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THE EFFECTS OF OXYGEN SATURATION AND CARBON DIOXIDE CONCENTRATION ON THE GROWTH AND FEED CONVERSION OF AQUACULTURE FISH

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ABSTRACT

The aim of the study was to gain knowledge on the effect of water quality on the performance of fish in aquaculture using Arctic charr as a model. The Arctic charr (251.45±18.0g mean initial body mass±SD) were reared at two different levels of O_2 availability (80% and 120% of air saturation) and three levels of CO_2 concentration (LOW, MED and HIGH) for 60 days. The availability of O_2 significantly affected condition factor (CF), oxygen consumption and food conversion ratio (FCR) while growth rate was not significantly affected. The final size of fish reared at 120% of air saturation did not differ significantly from ($p > 0.05$) groups reared at 80%, suggesting that increasing oxygen saturation from 80% to 120% does not improve the growth of Arctic charr. Carbon dioxide concentration of the rearing water significantly affected growth rate, FCR, O_2 consumption and CF. The final size of fish reared at HIGH group was significantly lower ($p < 0.0001$) than in groups reared at MED and LOW group, an indication that CO_2 limits the growth of the fish. There were no interactive effects of CO_2 and O_2 on growth of the fish, suggesting that hyperoxic conditions may not increase tolerance to CO_2 in Arctic charr. However there was a significant interaction of CO_2 and O_2 on O_2 consumption suggesting that the effect of CO_2 is dependent on O_2 saturation of the system. The O_2 and CO_2 concentrations affected the hematology of the fish indicating adaptations to both reduced oxygen saturation (increased hematocrit and blood hemoglobin levels) and increased CO_2 concentration (reduced Cl^- concentration). The current study suggests that the recommended maximum level of CO_2 to maintain the welfare and maximum growth of Arctic charr is between 10-20 mg L^{-1} with a limited advantage of increasing the oxygen availability above 80%.

Key words: *Oxygen saturation, Carbon dioxide concentration, Growth, Blood, Arctic charr (Salvelinus alpinus)*

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1 INTRODUCTION

Aquaculture in sub-Saharan Africa accounts for less than 1% of the total world production although its contribution has expanded from 4,243 mt in 1970 to 359,790 mt in 2010 as a result of rapid development in freshwater fish farming most notably in Nigeria, Uganda, Zambia, Ghana and Kenya (FAO, 2012). The contribution of sub-Saharan Africa to total global aquaculture productions relies mostly on the culture of tilapia.
While African capture fisheries have been exploited to their maximum and in some cases above sustainable levels (FAO, 2010), African demand for fish has grown. According to FAO statistics, approximately 800 million people in the developing world are food insecure, a quarter of them in sub-Sahara Africa (FAO, 2012). However, while the number of food insecure people globally is expected to decline to about 700 million by 2030, a baseline projection suggests that sub-Sahara Africa will have a 27 % increase in food insecurity (World Bank, 2006; Randall *et al.*, 2008). Already sub-Sahara is the developing region with the highest proportion of its population undernourished; by 2030 it could account for more than 40% of all undernourished in the world. Part of the cause (if not the principal cause) is the absence of economic growth in the region as a whole (World Bank, 2006). Average real per capita income in sub-Sahara is lower now than thirty years ago; so fewer people are able to access food, even if it is available (UNDP, 2005; Randall, 2008, FAO, 2012). Fish is one of the appropriate animal protein sources that needs to be considered to remedy this, particularly in regions like sub-Saharan Africa where about 60 % of the population is suffering from chronic malnutrition (West, 1996; FAO, 2012).

1.1 Status of aquaculture in Kenya

East Africa has so far relied heavily on capture fisheries with a tendency to marginalise aquaculture as far as resource allocation and manpower development is concerned. The countries in the region are not exceptions to the global trend of declining stocks of wild fish and capture fisheries alone can no longer meet demand for fish, both for local consumption and export. Fish processing plants around Lake Victoria, for example, are operating at less than 50% capacity while some have closed down (MoF, 2013). Therefore, the need for aquaculture to supplement capture fisheries cannot be overstated.
In Kenya, Lake Victoria is a major source of quality protein food in the form of fish of various species. The Lake Victoria fishery provides employment and income to communities living in the Lake region and other parts of the country. It is also a major source of foreign exchange through exports of fish, mainly Nile perch fillets (Abila, 2003; MoF, 2013).

The Lake Victoria fishery has over the last two decades shifted from a complex multi-species fishery to one dominated by only three fish species, namely Nile perch, Nile tilapia and *Rastrineobola argentea* ("dagaa"). Over the last three to five years, there has been evidence of decline in catch per unit effort and the average sizes of fish caught. At the same time, the fishing effort (in terms of fishers, fishing gear, and crafts) has been rising steadily (Othina *et al.*, 2003; MoF, 2013). Environmental threats also pose a great danger to fish production from the lake. As the scenario calls for prudent management of the fish stocks in the lake, there is need to augment fish production in the country through aquaculture.

While natural fish stocks in Lake Victoria are declining from overfishing among other factors, demand for fish protein has been on a gradual increase as a result of rapid human population growth (FAO, 2006; FAO, 2012). Aquaculture is viewed as an alternative to reducing the widening gap between fish demand and its supply. Recognizing aquaculture as one of the viable options for revamping the economy, the Kenya government initiated an Economic Stimulus

Program (ESP) targeting fish farming in two thirds of the country and especially targeting areas with high unemployment rates.

The aquaculture component of the stimulus package, the Fish Farming Enterprise Productivity Program (FFEPP), started in mid-2009. The program aimed at increasing production of farmed fish from 4000 tonnes to over 20,000 tonnes in the medium term and over 100,000 tonnes in the long term (Charo-Karisa & Gichuri, 2010). In the first year of the program, over 27000 ponds were dug; 200 ponds in each of 140 constituencies countrywide. This alone created a national short-term demand of about 28 million certified tilapia and catfish fingerlings and over 14,000 metric tonnes of formulated fish feeds. The multiplier effect resulting from farmers digging their own ponds is expected to increase the demand for fingerlings to over 100 million and the demand for fish feeds to 100,000 MT in the medium term (Charo-Karisa & Gichuri, 2010).

Presently the aquaculture sector is growing rapidly in Kenya with a 40.5% increase in production between 2009 and 2012 (MoF, 2012). Of the total farmed fish production, Nile tilapia contributed 75% (16,115 tonnes), African catfish 18% (3,868 tonnes), common carp 6% (1,289 tonnes) and rainbow trout 1% (214 tonnes) (Figure 1). This production was from 68,734 ponds with an area of 20,620,200 m^2 (2,062 hectares) with stocking density of 4 m^{-2}, 161 tanks (23,085 m^2) with a stocking density of 300 m^{-3} and 124 reservoirs (744,000 m^2) throughout the country. Over the last ten years, fish production has increased from as low as 1,012 tonnes in 2003 to the present production of 21,487 tonnes (Fig. 2). The FFEPP played a leading role in jumpstarting aquaculture economy hence it acted as an impetus to aquaculture development in the country (Musa *et al.*, 2012).

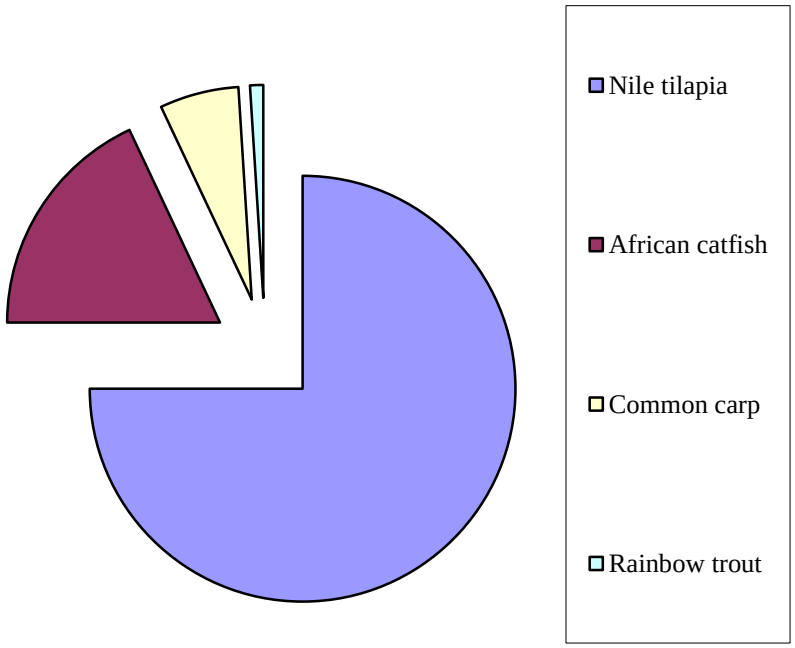


Figure 1. Percentage production by fish species in Kenya (MoF, 2012)

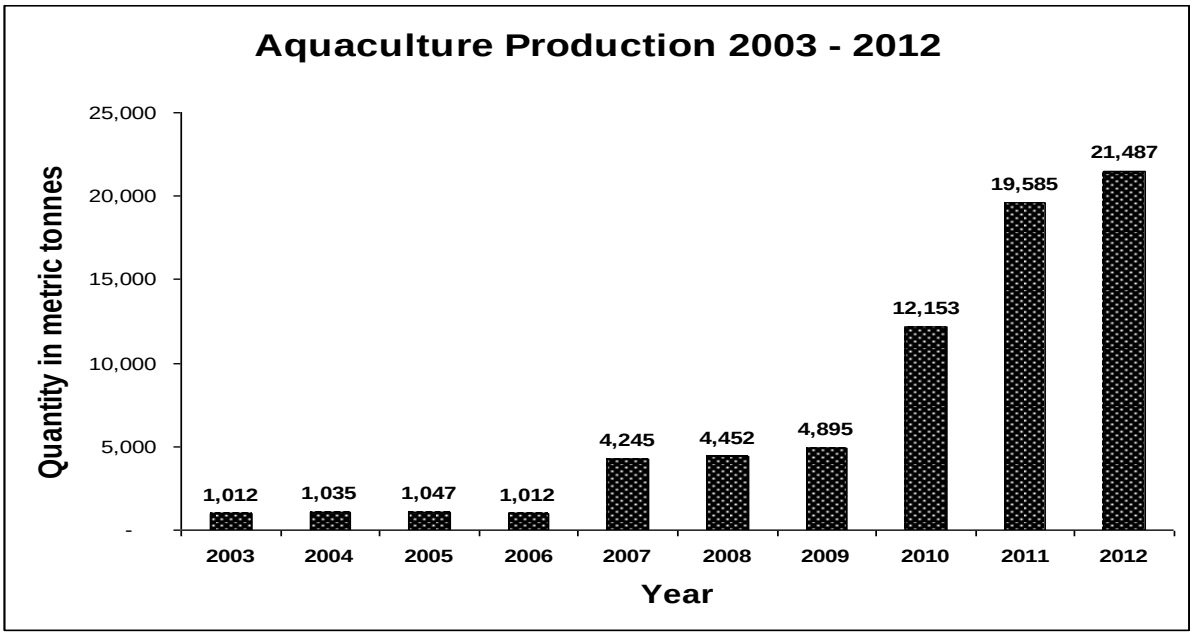


Figure 2. Aquaculture production for the last ten years (2003-2012) in Kenya (MoF, 2012)
If aquaculture production in the country grows as planned, the producers will put more emphasis on increased productivity per unit volume hence calling for better management practices of which water quality is a critical factor to put into consideration.

1.2 Motivation

Water quality and particularly oxygen (O_2), carbon dioxide (CO_2) and ammonia (NH_3) have an influences on feed consumption, metabolic rate and growth of fish (Brett, 1979; Elliott, 1982; Dutta, 1994; Bhikajee& Gobin, 1998; Pillay & Kutty, 2005). Feeding is the single largest cost in raising African catfish and Nile tilapia, often exceeding 60% of the operating expense (Craig & Helfrich, 2002). For aquaculture to be profitable it is important that the cultured organism's rates of feed intake and growth be both high and uniform (Brett, 1979; Elliott, 1982; Koskela *et al*., 1997). The first step towards making the aquaculture industry more profitable and viable is the mapping of the optimum CO_2 and O_2 for growth and feed conversion efficiency.
Currently in Kenya, there are intensive land-based farms that recycle water e.g. Jambo fish farm in Kiambu, Jasa fish farm in Thika, Kitengela fish farm in Kitengela, Baobab farm in Mombasa and Thinkcubator fish farms in maseno. However the main challenges these farms have been facing are the high mortality rates resulting in poor return from the production. Water quality could be a contributor to the high mortality rates. In recirculating aquaculture systems (RAS) the elevation of catabolites may become critical because of the continuing search for minimizing water exchange rates in an attempt to reduce environmental impact (Martins *et al*., 2009). To effectively exploit such systems, it is important to determine the optimum water quality parameters to maintain maximum growth of fish.

The objective of this study was to find the minimum O_2 saturation and maximum CO_2 concentration required to maintain maximum growth of fish. The knowledge gained will be transferred home in order to advise farmers on water quality management for improved productivity.

1.3 Objectives of the Study

The main objective of the study was to gain knowledge on water quality management for improved performance of fish in aquaculture.

1.3.1 *Goals of the Study*

1. To assess the effect of different saturation of O_2 and different concentration of CO_2 on the growth performance and feed conversion of Arctic Charr
2. To assess the effect of different saturation of O_2 and different concentration of CO_2 on oxygen consumption of Arctic charr.
3. To assess hematological response of Arctic charr to different concentration of CO_2 and O_2.
4. To assess the interactive effects of CO_2 and O_2 on growth, feed conversion, haematology and oxygen consumption of Arctic charr

1.3.2 *Research questions*

The main research questions answered include:

1. Do O_2 saturation and CO_2 affect the growth rate, condition, feed conversion, O_2 consumption, and haemotology of Arctic charr? If so, at what levels are these positively affected?
2. Is there interactive effect of CO_2 and O_2 on growth, condition, feed conversion, O_2 consumption, and haemotology of Arctic charr?

2 LITERATURE REVIEW

2.1 Water quality in aquaculture

Water quality refers to all physical and chemical qualities of water that affect the performance of aquaculture fish. Performance of aquaculture fish is dependent on the water quality. Favourable water quality is necessary for maintaining good growth, feed intake and reproduction (Wedemeyer, 1996). Both the physical and chemical aspects of the environment must be acceptable to the particular fish species requirements. The quality parameters that are of particular biological and ecological importance are O_2, NH_3 and CO_2 (Wedemeyer, 1996).

The problems of CO_2 and O_2 may be different depending on production practice. In semi-intensive culture O_2 increases during the day and decreases at night while CO_2 increases at night and decreases during the day. In intensive aquaculture both increase in concert (Boyd & Tucker, 1998). Therefore water quality management is one of the most important culture practices, especially in intensive culture system where metabolically-derived CO_2 may accumulate.

2.2 Respiration in fish

Fish, like other animals, take O_2 from the rearing water and excrete CO_2 (Groot *et al.*, 1995). Gas exchange occurs primarily through the gills in most fish species although some also use the skin or lung like structures in addition to gills. As water flows across the gills, the O_2 in the water diffuses into blood circulating through vessels in the filaments and lamellae. Simultaneously, CO_2 diffuses from blood to the water (Groot *et al.*, 1995).
Fish use two methods to ventilate their gills: buccal/opercula pumping (active ventilation) and ram ventilation (passive ventilation). In buccal/opercula ventilation the fish suck in water through the mouth (buccal chamber) and push it over the gills and out of the opercula chamber (where the gills are housed). At this time the pressure in the buccal chamber is kept higher than the pressure in the opercula chamber so as to allow the fresh water to be constantly flushed over the gills (Groot *et al.*, 1995). In ram ventilation, the fish swims with open mouth, allowing water to wash over the gills. This method of ventilation is common to fast moving fish (Boyd & Tucker 1998).
Most of the CO_2 excreted across the gills is carried in the blood as bicarbonate (HCO_3^-) but dehydrated in the red blood cells before diffusing out across the gills as dissolved CO_2 gas. Most of the O_2 taken up from the water is transported away from the gills bound to haemoglobin (Hb). More importantly, the excretion of CO_2 and dehydration of HCO_3 in the red blood cells is closely linked to the binding of oxygen to haemoglobin (Brauner *et al.*, 2000). Because Hb-oxygenation releases protons and HCO_3- dehydration consumes protons, there is an extensive interaction between O_2 and CO_2 transfer (Perry *et al.*, 1996; Powel & Perry, 1997; Brauner *et al.*, 2000). Acidification of the blood can affect oxygen binding to Hb via the Bohr Effect and the Root shift in many teleost fishes.

2.3 Diffusion of gases across gills

The solubility of dissolved gases in water depends on temperature, salinity and their individual partial pressure gradients across the surface (Timmons & Ebeling, 2007). Oxygen as a gas has a low solubility in water. While the O_2 content of the water sets the absolute availability of O_2 in the water, it is the O_2 partial pressure gradient that determines how rapidly O_2 can move from

the water into the fish's blood to support its metabolic rate. This is because O_2 moves by diffusion across the gills of fish (Thorarensen & Farell, 2011).

According to Fick's law of diffusion, the rate of diffusion of O_2 across the gills is determined by the gill area, the diffusion distance across the gill epithelia, the diffusion constant and the difference in partial pressure of O_2 across the gills (Piiper, 1990). Consequently, partial pressure of O_2 is the most appropriate term for expressing O_2 levels in aquaculture water (Thorarensen & Farell, 2011). However, O_2 concentration is the more commonly used term and for a given temperature and salinity, the partial pressure of O_2 and O_2 content in water are linearly related (Thorarensen & Farell, 2011). Another suitable method for expressing O_2 levels in aquaculture is % air saturation (often reduced to just % saturation) which is directly proportional to the partial pressure and is reported on most O_2 meters that have built in algorithms for temperature and salinity. In this study % saturation was used.

Acceptable levels of CO_2 should also be expressed in units of partial pressure because most of the CO_2 produced by the fish diffuses across the gills down a partial pressure gradient from plasma to water. However, since the concentration of CO_2 is directly proportional to its partial pressure at any given temperature and salinity, the aquaculture literature more commonly reports the levels of dissolved CO_2 gas in units of concentration, primarily as mg CO_2L^{-1} or ppm and in this study mgL^{-1} was followed.

2.4 Effects of oxygen and carbon dioxide levels on growth and feed conversion of fish

The growth rate of fish is influenced by factors such as feed availability, temperature, photoperiod and other environmental conditions (Boeuf & Payan, 2001; Nordgarden *et al.,* 2003). Several studies on different species of fish have indicated that growth is reduced under hypoxic (oxygen less than 100% of air saturation) conditions (Wang *et al.,* 2009); these include species in aquaculture such as Atlantic halibut (*Hippoglossus hippoglossus*) (Thorarensen *et al.,* 2010), rainbow trout (*Oncorhynchus mykiss*) (Pedersen, 1987; Dabrowski *et al.,* 2004), Atlantic salmon (*Salmo salar*) (Crampton *et al.,* 2003; Bergheim *et al.,* 2006), seabass (*Dicentrarchus labrax*) (Thetmeyer *et al.,* 1999; Pichavant *et al.,* 2001), southern flounder (*Paralichthys lethostigma*) (Taylor & Miller, 2001), spotted wolffish (*Anarhichas minor*) (Foss *et al.,* 2002), turbot (*Scophthalmus maximus*) (Pichavant *et al.,* 2000; 2001; Person-Le Ruyet *et al.,* 2003), Atlantic cod (Chabot & Dutil, 1999) and channel catfish (*Ictalurus punctatus*) (Buentello *et al.,* 2000). There is some evidence that moderate hyperoxia may improve the growth of fish (Foss *et al.,* 2003; Dabrowski *et al.,* 2004; Hosfeld *et al.,* 2008) while other studies have failed to find any improvement of growth in hyperoxia compared with normoxia (Edsall & Smith, 1990; Caldwell & Hinshaw, 1994; Person-Le Ruyet *et al.,* 2002). Although moderate hyperoxia may in some cases improve the growth rate of fish, there is evidence to suggest that too high levels of oxygen may be detrimental (Colt, 2006). Long term exposure to oxygen saturation of 140%–150% may cause oxidative stress, increased susceptibility to disease and increased mortality in salmonids (Lygren *et al.,* 2000; Ritola *et al.,* 2002; Fridell *et al.,* 2007). In addition, high oxygen levels may prove toxic to fish and concentrations above 25 mg L^{-1} should be avoided (Colt, 2006). High oxygen levels may also improve feed conversion (Crampton *et al.,* 2003; Bergheim *et al.* 2006).

Long-term exposure to sub-lethal but elevated levels of CO_2 may compromise growth and welfare of Atlantic salmon (Fivelstad et al., 1999). The long-term effects of exposure to high levels of CO_2 on fish include reduced growth rate, higher FCR and nephrocalcinosis (Smart et al., 1979; Smart, 1981; Fivelstad *et al.,* 1999; 2003; 2007). The growth rate of Atlantic salmon

was significantly reduced when exposed to ≥30 mg CO_2 L^{-1} at the parr and post-smolt stage (Fivelstad *et al.,* 1998; 2007). Some studies have reported reduced growth rate at 20 mg L^{-1} and minor effects were found even at 15 mg L^{-1} (Fivelstad *et al.,* 1999; 2003; Hosfeld *et al.,* 2008). Moreover, Fivelstad *et al.* (1999) found a significantly higher mortality rate at 19 and 32 mg CO_2 L^{-1} than at 7 mg CO_2 L^{-1}. The recommended maximum level of CO_2 to maintain the welfare and maximum growth of salmonids is 20 mg L^{-1} (Portz *et al.,* 2006; Timmons *et al.,* 2001). However, given the evidence to suggest that a maximum limit may be as low as 10 mg CO_2 L^{-1} (Wedemeyer, 1996; Fivelstad *et al.,* 1998). Wedemeyer (1997) suggested that the toxicity of CO_2 is probably increased when oxygen saturation is low.

2.5 Effects of oxygen and carbon dioxide levels on oxygen consumption by fish

Fish respond to a decrease in the levels of dissolved oxygen by increasing both ventilatory frequency and ventilation volume rate (Randall, 1982). The cost of ventilation has been estimated as being about 3–10% of the resting oxygen consumption at normoxia (Farrell & Steffensen, 1987; Rantin et al., 1992), but it may increase to 50% at hypoxia (Hughes, 1973). Any increase in ventilation will reduce the energy available for other metabolic processes such as growth (Brett & groves, 1979). During hypoxia, increased pumping will induce a rise in oxygen demand (Berschick et al., 1987). With declining oxygen tensions a point is reached, the critical oxygen tension, where the partial pressure of oxygen is reduced to levels where diffusion is not enough to support the oxygen demands of the fish. In severe cases, oxygen deficiency causes asphyxiation and fish will die, depending on the oxygen requirements of the species and, to a lesser extent, on their rate of adaptation.

In hyperoxic environments, fish hypo-ventilate and this may cause hypercapnia due to retention of CO_2 in the blood (Randall & Daxboeck, 1984). Similar results have been obtained for e.g. rainbow trout (Gilmour & Perry, 1994; Wood & Jackson, 1980), turbot (Person-Le-Ruyet, et al., 2002) and seabass (Checchini & Caputo, 2003) in hyperoxic water. Reduced ventilation in hyperoxia causes the increase of blood P_{CO2}, T_{CO2} and HCO_3^- while pH is initially reduced (Clairborne, 1997; Wood *et al.,* 1984). However, the fish compensate for the acidosis by increasing the blood HCO_{3-} concentration through Cl^-/HCO_3^- exchange (Clairborne, 1997; Wood *et al.,* 1984).

Prolonged exposures to high CO_2 levels can also directly affect O_2 consumption rates (Tang *et al.,* 2009). Elevating P_{CO2} was found to depress O_2 consumption rate at higher tensions (Basu, 1959), even though comparable reductions in water pH have been shown to elevate O_2 consumption rate by as much as 40% (Butler *et al.,* 1992).

2.6 Carbon dioxide, oxygen and fish haematology

The initial result of elevated plasma P_{CO2} is reduced plasma pH. Plasma pH is however generally restored close to control values within 2–7 days as a result of increased bicarbonate levels (Eddy *et al.,* 1977; Heisler, 1984, 1986). Other physiological effects on fish exposed to hypercapnia include increased epinephrine levels (Perry *et al.,* 1986), increased adrenaline levels (Perry *et al.,* 1986), hyperventilation (Janson & Randall, 1975; Smith & Jones, 1982; Fivelstad *et al.,* 1999; Hosfeld *et al.,* 2008), depression of the blood oxygen content (Eddy *et al*., 1977), reduced branchial chloride influx rates (Perry *et al.,* 1986a; Goss *et al.,* 1994) and reduced plasma chloride (Lloyd & White, 1967; Eddy *et al*., 1977; Fivelstad *et al.,* 1999, 2003). During acute carbon dioxide exposure elevated plasma cortisol levels have been observed (Petochi *et al*., 2011).

Unless CO_2 is removed from the rearing water (usually with water replacement or degassing), CO_2 excretion by the fish increases the concentration and partial pressure of CO_2 in the water. Any increase in ambient CO_2 partial pressure is rapidly reflected in blood CO_2 (Randall & Daxboeck, 1984). Thus, aquatic hypercarbia (an increase in the partial pressure of CO_2 in the water) causes hypercapnia (an increase in the partial pressure of CO_2 in the blood) in the fish and correspondingly decreases blood pH (acidemia) and this, in turn, shifts the equilibrium from HCO_3^- to CO_2 (Thorarensen & Farrell, 2011). However, over a period of few days fish can compensate for the reduced blood pH by excreting H^+ and taking up of HCO_3^- from the surrounding water through exchange of HCO_3^- and Cl^- (Heisler, 1984; 1986; Clairborne, 1997). Thus, fish subjected to hypercarbic environments may have near normal blood pH while being hypercapnic. However, the concentrations of CO_2 that reduce growth are not high enough to affect the oxygen affinity of haemoglobin. Even so, there is a limit to these compensatory mechanisms, which then sets the upper limit for CO_2 tolerance in fish.

Exposure to hyperoxia reduces respiration frequency, which causes accumulation of CO_2 in the blood and respiratory acidosis (Powell & Perry, 1997). Raising oxygen saturation to 500 %, which may occur during transport when pure O_2 is added directly to the water, can cause plasma ion imbalance, hypercapnia, respiratory acidosis and stress in Atlantic salmon smolts (Brauner *et al*., 2000 a, b).

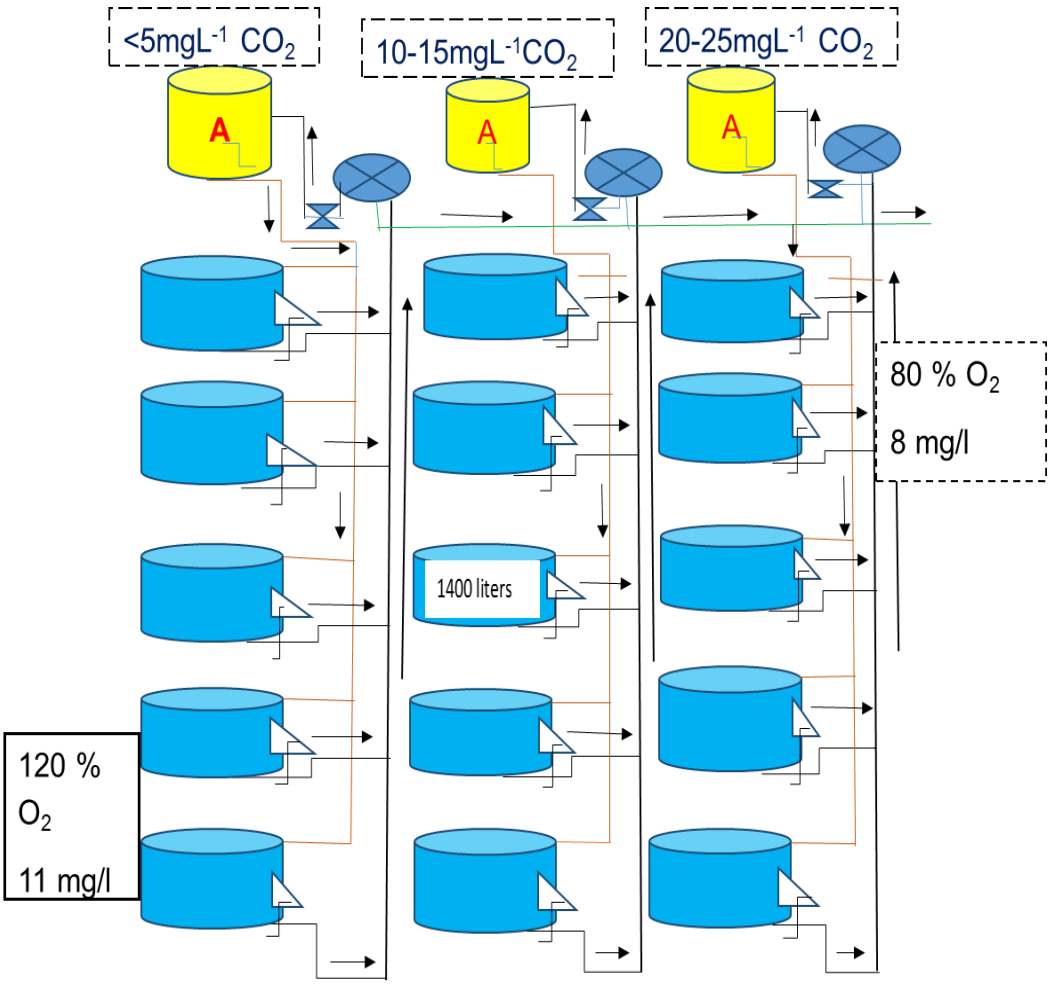
3 MATERIALS AND METHODS

3.1 Study area

This experiment was performed in an indoor environmental laboratory, at Verid the Holar University College research centre in Saudarkrokur from November 2013 to February 2014.

3.2 Experimental fish

The experimental fish were obtained from the Holalax fish farm. The fish were acclimatised to the tanks and conditions for one month under 80% O_2 saturation, 20pp salinity and 7.9 ºC temperature. At the start of the experiment, the fish were starved for two days before they were individually measured to obtain the initial weight. During the measurement, the fish were anaesthised using Tricaine methane sulfonate (MS-222) used in the ratio of 40-50 mg per litre of water. The fish were distributed at random among fifteen flow through circular fibreglass tanks (bottom area: 1.8 m^2; volume 1.4 m^3), 60 fish in each tank. Experimental set up is illustrated below in Figure 3.



LEGEND

A = Aerator and Low-head oxygenator

= Outlets

= Inlet

= Reservoir tank

= Water pump

= Direction of water

= Pellet collector plate

Figure 3: The arrangement of the tank system used in the experiment

3.3 Control of oxygen saturation, carbon dioxide concentration, temperature and salinity

The experiment was conducted as a three (CO_2 level) x two (O_2 level) factorial study (Fig. 3). The target levels of O_2 were 80% and 120% of air saturation while the target levels of CO_2 were < 5 mgL^{-1} (LOW), 10-15 mgL^{-1} (MED) and 20-25 mgL^{-1} (HIGH). Mean values for O_2 saturation in the six treatments were (mean±standard deviation) 80 ± 5%, 121 ± 12%, 82 ± 10%,

120 ±16%, 81 ± 5% and 120 ± 18% while mean values for CO_2 concentration were (mean±standard deviation) 4.70±0.15 mgL^{-1}, 4.61±0.12 mgL^{-1}, 14.4±0.28 mgL^{-1}, 14.60±1.13 mgL^{-1}, 20.60±0.53 mgL^{-1} and 21.2±0.51 mgL^{-1} (Table 1). The tested levels of CO_2 and O_2 were chosen from preliminary studies. Each treatment was tested in triplicate tanks except for the 120% of saturation treatment which was tested in two tanks.

Table 1. Mean water oxygen saturation [mgL–1], CO2 [mg L–1], pH and temperature [°C] during the experiment period (days 0 to 60)

	<5mgL^{-1}/80%	< 5mgL^{-1}/120%	10-15mgL^{-1}/80%	10-15mgL^{-1}/120%	20-25mgL^{-1}/80%	20-25mgL^{-1}/120%
O_2 (%)	80.0±0.02	121.0±0.03	82±0.01	120±0.05	81±0.01	120±0.05
CO_2 (mg L^{-1})	4.70±0.15	4.61±0.12	14.4±0.28	14.6±1.13	20.6±0.53	21.2±0.51
pH	7.28±0.01	7.30±0.01	6.76±0.01	6.78±0.01	6.61±0.01	6.60±0.01
Temperature (0C)	6.7±0.02	6.8±0.01	6.6±0.01	7.0±0.03	6.6±0.06	7.2±0.02

The tanks were in three separate systems with five tanks in each system. The LOW120% and LOW80% treatments were in the first system, the MED80% and MED120% treatment were in the second system and the HIGH80% and HIGH120% treatments were in the third system. The inflow water to all systems came from the same source and therefore, the temperature and salinity did not vary significantly between the tanks. The water level in the tanks was adjusted by varying the height of external stand pipes. The culture tanks have a central drainage system. The water leaving the tanks was passed through a wire mesh feed trap where the remaining feed pellets are collected. The water used in the experiemnt was partially reused. The total water flow into each tank was 17 L min^{-1} consisting of 12 L min^{-1} of reused water and 5 L min^{-1} of new water.

Each rearing system had a separate aerator and a low head oxygenator (Timmons *et al*., 2001) and the oxygen saturation in each system was adjusted by injecting pure oxygen gas into the LHO of each system (80% O_2 saturation) and by bubbling pure O_2 through the rearing water (120% oxygen saturation). Oxygen saturation was measured daily with a handheld oxygen meter (YSI 550A) each morning before the fish were fed and adjusted, if required, by adjusting the inflow of oxygen Salinity was measured using PAL (Japan) pocket refracot meter each morning. Alkalinity was determined as described in APHA (1990) while pH was measured by an OxyGuard® Handy pH-meter once per day in each of the experimental tanks. Carbon dioxide was monitored daily using OxyGuard CO_2 Analyser (Chicago, IL, USA).
The mean pH values differences were significant ($p < 0.05$) among the CO_2 treatments showing pH 6.60 to7.30 (Table 1). Notably, it was observed that there was an inverse relationship between CO_2 and pH (Figure 4).

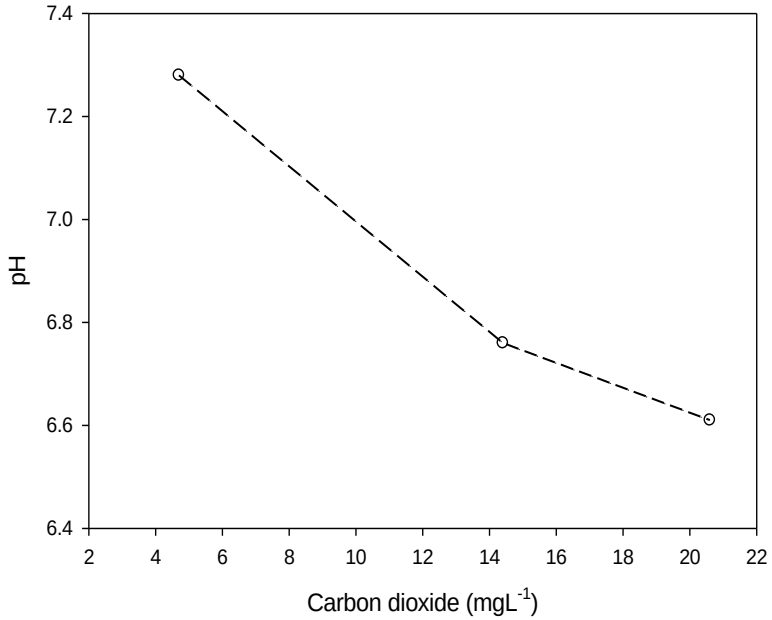


Figure 4. The relationship between carbon dioxide concentration and pH levels during the entire experiment period

3.4 Sampling and measurements

3.4.1 *Specific growth rate*

Weight measurements were taken monthly to obtain the specific growth rate (SGR). Prior to measurements, the fish were starved for one day. Both the initial weight and final weight of the fish was used to calculate the growth performance in terms of SGR. Specific growth rate was calculated according to the formula of Houde & Schekter (1981):

$$\mathrm{SGR} = 100\ \mathrm{X}\ \big(\mathrm{In(w2)} - \mathrm{In(w1)}\big)/(\mathrm{t2} - \mathrm{t1})$$

Where w1 is the initial body mass at time t1 and w2 is the body mass at the end of the growth period (at t2).

3.4.2 *Condition factor*

The condition factor (CF) was calculated from the formula adopted from Ayoade & Ikulala, (2007):

$$\mathrm{CF} = 100\mathrm{W}/aL^{b}$$

Where W is the weight of the fish, a is the intercept, b the slope and LT the corresponding total length

3.4.3 *Feeding and the collection of leftover feed*

The fish were handfed with dry pellets (LAXÁ, Akureyri, Iceland; size: 2.5–6 mm; containing 52% crude protein, 19% crude fat, and 9.0% crude ash) two times each day except Sundays when the fish were not fed. The first feeding was around 9 am and the second feeding around

2 pm, once a day. In addition, automatic feeders were used to provide feed continuously. The feed was presented until uneaten pellets were present on the bottom. Each tank was equipped with a feed trap to catch excess feed pellets from the water outlet. The feed presented each day was weighed and uneaten pellets were counted. The total mass of uneaten feed was estimated by multiplying the number of uneaten pellets with the mean weight of a sample of 100 pellets. The feed consumption in each tank was calculated by subtracting the weight of uneaten feed from the amount of feed presented.

3.4.4 *Feed conversion ratio (FCR)*

The total amount of feed consumed (C_T) was be calculated as:

$$\mathrm{CT} = \text{Amount of feed fed} - (\text{number of uneaten pellets x mean weight of pellet})$$

Feed conversion ratio (FCR) indicated how much feed was required for each unit gain in weight (Pillay and Kutty 2005) and was calculated as:

$$FCR = {}^{CT}/_{Increase\ in\ body\ mass\ during\ the\ same\ time}$$

Daily feeding rate (F) was calculated from:

$$F = {}^{CT}/_{W}$$

Where W is the mean daily fish weight over each of the experimental periods.

3.4.5 Oxygen consumption

Fish O_2 consumption was monitored using OXY-4 oxygen meter controlled with software, which also saves and visualizes the measured values. During the measurement of O_2 consumption, the inlets to all the tanks were turned off and oxygen consumption rate monitored using fiber optic oxygen mini-sensor for a duration of 30 minutes.

3.4.5 *Blood samples and analysis*

Blood samples were collected and analysed following the methods described in Thorarensen *et al.* 2010. To monitor the effects of different degrees of availability of O_2 and CO_2 on the blood physiology, blood samples (0.1–0.2 mL) were extracted with 1 mL syringes from the caudal vessels of ten fish (i.e., nonlethal sampling) from each experimental group on two occasions during the experimental period (days 0 and 60) and analyzed using an i-STAT® portable clinical analyzer (Abbott Inc., Illinois, USA). The fish were sampled at random from each tank. The analyzer was used in conjunction with 6+ disposable cartridges measuring blood and displaying calculated values of Na^+, Cl^-, K+, GLU, Hct and Hb.

3.5 Statistical analysis

Data was analyzed using R software package (Statsoft Inc., 2013, version 3.0.2). The results are given as mean ± SEM. The mean weight of the fish was compared at each sampling date with a nested mixed model ANOVA (tanks nested within treatment) with time and groups as factors and Tukey's test used for post hoc comparison of different treatments at $p < 0.05$. All

fish (60 in each tank) were used for these comparisons. The growth trajectories of different groups were also compared with a two-way (treatment×time) nested ANOVA (Ling, 2007). SGR were compared using one-way nested ANOVA. The blood values were also compared using nested ANOVA. The FCR for different treatments were compared with one-way ANOVA. Significant differences among means of the treatments (Tukey's HSD post hoc test; $p<0.05$) were indicated by different superscripts.

4 RESULTS

4.1 Growth

The O_2 saturation of the rearing water did not significantly affect the growth of the fish (Figure 5). There was no significant difference ($p > 0.05$) in the mean weight of the fish exposed to 120% and 80% of O_2 saturation at any time during the experiment. There were no significant differences ($p > 0.05$) between tanks within the treatments. For all figures presented in this section, significant differences are indicated with different superscript letters (Tukey's test, $p < 0.05$).

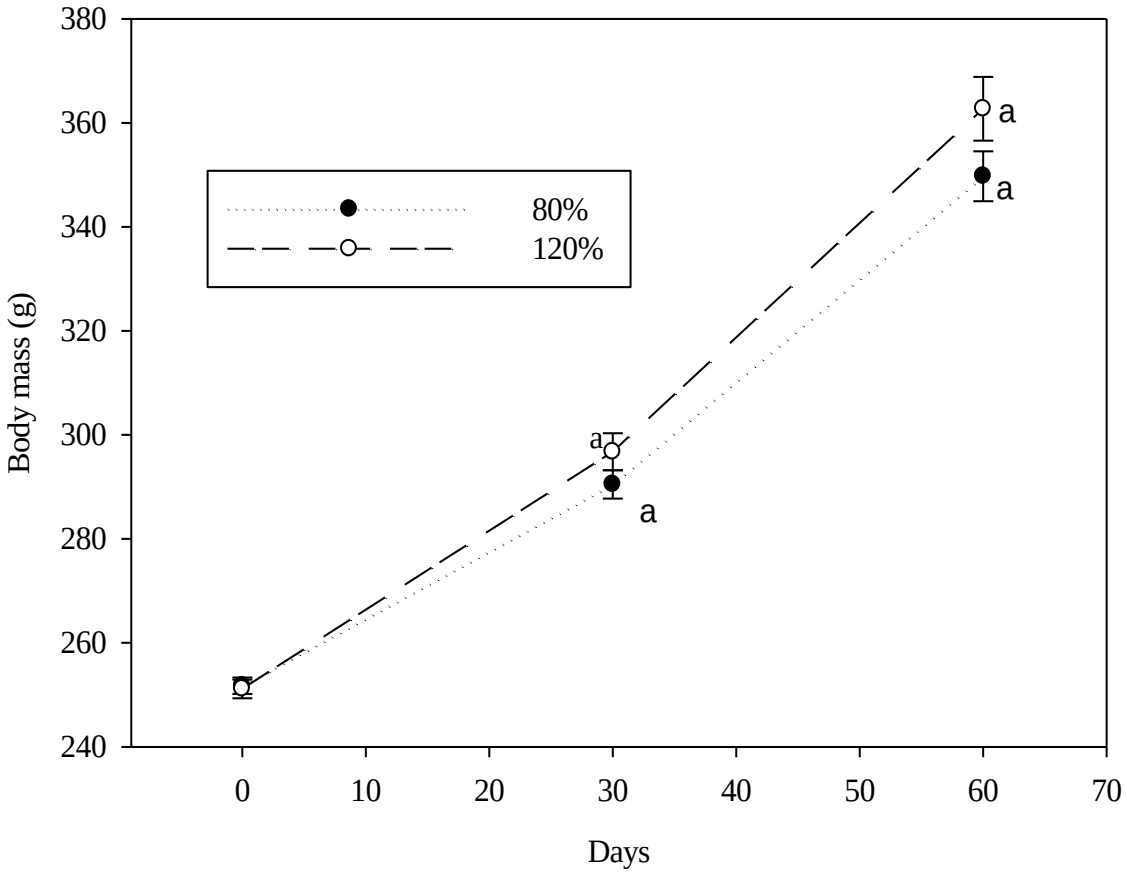


Figure 5. Mean body mass (±SEM) of fish reared at two levels of oxygen saturation.

The CO_2 concentration of the rearing water significantly affected the growth of the fish (Figure 6). On day 30 there was a significant difference ($p < 0.002$) in the mean size of the groups with the fish exposed to HIGH CO_2 concentration being significantly smaller than all other groups. The difference in the mean size increased during the experiment and on day 60 the group exposed to HIGH CO_2 concentration was significantly ($p < 0.0001$) smaller than all other groups. There was no significant difference ($p > 0.05$) in the mean weight of the fish exposed to LOW and MED CO_2 concentration at any time during the experiment. Notably, there were no significant differences ($p > 0.05$) between tanks within treatments.

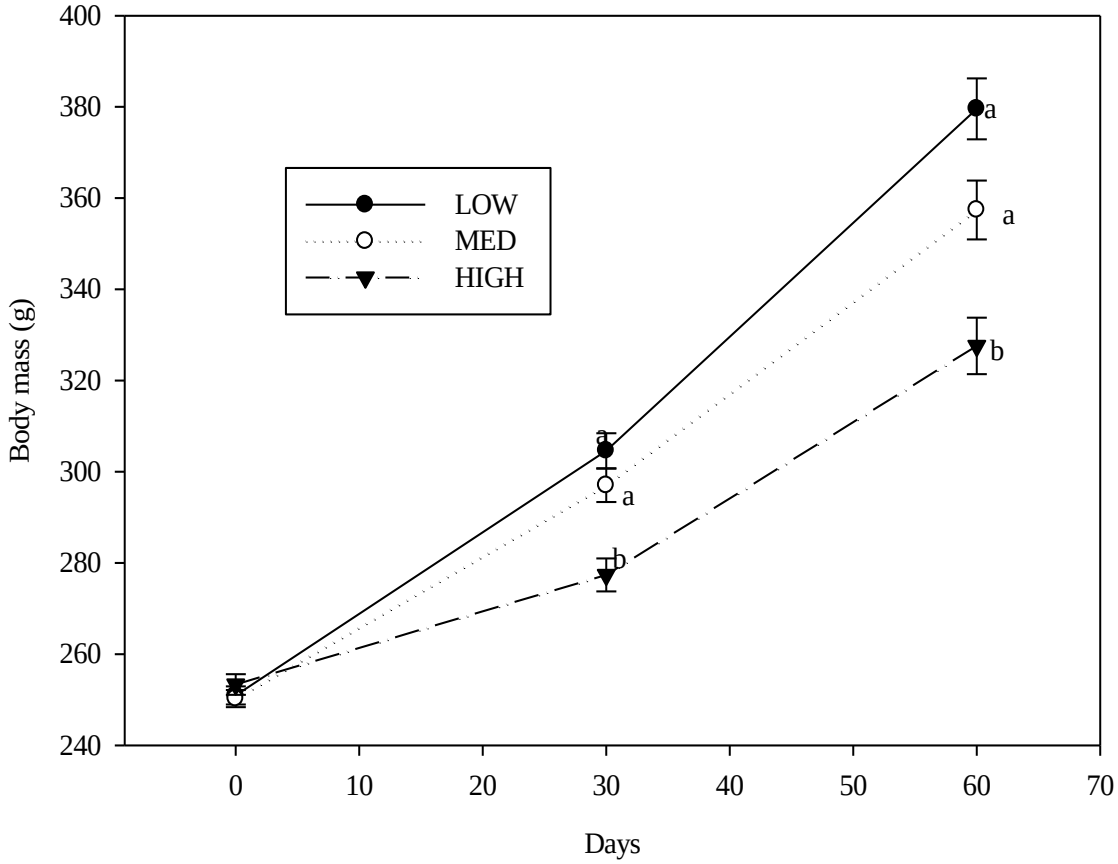


Figure 6. Mean body mass (±SEM) of fish reared at three levels of carbon dioxide concentrations.

In the first and second experimental period, there was no significant interaction between CO_2 concentration and O_2 saturation on growth (two-way nested ANOVA, $p > 0.05$, Figure 7). The mean size of the groups with the fish exposed to HIGH80% treatment was significantly smaller ($p < 0.02$) than all the groups at any time during the experiment. There was no significant difference ($p > 0.05$) in the mean weight of the fish exposed to HIGH80%, HIGH120%, MED80%, MED120%, and LOW120%. There were no significant differences ($p > 0.05$) between tanks within treatments.

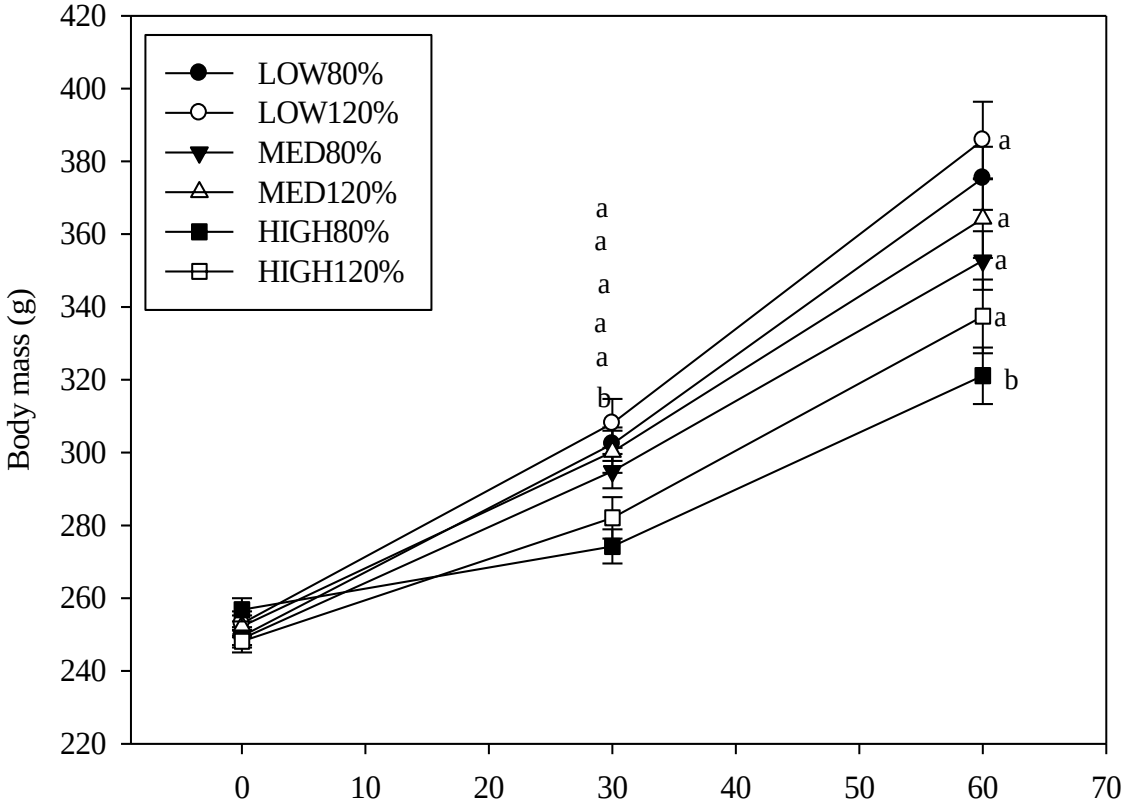


Figure 7. Mean body mass (±SEM) of fish reared at six levels of oxygen saturation and carbon dioxide concentration.

The addition of time as a factor into the ANOVA to compare the growth trajectories of the different groups showed significant interaction between CO_2, tank and time on growth at any time during the experiment (three-way nested ANOVA, $P < 0.0001$).

The mean SGR (Figure 8) was not significantly different among fish exposed to different levels of O_2 saturation during the first ($p > 0.05$) and second ($p > 0.05$) growth intervals. There was also no significant difference ($p > 0.05$) among the mean SGR during the entire experiment. The mean overall SGR in the group exposed to 80% of O_2 saturation was not significantly different ($p > 0.05$) from the groups exposed to 120% oxygen saturation.

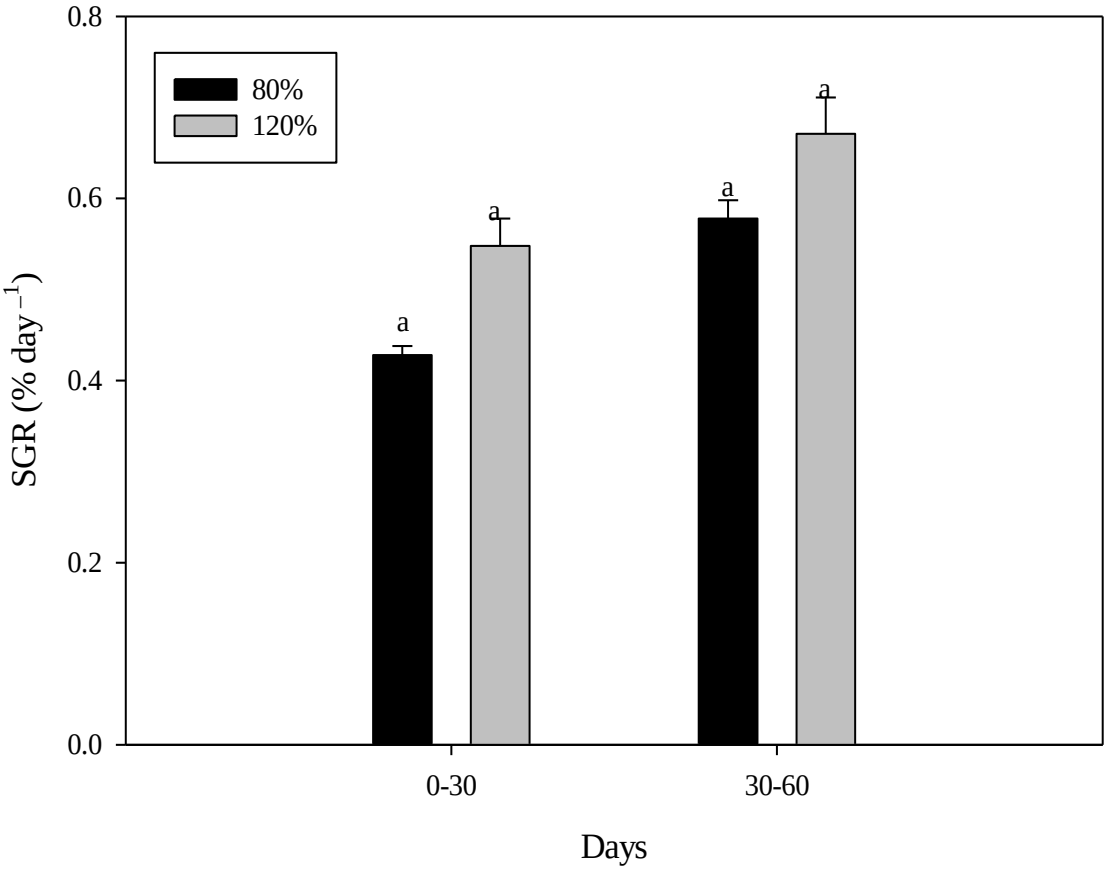


Figure 8. Mean specific growth rate (±SEM) of fish reared at two different levels of oxygen saturation.

The mean SGR (Figure 9) was significantly different among fish exposed to different levels of CO_2 concentration during the first ($p < 0.002$) and second ($p < 0.002$) growth intervals. There was also a significant difference among the mean SGR during the entire experiment. During the first and second growth intervals, there was no significant difference ($p > 0.05$) in the mean SGR of fish exposed to LOW and MED CO_2 concentration. However the mean SGR of fish exposed to HIGH CO_2 was significantly lower than LOW and MED groups ($p < 0.002$). The mean overall SGR in the group exposed to HIGH CO_2 concentration was significantly lower than in MED and LOW groups (Figure 9).

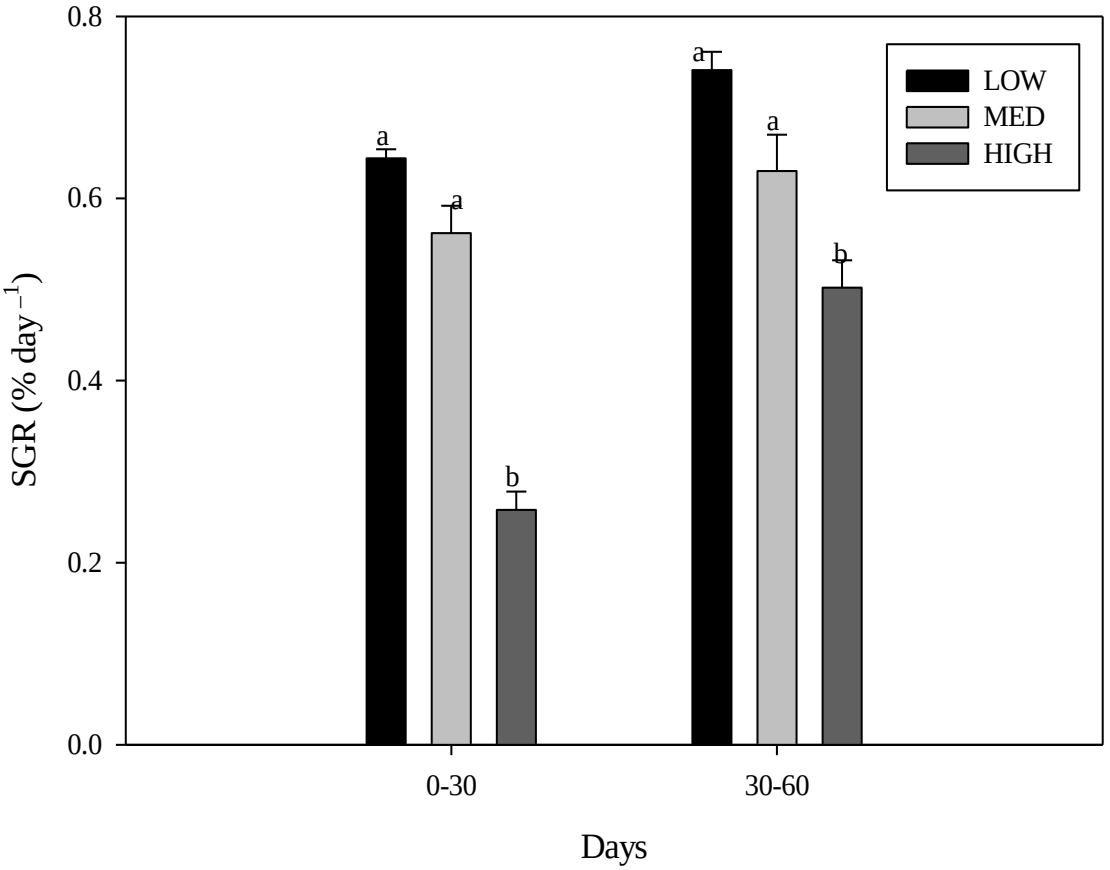


Figure 9. Mean specific growth rate (±SEM) of fish reared at three different levels of carbon dioxide concentration.

In the first and second experimental period, there was no significant interaction between CO_2 concentration and O_2 saturation on SGR (two-way nested ANOVA, $p > 0.05$, Figure 10). The mean size of the groups with the fish exposed to LOW120% treatment was significantly higher ($p > 0.002$) than the groups exposed to MED80%, MED120%, HIGH80% and HIGH120% during the first growth interval. However it was not significantly different ($p > 0.05$) from LOW80% during the same period. The mean SGR of fish exposed to LOW120% was significantly ($p < 0.002$) higher than all the other treatments during the second growth interval. Notably, the mean overall SGR in the group exposed to LOW%120 treatments was significantly higher than all the other treatments ($p < 0.002$). There were no significant differences ($p > 0.05$) between tanks within treatments.

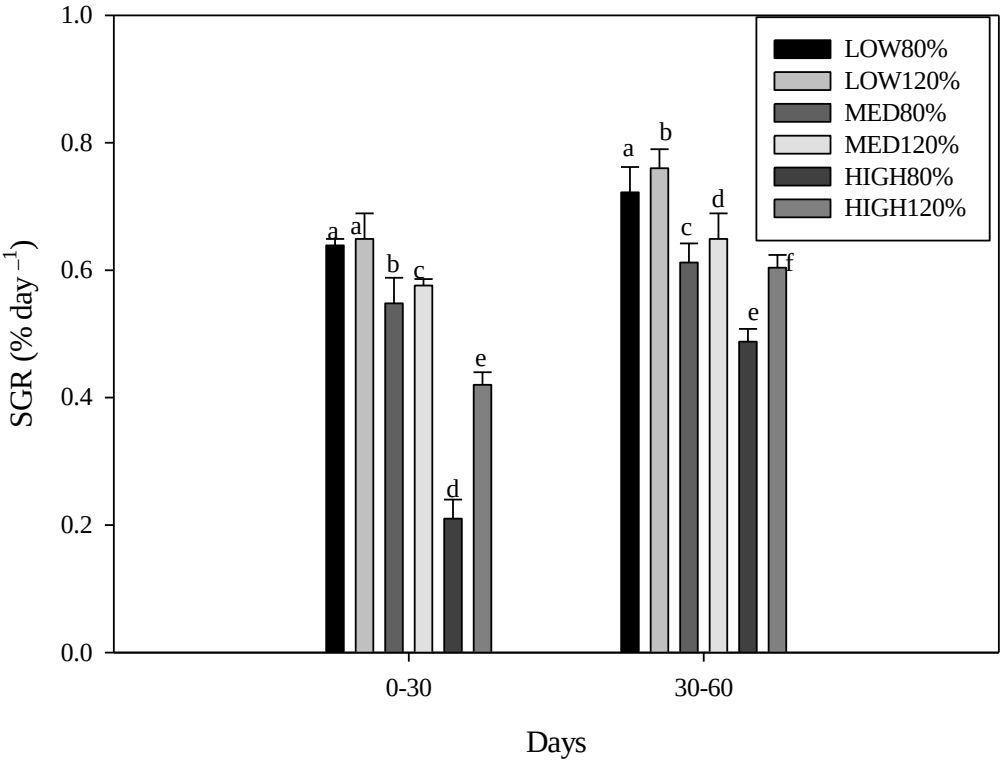


Figure 10. Mean specific growth rate (±SEM) of fish reared at six different levels of carbon dioxide and oxygen saturation.

4.2 Condition Factor

Covariance analysis was carried out to cancel out the effect of weight with all the L-W relationships having similar slope (3.3). Oxygen saturation of the rearing water significantly affected CF of fish (Figure 11). Condition factor of fish reared under 80% O_2 saturation was significantly higher ($p < 0.02$) than at 120% O_2 during the entire growth period.

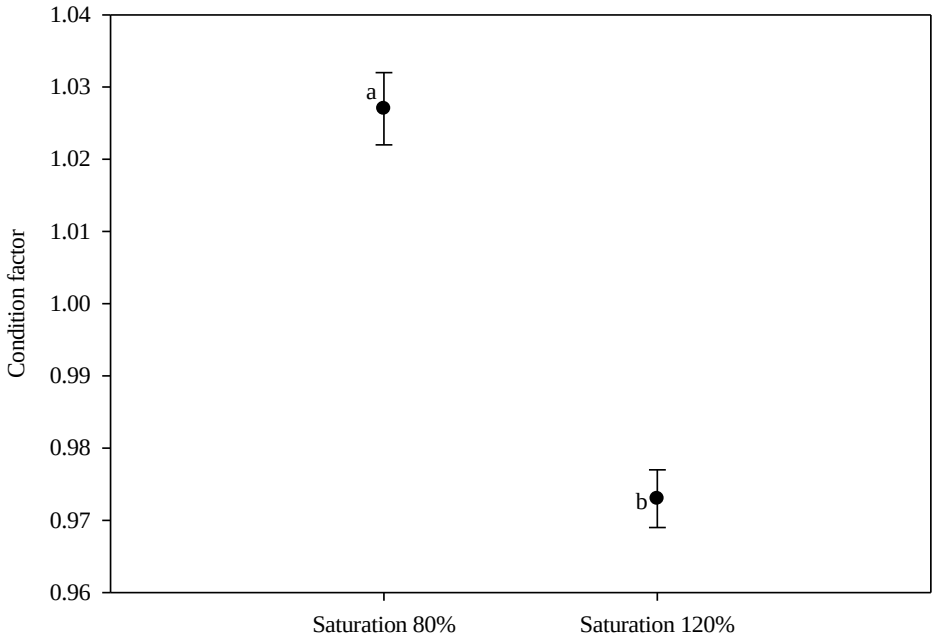


Figure 11. Condition Factor (CF) of Arctic charr reared under different oxygen saturation.

Carbon dioxide concentration of the rearing water significantly affected the condition factor (CF) of fish (Figure 12). Fish reared under LOW treatment had significantly higher ($p < 0.0001$) CF than MED and HIGH groups during the growth periods. Condition factor of fish under MED and HIGH groups did not differ significantly ($p > 0.05$).

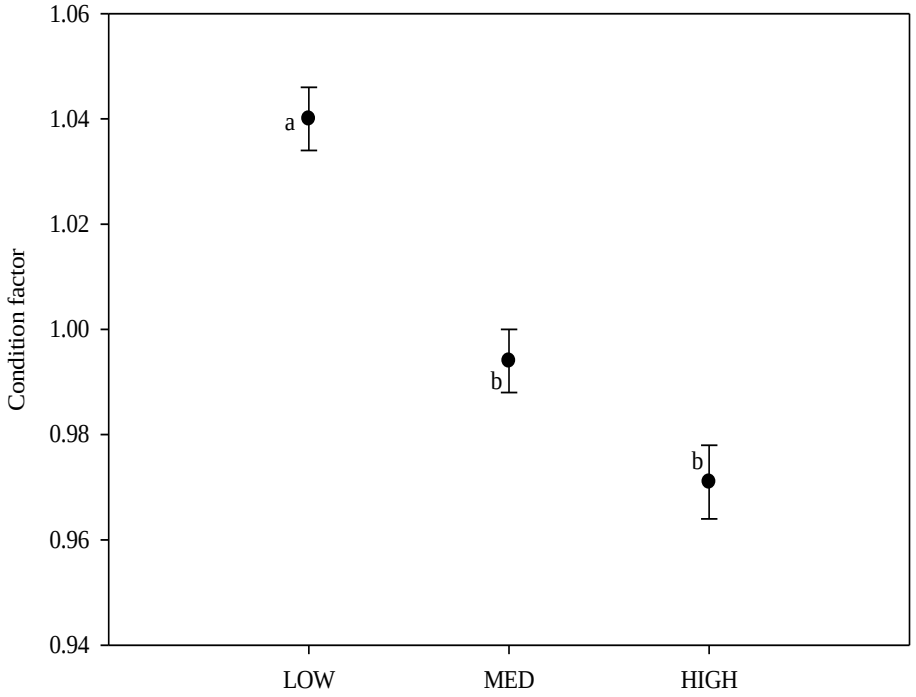


Figure 12. Condition factor (CF) of Arctic charr at different levels of Carbon dioxide concentrations.

In the first and second experimental period, there was no significant interaction between CO_2 concentration and O_2 saturation on CF (two-way nested ANOVA, $p > 0.05$, Table 2). Condition Factor was significantly higher ($p < 0.002$) in LOW80% as compared to the other treatments at any given time (Table 2).

Table 2. Condition Factor CF, (K) of Arctic charr under different saturation of oxygen and carbon dioxide concentrations.

	K	
TREATMENT	Day 30	Day 60
LOW80%	1.035 ± 0.008^{a}	1.050 ± 0.009^{a}
LOW120%	0.995 ± 0.009^{b}	1.044 ± 0.012^{b}
MED80%	0.990 ± 0.008^{b}	0.991 ± 0.009^{c}
MED120%	0.973 ± 0.009^{c}	0.998 ± 0.012^{d}
HIGH80%	0.976 ± 0.008^{d}	0.977 ± 0.010^{e}
HIGH120%	0.949 ± 0.009^{e}	0.962 ± 0.012^{f}

4.3 Feed conversion ratio

The oxygen saturation of the rearing water significantly affected feed ingestion rate (F) food conversion ratio (FCR) and total food consumption (CT). The F and CT of fish exposed to

120% O_2 saturation was significantly higher ($p < 0.001$) than 80% oxygen saturation. However FCR was significantly higher in 80% than in 120% group ($p < 0.005$). Daily feeding rate increased with O_2 saturation ($p < 0.002$; R^2: 0.98). Notably, daily increases in body mass increased linearly with the ingestion rate ($p < 0.001$, R^2: 0.88)

Table 3. Food conversion ratio (FCR), daily feeding rate (F) and total food consumption (CT) of Arctic charr reared at different saturation of oxygen.

Treatment DO (%)	FCR	F(g fish^{-1} day^{-1})	CT (g)
80	1.29[a]	1.95±0.03[a]	0.12±1.9[a]
120	1.11[b]	2.08 ±0.03[b]	0.14±2.4[b]

The CO_2 concentration of the rearing water significantly affected the F, CT and FCR of the fish (Table 4). Daily feeding rate and CT was significantly higher ($p < 0.002$) in LOW group as compared to MED and HIGH group. However FCR was significantly lower ($p < 0.001$) in fish exposed to LOW group as compared to MED and HIGH groups. There was an inverse relationship between CO_2 and F ($p < 0.004$, R^2: 0.98), CT ($p < 0.005$, R^2: 0.78). However, there was a direct relationship between CO_2 and FCR ($p < 0.001$, R^2: 0.89).

Table 4. Food conversion ratio (FCR), daily feeding rate (F) and total food consumption (CT) of Arctic charr reared at different saturation of carbon dioxide.

Treatment CO_2 (mg L^{-1})	FCR	F(g fish^{-1} day^{-1})	CT (Kg)
LOW	1.08a	2.33±0.04[a]	0.19±2.3[a]
MED	1.39b	2.14±0.04[b]	0.13±2.1[b]
HIGH	1.86c	1.58±0.04[c]	0.09±2.2[c]

There was no significant interaction (two-way nested ANOVA, $p > 0.05$, Figure 10) between O_2 saturation and CO_2 concentration on F and CT. Fish in treatment LOW120% had significantly higher ($p < 0.003$) F and CT than MED120%, MED180%, HIGH120% and HIGH80%. However the F and CT in LOW120% did not vary significantly ($p > 0.05$) from LOW80% (Table 6).

Table 5. Food conversion ratio (FCR), daily feeding rate (F) and total food consumption (CT) of Arctic charr reared at different saturation of oxygen and different concentration of carbon dioxide.

Treatment		FCR	F(g fish^{-1} day^{-1})	CT (Kg)
CO2 (mg L^{-1})	DO (%)			
LOW	80	1.10	2.32±0.05^{ac}	0.14±0.09^{ac}
	120	1.05	2.34±0.04^{ac}	0.14±.008^{ac}
MED	80	1.20	2.08±0.02^{bc}	0.12±0.01^{bc}
	120	1.15	2.19±0.02 ^{c}	0.13±0.02^{c}
HIGH	80	1.36	1.45±0.01 ^{d}	0.09±0.00^{d}
	120	1.17	1.71±0.01 ^{e}	0.10±0.01^{e}

4.4 Oxygen consumption

Oxygen saturation of the rearing water significantly ($p < 0.02$) affected O_2 consumption of fish (Figure 13). Mean O_2 consumption of the fish exposed to 120% O_2 saturation was significantly higher than 80% O_2 saturation.

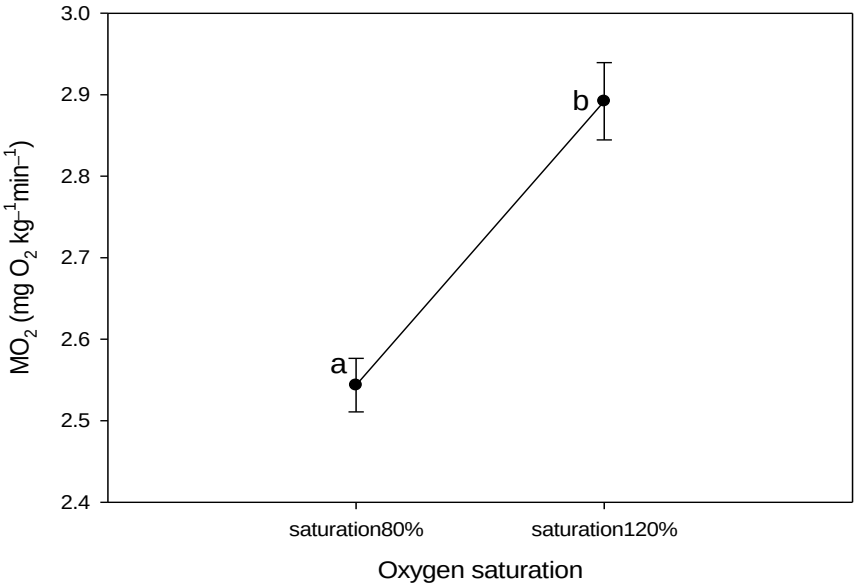


Figure 13. Oxygen consumption rate of Arctic charr at different levels of oxygen saturation.

Carbon dioxide concentration of the rearing water significantly affected O_2 consumption of fish (Figure 14). Oxygen consumption of fish in the HIGH group was significantly lower ($p < 0.002$) than all other treatments. However, the O_2 consumption in LOW and MED did not differ significantly ($p > 0.05$).

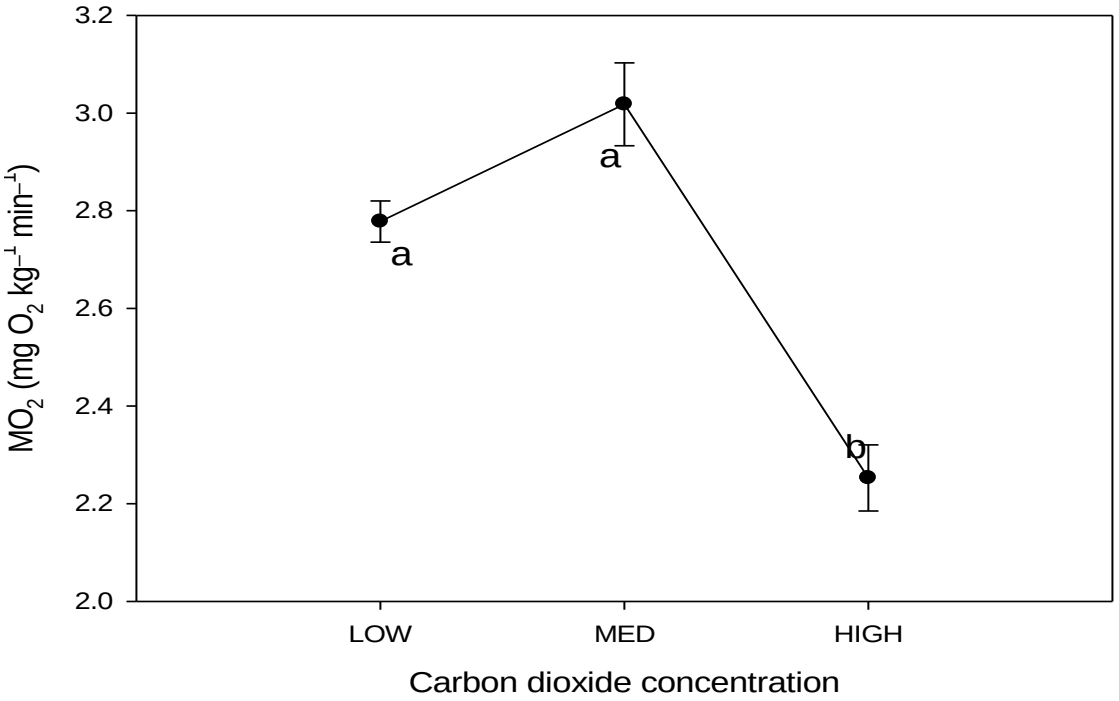


Figure 14. Oxygen consumption rate of Arctic charr at different levels of carbon dioxide concentration.

There was significant interaction between CO_2 concentration and O_2 saturation on O_2 consumption (two-way nested ANOVA, $p < 0.03$, Figure 15). Oxygen consumption in MED120% treatment was significantly higher ($p < 0.002$) than MED80%. Notably, oxygen consumption in LOW120% treatment was significantly higher ($p < 0.002$) than LOW80%.

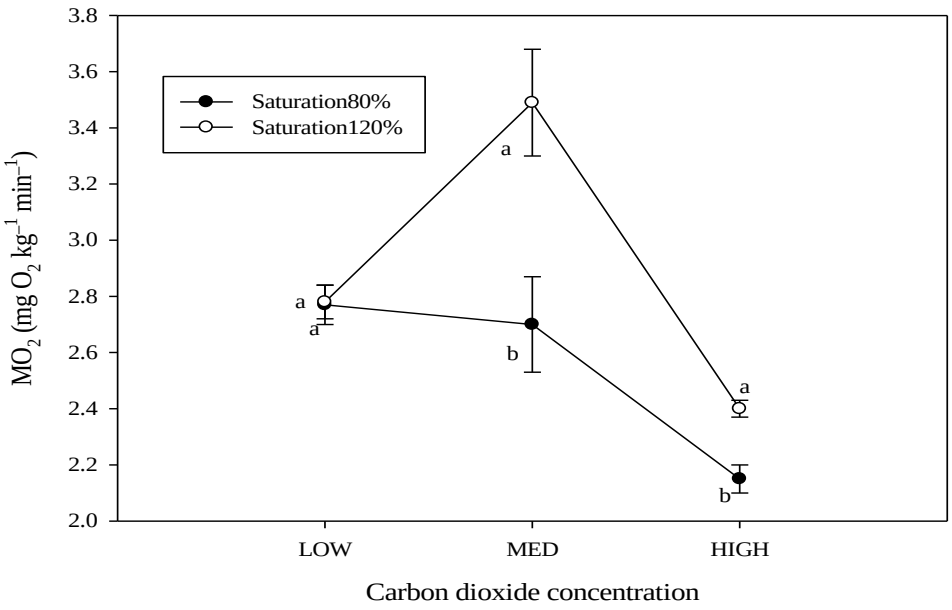


Figure 15. Maximum oxygen consumption rate of Arctic charr at different levels of oxygen and carbon dioxide concentration.

4.5 Hematology

The oxygen saturation significantly affected the hematology of the fish (Table 6). On day 60, the blood Hb and Hct was significantly higher ($p < 0.002$) in the 80% treatment groups than 120% O_2 saturation. There was no significant difference in mean Na^+, K^+, Cl^- and GLU among the treatment groups exposed to different levels of O_2.

Table 6. Blood Na+, K+, Cl-, GLU, Hct and Hb measured on day day 60 in the oxygen treatment. All results are presented as mean ± SEM.

Oxygen saturation	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	GLU (mg/dL)	Hct (% pcu)	Hb (g/dL)
80%	153.44±1.23a	3.10±0.13a	133.77±1.12a	74.49±3.07a	28.92±0.75a	9.83±0.25a
120%	152.66±1.07a	3.02±0.12a	132.84±0.979a	70.88±2.68a	26.11±0.65b	8.85±0.22b

The carbon dioxide concentration of the rearing water significantly affected the hematology of the fish (Table 7). The mean plasma Cl^- and K^+ concentration was significantly lower ($p < 0.002$) for both MED and the HIGH carbon dioxide groups as compared to LOW group on day 60. However, there was no significant ($p > 0.05$) difference in K^+ and Cl^- between MED and HIGH groups ($P > 0.05$). In addition, there was no significant difference ($p > 0.05$) in mean Na^+, GLU, Hct and Hb among the treatment groups.

Table 7. Blood Na+, K+, Cl-, GLU, Hct and Hb measured on day day 60 in carbon dioxide treatments. All results are presented as mean ± SEM.

Carbon dioxide (mgL^{-1})	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	GLU (mg/dL)	Hct (% pcu)	Hb (g/dL)
LOW	153.69±1.37a	3.43±0.15a	138.77±1.30a	73.07±3.56a	28.95±0.87a	9.84±0.30a
MED	150.60±1.34a	2.96±0.15b	131.80±1.27b	73.65±3.49a	26.35±0.85a	8.96±0.29a
HIGH	154.29±1.37a	2.76±0.15b	128.85±1.30cb	70.8±3.56a	26.45±0.87a	8.96±0.30a

No significant interaction terms between CO_2 concentrations and O_2 saturations were identified for any of the hematological parameters (two-way nested ANOVA, $p > 0.05$, Table 8).

Table 8. Blood Na+, K+, Cl-, GLU, Hct and Hb measured on day day 60. All results are presented as mean ± SEM.

TRMNT	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	GLU (mg/dL)	Hct (% pcu)	Hb (g/dL)
LOW80%	154.00±2.33a	3.57±0.50	139.73±1.67	75.07±12.34	31.93±4.63	10.86±1.58
LOW120%	153.77±8.18a	3.33±0.58	138.13±3.76	71.73±23.30	26.97±5.69	9.16±1.92
MED80%	151.90±2.51a	2.75±0.54	133.00±5.15	74.80±11.52	27.40±3.10	9.32±1.04
MED120%	149.30±3.77a	3.17±0.58	130.60±4.32	72.50±12.06	25.30±2.91	8.59±0.99
HIGH80%	154.43±7.07a	2.99±0.58	128.57±7.21	73.60±15.27	27.43±3.91	9.32±1.35
HIGH120%	153.80±7.64a	2.61±0.33	129.03±7.63	68.93±15.18	25.80±2.47	8.71±0.95

5 DISCUSSION

At the end of the 8th week experiment period, the mean body mass of the fish reared at 120% of oxygen saturation did not differ significantly from the group maintained at 80% saturation suggesting that oxygen saturation does not affect the growth rate of Arctic charr (Figure 5). Thus the minimum oxygen levels required to support the maximum growth of charr is less than 100% of air saturation. The result of the present study concurs with those of several other studies, which indicated that oxygen saturation under 100% of saturation are sufficient to support the maximum growth of fish (Lakani *et al.,* 2013). Pedersen (1987) reported that oxygen saturation of 70% (7 mg L^{-1}) will support the maximum growth of rainbow trout and his results are corroborated by the findings of Edsall & Smith (1990) and Caldwell & Hinshaw (1994). Studies performed by Pichavant *et al*. (2000) and Person-Le Ruyet *et al*. (2003) also suggest that the maximum growth rate of turbot is reached when the oxygen saturation is 64%–75%. All these findings suggest that 50%–75% of oxygen saturation is adequate to support the maximum growth of fish. However, there are also results indicating that oxygen saturation close to 100% or even higher is required to support the maximum growth of both Atlantic halibut (Thorarensen *et al,* 2010), Atlantic salmon (Crampton *et al*., 2003) and channel catfish (Buentello *et al*., 2000). Moreover, some studies have indicated that oxygen saturation above 100% may be required for the maximum growth of both Atlantic salmon (Hosfeld *et al*., 2008), rainbow trout (Dabrowski *et al*., 2004), spotted wolffish (Foss *et al*., 2002), sea bass (Sargolia *et al*., 1995), and turbot (Björn Björnsson, Marine Research Institute, Reykjavik, Iceland). It is not clear what causes this discrepancy. However, it is interesting that the reported critical oxygen levels appear to increase with time from those of Davis (1975) and Brett (1979) to recent reports of critical oxygen levels in the hyperoxic range (Foss *et al*., 2003; Dabrowski *et al*., 2004; Hosfeld *et al*., 2008). A number of reference texts in aquaculture suggest that 50%–80% oxygen saturation is enough to support maximum growth (e.g., Davis, 1975; Brett, 1979; Jobling, 1995; Wedemeyer, 1997; Timmons *et al*., 2001; Colt, 2006). The present study and some of those cited above also supports this finding.

Carbon dioxide concentration of rearing water affects the growth rate of Arctic charr. At the end of the 8th week experiment period, the mean body mass of the fish reared at MED and LOW CO_2 concentration was significantly higher than in the group maintained at HIGH CO_2. However there was no significant difference in mean body mass of the fish reared at MED and LOW CO_2. This could suggests that the CO_2 concentration must be kept under 15 mg L^{-1} to maintain maximum growth of Arctic charr, which is comparable to the safe levels of 10 mg L^{-1} suggested by Fivelstad *et al*. (1998). The result of the present study concur with those of several other studies which indicated that carbon dioxide above 15 mg L^{-1} of concentration compromises growth rate of fish. The growth rate of Atlantic salmon was significantly reduced when exposed to ≥30 mg CO_2 L^{-1} at the parr and post-smolt stage (Fivelstad *et al*., 1998; Fivelstad *et al*., 2007; Hosefeld, 2008). Some studies have reported reduced growth rate at 20 mg L^{-1} and minor effects were found even at 15 mg L^{-1} (Fivelstad *et al*., 1999, 2003; Hosfeld *et al*., 2008). Moreover, Fivelstad *et al*. (1999) found a significantly higher mortality rate at 19 and 32 mg CO_2 L^{-1} than at 7 mg CO_2 L^{-1}. Smart *et al*. (1979) found a slight reduction in mean weight for rainbow trout exposed to 22 mg^{-l} (P_{CO2}7 mm Hg) when compared to the mean CO_2 weight for trout held at 12 mg L^{-l}. For longer exposures during grow-out period, much lower levels of CO_2, such as 6 mg L^{-1}, may already have a negative impact (Fivelstad *et al*., 2003; Foss *et al.* 2003). High CO_2 increases the costs associated with acid–base balance (Pörtner & Farrell, 2008) which may result in less energy being available for growth. A number of reference texts in aquaculture suggest that the recommended maximum level of CO_2 to maintain the welfare and maximum growth of salmonids is 20 mg L^{-1} (Portz *et al*., 2006; Timmons *et*

al., 2001). However, given the evidence to suggest that a maximum limit may be as low as 10 mg CO_2 L^{-1} (Fivelstad *et al*., 1998; Wedemeyer, 1996). Review by Thorarensen & Farell (2011) suggested that precautionary approach might adopt this lower level. The present study and some of those cited above also concur with these finding.

There were no interactive effects of O_2 and CO_2 on growth of Arctic charr. This could indicate that the effect of the different levels of O_2 is independent of the level of CO_2 present in the rearing water. This contradicts the findings by Hosfeld *et al*. (2008) who found a significant interaction between O_2 and CO_2 on the performance of Atlantic salmon. Certain studies have also indicated that the toxicity of CO_2 is probably increased when O_2 saturation is low (Alabaster & Lloyd, 1982; Wedemeyer, 1997). When anesthetizing or euthanizing mammals with CO_2, oxygen is often added to the gas mixture (i.e. hyperoxic CO_2 anesthesia) to prevent hypoxemia and asphyxiation (and the sensation thereof) to reduce stress and suffering (Coenen *et al*., 1995; Kohler *et al*., 1999). Notably, toxicity of certain gases such as ammonia have been reported to decrease with increasing oxygen levels (Lloyd, 1961; Alabaster *et al*., 1979; Thurston *et al*., 1981; Wajsbrot *et al*., 1993, Foss *et al*., 2003). Lack of interaction between the two gases in the current experiment could possibly be due to the shorter period of the experiment and this was supported by statistical power indicating 82% chance of realizing an interaction of O_2 and CO_2 with time.

Oxygen saturation of the rearing water affects the condition of fish. Fish reared in 80% O_2 saturation had higher condition factor than fish reared under 120% O_2 saturation (Figure 11). The lower condition factor in 120% oxygen saturation could be as a result of reduced ventilation in such environment (Gilmour & Perry, 1994) which exposes the fish to acidosis (Clairborne, 1997). Similar results have been obtained for e.g. rainbow trout (Wood & Jackson, 1980; Gilmour & Perry, 1994), turbot (Person-Le Ruyet *et al*., 2002) and seabass (Checchini & Caputo, 2003) in hyperoxic water.

Carbon dioxide concentration of the rearing water also affected the condition factor of the fish. Condition factor varied significantly with CO_2 concentrations with HIGH groups having significantly lower condition factor than LOW and MED groups. Reduced condition factor observed in both high CO_2 groups in the present experiment appear to be a typical long-term effect of CO_2 (Fivelstad *et al*., 1999, 2003). The overall reduction in condition factor in the high CO_2 groups in the present investigation, can probably be related to reduced food intake (Smart, 1981; Crocker & Cech, 1996) or to chronic stress (Wedemeyer, 1997) and higher energy expenditure in the groups reared under such suboptimal water quality regimes.

There were no interactive effects of O_2 and CO_2 on condition of fish possibly suggesting the effect of O_2 is independent of the effect of CO_2 concentration in the rearing water. This contradicts the findings by Hosfeld *et al*. (2008).The shorter duration of the experiment could also have led to lack of interaction.

Oxygen saturation and carbon dioxide concentration of the rearing water affected F and CT and FCR of Arctic charr. There was a significant independent effect of O_2 on F, FCR and CT, as the mean CT and F of the groups exposed to 120% O_2 saturation was significantly higher than the groups exposed to 80% O_2 saturation. In addition, FCR in the groups exposed to 120% O_2 was significantly lower than the groups exposed to 80% O_2 saturation. The results of the current study concur with the findings of Crampton *et al.* (2003) and Bergheim *et al*. (2006) who noted that high O_2 levels may improve feed conversion and thus decrease the cost of production. However the current findings contradict other studies which have indicated no effects of oxygen

on FCR (Thorarensen *et al*., 2010). There was a significant strong effect of CO_2 on CT, FCR and F. The results indicated that high CO_2 reduce CT and F and this concurs with several other studies which have reported reduced feed intake and growth with high levels of CO_2 (>20–40 mg L^{-1}) (Crocker & Cech 1996; Fivelstad *et al.*, 1998; Lemarie´ *et al*., 2000; Foss *et al*., 2003). However the results of the present study contradict the findings by Nawicki *et al*. (2012) indicating that there was no effects of CO_2 on consumption rate. Other studies (Cecchini *et al*., 2001; Foss *et al*., 2003; Santos *et al*., 2013) also indicated that elevated CO_2 in isolation did not significantly affect food consumption of juvenile *Amphiprion melanopus*. Growth of fish is largely dependent on consumption of feed, its assimilation and conversion into body tissues (Nikolski, 1963; Brett & Groves, 1979; Dutta, 1994; Burel et al., 1996). It is clearly evident from the present data that growth rate was strictly dependent on CT. The slower growth rates in treatment of 80% saturation of oxygen and HIGH CO_2 groups were due mainly to lower CT. A similar relation of growth to rate of CT has been demonstrated in largemouth bass (Thompson, 1941), brown trout (Pentelow, 1939; Elliott, 1982), sockeye salmon (Brett & Shelbourne, 1975; Biette & Green, 1980), common carp (Huisman, 1974), margined sculpin (Davis & Warren, 1965), coho salmon (Stauffer, 1973), channel catfish (West, 1965; Andrews & Stickney, 1972) and striped bass (Cox & Coutant, 1981).

There was no significant interaction between CO_2 and O_2 on FCR, CT and F, probably indicating that the effect of CO_2 concentration on FCR, CT and F is independent of O_2 saturation.

Oxygen saturation and CO_2 concentration of the rearing water affects O_2 consumption (Figure 13, 14). This result contradicts previous findings that have failed to find significant difference in oxygen consumption under normaxic and hyperoxic environment (Dejours *et al*., 1977; Wilkes *et al*., 1981; Berschick *et al*., 1987; Person-Le Ruyet *et al*., 2002; Lakani *et al*., 2013). The O_2 consumption was also influenced by CO_2 concentration of the system decreasing at the highest CO_2. The high O_2 consumption in MED and LOW treatments could be as a result of energy requirements for digestion and absorption, biosynthesis and storage of nutrients as higher CT and F were observed in those treatments (Table 6). Mean daily O_2 consumption is usually observed to increase with increased food intake, as the metabolic costs associated with digestion will thus increase (Jobling, 1981; Timmons *et al*., 2001, Thorarensen & Farrell, 2006). Such results have recently been demonstrated for both turbot and sea bass, Dicentrarchus labrax L. (Pichavant *et al*., 2001). Notably, a higher swimming activity was observed, although not quantified, in the MED and LOW groups, which may account for an increase in O_2 consumption rates. The significant reduction in oxygen consumption rates for HIGH group is probably related to reduced food intake; therefore, a reduced metabolic rate (Fivelstad *et al.*, 1998)

There is interaction between CO_2 and O_2 on O_2 consumption, suggesting that the effect of CO_2 is dependent on O_2 concentration in the system. The result of the present study concurs with Basu (1959) and Saunders (1962) who found that the effects of increased water carbon dioxide concentration were more pronounced at low oxygen concentrations.

Oxygen saturation of the rearing water affected the hematology of Arctic charr. A significantly higher Hct and Hb were observed at 80% saturation than at 120% O_2 saturation, suggesting that the fish adapted to lower O_2 levels by increasing the oxygen carrying capacity of the cardiovascular system. Similar results have been reported in killifish (Greaney & Powers, 1978) and rainbow trout (Soivio *et al.*, 1980). However, no increase in Hct or Hb levels were observed in carp when exposed in a similar different oxygen levels (Lykkeboe & Weber, 1978; Jensen & Weber, 1985). Increasing haemoglobin concentration possibly improves preserving oxygen

delivery from gills to tissues. Besides, there was a steep increase for the haematocrit value which was significantly resulting possibly from a significant increase in number of red blood cells. This response has been observed in many marine and freshwater fish species (Muusze *et al.*, 1998; Smit & Hattingh, 1978; Soldatov 1996).

The CO_2 concentration of the rearing water also affected the hematology of Arctic charr. The plasma chloride was significantly reduced in MED and HIGH groups. These results are in accordance with result from other studies on the effect of increased levels of CO_2 (Lloyd & White, 1967; Eddy *et al*., 1977; Iwama *et al*., 1993; Dimberg & Høglund, 1987; Fivelstad *et al*., 1998, 1999). The reduction in plasma chloride levels may reflect an electroneutral ion exchange with HCO_3^- caused by high CO_2 (Goss et al., 1994). The K value was significantly lowered in MED and HIGH groups, indicating that ion regulatory capacity is negatively influenced by these CO_2 levels in Arctic charr. The findings are in line with previous research findings on effect of CO_2 on physiology of fish (Fivelstad *et al.*, 1999, 2003b).
There was no significant interaction of O_2 and CO_2 on hematology. This could be an indication that the effects of CO_2 are independent on O_2 concentration in the environment. This could be due to ventilation drive in fish which is regulated based on the partial pressure of oxygen rather than CO_2 or pH as in terrestrial animals (Gilmour, 1997).

6 CONCLUSIONS AND RECOMMENDATIONS

The results of this study showed that there were no interactive effects of oxygen and carbon dioxide on performance of Arctic charr but carbon dioxide has a strong independent effect on performance of Arctic charr. No difference was realised in growth performance of arctic charr reared in 80% and 100% oxygen saturation. The results of this and other studies suggest that fish farms should utilize oxygen saturation 80% for Arctic charr of this size in order to maximize profits. It is suggested that the maximum acceptable level of CO_2, where the growth and welfare of Arctic charr are not compromised is between 10-20 mg L^{-1}. The results indicated an inverse relationship between CO_2 and pH. Therefore the results of the current study can be attributed to both a CO_2 and/or pH effect. Further studies are recommended to investigate the relationship/interaction of pH and CO_2 and should include long-term studies on Arctic charr. The increased CO_2 tolerance of fish experiencing hyperoxic conditions demonstrated in the present experiment should also be more thoroughly investigated, as this could be of great importance in intensive fish farming facilities. Further studies should include more detailed observations on overall activity as well as map the O_2 consumption rate on a wider time and fish size scale.

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