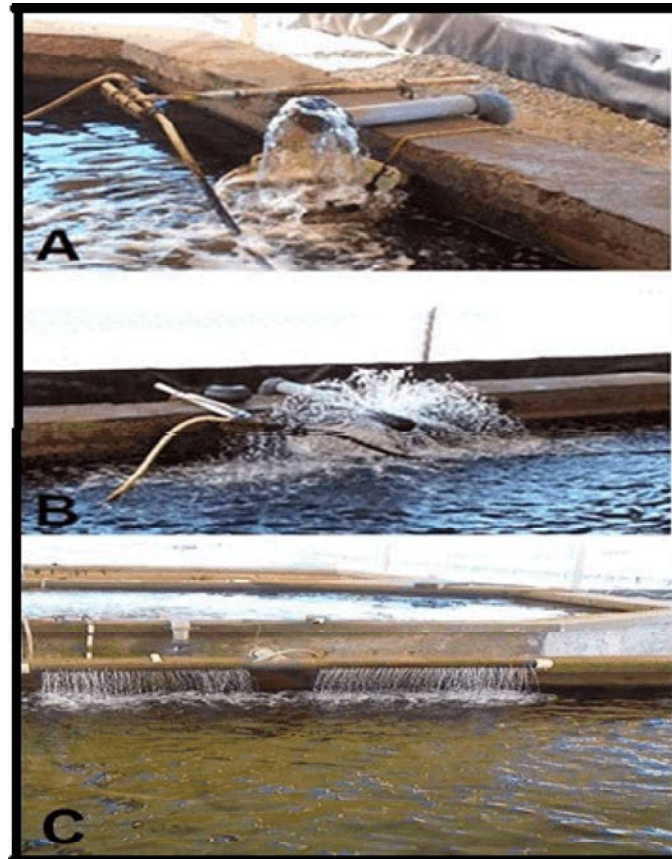


Plate 5: Showing (A) Introduction of sea water to the pond through the pipes directed water up and released down in plastic bucket for releasing of excesses gases (notice that the more excesses of gas bubbles around the bucket in the pond), (B& C) Other treatments introducing sea water to the pond through perforated pipes may be take different forms for releasing injected gas.

DISCUSSION

One of the most important of all diseases of cultured fish is the condition generally known as gas-bubble disease. First described in aquarium fishes in 1898 by Gorham, It has now been observed clinically in a wide variety of farmed species and under a number of different circumstances; outbreaks in wild fish are less common [3-5]. It was originally perceived as a problem principally for fish downstream of an entrained hydro-electricity system. It has led to wide interest in its pathophysiology.

Gas bubble disease is a surprisingly common condition in fish although it can affect many different species. It is particularly common in newly imported animals and as the bubbles, which are easily visible to the naked eye. True gas bubble disease is a non-infectious condition and it cannot be transmitted between fish, although complications can occur if the wounds become secondarily infected with bacteria.

The present study aimed to investigate most circumstances and causes of gas bubble disease in certain Red Sea cultured fish species; Asian sea bass *Lates calcarifer*, *Oreochromis spilurus* and Hamour, *Epinephelus coioides* in Fisheries Research Center, Jeddah, Saudi Arabia.

Regarding the clinical signs of gas bubble disease, the present study revealed that death may occur suddenly with large and small external gas filled bubbles easily visible to the naked eye. The bubbles are located and distributed on the external body surface at the fin margins and body skin, the bubbles may be so large that small fish may lose their balance and float uncontrollably in the water due to the large amount of gas contained within the bubbles. This is not usually a fatal condition, but eyes and fins be damaged quite severely, exophthalmia and open mouth such findings are similar with the results given by Noga [6].

Concerning the causes of gas bubble disease, the present study nearly coincide with that obtained by Roberts [1] who reported that, in a mixture of gases such as air, each gas dissolves in water according to its solubility. This in turn is controlled by the following: The total air pressure and the partial pressure of the gas in the air mixture in contact with water. In air the partial pressures of nitrogen and oxygen are 0.78 and 0.21, respectively with pumped water supplies, air and water may be drawn into the pump together so that the air is compressed by the pump, resulting in greater solution of oxygen and nitrogen gases [13, 10]. In aquaria and hatcheries the condition may be caused by leaks in pump or valve systems or by sudden temperature gradients. It

has been associated with altitude changes of fish being transported by air [14]. Dissolved salt content, as a general rule, oxygen and nitrogen and indeed most gases, are less soluble in water containing dissolved salts. The effect of increasing concentrations of sea-water salts decreases oxygen solubility. Also increasing temperature decreases the solubility of most gases in water.

Regarding the prevalence and body weight of fish affected with gas bubble disease, the present study showed that the three infected fish species; Asian Seabass (*L. calcarifer*), Hamour *E. coioides* and *O. spilurus* were showed different prevalence and susceptibility for the disease. *O. spilurus* was highest prevalence 31.91% followed by Asian seabass 25.52% then Hamour *E. coioides* 19.23%. Asian sea bass and *Oreochromis spilurus* affected fish were less than 5 kg body weight, on contrast infected Hamour was of body weight more than 5 kg. The results nearly agree with most of studies Espmark Asa Maria *et al* [8] and Noga [8] who reported that approximately 100% incidence of GBD in small fish, while the ratio decreased with large fish according also to species difference. In general, levels of about 110% saturation are considered dangerous for fish. However, this varies with the species and with the age of the fish. For example, even low levels (101 – 105%) affect salmonid sac fry, while adult salmonids often tolerate over 125% saturation [15], in addition Meyers *et al.* [16] who reported that the disease can affect any fish or invertebrate anywhere in supersaturated waters. Levels of gas super saturation causing pathological changes or mortality vary for different fish species and ages of fish.

Present study revealed that all water parameters salinity, Ammonia, Nitrate, Nitrite, dissolved oxygen and pH. approximately normal except gas saturation and temperature of water during week at the time of the disease appearance (GBD) in investigated fish; *Oreochromis spilurus*, Asian sea bass *Lates calcarifer* and Hamour *Epinephelus coioides* the nearly confirmed by Pauley and Nakatani [17] who reported that the stress and prognosis depend on the size and species of affected fish, the degree and duration of the super saturation and the water temperature and Hauck [14] who mentioned that in aquaria and hatcheries the condition may be caused by leaks in pump or valve systems or by sudden temperature gradients. It has been associated with altitude changes of fish being transported by air. Furthermore decreasing salinity may be additive cause for the disease where, the incidence of the disease increased in low salinity as dissolved salt content. As a general rule, oxygen and nitrogen and most gases, are less soluble in water containing more dissolved salts [1].

Regarding the control the present study revealed that, introducing sea water to the pond through the pipes directed water up and released down in plastic bucket for releasing of excesses dissolved gases, other treatment introducing sea water to the pond through perforated pipes for releasing injected gas. Results nearly confirmed by Meyers *et al.* [16] Noga [6] and Roberts [1].

Concerning the histopathological study, present study revealed that several large and small gas emboli embedded in the branchial vessels of gills, edema of the secondary lamellae of the gills and occlusion of the large branchial vessels. Large and small gas emboli embedded in large branchial blood vessels of gills, swollen gill lamellae with congestion of blood capillaries. Hyalinized muscle bundles with infiltration of inflammatory cells. Liver suffered from necrosis, vascular degeneration of hepatocytes. Intestine suffered from detachment of villi with infiltrations of inflammatory cells, the results nearly agree with Pauley and Nakatani [17] and Speare [18-20]. Edsall and Smith [10] who confirmed present description in a small number of chinook salmon mentioned that the major consistent histological feature observed is edema of the secondary lamellae of the gills with concomitant degeneration of the overlying respiratory epithelium showed that intravascular gas emboli led to occlusion of large branchial vessels and that this was a principal cause of acute mortality. Other lesions, including edema and bulbous disruption of the buccal and intestinal mucosa and vacuolar degeneration of the renal tubular epithelium, were described as part of a general syndrome which also included hepatic and muscular changes. [17] Who reported that histopathology of gas bubble disease has been reported to include edema of the gill secondary lamellae, with accompanying degeneration of the overlying epithelium. Other lesions include edema and embolic disruption of buccal and intestinal mucosa, as well as vacuolar degeneration of the renal tubular epithelium. Lesions may also occur in the liver and muscle. Tissue hemorrhage and brain damage have been postulated to cause death [21].

From the present study it was concluded that gas bubble disease is a non-infectious disease caused by environmental problems, the main reason for disease in the present study may be sudden lowering of water temperature which help in containing oxygen and other gases in water in addition that colder water can capture and maintain more gases in it in addition of low salinity. The disease infects Asian sea bass *Lates calcarifer*, *Oreochromis spilurus* and Hamour, *Epinephelus coioides* with more prevalence in younger fish. The disease often looks disastrous for the affected animal, in many cases the

bubbles resolve with few simple procedures for the affected fish. The most common adverse outcomes are loss of an eye, brain and bacterial or protozoal infections of the lesions. However, it is treatable and can be well easily managed in aquaculture systems. Gas bubble disease can be prevented by slowly heating up water when it is added to the pond. Do not submerge the hose when filling up a pond. Instead, spray water from above, as this will allow all the gases to harmlessly release into the air in addition of vigorous aeration to volatilize excess gas.

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REFERENCES

1. Roberts, R., 2012. Fish Pathology 2nd ed. Elsevier Health Sciences.
2. Alikunhi, K.H., V. Ramachandran and H. Chaudhuri, 1951. Mortality of carp fry under supersaturation of dissolved oxygen in water. Proc. Natl. Inst. Sci. India, 17: 261-264.
3. Harvey, H.H. and A.C. Cooper, 1962. Origin and treatment of supersaturation. Int. Pac. Salmon Fish. Comm., Prog. Rep., 9: 1-19.
4. Rucker, R.R., 1975. Mortalities of coho salmon, *Oncorhynchus kisutch*, in water with constant total gas pressure and different oxygen-nitrogen ratios. U.S. Fish. Wildl Serv. Fish. Bull., 73: 915-18.
5. Saeed, M.D. and S.A. Al-Thobaiti, 1997. Gas bubble disease in farmed fish in Saudi Arabia. Vet. Rec., 140: 682- 4.
6. Noga, E., 2010. Fish disease: diagnosis and treatment John Wiley and Sons.
7. Feng Jing-jie, L.I. Ran, Hui-xia YANG and L.I. Jia 2013. A laterally averaged two-dimensional simulation of unsteady supersaturated total dissolved gas in deep reservoir, 25(3): 396-403.
8. Espmark Åsa Maria, Kirsti Hjelde and Grete Baeverfjord, 2010. Development of gas bubble disease in juvenile Atlantic salmon exposed to water supersaturated with oxygen Volume 306, Issues 1-4, 15 August, Pages 198-204.

9. Lemarié Gilles, Camilla Diesen Hosfeld, Gilles Breuil and Sveinung Fivelstad 2011. Effects of hyperoxic water conditions under different total gas pressures in European sea bass (*Dicentrarchus labrax*) Aquaculture Volume 318, Issues 1-2, 27, pp: 191-198.
10. Edsall, D.A. and C.E. Smith, 1991. Oxygen induced gas bubble disease in rainbow trout, *Oncorhynchus mykiss*. (Walbaum). Aquacul. Fish. Manage., 22: 135-40.
11. Lucky, Z., 1977. Methods for the diagnosis of fish diseases, Amerind Publishing Co., P V T. Ltd., New Delhi, Bombay, India.
12. Bancroft, J.D. and A. Stevens, 1996. Theory and practice of histological techniques. Fourth edition, Churchill living stone, Edinburgh London, Melbourne. pp: 304-307.
13. Weitkamp, D.E. and M. Katz, 1980. A review of dissolved gas super saturation literature. Transactions of the American Fisheries Society, 109: 659-702.
14. Hauck, A.K., 1986. Gas-bubble disease due to helicopter transport in young pink salmon. Trans. Am. Fish. Soc., 115: 630-5.
15. Wood, J.W., 1974. Diseases of Pacific Salmon: their prevention and treatment, 2nd ed., State of Washington Department of fisheries Hatchery Division, pp: 82.
16. Meyers Theodore, Tamora Burton, Collete Bentz and Norman Starkey 2008. Common Diseases of Wild and Cultured Fishes in Alaska. Southfork Graphic Services 4th ed.
17. Pauley, G.B. and R.E. Nakatani, 1967. Histopathology of gas - bubble disease in salmon fingerlings. Journal of the Fisheries Research Board of Canada, 24: 867-870.
18. Speare, D.J., 1990. Histopathology and ultrastructure of ocular lesions associated with gas bubble disease in salmonids Journal of Comparative Pathology, 103(4): 421-432.
19. Speare, D.J., 1991. Endothelial lesions associated with gas bubble disease in fish Journal of Comparative Pathology, 104(3): 327-335.
20. Speare, D.J., 1998. Disorders associated with exposure to excess dissolved gases. In *Fish disease and disorders*, ed. J.F. Leatherland & P.T.K. Woo, pp: 207-24. London: CABI Press.
21. Ferguson, H.W., R.T. Kongtorp and Taksdal, 2005. An outbreak of disease resembling heart and skeletal muscle inflammation in Scottish farmed salmon, *Salmo salar* L., with observations on myocardial regeneration. J. Fish Dis., 28: 119-23.