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Assessment of two feeds on survival, proximate composition, and amino acid carbon isotope discrimination in hatchery-reared Chinook salmon

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ABSTRACT

Chinook salmon (*Oncoryhnchus tshawytscha*) populations along the North Pacific coast have declined dramatically in recent decades. Hatchery-based experimental diet studies have the potential to inform Chinook salmon aquaculture practices, aid in stock enhancement, and determine species-specific parameters which can be applied to studies of wild fish. We fed hatchery-reared juvenile Chinook salmon two diets – a fishmeal-based diet (BioVita™) and an alternative diet (BioClark's™) made partially from terrestrially derived protein. We measured a suite of fish survival, growth, and nutritional performance metrics to determine if the diets had differing effects on fish development. We also determined amino acid ^{13}C trophic discrimination factors (TDFs) between diet and tissue for liver, muscle, and whole body homogenate. We found no difference in freshwater survival, marine survival, or proximate composition between fish from the two diet treatments. Within the amino acid ^{13}C TDFs, we found that the essential amino acids phenylalanine, leucine, and isoleucine had no isotopic discrimination between diet and any of the Chinook salmon tissues analyzed, making those amino acids the best candidates for determining nutrient and carbon sources in wild fish. This is the first time amino acid ^{13}C TDFs have been determined for a Pacific salmon species, thus contributing a critical step in understanding the implications of any future research conducted on amino acid isotopic biogeochemistry and carbon source dynamics in these coastal-oceanic species. Additionally, these results show that the alternative terrestrially derived feed may be as effective in rearing juvenile Chinook salmon as the more expensive fishmeal-based feed.

1. Introduction

Chinook salmon (*Oncorhynchus tshawytscha*) returns have declined along much of the North Pacific coast in recent decades. In Alaska, Chinook returns have plummeted in many regions, causing closures of fisheries from Southeast Alaska to the Yukon-Kuskokwim River system (ADFG, 2013). Chinook salmon are a major commercial and subsistence resource for the people of Alaska and are one of the most popular species for sport fishing. Subsistence harvest of Chinook salmon averaged approximately 167,000 fish from 1989 to 2006 and sport fishing landings were nearly equal, but both have declined significantly during the poor return years of the last decade (Fall et al., 2017). The current commercial Chinook salmon fishery in Alaska is valued at approximately $16.3 million per year (ADFG, 2019). The causes of declines in Chinook salmon escapement are not fully understood, but research points to mortality during early freshwater residence (Neuswanger et al., 2015) or declines in early marine survival (Schindler et al., 2013), possibly due to changes in dietary ecology (Hertz et al., 2016). Prey abundance, quality, and phenology may be shifting, but quantifying changes in resource utilization and trophic ecology is difficult in this highly migratory and widely distributed species. Tools and techniques which can help to detect influential changes in feeding ecology or trophic position will help to isolate the factors which are causing Chinook population declines. Additionally, studies informing hatchery-based production of Chinook salmon can help aid in stock enhancement and conservation in the face of such declines.

Controlled feeding trials of hatchery-raised stocks can provide information about the efficacy of different feeds for fish development and growth. Aquaculture is one of the fastest-growing food production sectors globally, and fishmeal-based feeds are placing increasing demands on pelagic fisheries and small forage fish such as anchovy, herring, and mackerel (Naylor et al., 2005). The National Oceanic and Atmospheric Administration's (NOAA) National Marine Fisheries Service (NMFS) has funded research into alternative feeds in order to determine if non-fishmeal-based feeds can produce hatchery-raised fish of equal health and quality to those raised on fishmeal-based diets. In

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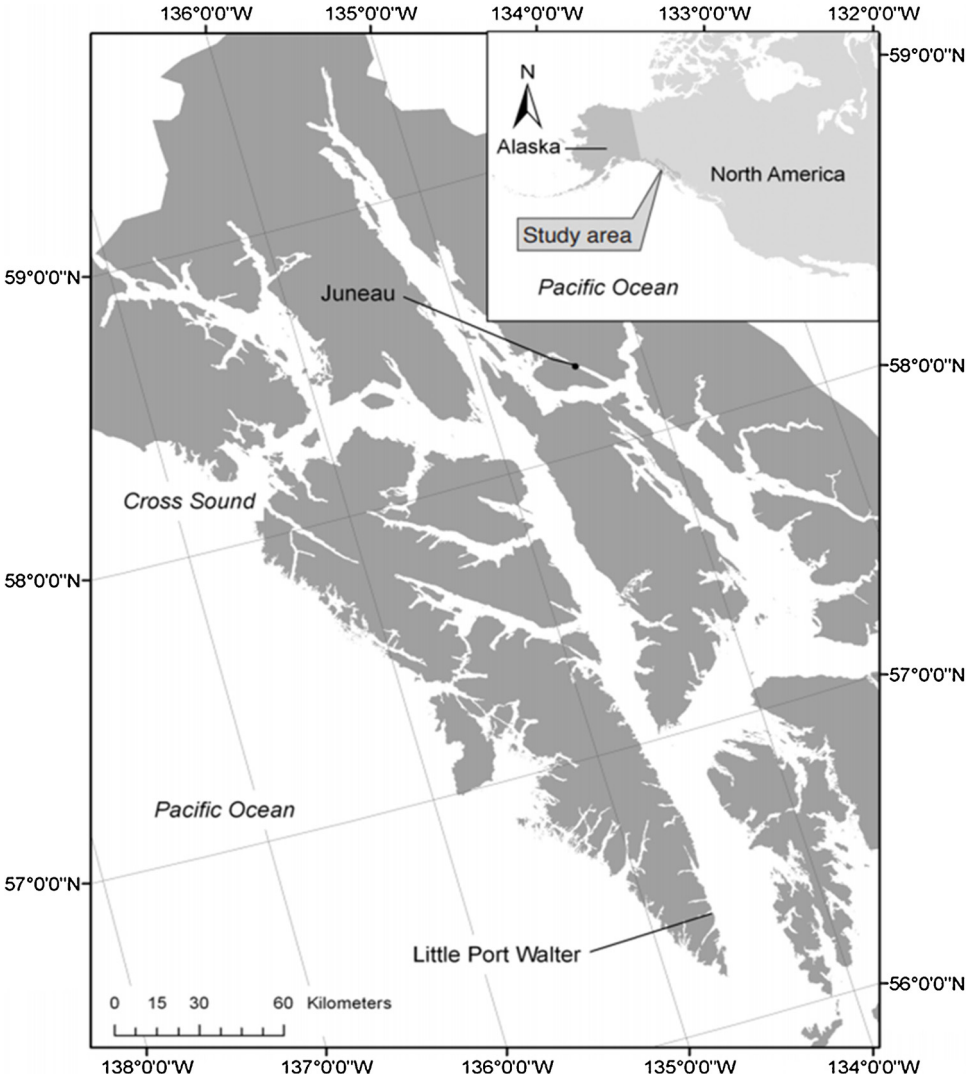

Fig. 1. Location of Little Port Walter Hatchery, Southeast Alaska, USA.

particular, feeds that derive protein from terrestrially-based sources have the potential to be less expensive, have a lower impact on water quality from food waste (Davidson et al., 2016), and reduce pressure on pelagic fisheries that provide fishmeal. Hatchery feeding trials also provide information that is useful for assessing wild stocks, such as determining freshwater and marine survival, optimal growth conditions, and stable isotope diet-to-tissue discrimination factors (Gwak et al 2003, Le Vay and Gamboa-Delgado 2011, Skinner et al., 2017). Despite the extensive use of hatcheries for stock enhancement of Pacific salmonids (Flagg, 2015), few hatchery-based feed studies for Chinook salmon exist.

Among the most fundamental metrics of fish performance are survival, growth, and proximate composition (Shearer, 1994). Survival metrics in fresh water and adult returns of hatchery fish are ultimately two of the most important measures of juvenile dietary efficacy since recruitment into the spawning class is one of the primary goals of stock enhancement and conservation. Proximate composition is the measure of the body weight percentage of each major biochemical component that makes up body mass, namely moisture, protein, lipid, carbohydrate, and ash (Shearer, 1994). Proximate composition has been shown to affect ontogenetic timing in juvenile salmonids, and threshold levels of lipid storage in particular have been linked to multiple important changes in salmonid development including smoltification and maturation (Thorpe, 1986; Rowe et al., 1991). Determining the effect of diet on proximate composition in hatchery-reared fish has been accomplished across many species and remains one of the most simple and effective means of assessing dietary quality (e.g. Bagheri et al., 2016; Barron et al., 2016; Craft et al., 2016; Grigorakis et al., 2002).

Compound-specific stable isotope analysis (CSIA) is another means by which researchers can improve our understanding of the effects of diet composition on fish productivity. The use of stable isotope data and associated analytical tools to study food web ecology and trophic structure has increased steadily in recent years (Layman et al., 2012). Most initial studies focused on stable isotope ratios of bulk tissue samples, but recent advances in analytical techniques have allowed for the measurement of stable isotope ratios of specific biochemical compounds, such as amino acids and fatty acids. Compound-specific isotope analysis of carbon isotopes in amino acids of wild-caught fish has the potential to help determine carbon sources in food webs (Larsen et al., 2013; McMahon et al., 2016), examine site fidelity and migration patterns (McMahon et al. 2013), and improve diet-modeling exercises (Pethybridge et al., 2018). Amino acid CSIA studies must first be parameterized by determining compound-specific trophic discrimination factors (TDFs), a measure of the change in isotopic ratios from diet to consumer as food is absorbed, metabolized, and used to build the consumer's tissues. Feeding trials in hatchery-raised fish provide an excellent opportunity to understand diet-to-tissue stable isotope TDFs. Many controlled feeding studies have determined isotopic TDFs for bulk

fish tissues such as muscle and liver (Vander Zanden et al., 2015), but far fewer studies have been performed to determine TDFs for individual biochemical compounds within those tissues such as amino acids

Determining stable isotope TDFs for individual tissues also allows for diet estimation over different time frames, as different tissues have different isotopic turnover rates. Liver has a rapid turnover rate of days to weeks, whereas muscle takes multiple months for the tissue to reach isotopic equilibrium (Boecklen et al., 2011). Knowing TDFs for tissues with differing turnover rates allows for insight into multiple temporal windows of dietary nutrient incorporation and can aid in the determination of seasonal dietary changes.

We investigated the effects of two diets: one a traditional fishmeal-based feed (BioVita™, BioOregon, WA, USA) and the other a less expensive terrestrial/plant-derived protein-based feed (BioClark™, BioOregon, WA, USA), on a suite of growth, survival, and nutritional performance metrics. We measured freshwater and marine survival, length, weight, whole-body proximate composition, and amino acid ^{13}C TDFs (muscle, liver and whole-body homogenate) for Chinook salmon raised in a hatchery to determine if there were differences in fish development between the two dietary treatments.

2. Methods

2.1. Study design & fish rearing

In August of 2011, gametes were harvested from male and female Chinook salmon returning to the NMFS Little Port Walter Marine Research Station (LPW) located on southern Baranof Island in Southeast Alaska (Fig. 1). The returning salmon were composed of both Unuk and Chickamin river broodstocks which have been raised and released from LPW's experimental hatchery since 1976. The gametes were collected, fertilized, and seeded into Marisource vertical incubator heath trays where they were kept through the winter. The following spring (2012), the emergent fry were ponded into shallow, 12.9 m^3, freshwater vertical raceways (VRs) (Heard and Martin, 1979) and segregated by stock (Unuk or Chickamin) in their respective VRs. Families within a stock were balanced across ponding VRs. When fry reached an average size of 2.3 g (July 2012) they were moved into ten deeper, 23.0 m^3 VRs. The deeper VRs were stocked with approximately 15,000 fry each. Five VRs were randomly assigned to one feeding group (75,000 fry,) and five VRs were assigned to a second feeding group (75,000 fry). Each VR was treated as a replicate of its respective feeding group treatment.

A marine fish-based protein feed (BioVita™) was used for the first feeding treatment (hereafter "fishmeal") and a terrestrially based protein feed (BioClark's™) was used for the second feeding treatment (hereafter "alternative"). BioClark's™ feed was less calorically dense than the BioVita™, so 5.6% more food by weight was applied to the alternative treatment so that the diets were calorically equivalent. The energetic values of the diets were based on nutritional content information provided by the manufacturer.

The different stocks and treatments were counted, adipose finclipped, and tagged with coded-wire tags (CWTs) in October 2012. In April of 2013, tagged fish from both treatment groups were checked for tag retention. The mean tag retention was 96% for the fishmeal treatment and 99% for the alternative treatment. After tag retention estimates were completed, all treatment groups were transferred to saltwater net pens for pre-release saltwater acclimatization. A total of 132,965 CWT-tagged juvenile Chinook (74,101 fishmeal fed and 58,864 alternative fed) were released into the marine environment on May 15 and 16 of 2013 (Table 1).

Released fish were recovered from commercial, sport, and ocean fisheries and from maturing Chinook returning to LPW. The recoveries took place over a 4-year period from August 2013 to August 2017. Fisheries recoveries occurred during sampling conducted by the Alaska Department of Fish and Game (ADF&G). Maturing Chinook returning to LPW were collected and held in marine net pens until processing in August of each year. Returning fish CWTs were extracted and decoded to determine which release groups they originated from.

Table 1
Freshwater survival for both BioVita™ and BioClark™ diet treatments. No statistically significant differences ($p > 0.05$) in freshwater survival were found between the two diet treatments.

Feed Type	Stock	Total Freshwater Survival (%)
BioVita	Unuk	99.00
	Chickamin	98.80
BioClark's	Unuk	98.21
	Chickamin	98.82

2.2. Freshwater and marine survival

Freshwater survival (*FS*) for a particular CWT code was calculated as

$$FS = \frac{F_i - F_m}{E_r}$$

where F_i is the initial number of fish cultured per VR, F_m is the cumulative freshwater mortality per VR, and E_r is the number of tagged smolts released (Table 2).

Marine survival (*MS*) for a particular CWT code was calculated as

$$MS = \frac{E_f + E_h}{E_r}$$

where E_f is the estimated number of fish caught in commercial and sport fisheries expanded by a sampling expansion factor to account for different sampling rates of CWT-tagged fish in different time-area-gear strata (Nandor et al., 2010), E_h is the number of tagged fish returning to LPW, and E_r is the number of tagged smolts released with a particular CWT code.

We analyzed freshwater and marine survival between the two feed groups using mixed effects logistic regression (Zuur et al., 2009) with replicates as a random effect and the feed type (fishmeal vs. alternative) as the independent variable. All statistical analyses were performed in R (Version 3.3.2, R Core Development Team).

2.3. Growth and proximate composition

Chinook smolts from each treatment group (N = 25) were collected and frozen on May 15, 2013 before the treatment groups were released into the marine environment. Whole frozen Chinook smolts were measured for fork length (FL) and wet mass. Fish mass was measured to the nearest 0.1 g on a Sartorius CPA323S balance (Sartorius, Göttingen, Germany). A muscle plug weighing 100 mg or no more than 5% total body mass (whichever was less) and the whole liver were dissected from the fish and frozen at -20 °C. The muscle plug and liver samples were then freeze-dried and homogenized using a BioSpec BeadBeater. The remaining whole fish sample was homogenized using a Fisher

Table 2
Marine survival for both BioVita™ and BioClark™ diet treatments. No significant differences ($p > 0.05$) in marine survival were found between the two diet treatments.

Feed Type	Stock	# Released	Total Returns	Total Marine Survival (%)
BioVita	Unuk	44,465	352	0.79
	Chickamin	29,636	128	0.43
	Total	**74,101**	**480**	**0.65**
BioClark's	Unuk	29,219	230	0.79
	Chickamin	29,645	133	0.45
	Total	**58,864**	**363**	**0.62**

Scientific Tissuemiser and frozen at −20 °C. A small subsample (~10 mg) of dried muscle plug and liver was taken for CSIA and the remaining dried sample was later returned to the dried whole fish homogenate for lipid analysis, protein analysis, and CSIA.

A LECO Thermogravimetric Analyzer TGA 601 LECO Inc., St. Joseph MI) was used to measure moisture and ash content gravimetrically using a temperature of 135 °C for moisture content and 600 °C for ash content. Moisture and ash values obtained from the two methods varied by less than 1%. Moisture analyses were duplicated to ensure the coefficient of variation (CV) was ≤ 1 standard deviation (SD).

Nitrogen content was measured with a LECO nitrogen analyzer (TruSpec CN, LECO Inc., St. Joseph MI) following the Dumas method (Sweeney and Rexroad, 1987). Protein content was estimated using a standard method of multiplying total nitrogen content by a conversion factor of 6.25, the nitrogen content of protein in animal tissues (Craig et al., 1978). All samples were run in duplicate to ensure the CV for estimated nitrogen content was ≤ 1 standard deviation (SD). Quality assurance samples included with each batch of 17 samples included a blank reference consisting of cane sugar and a standard reference material (NIST 1546) obtained from the US National Institute of Standards and Technology (NIST). If quality assurance samples exceeded prescribed limits (≥ 15% variation for reference samples, 0.1% protein for blanks), then the samples were re-analyzed.

For total lipid analysis, a modified version of the protocol described by Van Handel (1985) was used. First, 10–30 mg of homogenized dry fish tissue was placed into a glass vial. A chloroform-methanol solution was then added (2 mL of 2:1 v/v), then vials were capped and sonicated in a water bath for 30 min. From the sonicated sample, 1 mL of a 1:10 dilution of each sample was made. Next, 100 µL of the 1:10 dilution was added to a glass 96-well plate, with each sample being run in triplicate. The solvent was evaporated by placing the glass 96-well plate on a temperature-controlled heated block at 100 °C for 10 min. Twenty microliters of concentrated sulfuric acid was then added to each well and the samples were allowed to incubate at 100 °C for 10 min, after which the plate was allowed to cool to room temperature. Two-hundred and eighty µL of SPV (6.8 mM vanillin, 2.6 M phosphoric acid) reagent was added and the samples were incubated at room temperature with gentle shaking for 30 min. The absorbance at 490 nm was recorded using a Victor3 1420 Multilabel Counter (Perkin-Elmer, Wellesley, MA). Total lipid was calculated by comparison of the absorbance values to a calibration curve generated using menhaden (*Ethmidium maculatum*) oil. Quality of proximate composition values were also evaluated by summing all of the components (protein, lipid, moisture, and ash). If the sum deviated from 100% by more than 1.5%, samples were reanalyzed if additional homogenate was available.

Measures of fish size and proximate composition were tested for normality using the Shapiro-Wilk test. Data with normal distributions were tested for significant differences across diet treatments using a two-sided t-test. Fish size and proximate composition data with non-normal distributions were tested for significant differences using both the Kruskal-Wallis test and the Pearson Chi-square test. All statistical analyses were performed in R (Version 3.3.2, R Core Development Team).

2.4. Amino acid $\delta^{13}C$ analysis

For amino acid $\delta^{13}C$ analysis, a 10 mg sample of freeze-dried and homogenized tissue was acid-hydrolyzed in a 6 M HCl solution for 70 min and then dried down in a temperature-controlled heating block at 60 °C under a stream of purified nitrogen gas. The hydrolyzed amino acids were then derivatized in a solution of methanol, pyridine, and methyl chloroformate (Sigma Aldrich, St. Louis, MO, USA) using a one-step rapid derivatization method (Walsh et al., 2014). Compound-specific ^{13}C amino acid analysis was performed using a Trace 1310 gas chromatograph (Thermo Electron, Bremen Germany) on a DB-23 column (Agilent Technologies). The gas chromatograph was coupled to a Delta V Advantage isotope ratio mass spectrometer via an Isolink II combustion interface (Thermo Electron, Bremen, Germany) at a reactor temperature of 1000°C. For each analysis, a 0.5 µl aliquot of derivatized sample was injected into a deactivated splitless liner (Restek Corporation, Bellefonte, PA) at 250 °C with a helium flow rate of 1.2 ml/ min. For each sample, the $^{13}C/^{12}C$ ratio was determined and calibrated to the international reference standard scale, Vienna Pee Dee Belemnite (V-PDB). Each sample was arithmetically corrected for the addition of carbon during derivatization by running purified and derivatized amino acids of a known isotopic value throughout the analytical sequence and applying a correction factor according to the method described by Docherty et al., 2001Docherty et al. (2001). Amino acid carbon discrimination factors were tested for differences across diets and tissue combinations within each amino acid using a one-way ANOVA ($\alpha = 0.05$). If significant differences were detected, Tukey's HSD test was used to determine which means differed.

2.5. Feed amino acid composition

We analyzed each of the feed samples for amino acid concentrations in order to determine if the two feeds provided equivalent rations of individual amino acid species. The feeds were analyzed in replicate by the University of California Davis Proteomics Center using an L8800 Hitachi Amino Acid Analyzer (Sigma, A-9906). Amino acids (AAs) were quantified using ion-exchange chromatography followed by treatment with ninhydrin and colorimetric analysis of the ninhydrin-treated AAs (Ozols, 1990). An amino acid standards solution for protein hydrolysate was used to determine response factors and thus calibrate the analyzer for each amino acid. In addition, this standard has been verified against the NIST standard reference material 2389a. Each injection contains norleucine as an internal standard to allow correction of the results for variations in sample volume and chromatography.

3. Results

3.1. Freshwater and marine survival

Freshwater and marine survival results are summarized in Tables 1 and 2, respectively. Analyses indicated no significant difference in freshwater or marine survival between the fishmeal and alternative feeding groups ($P > 0.05$).

Table 3
Mean (± SD) of Chinook salmon length, mass, and proximate composition measurements by diet treatment. No statistically significant differences ($p > 0.05$) were found between the two experimental diet treatments. The BioVita™ diet is composed primarily of fishmeal, while the BioClark's™ diet is composed of various terrestrially-derived proteins.

	N	Length (mm)	Weight (g)	Prox. ED (kJ/g)	% Protein	% Lipid	% Ash
BioClark's™	20	101.5 (8.2)	15 (3.7)	6.4 (1.3)	15.8 (0.6)	8.9 (3.2)	2.3 (0.2)
BioVita™	23	101.1 (9.8)	15.9 (4.8)	6.3 (1)	15.7 (0.6)	8.5 (2.6)	2.3 (0.3)

Table 4
Mean (+/- 95% confidence interval) values of amino acid ^{13}C trophic discrimination factors from diet (BioClark™; N = 20, and BioVita™; N = 23) to fish tissues and whole body homogenate. Significant differences ($p < 0.05$) within an amino acid are denote by letter groupings. Significant differences do not apply across different amino acids.

		AA13CTrophic Discrimination Factors (‰)										
		Non-Essential					Essential					
Tissue	Diet	Ala	Asp	Glu	Gly	Pro	Ile	Leu	Lys	Met	Phe	Val
Muscle	BioClark	−1.3 (0.6)^{a}	0.7 (0.6)^{ab}	−1.3 (0.6)^{c}	5.7 (0.6)^{b}	1.2 (0.7)^{b}	0.1 (0.3)^{bc}	−0.3 (0.4)^{b}	1.4 (0.9)^{b}	−3.1 (0.5)^{b}	−0.7 (0.6)^{b}	0.7 (1.3)^{ab}
	BioVita	−2.2 (0.5)^{ab}	−0.6 (0.6)^{b}	0.9 (0.4)^{b}	2.6 (0.8)^{d}	1.6 (0.2)^{b}	−0.2 (0.3)^{c}	0.1 (0.3)^{ab}	1.9 (1)^{b}	−1.6 (0.4)^{a}	0 (0.4)^{ab}	1.2 (0.3)^{ab}
Liver	BioClark	−1.7 (0.5)^{ab}	1.3 (1.4)^{ab}	0.7 (0.8)^{b}	8.2 (1.2)^{a}	2.2 (0.6)^{ab}	0.9 (0.5)^{a}	0.3 (0.4)^{ab}	4.3 (1.2)^{a}	−1.8 (0.5)^{a}	0.2 (0.6)^{ab}	2.5 (1.1)^{a}
	BioVita	−2.7 (0.6)^{b}	0.2 (1.1)^{ab}	2.6 (0.5)^{a}	5.3 (1.2)^{bc}	3.1 (0.6)^{a}	0.3 (0.5)^{abc}	0.6 (0.3)^{a}	2.2 (1.1)^{b}	−1.3 (0.5)^{a}	0.5 (0.3)^{a}	2 (0.4)^{a}
Whole Fish	BioClark	−1 (0.8)^{a}	1.5 (0.7)^{a}	−1.3 (0.4)^{c}	5.9 (0.5)^{b}	1.7 (0.5)^{b}	0.6 (0.3)^{ab}	0.2 (0.4)^{ab}	1.6 (0.7)^{b}	−3.1 (0.6)^{b}	0.9 (0.6)^{b}	−0.9 (1.3)^{b}
	BioVita	−1.7 (0.7)^{ab}	1.1(0.7)^{ab}	0.8 (0.2)^{b}	3.9 (0.6)^{cd}	3.2 (0.3)^{a}	0.3 (0.2)^{abc}	0.6 (0.2)^{a}	0.7 (0.9)^{b}	−2 (0.5)^{a}	−0.5 (0.4)^{b}	−0.3 (1.2)^{b}

3.2. Growth and proximate analysis

Results from fish size measurements and proximate analyses are summarized in Table 3. We found no significant differences ($p > 0.05$) in mean length or fish mass between the two feed treatments. Furthermore, we found no significant differences ($p > 0.05$) in any of the measures of proximate composition. All measures of fish size and proximate composition fell within previously observed ranges for this species and size class based on records from NMFS.

3.3. Amino acid stable isotope trophic discrimination factors

Amino acid ^{13}C trophic discrimination factors are summarized in Table 4. Three of the essential amino acids (Ile, Leu, and Phe) had TDFs that averaged near zero for all tissues, with isoleucine (Ile) and leucine (Leu) having some of the smallest variance and the smallest TDF deviations from zero. Phenylalanine (Phe) also had mean TDFs near zero for all tissues but had a larger variance across all tissues. Three of the essential amino acids analyzed (Met, Lys, and Val) had TDFs that were significantly different ($p < 0.05$) than zero. Methionine (Met) was consistently depleted in ^{13}C relative to diet by as much as -3.1‰. Lysine (Lys) was consistently enriched in ^{13}C relative to diet, particularly in liver tissue. Lysine also had some of the largest variance in TDF across all amino acids and tissues. The other essential amino acid analyzed, Valine (Val), was enriched in ^{13}C relative to diet in both muscle and liver but was slightly depleted in ^{13}C in whole fish homogenate. Liver TDFs were consistently more positive than both muscle and whole body homogenate with some exceptions (Ala). Whole body homogenate and muscle TDFs were similar and statistically indistinguishable within individual amino acids for both diet treatments.

3.4. Feed amino acid composition

Amino acid molar percent composition of the two feeds are shown in Fig. 2. Nine of the 11 amino acids were found to have significantly different ($p < 0.05$) molar percent compositions (Ala, Glx, Ile, Leu, Lys, Phe, Ser, Thr, Tyr, Val). The amino acids with the largest differences in molar percent composition were Glx (4.0%), Leu (2.2%), Ser (1.4%), and Lys and Ala (1.3%). All other significant differences in molar percent composition differed by less than 1 percent.

4. Discussion

Controlled feeding trials of hatchery-raised fish provide an excellent opportunity for evaluating the effects of feed on fish growth, health, and survival. Hatchery-based feeding trials also allow for the determination of parameters, such as stable isotope trophic discrimination factors, which can aid in ecological studies of wild fish populations. Measures of size and proximate composition of juvenile hatchery-reared fish are necessary to determine if differing diets lead to differences in growth or nutrient provisioning, and ultimately survival. Our results show that the two diets tested had no difference in their effect on the final size or proximate composition of the juvenile Chinook salmon raised in this study. The brood year of fish from this experiment were marked with coded wire tags (CWTs) and released and into the ocean environment for up to four years. We found no difference in survival rates across the two dietary treatments. Adult returns of these hatchery fish are an important measure of juvenile dietary efficacy, since recruitment into the spawning class is one of the primary goals of stock enhancement. Additionally, this is the first study to determine amino acid carbon isotope TDFs in a salmonid. The amino acid carbon isotope TDFs determined in this study can be applied to a wide range of ecological studies on salmonids such as determination of dietary composition, tracking movement, tracing carbon flow through marine ecosystems, and estimation of terrestrial carbon use by juvenile salmon in near-shore habitats.

4.1. Survival, growth and proximate analysis

The two diets did not vary significantly in their effect on fish survival or proximate composition. This is an encouraging economic and environmental impact result for the use of alternative feeds in Chinook salmon hatchery production and potentially in other salmonid species. The BioClark's™ diet has a lower cost compared to the fishmeal-based BioVita™, which has the potential to lower overall production costs of hatchery-reared Chinook. In this study, the cost per kilogram of growth was $5.54 for the BioVita™ diet and $4.79 for the BioClark's™ diet. Due to differing nutrient density (Appendix 1), approximately 5% more (by weight) of the BioClark's™ diet was required to make the dietary treatments calorically equal, which offset some cost savings of that diet. The difference in cost between the two feeds was still great enough to make the alternative diet less expensive overall. The terrestrially-derived alternative feed may have a lower impact on water quality relative to other animal protein-based feeds (Davidson et al., 2016) and reduce the need for fishmeal-based ingredients resulting in less demand on wild forage fish stocks, providing environmental benefits in addition to cost savings.

Aquaculture production has risen rapidly in recent decades, nearly doubling every ten years

(Food and Agricultural Organization [FAO], 2010), with the need for feed protein imposing significant pressures on other fisheries such as pelagic forage fish. Although Chinook salmon are primarily raised for wild stock enhancement purposes of conservation and fisheries, other salmon species are raised in aquaculture facilities for direct human consumption. Salmon production has grown more quickly than other sectors and species of global finfish aquaculture production for a host of reasons including controlling feed costs, better production practices, and better economies of scale (Asche, 2008), with Norway and Chile accounting for approximately 80% of global production (Asche et al., 2013). Due to all of these factors, information that provides a means to

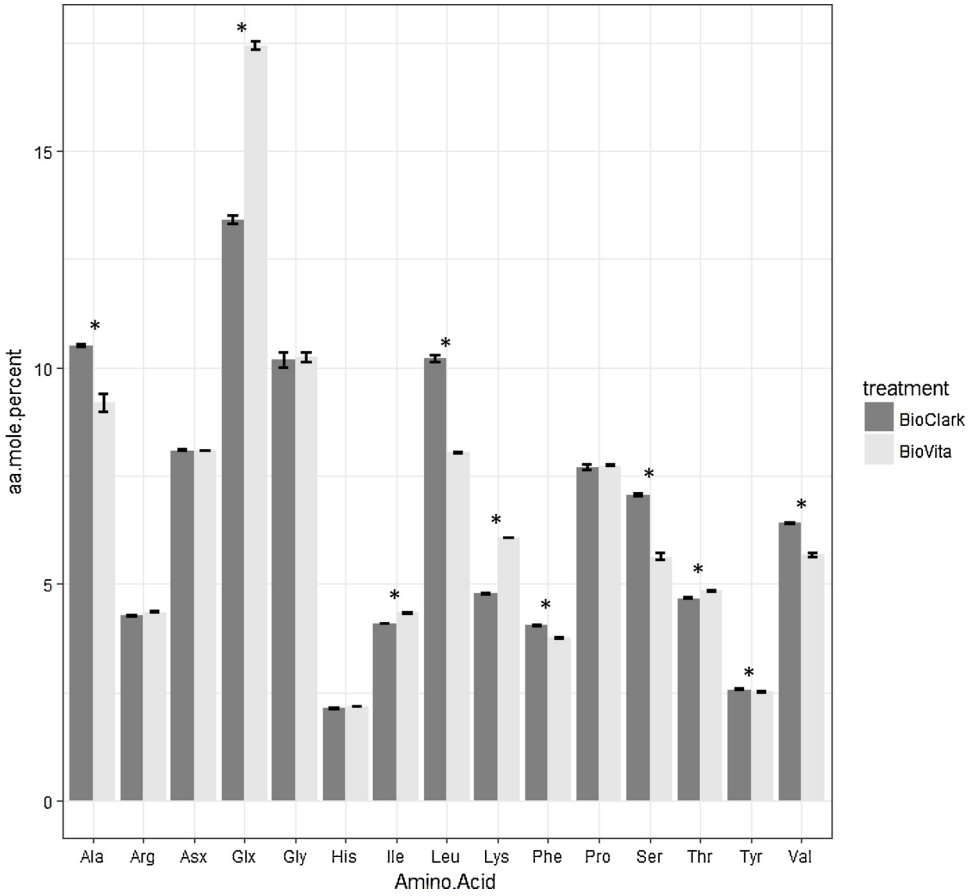

Fig. 2. Mean (± SD) amino acid molar percent compositions of two Chinook salmon diets. Asterisks denote significantly different ($p < 0.05$) molar percent composition between diets within a particular amino acid.

decrease the cost of salmon production, reduce water quality impacts, and alleviate pressure on wild forage fish used to make fishmeal have the potential to yield a substantial economic and environmental benefit for salmon production. The alternative diet incorporates some fishmeal and fish oil into the diet, but lessens the need for these ingredients by utilizing alternative protein sources and raw materials.

Further study is needed to determine if alternative feeds have negative effects on fish development. In a study of alternative plant-based feeds on sablefish (*Anoplopoma fimbria*) development, Rhodes et al. (2016) found that fish receiving alternative feeds expressed lower weight gain, shorter length, and significant histopathology of the liver and gastrointestinal tract. The alternative feeds in that study, however, incorporated plant-based oils in place of fish oil and had significantly different fatty acid profiles than a fishmeal-based diet, which may have contributed to the observed differences in growth and histopathology. The alternative diet in this study uses fish oil for the fatty acid component of the feed and therefore possibly prevents any dietary deficiencies in essential fatty acids.

4.2. Feed amino acid composition and amino acid stable isotope trophic discrimination factors

Stable isotope trophic discrimination factors (TDF) are essential for the interpretation of stable isotope data collected from free-living animals. General patterns of trophic discrimination are widely known from bulk stable isotope analyses, such as the enrichment of about 3‰ in the heavy isotope of nitrogen relative to diet when assimilated by a consumer. There is, however, substantial TDF variability across taxa, tissues, and diet quality, which necessitates controlled studies to determine TDFs that are tailored for a particular species or tissue (del Rio et al., 2009). The number of studies using compound-specific isotopic analysis is growing quickly, yet very few studies have investigated species-specific TDFs for ^{13}C in amino acids. Our study is the first to present these data for a salmonid species. The TDFs determined in this study can be used to help understand the ecology of wild salmonids and juvenile Chinook salmon in particular, which can inform a broad range of research targeted toward conservation and ecosystem-based fisheries management.

The patterns of amino acid isotopic discrimination observed in this study are similar to those found in McMahon et al. (2010), one of the few other studies of amino acid carbon isotope discrimination in a marine teleost and in which the same fishmeal feed was also used for one of their dietary treatments. Few researchers have investigated TDFs for multiple tissues in the same study. The TDFs for phenylalanine, leucine, and isoleucine were not significantly different from zero across tissues and treatments in both McMahon et al. (2010) and in this study. The amino acids Ile, Leu, and Phe have been found in other work to be incorporated into consumer tissues with little or no ^{13}C isotopic fractionation (Howland et al., 2003; Jim et al., 2006). This illustrates one of the most useful applications of compound-specific amino acid analysis: the ability to determine the carbon isotope signature of primary producers from any sample of a consumer in a food web.

In this study as well as McMahon et al. (2010), the non-essential amino acids showed a wide range of TDFs. In both studies, alanine was depleted in ^{13}C relative to diet for all treatments. Aspartic acid and glutamic acid averaged near-zero ^{13}C discrimination relative to diet when considering mean values across all treatments in both our study and McMahon et al. (2010), but both amino acids showed substantial deviation from zero within individual treatments and tissues.

Glycine had the largest isotopic discrimination in our study and all

fish tissues analyzed were significantly enriched in ^{13}C relative to diet, with TDFs averaging approximately 6‰ across all treatments. Liver tissue in the alternative treatment fish was 8.2‰ enriched in ^{13}C relative to diet, the largest TDF observed in this study. While liver tissue was the most ^{13}C enriched in glycine, both muscle and whole fish homogenate were also enriched by approximately 3–6 ‰ relative to diet, with the alternative treatment 2–3 ‰ higher than the fishmeal treatment in all tissues. Interestingly, fish fed the fishmeal diet in McMahon et al. (2010) were significantly depleted in glycine ^{13}C by approximately 8‰, and only one of four diet treatments in that study produced fish tissues with ^{13}C-enriched glycine (˜ 3‰). Bulk carbon isotope discrimination from diet to tissues is generally assumed to be small (˜ 1‰) but can be quite variable (see Caut et al., 2009 for a summary of bulk TDFs across species). Determining the amino acids with the largest TDF magnitudes and variability, such as glycine in this study, and understanding the biochemical causes of fractionation will contribute greatly to understanding the variability of bulk carbon isotope discrimination across tissues and species.

Differences in feed amino acid percent composition are one potential source of the observed differences in carbon isotope discrimination across the two diets (McMahon et al., 2015). Although the amino acid molar percent composition results showed high precision, the comparison is based on a sample size of two for each feed and should be interpreted with this constraint in mind. This scenario can be explained through a mass balance perspective; if one diet has a higher concentration of a specific amino acid (i.e. alanine), then the fish on that diet would not need to produce as much alanine *de novo* relative to the other diet. This would result in a lower discrimination factor due to less deamination to scavenge nitrogen from other sources and the resultant isotopic enrichment that occurs during that process. The non-essential amino acids with the largest differences in percent composition between the two diets, glutamic acid and alanine, both had consistent differences in carbon isotope discrimination between the two diets. The alternative diet had a higher abundance of alanine relative to the fishmeal feed, while there was significantly more glutamic acid in the fishmeal feed. Fish raised on the alternative diet were 1‰ more enriched in alanine in all tissues relative to fish on the fishmeal diet. Conversely, glutamic acid was 2‰ heavier relative to diet across all tissues in fish fed the fishmeal treatment compared to the alternative diet. Whole-body alanine percent composition for finfish averages approximately 6.2% (Kaushik and Seiliez, 2010), so both diets likely provided sufficient alanine to meet demands for tissue growth, which is evidence that the mass balance scenario may not be causing the observed differences. The same evidence applies to glutamic acid as well, and further work is necessary to determine the cause of differences in TDF between the two diet treatments.

5. Conclusions

Our results demonstrate that a terrestrially-derived alternative feed is equally as effective in culturing juvenile Chinook salmon as fishmeal-based feed. If stock enhancement becomes a more important component of maintaining Chinook salmon populations, these results can help inform aquaculturists and potentially lessen production costs. The use of a feed with reduced fishmeal content can also help to ease pressure on global forage fish stocks used for making fishmeal. Additionally, the determination of amino acid ^{13}C trophic discrimination factors is a vital step in applying amino acid CSIA to wild fish populations, which may provide insight into changes in the marine food web and the drivers of declines in Chinook salmon survival and overall abundance. Our results demonstrate that the baseline ^{13}C signature of diet is preserved in phenylalanine, leucine, and isoleucine in Chinook salmon tissues and can be applied to diet modeling efforts, which can help in linking mortality to drivers of ocean productivity and trophic ecology during a critical stage in their life history.

Trade names disclaimer

Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fishres.2019.06.001.

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