Improved system to normalize the oxygen distribution in the tank water in aquaculture

Panangiotis Angelidis

Laboratory of Ichthyology, Veterinary Medicine School, Aristotle University of Thessaloniki, 541 24 Greece, E - mail: panangel@vet.auth.gr

Abstract. Oxygen control systems usually regulate the oxygen diffusion in the fish farm water through an electromagnetic valve. This valve alternates its «open/close» function mode according to the detected dissolved oxygen (DO) level in the water. During the «open» mode, oxygen diffusion take place and the DO can rise to high values (over saturation) until the desired oxygenated water is detected by the oxygen probe. In the improved system presented in this study the oxygen over saturation of the farm water is avoided by a twin timer which is activated by the oxygen meter transmitter. It is manually adjustable and starts operating automatically when the DO is low in the water. The «on» and «off» mode are alternated according to its manual adjustment. During the «off» time no oxygen is diffused and the batch of oxygen that was diffused during the «on» time is allowed to be distributed in the water.

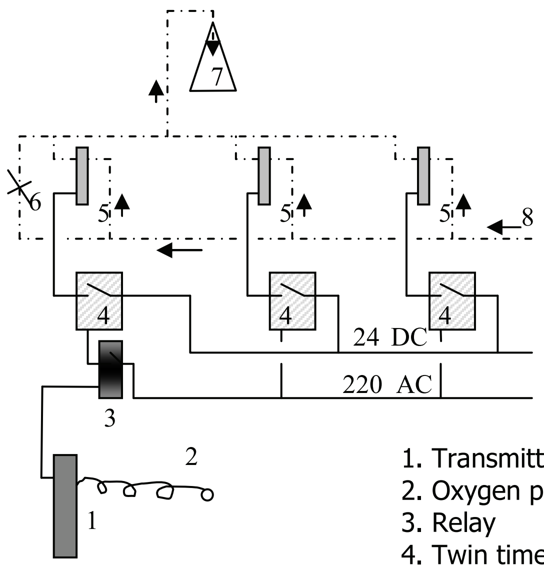
Key words: fish, oxygen, twin timer, oxygen device.

1 Introduction

Intensive fish farming systems, especially the recirculating aquaculture systems (RAS), operate in high stocking density of fish in the tanks. In these conditions the dissolved oxygen (DO) of the water can be depleted in a very short time and the fish undergo anoxia conditions that very shortly could stress the fish and lead to fish death. The enrichment of the farm water with oxygen by air diffusion is not efficient in the intensive fish farming systems. To reach high oxygen concentration by air diffusion in the water very high energy is needed, proportionally (Timmons and Ebeling 2007). It was reported that the standard aeration efficiency (SAE) is between 0.2-2 kg/kwh (Boyd and Watten 1989). In all fish farming systems which are operated in high fish stocking densities almost pure oxygen is diffused in the water. To get high oxygen dilution levels both water and oxygen are mixed in pressurized oxygen reactors usually at 2 - 8.6 bar (28-125 psig, Watten 1994). By these systems, it is easy to reach oxygenated water at concentration as high as 20 – 50 mg/l. To adjust the DO concentration online devises (oxygen meters) are used (Grecay and Stierhoff 2002). Hassell et al. (2009) developed a simple system to keep water oxygenated. These commonly used devices not only display the value of the oxygen

In: M. Salampasis, A. Matopoulos (eds.): Proceedings of the International Conference on Information and Communication Technologies
for Sustainable Agri-production and Environment (HAICTA 2011), Skiathos, 8-11 September, 2011.

concentration but can also control electromagnetic valves and adjust the amount of the oxygen that is entering in the water. When the oxygen concentration in the water fells below the programmed value, automatically oxygen is diffused in the water until its concentration reaches the desired one. Better results can be achieved when an oxygen control system is connected to each fish tank. In most farms the oxygen meter probe is located in the central effluent tank to avoid high equipment cost. The main disadvantage of this system is that oxygen is constantly diffused in the water mass until the concentration of the DO in the water which passes by the oxygen meter probe reaches the desired value. During this time, from the start of the oxygen diffusion until the detection of the desired value, oxygen is constantly diffused in the water resulting in much higher DO concentration levels in the fish tank water. These levels temporarily could be in the range of 20-30 mg/l. If these time intervals are prolonged, apart from the oxygen losses, bubble disease could affect the fish. Very often the fish are affected by a sub acute form of the disease that provokes low



1. Transmitter
2. Oxygen probe
3. Relay
4. Twin timer
5. Electromagnetic valve
6. Manual valve
7. Oxygen reactor
8. Oxygen stor tank

Continue line: electrical cables
Discontinue line: oxygen tubes

Fig. 1. Electrical connections and lay out.

mortality over long periods. The fish often suffer from gill necrosis (personal observation). To avoid high oxygen concentration, RAS equipment constructors regulate the oxygen pressure or the oxygen quantity diffused by means of a manually adjusted oxygen flow meter. Unfortunately, these interventions do not always satisfy the oxygen needs of the fish. The oxygen concentration in the water fluctuates from low to high levels depending mainly on fish appetite, fish biomass and mass of the water.

In the system designed in this experiment, the oxygen diffusion is electronically adjusted. When the oxygen meter produces the signal to diffuse oxygen in the water, this electronic signal does not control directly the electromagnetic oxygen valve but activates an electronic twin timer that starts to control the electromagnetic oxygen valve. This twin timer is adjustable and the time of the valve open/close (on/off) is adjusted manually to alternate its function according to the fish needs for oxygen. During the time interval the valve is closed, the oxygen has enough time to be diluted in the water and then the oxygenated water to be dispersed in the fish tank water. To enhance the safety of the system one or two additional twin timers could control one or two additional electromagnetic valves respectively without any connection to the oxygen meter transmitter. These additional twin timers/valves could give the normal oxygen required by the fish and the oxygen transmitter/twin timer can give only the excess of oxygen required, for example during the feed digestion by the fish. On the other hand, each adjusted twin timer/valve is able to give all the oxygen required by the fish and therefore the system reaches a very high level of safety and viability.

2 Material and Methods

2.1 Description of the electronic set up and its function

An electrical panel was constructed and it was supplied with 230 Volt (V) alternating electrical current (AC) at 50 Hz. The current after its entrance in the panel was conducted through an interrupter and an automatic fuse [2 Ambers (A)]. From the 230 V fuse, the current was addressed to the stabilized transformer [230 V-AC: 24 V direct current (DC)] and to other 230 V relays and timers of the panel. The stabilized transformer supplied 24 V DC to the 24 V relays and to the oxygen meter transmitter. Just after the transformer a 24 V fuse was interposed to secure the 24 V circuit. The 11 pin oxygen meter transmitter (OxyGuard - Denmark) was alimented from the 24V DC transformer and it was connected to the oxygen meter probe (OxyGuard - Denmark), to the control oxygen 24V DC relay, to the low and high level oxygen relays (24V DC) and to the oxygen value display (Power Electronic Control - Greece).

When the oxygen meter transmitter activated the control oxygen relay (according to the DO concentration value in the RAS water and to the pre set control value on the transmitter), this relay started to aliment with 230 V AC a twin timer (DHC,

Wenzhou China). On to this twin timer a 24 V DC electromagnetic valve normal closed (NC) was connected to diffuse oxygen in the oxygen reactor of the RAS (Fig. 1). The adjustment of the twin timer is performed manually into two set up. One concerns the desired time “on” and the other one the desired time “off” in seconds. This pre set time durations alternate the function of the electromagnetic valve and consequently the quantity of the oxygen diffused in the water.
In this experiment the pre set DO control value on the oxygen control device was 7.3 mg/l and the regulation on the twin timer was time «on» two seconds and time «off» eight seconds. This regulation on the twin timer set after preliminary observations.
The high level oxygen relay activates an optical, acoustic and electronic alarm system to inform the farmer when the oxygen concentration value passes over the pre set value on the transmitter.
The low level oxygen relay activates an optical, acoustic and electronic alarm system to inform the farmer when the oxygen concentration value is lower than the pre set one on the transmitter. In addition, it stops the alimentation of one 24V DC normal open (NO) electromagnetic valve. This valve is kept close when it is alimented with electricity and allows the oxygen flow only when no current aliments it. The oxygen that flow through the valve is not conducted in the oxygen reactor but it is conducted directly into the emergency oxygen diffusion tubes which are placed on the bottom of each fish tank. In case of emergency this valve opens and oxygen is directly diffused in the fish tank water. This NO electromagnetic valve opened not only when the oxygen meter detects low oxygen in the water but also when an electrical failure occurs in the farm. This electromagnetic valve can also be controlled by other relays, in serial connection, those are activated by level indicators of the tank water or pressure detectors in the pressurized pipes of the RAS. These set up are additional emergency back up.
Another two twin timers were alimented with 220 V AC. To these twin timers, two 24 V DC electromagnetic valves normal closed (NC) were connected respectively to diffuse oxygen in the oxygen reactor of the RAS. The set up of the twin timers is performed with two manual regulations. One concerns the desired time «on» and the other one the desired time «off» in seconds. This pre set time durations alternates the function of the electromagnetic valves and consequently the quantity of the oxygen diffused in the water. These two twin timers set up, in parallel with the oxygen meter transmitter twin timer, are an addition oxygen diffusion system. These two twin timers set up could be pre set to give the base oxygen demanded by the fish and the oxygen meter transmitter twin timer to provide the excess of oxygen demanded in extreme conditions. Nevertheless all the three twin timers, if they are set adequately, are able to supply the oxygen needed even if they operate alone. This system increases by 300% the safety level of the oxygen control installation.
A manual oxygen valve is recommended to be connected in parallel to the other electromagnetic valves to give the possibility to the farmer to supply oxygen in the oxygen reactor when the entire oxygen control panel is out of order. When the manual valve is open, the flow of the oxygen in the oxygen reactor is continued (no possible normalization). This manual valve is recommended to be a needle valve for fine adjustment of the oxygen flow.

Table 1. Mean individual weight of the fish in each fish tank

Fish tank No	n of fish weighted	Mean individual fish weight (g) $\pm$ SD	Biomass (kg)	Estimated n of fish/tank
1	30	255.2$\pm$ 42.6	1,790	7,013
2	30	267.7$\pm$ 52.4	1,512	5,649
3	30	288.8$\pm$ 38.2	1,548	5,360
4	30	166.2$\pm$ 32.9	735	4,422
5	30	174.4$\pm$ 24.3	875	5,018
6	30	134.5$\pm$ 27.4	680	5,056
7	30	124.7$\pm$ 25.4	570	4,572
8	30	83.3$\pm$ 22.0	512	6,149
9	30	83.5$\pm$ 16.2	338	4,048
	TOTAL		8,560	27,463

2.2 Description of the recirculating fish farm system

The experiment took place in a RAS eel farm in northern Greece. Eels of a total biomass of 8,560 kg farmed in a RAS of 9 circular fish tank, 5m diameter - 20m^3 each. All the RAS water [fish tank water (180m^3) and mechanical – biological filter tank water (about 70m^3)] recirculated twice per hour through the mechanical filter (Hydrotech, 60μ mesh), the biological trickling filter (150m^3 plastic network tubes of about 200m^2/m^3) and the oxygen reactor (3m^3). The recirculation of the water was assured by four centrifuge pumps. The water temperature was maintained during the experiment at 19$\pm$0.5^o C by a heat exchanger. The mean individual weight of the fish in each fish tank is shown in table No 1. Two days prior to the experiments the fish were starved.

2.3 Oxygen balance- sample collection

To compare the mode of the oxygen dilution into the fish tank water two experiments were carried out. In the first one the oxygen was diffused into the oxygen reactor by the system developed for this experiment and described above (twin timer). In the second experiment the oxygen was diffused into the oxygen reactor by an electromagnetic valve which was controlled by the oxygen meter transmitter (classical method). The electromagnetic valve stayed open for the whole period during which the oxygen meter detected low oxygen according to the pre set control value. In the twin timer system, once the oxygen meter transmitter produced a signal of oxygen lack a twin timer was activated in stated of the direct control of the electromagnetic valve. The electromagnetic valve started to close and open according to the manual regulation of the «on» and «off» duration of the twin timer (2 seconds «on» and 8 seconds «off»). To secure a more advantageous oxygen diffusion, two additional twin timers were added. They can operate in parallel and independent of the twin timer that was connected to the oxygen meter transmitter. To be able to compare the mode of oxygen dilution of the system that was developed for this experiment to the classic system, the two additional timers were switched off during the two experiments. Both experiments were performed on the same day, in the same fish farm, in the same fish tank – fish which were starved for two days before the experiment.

The control value of the oxygen concentration was pre set for both experiments at 7.3 mg/l. When the oxygen meter detected oxygen concentrations lower than 7.3 mg/l it activated through its transmitter the electromagnetic valve in the classical system or the twin timer and then the electromagnetic valve in the new system. When the oxygen concentration reached values higher than approximately 8.0 mg/l, the oxygen meter transmitter stopped activating the electromagnetic valve or the twin timer respectively and consequently the diffusion of the oxygen in the oxygen reactor.

Prior to the beginning of the experiments the oxygen concentration in the fish tank water was 6.7 mg/l. During the experiments the DO concentration values were recorded. They were recorded in the central effluent tank (mechanical filter tank) of the nine fish tanks by the oxygen meter and by a hand oxygen meter (OxyGard hand meter gamma) in the water of each fish tank. The oxygen values were recorded every 15 minutes for three hours in each experiment.

In all nine fish tanks the mortality was noted during the seven days after the end of the experiment. To investigate the appearance of air bubbles in the gill vessels, first and third day after the end of the experiment three fish from of each tank were sampled and their gills observed under an optical microscope.

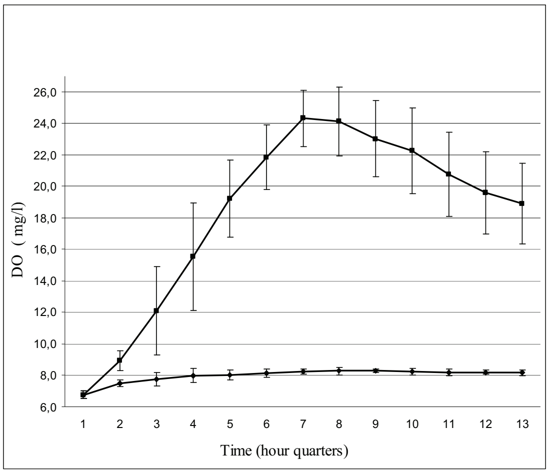


Fig. 2. Mean DO values (± SD) in the nine fish tanks. ■Classical oxygen device,
● Oxygen device with the twin timer

2.4 Statistic analysis

The mean values and the standard deviations of the means of the DO values were calculated in the nine fish tanks on each time on which the DO measurements took place. The comparison of the mean values was tested for significant difference by the t test for $P \leq 0.01$.

3 Results

A slow increase of the DO values was observed in the first experiment where the twin timer system was applied. The start value of the DO was 6.7 mg/l in the central effluent tank (mechanical filter tank) and its mean value in the nine fish tanks was

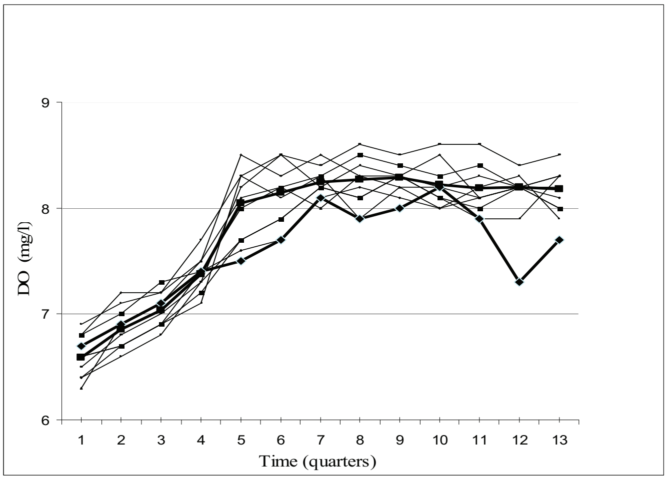


Fig. 3.
DO values in fish tanks with twin relay
Thick line and ■ indicate the mean DO values of the nine fish tanks
Thick line and ♦ indicates the DO values in the central effluent tank
Fine lines indicate the DO values in each fish tank

6.6 mg/l. During the first half hour the mean DO value reached 7.0 mg/l and finally 8 mg/l by the end of the first hour. During the second and third hour the DO remained stable and about 8.3 mg/l in the fish tanks but also in the central effluent tank. (Fig. 2

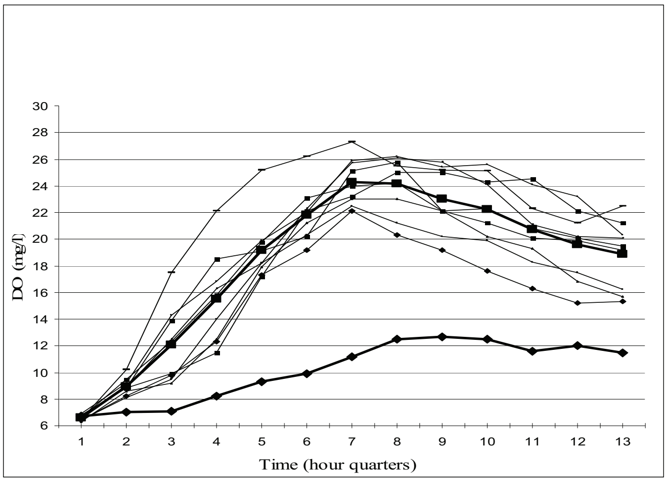


Fig. 4.
DO in fish tank with the classical oxygen device
Thick line and ■ indicate the mean DO values of the nine fish tanks
Thick line and ♦ indicates the DO values in the central effluent tank
Fine lines indicate the DO values in each fish tank

& 3).
In contrast, in the second experiment the DO values started to rise very quickly in the fish tank when the classical oxygen meter detected the low DO value (6.7 mg/l) in the central effluent tank (mechanical filter tank) and started the electromagnetic valve to diffuse oxygen in the nine fish tanks. During the first hour the mean DO value reached 15.5 mg/l and finally 19.2 mg/l after the end of the first hour and attained its peak value (24.3 mg/l) after two hours, approximately. It remained high (24.1 – 18.9 mg/l) until the end of the second experiment (three hours) (Fig. 2&4).

The DO values in the central effluent tank water (mechanical filter tank) augmented slower and they get its maximum (12.7 mg/l), two hours after the onset of the experiment.

The mean DO values are significantly higher ($P< 0.01$) in the nine fish tanks when the diffused oxygen controlled by the classic oxygen control system compared to the system that used the twin timer on each measure time (Fig. 2).
No mortality among the fish was noted during the experiment and during seven days after its end. No air bubbles were observed in the sampled gill tissues on the first and third day after the end of the experiment.

4 Discussion

Dissolved oxygen in the water is crucial for success of the aquaculture, especially in the RAS, where the fish are farmed in high number/biomass of fish per volume unit of water (stocking density). In these systems oxygen has to assure not only the viability of the fish but their welfare and their productivity (Santos et al. 2010; Richards 2009; Shultz et al. 2011; Nieuwegiessen et al. 2008). Brandt et al. (2009) reported that growth was related to DO as well as to the interaction between temperature and DO in striped bass farming. Health disorders, affects the welfare of the fish and high feed convertion rate (FCR) can be recorded by low or high dissolved oxygen levels (Santos et al. 2010). Wu (2009) reported that hypoxia can affect courtship behaviors, mate choice, and reproductive efforts in fish. Thorarensen et al. (2010) demonstrated that the Atlantic halibut (*Hippoglossus hippoglossus* L.) reduced its growth at low oxygen levels but the FCR was not influenced by the low (57% air saturation) or high (150% air saturation) oxygen level. The 150% air saturation applied by Thorarensen et al. (2010) is not so high compared to the 24.3 mg/l used in our experiment at $19\pm0.5^{o}C$ in fresh water (260% oxygen saturation, oxygen saturation of the fresh water at $20^{o}C$ is 9.1mg/l). For each temperature and salinity the saturation value of the farm water with dissolved oxygen is considered as optimal DO value (Lushchak and Bagnyukova 2006). As the DO values of the farm water deviate from the saturation value the fish suffer stress, change their metabolism mode and became more vulnerable to pathogenic factors (Weyts et al. 1999). In case of low DO in the water the fish accelerate their respiratory function, suffer stressful conditions, secret cortisol, their immune system becomes weaker, the fish becomes vulnerable to the pathogens and finally they show anorexia, anoxia and they can quickly die (Bowden 2008; Fast et al. 2008; Peter 2011). In the case of high DO levels the fish apart from stress can also be affected by the bubble disease. The bubble disease often provokes respiratory problems, gill necrosis, and death. Espmark et al. (2010) observed bubble disease on Atlantic salmon after 14 days exposure to 160-220% oxygen saturation. These salmons displayed more panic episodes compared to the controls, indicating physiological stress and possibly pain.
In our experiment, we observed high rise of the DO values in the farm water when the oxygen diffusion was controlled by the classical system. Once the classical oxygen control device detected the pre set low control DO value in the effluent tank,

automatically the electromagnetic valve was opened and allowed the oxygen diffusion into the oxygen reactor. The oxygenated water excited the oxygen reactor and started to be distributed in the nine fish tanks. The total water mass in these nine fish tanks was about 180tn (180m^3). During the diffusion of the oxygenated water in the fish tanks the electromagnetic valve remained open and it continued to diffuse oxygen in the fish tanks water through the oxygen reactor. The electromagnetic valve was closed and stopped the additional diffusion of oxygen in the fish tanks, only when the classical control oxygen device detected DO value equal or higher of the pre set control value in the central effluent tank. Because of the important mass of the farm water (about 200 tones, fish tank plus mechanical filter tank) this time was long enough. During this time, the electromagnetic valve continued to diffuse oxygen in the farm water. The consequence of this continues and long lasting diffusion of oxygen was the high level of the DO values in the fish tank, (a mean value rising to 24.3mg/l). The DO values were not the same in the nine fish tank because these tanks contained unequal numbers of fish, eels with different body size and different biomass. Additionally, the water inlet in each tank was manually adjusted. In this experiment the period in which the oxygen allowed to be diffused in the water lasted about four hour quarters. As it is shown in Fig. 4 the pre set control DO value (7.3mg/l) in the oxygen device gained about four hour quarters after the start of the oxygen diffusion. In the same figure it is shown that the DO values continued to rise in the farm water until the 8th hour quarter despite that the oxygen diffusion was stopped on the 4th hour quarter. The same evolution of the DO values of the fact in a less magnification was observed in the central effluent tank water. This phenomenon showed that the amount of oxygen that was diffused in the fish tank water during the «on» mode of the oxygen device was too much. It was large enough to increase the mean DO value up to 24.3 mg/l and continued to rise and remain in high levels (10 – 12.7 – 11.5mg/l) in the central effluent tank water from the 6th until the 12th hour quarter (Fig. 4). The fish under high DO amounts could suffer damages in the respiratory tissue by the bubble formation in the gill blood vessels. Usually in this system the duration of the high DO amount in the water does not last too long. This time period was about 3 hours in our experiment. No eel mortality or specific lesions in the gills were observed after this high DO amount in the farm water. Our previous unpublished data suggested that the duration of the application of these high DO values was critical. When DO values of about 25 mg/l were applied for about 5 – 7 days on the eels of another recirculating water farm, the fish developed the bubble disease in the gills. In the eels of this farm necrosis of the gills tissue was observed and about 4-5% of the fish died within two months. We have also previously observed that shorter high DO exposure time could lead to glass eels developing bubble disease. Oxygen bubbles were observed in the surrounding soft tissue of the eyes of the glass eels. These glass eels swim in vertical position. Their head was just under the water surface and some fish were forced by the oxygen bubbles to keep the end of their head outside the water. They did not take any food and died in a few hours or days. Because of the short exposure time to the high DO levels, mortality was not observed among the eels in our experiments but too much oxygen escaped in the atmosphere. It is considered certain that the fish organisms and their health were stressed by these high oxygen levels. Stressed fish usually show higher vulnerability

to pathogenic agents and to other environmental changes (Angelidis et al., 1987; Ellis et al. 2002).
The improved system that was operated with the twin timers showed a very soft oxygen distribution in the whole mass of the water. Whilst the time «on» of the oxygen device the adjustable alternated function of the twin timer proved to be very efficient for the normal oxygen distribution in the entire farm water. The manual regulation of the «on» (two seconds) and «off» (eight seconds) time on the twin timer gives the possibility to the farmer to adjust very easily the diffused oxygen amount in the farm water. The oxygen is allowed to be diffused in the oxygen reactor water in small batches. The «off» time intervals between the «on» modes give the possibility to oxygen to be normally diffused in the oxygen reactor water and then to be normally dispersed in the whole water mass of all the fish tanks. By this system, this time delay and the small batches of oxygen diffusion allows the oxygenated water to fill the fish tank and then to return into the central effluent tank. In this central tank the oxygen level will be detected by the oxygen probe and if it was raised to the control pre set value, it will switch the oxygen device and the twin timer off and then the electromagnetic valve will stop the oxygen diffusion in the farm water. The evolution of the DO values is very similar in the fish tanks but also in the central effluent tank (Fig. 3). By this system the DO in the water remains always very close to the pre set control value, the fish do not suffer stressful conditions and no oxygen is lost in the air. The manipulation of the system is manual and the farmer can adjust the DO amounts in the farm water very easily. For example, if he wishes to double the diffused amount of oxygen in the farm water he has only to double the «on» time or to shorten the «off» time to its half on the twin timer. To set the optimal regulation on the twin timer preliminary experiments has do be done by the farmer.

References

1. Angelidis, P., Baudin-Laurencin, F., Youinou, P. (1987) Strees in rainbow trout *Salmo gairdneri*: effects upon phagocyte chemiluminescence, circulating leucocytes and susceptibility to *Aeromonas salmonicida*. Journal of Fish Biology 31 (sup. A), p.113-122.
2. Brandt, S. B., Gerken, M., Hartman, K. J., Demers, E. (2009) Effects of hypoxia on food consumption and growth of juvenile striped bass (*Morone saxatilis*). Journal of Experimental Marine Biology and Ecology, 381, p.143–149.
3. Bowden, T. J. (2008) Modulation of the immune system of fish by their environment. Fish & Shellfish Immunology 25, p.373-383.
4. Boyd, C.E. and Watten, B.J. (1989). Aeration systems in aquaculture. CRC Crit. Rev. Aq. Sci. 1, 425-472.
5. Ellis, T., North, B., Scott, A.P., Bromage, N.R., Porter, M., Gadd D. (2002) The relationships between stocking density and welfare in farmed rainbow trout. J. Fish Biol. 61,p. 493-531.

6. Espmark, Å. M., Hjelde, K., Baeverfjord, G. (2010) Development of gas bubble disease in juvenile Atlantic salmon exposed to water supersaturated with oxygen Aquaculture, 306 (1-4), p. 198-204.
7. Fast, M. D., Hosoya, S., Johnson, S. C., Afonso, L.O.B. (2008) Cortisol response and immune-related effects of Atlantic salmon (*Salmo salar* Linnaeus) subjected to short- and long-term stress. Fish & Shellfish Immunology 24, p. 194-204.
8. Grecay, P. A. and Stierhoff, K. L. (2002) A device for simultaneously controlling multiple treatment levels of dissolved oxygen in laboratory experiments. Journal of Experimental Marine Biology and Ecology, 280(1-2), p. 53-62.
9. Hassell, K.L., Coutin, P.C., Nugegoda, D. (2009) A novel approach to controlling dissolved oxygen levels in laboratory experiments. Journal of Experimental Marine Biology and Ecology, 371, p. 147–154.
10. Lushchak, V.I. and Bagnyukova, T.V. (2006) Effects of different environmental oxygen levels on free radical processes in fish. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 144(3), p.283-289.
11. Nieuwegiessen van de, P. G., Boerlage, A. S., Verreth ,J. A.J., Schrama, J. W. (2008) Assessing the effects of a chronic stressor, stocking density, on welfare indicators of juvenile African catfish, *Clarias gariepinus* Burchell. Applied Animal Behaviour Science, 115(3-4), p. 233-243.
12. Peter, M.C. S. (2011) The role of thyroid hormones in stress response of fish. General and Comparative Endocrinology, 172(2), p. 198-210
13. Richards, J. G. (2009) Metabolic and Molecular Responses of Fish to Hypoxia. Fish Physiology, 27, p. 443-485.
14. Santos, G.A., Schram, J.W., Mamauag, R.E.P, Rombout, J.H.W.M., Verreth, J.A.J. (2010) Chronic stress impairs performance, energy metabolism and welfare indicators in European sea bass (*Dicentrarchus labrax*): The combined effects of fish crowding and water quality deterioration. Aquaculture, 299,(1-4), p. 73-80.
15. Shultz, A. D., Murchie, K.J., Griffith, C., Cooke, S.J., Danylchuk, A.J., Goldberg, T.L., Suskia, C.D. (2011) Impacts of dissolved oxygen on the behavior and physiology of bone fish: Implications for live-release angling tournaments. Journal of Experimental Marine Biology and Ecology, 402, p. 19–26.
16. Thorarensen, H., Gústavsson, A., Mallya, Y., Gunnarsson, S., Árnason, J., Arnarson, I., Jónsson, A.F., Smáradóttir, H., Zoega,, G.Th., Imsland, A.K. (2010) The effect of oxygen saturation on the growth and feed conversion of Atlantic halibut (*Hippoglossus hippoglossus* L.). Aquaculture 309, p. 96–102.
17. Timmons, M.B. and Ebeling, J.M. (2007) Gas transfer. In Recirculating Aquaculture. Ed.Cayuga Aqua Ventures, Ithaca, NY, p.397-438.
18. Watten, B.J. (1994) Aeration and oxygenation. In. Aquaculture water reuse systems: Engineering design and management. Timmons, M.B. and Losordo, T.M., Ed. Elsevier, Amsterdam.

19. Weyts, F.A., Cohen, N., Flik, G., Verburg-Van Kemenade, B.M. (1999) Interaction between the immune system and the hypothalamo-pituitary-interrenal axis in fish. Fish and Shellfish Immunol. 9, p.1-20.
20. Wu, R.S.S. (2009) Effects of Hypoxia on Fish Reproduction and Development. Fish Physiology, 27, p.79-141.