Use of avoidance response by rainbow trout to carbon dioxide for fish self-transfer between tanks

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Abstract

Convenient, economical, and reduced labor fish harvest and transfer systems are required to realize operating cost savings that can be achieved with the use of much larger and deeper circular culture tanks. To achieve these goals, we developed a new technology for transferring fish based on their avoidance behavior to elevated concentrations of dissolved carbon dioxide (CO$_2$). We observed this behavioral response during controlled, replicated experiments that showed dissolved CO$_2$ concentrations of 60–120 mg/L induced rainbow trout (\textit{Oncorhynchus mykiss}) to swim out of their 11 m$^3$ “growout” tank, through a transfer pipe carrying a flow with $\leq$ 23 mg/L dissolved CO$_2$, into a second 11 m$^3$ “harvest” tank. The research was conducted using separate groups of rainbow trout held at commercially relevant densities (40–60 kg/m$^3$). The average weight of fish ranged from 0.15 to 1.3 kg during the various trials. In all trials that used a constant flow of low CO$_2$ water ($\leq$ 23 mg/L) entering the growout tank, the transfer pipe was restricted to the area of relatively low CO$_2$ water at the entrance of the transfer pipe. However, the rate of fish transfer from the growout tank to the harvest tank was more than doubled when the diameter of the transfer pipe was increased from 203 to 406 mm. To consistently achieve fish transfer efficiencies of 99%, water flow rate through the fish transfer pipe had to be reduced to 10–20% of the original flow just before the conclusion of each trial. Reducing the flow of relatively low CO$_2$ water near the end of each fish transfer event, restricted the zone of relatively low CO$_2$ water about the entrance of the fish transfer pipe, and provided the stimulus for all but a few remaining fish to swim out of the growout tank. Results indicate that the CO$_2$ avoidance technique can provide a convenient, efficient, more economical, and reduced labor approach for fish transfer, especially in applications using large and well mixed circular culture tanks.

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1. Introduction

Larger production systems that include bigger culture tanks can impart economies of scale that reduce fixed and variable costs in tank-based recirculating aquaculture systems (Wade et al., 1996; Summerfelt et al., 2004). Technologies for fish transfer and grading within large, deep circular culture tanks (e.g. 1.5–5.0 m
depth; 100–10007 m3/tank) must be developed and assessed to better achieve the labor savings potential of these large tanks. A new approach that takes advantage of a fish’s natural behavioral response could potentially allow fish to be herded to harvest in the culture environment.

Avoidance and/or “flight” responses and behavioral changes in fish have been documented for sound (Knudsen et al., 1997; Maes et al., 2004; Taylor et al., 2005), smell (Berejikian et al., 1999), and chemicals (Summerfelt and Lewis, 1967; Exley, 2000). Excluding fish from hazardous areas such as water intakes in natural waterways using sound has recently been tested and found effective for some species (Knudsen et al., 1997; Maes et al., 2004). Olfactory triggers have been shown to cause a “fright reaction” in many species, especially those of cyprinids (Pfeiffer, 1977). Behavioral changes of salmonids due to perceived predators via an olfactory stimulus have been noted, but a true “fright reaction” or flee response due to such a stimulus has not been reported (Pfeiffer, 1977).

Recent research suggests that rainbow trout (Oncorhynchus mykiss), Atlantic salmon (Salmo salar), and dogfish (Squalus acanthias) have external chemoreceptors that sense dissolved carbon dioxide (CO2) and/or acidity (H+) and mediate cardio-respiratory adjustments during hypercapnia (McKendry and Perry, 2001; Perry and McKendry, 2001). Research by Summerfelt and Lewis (1967) and research that they referenced (Shelford and Allee, 1913; Shelford and Powers, 1915; Wells, 1915, 1918; Collins, 1952) indicate that fish can sense and avoid areas with elevated concentrations of dissolved CO2. More recent fish behavior research has also suggested that fish can sense and avoid areas with elevated concentrations of dissolved CO2 (Bil’ko and Kruzhilina, 2000; Ross et al., 2001; Oelssner et al., 2002). Taking advantage of the CO2 avoidance response could lead to new technology that would increase efficiency and reduce labor during harvest. Therefore, we designed a study to test the feasibility of using such a method as a stimulus for passive transfer of fish.

CO2 is a by-product of respiration, naturally excreted by the fish through their gills, and is present in all natural waters. Dissolved CO2 in excess of 20 mg/L, however, can begin to impair the transport of oxygen (O2) in the fish’s blood due to the Bohr effect (Colt et al., 1991; Wedemeyer, 1996). Reduced O2 transport to tissues and organs can create cerebral hypoxia, which produces the anesthetic effect reported by Gelwicks et al. (1998) at higher CO2 concentrations. Dissolved CO2 solutions of 155 mg/L induce anesthesia in rainbow trout in less than 3 min of exposure at 14 °C (Gelwicks et al., 1998); the CO2 anesthetized fish recovered normal swimming activity in <10 min with no mortality, when total exposure times were <15 min. When rainbow trout anesthetized with CO2 are returned to a low CO2 environment, the CO2 diffuses out of the gills and the fish rapidly recover from hypercapnia (Eddy et al., 1977). However, exposing rainbow trout to CO2 concentrations of 300–320 mg/L for a 15 min interval was found to kill 20% and 93% of the rainbow trout at water temperatures of 14 and 19 °C, respectively (Gelwicks et al., 1998). Chronic exposure to more moderate concentrations of dissolved CO2 (e.g., 34.5 ± 3.8 and 48.7 ± 4.4 mg/L) have been reported to reduce rainbow trout growth compared to control fish cultured at 22.1 ± 2.8 mg/L of CO2, although survival remained at or close to 100% for all treatment groups (Danley et al., 2005). Somewhat analogous results were reported in post-smolt Atlantic salmon cultured in freshwater (Martens et al., 2006) and seawater (Fivelstad et al., 1998). However, Fivelstad et al. (1999) reported that condition factor of Atlantic salmon smolt was significantly reduced with chronical exposure to CO2 concentrations of 15–20 mg/L. Nephrocalcinosis has also been reported in fish chronically exposed to moderate concentrations of CO2 (Landolt, 1975; Smart et al., 1979; Fivelstad et al., 1999). However, short-term exposure of fish to low to moderate concentrations (e.g., 5–12 mg/L) of dissolved CO2 does not appear to have long-lasting, detrimental effects on fish health.

The U.S. Food and Drug Administration (FDA) has designated CO2 a low regulatory priority drug for anesthesia of cold, cool, and warmwater fish. Therefore, CO2 may be used as an anesthetic as long as FDA guidelines for LRP drugs are followed. Additionally, because CO2 leaves no toxic by-products, fish that have been exposed to dissolved CO2 can be consumed immediately, without any requirements for a withdrawal period to flush CO2 from live fish. The withdrawal period is the interval between the time of the last administration of a regulated compound and the time when the animal can be safely slaughtered for food. In addition, commercially farmed salmonids are sometimes anesthetized in an ice-slurry containing high concentrations (200–1000 mg/L) of dissolved CO2 during harvest (Erikson et al., 1997; Mørkøre et al., 2002; Robb and Kestin, 2002; Jitinandana et al., 2005; Poli et al., 2005). Use of moderate CO2 concentrations (37–80 mg/L) immediately following live chilling and just before cutting the gill arches has been reported to reduce handling stress for Atlantic salmon in a commercial slaughter line (Erikson et al., 2006). They
reported that exposing fish to moderate CO₂ concentrations immediately following live chilling produced a carcass with a long pre-rigor period (about 24 h). This period provided ample time for pre-rigor processing and was considered to be superior to the short pre-rigor period produced by the traditional method that uses very high levels of CO₂ in a relatively crowded tank (Erikson et al., 2006).

CO₂ is an extremely soluble gas that is commercially available and easy to dissolve in water. CO₂ gas is sometimes added to drinking water, industrial waters, and wastewaters in a process termed ‘recarbonation.’ Because CO₂ gas is much more soluble than O₂ gas, CO₂ gas can be transferred effectively into water within the same unit processes used to dissolve pure O₂ gas. In addition, stripping excess dissolved CO₂ is practical using conventional gas transfer equipment, as long as high volumetric flows of air:water are maintained (Grace and Piedrahita, 1994; Summerfelt et al., 2000, 2003).

The objective of this work was to test whether rainbow trout that avoid water containing elevated concentrations of dissolved CO₂ could be induced to swim through a CO₂ gradient and to an area containing a relatively low concentration of CO₂, such as a pipe leading to another culture tank or a harvest tank.

2. Materials and methods

Four experiments were conducted to test the hypothesis that the avoidance behavior of rainbow trout to high concentrations of dissolved CO₂ can be used to transfer fish between tanks. A fifth experiment was conducted to determine the time required for rainbow trout to lose their equilibrium at dissolved CO₂ concentrations of 90, 120, and 150 mg/L, when the fish were not provided with the option of moving to an environment containing a lower dissolved CO₂ concentration.

2.1. Water quality monitoring

At time 0 for each trial in all experiments, samples for dissolved CO₂ concentration were taken from both tanks using a peristaltic pump or siphon tube to reduce agitation and loss of dissolved gas. Dissolved CO₂ was measured according to standard methods (APHA, 1998). Dissolved O₂, pH, and temperature levels were measured in both tanks every 10 min. In the growout tank, a Hach SC100 Universal Dual-Channel Controller (Hach Company, Loveland, Colorado) was used with Hach digital pH sensor and digital low dissolved O₂ probe with temperature sensor. In the harvest tank, an OxyGuard 1 Single Channel Oxygen Meter (OxyGuard International A/S, Birkerod, Denmark) with temperature-calibrated dissolved oxygen probe (Hach Company) was used, and pH and temperature monitored using a YSI Model 60 Hand-Held pH Meter (YSI Inc., Yellow Springs, Ohio).

2.2. Research system description

Two 3.7 m diameter (11.1 m³ volume at 1.07 m depth) circular culture tanks were used for the experiments. One tank represented a growout tank and the other represented a harvest tank (Fig. 1). The tanks were connected by a transfer pipe that was 1.35 m long × 203 mm diameter in Experiments 1 and 2 and 1.35 m long × 406 mm diameter in Experiments 3 and 4 (Fig. 2). Rainbow trout density in the growout tank
was 40–60 kg/m³. When the experiments were not occurring, water flowed at approximately 370 L/min (98 gpm) through the growout tank in a single-pass, which provided a mean hydraulic exchange rate of one tank volume every 30 min. Dissolved O₂ concentration in the growout tank was maintained at 100–120% saturation by controlling the addition of pure O₂ gas in a down flow bubble contactor located on a 300 L/min side-stream flow pumped from the tank’s sidewall discharge box and returned to the tank at a different location (Fig. 1). This oxygenated and recarbonated (during CO₂ avoidance trials) side-stream flow was re-introduced into the culture tank through a 90° elbow that was (a) oriented horizontally, i.e., the inlet was parallel to the floor of the culture tank, (b) pointed 45° past the tank wall tangential, i.e., directed between the center of the tank and the adjacent wall, and (c) located on the opposite side of the culture tank from the transfer pipe. The location and orientation of the inlet flow was selected to produce circular rotating flow and optimize mixing across the growout tank. Dissolved CO₂ concentration in the harvest tank was maintained near 10 mg/L (initially at 6–9 mg/L before fish entered the tank, but increasing to 10–15 mg/L when the tank filled with fish) by pumping a sidestream of water from the tank’s sidewall box, through a 38 cm diameter x 2 m tall column packed with 5 cm diameter random packing. This sidestream flow was then returned to the harvest tank (Fig. 1). Before this sidestream stripping column was added during Experiment 1, water exiting the harvest tank contained up to 23 mg/L of dissolved CO₂. Feed was withheld from fish 12–24 h before each trial. They were not fed during the trials to keep feeding response from biasing results.

The following procedures were used to begin a trial:

- Water flow rate for the harvest tank was set to approximately 370 L/min.
The plug over the transfer pipe (Figs. 1 and 2) was removed. The bottom drain stand-pipe on the harvest tank was capped. All the water entering the harvest tank then flowed through the fish transfer pipe and into the growout tank (Fig. 1). During Experiments 1 and 2, the velocity was approximately 19 cm/s through the 203 mm diameter connection pipe. This velocity provided approximately 1/2-body length per second swimming speed for a 38 cm long fish. During Experiments 3 and 4, velocity was approximately 5 cm/s through the 406 mm diameter connecting pipe (approximately 1/8-body lengths per second).

- The single-pass water that normally entered the growout tank was turned off. This water flow requirement was now being met by water flowing from the harvest tank.

- The valve was opened to add pure CO₂ gas at the down flow bubble contactor treating the sidestream flow from the growout tank.

Time zero \((T = 0)\) occurred when the fish began crowding and moving through the ‘fish transfer’ pipe at a pH of approximately 6.7–6.8 \((\text{CO}_2 \text{ beginning to exceed } 50 \text{ mg/L})\).

During each trial, control valves were used to manually adjust the amount of O₂ and CO₂ gas that were added to the down flow bubble contactor to achieve the desired dissolved O₂ concentration and pH, respectively, within the growout tank. The pH and dissolved O₂ probes were placed near the growout tank’s sidewall drain (Fig. 1). The pH was continuously monitored to provide a real time approximation of the dissolved CO₂ concentration in water. Dissolved CO₂ is controlled by the acid–base equilibrium within the total carbonate carbon system, i.e., CO₂, carbonic acid \((\text{H}_2\text{CO}_3)\), bicarbonate \((\text{HCO}_3^-)\), and carbonate ions \((\text{CO}_3^{2-})\) concentrations according to

\[
\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3, \quad \text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- + \text{H}^+, \quad \text{HCO}_3^- \leftrightarrow \text{CO}_3^{2-} + \text{H}^+
\]

The dissolved inorganic carbon system \((\text{CiCO}_3)\) is defined as

\[
[\text{CiCO}_3] = [\text{CO}_2] + [\text{H}_2\text{CO}_3] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}]
\]

with each \([\ ]\) species represents a molar concentration.

Because water is more commonly classified based on its alkalinity, rather than on its CiCO₃, the dissolved CO₂ concentration can be calculated as a function of the water’s pH, alkalinity, and temperature, where temperature affects the equilibrium constants \(K_w, K_o, K_1, K_2\) (defined elsewhere; Summerfelt et al., 2000):

\[
\text{CO}_2 (\text{mg/L}) = 44,000 \left\{ \frac{\text{Alk}(\text{mg/L}\text{CaCO}_3)}{50,000} - 10^{(p\text{H} - pK_w)} - 10^{(-p\text{H})} \right\}
\times \left\{ \frac{1}{10^{(p\text{H} - p(K_wk_1))} + 2 \times 10^{(2p\text{H} - p(K_wk_1)pK_2)}} \right\}
\]

Because the alkalinity and temperature of the single-pass system water were relatively constant during the study, pH could be used as an accurate measure of dissolved CO₂ concentration. Titrated CO₂ levels were

Fig. 2. Fish transfer pipes that were used to connect the growout tank and harvest tank in Experiments 1 and 2 (203 mm diameter pictured above) and in Experiments 3 and 4 (406 mm diameter pictured below). The air burst fitting is also shown.
plotted against pH, allowing us to predict CO₂ concentration from pH (Fig. 3).

Fish survival and behavior were monitored during, and for the 48 h following, each trial. At the end of each trial the transfer pipe was plugged, and the stand-pipe drain in the harvest tank was uncapped. The fish that swam from the growout tank to the harvest tank during the trial were then counted or weighed (depending on the group) and returned to the growout tank. When less than approximately 85% of the fish swam from the growout tank to the harvest tank, fish were weighed back into the growout tank. Using the known number of fish present at the start of the trial (this number was obtained either by counting or bulk weights), we were then able to calculate the total number of fish that had moved during the trial. When greater than 85% of the fish moved during the trial, the fish remaining in the growout tank were counted, allowing us by subtraction to determine the total number of fish that had moved. Counting was done either visually or by counting fish in pictures taken from high angles. Subsequent trials were conducted after a ≥48 h recovery period.

2.3. Experiment 1

Eight trials were performed to help identify the best experimental conditions for fish transfer. One group of rainbow trout at a culture density of approximately 60 kg/m³ with average size of 1.3 kg/fish were exposed, first, to replicate 2 h trials with no increase in dissolved CO₂ (control), second, to replicate 2 h trials at a dissolved CO₂ concentration of 45 mg/L, and, third, to repeated 2 h or longer trials at a dissolved CO₂ concentration that ranged from 60 to 120 mg/L. In these latter trials, CO₂ concentration was quickly increased to 60 mg/L, until fish began moving steadily. For the remainder of each trial, CO₂ concentration was gradually increased to approximately 110–120 mg/L. Each condition was replicated three times. During each trial, water from the harvest tank flowed through the fish transfer pipe into the growout tank (Figs. 1 and 2). This 370 L/min flow contained a dissolved O₂ concentration of approximately 110–130% saturation and a dissolved CO₂ concentration that started out at <6–9 mg/L (when the harvest tank was empty) but increased to 10–15 mg/L as the tank filled with fish.

Using the same fish repeatedly could train them to swim out of the tank when they encounter changes in dissolved gas concentration. Therefore, all trials using the single group of rainbow trout were conducted in order of increasing CO₂ concentration. Ambient control trials were conducted first, 45 mg/L CO₂ trials were conducted second, and the repeated trials at 60–120 mg/L CO₂ were conducted last to protect the fish from the worst conditions until the end and to reduce the possible effect of training.

After determining that rainbow trout avoided and swam out of CO₂ concentrations of approximately 60 mg/L (Fig. 4), the fish were exposed to eight 2 h or longer trials, where the dissolved CO₂ concentration in each trial was gradually increased to 60 mg/L and then to 110–120 mg/L by the end of each trial (Fig. 5).

After fish entered the transfer pipe, many seemed hesitant about moving through it and into the harvest tank, so a slight change was made between each trial in an attempt to identify the best conditions. Before Trial 3 began, a heavy (10 mm), grey tarp was used to cover the harvest tank to create a dark, possibly less intimidating environment. Also, before the start of the fourth trial, air bursts were added to the transfer pipe 15 cm from the growout tank to further encourage fish to swim out of the pipe and into the harvest tank (Fig. 2). Air bursts were supplied by compressed air that was released by a solenoid valve that opened for 1 s every 1 min. Before beginning Trial 5 of Experiment 1, a gas-stripping column was placed over the harvest tank (Fig. 1) to reduce CO₂ concentrations to ≤15 mg/L in the water flowing through the transfer pipe.

2.4. Experiment 2

Experiment 1 used the same group for each trial. At least partially confounded results could have been produced as the fish learned to avoid the increased CO₂ environment. Therefore, in Experiment 2, the lowest CO₂ concentrations at which the fish had responded were used with three groups of naïve rainbow trout. For each group of naïve fish, the treatment was replicated...
three times. These trials on naïve fish determined the effect repeated exposure to changes in dissolved gas concentrations had on the rate of fish transfer. Another objective of Experiment 2 was to determine the approximate percentage of tank evacuation a culturist could expect the first time a naïve group of fish is exposed to increased CO₂.

Rainbow trout used in Experiment 2 were held in the growout tank at densities of 41–47 kg/m³ and had a mean weight of 150–250 g/fish. The first group tested was smallest and the last group tested was largest, because the fish grew while they were being held for the trials.

The time period for each trial ranged from 6 to 7 h, which provided more time for fish to swim from the growout tank to the harvest tank than in Experiment 1. Fish movement was at or near zero by the end of these time periods.

A mass flow meter (Aalborg, Orangeburg, NY) was used to determine CO₂ gas supplied during fish transfer trials. The flow meter was attached to the CO₂ inflow line. The flow meter was factory calibrated for use with CO₂ and was manually calibrated to “zero” after a 15 min warm-up period with no gas flow. This flow meter was used first during the last trial of Experiment 2, but was used in all subsequent experiments.

2.5. Experiment 3

The small diameter (203 mm) fish transfer pipe used in Experiments 1 and 2 appeared to affect the rate at which fish were able to move from the growout tank to the harvest tank, which caused the fish to pile-up in the growout tank at the entrance to the fish transfer pipe (Fig. 4). To determine if exit rate was impeded by...
the small pipe, Experiment 3 used a larger diameter (406 mm) fish transfer pipe (Fig. 2).

Three trials were conducted with a group of naïve fish (mean weight = 400 g) held at similar densities as Experiment 2. During these trials we used similar methods as in Experiment 2. The air bursts in the transfer pipe were not used because they appeared to be ineffective. Trial times were variable and dependent on fish movement. Trials were conducted for 3 h or until fish movement through the channel was zero. Trial time was shorter during this experiment because fish moved more quickly through the larger diameter transfer pipe.

2.6. Experiment 4

In the previous experiments, approximately 10–20% of the fish remained in the growout tank at the end of transfer. The remaining fish were primarily located within the plume of relatively low dissolved CO2 adjacent to the ‘fish transfer pipe.’ Experiment 4 was conducted to determine if the percentage of fish transferred could be increased by restricting the low CO2 water flow into the growout tank. After approximately 80% of the fish had moved from the growout tank to the harvest tank, the inflow to the harvest tank was reduced from 370 to 64 L/min. When less than 5% of the fish remained in the growout tank, flow was again reduced to 38 L/min.

One group of fish was used. This group of fish was used for three replicated CO2 avoidance tests. Mean fish weight was 430–490 g/fish. Trials were stopped when, after the flow was reduced to 38 L/min, fish transfer had ceased, or, if loss of equilibrium was apparent in the remaining population.

A fourth trial was also conducted with this group of fish. It was performed without a tarp covering the harvest tank in order to determine if the tarp actually aided in transfer efficiency.

2.7. Experiment 5

As trial time and CO2 concentration increased, the ability of fish to physically swim from the growout to the harvest tank was negatively affected. Therefore, Experiment 5 was done to determine how quickly various dissolved CO2 concentrations would affect rainbow trout equilibrium. Fish were exposed to 2 h of elevated dissolved CO2 concentrations of 90, 120, and 150 mg/L. Fish were not provided an opportunity to move to an area of lower dissolved CO2 concentration. Each treatment was replicated three times, using a total of nine groups of fish. In each replicate, approximately 65 large fish (mean weight 1.3 kg), and 65 small fish (mean weight 0.150 kg) were used. The larger trout were the same fish used in Experiment 1. The small trout were naïve fish that, up until this point, had not been exposed to artificially high levels of CO2. Both groups (small and large) were placed into the experimental tank at the same time.

For each replicate, ambient dissolved CO2 concentration was increased after the large and small trout were put into the growout tank. The rate of CO2 addition (L/min) was standardized across all treatments. Dissolved CO2 was added slowly to the tank. When the target level was reached, CO2 gas flow was turned off. Additional CO2 gas was unnecessary, because there was no water flowing through the tank. This condition was maintained for 120 min. Dissolved O2 was maintained at 10 ± 1 mg/L using a combination of the down-flow bubble contactor and O2 diffusers which turned on at a set point of 10.5 mg/L.

Fish behavior was monitored throughout each trial. When a fish lost equilibrium and rolled, with ventral side up, it was removed from the tank, and time and fish size were recorded. The fish was then placed into a recovery tank containing water with a dissolved CO2 concentration less than 15 mg/L. The order of the nine trials was randomized to avoid effects related to the day of the trial.

2.8. The CO2 mass balance

A non-steady state mass balance on CO2 can prove a critical tool for predicting how much CO2 gas should be added to achieve a specific dissolved CO2 concentration within a specific water flow and culture tank volume. A non-steady state mass balance can be derived to estimate CO2 concentration that will accumulate within a continuous-flow, homogeneously mixed fish culture tank, i.e.:

\[
\left( \frac{dC}{dt} \right)_{\text{Accumulation}} = \left( M_{\text{gas, in}} \right)_{\text{Inflow}} - \left( Q \right)_{\text{Outflow}} + \left( r_{\text{CO2}} \right)_{\text{Generation}}
\]

where \( C \) is the change in concentration of CO2 in a mixed culture tank (g/m³) (same as mg/L), \( V \) the operating volume of culture tank (m³), \( Q \) the volumetric flow rate of water (m³/s), \( r_{\text{CO2}} \) the rate of CO2 reaction in the culture volume (g/(s m³)), and \( M_{\text{gas, in}} \) is the mass flow rate of CO2 gas into the culture tank (g/s).

When applying this mass balance, it requires accepting and recognizing several assumptions that are only approximately correct, e.g.:
• The culture tank water volume (V) can be assumed to be homogeneously mixed. This is not entirely correct, because the dissolved CO₂ concentration was relatively low in the vicinity of the low CO₂ water flow entrance and the dissolved CO₂ concentration was relatively high in the vicinity where the high CO₂ water flow enters the tank.

• The rate of CO₂ reaction (rCO₂) in the culture volume is neglected. The mass balance does not account for dissolved CO₂ concentration shift produced by acid–base equilibrium within the dissolved inorganic carbon system. Rather, the mass of CO₂ dissolved into the water is equal to the difference between in tank influent and effluent concentrations of total carbonate carbon (Grace and Piedrahita, 1994). Dissolving CO₂ gas within the down flow bubble contactor results in a shift in pH and a new fractionation of the total carbonate carbon system into its various forms, i.e., CO₂, H₂CO₃, HCO₃⁻, and CO₃²⁻. Acid–base equilibrium sets the relative fraction of each component as a function of the water’s pH and temperature.

• The mass of CO₂ gas that volatilizes and degasses out at the tank’s air–water interface can be assumed to be negligible, because volatilization would be minimal compared to the Mgas,in.

• The volumetric flow rate of water (Q) through the culture tank can be assumed to remain constant.

Now that the imprecision inherent in the use of the CO₂ mass balance approach has been recognized, the mass balance can be used to better understand how Mgas,in, Q, and V affect the CO₂ concentration change as a function of time (t). Integrating Eq. (3) provides an estimate of the Mgas,in that must be injected into the down flow bubble contactor in order produce the desired CO₂ concentration change, for a given Q and V, within the culture tank after a given time, i.e.:

\[ C = \left( \frac{M_{\text{gas,in}}}{Q} \right) \left( 1 - e^{-\frac{t}{Q/V}} \right) \]  

(4)

The concentration of dissolved CO₂ (C) in the culture tank at any given time (t) after CO₂ gas injection begins can then be estimated by accounting for the CO₂ concentration change produced by CO₂ gas injection (C; Eq. (4)), the CO₂ concentration entering the culture tank (Cin), and the CO₂ concentration produced by fish respiration (Cresp), i.e.:

\[ C_t = C + C_{\text{in}} + C_{\text{resp}} \]  

(5)

or

\[ C_t = C_{\text{in}} + C_{\text{resp}} + \left( \frac{M_{\text{gas,in}}}{Q} \right) \left( 1 - e^{-\frac{t}{Q/V}} \right) \]  

(6)

Note: C_in would be constant in a flow-through aquaculture application, but would increase with time in a water reuse system; C.resp is proportional to the consumption of dissolved oxygen across the culture tank at any given time (Colt et al., 1991); C.resp can be estimated by measuring the CO₂ concentration change across the culture tank (under steady state conditions) before CO₂ gas injection begins; and C.resp can be calculated (under steady state conditions) if the rate of CO₂ generation due to fish metabolism (mCO₂) in the culture volume is known, i.e.:

\[ C_{\text{resp}} = \frac{m_{\text{CO}_2} V}{Q} \]  

(7)

where mCO₂ is the rate of CO₂ generation due to fish metabolism in the culture volume (g/(s m³)).

After CO₂ gas injection has occurred for a time sufficient to achieve steady-state conditions, there would be no change in culture tank CO₂ concentration (i.e., no CO₂ accumulation), thus, CO₂ input would equal CO₂ output and the exponential term in Eqs. (4) and (6) would go to zero, i.e.:

\[ C_{t=\infty} = C_{\text{in}} + C_{\text{resp}} + \left( \frac{M_{\text{gas,in}}}{Q} \right) \]  

(8)

Once the steady state CO₂ concentration has been achieved, if the flow of CO₂ gas is stopped, Eq. (9) can be used to predict the time it would take to dilute CO₂ back to a given concentration throughout the tank, i.e.:

\[ C_t = C_{\text{in}} + C_{\text{resp}} + \left( \frac{M_{\text{gas,in}}}{Q} \right) e^{-\frac{t}{Q/V}} \]  

(9)

where t is now the time after the flow of CO₂ gas is terminated.

2.9. Statistical analysis

All statistical tests were performed using R (R Development Core Team, Vienna, Austria). To analyze the difference between control treatments and experimental treatments, a t-test was used. Using the Bonferroni correction factor, significant α = 0.016 for these tests. Analysis of variance (ANOVA) was used to test differences among Experiments 2–4 and within Experiment 2 with a significant α = 0.05. If the ANOVA showed a significant difference, Scheffe’s multiple comparison test (α = 0.05) was then used to
analyze significant differences between experimental treatments.

3. Results

3.1. Experiment 1

Less than 1% of the fish (0–3 fish) moved from the growout tank to the harvest tank within a 2 h period during each of the replicated control trials. Results were the same for the two trials at a 2 h sustained CO2 concentration of approximately 45 mg/L.

During the remaining trials for Experiment 1, CO2 was added at a rate of 6–14 L/min to avoid acclimation and to induce the fish to respond. Fish movement began to occur when the dissolved CO2 concentration in the growout tank reached 60 mg/L. When fish first began the CO2 avoidance response, they congregated in front of the transfer pipe inlet supplying low dissolved CO2 water to the growout tank (Fig. 4). When they started to move through the channel at a high rate, the dissolved CO2 concentration was temporarily held constant, typically for a time period of 20–50 min. When the rate of fish movement slowed, CO2 concentrations were gradually increased at intervals throughout the trial (Fig. 5) to reduce fish acclimation and to maximize fish movement through the transfer pipe. Using this technique, the best range of fish movement was produced when the dissolved CO2 concentration was gradually increased from 60 to 120 mg/L (pH ~ 6.72–6.41), and by the end of Trial 5, over 80% of the fish were voluntarily swimming to the harvest tank within a 4 h exposure when CO2 concentration was 60–100 mg/L.

During Trials 6 through 8, the maximum CO2 concentration was 120 mg/L and 90–94% of the rainbow trout swam into the harvest tank within 4 h (Fig. 6). Almost all of the fish remaining in the growout tank were congregated in front of the inlet to the transfer pipe. A profile of the dissolved CO2 concentration across a vertical cross-section that bisects the 11 m³ circular culture tank (Fig. 7) indicates that a narrow

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Quick CO2 Addition, 2-h trial period with thin tarp over harvest tank to exclude light</td>
</tr>
<tr>
<td>2</td>
<td>Quick CO2 Addition, 2-h trial period but leveled off more quickly with thin tarp over harvest tank to exclude light</td>
</tr>
<tr>
<td>3</td>
<td>Quick CO2 Addition, 2-h trial period but leveled off more quickly, increased as fish acclimated, single heavy tarp - harvest tank very dark</td>
</tr>
<tr>
<td>4</td>
<td>Same as trial #3, 2-h trial period but used a burst of air in the channel to encourage fish to move through quicker</td>
</tr>
<tr>
<td>5</td>
<td>Same as trial #4, but used a longer (4-h) trial period and began using a CO2 stripping column in the harvest tank to bring CO2 down to 6-9 mg/L in the ‘fish transfer’ pipe</td>
</tr>
<tr>
<td>6</td>
<td>Same as trial #5</td>
</tr>
<tr>
<td>7</td>
<td>Same as trial #5, but run shorter because fish were done moving (94% moved, remaining fish wouldn’t move)</td>
</tr>
<tr>
<td>8</td>
<td>Same as trial #5, but run shorter because fish were done moving (94% moved, remaining fish wouldn’t move)</td>
</tr>
</tbody>
</table>

Fig. 6. Transfer rate and percent of rainbow trout transferred from the growout to the harvest tank in each trial of Experiment 1. The different conditions tested in each trial are described in the accompanying table.
zone of relatively low CO2 water is confined to an area immediately in front of the relatively low CO2 inlet at the channel connecting the two tanks. Yet, the dissolved CO2 concentration across the remainder of the tank is relatively constant at approximately 120–130 mg/L (Fig. 7). The remaining fish were, by-and-large, remaining within the area containing the low concentration of dissolved CO2 and did not appear to have any incentive to move through the transfer pipe without a large number of fish pushing them from behind. From a harvest stand-point, these remaining fish were located in a position where they could be removed with a fish pump or a brail net. Alternatively, an engineering solution could be developed to help crowd these fish into the transfer pipe. Five fish (less than 1% of the total number of fish) died within 48 h of the end of Trial 1. As mortality only occurred during the first exposure of this group to the elevated CO2 conditions, these fish may have died from sub-clinical fish health problems when exposed to elevated CO2 concentrations. In subsequent high CO2 trials, an occasional 1–2 fish would lose equilibrium during each trial, but no more than 1–2 (less than 0.50% of total fish) deaths occurred within the 48 h post-trial period.

3.2. Experiment 2

In Experiment 1, the same group of fish had been used for all trials. In each successive trial, fish became more responsive to the changing CO2 concentrations and moved out of the tank more quickly than in the previous trial. This behavior suggested that fish were learning from experience in previous trials. Experiment 2 used naïve groups of fish to prevent learned behavior from affecting the results.

The mean fraction of fish moving during Experiment 2 was significantly greater than control trials \( (P < 0.0001) \). In Experiment 2, approximately 80% of the rainbow trout swam from the growout tank, through the transfer pipe, and into the harvest tank within a 7 h period during their first exposure to high CO2 conditions (Fig. 8). There was no significant difference in the fraction \( (P = 0.59) \) of the fish that moved among all nine trials (Fig. 8) in this experiment. The only irregularity in the data occurred during the first trial with the first group of fish. Compared to the other trials, movement of fish in Trial 1 of naïve Group 1 through the channel was not maximized, most likely due to a suboptimal carbon dioxide concentration. As compared to larger fish, smaller fish appeared to have increased tolerance for high levels of dissolved CO2, which was tested later in Experiment 5. This increased tolerance increased the effective range of dissolved CO2 higher than expected. In subsequent trials, CO2 concentrations were increased to account for this observation. Since this was an anomalous data point...
and treatment differed slightly from trial 1 in subsequent fish groups from this point forward in the analysis, this single point was not included.

Time interval for each exposure varied (7, 6.5, and 6 h), and the number of fish in the system varied from group to group. Therefore, the percentage of fish that moved through the channel per hour was estimated, but this data is not shown. There seemed to be a general increase in the rate of fish movement during subsequent exposures, but this difference was not statistically significant ($P = 0.21$).

The volumetric flow of CO$_2$ that was added to achieve the desired dissolved CO$_2$ levels within the growout tank was recorded (Fig. 5). The dissolved CO$_2$ concentration increased in proportion to increases in the volumetric flow of CO$_2$ gas added, as would be expected from Eqs. (4) and (6).

During each of the nine trials in Experiment 2, 3.75–3.95 m$^3$ of CO$_2$ was added to the growout tank to obtain the desired CO$_2$ concentrations. A liquid CO$_2$ dosers bottle with a unit cost of $1/m^3$ of CO$_2$ (at standard conditions) produces a total CO$_2$ cost of less than $4 per fish transfer event in the 11,100 L tank. That is a price of approximately $0.36 per 1000 L of tank capacity per fish move.

By the end of Experiment 2, it was apparent that the fish congregating in front of the transfer pipe entrance were impeded from exiting the growout tank by the relatively small (203 mm) pipe diameter, reducing the rate that fish exited the tank. Therefore, a larger (406 mm) diameter transfer pipe was used in Experiment 3.

### 3.3. Experiment 3

Percentage of fish movement again was significantly greater than control trials ($P = 0.010$). During the trials that were conducted with the 406 mm pipe (Fig. 9), there was no significant increase in the percentage of fish that moved ($P = 0.075$). However, the fish moved in about half the time that was required with the use of the 203 mm pipe, i.e., fish transfer was complete in 3 h with the larger pipe compared to 6–7 h with the smaller diameter pipe. This hourly transfer rate was statistically significant at the $P = 0.05$ level (Fig. 9). This increased transfer rate reduced total transfer time, which reduced exposure time to high levels of dissolved CO$_2$.

### 3.4. Experiment 4

When the flow of low CO$_2$ water entering the growout tank was reduced near the end of each trial, there was a significant increase in the percentage of fish that moved through the addition of CO$_2$ to the growout tank compared to control trials ($P < 0.0001$). Nearly all of the fish (>99%, Fig. 10) swam into the harvest tank when the flow of low CO$_2$ water entering the growout tank was reduced near the end of each trial. Apparently, the fish were induced to swim out of the growout tank and through the 406 mm diameter pipe as the zone of relatively low CO$_2$ water (Fig. 7) began to disappear. During this experiment, both the percentage and rate of fish transfer (Fig. 10) was significantly different from the percentage and rate encountered during Experiments 2 and 3 based on Scheffe’s test ran at $P = 0.05$. The remaining fish (circa 1%) within the growout tank looked lethargic and likely to be easily netted. During Trial 4 of Experiment 4, the tarp was not used to cover the harvest tank. Approximately 99% of the fish swam from the growout tank to the harvest tank within 3 h.
3.5. Experiment 5

During each exposure period, both large (1.3 kg) and small (0.15 kg) fish lost equilibrium during the 120 min trial period (Fig. 11) when a low CO₂ environment was not accessible. However, for a given concentration of dissolved CO₂, the large fish were more likely to lose equilibrium than small fish (Fig. 11). The lowest percentage of fish losing equilibrium occurred at the 90 mg/L concentration, when about 5% of the small fish lost equilibrium within the 120 min exposure period. The highest percentage of fish losing equilibrium occurred during the 150 mg/L trials where more than 99% of the large fish lost equilibrium (Fig. 11).

Minimal delayed mortality was associated with CO₂ exposure. Within 24 h of these toxicity trials, 1 to 3 large fish died after the higher (120 and 150 mg/L) treatment levels, but none of the small fish died during Experiment 5.

4. Discussion

4.1. Fish transfer system

Avoidance of certain concentrations of dissolved CO₂ has been previously demonstrated (Summerfelt and Lewis, 1967; Bil’ko and Kruzhlina, 2000; Ross et al., 2001; Oelssner et al., 2002). Ours is the first attempt to use this avoidance behavior as a mechanism to promote fish self-transfer.

Our results indicate that using dissolved CO₂ can provide an efficient means of transferring fish in deep, circular culture tank systems that can be thoroughly mixed. The majority of the fish present in the growout tank swam into the harvest tank, even during initial trials with less than ideal conditions. The transfer rate
was doubled by using a larger diameter transfer pipe, and the overall percentage of fish transferred was increased to nearly 100% by reducing the flow of low CO₂ water into the growout tank near the end of the trial. This reduction in transfer time reduces cost and most likely fish stress, although fish stress during this procedure still needs to be quantified. By achieving nearly 100% transfer over a 3 h period that ended with reduced flow, we demonstrated that the CO₂ avoidance method for fish transfer could be quick, effective, and require little labor, even when relying on the fish to voluntarily swim out of the culture tank.

Both transfer pipes were opaque and the harvest tank was darkened by covering the tank with a heavy tarp. Covering the harvest tank with a tarp seemed to overcome some of the hesitancy of fish that had swum through the transfer pipe and arrived at the entrance into the harvest tank. In fact, many fish would not leave the transfer pipe until they were pushed from behind by other fish. Hesitation to enter the harvest tank was reduced when the tarp was used. When the tarp was partly removed from the harvest tank most of the fish stayed in the area that was covered. This observation was contrary to results of the 4th trial in Experiment 4, where 99% of fish moved despite the absence of the tarp on the harvest tank. This result indicates that either the fish were so well trained that they swam across into the uncovered tank anyway or that the light intensity in the harvest tank did not affect fish behavior during transfer. Further research would be required to determine if light intensity actually influences the rate of fish movement into the harvest tank.

The air burst used in the smaller diameter transfer pipe increased the rate of movement through to the harvest tank. When the air burst was applied, air bubbles moved in both directions along the pipe because it was always completely filled with water. The bubbles stimulated the fish in the pipe to move toward the harvest tank. A few of the fish that were at the entrance to the transfer pipe would retreat, but only momentarily. The air burst used with the larger diameter transfer pipe was ineffective. The larger pipe was never completely filled with water, i.e., the top 3 cm was not submerged, so all the air escaped the water directly at the site of the air burst.

Fish transfer with the aid of dissolved CO₂ also proved to be very cost efficient. Carbon dioxide can be introduced into the system via existing down flow bubble contactors with only minimal modifications. During the trials using the larger pipe (best conditions), approximately 3.75–3.95 m³ of CO₂ was added to obtain the necessary CO₂ concentrations within the growout tank. The unit cost of CO₂ we used was $1/m³. This means that the cost of CO₂ for a single fish transfer was less than $4 for our 11,100 L tank, and that the price of CO₂ per 1000 L of tank capacity is only $0.36 per transfer event.

These experiments proved the concept that CO₂ avoidance can be used to transfer fish out of a relatively modest sized 11 m³ culture tank. However, in spite of this preliminary success, considerable design ingenuity and optimization may be required to successfully apply this technology and achieve acceptable harvest times when using larger commercial-scale culture tanks, e.g., those ranging in size from 50 to 1000 m³ per tank. To achieve more rapid fish transfer rates in larger culture tanks, we suggest coupling the CO₂ avoidance method for fish transfer with a fish pump or brail net. Because fish congregate in front of where the low CO₂ flow enters the tank, a fish pump or brailing operation placed at this location would simplify and speed the task of transferring the fish from a large culture tank to another location. However, whether or not the fish are swum, pumped, or brailed out of the tank, a sufficiently large flow of low CO₂ water flow must be supplied to create sufficient volume of relatively low CO₂ water where fish can congregate as they move (or are moved) out of the tank. Also, the CO₂ avoidance method for fish transfer requires equipment to transfer sufficient CO₂ gas (and possibly O₂ gas) into the tank, such as down flow bubble contactors or micro-bubble diffusers. Furthermore, the culture tank design must create a circular water rotation that will, in turn, create relatively thorough water mixing, except for in the tank region immediately in front of the low CO₂ water inlet. Although the CO₂ avoidance technique could be applied in static ponds or plug-flow applications in raceways and ocean net pens, additional work will be required to develop an appropriate procedure for environments that are not mixed.

4.2. Behavioral observations

The adverse effects of CO₂ on fish health and physiology in fish culture are well known (Smart et al., 1979; Wedemeyer, 1996; Gelwicks et al., 1998; Fivelstad et al., 1999; Danley et al., 2005; Martens et al., 2006). Rainbow trout can restore physiological homeostasis and acclimate to relatively moderate increases in CO₂ (Eddy et al., 1977; Gelwicks et al., 1998). However, elevated levels of environmental CO₂ can result in increases in blood CO₂, i.e., hypercapnia, and reduce the O₂ carrying capacity of hemoglobin due to the Bohr effect. Elevated CO₂ levels cause a drop in
blood pH, which results in a decrease of the hemoglobin-oxygen binding affinity, leading to respiratory distress. In severe cases, low blood pH can decrease the maximum capacity of hemoglobin to bind O₂ (Root effect). Even at high levels of O₂, the Root effect will prevent hemoglobin from fully binding to O₂. Fish under respiratory distress will actively seek out more favorable environmental conditions or, if that option is not available, reduce activity in an effort to decrease O₂ requirements (Schjolden et al., 2005; van Raaij et al., 1996; Wedemeyer, 1996).

Anras and Lagardere (2004) reported that rainbow trout held at a density of 80 kg/m³ exhibited both stationary and circular swimming trajectories. Fish densities in our study ranged from 41 to 60 kg/m³ and swam into the water current as it rotated about the circular tank. Under normal (i.e. no CO₂ addition, >100% saturation O₂) conditions, fish were observed holding position in the current but in general were more likely to slowly swim against the current in a circular pattern around the tank. Fish swimming behavior changed when CO₂ addition began. As CO₂ concentrations rose from 35 to 60 mg/L (pH 7.20–6.95), the fish began to break out of the uniform circular swimming pattern and display more chaotic swimming behavior, apparently similar to what Anras and Lagardere (2004) reported for fish held at very high densities (i.e., 136 kg/m³). However, when CO₂ concentrations were maintained at 45 mg/L throughout the culture tank, fish did not congregate at the entrance of the fish transfer pipe, even when low CO₂ inflow occurred.

Swimming behavior changed again when dissolved CO₂ increased to levels >60 mg/L and fish began to congregate at the entrance of the transfer pipe (Fig. 4) where low CO₂ inflow occurred (Fig. 7). As dissolved CO₂ in the growout tank increased, fish crowded into the small area (Fig. 4) containing water with low levels of dissolved CO₂ (Fig. 7), eventually pushing their way into the flow of low CO₂ water flowing through the fish transfer pipe. Due to the bottleneck created at this point, some fish were pushed away from the pipe inlet. These displaced fish would drift back with the current before repositioning themselves toward the clean water inlet. During each trial, a small number of fish (approximately 5–20) tended to swim lazily just under the surface of the water in the middle of the tank or drift with the current. Occasionally, a few fish would also lay motionless, but upright on the bottom of the tank. None of these relatively listless fish would crowd into the clean water inlet and, at the end of Experiment 4, these fish were the approximately 1% of the original tank population that could not be induced to swim out of the tank.

The observed behavioral responses to increased CO₂ levels tended to follow the two different stress-coping strategies shown in animals. These two stress-coping strategies are identified by a marked difference in locomotion and overall energy expenditure when an animal is exposed to a stressor. With the active strategy, animals use increased motion (i.e., swimming behavior) to try to seek out a more favorable environment, while with the passive, or “wait and see” strategy, animals conserve energy by becoming motionless in an attempt to allow conditions to improve (Pottinger et al., 1994; van Raaij et al., 1996; Schjolden et al., 2005). Outside of an occasional and brief darting response, fish exhibiting the active strategy would swim normally (i.e., aerobic metabolism) rather than vigorously (i.e., anaerobic metabolism). In Experiment 4, approximately 99% of the fish exhibited the active strategy and crowded into the low CO₂ inflowing water in the transfer pipe to avoid the high CO₂ portions of the growout tank. In contrast, the passive or “wait and see” strategy was most likely exhibited by the approximately 1% of the fish that swarm lazily on the surface or lay motionless on the bottom in regions of the growout tank that contained relatively high CO₂ concentrations. Additional research could be performed to analyze whether there is a difference in the plasma cortisol response levels between these two groups of fish. Further research could also be used to compare the stress response of the CO₂ avoidance technique to more traditional harvest technologies. Although fish feed was not supplied during these trials, the fish that were congregating and swimming out of the growout tank would readily eat feed pellets when provided, which we think is a good indication that the fish were not unduly stressed.

4.3. Toxicity study

The results of the toxicity study show that rainbow trout of varying size have variable thresholds for CO₂ tolerance. This indicates that different CO₂ concentrations may be required for effective transfer of differently sized fish. However, more detailed studies are required to quantify the most effective CO₂ concentrations needed for fish transfer across all size ranges of fish, as well as for different species.

The results also indicate that exposing rainbow trout to dissolved CO₂ concentrations of 90, 120, and 150 mg/L caused these fish to lose equilibrium and in some instances die, when fish were not provided ready access to an environment containing a relatively low
concentration of dissolved CO₂. In comparison, Gelwicks et al. (1998) reported that 155 mg/L of dissolved CO₂ induced anesthesia in rainbow trout in less than 3 min of exposure at 14 °C, which was somewhat faster than observed in the present study (Fig. 11) at almost the same temperature. However, Gelwicks et al. (1998) also reports that anesthetized fish rapidly (i.e., <10 min) recovered normal swimming activity with no mortality when total exposure times were brief (i.e., <15 min), which is comparable to what was observed in the present study. In addition, Gelwicks et al. (1998) report that increased mortality occurred at warmer temperatures (i.e., 19 °C versus 14 °C), which suggests that caution would be prudent if the CO₂ avoidance method is used to move rainbow trout at water temperatures >14 °C.

4.4. CO₂ gas addition and CO₂ accumulation/flushing

Quasi-steady-state conditions were approached during fish transfer events when the slope of the CO₂ concentration versus time curve began to flatten out (Fig. 5), which occurred after the mass of CO₂ gas injected into culture tank water was held constant for approximately 100 min. Substituting the conditions tested during this study, i.e., \( C_{\text{in}} = 10 \, \text{g/m}^3 \) (i.e., 10 mg/L), \( C_{\text{resp}} = 3 \, \text{g/m}^3 \) (i.e., 3 mg/L), \( Q = 0.0062 \, \text{m}^3/\text{s} \) (370 L/min), \( V = 11 \, \text{m}^3 \), and \( M_{\text{gas, in}} = 0.49 \, \text{g/s} \) (15 L/min at standard conditions), into Eq. (8) provides an estimate of the dissolved CO₂ concentration produced in the culture tank after a quasi-steady state has been achieved, i.e., approximately 90 mg/L. According to Eq. (6), it would take approximately 65 min to come within 10% of the steady-state condition and approximately 135 min to come within 1% of the steady-state conditions in a culture tank operated in a flow-through manner. Alternately, if all inlet conditions were held constant for 30 or 60 min, then the CO₂ concentration produced in the culture tank at the end of each of those periods would be within approximately 67 or 88%, respectively, of the steady state value. Conversely, once the steady state CO₂ concentration has been achieved, if the flow of CO₂ gas is stopped, Eq. (9) can be used to predict the time it would take to dilute CO₂ back to a given concentration throughout the tank. For example, it would take approximately 73 min to dilute the mean dissolved CO₂ concentration back to 20 mg/L (i.e., 20 g/m³), once steady state had been reached under the conditions described above and the flow of CO₂ gas had been turned off.

Note that in a water reuse system, \( C_{\text{in}} \) (Eq. (6)) would increase with time, which in turn would change the time required to reach quasi-steady state conditions.

Eq. (4) can also be used to provide an estimate of the \( M_{\text{gas, in}} \) that must be injected into the down flow bubble contactor in order produce a desired CO₂ concentration within the culture tank after a given time, even when it is desired to change conditions, such as \( Q \), in order to decrease the zone of relatively low CO₂ water occurring in front of the fish transfer pipe. For example, if \( Q \) is reduced by 50%, then the \( M_{\text{gas, in}} \) can also be reduced proportionally (i.e., by 50%) in order to maintain the desired CO₂ concentration in the culture tank.

5. Conclusions

Dissolved CO₂ concentrations of 60–120 mg/L can stimulate rainbow trout to swim from a high carbon dioxide environment into an area that contains lower concentrations of dissolved CO₂. This response was used to induce the majority of rainbow trout to voluntarily swim from a “growout” tank, through a fish transfer pipe carrying water containing a CO₂ concentration of ≤10–15 mg/L and a dissolved O₂ concentration of 10–15 mg/L. This response was rapid (i.e., <10 min) recovered normal swimming activity with no mortality when total exposure times were brief (i.e., <15 min), which is comparable to what was observed in the present study. In addition, Gelwicks et al. (1998) report that increased mortality occurred at warmer temperatures (i.e., 19 °C versus 14 °C), which suggests that caution would be prudent if the CO₂ avoidance method is used to move rainbow trout at water temperatures >14 °C.

We conclude that the CO₂ avoidance technique can provide a convenient, efficient, relatively low cost, and reduced labor approach for fish transfer, especially in applications using large and deep circular culture tanks. We suspect that the technique produces relatively little stress on the fish, but further research is required to determine the stress response. However, more work will be required to successfully apply this technology to large commercial-scale culture tanks and more research is required to establish how the use of the CO₂ avoidance technique influences fish health and well-being in comparison with other fish transfer and handling techniques.

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References


