Evaluation of a Wisconsin-type bioenergetics model to estimate brook charr (*Salvelinus fontinalis*) growth and food consumption under two salinity conditions.

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Abstract

In Québec, Canada, brook charr (*Salvelinus fontinalis*) is the most sought-after species for recreational fisheries, which makes it economically important. To improve population monitoring and better anticipate climate change impacts on brook charr, bioenergetics models can be useful. The objective of this research was to evaluate the performance of a resident brook charr Wisconsin Energy Budget (WEB) model applied to an anadromous strain under two salinity treatments. Growth and food consumption were predicted by the model and compared to the observed values obtained after a 60-day experiment in the laboratory on fish reared in fresh or brackish water. Predictions for fish reared in fresh water better estimated growth rate and consumption than for fish reared in brackish water, for which growth was overestimated and consumption was underestimated. Overall, these results suggest that there is a difference for the WEB model's predictions depending on the salinity and that observed food consumption is predicted more accurately than growth.

Keywords: Brook charr, Bioenergetics model, Metabolism, Salinity, Growth, Consumption, Temperature, Anadromy

Introduction

Brook charr is the most sought-after species for recreational fisheries in Québec, Canada, representing approximately 30 % of the total fishing effort (Gagné 2023). Populations inhabiting coastal rivers of eastern Canada have two ecotypes: anadromous and freshwater resident (Power 1980; Hendry et al. 2004). Anadromous populations have been declining since the early 2000s due to anthropogenic pressures such as overfishing, habitat degradation, the introduction of non-native competing species, as well as climate change (Ministère des Forêts, de la Faune et des Parcs 2020). Moreover, no provincial licence is needed to fish in the St. Lawrence estuary where the anadromous strain is found making them more vulnerable to recreational fisheries (Gagné 2023). The need to ensure proper management of coastal populations is particularly important as brook charr, whose metabolism is generally optimal between 10 and 19 °C for growth and gonad development (Hokanson et al. 1973, Jourdain-Bonneau et al. 2023), may be negatively affected the rise in freshwater temperature. Indeed, temperatures close to 20-24 °C have been observed in some rivers in Québec (Daigle et al. 2019). Ectotherm organisms, such as fish, see their metabolic rate fluctuate according to ambient temperature (Brett 1972). Under sub-optimal conditions, mortality rates increase while reproduction efforts decrease compromising species resilience (McCormick et al. 1972). It is therefore important to take an interest in brook charr coastal populations to ensure their conservation and stock management.

With the aim of improving brook charr population monitoring, the use of bioenergetics models may improve our understanding of environmental effects on growth, reproduction, and food consumption of a species (Kitchell et al. 1977; Ney 1993; Rudstam et al. 1994; Railsback and Rose 1999)). They have also been used to evaluate stocking strategies by identifying more effective stocking locations and limiting environmental factors (Stewart et al. 1981; Al-Chokhachy et al.

2009) while they have also been used to evaluate the impacts of increasing temperatures on fish energetics (Lyons 1997; Bevelhimer and Bennett 2000; Bevelhimer 2002). Using the principles of mass conservation, energy acquired through food consumption is distributed into four physiological components at the individual level: metabolism (standard metabolism, activity and energy required for digestion), production of metabolic waste (equivalent to the total of energy egested as feces and excreted as urea and ammonia), growth, and reproductive effort (Ney 1993; Deslauriers et al. 2017). The Wisconsin Energy Budget (WEB) model estimates consumption as a proportion of the maximum ration available for the fish (Ney 1993) wherein growth is controlled by consumption and respiration that are both influenced by fish size and environmental temperature (Kitchell et al. 1977).

The WEB model explored in this study was developed by Hartman and Sweka (2003) and refined by Hartman and Cox (2008) on a juvenile and adult freshwater brook charr resident strain. The goal of this model was to improve fish community management in eastern streams where brook charr are native, and in western streams, where they are considered a threat to other native salmonids of the United States (Hartman and Sweka 2003; Hartman and Cox 2008). This specific bioenergetics model was first developed to assess fish thermal limits to determine streams where brook charr could become vulnerable due to climate change (Hartman and Sweka 2003). According to Ney (1993), the maximum error rates, calculated by dividing the difference between model predictions and observed data by the observed data, should be between 30-50 % for a model to be validated and used for management applications. The brook charr bioenergetics model was within 20% of observed consumption rates in a laboratory experiment and was thus considered valid for field applications (Hartman and Sweka 2003). Hartman and Cox (2008) further refined the parameters for metabolism and activity rate because they assumed that gastric evacuation rates for

brook charr were the same as for other charr species whilst it has been shown to be lower in brook charr (Sweka et al. 2004) and that the activity rate multiplier was for routinely active fish. The refinement of the model provided predictions within 2.3 % of the observed growth, final mass and consumption which makes this WEB model one of the best-validated one (Hartman and Cox 2008). Since then, this bioenergetics model has been used for different purposes, including predictions of the effects of climate change on seasonal growth and fish energy demands (Christianson and Johnson 2020) or the evaluation of time and temperature regime on respiration and ammonia excretion (Hansen and Rahel 2015). However, the performance of this WEB model has never been tested on an anadromous strain, which could introduce bias as their metabolism has been shown to differ from the resident ecotype (Morinville et Rasmussen 2003).

The objective of this research project was to evaluate the performance of the freshwater brook charr WEB model applied to an anadromous strain. Growth and food consumption were predicted by the model and compared to the observed values obtained after a 60-day experiment in the laboratory under two salinity conditions: fresh and brackish water. We hypothesized that WEB predictions would provide better estimates for fish reared in fresh water rather than in brackish water because transfer into brackish water has been shown to generate a systemic response (ex. stress, osmoregulatory response, endocrine response, and/or modifications of appetite regulation (new habitat, new prey) (Rao 1968; McCormick 1985; McCormick 1994)), for which overall costs are difficult to estimate and are not taken into account in a model developed for freshwater fish.

Materials and methods

Fish husbandry

In accordance with the Canadian Council on Animal Care guidelines, all experimental protocols were approved by the UQAR Animal Care Committee (Certificate # CPA-88-22-241).

Anadromous brook charr used for the experiment were held at the ISMER-UQAR (Institut des sciences de la mer – Université du Québec à Rimouski) aquaculture station in Pointe-au-Père (Québec, Canada). Broodstock used to produce these fish originated from the Laval River, a tributary of the St. Lawrence Estuary (Bastien et al. 2011). The stock has been divided into two lines over six generations; the control line, whose individuals are randomly chosen as broodstock after each new generation is produced, and the selected line, whose broodstock are chosen based on two traits: 1. the absence of early gonad development, and 2. larger individuals within fish not showing early gonad development. Selection takes place in the fall at age 1+. Spawning takes place at the aquaculture station when females are ready to release their eggs, generally between November and December. For each generation, both lines are composed of at least 20 families. Each family is created using unique male-female combinations within each line avoiding full-sib crosses. Eggs hatch between January and February depending on spawning date and water temperature. At a temperature of 8 °C, it takes approximately four weeks for the fingerlings to absorb their yolk sac. For fingerlings and adults, maximal biomasses within rearing tanks are kept below 30 kg m^{-3}. Brook charr remain in freshwater throughout the year until they reach age 2+.

Experimental design

During the summer of 2022, a 60-day growth experiment was conducted in twelve 500-liter flow-through tanks. For the experiment, treatments were randomly distributed. There were three replicate tanks for each line per water treatment. Three families from each line and ~five fish per family were used to ensure that the observed effects were not due to parental effects, but to the treatment and/or the genetic line. Fish were randomly chosen from ~200 individuals available for each family. 174 brook charr were used: 88 from the control line and 86 from the selected line, but 172 were used for the analyses: 86 from the control line and 86 from the selected line. One control

fish was misplaced in the wrong tank at the beginning of the experiment and was withdrawn from all analyses. One fish (control line) died on day 59 of the experiment. Half of the fish were reared in fresh water (0 ppt; 6 tanks) and the other half reared in brackish water (14 ppt; 6 tanks). Each tank contained a maximum of 15 fish with both lines (control and selected) kept separately (in average 3.84 ± 0.46 Kg m^{-3}). Brook charr were of age 2+ at the time of the experiment, which infers that they had developed physiological aptitudes necessary for life in salt water and would be sexually mature to reproduce in the fall (Boula et al. 2002). One day before the beginning of the experiment, fish were anesthetized (Ethyl 3-aminobenzoate methanesulfonate [MS-222] 0.16 g/L, Sigma-Aldrich co., Missouri, USA) for the measurement of fork length (mm), mass (g), and implantation of Passive Integrated Transponder (PIT tags, AVID, California, USA) into the epaxial muscle near the dorsal fin for individual identification. Brook charr were not fed 24 hours prior to anesthesia.

Fresh water was supplied by dechlorinated tap water from the City of Rimouski whilst brackish water was obtained by mixing City of Rimouski fresh water and salt water obtained from an underwater pumping system with the water source located 1 km offshore in the St. Lawrence Estuary. Temperature was measured once daily because it is stable over the course of 24 hours. Salt water is kept in large holding tanks (3 days autonomy; 1 million L) which stabilizes water temperature over short periods of time (days). Once the fish were evenly distributed in the tanks, salinity was increased by 2 ppt per day until 14 ppt was reached following a standardized protocol from the aquaculture station because it has been shown that transferring brook charr directly in brackish water generally increase significantly cortisol levels when temperature differs between the two environments (Claireaux and Audet 2000).

Photoperiod was maintained to reflect natural conditions at the Pointe-au-Père latitude (48°30’59.99” N). Temperature, salinity, and dissolved oxygen were measured in each tank once daily (Table 1) using a YSI probe (Professional Plus (Pro Plus) Multiparameter instrument, YSI Inc., Ohio, USA).

A failure in the saltwater supply system occurred on day 24 of the experiment. The saltwater reserves of the aquaculture station were therefore used until day 32 after which a temporary supply system was installed for the remainder of the experiment. Water flow rates were reduced on day 27 for each tank, from 10 L min^{-1} to 5 L min^{-1}, to save water and allow for the temporary system to operate correctly. To ensure homogeneity between the treatments, water flow in freshwater tanks was also reduced to the same rate. The change in water flow rates did not impact temperature, salinity, or dissolved oxygen. However, the use of a temporary system increased the temperature variability for the brackish water treatment as water was pumped closer to the St. Lawrence River coastline. Nonetheless, it was assumed that no impact on WEB model predictions would occur given that the model takes daily temperature into account. As a result of this event, fish reared in brackish water did not eat for a whole day (day 33) during the installation of the temporary system because of high turbidity. This observation was considered in the growth rate simulations as daily food consumption values were provided to the model.

The feed used was the same for each tank (3 mm Floating fish (salmonid) feed, Corey Aquafeeds, New Brunswick, Canada). The food ration represented 1.5 % of the tank initial biomass per day. The feed was given in two 20 min intervals to allow all fish to eat. The uneaten wet feed was weighted, converted into dry mass feed, and subtracted from the initial ration. To convert into dry mass feed, 20 g of feed was placed into fresh and brackish water tanks for 20 min. The recovered pellets were weighted to calculate the amount of water absorbed and used for the

conversion into dry mass feed. This was made in triplicate both for fresh and brackish water. The number of days before the ration was completely eaten was noted. Prior to the experiment, fish were fed the same food at the same 1.5% ration every day in 500-L tanks at a density of 200 fish/tank. At the end of the experiment, the individuals were anesthetized with MS-222 (0.2 g L^{-1}) before being measured (mm) and weighted (g) again, and ultimately sacrificed. Epaxial muscles were sampled on the left side over the lateral line to measure relative energy content. If the fish was too small, the right side was also sampled. Gonads and liver were also weighted to calculate somatic indices.

Growth rate and Fulton's condition factor

Growth rate was calculated using fish initial and final mass during the 60-day experiment following the equation:

(1) Growth rate = (Final mass (g) - Initial mass (g)) / 60 days

Fulton's condition factor (K) and its delta (ΔK) were calculated for each fish following the equations:

(2) $K = \text{Body mass (g)} / \text{Fork length (cm)}^3 * 100$

(3) $\Delta K = \text{Final K} - \text{Initial K}$

Gonadosomatic and hepatosomatic index

Gonadosomatic index (GSI) and hepatosomatic index (HSI) were calculated following the equations:

(4) GSI = Gonad mass (g) / Total body mass (g) * 100

(5) HSI = Liver mass (g) / Total body mass (g) * 100

Energy density

Bomb calorimetry (Parr 6100 Compensated Calorimeter, Parr Instruments, Illinois, USA) was used to quantify energy density (ED, J g^{-1} of wet mass) for the muscle of a subset of fish (n = 72; two per family per tank for a total of six fish per tank). Muscle samples were desiccated in a drying oven for 72 hours at 60°C until the dry mass was constant. The dried samples were powdered down using a mortar and pestle and then made into ~1 g pellets. The pellets were combusted at 370 psi oxygen (1 psi = $6.894\,747 \times 10^3$ Pa). The nitric acid resulting from combustion was neutralised by 0.1 M NaOH. Three benzoic acid controls were made at the beginning of each day to reduce experimental error. A linear regression model between dry-to-wet mass ratio and muscle energy density estimated through bomb calorimetry was created with this subset of fish to estimate the final energy density of all other fish (n = 72). Thirty-six fish were sacrificed on day 1 of the experiment to estimate initial energy density values for each family used. The family average was calculated and used as the initial value for each fish within a given family.

Energy density was also estimated by bomb calorimetry for the feed found in the two bags (25 kg of feed per bag) used during the experiment. Three replicates of 10 g per bag of feed given to the fish were used to quantify energy density. The mean energy density of the feed was calculated to obtain only one value per bag and the two values were used in the model as input.

Bioenergetics modeling

The WEB model simulations were performed using Fish Bioenergetics 4 (FB4) v1.1.1 platform in R (Deslauriers et al. 2017). The parameters for consumption, growth and metabolism used in the WEB model were developed with Appalachian brook charr from the Bowden State Fish Hatchery, Bowden, West Virginia (Hartman and Sweka 2003; Hartman and Cox 2008). Egestion, excretion and specific dynamic action were borrowed from a Brown Trout (*Salmo trutta*) model

(Elliott 1976). The activity rate multiplier (ACT), set as 2.89 by Hartman and Cox (2008) in their refinement of the WEB model, was also used. Additional input data were provided in a design file to FB4 (see instructions here: fishbioenergetics.org) and included initial fish mass, initial and final fish energy density, mean feed energy density, daily tank temperature and daily food consumption (in g) over the 60 days. The oxycalorific coefficient, which converts oxygen consumption data to energy units, was 13 560 J g^{-1} O_2 (Elliott and Davison 1975; Hartman and Sweka 2003). Two approaches were used to obtain WEB predictions: estimating growth providing food consumption and estimating total consumption providing the final mass of the fish. Given that food consumption was measured per tank and not per individual, growth predictions were made using mean observed initial mass for the 15 fish in the tank, mean energy density at the beginning and end of the experiment and daily food consumption. The FB4 code was modified to allow for the use of actual daily food rations as the consumption input. Total consumption predictions were made for each individual fish using initial and final fish mass as input. Individual consumption values were then summed together by tank to compare with the observed tank food consumption. Error rates of final mass and the consumption predictions made by the model were calculated as follows:

(6) Error rates = | Observed value – Predicted value | / Observed value

Statistical analysis

An independent t-test was carried out on the diet energy density to determine whether it was significantly different between the two bags of feed used for the experiment. The effects of number of days until the ration was completely eaten, the overall food consumption, the initial fish mass and the final fish mass were also tested between the treatments using multiple independent t-tests.

For ΔK, growth rate, GSI and HSI, normality of residuals was tested using a Shapiro-Wilk test and homogeneity of variances was tested using a Levene test. Salinity and genetic line effects on growth rate and ΔK were tested using a two-way analysis of variance (ANOVA). A square root transformation was made on the growth rate to achieve normality of residuals. For ΔK, normality was not achieved following data transformation so a Kruskal-Wallis test was used with lines and treatments combined as one factor. For the growth rate, in presence of significant effects, Tukey HSD comparison of mean was performed.

Male and female fish were tested separately for GSI and HSI. Female GSI and HSI were tested using a two-way ANOVA. A Games & Howell test was performed in presence of a significant effect because variances were heterogeneous for the GSI while a Tukey HSD was used for the HSI. For male GSI and HSI, a treatment effect was tested using a Kruskal-Wallis test. Selected and control fish were tested separately as residuals were not normal and variances were heterogeneous. A posteriori LSD rank test was performed in the presence of significant differences.

An ANOVA was used to test if the family had an effect on the initial fish muscle energy density. In the absence of a family effect, the line average could be used as the initial energy density value provided to the model. The WEB model interpolates a constant change in energy density over the 60-day experimental period.

To determine the WEB model performance, final mass and food consumption predictions made at the tank level were plotted against final mass and consumption observations to test if the intercept and slope deviated significantly from 0 and 1, respectively.

All statistical analyses were performed with R version 4.2.3 (2023-03-01) (R Core Team 2023).

Results

Food consumption

The 1.5 % ration was completely eaten after 13.8 ± 4.4 (mean ± S.D.) days in fresh water (FW) and 17.3 ± 3.3 days in brackish water (BW) with no significant difference among treatments (Welch's *t* test; df = 9.35; p = 0.15). The number of days before food ration was completely eaten also did not differ between lines: 15.2 ± 4.3 and 16.0 ± 4.3 days for the control and selected lines (Welch's *t* test; df = 10.00; p = 0.74), respectively. The total food consumption over the 60-day experimental period was not significantly different between fresh and brackish water with values of 1576.6 ± 212.8 g per tank and 1546.7 ± 228.1 g per tank respectively (Welch's *t* test; df = 10.00; p = 0.83) but was significantly different between lines (Welch's *t* test; df = 8.64; p = 0.004) with 1417.2 ± 168.4 g for the control line and 1706.1 ± 139.3 g for the selected line.

Growth rate and Fulton's condition factor

No interaction was found between the treatment and genetic line for the absolute growth rate ($F_1 = 0.16$; p = 0.69; Fig. 1). The salinity treatment had no influence on the absolute growth rate ($F_1 = 1.70$; p = 0.19; Fig. 1). However, the absolute growth rate was significantly lower in the control line than in the selected line ($F_1 = 9.12$; p = 0.003; Fig. 1). Once standardized by size, growth rates were significantly higher in the control line (FW 15.0 ± 3.6 g/g/d, BW 15.0 ± 4.5 g/g/d) than in the selected line (FW 14.3 ± 3.4 g/g/d, BW 13.2 ± 3.3 g/g/d) ($F_1 = 4.35$; p = 0.04). The salinity treatment had no influence ($F_1 = 1.38$; p = 0.24). Initial fish mass was significantly lower in the control line than in the selected line (Welch's *t* test; df = 167.25; p < 0.001; Table 2), with the mean mass being 119 ± 53 g and 149 ± 59 g, respectively. Final fish mass also differed between both lines (Welch's *t* test; df = 164.03; p < 0.001; Table 2). The mean final fish mass for the control line (219 ± 83 g) was significantly lower than for the selected line

(268 ± 99 g). ΔK was not significantly different between the line-treatment combinations ($X^2_3 = 2.55$; $p = 0.47$; Table 2).

Gonadosomatic index

Two initial fish were withdrawn from the GSI and HSI analyses because their sex was not determined with certainty. As fish selection to be used in the experiment was performed randomly, sampling of pre-experimental control males was low (n = 3) compared to post-experimental control males (brackish water n = 25, fresh water n = 26).

For the females, a significant difference was found for the treatment ($F_2 = 10.73$; $p < 0.001$; Table 3) but not for the line ($F_1 = 0.65$; $p = 0.42$; Table 3). A treatment effect was observed for male GSI in the control ($X^2_2 = 8.87$; $p = 0.01$; Table 3) and in the selected line ($X^2_2 = 7.13$; $p = 0.03$; Table 3). For both lines, the initial treatment was significantly lower than the two experimental treatments. No significant difference was observed between fresh and brackish water.

Hepatosomatic index

No interaction was found between treatments and genetic line for female HSI ($F_2 = 0.92$; $p = 0.40$; Table 3). Female brook charr from the selected line had significantly lower HSI than those from the control line ($F_1 = 16.85$; $p < 0.001$; Table 3). Significantly lower initial HSI was found compared to fresh ($p < 0.001$; Table 3) and brackish water ($p < 0.001$; Table 3). For male HSI, a treatment effect was observed only in the selected line ($X^2_2 = 8.15$; $p = 0.02$; Table 3) where initial males had significantly lower HSI than those reared in brackish water but not with fish reared in fresh water. Therefore, there was a significant difference between fresh and brackish water treatments for males.

Energy density

There was no significant difference for muscle energy density between families at the beginning of the experiment ($F_6 = 1.27$; $p = 0.30$). Therefore, mean muscle energy density was used for each genetic line as an input for the WEB model without considering family effects. However, muscle energy density differed significantly between the initial values measured and both salinity treatments ($p < 0.001$; Fig. 2).

The relationship between dry to wet mass ratio (DWR) and energy density (ED) for age-2+ brook charr was calculated in fresh (ED = 30382.3 x DWR – 1766.26; $r^2 = 0.88$; Fig. 3) and brackish water (ED = 32690.4 x DWR – 2315.08, $r^2 = 0.90$; Fig. 3) for 36 fish per treatment. For the remaining hundred fish whose ED was not estimated by bomb calorimetry, these regressions were used to estimate ED depending on the treatment fish were reared in and their respective DWR since they vary depending on the environmental conditions and the species (Hartman and Brandt 1995; Johnson et al. 2017).

Feed energy density did not significantly differ between the two bags used in the experiment (n = 3 per bag, Welch's *t* test; df = 3.86; p = 0.1942). The mean energy density for the first bag of feed was of 20 244 ± 543 J g^{-1} of wet mass while it was of 21 017 ± 657 J g^{-1} of wet mass for the second one. Even without significant difference, both values were still used in the WEB model simulations to represent reality more accurately.

Bioenergetics modeling

Linear model confidence intervals of WEB final mass predictions against brook charr final mass observations did include the values of 1 for the slope [CI 95% 0.89, 1.49] and 0 for the intercept [CI 95% -113.64, 36.94] for the fresh-water treatment (Fig. 4). Confidence intervals for the brackish water treatment included the value of 0 for the intercept [CI 95% -103.89, 13.02] but

not the value of 1 for the slope [CI 95% 1.10, 1.59] (Fig. 4). Final mass prediction error rates in fresh and brackish water were of 3.38 ± 3.64 % and 15.1 ± 3.91 % respectively (Fig. 4). The absolute difference between the final mass predictions and the observed value was of 9.16 ± 10.9 g in fresh water and of 37.0 ± 14.5 g in brackish water.

Overall food consumption was also estimated with the WEB model by fitting it to the final mass of each fish. The confidence intervals of the linear models for predicted and observed mass in fresh and brackish water both include the value of 1 for the slope and 0 for the intercept (FW 0.75 [CI 95% 0.25, 1.25], 26.30 [CI 95% -28.86, 81.29]; BW 0.75 [CI 95% 0.33, 1.17], 10.94 [CI 95% -34.92, 56.81]), Fig. 5). Food consumption error rates were of 3.2 ± 4.2 % in fresh water and of 14.4 ± 3.2 % in brackish water (Fig. 5). The absolute difference between food consumption predictions and the observations was of 56.7 ± 82.3 g in fresh water and 225.0 ± 67.0 g in brackish water.

Discussion

Altogether, our results showed that the brook charr WEB model better predicted growth variations occurring in fresh water compared to brackish water. Indeed, the WEB model better performed at estimating mass in fresh water. Ney (1993) suggested a maximum error rate between 30 and 50 % to validate the model in the laboratory which means that even if the slope confidence intervals did not include 1 in brackish water, the brook charr WEB model is still validated in fresh and brackish water with 3.38 ± 3.64 % and 15.1 ± 3.91 % error rates, respectively. While we validated the model with an anadromous strain, Hartman and Cox (2008) showed that predictions were within 1.7 ± 5.1 % of the measured final mass after refining parameters for metabolism and activity rate. This implies that the model performance varies depending on the environmental conditions, the fish rearing density, the energy density of the feed and the brook charr strain being

used. Indeed, the greater energy density of the feed compared to the natural prey used by Hartman and Cox (2008) could exacerbate errors in the predictions because of its impact on consumption and, ultimately, growth.

We had initially hypothesised that the WEB model should have underestimated growth in brackish water, but the opposite was observed in this study. While we expected increased growth in brackish water because salinity enhances the production of a growth hormone in anadromous brook charr (McCormick 1994), no difference was observed during this experiment and it might explain why predictions in brackish water were not underestimated. Furthermore, considering that selected fish had a greater initial mass and ate the same ration as the control fish, we may presume that this could have limited their growth potential, which indicates that size differences may not account for the resulting higher growth observed in the selected fish. Adaptations in the migratory behaviour of this species may also imply a variation in energy demands between populations (Bernatchez and Dodson 1987), variations that are not considered by the WEB model. This particularity could explain the higher error rates in comparison to the original model by Hartman and Cox (2008). It has also been shown that growth efficiency is lower in anadromous brook charr a year before the onset of migration compared to the residents inferring that their metabolic costs are higher (Morinville and Rasmussen 2003). Trudel et al. (2004) showed that standard metabolic rates are underestimated for steelhead in salt water, which only confirms the need to consider salinity in bioenergetics models. Even though the ACT parameter was refined from 1 to 2.89 because fish were more active than what had been originally anticipated (Hartman and Cox 2008), further experiments should aim to refine this parameter for an anadromous strain considering that bioenergetics models are sensitive towards parameters associated with metabolism (Hartman and Brandt 1993, Ney 1993).

Given that there was no significant difference between brackish and freshwater treatments in any of the physiological factors such as growth rate, Fulton's condition factor, HSI (except for male) and GSI, temperature was one factor that could influence the WEB predictions. Indeed, ambient temperature conditions that were used in the experiment resulted in a colder environment in brackish water, where the mean temperature was ~1.5 °C lower than the freshwater treatment. Both treatments ate the same ration, so colder temperature for the brackish water group could suppose lower metabolic costs leading to a surplus of available energy allowing the WEB model to overestimate the final mass. By re-running the brackish treatment fish simulations with the mean temperature from the freshwater treatment, it was shown that the slope and intercept of the brackish water treatment was closer to the freshwater linear model (Fig. S1). The same was observed when using mean brackish water temperature, whereas the freshwater treatment slope and intercept were closer to the brackish water we initially had. Salinity is not an input in this bioenergetics model while other applications have shown that salinity has an effect on physiological rates and oxygen consumption (Atlantic sturgeon (*Acipenser oxyrinchus*), Niklitschek and Secor 2009). This experiment also suggests that WEB model's predictions are affected by salinity as, in brackish water, growth was overestimated while food consumption was underestimated.

While the WEB model respectably predicted brook charr final mass in both water treatments, it performed better at predicting overall food consumption for the 60 days as it has been shown in other studies (Rice and Cochran 1984, Wahl and Stein 1991). Brook charr is known to display dominance in certain situations and it has already been established that dominance in salmonids is frequently linked to the size of individuals and population density (Grossman and Simon 2020) while prey capture success is strongly influenced by the dominance status (Sliger and Grossman 2021). Therefore, this may explain why the model did not perform well to predict final fish mass

as the original model was developed using fish reared separately while we used 15 fish per tank. Indeed, it has been shown in striped bass (*Morone saxatilis*) that as fish density increased, consumption also increased while growth rates declined due to higher activity (Hartman and Brandt 1993). Brook trout activity rates also increase when fish density doubles (Marchand and Boisclair 1998), which could be attributed to the increase of spontaneous swimming (Tang and Boisclair 1993) resulting in an underestimation of fish consumption rates predicted by the WEB model (Tang et al. 2000), and for which the potentially higher swimming costs caused by the increased fish density used in this study is not considered. It is also why both lines were kept separately in the study design given that it was presumed that individuals from the selected line were more voracious than the control line individuals. Because brook charr from the selected line had significantly greater initial and final mass and that dominance is often size-dependent, this also explains the rationale behind the separation of the lines. To improve consumption measurements, future studies should aim to run their experiments on fish kept separately to accurately validate bioenergetic models.

As there were no significant differences in food consumption between salinity treatments, there was one between lines whereas brook charr from the selected line ate more than those from the control line. However, the 1.5 % ration was chosen according to the initial tank biomass. Brook charr from the selected line had significantly higher initial mass than those from the control line. Therefore, even if there was a significant difference in overall food consumption, it could not explain the difference in WEB predictions. In contrast, it could have influenced brook charr growth rate which was significantly lower for the control line. This is in line with our assumption because these individuals were not selected for their growth characteristics. Observed Brook charr lower growth rates from the control line could have occurred because it has been shown that gonad

development can take 8 to 15 % of the total body energy in rainbow trout (Kaushik and Médale 1994). However, our experiment was conducted from June to July, and age 2+ anadromous brook charr do not start investing in reproduction until the end of August (De Montgolfier et al. 2009), which infers that gonads were undeveloped at the time of our experiment. Hence, the 1.5 % ration could have been the limitation to growth for the control line since smaller fish have higher specific consumption rates than larger fish at a given temperature.

In this study, predictions for fish reared in fresh water better estimated growth rate and mass gain than for fish reared in brackish, for which growth was overestimated even though we had predicted the opposite. This experiment suggests that there is a difference for the WEB model's prediction depending on the water temperature and salinity, which means that for conservation and management purposes, it is important to consider both. Indeed, this bioenergetics model can be used to predict final mass and food consumption for anadromous brook charr populations in Québec, Canada, to evaluate stocking impacts but there would be a need to refine the ACT parameter to improve the predictions and reduce the error rates of the WEB model for the strains considered. There is also a need to refine the respiration and consumption submodels for the anadromous ecotype as metabolic rates are approximately 30 % higher in salt water than in fresh water (Rao, 1968) and because consumption rates can vary depending on the diet.

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Competing interests

The authors declare there are no competing interests.

Data availability

Data files and scripts used for this study are publicly available from the Université du Québec à Rimouski's Dataverse Collection within the Borealis Repository (https://doi.org/10.5683/SP3/5A4LHI).

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579 **Tables**

580 **Table 1.** Mean (± S.D., n = 6) experimental water parameters for brook charr (*Salvelinus*
581 *fontinalis*) reared in fresh or brackish water for 60 days. The salinity value does not include
582 the seven days of acclimation to brackish water.

Treatment	Temperature (°C)	Salinity (PSU)	Oxygen (mg/L)
Brackish water	10.9 ± 1.3	13.98 ± 1.23	9.41 ± 1.77
Fresh water	12.4 ± 1.6	0.19 ± 0.04	8.06 ± 2.48

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Table 2. Summary of mean (± SD) initial and final mass, fork length, energy density, and delta Fulton's condition factor for 172 brook charr (*Salvelinus fontinalis*) reared in fresh or brackish water for 60 days.

Treatment	Line	n	Initial mass (g)	Final mass (g)	Initial fork length (mm)	Final fork length (mm)	Initial energy density (J g^{-1} of wet mass)	Final energy density (J g^{-1} of wet mass)	ΔK
Brackish water	Selected	43	148 ± 55	261 ± 90	240 ± 28	276 ± 29	5182 ± 161	5874 ± 427	0.18 ± 0.07
	Control	43	118 ± 57	215 ± 86	214 ± 35	251 ± 34	5181 ± 256	5794 ± 324	0.18 ± 0.09
Fresh water	Selected	42	151 ± 63	276 ± 107	241 ± 33	279 ± 38	5182 ± 161	5766 ± 392	0.24 ± 0.48
	Control	44	120 ± 49	222 ± 81	217 ± 32	256 ± 35	5181 ± 256	5794 ± 342	0.16 ± 0.06

589 **Table 3.** Mean (± S.D.) hepatosomatic index (HSI) and gonadosomatic index (GSI) for male and female age-2+ brook charr
590 (*Salvelinus fontinalis*) reared in fresh or brackish water for 60 days. The initial treatment represents fish sacrificed on day 1
591 while fresh and brackish water treatments represent fish sacrificed on day 60. Superscript letters indicate significant differences
592 between treatments. Lowercase superscript letters represent significant differences between the genetic lines. n is the number
593 of fish sampled for each treatment.

Line	Treatment	HSI		GSI		N	
		Male	Female	Male	Female	Male	Female
Control	Initial	1.91 ± 0.27 ^{a}	1.79 ± 0.37 ^{A}	0.15 ± 0.02 ^{a}	0.63 ± 0.20 ^{a}	3	12
	Fresh water	1.79 ± 0.24 ^{a}	2.18 ± 0.22 ^{B}	1.41 ± 0.83 ^{b}	0.87 ± 0.49 ^{b}	26	18
	Brackish water	1.85 ± 0.23 ^{a}	2.28 ± 0.31 ^{B}	0.22 ± 0.26 ^{b}	1.11 ± 0.50 ^{b}	25	18
Selected	Initial	1.24 ± 0.29 ^{a}	1.30 ± 0.38 ^{a}	0.11 ± 0.07 ^{a}	0.53 ± 0.08 ^{a}	9	10
	Fresh water	1.46 ± 0.28 ^{ab}	1.93 ± 0.30 ^{b}	0.63 ± 0.54 ^{b}	0.96 ± 0.32 ^{b}	24	18

	Brackish water	1.58 ± 0.20 [b]	1.92 ± 0.36 [b]	0.68 ± 0.62 [b]	0.85 ± 0.26 [b]	18	25

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Figure Captions

Figure 1. Box plot representing the growth rate (g day^{-1}) of 172 age-2+ brook charr (*Salvelinus fontinalis*) reared in fresh or brackish water for 60 days. The middle hinge of the boxplot is the median, the lower and upper hinges correspond to the first and third quartiles, and the lower and upper whisker extend at most 1.5 x the inter-quartile range from the hinges. Dots represent outliers. The value on top of each box is the sample size. All data points were used for statistical analysis. Letters at the top of the figure indicate the significant differences between treatments and lines.

Figure 2. Energy density (J g^{-1} of wet mass) of age-2+ brook charr (*Salvelinus fontinalis*) at the beginning and end of the experiment where fish were reared in fresh or brackish water for 60 days. The initial treatment represents fish sacrificed on day 1 of the experiment while fresh and brackish water treatments represent fish sacrificed on day 60. The middle hinge of the boxplot is the median while the lower and upper hinges correspond to the first and third quartiles and the lower and upper whisker extend at most 1.5 x the inter-quartile range from the hinges. Dots represent outliers. Values at the top of each box is the sample size.

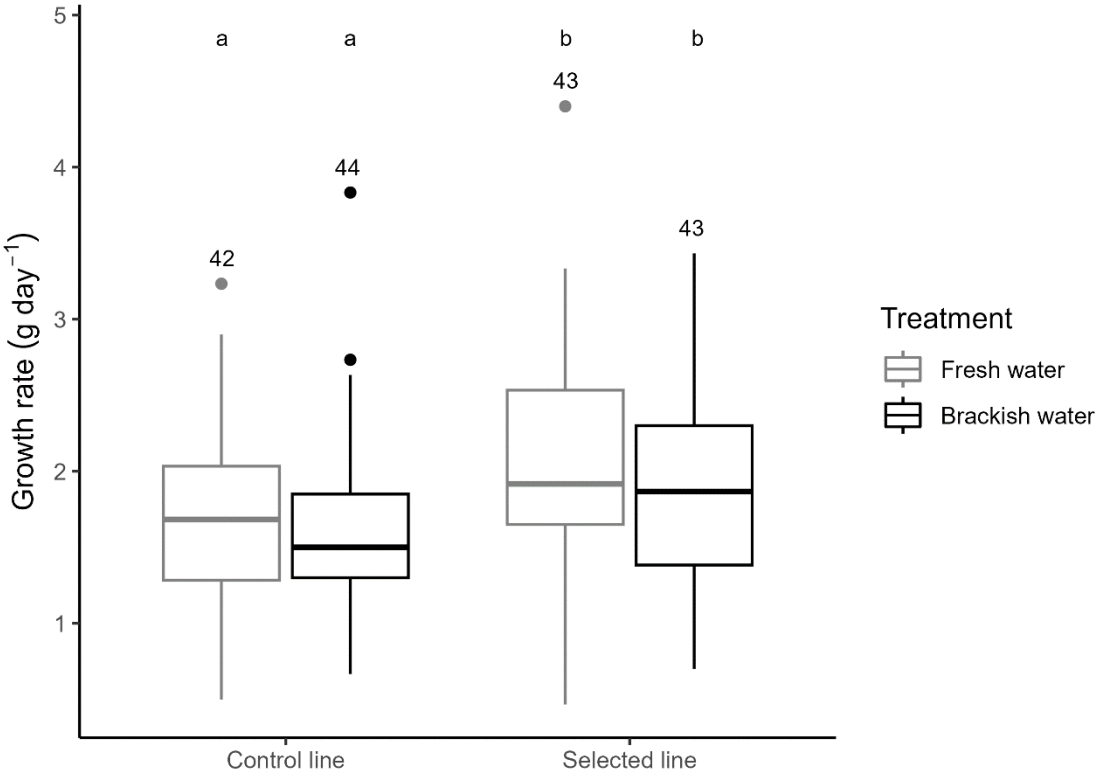
Figure 3. Relationship between dry to wet mass ratio and energy density (J g^{-1} of wet mass) measured in 72 age-2+ brook charr (*Salvelinus fontinalis*) at the end of the 60-day experiment: 36 in fresh water and 36 in brackish water. The solid line represents the regression through the actual data for both treatments. The shaded area represents the confidence intervals.

Figure 4. Predicted mass by the WEB model (g) compared to the mass observed experimentally (g) per tank of age-2+ brook charr (*Salvelinus fontinalis*) reared in fresh or brackish water for 60 days. The solid lines represent the regression through the actual data for both treatments. The shape of the points represents the genetic line. The shaded area represents the confidence intervals. The dashed line represents a slope of 1 and an intercept of 0.

Figure 5. Predicted tank total consumption by the WEB model (g) compared to consumption observed experimentally (g) where age-2+ brook charr (*Salvelinus fontinalis*) were reared in either fresh or brackish water for 60 days. The points shape represents the line. The shaded area represents the confidence intervals. The dashed line represents a slope of 1 and an intercept of 0.

627 **Figures**

628 **Figure 1.**



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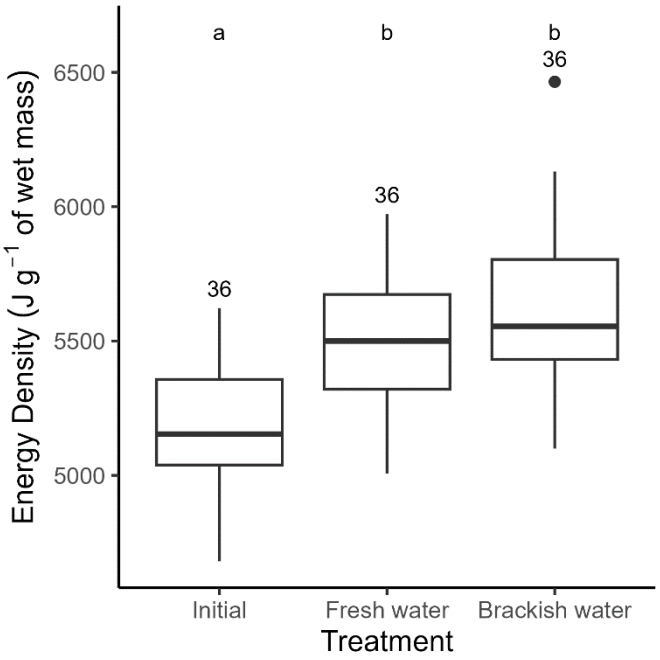
631 **Figure 2.**



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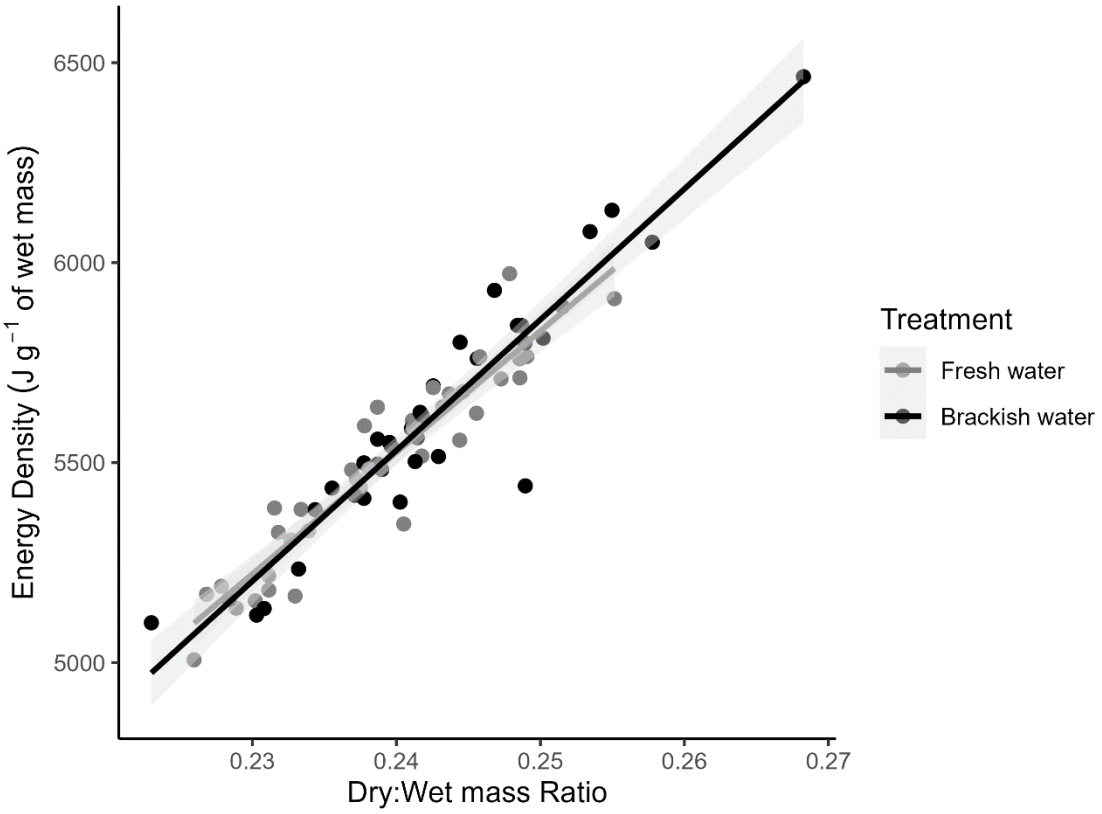
634 **Figure 3.**



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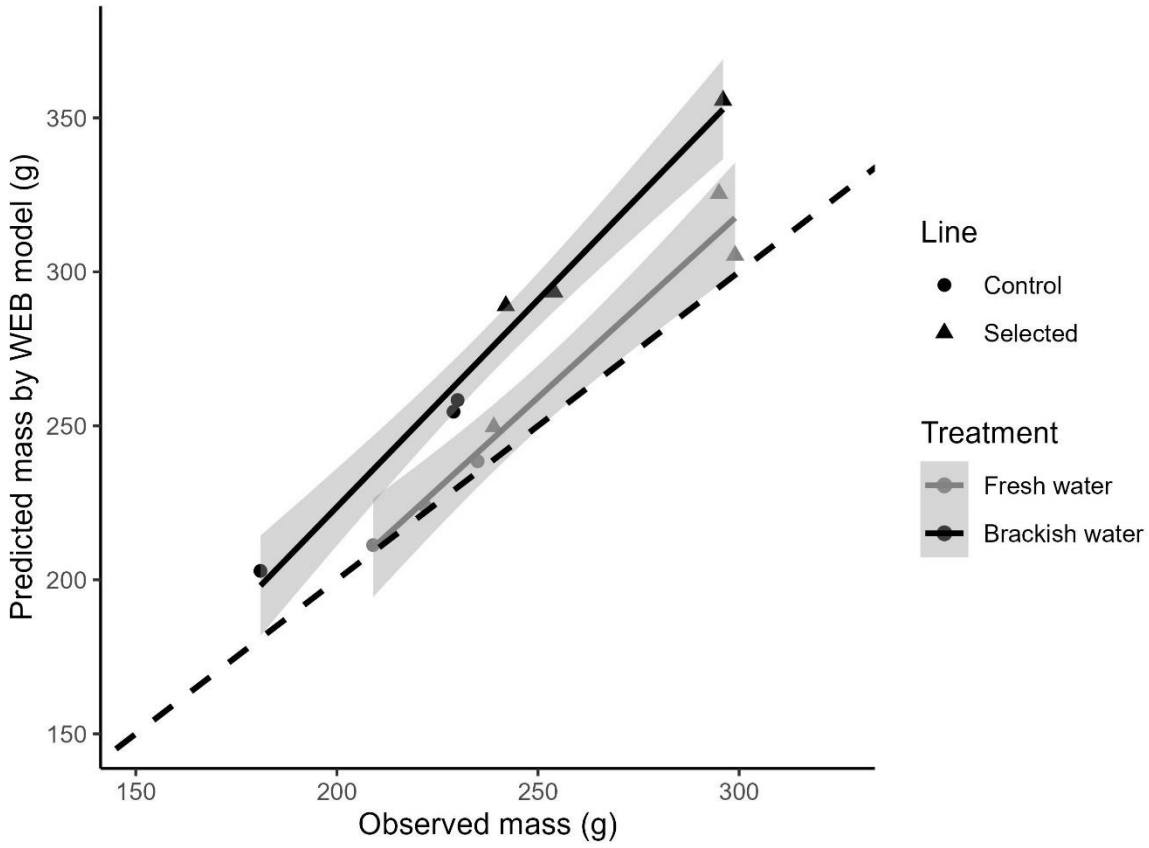
637 **Figure 4.**



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641 **Figure 5.**

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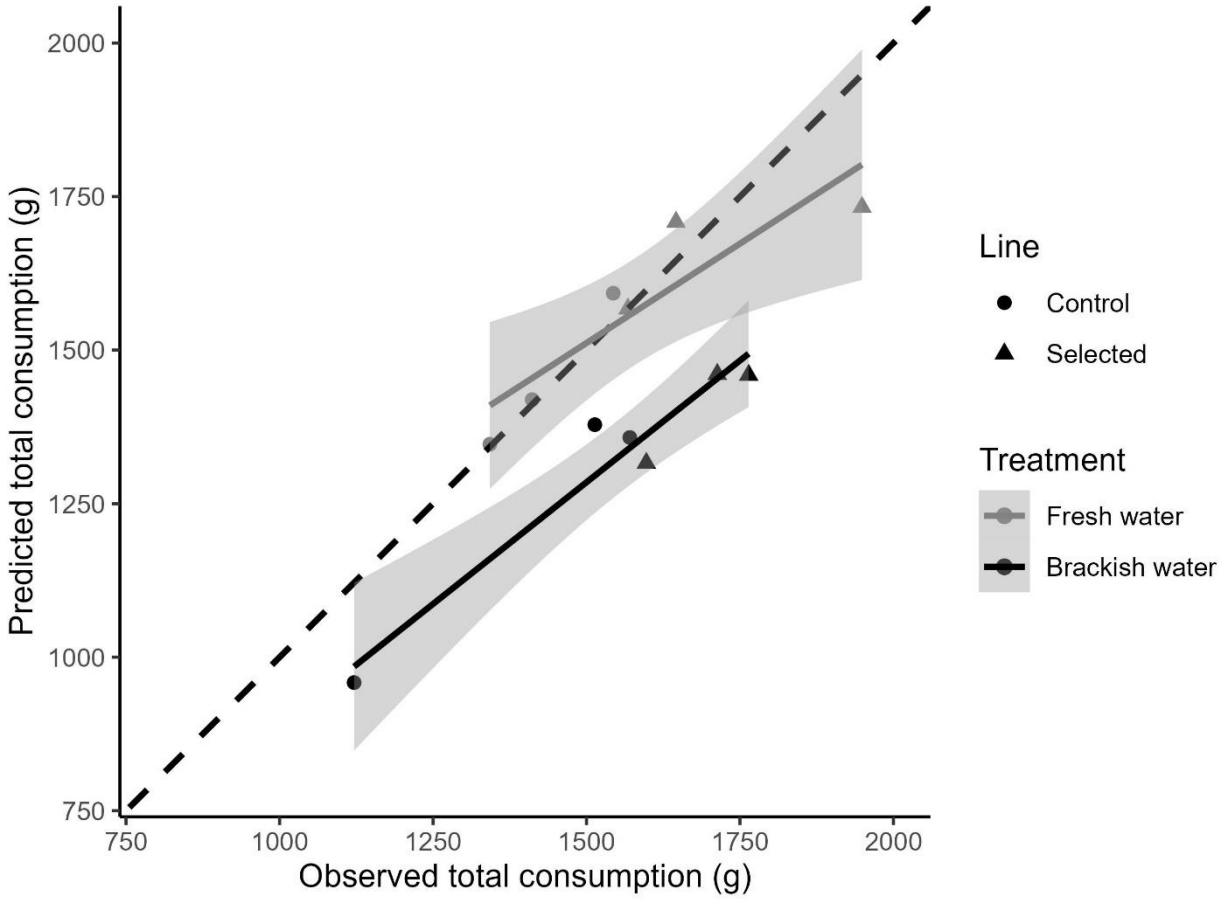

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