

## 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<sub>2</sub>). We observed this behavioral response during controlled, replicated experiments that showed dissolved CO<sub>2</sub> concentrations of 60–120 mg/L induced rainbow trout (*Oncorhynchus mykiss*) to swim out of their 11 m<sup>3</sup> “growout” tank, through a transfer pipe carrying a flow with ≤23 mg/L dissolved CO<sub>2</sub>, into a second 11 m<sup>3</sup> “harvest” tank. The research was conducted using separate groups of rainbow trout held at commercially relevant densities (40–60 kg/m<sup>3</sup>). 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<sub>2</sub> water (≤23 mg/L) entering the growout tank from the harvest tank, approximately 80–90% of the fish swam from the growout tank, through the transfer pipe, and into the harvest tank after the CO<sub>2</sub> concentration in the growout tank had exceeded 60 mg/L. The fish that remained in the growout tank stayed within the area of relatively low CO<sub>2</sub> 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<sub>2</sub> water near the end of each fish transfer event, restricted the zone of relatively low CO<sub>2</sub> 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<sub>2</sub> 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

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depth; 100–1000+ m<sup>3</sup>/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 (CO<sub>2</sub>) and/or acidity (H<sup>+</sup>) 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 CO<sub>2</sub>. More recent fish behavior research has also suggested that fish can sense and avoid areas with elevated concentrations of dissolved CO<sub>2</sub> (Bil'ko and Kruzhilina, 2000; Ross et al., 2001; Oelssner et al., 2002). Taking advantage of the CO<sub>2</sub> 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.

CO<sub>2</sub> is a by-product of respiration, naturally excreted by the fish through their gills, and is present in all natural waters. Dissolved CO<sub>2</sub> in excess of 20 mg/L, however, can begin to impair the transport of oxygen (O<sub>2</sub>) in the fish's blood due to the Bohr effect (Colt et al., 1991; Wedemeyer, 1996). Reduced O<sub>2</sub> transport to tissues and organs can create cerebral hypoxia, which produces the anesthetic effect reported by Gelwicks et al. (1998) at higher CO<sub>2</sub> concentrations. Dissolved CO<sub>2</sub> 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 CO<sub>2</sub> anesthetized fish recovered normal swimming activity in <10 min with no mortality, when total exposure times were <15 min. When rainbow trout anesthetized with CO<sub>2</sub> are returned to a low CO<sub>2</sub> environment, the CO<sub>2</sub> diffuses out of the gills and the fish rapidly recover from hypercapnia (Eddy et al., 1977). However, exposing rainbow trout to CO<sub>2</sub> 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 CO<sub>2</sub> (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 CO<sub>2</sub>, 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 chronic exposure to CO<sub>2</sub> concentrations of 15–20 mg/L. Nephrocalcinosis has also been reported in fish chronically exposed to moderate concentrations of CO<sub>2</sub> (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 CO<sub>2</sub> does not appear to have long-lasting, detrimental effects on fish health.

The U.S. Food and Drug Administration (FDA) has designated CO<sub>2</sub> a low regulatory priority drug for anesthesia of cold, cool, and warmwater fish. Therefore, CO<sub>2</sub> may be used as an anesthetic as long as FDA guidelines for LRP drugs are followed. Additionally, because CO<sub>2</sub> leaves no toxic by-products, fish that have been exposed to dissolved CO<sub>2</sub> can be consumed immediately, without any requirements for a withdrawal period to flush CO<sub>2</sub> 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 CO<sub>2</sub> during harvest (Erikson et al., 1997; Mørkøre et al., 2002; Robb and Kestin, 2002; Jittinandana et al., 2005; Poli et al., 2005). Use of moderate CO<sub>2</sub> 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

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