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# Impact of carbon dioxide level, water velocity, strain, and feeding regimen on growth and fillet attributes of cultured rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

Production and management variables such as carbon dioxide ($CO_2$) level, water velocity, and feeding frequency influence the growth and fillet attributes of rainbow trout (*Oncorhynchus mykiss*), as well as cost of production. More information is needed to determine the contributions of these variables to growth and fillet attributes to find the right balance between input costs and fish performance. Two studies, of 84 and 90 days duration, were conducted to determine the effects of $CO_2$ level, water velocity, and feed frequency on rainbow trout growth, fillet yield, and fillet quality. In the first study, two $CO_2$ levels (30 and 49 mg/L) and two velocity levels (0.5 and 2.0 body lengths/s) were tested. In the second study two $CO_2$ levels (30 and 49 mg/L) and two feeding regimens (fed once daily to satiation or three times daily to satiation) were tested. In the first study, after 84 days, fillet weight from high $CO_2$ tanks was 13.5% lower than the fillet weights of fish from low $CO_2$ tanks. Percent fat of fillets was higher in low $CO_2$ fish ($P = 0.05$) after 84 days and, fish from the low $CO_2$ treatment were larger ($P < 0.01$). Both studies had similar results in regards to fat content and weight of fillets in response to elevated $CO_2$ levels. Velocity had little affect on either whole wet weight or fillet attributes of rainbow trout in this study. Muscle tissue contained more ($P < 0.01$) fat when fish were fed three times daily (7.3%; day 90) compared to once daily (5.4%; day 90). Also, fish were larger ($P < 0.05$) when fed 3 times per day (1079 g; day 90) in comparison to only one daily feeding (792 g; day 90). Fish in high feed/high $CO_2$ tanks were larger and had more fillet fat than fish from low feed/low $CO_2$ tanks. To maximize rainbow trout growth at aquaculture facilities, management strategies should attempt to keep $CO_2$ levels below 30 mg/L when cost efficient. However, feeding 2–3 times daily should reduce production losses if $CO_2$ cannot be minimized. The effect of strain and velocity were minimal over the range we tested in comparison to the effects of $CO_2$ and feeding regimen.

## 1. Introduction

With increasing demand for aquatic foods and with a concurrent interest in expanding production capabilities through more intensive culture, studies are warranted that evaluate critical production parameters and their effect on the quality of aquatic foods. Carbon dioxide ($CO_2$) is an important production parameter that can potentially influence growth or fillet attributes. Danley et al. (2005) evaluated the effect of different levels of carbon dioxide ($CO_2$) (22.1, 34.5, and 48.7 mg/L) on physiological responses, growth, and fillet quality of both fresh and smoked product of rainbow trout (*Oncorhynchus mykiss*). These authors found that increasing $CO_2$ levels resulted in decreased growth rates, which corresponded with smaller fresh and smoked fillet weights. Good et al. (2010) also evaluated the influence of $CO_2$ at two concentrations, 8 and 24 mg/L, on growth and survival of rainbow trout. They reported that there was no difference in growth or survival between the two treatment groups.

In addition to $CO_2$ as an environmental consideration, water velocity in the production system and feeding frequency may have some bearing on the management decisions related to $CO_2$ levels. It is recognized that most salmonids held in water moving at a rate of 1 to 2 body lengths per second demonstrated increased growth with less fish-to-fish competition than fish held in static conditions (Christiansen et al., 1992; Davison, 1997; Jobling et al., 1993a, 1993b).

An additional factor of interest to aquaculture production facilities is the effect of genetic strain. Smith et al. (1988) determined that there were differences in both growth rates and carcass composition among ten different strains of rainbow trout tested. Valente et al.

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(2001) also reported that growth rates for two strains of rainbow trout, fed using self-feeders, differed significantly. Further evidence of a strain effect on growth rates is provided by Silverstein et al. (2005) who demonstrated that there was a significant genetic component in the residual feed intake, which is correlated to growth rates, of six different strains of rainbow trout. The effects of strain under management conditions including $CO_2$ levels, feeding frequency, and swimming speed have not been examined.

Regarding feeding frequency, considerable research has been directed at optimizing feeding regimen of salmonid fishes (Cho, 1992; Grayton and Beamish, 1977; Houlihan et al., 2001; Ruohonen et al., 1998). Contrary to aggressive and rapid feeding behavior often associated with trout, rainbow trout grown in water with 48.7 ± 4.4 mg/L free $CO_2$ displayed lethargic and intermittent feeding behavior (Danley, 2001) when fed a standard 2% daily ration twice per day. Under elevated $CO_2$ conditions, increased respiratory demands associated with aggressive and once daily feeding would be difficult to meet. Therefore, it is possible that with high $CO_2$, it may be necessary to increase meal frequency and decrease the individual meal size to reduce negative impacts on efficiency and growth related to increased respiratory demands.

The aforementioned studies demonstrate that water velocity and feeding frequency are important considerations in the design of food fish production systems. Because of limited information in this area, our studies were designed to evaluate the interaction between $CO_2$ level and water velocity as well as $CO_2$ level and feeding frequency on two different strains of rainbow trout in the context of fillet processing and quality attributes.

## 2. Methods

### 2.1. Carbon dioxide and water velocity experiment

Two strains of rainbow trout were used for this study. One commercial strain was derived from Kamloops and Puget Sound (Kamloops) steelhead and the other strain was a stock derived from Alpine lakes in the Cascade Range (Cascade). A total of 60 to 65 fish of each strain were PIT tagged and stocked in each 1000 L tank and allowed to acclimate to the flow through system (input to the system was maintained at 36–40 L/min) for one month. During the acclimation period fish were held at the ambient conditions of the flow through system and fed to satiation once daily (velocity = 0.5 body lengths/s, 30 ± 1 mg/L free $CO_2$, Table 1). High velocity (HV) and low velocity (LV) as well as high carbon dioxide (HC) and low carbon dioxide (LC) levels were tested. The high and low velocity treatments used rotational velocities of 2.0 and 0.5 body lengths/s, respectively. The high and low $CO_2$ levels were approximately 49 ± 1 and 30 ± 1 mg/L free $CO_2$, respectively.

Carbon dioxide treatments were maintained by diffusing liquid $CO_2$ directly into the experimental tanks via micropore diffusers. Gas flow for each tank was adjusted as needed through a remote flow meter, to maintain treatment concentrations. Treatment $CO_2$ levels were measured daily using tank pH, water temperature, and a standard nomogram (APHA, 1998). Carbon dioxide concentrations were measured weekly using a sodium hydroxide titration technique to verify results of the nomogram (Hach Co., Loveland, Colorado). Velocity was adjusted based on measurements taken every 3–4 days with a Marsh-McBirney Corp. flow meter (model no. 201-D) from four quadrants at three depths in each tank.

**Table 1**
Summary of water quality attributes measured over the course of the $CO_2$/velocity study in 2005 and the $CO_2$/feeding regimen study in 2006.

| | Temp. (°C) | pH | $NH_4$–N (mg/L) | Un-ionized $NH_4$–N (mg/L) | $NO_2$–N (mg/L) | $NO_3$–N (mg/L) | Hardness ($CaCO_3$ mg/L) |
|---|---|---|---|---|---|---|---|
| Average | 15.3 | 7.19 | 0.81 | 0.003 | 0.028 | 2.87 | 285 |
| Minimum | 13.7 | 6.89 | 0.35 | 0.001 | 0.001 | 2.02 | 169 |
| Maximum | 17.1 | 7.57 | 1.45 | 0.009 | 0.210 | 3.69 | 335 |

Each $CO_2$ × velocity treatment combination was replicated three times. This arrangement resulted in a design of 12 tanks with three tanks of each of the following treatment combinations: HV/HC, HV/LC, LV/HC, and LV/LC. After the one month acclimate period, five fish from each strain were sampled from each tank (Day 0). Carbon dioxide and velocity treatments were initiated 24 h after the first sample. Subsequently, fish samples were collected at 28, 56, and 84 days.

At sampling, five trout from each strain were sampled from each tank, and fish were percussively stunned. Hematocrit and plasma chloride levels were measured following the methods of Danley et al. (2005). Each trout was eviscerated; the head, bones, and fins were removed (butterfly filleted); and the butterfly fillet was weighed to determine fillet yield (%):

$$fillet\ yield = [(g\ raw\ fillet)/(g\ whole\ fish)] * 100$$

Fillets were rinsed and chilled in an ice slurry (2:1 ice to water with 0.1% NaCl) and randomly sorted into either fresh or smoked (cooked) assessment groups for subsequent analyses. Fillets designated for fresh analyses were placed in a 3 °C cooler to drain overnight. Moisture, lipid, protein, and ash content were determined using one randomly chosen side of each fresh butterfly fillet. This fillet half was skinned, frozen in liquid nitrogen, and powdered for 45 s. Powdered samples were stored at −20 °C until analyzed. Powdered samples were analyzed for proximate composition using standard procedures (AOAC, 1990).

Fillets designated for smoked processing were brined (1.4 L brine per 450 g fish) in 8.7% NaCl and 6.1% brown sugar for 1.5 h. Brined fillets were placed skin side down on stainless steel expanded metal racks, and were drained overnight at 3 °C to allow brine equilibration and pellicle formation. All fillets were covered with polyethylene wrap 4 h after rinsing to prevent excessive drying.

Fillets were smoked with skin on in a microprocessor-controlled smoke oven (Model CVU-490; Enviro-Pak, Clackamas, Oregon, USA) to an internal temperature of 65.5 °C and held for 50 min (Federal Register, 1995). Smoked fillets were cooled for 30 min at ambient temperature then transferred to 3 °C. Cook yield was calculated as:

$$cook\ yield = [(g\ fillet\ after\ smoking)/(g\ fillet\ before\ smoking)] * 100$$

Each smoked fillet was used to assess texture. For determination of Kramer shear force, a 6.5 × 3 cm section was removed from the cranial end of the fillet, dorsal to the lateral line. Peak force per fillet section was measured using a 5-blade, Kramer shear cell attached to a texture analyzer. The texture analyzer (Model TA-HDi; Texture Technologies Corporation, Scarsdale, New York, USA) was equipped with a 50-kg load cell, and analyses were performed at a crosshead speed of 2.08 mm/s. Samples, approximately 2-cm thick, were sheared perpendicular to the orientation of the muscle fibers. Peak force (g) was then divided by the weight (g) of each smoked fillet section, and values were reported as g force/g of fillet section.

### 2.2. Carbon dioxide and feeding regime experiment

Both Kamloops and Cascade strains of fish were used for this experiment. Fifty fish from each strain were PIT tagged and stocked into 12 individual 1000 L tanks and allowed to acclimate to the flow through system (input to the system was maintained at 36–40 L/min) for one month (velocity = 0.5 body lengths/s, 30 ± 1 mg/L free $CO_2$, fed to satiation once daily, Table 1). At the start of the experiment 20 fish (10 from each strain) were sampled at random for proximate composition, fillet yield, cook yield, Kramer shear force, hematocrit, and chloride. One hour after the first sampling event, treatments commenced. High carbon dioxide (HC) and low carbon dioxide (LC) levels as well as high and low feeding regimen were the treatment, main effects. High carbon dioxide ($CO_2$) treatment was approximately 49 ± 1 mg/L and low $CO_2$ was approximately 30 ± 1 mg/L free $CO_2$. Carbon dioxide was measured and adjusted using the same methods as the $CO_2 \times$ velocity experiment. For the high feed (HF) treatment, fish were fed to satiation three times daily while fish in the low feed (LF) treatment group were fed to satiation once daily. Similar to the $CO_2$ and velocity experiment described earlier, each treatment combination was replicated three times resulting in 12 total tanks that included three of each of the following treatment combinations: HF/HC, HF/LC, LF/HC, and LF/LC. Three fish from each strain were sampled from each treatment tank on days 45 and 90. Fish were analyzed for proximate composition, fillet yield, cook yield, Kramer shear force, hematocrit, and plasma chloride following the same methods as the aforementioned $CO_2 \times$ velocity experiment.

### *2.3. Data analysis*

Data from both experiments were analyzed using analysis of variance (ANOVA) procedures in program R (R Development Core Team, 2009). ANOVA was used to determine if differences in measured values of proximate composition, fillet yield, cook yield, or Kramer shear force were affected by treatment groups ($CO_2$ and velocity level or $CO_2$ and feeding regime), strain or day of experiment. We also tested for interactions between treatment groups ($CO_2 \times$ velocity level or $CO_2 \times$ feeding regime). An alpha level of <0.05 was used to establish statistical significance. Nonnormal data was normalized by applying the Box–Cox transformation, a procedure that selects the best power transformation to normality (Sokal and Rohlf, 1995).

## 3. Results

### *3.1. Carbon dioxide and velocity experiment*

Rainbow trout raised in HC treatment tanks had significantly lower wet weights than fish from low LC tanks ($P < 0.01$; Fig. 1). Although fish from both $CO_2$ levels did increase in weight during the study, the average weight of LC fish was 104 g (14%) greater than HC fish after 84 days. Velocity had no effect on wet weight of fish during the study ($P = 0.84$). Strain had a significant influence on fish wet weights ($P = 0.03$). The Cascade strain was 15 g lighter than the Kamloops strain at the start of the study, but was 55 g larger, on average, after 84 days (Table 2).

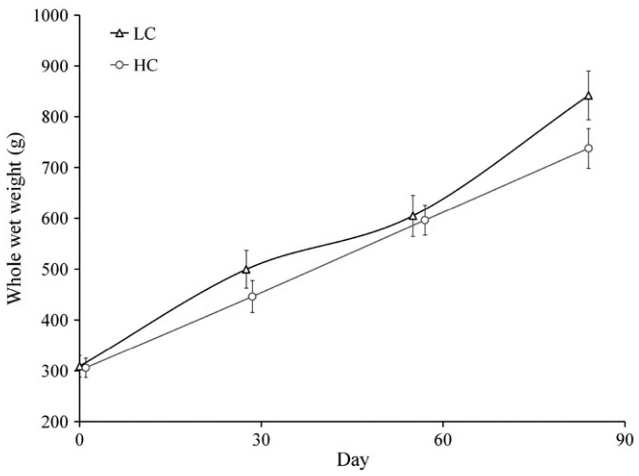

**Fig. 1.** Average whole wet weight for fish raised in low (LC) and high (HC) $CO_2$ treatment tanks during the $CO_2$-veleocity study in 2005. Samples were collected on days 0, 28, 56, and 84. Black bars represent ±2SE. Data points for days 0, 28, and 56 are offset so SE bars can be clearly seen.

**Table 2**
Wet weight and fillet yield for the Cascade and Kamloops strains of fish on days 0 and 84 of the $CO_{2/}$velocity study in 2005 and days 0 and 90 of the $CO_{2/}$feeding regimen study in 2006. Values represent averages ± 2 standard errors. Strain had a significant influence on both wet weight and fillet yield in the 2005 study and only on wet weight in the 2006 study.

| Year | Strain | Day | Wet weight (g) | Fillet yield (%) |
|---|---|---|---|---|
| 2005 | Cascade | 0 | 302 ± 19 | 65.1 ± 1.7 |
| | | 84 | 809 ± 49 | 65.1 ± 0.8 |
| | Kamloops | 0 | 317 ± 18 | 66.3 ± 2.1 |
| | | 84 | 754 ± 39 | 65.4 ± 0.5 |
| 2006 | Cascade | 0 | 505 ± 53 | 69.0 ± 1.6 |
| | | 90 | 866 ± 92 | 67.5 ± 0.6 |
| | Kamloops | 0 | 560 ± 91 | 67.6 ± 0.8 |
| | | 90 | 1036 ± 87 | 67.8 ± 0.7 |

Carbon dioxide concentration, velocity, and strain did not influence protein levels of fish during this study; however, average percent protein increased from 19.5 to 20.2% as fish grew (Table 3; $P < 0.01$). Percent ash was significantly lower in fillets of fish from high $CO_2$ treatment tanks ($P = 0.02$). The Kamloops strain had higher ash content than the Cascade strain ($P = 0.04$) and percent ash decreased on average over the duration of the study ($P < 0.01$). Fish from the HC treatment had higher percent moisture ($P < 0.01$) and decreased percent fat ($P = 0.04$) in comparison to LC treatment fish (Table 3). The Cascade strain had higher percent moisture ($P < 0.01$) and lower percent fat ($P < 0.01$) than the Kamloops strain, and, on average, both strains had decreased percent moisture ($P < 0.01$) and increased percent fat ($P = 0.03$) over the course of the study.

Fillet yield was influenced by $CO_2$ ($P < 0.01$), velocity ($P = 0.05$), strain ($P < 0.01$), and day ($P < 0.01$). Fillet yield was highest in fish from the LC treatment, LV treatment (Table 4), Kamloops strain, and on the last day of the study. Cook yield of LC fish fillets (81.0%) was higher than HC fish fillets (78.9%; $P < 0.01$). Over the course of the study cook yield increased ($P < 0.01$) from 79.0 to 81.9%. $CO_2$, velocity, and strain had no effect on Kramer shear force (all $P > 0.10$), but Kramer shear force did increase linearly as the study progressed ($P < 0.01$; Kramer shear force $= 1.4112(\text{day}) + 218.08$; $R^2 = 0.99$). Hematocrit levels were 37.2% in the Kamloops strain in comparison to 36.3% in the Cascade strain ($P = 0.04$) and hematocrit levels, on average, increased from 35.8 to 38.7% over the course of the study ($P < 0.01$). LC fish had higher plasma chloride levels than HC fish ($P < 0.01$). Plasma chloride also differed on the various sample dates ($P = 0.02$), but no clear trend was associated with day. Hematocrit and plasma chloride levels for fish sampled the end of the study are reported in Table 5.

The interaction between $CO_2$ and velocity had very little influence the dependant variables measured in this study. Plasma chloride was the only dependant variable in which there was a significant interaction between $CO_2$ and velocity (Table 6). At the HC treatment level

**Table 3**
Proximate composition estimates for filets of fish grown in high $CO_2$ (HC) treatment tanks in comparison to fillets of fish from low $CO_2$ (LC) treatment tanks on day 84 of the $CO_{2/}$velocity study in 2005 and day 90 of the $CO_{2/}$feeding regimen study in 2006. Values represent averages ± 2 standard errors. Superscripts $^{a}$ and $^{b}$ are used to demonstrate statistical significance in 2005 and superscripts $^{c}$ and $^{d}$ are used for 2006. Values in the same year and column with different superscripts indicate that there was a statistically significant treatment effect.

| Year | Treatment | % moisture | % fat | % ash | % protein |
|---|---|---|---|---|---|
| 2005 | HC | 74.07 ± 0.34$^{a}$ | 5.43 ± 0.51$^{a}$ | 1.25 ± 0.03$^{a}$ | 20.04 ± 0.20$^{a}$ |
| | LC | 72.91 ± 0.35$^{b}$ | 6.01 ± 0.49$^{b}$ | 1.31 ± 0.03$^{b}$ | 20.33 ± 0.20$^{a}$ |
| 2006 | HC | 73.73 ± 0.43$^{c}$ | 5.94 ± 0.48$^{c}$ | 1.26 ± 0.02$^{c}$ | 19.85 ± 0.29$^{c}$ |
| | LC | 73.20 ± 0.37$^{d}$ | 6.37 ± 0.45$^{d}$ | 1.32 ± 0.03$^{d}$ | 20.28 ± 0.25$^{c}$ |

**Table 4**
Fillet yield (%), cook yield (%), and Kramer shear force (g/g) estimates for fish reared in high $CO_2$ (HC), low $CO_2$ (LC), high velocity (HV), and low velocity (LV) treatment conditions during the $CO_2$/velocity study in 2005 and HC, LC, high feed (HF), and low feed (LF) treatment conditions during the $CO_2$/feeding regimen study in 2006. Values represent averages (±2 standard errors) for the fish sampled on the final day of each study. Superscripts $^a$ and $^b$ are used to demonstrate statistical significance in 2005 and superscripts $^c$ and $^d$ are used for 2006. Values in the same year, column, and treatment type (e.g. $CO_2$) with different superscripts indicate that there was a statistically significant treatment effect.

| Year | Treatment | % fillet yield | % cook yield | Kramer shear (g/g) |
|---|---|---|---|---|
| 2005 | HC | $65.70 \pm 0.58^a$ | $81.45 \pm 0.67^a$ | $339 \pm 28^a$ |
| | LC | $66.16 \pm 0.77^b$ | $82.62 \pm 0.87^b$ | $353 \pm 27^a$ |
| | HV | $65.49 \pm 0.69^a$ | $82.02 \pm 0.86^a$ | $338 \pm 31^a$ |
| | LV | $66.18 \pm 0.63^b$ | $81.72 \pm 0.73^a$ | $347 \pm 28^a$ |
| 2006 | HC | $67.46 \pm 0.65^c$ | $78.96 \pm 0.72^c$ | $299 \pm 36^c$ |
| | LC | $67.90 \pm 0.68^c$ | $80.19 \pm 0.76^d$ | $300 \pm 24^c$ |
| | HF | $67.73 \pm 0.60^c$ | $80.77 \pm 0.70^c$ | $292 \pm 30^c$ |
| | LF | $67.62 \pm 0.72^d$ | $78.38 \pm 0.61^d$ | $307 \pm 32^c$ |

plasma chloride levels decreased as velocity increased, however, at the LC treatment level the opposite occurred (Fig. 2). A summary of all ANOVA results (p-values) for the $CO_2$ and velocity experiment are reported in Table 6.

### 3.2. Carbon dioxide and feeding regime experiment

Both LC and HF treatments resulted in increased whole wet weights compared to alternative treatments (both $P<0.01$; Fig. 3). Furthermore, strain (Kamloops > Cascade) and day (weight increased during study) had significant affects on wet weights (both $P<0.01$). After ninety days, fish that were raised in LC/HF treatment tanks had wet weights that were 515 g (71%) heavier than fish raised in HC/LF treatment tanks (Fig. 3). Additionally, wet weight was the only dependant variable from this portion of the study in which there was a significant interaction between $CO_2$ and feeding regime (Table 7). At the LC treatment level, increasing feed frequency had a larger positive influence on wet weight than it did at the HC treatment level (Fig. 4).

Muscle protein levels were unaffected by $CO_2$ level, feeding regimen, and strain (all $P>0.30$); however, protein on average decreased from 21.1 to 20.1% during the 90 day study ($P=0.05$). Percent ash was higher in fish from LC treatment groups (Table 3) and decreased as fish grew (both $P<0.01$). Percent ash was unaffected by feeding regimen or strain (both $P>0.40$). Moisture content was lower in LC and HF treatment groups (both $P<0.01$; Fig. 5) but was unaffected by strain or day (both $P>0.11$). Percent fat was higher in LC ($P=0.05$) and HF ($P<0.01$; Fig. 5) treatment tanks but was unaffected by strain or day (both $P>0.11$).

Fillet yield was higher in HF treatment fish ($P=0.01$), and fillet yield decreased as the study progressed ($P<0.01$). Strain and $CO_2$ level had no effect (both $P>0.40$) on fillet yield. Cook yield was affected ($P<0.01$) by $CO_2$ level and feeding regimen; LC and HF treatment groups had higher cook yields (Table 4) compared to alternative treatments, and cook yield was the lowest at the end of the study ($P<0.01$). $CO_2$ level, feeding regimen, strain, and day had no affect on Kramer shear force (all $P>0.07$). Hematocrit levels were higher in the Cascade strain compared to the Kamloops strain ($P<0.01$), and HF fish had lower hematocrit levels ($P=0.03$) than LF fish. Plasma chloride levels were higher in the LC fish (Table 5; $P<0.01$), and they were significantly different on the three sampling dates ($P<0.01$). Nonetheless, there was no clear trend in chloride levels; they were highest on day 0, dropped slightly on day 45, and then increased again on day 90. Chloride levels were higher in LF fish compared to HF fish ($P=0.03$). A summary of all ANOVA results (p-values) for the $CO_2$ and feeding regime study are reported in Table 7.

**Table 5**
Hematocrit (%) and chloride concentration (mEq/L) estimates for fish reared in high $CO_2$ (HC), low $CO_2$ (LC), high velocity (HV), low velocity (LV), Kamloops strain, and Cascade strain treatment conditions during the $CO_2$/velocity study in 2005 and HC, LC, high feed (HF), low feed (LF), Kamloops strain, and Cascade strain treatment conditions during the $CO_2$/feeding regimen study in 2006. Values represent averages (±2 standard errors) for the fish sampled on the final day of each study. Superscripts $^a$ and $^b$ are used to demonstrate statistical significance in 2005 and superscripts $^c$ and $^d$ are used for 2006. Values in the same year, column, and treatment type (e.g. $CO_2$) with different superscripts indicate that there was a statistically significant treatment effect.

| Year | Treatment | Hematocrit (%) | Chloride (mEq/L) |
|---|---|---|---|
| 2005 | HC | $37.9 \pm 0.8^a$ | $97.7 \pm 1.6^a$ |
| | LC | $39.1 \pm 0.9^a$ | $108.9 \pm 1.0^b$ |
| | HV | $37.9 \pm 1.0^a$ | $100.7 \pm 2.4^a$ |
| | LV | $38.8 \pm 0.8^a$ | $103.6 \pm 1.7_a$ |
| | Kamloops | $38.5 \pm 0.9^a$ | $101.9 \pm 1.9^a$ |
| | Cascade | $38.3 \pm 0.9^b$ | $102.8 \pm 2.2^a$ |
| 2006 | HC | $38.0 \pm 1.9^c$ | $104.5 \pm 2.6^c$ |
| | LC | $38.1 \pm 1.6^c$ | $118.9 \pm 2.3^d$ |
| | HF | $36.7 \pm 1.7^c$ | $108.5 \pm 3.0^c$ |
| | LF | $39.3 \pm 1.7^d$ | $112.3 \pm 2.9^d$ |
| | Kamloops | $37.7 \pm 2.0^c$ | $109.5 \pm 3.0^c$ |
| | Cascade | $38.4 \pm 1.5^d$ | $111.3 \pm 3.0^c$ |

**Table 6**
Results from all ANOVA tests for the $CO_2$ velocity experiment done in 2005. Values presented are the p-values for each source of variation (factor) and interaction term. An example an ANOVA model tested would be: $Protein = CO_2 + Velocity + Strain + Day + CO_2 * Velocity$. Values in bold are significant (<0.05).

| Dependent variables | Source of variation | | | | |
|---|---|---|---|---|---|
| | $CO_2$ | Velocity | Strain | Day | Interaction |
| | | | | | $CO_2 \times$ Velocity |
| Proximates | | | | | |
| Protein | 0.09 | 0.28 | 0.76 | <**0.01** | 0.14 |
| Ash | **0.02** | 0.68 | **0.04** | <**0.01** | 0.49 |
| Water | <**0.01** | 0.26 | <**0.01** | <**0.01** | 0.43 |
| Fat | **0.04** | 0.83 | <**0.01** | **0.03** | 0.98 |
| Production attributes | | | | | |
| Wet weight | <**0.01** | 0.84 | **0.03** | <**0.01** | 0.67 |
| Fillet yield | <**0.01** | **0.05** | <**0.01** | <**0.01** | 0.73 |
| Cook yield | <**0.01** | 0.66 | 0.15 | <**0.01** | 0.55 |
| Kramer shear | 0.10 | 0.94 | 0.26 | <**0.01** | 0.42 |
| Blood measurements | | | | | |
| Hematocrit | 0.23 | 0.06 | **0.04** | <**0.01** | 0.66 |
| Plasma chloride | <**0.01** | 0.98 | 0.45 | **0.01** | <**0.01** |

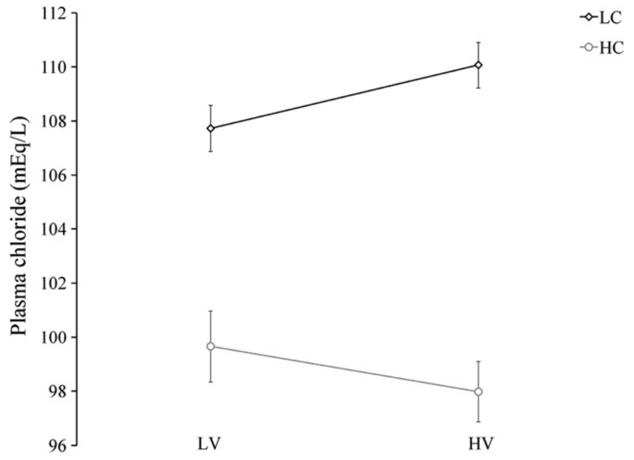

**Fig. 2.** Average plasma chloride levels separated by low $CO_2$ (LC), high $CO_2$ (HC), low velocity (LV), and high velocity (HV) treatment groups to demonstrate interaction effects. Black bars represent ±2SE.

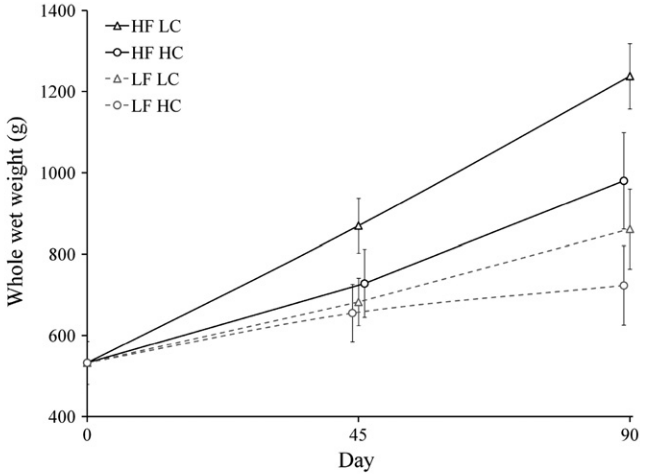

**Fig. 3.** Average whole wet weight for fish raised in high feed-low $CO_2$ (HF LC), high feed-high $CO_2$ (HF HC), low feed-low $CO_2$ (LF LC), and low feed-high $CO_2$ (LF HC) treatment tanks during the $CO_2$-feeding regime study in 2006. Samples were collected on days 0, 45, and 90. Black bars represent ±2SE. Data points for days 45 and 90 are offset so SE bars can be clearly seen.

## 4. Discussion

### 4.1. $CO_2$ effects

During the present study, elevated $CO_2$ levels resulted in decreased growth, lower fillet fat and higher fillet moisture in rainbow trout. This provides evidence to suggest that minimizing $CO_2$ will result in larger fillets with greater fat content. At the same time, $CO_2$ level did not influence Kramer shear force, suggesting that size and fat content in the range examined did not affect texture. Because fat serves as a lubricant (Miller, 2004) shear force was expected to decrease in fillets with more fat content and evidence for this trend has been reported in recent literature (Aussanasuwannakul et al., 2011). However, age/size effects are also known to influence shear force (Aussanasuwannakul et al., 2011) and it can be difficult to separate these affects. Inability to separate age/size effects is a potential explanation as to why $CO_2$ level did not influence Kramer shear force in this study. Previous researchers have reported that $CO_2$ levels have an effect on salmonid growth. Fivelstad et al. (1998) reported that growth rates of Atlantic salmon (*Salmo salar* L.) decreased substantially when $CO_2$ levels were increased from 26 to 44 mg/L. Reduced plasma chloride levels in freshwater fish is an indicator of stress. Reduced plasma chloride levels often occur when $CO_2$ levels increase because of an electroneutral ion exchange with $HCO_3^-$ (Fivelstad et al., 1998, 2003a; Goss et al., 1994). During the present study, the high and low $CO_2$ treatment levels were approximately 49 and 30 mg/L of free $CO_2$, respectively. The results indicated that when $CO_2$ was increased from 30 to 49 mg/L, there was a significant decrease in plasma chloride and fillet weight decreased by 13.5% after 84 days. Danley et al. (2005) reported a 23.7% decrease in rainbow trout fillet weight when $CO_2$ levels were increased from 22 to 49 mg/L. However, Good et al. (2010) reported that there was no difference in rainbow trout growth or survival when reared in $CO_2$ concentrations of 8 or 24 mg/L. The results of the present study, as well as those from previous literature suggest that in order to maximize growth rates aquaculture facilities culturing rainbow trout or other salmonid species should maintain $CO_2$ levels below 30 mg/L and realize that when levels exceed 40 mg/L losses in production and reduced fillet fat content can occur.

**Table 7**
Results from all ANOVA tests for the $CO_2$ feeding regime experiment done in 2006. Values presented are the p-values for each source of variation (factor) and interaction term. An example an ANOVA model tested would be: Protein = $CO_2$ + Feed + Strain + Day + $CO_2$ ∗ Feed. Values in bold are significant (<0.05).

| Dependent variables | Source of variation | | | | |
|---|---|---|---|---|---|
| | $CO_2$ | Feed | Strain | Day | Interaction |
| | | | | | $CO_2$ × Feed |
| Proximates | | | | | |
| Protein | 0.31 | 0.62 | 0.77 | **0.05** | 0.06 |
| Ash | **<0.01** | 0.43 | 0.47 | **<0.01** | 0.70 |
| Water | **<0.01** | **<0.01** | 0.11 | 0.40 | 0.09 |
| Fat | **0.05** | **<0.01** | 0.43 | 0.11 | 0.12 |
| Production attributes | | | | | |
| Wet weight | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **0.04** |
| Fillet yield | 0.79 | **0.02** | 0.26 | **0.03** | 0.37 |
| Cook yield | **<0.01** | **<0.01** | 0.23 | **<0.01** | 0.49 |
| Kramer shear | 0.60 | 0.07 | 0.23 | 0.46 | 0.39 |
| Blood measurements | | | | | |
| Hematocrit | 0.43 | **0.03** | **<0.01** | 0.21 | 0.38 |
| Plasma chloride | **<0.01** | **0.01** | 0.08 | **<0.01** | 0.27 |

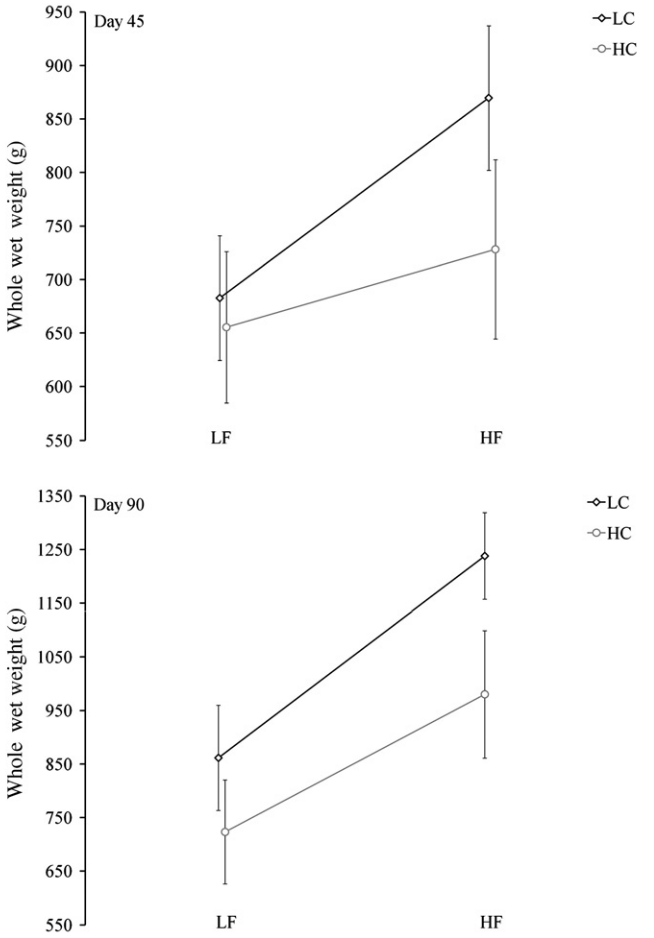

**Fig. 4.** Average wet weight on days 45 and day 90, separated by low $CO_2$ (LC), high $CO_2$ (HC), low feed (LF), and high feed (HF) treatment groups to demonstrate interaction effects. Black bars represent ±2SE.

Fish reared in elevated $CO_2$ conditions in this study had decreased levels of ash, which suggests that increased $CO_2$ levels are capable of disrupting the mineral balance of rainbow trout. Several other studies have also indicated that elevated $CO_2$ levels are capable of affecting the mineral balance of fish (Fivelstad et al., 2003b; Graff et al., 2002). This phenomenon is likely caused when elevated $CO_2$ levels forced fish to use calcium (Ca) and phosphorus (P) present in the body to buffer against decreases in blood pH (Helland et al., 2005; Meghji et al., 2001).

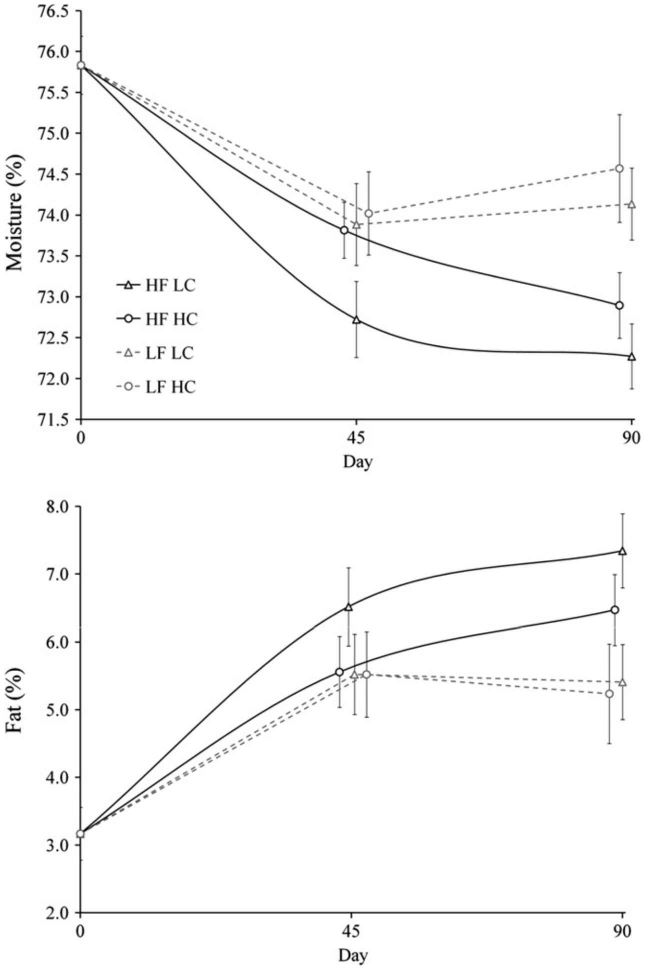

**Fig. 5.** Average % fillet fat and moisture for fish raised in high feed-low $CO_2$ (HF LC), high feed-high $CO_2$ (HF HC), low feed-low $CO_2$ (LF LC), and low feed-high $CO_2$ (LF HC) treatment tanks during the $CO_2$-feeding regime study in 2006. Samples were collected on days 0, 45, and 90. Black bars represent ±2SE. Data points for days 45 and 90 are offset so SE bars can be clearly seen.

### 4.2. Velocity effects

The velocities evaluated during the present study (0.5 and 2.0 body lengths/s; 14 and 57 cm/s) had no affect on whole wet weight or fillet proximate composition. The only attribute that was influenced by velocity was fillet yield, which was higher in fish from the low velocity treatment (LV = 65.6%, HV = 63.6%). Previous research has demonstrated that rainbow trout grow faster with some water velocity present (Farrell et al., 1990; Houlihan and Laurent, 1987) or with prolonged exercise training (Jobling et al., 1993a, 1993b). Exercise training in fish can lead to reduced oxygen consumption rates, more efficient swimming modes, and increased aerobic activity (Jobling et al., 1993a, 1993b). These changes often result in improved food conversion efficiency and improved growth rates. In addition to exercise related results, velocity levels are also related to energy used for aggressive behavior. Farrell et al. (1990) reported that rainbow trout held at 30 cm/s were 13% larger after 28–52 days compared to fish raised at <1 cm/s. When rainbow trout were reared at 1 body length/s for six weeks they grew twice as fast as control fish raised in still water (Houlihan and Laurent, 1987). Decreased growth rates in still water are likely caused by aggressive activities used to establish hierarchies (Davison, 1997). As water velocity increases, these aggressive behaviors of salmonids are minimized (Adams et al., 1995; Christiansen and Jobling, 1990); however, the energy required to swim increases. These findings suggest that there should be an optimum water velocity where energy losses from aggressive behavior and swimming are minimized. Our results indicate that increasing water velocity from 0.5 to 2.0 body lengths/s (14 to 57 cm/s) had little affect on rainbow trout growth. The most likely explanation for our results is that the energy gained from decreases in aggressive behavior at the higher water velocity was equivalent to the extra energy used during swimming.

### 4.3. Strain effects

Although strain did have a significant influence on growth, the results were not consistent between our two studies. In the first study the Cascade strain grew faster while in our second study the Kamloops strain grew faster. Similar inconsistencies in the results of our two studies occurred for fillet attributes. Compared to the consistent effects of feeding frequency and $CO_2$ treatments, we conclude that the strain related differences in growth and fillet attributes in this study were minor and not of production significance. Because the two strains used in this study did not have consistent differences in growth rates or fillet attributes under the conditions investigated in this study, we infer that while growth rate can differ by genetic strain (Silverstein et al., 2005; Smith et al., 1988; Valente et al., 2001) effects of $CO_2$ and feeding frequency should be similar across strains.

### 4.4. Feeding regime effects

This research adds to a growing body of literature that suggests multiple feedings to satiation per day will increase growth rate of rainbow trout. During the present study, rainbow trout raised in low $CO_2$ conditions and fed to satiation three times daily had wet weights 43.7% greater than fish fed to satiation once daily. These results are similar to those of Ruohonen et al. (1998) who suggested that rainbow trout should be fed at least three times per day in order to maximize growth rates. Results of the present study were also similar to those of Grayton and Beamish (1977) who reported that growth and food intake was maximized with two feedings to satiation per day.

Grayton and Beamish (1977) suggested that body fat levels of rainbow trout will increase with the number of daily feedings. Rainbow trout from our study, fed to satiation three times daily, had higher percent fat and lower percent moisture in their fillets than fish that were fed to satiation once daily. Tidwell et al. (1991) reported slightly different results, indicating that percent body fat did not increase when rainbow trout were fed to satiation instead of according to a size/water temperature chart or with a demand feeder. This finding is unexpected considering that fish fed to satiation consumed a much greater amount of feed and had increased growth rates. It is possible that because fish from Tidwell et al. (1991) were raised in ponds where space is not limited, excess swimming could have burned off fat reserves. Albeit it seems clear that when rainbow trout are raised in tanks and fed to satiation multiple times per day percent body and fillet fat will increase and percent body and fillet moisture will decrease.

Fish reared in HC/HF tanks grew larger and had more fillet fat than fish reared in LC/LF tanks indicating that feeding regimen is a more important factor than $CO_2$ level over the range tested in this study. This is important because the cost of minimizing $CO_2$ levels can be large and may be greater than the cost of providing feed more frequently. Feeding more frequently to overcome the problems caused by elevated $CO_2$ levels should be considered as a viable management strategy when attempting to maximize aquaculture production in the most financially efficient manner.

### 4.5. Interactions

The majority of the dependant variables measured in this study were uninfluenced by interactions between $CO_2$ and velocity or $CO_2$ and feeding regime. However, there was a significant $CO_2$ × velocity interaction effect on plasma chloride levels. At the high $CO_2$ treatment level plasma chloride levels decreased as velocity increased, however, at the low $CO_2$ treatment level the opposite occurred. This suggests at the high $CO_2$ level tested in this study (49 mg/L) the fish were more comfortable at the low treatment velocity (0.5 body lengths/s). Conversely, at the low $CO_2$ treatment level (30 mg/L) fish performed slightly better at higher velocities (2.0 body lengths/s). There is substantial evidence that suggests rainbow trout perform better when some velocity is present (Farrell et al., 1990; Houlihan and Laurent, 1987), however, this is the first study that demonstrates production related attributes can be influenced by velocity and $CO_2$. The interaction that we detected in this study suggests that aquaculture facilities may need to adjust the velocity according to the $CO_2$ levels present in the system in order to maximize production. More research over a wider range of $CO_2$ and velocity levels is warranted.

The $CO_2$ feeding regime study provided evidence to suggest that there was a significant $CO_2$ × feeding regime interaction effect on wet weight. When $CO_2$ was low (30 mg/L), increasing the number of daily feedings had a large positive influence on wet weight. At a high $CO_2$ level (49 mg/L) increasing the number of daily feedings also resulted in heavier fish however, the increase in fish wet weight was not as large as the increase that occurred at the low $CO_2$ treatment level. Previous researchers have demonstrated that food conversion efficiency is related to water quality attributes (Altinokand and Grizzle, 2001; Smart, 1981) and this is a possible reason we detected a significant interaction between $CO_2$ level and feeding regime in our study. Aquaculture facilities may be able to offset losses in production due to elevated $CO_2$ levels by increasing the number of daily feedings, however, food conversion efficiency may be lower.

## 5. Conclusions

Controlling $CO_2$ levels and optimizing feeding regimen are of utmost importance when maximizing growth of rainbow trout. Based on this research and a review of previous literature, we suggest that $CO_2$ levels should be closely monitored and kept below 30 mg/L when possible. If maximizing growth is important, feeding rainbow trout to satiation 2–3 times daily will substantially increase growth rates and fat levels in comparison to one feeding/day. Also, if the cost of minimizing $CO_2$ levels becomes too great, the negative influence of $CO_2$ can be partially overcome by feeding to satiation 2–3 times daily. Lastly, the influence of velocity in the range of 0.5–2.0 body lengths per second is minimal in comparison to feeding regimen and $CO_2$ levels. Nonetheless, previous literature suggests that some flow should exist to minimize aggressive behavior related to hierarchy establishment.

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