



Effects of feeding rate on growth performance and nutrient partitioning of young-of-the-year white sturgeon (*Acipenser transmontanus*)

S. LEE¹, L.Y. HALLER¹, N.A. FANGUE², J.G. FADEL¹ & S.S.O. HUNG¹

¹ Department of Animal Science, University of California, Davis, CA, USA; ² Department of Wildlife, Fish and Conservation Biology, University of California, Davis, CA, USA

Abstract

A 10-week growth trial was performed to evaluate the effects of feeding rate on growth performance, nutrient partitioning, and to determine the optimum feeding rate for young-of-the-year white sturgeon. Three tanks with 30 fish per tank each were assigned to five feeding rates, 0.4–2.0% body weight per day (BW day⁻¹). Weight gain significantly ($P < 0.05$) increased with increasing feeding rate up to 1.2% BW day⁻¹. Feed efficiency exhibited an inverse relationship to weight gain. Hepatosomatic and gonadal-fat-body (GFB)-somatic indices were significantly affected by feeding rate, and the lowest values were observed at 0.4% BW day⁻¹. The increased feeding rate significantly affected whole-body and carcass lipid and moisture contents. Protein gain in the carcass, but not in the GFB, was significantly influenced by feeding rate. Lipid gain in the carcass and GFB was also significantly affected by feeding rate. Four regression models were tested for the estimation of optimum feeding rate, including one-slope straight broken-line, two-slope straight broken-line, quadratic broken-line and quadratic models. Adjusted coefficient of correlation and corrected Akaike information criterion were used to compare model performance. The quadratic broken-line model was chosen as the best based on the criteria. The estimated optimum feeding rate for young-of-the-year white sturgeon (360 g) is 1.5% BW day⁻¹.

KEY WORDS: broken-line, energy storage, gonadal-fat-body, optimum feeding rate, polynomial

Received 19 June 2014; accepted 6 October 2014

Correspondence: S.S.O. Hung, Department of Animal Science, University of California, One Shields Ave, Davis, CA, USA. E-mail: sshung@ucdavis.edu

Introduction

White sturgeon are a commercially important aquaculture species, providing meat and caviar for human consumption. The USA is one of the major global producers of caviar (Bronzi *et al.* 2011), and this caviar production mostly comes from white sturgeon (F. S. Conte, University of California, Davis, CA, USA, personal communication).

In general, when fish are fed at a higher rate than needed for maintenance requirement, excessive energy accumulates in storage tissues, such as the muscle, liver and viscera, mainly in the form of lipid. To our knowledge, white sturgeon do not utilize viscera as a storage tissue but utilize a gonadal-like adipose tissue, also called the gonadal-fat-body (GFB) by Scarnecchia *et al.* (2007). Gonadal-fat-bodies were observed in young white sturgeon (less than 1 year old; S. Lee, personal observation); however, there is currently no published information regarding the composition of GFBs and its role with respect to this species. Scarnecchia *et al.* (2007) reported that Northern Great Plains paddlefish (*Polyodon spathula*), an Acipenseriform fish closely related to sturgeon (Grande & Bemis 1991), exhibited an accumulation of energy reserves in GFBs. Gonadal-fat-bodies were also observed in other Acipenseriform species, including shortnose sturgeon (*Acipenser brevirostrum*; Ryder 1890), Chinese paddlefish (*Psephurus gladius*; Chenhan *et al.* 1995), Adriatic sturgeon (*Acipenser naccarii*; Garcia-Gallego *et al.* 1999) and hybrid bester (*Huso huso* × *Acipenser ruthenus*; Steffens & Jahnichen 1995).

Feeding rate is an important factor affecting growth of fish (Brett & Groves 1979), and the determination of optimum feeding rate (OFR) is a critical component for the success of aquaculture operations. Thus, a series of feeding rate studies for white sturgeon varying from 0.05 to 764 g were conducted in our laboratory (Hung & Lutes 1987; Hung *et al.* 1993a, 1995; Deng *et al.* 2003; De Riu *et al.*

2012). These data have recently been used to develop an OFR prediction model, but following these initial OFR estimates, an important gap of fish between 62 and 764 g was detected (see Fig. 1). To develop a better OFR prediction model, white sturgeon of approximately 360 g in weight were chosen for the present study, and the outcomes were incorporated in an elaboration of an OFR prediction model (Lee *et al.* 2014).

For the estimation of OFR and/or nutrient requirements, various statistical methods, such as analysis of variance (ANOVA) with multiple range tests and regression models, including broken-line and quadratic (also called second-order polynomial) models, have been used. In the previous feeding rate studies from our laboratory (Hung & Lutes 1987; Hung *et al.* 1989, 1993a,b, 1995; Deng *et al.* 2003; De Riu *et al.* 2012; Zheng *et al.* 2015), either ANOVA with multiple range tests or a two-slope straight broken-line model was used for the estimation of OFR. However, these statistical methods may not give the most accurate estimates because no justification was given for the chosen model being the best to fit the set of observations. In his critique, Shearer (2000) pointed out that the design for that particular experiment and the resulting variations in the responses can lead to the selection of an inappropriate model. For the choice of the most accurate OFR, therefore, testing of various statistical models, followed by appropriate comparisons of model performance on the estimation, must be performed.

The objectives of this study were (i) the evaluation of the effects of feeding rate on growth performance and nutrient

partitioning in young-of-the-year (YOY) white sturgeon (360 g) and (ii) the determination of an accurate OFR through testing of various regression models with model comparisons.

Materials and methods

Animal acquisition and maintenance

White sturgeon larvae (3 days posthatch; DPH) donated by a local farm (Lazy Q Fish Ranch LLC, Dixon, CA, USA) were transported (23 April 2012) to the Center for Aquatic Biology and Aquaculture at the University of California, Davis, CA, USA. Fish were reared in circular fibreglass tanks (150 cm diameter, 45 cm height, ca. 675 L water volume) supplied with flow-through degassed well water (18–19 °C) throughout the rearing period. Once the larvae started exogenous feeding (10–14 DPH), they were fed a commercial salmonid starter feed (Soft-Moist #0 crumble, Rangen, Buhl, ID, USA) with 24-h automatic feeders (Lifeguard Automatic Fish Feeder, Lifeguard Aquatics, Cerritos, CA, USA). Fish were fed a variety of salmonid feeds (Soft-Moist #1, #2 and #3 crumble, Rangen; SCD 1.0-, 2.0- and 3.0-mm sinking pellet, Skretting, Tooele, UT, USA) until they reached the desired size (approximately 360 g) for the experiment. Fish were maintained according to the animal protocol approved by the Campus Animal Care and Use Committee (Protocol Number 16541).

Experimental design

A random distribution of 450 YOY white sturgeon (360 ± 12 g; mean \pm SD; 269 DPH) into the 15 fibreglass tanks (ca. 675 L) was made, resulting in 30 fish per tank. During a 1-week acclimation period, fish were fed at 1.4% body weight per day (BW day^{-1}) with the same commercial feed (SCD 3.0-mm sinking pellet, Skretting) used for the current growth trial. Proximate composition of the feed (g kg^{-1}), as determined through the AOAC method (Jones 1988), was 40 moisture, 418 crude protein and 190 crude lipid. At the end of the acclimation week, the 15 experimental tanks were randomly assigned to one of five feeding rates (0.4, 0.8, 1.2, 1.6 and 2.0% BW day^{-1}), resulting in three tanks per treatment, and the growth trial was carried out for 10 weeks. Every 2-week period all fish in each tank were weighed, the weights from each tank were averaged, and the amount of feed per tank was adjusted accordingly. Every morning (between 09:00 and 10:00 AM) the feed was

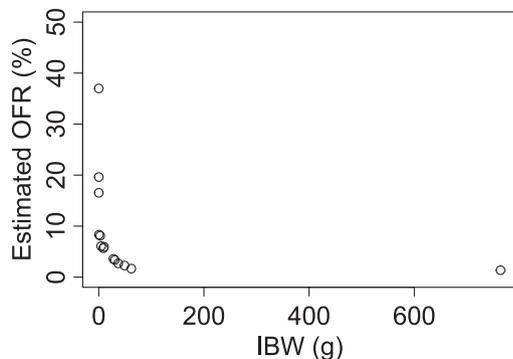


Figure 1 The scatter plot of the estimated optimum feeding rates (OFR) obtained from the published data (Hung & Lutes 1987; Hung *et al.* 1993a, 1995; Deng *et al.* 2003; De Riu *et al.* 2012) by the quadratic broken-line model analysis, plotted against the corresponding initial mean body weights (IBW) (see Lee *et al.* 2014 for more details). Data sets from the current study were not included in this plot.

loaded in a 24-h belt feeder (Zeigler Brothers Inc., Gardners, PA, USA) located on top of the tank. The tanks were covered, but each cover had a rectangular hole (ca. 10 cm width, 33 cm length) for the feed to fall through. Water inside the tank was rapidly drained once a day to ca. 50% of total volume to remove fecal matter and uneaten feed. All experimental tanks were supplied with degassed well water at 10–15 L min⁻¹. Water temperature and dissolved oxygen were recorded daily, and the ranges were 18.1 ± 0.3 °C (mean ± SD) and 8.1 ± 0.4 mg L⁻¹, respectively. Ammonia and pH were measured weekly, and the values were 0.1 ± 0.1 mg L⁻¹ and 7.8 ± 0.2, respectively. The experimental tanks were located outdoors, exposing the fish to a natural photoperiod through the feeding hole. The growth trial started on 14 Jan 2013 and ended on 25 Mar 2013.

Measurements

After the 10-week growth trial, all fish in each tank were weighed for the calculation of weight gain (WG) and feed efficiency (FE). Weight gain was calculated as $100 \times (\text{FBW} - \text{IBW}) / \text{IBW}$, and FE was calculated as $100 \times (\text{FBW} - \text{IBW}) / \text{TF}$, where the FBW, IBW and TF indicated the final body weight, initial body weight and total amount of feed given, respectively. After final weighing and prior to final sampling, feeds were withheld from the fish for 24 h. Three fish from each tank were captured randomly and euthanized with an overdose (0.5 g L⁻¹) of tricaine methanesulfonate (MS-222, Argent Inc., Redmond, WA, USA) and kept at -20 °C for whole-body proximate analysis. Additional three fish were randomly captured and euthanized with the overdose of MS-222 to collect blood with a 22-G needle and vacutainer (BD Vacutainer® Ref # 36664, Franklin Lakes, NJ, USA) through a puncture of the caudal vein. The blood sample was centrifuged at 4500 g for 5 min at room temperature to obtain plasma samples for metabolites (protein, glucose and triacylglyceride) determinations. The fish was then dissected, and the weights of the liver for hepatosomatic index (HSI), viscera (consisting of digestive tract from the lower esophagus to the anus) for viscerosomatic index (VSI), and GFB for GFB-somatic index (GFBSI) were obtained. Collected tissues were frozen on a flat-end tong prechilled in liquid nitrogen, put into Whirl-Pak® bags (Nasco, Ft. Wilkinson, WI, USA) and kept at -80 °C until sample processing. Each frozen tissue (liver, GFB and viscera) was ground using a liquid nitrogen-cooled mortar and pestle. Ground samples were put in aluminium bags and freeze-dried for

5 days. The freeze-dried samples were then thoroughly mixed, put into the Whirl-Pak® bags and kept in -20 °C for proximate analysis. The carcass, consisting of the eviscerated fish with gills and heart, was put in a 15-L plastic bag and kept at -20 °C for later proximate analysis. The proximate analysis was performed through the AOAC method (Jones 1988). Protein and lipid gains in the carcass, GFBs and viscera were calculated using the equation, $[(\text{FTW} \times \text{FNC}) - (\text{ITW} \times \text{INC})] / 100$, where the FTW and ITW were the final and initial tissue weights (g), respectively, and the FNC and INC were the final and initial nutrient compositions (%) in the tissue, respectively. Plasma protein, glucose and triacylglyceride concentrations were determined with commercially available kits (Total Protein Kit (Product Code: TP0200), Micro-Lowry, Onishi & Barr Modification; Glucose Assay Kit (Product Code: GAGO20); Serum Triglyceride Determination Kit (Product Code: TR0100), Sigma-Aldrich, St. Louis, MO, USA).

Estimation of OFR

Commonly used regression models for the estimation of OFR and/or nutrient requirement levels, including one-slope straight broken-line (one-slope BL), two-slope straight broken-line (two-slope BL), quadratic broken-line (quadratic BL) and quadratic (quadratic) models (Zeitoun *et al.* 1976; Robbins *et al.* 1979, 2006; Shearer 2000; Pesti *et al.* 2009; Lee *et al.* 2014), were tested. Functional equation forms and graphical illustrations of these models and codes for fitting each model to a set of observations (WG and feeding rate were considered as dependent and independent variables, respectively) can be found in the literature (Lee *et al.* 2014). Specific model selection criteria, including the adjusted coefficient of correlation (R_{adj}^2) and corrected Akaike information criterion (AICc), were calculated for comparisons of the model performance on the estimation of OFR. The larger R_{adj}^2 and smaller AICc values indicate a better model for its performance. Both criteria are corrected for the number of parameters (see Lee *et al.* 2014 for details). R 3.0.1 (R Development Core Team 2013) was used for the analyses.

Statistical analysis

All results, except for the OFR estimation, were analysed by one-way analysis of variance (ANOVA) accounting for the five feeding rate treatments, and comparisons between treatment means were performed through the Tukey's studentized range test. Statistical analyses were conducted

using SAS (version 9.3, SAS Institute, Cary, NC, USA), and significance was tested at $P < 0.05$.

Results and discussion

Growth performance and morphological changes

The growth performance values for YOY white sturgeon fed at the various feeding rates for 10 weeks, including WG, FE, HSI, GFBSI and VSI, are shown in Table 1. Weight gain significantly increased with increasing feeding rate up to 1.2% BW day⁻¹ and plateaued at higher feeding rates. Fish fed above 1.2% BW day⁻¹ for 10 weeks doubled their body weights. A similar growth rate was reported in white sturgeon (250 g) fed at 1.5% BW day⁻¹ for 8 weeks at the same temperature (Hung *et al.* 1989). On the other hand, FE exhibited an inverse relationship with respect to WG. This inverse relationship was also observed in previous feeding rate studies on white sturgeon (Hung *et al.* 1993a; Deng *et al.* 2003; De Riu *et al.* 2012).

The lower FE observed in white sturgeon fed at a higher feeding rate may be attributed to a single reason or a combination of reasons. Due to the unique feeding behaviour of white sturgeon, a continuous feeder eating small amounts of feed continuously (Cui *et al.* 1997), feed wastes were observed throughout the feeding trial, resulting in decreasing FE at higher feeding rates. The rate of nutrient accumulation with increasing feeding rate could decrease as white sturgeon approach satiation. Thus, low FE seems

unavoidable in the achievement of a maximum growth; however, it may still be cost-effective to feed young and small fish at the optimum level, even with lower FE because it will provide better nutritional status, which will likely outweigh the higher feed costs.

Hepatosomatic index and GFBSI of fish fed at the various feeding rates were similar to that for WG. Viscerosomatic index was, however, not significantly affected by feeding rate. In Arctic charr (*Salvelinus alpinus*; Miglavs & Jobling 1989), rainbow trout (*Oncorhynchus mykiss*; Storebakken *et al.* 1991), striped bass (*Morone saxatilis*; Hung *et al.* 1993b), Atlantic salmon (*Salmo salar*; Einen *et al.* 1999), gilthead sea bream (*Sparus aurata*; Company *et al.* 1999), tropical bagrid catfish (*Mystus nemurus*; Ng *et al.* 2000), European sea bass (*Dicentrarchus labrax* L.; Erolđođan *et al.* 2004) and Atlantic cod (*Gadus morhua* L.; Hatlen *et al.* 2007), HSI and VSI were highly correlated with feeding rate because of their roles as energy storage sites. Changes in HSI in a previous feeding rate study of white sturgeon were also observed (Hung & Lutes 1987); however, data regarding the effect of feeding rate on VSI in white sturgeon are not available, except for the current study, which shows no significant changes. These results suggest that the viscera in white sturgeon do not function as an energy storage site. Conversely, GFBSI was significantly affected by feeding rate, and this index for fish fed above 1.2% BW day⁻¹ was almost twice as high as that for fish fed at 0.4% BW day⁻¹, indicating that the GFB accumulated more fat energy when it was in excess. Hageman *et al.* (1986) and Scarnecchia *et al.*

Table 1 Growth and morphological parameters of young-of-the-year white sturgeon fed at the various feeding rates for 10 weeks¹

Measurements	Feeding rate (% body weight per day)					P-value
	0.4	0.8	1.2	1.6	2.0	
IBW ² (g)	368.1 ± 6.7	362.2 ± 7.8	360.4 ± 2.9	354.5 ± 7.2	354.4 ± 8.7	0.6193
FBW ³ (g)	495.5 ± 7.8 ^c	681.6 ± 10.1 ^b	750.3 ± 18.1 ^a	767.8 ± 16.6 ^a	771.7 ± 14.4 ^a	<0.0001
WG ⁴ (%)	34.8 ± 4.4 ^c	88.3 ± 3.5 ^b	108.2 ± 4.5 ^{ab}	117.0 ± 9.2 ^a	117.9 ± 5.5 ^a	<0.0001
FE ⁵ (%)	114.6 ± 11.1 ^a	118.3 ± 3.1 ^a	87.8 ± 2.6 ^b	68.8 ± 2.2 ^{bc}	54.8 ± 0.9 ^c	<0.0001
HSI ⁶	1.38 ± 0.13 ^b	1.78 ± 0.11 ^{ab}	1.88 ± 0.06 ^{ab}	2.09 ± 0.13 ^a	1.91 ± 0.08 ^a	0.0096
GFBSI ⁷	1.11 ± 0.12 ^b	2.03 ± 0.25 ^{ab}	2.17 ± 0.27 ^{ab}	2.17 ± 0.40 ^{ab}	2.35 ± 0.10 ^a	0.0375
VSI ⁸	4.11 ± 0.14	4.70 ± 0.29	5.06 ± 0.08	4.86 ± 0.38	4.72 ± 0.10	0.1180

¹ Means ± SEM ($n = 3$) with different superscripts within each row are significantly ($P < 0.05$) different by Tukey's studentized range test.

² IBW: average initial body weights.

³ FBW: average final body weights.

⁴ WG: weight gain = $100 \times (\text{FBW} - \text{IBW}) / \text{IBW}$.

⁵ FE: feed efficiency = $100 \times (\text{FBW} - \text{IBW}) / \text{TF}$ where the TF indicated the total amount of feed given.

⁶ HSI: hepatosomatic index = $100 \times (\text{liver weight} / \text{FBW})$.

⁷ GFBSI: gonadal-fat-body-somatic index = $100 \times (\text{gonadal-fat-body weight} / \text{FBW})$.

⁸ VSI: viscerosomatic index = $100 \times (\text{viscera weight} / \text{FBW})$.

(2007) reported that the GFB weight in paddlefish decreased as the gonadosomatic index increased, suggesting its role in the energy supply for gonadal development.

Nutrient partitioning

Proximate composition changes Whole-body and carcass lipid and moisture contents were significantly affected by feeding rate; however, these values showed an inverse trend as lipid content increased with increasing feeding rate, while moisture content decreased (see Table 2). Although this inverse relationship was also observed in previous feeding rate studies (Hung & Lutes 1987; Hung *et al.* 1993a,b; Deng *et al.* 2003; De Riu *et al.* 2012; Zheng *et al.* 2015), the underlying reason is not yet clearly known. McCue (2010) proposed a possible explanation stating that 'cells somehow replace lost organic matter with water to maintain their size and ultimately functionality'. However, whole-body and carcass protein contents were not significantly affected by the feeding rate. This observation is supported from the aforementioned feeding rate studies, showing no changes in protein content as long as fish were fed above maintenance requirements.

Proximate composition of the GFB and viscera was not significantly affected by feeding rate (data were not shown in Table 2; liver composition data are not available due to lipid leakage during the freeze-drying process). Despite no changes observed in these tissues, the average lipid content in the GFB and viscera in white sturgeon (900 g kg^{-1} and 35 g kg^{-1} on wet matter basis, respectively) is quite unique compared with those of other fish species. First of all,

possession of the GFB is observed only in Acipenseriform species (Scarnecchia *et al.* 2007). Second, such a high lipid content in the GFB was not observed in other energy storage tissues, including the liver, muscle and viscera. Conversely, the lipid content in the viscera was very low relative to other species, including rainbow trout (Storebakken *et al.* 1991), striped bass (Hung *et al.* 1993b) and tropical bagrid catfish (Ng *et al.* 2000), showing at least 10-fold higher lipid content. Although the liver composition data from the current study are not available, the lipid content (340 g kg^{-1}) from the initial fish samples can be used for comparison with what thus infer from rainbow trout (82 g kg^{-1} ; Storebakken *et al.* 1991) and Arctic charr (59 g kg^{-1} ; Miglavs & Jobling 1989), which show a relatively lower lipid content in their livers, indicating a different energy storage strategies for white sturgeon compared to these two species. However, the whole-body composition of white sturgeon fed above $1.2\% \text{ BW day}^{-1}$ was very similar to that of other fish species, including Atlantic salmon (Storebakken & Austreng 1987), gilthead sea bream (Company *et al.* 1999), rainbow trout (Storebakken *et al.* 1991; Dumas *et al.* 2007), striped bass (Hung *et al.* 1993b), tropical bagrid catfish (Ng *et al.* 2000), all fed at optimum levels. Taken together, results suggest that white sturgeon possess a distinct nutrient partitioning strategy in the liver, GFB and viscera tissues in comparison with other fish species.

Protein and lipid gains Protein and lipid gains in the carcass, GFB and viscera of fish fed at the various feeding rates for 10 weeks are shown in Table 3. These results

Table 2 Proximate composition of whole-body and carcass of young-of-the-year white sturgeon fed at the various feeding rates for 10 weeks¹

	Feeding rate (% body weight per day)					P-value
	0.4	0.8	1.2	1.6	2.0	
Whole-body (g kg^{-1}) ²						
Moisture	$746 \pm 6^{a,+}$	732 ± 1^{ab}	717 ± 4^{bc}	723 ± 6^{bc}	711 ± 1^c	0.0022
Crude protein	$139 \pm 5^+$	138 ± 2	141 ± 2	147 ± 3	138 ± 3	0.2189
Crude lipid	$66 \pm 2^{b,+}$	82 ± 3^{ab}	101 ± 5^a	87 ± 11^{ab}	106 ± 1^a	0.0121
Carcass (g kg^{-1}) ³						
Moisture	755 ± 3^a	736 ± 5^b	726 ± 2^b	732 ± 3^b	731 ± 4^b	0.0013
Crude protein	151 ± 4	153 ± 3	148 ± 3	149 ± 3	150 ± 4	0.8677
Crude lipid	51 ± 2^b	70 ± 4^a	83 ± 2^a	75 ± 3^a	78 ± 5^a	0.0007

¹ Values are means \pm SEM ($n = 3$; $^+n = 2$ due to the outlier omission). Means with different superscripts within each row are significantly ($P < 0.05$) different by Tukey's studentized range Test.

² Initial proximate composition (g kg^{-1}) of whole-body (mean \pm SEM; $n = 3$) was moisture 727 ± 9 , crude protein 142 ± 10 and crude lipid 112 ± 5 .

³ Initial proximate composition (g kg^{-1}) of carcass (mean \pm SEM; $n = 3$) was moisture 743 ± 5 , crude protein 151 ± 2 and crude lipid 85 ± 3 .

Table 3 Protein and lipid gains in the carcass, gonadal-fat-body (GFB) and viscera of young-of-the-year white sturgeon fed at the various feeding rates for 10 weeks¹

	Feeding rate (% body weight per day)					P-value
	0.4	0.8	1.2	1.6	2.0	
Composition of gain (g)						
Carcass protein	16.6 ± 3.9 ^c	38.4 ± 3.7 ^b	47.8 ± 1.1 ^{ab}	56.8 ± 1.8 ^a	46.4 ± 6.0 ^{ab}	0.0002
Carcass lipid	-6.6 ± 1.6 ^c	11.5 ± 3.3 ^b	26.3 ± 1.8 ^a	25.3 ± 2.7 ^{ab}	21.9 ± 4.9 ^{ab}	<0.0001
GFB ² protein	-0.2 ± 0.0	-0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2259
GFB lipid	-4.3 ± 0.7 ^b	2.8 ± 1.7 ^{ab}	5.9 ± 2.0 ^a	6.8 ± 3.2 ^a	5.9 ± 0.4 ^a	0.0101
Viscera ³ protein	-0.2 ± 0.1 ^b	0.4 ± 0.2 ^{ab}	0.9 ± 0.2 ^a	1.1 ± 0.2 ^a	0.6 ± 0.2 ^{ab}	0.0065
Viscera lipid	-0.1 ± 0.0 ^b	0.0 ± 0.1 ^{ab}	0.3 ± 0.1 ^a	0.3 ± 0.1 ^a	0.2 ± 0.1 ^{ab}	0.0079

¹ Values are means ± SEM ($n = 3$). Means with different superscripts within each row are significantly ($P < 0.05$) different by Tukey's studentized range test. The gain was calculated using the equation, $[(FTW \times FNC) - (ITW \times INC)]/100$, where the FTW and ITW were the final and initial tissue weights (g), respectively, and the FNC and INC were the final and initial nutrient compositions (%) in the tissue, respectively. Data for liver were not available because the proximate analysis failed due to lipid leakage from the tissue during the freeze-drying process.

² The proximate composition (g kg^{-1}) of initial GFBs (mean ± SEM; $n = 3$) was moisture 106 ± 6 , crude protein 3 ± 1 and crude lipid 890 ± 6 .

³ The proximate composition (g kg^{-1}) of initial viscera (mean ± SEM; $n = 3$) was moisture 759 ± 19 , crude protein 182 ± 16 and crude lipid 45 ± 3 .

indicate absolute protein and lipid gains in different body parts. Feeding rate significantly influenced protein gain in the carcass but not in the GFB. Lipid gain in the carcass and GFB was significantly affected by feeding rate. In absolute terms, the average gains in the carcass protein (50.3 ± 7.4 g; mean ± SD) and the carcass lipid (24.5 ± 5.5 g) of fish fed above $1.2\% \text{ BW day}^{-1}$ were much pronounced in comparisons with the average gains in the GFB protein (0 ± 0 g) and the GFB lipid (6.2 ± 3.3 g) and in the viscera protein (0.9 ± 0.4 g) and the viscera lipid (0.2 ± 0.1 g) of fish fed at the same rate. These results indicate that the weight increase of white sturgeon was mainly associated with gains in protein and lipid in the carcass and, partially, with the gain in lipid in the GFB. Conversely, Storebakken *et al.* (1991) reported that the gain in the viscera lipid (30.4 g) in rainbow trout fed at the optimum level was similar to the gain in the carcass lipid (26.5 g). This difference of nutrient partitioning in the viscera tissue of the two species supports our aforementioned findings.

Plasma metabolites

Plasma metabolites (24 h postprandial), specifically protein, triacylglyceride (TAG) and glucose, of fish fed at the various feeding rates for 10 weeks are shown in Table 4. Plasma metabolites can be used as an indicator of the nutritional status of fish; however, caution should be exercised because their levels are readily affected by

exogenous (e.g. stressors, feeding) and endogenous (e.g. hormones, nutrient metabolism) factors (Congleton & Wagner 2006; McCue 2010). Fish fed at $0.4\% \text{ BW day}^{-1}$ exhibited the lowest levels of plasma protein and TAG; however, there was no significant effect of feeding rate on the plasma glucose level. This lack of change in the glucose level despite the overall nutritional status of the fish fed at $0.4\% \text{ BW day}^{-1}$ being relatively low may be attributed to the importance of maintaining glucose homeostasis. Gluconeogenic pathways are critical in fish because glucose is an essential metabolic fuel for vital tissues such as the nervous system (Cahill 1986; Moon & Foster 1995). This explanation is supported by the observed lower plasma protein and TAG levels because gluconeogenesis uses metabolic substrates such as glycerol released from mobilization and degradation of TAG, and from amino acids (alanine and glutamine are thought to contribute the most to protein-derived gluconeogenesis; see a review by McCue 2010).

Estimation of OFR

The one-slope BL, two-slope BL, quadratic BL and quadratic models were used for the estimation of OFR for YOY white sturgeon, and the estimated OFRs are shown in Fig. 2. For the comparison of model performances on the estimation of OFR, the specific model selection criteria, R^2_{adj} and AICc, were calculated (see Table 5). The OFR estimates were not identical, indicating that each model

Table 4 Plasma metabolites of young-of-the-year white sturgeon fed at the various feeding rates for 10 weeks¹

Plasma metabolites(24 h postprandial)	Feeding rate (% body weight per day)					P-value
	0.4	0.8	1.2	1.6	2.0	
Protein (g L ⁻¹)	21.4 ± 0.7 ^b	27.4 ± 1.2 ^a	27.3 ± 0.9 ^a	27.3 ± 1.1 ^a	26.6 ± 1.0 ^a	0.0141
Triacylglyceride (g L ⁻¹)	7.9 ± 0.5 ^b	10.9 ± 0.8 ^{ab}	13.0 ± 0.8 ^a	12.8 ± 0.7 ^a	11.8 ± 0.5 ^a	0.0022
Glucose (mg L ⁻¹)	766.4 ± 6.7	804.1 ± 32.3	848.7 ± 29.4	874.7 ± 13.3	837.0 ± 32.6	0.0869

¹ Values are means ± SEM (*n* = 3). Means with different superscripts within each row are significantly (*P* < 0.05) different by Tukey's studentized range test.

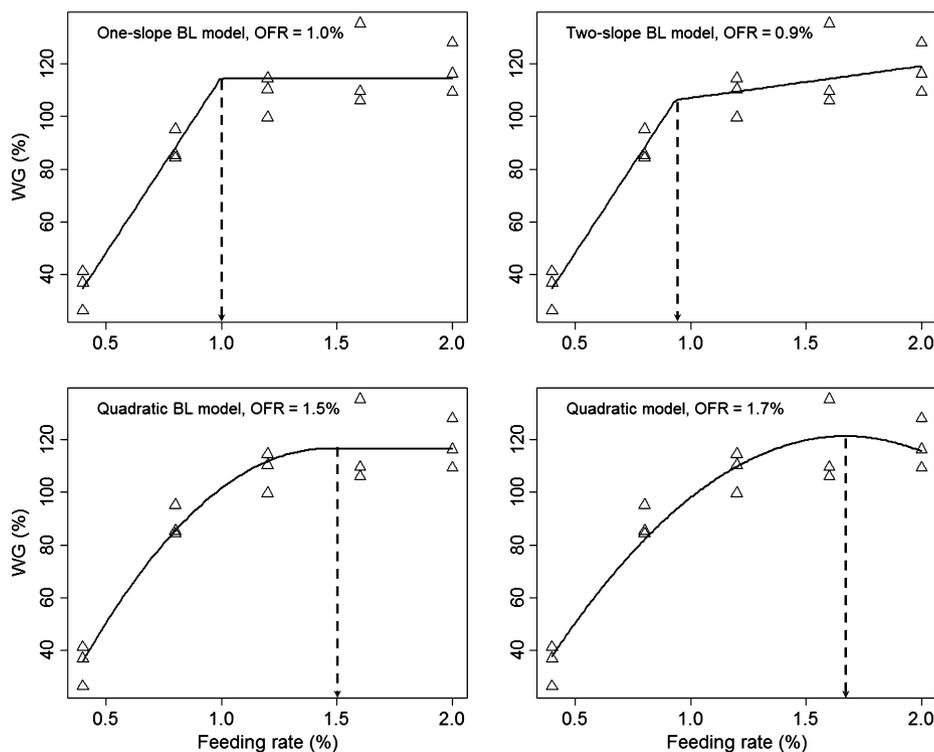


Figure 2 The plots of the line/curve fits to the observations from the 10-week growth trial of young-of-the-year white sturgeon fed at the various feeding rates (% body weight per day) performed by the one-slope broken-line (one-slope BL; top left), two-slope broken-line (two-slope BL; top right), quadratic broken-line (quadratic BL; bottom left) and quadratic (quadratic; bottom right) models. The symbol (Δ) indicated the growth response, weight gain (WG; %), responding to the respective feeding rate. The pointing arrow indicates the optimum feeding rate (OFR) estimated by each model.

estimated the OFR differently. Based on the R_{adj}^2 and AICc values, the quadratic BL model performed best.

The analysis of variance (ANOVA) with multiple range tests and regression models, such as broken-line and second-order polynomial models, has been applied in many nutrient requirement and feeding rate studies for the estimation of optimum levels. In spite of simplicity and convenience of using the ANOVA with multiple range tests, researchers have relied more on the regression models because they are better at accounting for the

dose–response relationship (Zeitoun *et al.* 1976; Robbins *et al.* 1979, 2006; Shearer 2000; Pesti *et al.* 2009). In many cases, a single regression model, either the two-slope BL or quadratic model, has been tested. However, it may be inappropriate to use a single model to achieve the most accurate estimate because Shearer (2000) stressed that the design for that particular experiment and the resulting variations in the response can contribute to the selection of an inappropriate model. Thus, a best-fit model to a set of observations is required because

Table 5 The model selection criteria values calculated from the adjusted coefficient of correlation (R_{adj}^2) and corrected Akaike information criterion (AICc) for the comparisons of the model performances on the estimation of optimum feeding rate of young-of-the-year white sturgeon

Criteria	Models(estimated optimum feed rate: % body weight per day)			
	One-slope BL ¹ (1.0)	Two-slope BL ² (0.9)	Quadratic BL ³ (1.5)	Quadratic ⁴ (1.7)
R_{adj}^2 ⁵	0.9038	0.9070	0.9119	0.9003
AICc ⁵	117.1	118.1	115.8	117.7

¹ One-slope broken-line model.

² Two-slope broken-line model.

³ Quadratic broken-line model.

⁴ Quadratic model.

⁵ The larger R_{adj}^2 and smaller AICc values indicate a better model for its performance. Both criteria are corrected for the number of parameters (see Lee *et al.* 2014 for details).

a less accurately estimated OFR will result in significant consequences for aquaculture operations with respect to production and feed costs. Achieving the most accurate estimate, therefore, requires testing of various statistical models, followed by appropriate model evaluation for comparisons of model performances on OFR estimation, as we performed in the present study.

The best-chosen model, the quadratic BL model, rarely has been tested for the estimation of OFR; however, this model represented the feeding rate–response relationship well, as shown in Fig. 2. In addition, the quadratic BL model was also selected as the best model for the development of an OFR prediction model (Lee *et al.* 2014). This quadratic BL model's feature, representing a single break point (indicating an OFR) which is the intersection of a second-order polynomial line and a plateau line, meets a typical feeding rate–response curve. This typical curve shows that a rate of growth with increasing feeding rate decreases as feeding rate approaches its maximum level and then plateauing at a higher feeding rate (see Lee *et al.* 2014 for more details). Although choice of a best-fit model is critical, selection of dietary input levels and the production of high quality data are even more important. Inappropriately selected levels and data with large variability, resulting in an atypical feeding rate–response curve, cannot be salvaged even by the best statistical test (see a critical review by Shearer 2000).

Summary

Measurements, including growth performance, proximate composition, protein and lipid gains, and plasma metabolites, of YOY white sturgeon were significantly affected by the various feeding rates. In comparison with other species,

especially rainbow trout (known as a model species for fish nutrition), white sturgeon possess a distinct nutrient partitioning strategy in the utilization of GFB, liver and viscera tissues for energy storage purposes. Furthermore, to estimate an accurate OFR, the one-slope BL, two-slope BL, quadratic BL and quadratic models were tested, followed by comparisons of model performances on the estimation of OFR, with the specific model selection criteria (R_{adj}^2 and AICc). Given the largest R_{adj}^2 and smallest AICc values, the quadratic BL model was chosen as the best model among those tested. Feeding at the estimated OFR, 1.5% BW day⁻¹ will provide the maximum growth for YOY white sturgeon.

Acknowledgements

This work was supported by the California Department of Fish and Wildlife, Ecological Restoration Program (Grant # E1183017). We would like to thank Dr. P. B. Lutes and Mr. E.F. Hallen at the Center for Aquatic Biology and Aquaculture at the UCD for their assistance on our growth trial. The infrastructure support of the Department of Animal Science and the College of Agriculture and Environmental Sciences of the UCD was also acknowledged. The authors also would like to acknowledge Palma Lower for her assistance in editing this document, Daniel S. Bai for his assistance in analysing samples and Mr. Joel Van Eenennaam for providing valuable inputs.

References

- Brett, J.R. & Groves, T.D.D. (1979) Physiological energetics. In: Fish Physiology. Bioenergetics and Growth. Vol. VIII (Hoar, W.S., Randall, D.J. & Brett, J.R. eds), pp. 279–352. Academic Press, New York.

- Bronzi, P., Rosenthal, H. & Gessner, J. (2011) Global sturgeon aquaculture production: an overview. *J. Appl. Ichthyol.*, **27**, 169–175.
- Cahill, G.F.J. (1986) Physiology of gluconeogenesis. In: *Hormonal Control of Gluconeogenesis*. Vol. 1 (Kraus-Friedman, N. ed), pp. 354–361. CRC Press, Boca Raton.
- Chenhan, L., Todd, A.G., Diao, X. & Liu, J. (1995) Biology of the Chinese paddlefish, *Psephurus gladius* (Martens). In: *Proceedings of the International Symposium of Sturgeons* (Gershanovich, A.D. & Smith, T.I.J. eds), pp. 13–21. VNIRO Publishers, Moscow.
- Company, R., Caldich-Giner, J.A., Kaushik, S. & Pérez-Sánchez, J. (1999) Growth performance and adiposity in gilthead sea bream (*Sparus aurata*): risks and benefits of high energy diets. *Aquaculture*, **171**, 279–292.
- Congleton, J.L. & Wagner, T. (2006) Blood-chemistry indicators of nutritional status in juvenile salmonids. *J. Fish Biol.*, **69**, 473–490.
- Cui, Y., Hung, S.S.O., Deng, D.F. & Yang, Y. (1997) Growth performance of juvenile white sturgeon as affected by feeding regimen. *Prog. Fish-Cult.*, **59**, 31–35.
- De Riu, N., Zheng, K.K., Lee, J.W., Lee, S.H., Bai, S.C., Moniello, G. & Hung, S.S.O. (2012) Effects of feeding rates on growth performances of white sturgeon (*Acipenser transmontanus*) fries. *Aquac. Nutr.*, **18**, 290–296.
- Deng, D.F., Koshio, S., Yokoyama, S., Bai, S.C., Shao, Q., Cui, Y. & Hung, S.S.O. (2003) Effects of feeding rate on growth performance of white sturgeon (*Acipenser transmontanus*) larvae. *Aquaculture*, **217**, 589–598.
- Dumas, A., de Lange, C.F.M., France, J. & Bureau, D.P. (2007) Quantitative description of body composition and rates of nutrient deposition in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **273**, 165–181.
- Einen, O., Mørkøre, T., Rørå, A.M.B. & Thomassen, M.S. (1999) Feed ration prior to slaughter – a potential tool for managing product quality of Atlantic salmon (*Salmo salar*). *Aquaculture*, **178**, 149–169.
- Eroldoğan, O.T., Kumlu, M. & Aktaş, M. (2004) Optimum feeding rates for European sea bass *Dicentrarchus labrax* L. reared in seawater and freshwater. *Aquaculture*, **231**, 501–515.
- García-Gallego, M., Sanz, A., Domezain, A. & De la Higuera, M. (1999) Age-size influence on tissue-lipid quality of the sturgeon *Acipenser naccarii*. *J. Appl. Ichthyol.*, **15**, 261–264.
- Grande, L. & Bemis, W.E. (1991) Osteology and phylogenetic relationships of fossil and recent paddlefishes (Polyodontidae) with comments on the interrelationships of Acipenseriformes. *J. Vert. Paleontol.*, **11**(Suppl. 1), 1–121.
- Hageman, J.R., Timpe, D.C. & Hoyt, R.D. (1986) The biology of paddlefish in Lake Cumberland, Kentucky. *Proc. Annu. Conf. Southeast. Assoc. Fish and Wildl. Agencies*, **40**, 237–248.
- Hatlen, B., Helland, S.J. & Grisdale-Helland, B. (2007) Energy and nitrogen partitioning in 250 g Atlantic cod (*Gadus morhua* L.) given graded levels of feed with different protein and lipid content. *Aquaculture*, **270**, 167–177.
- Hung, S.S.O. & Lutes, P.B. (1987) Optimum feeding rate of hatchery-produced juvenile white sturgeon (*Acipenser transmontanus*): at 20 & #xB0;C. *Aquaculture*, **65**, 307–317.
- Hung, S.S.O., Lutes, P.B., Conte, F.S. & Storebakken, T. (1989) Growth and feed efficiency of white sturgeon (*Acipenser transmontanus*) sub-yearlings at different feeding rates. *Aquaculture*, **80**, 147–153.
- Hung, S.S.O., Lutes, P.B., Shqueir, A.A. & Conte, F.S. (1993a) Effect of feeding rate and water temperature on growth of juvenile white sturgeon (*Acipenser transmontanus*). *Aquaculture*, **115**, 297–303.
- Hung, S.S.O., Conte, F.S. & Hallen, E.F. (1993b) Effects of feeding rates on growth, body composition and nutrient metabolism in striped bass (*Morone saxatilis*) fingerlings. *Aquaculture*, **112**, 349–361.
- Hung, S.S.O., Conte, F.S. & Lutes, P.B. (1995) Optimum feeding rate of white sturgeon, *Acipenser transmontanus*, yearlings under commercial production conditions. *J. Appl. Aquacult.*, **5**, 45–52.
- Jones, C.E. (1988) Animal feed. In: *Official Methods of Analysis of the Association of Official Analytical Chemists*, 14th ed (Williams, S. ed.), pp. 152–160. AOAC, Arlington, VA.
- Lee, S., Wang, Y., Hung, S.S.O., Strathe, A.B., Fanguie, N.A. & Fadel, J.G. (2014) Development of optimum feeding rate model for white sturgeon (*Acipenser transmontanus*). *Aquaculture*, **433**, 411–420.
- McCue, M.D. (2010) Starvation physiology: Reviewing the different strategies animals use to survive a common challenge. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.*, **156**, 1–18.
- Miglavs, I. & Jobling, M. (1989) The effects of feeding regime on proximate body composition and patterns of energy deposition in juvenile Arctic charr, *Salvelinus alpinus*. *J. Fish Biol.*, **35**, 1–11.
- Moon, T.W. & Foster, G.D. (1995) Tissue carbohydrate metabolism, gluconeogenesis and hormonal and environmental influences. In: *Metabolic Biochemistry. Biochemistry and Molecular Biology of Fishes*. Vol. 4 (Hochachka, P.W. & Mommsen, T.P. eds), pp. 65–100. Elsevier Science, Amsterdam.
- Ng, W.K., Lu, K.S., Hashim, R. & Ali, A. (2000) Effects of feeding rate on growth, feed utilization and body composition of a tropical bagrid catfish. *Aquacult. Int.*, **8**, 19–29.
- Pesti, G.M., Vedenov, D., Cason, J.A. & Billard, L. (2009) A comparison of methods to estimate nutritional requirements from experimental data. *Br. Poult. Sci.*, **50**, 16–32.
- R Development Core Team (2013) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.
- Robbins, K.R., Norton, H.W. & Baker, D.H. (1979) Estimation of nutrient requirements from growth data. *J. Nutr.*, **109**, 1710–1714.
- Robbins, K.R., Saxton, A.M. & Southern, L.L. (2006) Estimation of nutrient requirements using broken-line regression analysis. *J. Anim. Sci.*, **84**(ESuppl), E155–E165.
- Ryder, J.A. (1890) The sturgeons and sturgeon industries of the eastern coast of the United States, with an account of experiments bearing upon sturgeon culture. *Bulletin of the United States Fish Commission*, Vol. 8 (for 1888). U.S. Government Printing Office, Washington, D.C.
- Scarnecchia, D.L., Ryckman, L.F., Lim, Y., Schmitz, B.J. & Firehammer, J.A. (2007) Life history and the costs of reproduction in Northern Great Plains paddlefish (*Polyodon spathula*) as a potential framework for other Acipenseriform fishes. *Rev. Fish. Sci.*, **15**, 211–263.
- Shearer, K.D. (2000) Experimental design, statistical analysis and modeling of dietary nutrient requirement studies for fish: a critical review. *Aquac. Nutr.*, **6**, 91–102.
- Steffens, W. & Jahnichen, H. (1995) Rearing and production of bester (*Huso huso* (L.) x *Acipenser ruthenus* (L.)) in Germany. In: *Proceedings of the International Symposium of Sturgeons* (Gershanovich, A.D. & Smith, T.I.J. eds), pp. 342–349. VNIRO Publishers, Moscow.

- Storebakken, T. & Austreng, E. (1987) Ration level for salmonids. I. Growth, survival, body composition, and feed conversion in Atlantic salmon fry and fingerlings. *Aquaculture*, **60**, 189–206.
- Storebakken, T., Hung, S.S.O., Calvert, C.C. & Plisetskaya, E.M. (1991) Nutrient partitioning in rainbow trout at different feeding rates. *Aquaculture*, **96**, 191–203.
- Zeitoun, I.H., Ullrey, D.E. & Magee, W.T. (1976) Quantifying nutrient requirements of fish. *J. Fish. Res. Board Can.*, **33**(1), 167–172.
- Zheng, K.K., Deng, D.F., De Riu, N., Moniello, G. & Hung, S.S.O. (2015) The effect of feeding rate on the growth performance of green sturgeon (*Acipenser medirostris*) fry. *Aquac. Nutr.*, **21**, 489–495.