Journal of Fisheries and Aquaculture
Journal of Fisheries and Aquaculture
ISSN: 0976-9927 & E-ISSN: 0976-9935, Vol. 2, Issue 2, 2011, PP-23-28
http://www.bioinfo.in/contents.php?id=68

FISH ADAPTATION TO OXYGEN VARIATIONS IN AQUACULTURE FROM HYPOXIA TO HYPEROXIA

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Received: October 05, 2011; Accepted: November 02, 2011

Abstract- Hypoxia refers to low dissolved oxygen (DO) in an environment while the hyperoxia occurs when DO is beyond saturation in water. This paper reviews how fish adapt to environmental hypoxia and hyperoxia, and describes fish behavioral and physiological changes under these conditions. No adverse effects and abnormal behavior are found when fish are exposed to hyperoxia (<200% saturation), though there are some changes in the acid balance of fish blood. The abilities of fish to tolerance temperature, salinity, stocking density and ammonia in different O_2 conditions are discussed. Although the growth and physiological changes of fish in hypoxia have been thoroughly studied, research on how fish cope with hyperoxia is rare. In general, environmental hyperoxia is beneficial to fish growth especially at high ammonia and stocking density. However, it is necessary to further examine the implication of hyperoxia in fish culture in an attempt to improve aquaculture production efficiency. This review suggests future research be focused on the interaction between hyperoxia and other environmental factors to explore the feasibility of using hyperoxia in aquaculture.
Keywords: hypoxia, hyperoxia, behavior, physiological change, abiotic factors, biotic factors

Introduction

In fish farming, dissolved oxygen (DO) is vitally important for fish survival, growth, and reproduction [1]. Hypoxia or hypoxiation refers to DO content of a body of water becomes detrimental to aerobic organisms. It occurs as DO concentrations decline below the level required by aquatic animals when the supply of O_2 is cut off or O_2 consumption exceeds supply. In contrast, hyperoxia means that O_2 in water or body tissues is over saturated. A period of environmental hypoxia is likely to occur due to both natural causes such as diurnal oscillations in oxygen production due to high algal biomass, seasonal flooding, thermal stratification, ice cover, and dense vegetation, and anthropogenic causes such as eutrophication [2]. Hyperoxia occurs in noon or early afternoon due to strong photosynthesis [2]. In the past a few decades, studies on O_2 have focused on hypoxia because mortality or other damages associated with O_2 in fish mainly occur at low DO.

In intensive aquaculture, fish are reared at high density. Such a system requires water treatment to remove metabolic wastes and to reduce the risk of O_2 depletion. It is therefore a common practice to install O_2 supplementation systems on land-based fish farms to increase fish carrying capacity [3]. A conventional aeration method can only keep DO close to atmospheric saturation [4]. However, under an emergency condition such as accidental power failure, DO can quickly reach hypoxia. Considering low efficiency of aeration in intensive fish culture, fish farmers inject pure O_2 to fish tanks to increase fish biomass carrying capacity. Technological advance has made the use of pure O_2 injection more economically feasible in fish culture [5]. Under this scenario, fish in intensive aquaculture are likely to be exposed to hyperoxia.

It is hypothesized that the O_2 supersaturation can enhance fish tolerance to ammonia, food intake and food conversion efficiency. Foss et al. (2003) found that injection of O_2 could increase the carrying capacity of a fish culture system, providing that O_2 is a most limiting factor [6]. In fish hatchery management, pure O_2 has also been used as a means to improve larval fish survival and growth in the early stage [7]. However, little is known about the mechanism why hyperoxia can benefit fish farming. The objective of this paper is to review the effects of hypoxia and hyperoxia on fish growth, and to describe fish performance and physiological changes under these conditions. Because fish growth is highly dependent on environmental factors, there is a need to understand how fish cope with O_2 change at different temperature, salinity, stocking density, ammonia, and fish size. This review also discusses the feasibility of using environmental hyperoxia in commercial fish culture.

Definition of hypoxia and hyperoxia

Aquatic hypoxia is commonly defined as DO concentrations below 2-3 mg/L in marine and estuarine

waters and below 5-6 mg/L in fresh water. However, the threshold of hypoxia varies among fish species [8]. Although the current literature defines the range of hypoxia from 0.2 to 4.0 mg O_2/L, with a mean of 2.1 mg O_2/L, it still fails to adequately predict the threshold for some O_2 sensitive species [2]. Vaquer-Sunyer and Duarte (2008) reported that environmental thresholds for sublethal responses to hypoxia in fishes range from 2 to 10 mg/L [9]. The functional hypoxic level for one species usually does not apply for other species. Therefore, the definition of hypoxia should be based on the response of an organism rather than O_2 in the environment.

In a broad context, environmental hypoxia can be defined as the partial pressure of O_2 (PO_2) in water when physiological function is first compromised and a sublethal effect in a toxicological term occurs [2]. According to Wells (2009), environmental hypoxia is defined as any water PO_2 that decreases the arterial-blood O_2 transfer rate [10]. At the threshold of hypoxia in water, fish is limited in its capacity to acquire O_2 from the environment and its blood becomes hypoxemic. Functional hypoxia represents a situation where tissue O_2 demands exceed circulatory supply. On the other hand, little study has been done on hyperoxia. Hyperoxia is commonly defined as a higher than normal O_2 tension in water. In aquaculture practice, DO beyond the level of air saturation is considered hyperoxia.

Oxygen transport in blood and acid balance

Oxygen is brought into close contact with gills by a bulk of flowing water. Oxygen diffuses across gills into the blood with a gradient between 40 mm and 100 mm Hg PO_2 under a normal atmospheric condition [11]. Oxygen is transported in the blood from gills to capillaries, and it then diffuses across the capillary wall into tissues [12]. The property of blood that is of great importance to O_2 transport is the reversible binding of O_2 to the hemoglobin (Hb) molecule. This binding can be expressed as an ordinary reversible chemical reaction:

$Hb + O_2 == HbO_2$

Under a hypoxia condition, O_2 uptake requires Hb with a high O_2 affinity, but this means that most O_2 remains bound to Hb even at low O_2 partial pressure. This phenomenon is found in goldfish *Carassius auratus* and *crucian carp Carassius carassius* [13,14]. In contrast, salmon is highly active and hypoxia sensitive, but its Hb has relatively low O_2 affinities [15,16]. Under a hypoxic condition, fish have the ability to modulate O_2 affinity to Hb because fish Hb is also sensitive to other environmental factors such as temperature and pH. Reduction in temperature, hydrogen ions, and CO_2 will increase O_2 affinity to Hb, and vice versa [11]. If given the opportunity, a fish will move to cooler water during hypoxia [17,18]. At low temperature, fish will not only increase blood O_2 affinity but also reduce the use of whole-body adenosine triphosphate (ATP) due to the Q^{10} effect.

Hypoxia in fish is often accompanied by a reduction in blood pH due to increased anaerobic metabolism. The increase in blood H+ will reduce O_2 affinity to Hb by inducing Bohr and Root shifts that appear maladaptive in the hypoxic situation. In teleosts, hormones released by the adrenal glands can also play a protective function in response to low O_2 stress, as they activate an adrenergic Na^+/H^+ exchanger in the erythrocyte membrane that strives to increase intracellular pH [19].

At a hyperoxic condition, Hb combines O_2 to form oxy-hemoglobin (HbO_2) and the reaction goes to the right side of the Hb, O_2, and HbO_2 reaction equation [11]. Obviously, a sustained environmental hyperoxia condition can accelerate the above process and increase arterial O_2 and venous O_2 tensions [20]. With increasing O_2 concentration in water, blood O_2 transport can entirely rely on a physical process to supply sufficient O_2 to cells and tissues. On the other hand, CO_2 is 30 times more soluble in water than O_2 [11]. Thus, under a high tension of O_2, CO_2 accumulation results in an increase in plasma pressure of CO_2 (PCO_2) and a decrease in blood pH. Because hemoglobin oxygenation releases protons and HCO_3^- dehydration consumes protons, there is an extensive interaction between O_2 and CO_2 transfer. This interaction occurs in blood, centered in red blood cells, in both tissues and respiratory epithelia. Acidification in blood affects O_2 binding to Hb via the Bohr effect and the Root shift in many teleost fishes [21].

Some changes in acid-base balance are observed in species such as turbot *Scophthalmus maximus* L., rainbow trout *Oncorhynchus mykiss*, snake river cutthroat trout O. *clarki*, and white suckers *Catostomus commersoni* exposed to O_2 supersaturation [3, 7, 20]. These studies demonstrate that the hyperoxic acidosis can be buffered by the accumulation of HCO_3^-. For example, the pH buffering starts within 4 h in white suckers to prevent a significant rise in plasma HCO_3^-, and the further increase of PCO_2 does not reduce pH because of a simultaneous accumulation of HCO_3^- in blood [20]. In turbot, any reduction of blood pH is buffered within a day, as a result of HCO_3^- accumulation [3]. Up to present, no damage has been reported in most commercial species due to exposure to supersaturated oxygen.

Relationship between hemoglobin and haematocrit

Hemoglobin concentration and functional hypoxia are correlated in fishes. Wells and Baldwin (1990) demonstrated active fishes contain more Hb than benthic reef fishes [22]. Fish living at low temperature have a low metabolic rate and low Hb contents [23]. Exposure to environmental hypoxia in rainbow trout results in an increase in Hb concentration through the release of erythrocytes from the spleen, but under persistent hypoxia, an erythropoietin-mediated synthesis of new erythrocytes can increase oxygen-carrying capacity in the blood [24]. Oxygen-carrying capacity may also increase following a brief exposure to environmental hypoxia (about 30% saturation) via regulating Hb concentration [25]. Therefore, fish living permanently in a low-oxygen environment appear to have high Hb contents. During hypoxia, an increase in haematocrit due to red blood cell swelling and the release of red blood

cells from the spleen may occur within minutes or hours [26], which is presumably related to hypoxia inducible transcription factors [27].

Due to sufficient O_2 supply, a tendency shows low haematocrits and hemoglobins in fish held in O_2 supersaturated water. This trend is expected, since fish have low demand for O_2 transport capability when environmental O_2 is high [7].

Behavior

Oxygen is considered a limiting factor for fish growth because the availability of oxygen can regulate fish behavior, feeding, mating and other biological activities [28]. In order to maintain the same O_2 supply to tissues at low O_2, more energy must be allocated to respiration, thus increasing total energy expenditure [29]. A common response of fish to aquatic hypoxia is to increase the volume of water ventilated, while bimodal breathers increasingly rely upon air-breathing [30]. For example, both ventilation frequency and volume simultaneously increase as O_2 falls in common dentex *Dentex dentex* [31]. Another common response is that fish gulp air from surface water by gathering below water surface where more O_2 is available [32]. Doudoroff and Shumway (1970) reported that feeding activity, digestion and assimilation are strongly associated with O_2 availability [33]. In addition, hypoxia also impairs a number of key reproductive processes including gametogenesis, quality of sperm and eggs, reproductive behavior, hatching, and larval survivorship [34].

Comparing to hypoxia, our understanding on the impact of hyperoxia on fish behavior is still limited. Ventilation frequency is perhaps the well-documented activity in response to O_2 supersaturation. Typically, fish increase ventilation frequency and amplitude with falling O_2 [35]. However, under an O_2 supersaturated condition, fish decrease ventilation frequency and ventilation volume. In white suckers, ventilation volume declined by 50% due to reduction in ventilatory stroke volume under 300% O_2 saturation, but overall O_2 consumption was unaffected [20]. Dong (unpublished) observed that Japanese flounder *Paralichthys olivaceus* was quieter under O_2 supersaturated condition (16 mg/L) than in normal O_2 condition (6.5 mg/L) and there were no significant behavior changes in food intake and swimming. These results are similar to those reported by Peyraud & Serfaty (1964) and Dejours et al. (1977) [36,37]. Unfortunately, very little is known about the behavior in mate choice and reproduction when fish are exposed to O_2 supersaturation.

Abiotic factors

Temperature

Temperature is a major factor directly influencing metabolism and other physiological processes in fish such as food intake and nutritional efficiency [38-40]. Knowledge of temperature and O_2 interactions is very important for effective management of aquaculture systems [41]. In natural water, DO is relatively low and its solubility is inversely related to temperature [42]. Fry (1947) recognized that metabolism is the means by which organisms perform all activities while O_2 operates as a limiting factor to determine the maximum metabolic rate [43]. Toro-Silva et al. (2008) reported that O_2 limitation has its greatest impact near the optimum temperature for the growth of southern flounder *Paralichthys lethostigma* [43]. In their study, at 29 ^oC a reduction from 6.00 to 4.00 mg/L caused a 50% reduction in growth rate while at 27 oC, and such a reduction had no significant effect on growth rate. At low temperatures, a reduction in growth rate can be interpreted by Fry's (1947) framework that standard metabolism approaches maximum metabolic rate. In turbot, when O_2 concentrations are constant, fish consume more O_2 at higher temperatures over the temperature range tested [44]. Although the interacting effects of temperature and DO on growth have long been recognized, little information is available on the interactive effects of these factors on fish growth [43, 45].

Salinity

Salinity influences fish growth through osmoregulation [46, 47]. Osmoregulation can use 20 - 50% of the total energy expenditure, which is equivalent to 100 - 150 ml/h/kg O_2 depending on ambient salinity [47, 48]. The energy expenditure on osmoregulation inevitably affects other physiological processes in fish. In sea bass Dicentrarchus labrax fingerlings, an acute salinity change from 37‰ to 50‰ induces a transient increase up to 80% in routine O_2 consumption [49]. In white mullet *Mugil curema* fingerlings, oxygen consumption is 0.09 mg/L/g/h at 8.5‰ salinity, but oxygen consumption increases to 0.14 mg/L/g/h at 1.7‰ salinity [50]. The possible mechanism of salinity influence on O_2 consumption is via the changes of basic metabolism, osmoregulation and locomotion.

Ammonia

Ammonia is a major nitrogen metabolite in teleosts and is formed primarily as a result of protein catabolism [51]. Ammonia becomes more toxic when dissolved oxygen is low [52-54]. Thurston et al. (1981) found a positive linear correlation between DO and ammonia toxicity in rainbow trout and the toxicity of ammonia at 30% DO saturation is 1.9 times higher than at 80% DO saturation [55]. Foss et al. (2003) reported that wolfish *Anarhichas minor* are more tolerable to ammonia when DO is above saturation [6]. Similar results are also found in turbot and Atlantic cod [6, 51]. However, the underlying mechanisms causing high ammonia tolerance under hyperoxic conditions have not been thoroughly investigated yet. The possible explanations are the reduction in ventilation or the increase of gas diffusion distance in gills as observed in sea bass at hyperoxic conditions [56].

Biotic factors

Fish species and body size

Fish tolerance to hypoxic and hyperoxic conditions depends upon life stage and varies among species. Most fish species require DO >4.6 mg/L [9]. However, for

oxygen sensitive species such as salmonids, growth can be limited even at O_2 saturation at high temperatures [8]. On the other hand, for oxygen insensitive species, such as cod, fish can tolerate oxygen at 5% saturation at 5 ^oC [57].

As pointed out by Colt et al. (1991), O_2 toxicity to an organism depends on species, life stage, environmental conditions, physiological condition and nutritional history, and the hyperoxic toxic threshold varies among organisms [58]. In coho salmon Oncorhynchus kisutch, exposure of smolts to hyperoxia in fresh water can affect their ability to osmoregulation in seawater and thereby gives rise to mortality following transfer to sea water [59]. Eel *Anguilla Anguilla* can only survive in hyperbaric oxygenation for a few hours because the combination of hyperoxia and high pressure causes severe gill damage [60].

Most fish grow fast at early stage and its body mass can increase from less than 1 mg to over 1 g in a few weeks [61]. The scaling effects of body size on O_2 consumption and gill surface area can constrain hypoxia tolerance in fish. However, body size has little impact on the ability for fish to take up O_2 during hypoxic conditions, primarily because the respiratory surface area matches metabolic rate over a wide size range [62].

Under a hypoxic condition when oxygen is below the critical threshold, the ability to tolerate to hypoxia gives larger fish a clear advantage over smaller ones. In damselfish *Dascyllus aruanus*, when held in a closed respirometer where O_2 falls steadily, small individuals (10 mg) lose equilibrium quicker than large ones (40 g) [63]. If fish are at all able to compensate for the loss of aerobic respiration with anaerobic glycolysis, the high metabolic rate of small fish means that fish can rapidly use up their glycogen storage or fill up with anaerobic end products (lactate and H^+) [63]. Also in the case where the glycolytic rate is not high enough to maintain ATP levels in anoxia or severe hypoxia, ATP is likely to be used up faster in a small fish than in a large one due to a higher rate of ATP consumption in small fish [63].

Fish density

In intensive fish culture, the increase of stocking density is one way to optimize productivity [64]. However, high stocking density itself is a chronic stressor because it aggravates water quality and causes hypoxia [65, 66]. At high stocking density, fish frequently exhibit hyperventilation [67]. High stocking density results in a series of hematological changes [68]. For instance, juvenile gilthead seabream *Sparus aurata* show higher concentrations of haematocrit, Hb and red blood cells (43.87%, 10.76 g/dl and 3.36 × 106 mm^{-3}, respectively) at high stocking density (10.56 kg/m^3) than those (37. 21%, 9.32 g/dl and 2.82× 106 mm^{-3} respectively) at a low stocking density (2.64 kg/m^3) [68]. The change of blood physiological parameters is possibly an adaptation of fish to a short period of hypoxic condition.

It is a useful attempt to use hyperoxia to alleviate the stress caused by high stocking density in aquaculture. Physiologically, high stocking density can increase the amount of haematocrit and Hb, exerting stress on fish growth [68]. On the other hand, hyperoxia can decrease the volume of haematocrit, increase arterial O_2 and venous O_2 tensions and bring sufficient O_2 supply [20]. From the perspective of energetics, stocking density and DO appear to be critical to determine energy allocations. High stocking density results in a low proportion of energy allocation to growth and a high proportion to metabolic energy and energy lost in nitrogen excretion [67]. For carnivorous fish species, feces and nitrogenous excretion only account for a small portion of food energy and do not significantly influence the amount of food energy channeled to growth, but metabolism, usually accounts for a large portion on energy intake [69]. On the other hand, O_2 uptake by fish through gill surface is limited at high stocking density. Therefore, under a hyperoxic condition, high DO can enhance the O_2 uptake capacity of the fish and reduce the proportion of metabolic energy and channel more energy to growth [67].

Growth

Hypoxia causes significant reduction in appetite and results in poor food ingestion and growth [70]. Acute exposure to hypoxia quickly affects food digestion and nutrient absorption. For example, Atlantic cod immediately void their stomach when exposed to hypoxia [71]. As such, the vomiting response may be viewed as a stress response to a hypoxic challenge. As a result, hypoxia inhibits fish appetite and reduce food uptake, leaving less energy available for growth. In addition, hypoxia can also reduce assimilation efficiency and increase energy lost in feces [70].

The impact of hyperoxia on fish growth is clearly shown in rainbow trout and cutthroat trout O. clarki [7, 72]. In their study, when rainbow trout were fed to satiation, mean weight gain was 34% greater when fish were reared in 187% oxygen saturation than those reared at 95% saturation. Foss et al. (2003) found that the growth of spotted wolfish in the hyperoxia/NH3 group (14.5 mg/L O_2 with 0.17 mg/L unionized ammonia) was significantly faster than that in the nomoxic/NH3 group (9.6 mg/L O_2 with 0.17 mg/L unionized ammonia), suggesting that hyperoxic conditions can benefit fish growth despite high levels of ammonia in water [6].

The possible mechanisms of growth enhancement at O_2 supersaturation are as follows: (1) oxygen consumption - oxygen consumption is significantly high in fish experiencing hyperoxic conditions, indicating a higher metabolism rate [6]; (2) ventilation frequency - at high oxygen, fish slow down ventilation frequency and reduce energy cost for oxygen acquisition [28]; (3) food intake: fish ingest more food under hyperoxia than at normoxia (Dong, 2009 unpublished); and (4) energy allocation: fish shows high proportion of energy allocation to growth and low proportion to metabolic energy and nitrogen excretion loss [67].

Conclusion

Past research on the impact of oxygen on fish has focused on the responses of fish morphology, behavior, physiology, biochemistry, cell structure, molecular function to hypoxia exposure. However, literature on the impact of fish response to O_2 supersaturation in fish culture is sparse. The use of O_2 supersaturation in commercial fish culture is under-explored. Despite some merits of using hyperoxic condition in intensive fish farming, the use of O_2 saturation operation should be cautious to avoid unnecessary operation cost for using pure oxygen. Although the measurements of growth, food intake, food conversion efficiency, and energy allocation are important, studies on functional responses and mechanisms underlying the use of hyperoxia in fish farming should also be conducted.

Future development on the use of hyperoxia in fish farming should be based on the research in following areas: (1) physiological mechanisms that drive the superior fish growth performance in hyperoxia; (2) interactive effects between DO and other factors including temperature, salinity, stocking density and ammonia on growth, food intake and food conversion efficiency under hyperoxic and normoxic conditions; (3) behavioral changes when fish are reared under hyperoxic and normoxic conditions; and (4) cost-effective analysis of using hyperoxia in aquaculture settings.

List of abbreviations

Dissolved oxygen (DO)
Pressure of O_2 (PO_2)
Pressure of CO_2 (PCO_2)
Adenosine triphosphate (ATP)
Haemoglobin (Hb)

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