

1 Novel feed from invasive species is beneficial to walleye aquaculture

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## 29 **Abstract**

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31 Carnivorous fishes, such as walleye (*Sander vitreus*) are nutritionally demanding for fish  
32 meal. A promising alternative to marine-origin fish meal, the supply of which has been stagnant in  
33 recent decades, is fish meal derived from undesirable freshwater species, such as the White sucker  
34 *Catostomus commersoni*. To evaluate the relative value of such ingredients, we examined the  
35 growth performance of Walleye juveniles. Two dietary treatments were tested: an experimental  
36 diet (EXP) that was manufactured using White sucker as fish meal in comparison with a  
37 commercial (COM) diet, EWOS micro (EWOS Canada Ltd). The protein content was 50.4% and  
38 57.6% for EXP and COM diets, respectively. The energy content was  $5,098.76 \pm 9.23$  cal/g (mean  
39  $\pm$  SD) for the EXP diet and  $5,134.47 \pm 10.05$  cal/g for the COM diet. Starting at 27 d posthatch,  
40 Walleye juveniles (initial weight [mean  $\pm$  SD] =  $0.03 \pm 0.008$  g; initial length =  $15.7 \pm 1.5$  mm)  
41 were reared for 6 weeks in three replicate tanks for each treatment. Condition factor (0.83), final  
42 weight ( $1.12 \pm 0.3$  g), and weight gain ( $1.09 \pm 0.06$  g) were higher in EXP fish. Similarly, the  
43 energetic lipid content of fish in the EXP treatment group (mean  $\pm$  SD =  $5.01 \pm 0.45$  g/kg) was also  
44 higher than that of fish fed the COM diet ( $3.30 \pm 0.53$  g/Kg). Although the polar lipid content  
45 (membrane lipids) was similar in fish from the two treatments, the nutritional ratio for COM  
46 juveniles was over 1.5 for arachidonic acid and docosahexaenoic acid, indicating selective  
47 incorporation by juveniles and a potential diet  $\pm$ imbalance of these fatty acids. Furthermore, the  
48 higher observed selective incorporation of oleic acid in juveniles fed the EXP diet suggested that a  
49 higher value of this fatty acid in the EXP feed could have increased Walleye growth performance.  
50 Threonine was the main essential amino acid (AA;  $> 18.5\%$  of total AAs) while serine and glycine  
51 contributed the highest percentages of the nonessential AAs ( $> 31\%$  and  $8.5\%$  of total AAs,

52 respectively). All three AAs, often considered limiting ingredients, are important to support growth  
53 and are involved in metabolic processes in some fish species. Our results demonstrate that feed  
54 pellets made with white sucker fish meal improved growth in walleye juveniles and can be suitable  
55 and probably lower-cost alternative to marine fish meal in feeds for carnivorous fishes.

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58 **Key words:** Walleye (*Sander vitreus*), white sucker (*Catostomus commersoni*), invasive species,  
59 juveniles, diet, fatty acid, amino acid.

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67 **Introduction**

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69           Traditional marine-based protein sources derived from pelagic marine fisheries are no  
70 longer available in quantities that meet the current increased demands of intensive fish-farm  
71 production (FAO, 2016). Stagnant supply, greater demand, and rising prices have prompted the  
72 search for substitutes. Indeed, fish meal prices doubled from US\$694 to \$1,379 per metric ton  
73 between 2007 and 2008 (Tacon and Metian, 2008).

74           A potential alternative to marine-origin fish meal is a freshwater meal rendered from invasive  
75 and/or undesirable species, such as the White sucker *Catostomus commersonii*. Previous studies  
76 have evaluated the potential of Asian carp *Hypophthalmichthys* spp. as fish meal in pelleted feeds  
77 (Bowzer et al., 2013). Invasive Asian Carp were demonstrated to be a cost-effective alternative  
78 protein source (US\$600 per metric ton; Bowzer and Trushenski, 2015) to traditional marine origin  
79 fish meal in the diets of carnivorous fishes, such as hybrid Striped Bass (White Bass *Morone*  
80 *chrysops* × Striped Bass *M. saxatilis*), Rainbow Trout *Oncorhynchus mykiss*, and Cobia  
81 *Rachycentron canadum* (Bowzer et al., 2013; Bowzer and Trushenski, 2015).

82

83           The White Sucker is widespread in Canada, the U.S. Midwest, and the U.S. East Coast. It  
84 is both a predator and prey of Walleye *Sander vitreus* (Barton, 2011). White Suckers were  
85 introduced in Quebec, Canada, by sport fishermen who used it as a baitfish to capture Walleyes or  
86 Brook Trout *Salvelinus fontinalis* (Magnan et al., 1990; Duchesne, 1994; St-Laurent, 2002). White  
87 Suckers are found in small streams, rivers, and lakes, where they feed on worms, eggs, and fish  
88 larvae (e.g., Walleye larvae). The species is tolerant of turbid and polluted waters that are often  
89 unsuitable for other fish species (Beamish, 1973; Trippel and Harvey, 1987). This ability allows  
90 White Sucker populations to reach high abundances and increase their dispersal. It has been  
91 demonstrated that White Sucker is a pest species for Brook Trout and a competitor of Yellow Perch  
92 *Perca flavescens* (Duchesne, 1994). White Suckers are found on the same spawning grounds as  
93 Walleyes and breed at the same time of the year (Barton, 2011). In 1995, Forêt Faune et Parcs  
94 Québec initiated a control program aimed at the mass removal of adult White Suckers in five  
95 Quebec lakes (Magnan et al., 1990; Duchesne, 1994; St-Laurent, 2002). Although we do not have  
96 a precise estimate for the price per metric ton for this species, we think it is comparable or lower  
97 to Asian carp (US\$600 per metric ton), which is considerably lower than current pricing for marine-  
98 origin fish meal (US\$1,488 per metric ton, estimated to be 13% higher in 2030) (WBG, 2013).  
99 Additionally, an industry must be established to produce the product to supply the market. Due to  
100 the initial high risk of investment in a new product, White Sucker meal production facilities have  
101 not yet been developed. Based on the potential volumes and the facilities necessary to process  
102 White Suckers, it will likely only be a regional alternative to traditional fish meal sources.

103           The Walleye is an important freshwater sport and commercial fish in North America  
104 (Hartman, 2009; Johnson and Summerfelt, 2015). Only one available commercial grower feed  
105 (Walleye Grower [WG-9206]) has been developed for Walleye larvae and juveniles, and  
106 knowledge about their nutrient requirements is still very scarce. Walleyes have been farmed and

107 juveniles have been used in stocking programs for almost 100 years (Webster, 1978). The culture  
108 of Walleye juveniles by using pelleted feeds has a much shorter history. Walleyes were first  
109 cultured with pelleted feeds in the 1970s using Abernathy Salmon diet and trout granules in private  
110 hatcheries and universities in the USA (McCauley, 1970; Beyerle, 1975). The earliest open-  
111 formula diet (W series; W7, 14, 15, 16) were developed by the U.S. Fish and Wildlife Service in  
112 the 1970s (Beyerle, 1975). The W-16 diet has been used as starter, conversion, and grower diet  
113 (Summerfelt and Clayton, 2007, Kuipers and Summerfelt 1994). To date, the grower diet, WG-  
114 9206, developed in the early 1990s (Barrows and Lellis, 1996), is the only commercially  
115 manufactured diet for walleyes (Nelson & Sons, Inc., Murray, Utah) and has been used  
116 continuously to the present at Iowa Department of Natural Resources hatcheries in the USA  
117 (Summerfelt and Clay, 2007). Most W-series are available for importation in Canada. However,  
118 the cost of importation and the sometimes uncertain availability of pelleted feeds for exportation  
119 (i.e. WG 9206) make it hard for farmers in Quebec, Canada, to secure a stable access of Walleye  
120 grower feeds. For instance, in 2001, bovine spongiform was found in beef cattle in Japan, and U.S.  
121 importation of the BioKyowa FFK formulation (which had been used as habituation diet for  
122 Walleyes for 12 years) has halted indefinitely. The same happened with Otohime C2 pellets (used  
123 as habituation food for Walleyes) in 2016, where Canadian importation was stopped for an  
124 unknown reason. At Most farms in Quebec, Walleye juveniles and broodstock are given frozen  
125 fish, and sometimes pelleted diet formulations for salmonids are used to feed juvenile Walleyes  
126 (Mejri et al. 2014; *Pêche sportive du Réservoir Baskatong*, Grand Remous , Quebec; personal  
127 communication). Thus, development of a pelleted diet is necessary for Walleye production in  
128 Canada to increase. Ideally, such a diet should not only be more sustainable, but should also allow  
129 faster and more efficient weight gain than the diets currently in use. We tested the potential use of  
130 an undesirable species (the White Sucker) as fish meal for the culture of an economically important

131 fish species (the Walleye). The objective of this study was to test whether White Sucker can be  
132 used as a main ingredient in feed for Walleye juveniles, thus making use of the removed fish and  
133 creating a more sustainable approach. We tested the hypothesis that an artificial diet based on White  
134 Suckers can support or improve juvenile Walleye development compared to the commercial diet  
135 developed for salmonids that is currently used in some Canadian farms. This approach would make  
136 it possible not only to valorize an undesirable species that competes with species of high economic  
137 importance but also to produce an affordable and nutrient-dense feed.

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## 139 **Materials and Methods**

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### 141 **Fish culture**

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143 All experimental procedures were evaluated and approved by the Ethical-Scientific  
144 Committee for Animal Experimentation of the Université du Québec à Rimouski (Certificate  
145 number CPA-65-16-176).

146 The 6-week experiment was carried out at the PSRB aquaculture facility, Grand Remous,  
147 Quebec. Eggs were collected from wild broodstock in the *Philomène* River, Grand Remous, and  
148 then were incubated and hatched at PRSB facilities. Larvae started feeding at 3-4 d post hatch (dph)  
149 on Otohime B-1, B-2, C-1, and C-3 (premium Japanese fish nutrition, Japan; pellets used here were  
150 imported by PSRB in 2015, with some left to be used in 2016; Canadian importation of Otohime  
151 was halted in 2016]). The fish were then switched to EWOS Micro (0.5, 0.7 mm, complete fish  
152 feed for salmonids; EWOS Canada Ltd) for 3 weeks. Juveniles used in this experiment were 27

153 dph when the feeding trial started; and initial average weight was  $0.03 \pm 0.01$  g (mean  $\pm$  SD) and  
154 initial average length was  $15.7 \pm 1.5$  mm.

155 At the start of the trial, approximately 200 fish/ tank were randomly distributed into six  
156 900-L circular tanks to obtain three replicate tanks for each dietary treatment. Tanks were provided  
157 with natural freshwater from an external pond and connected to a recirculation system, where water  
158 was drum filtered, sand filtered, and vacuum degassed before use. The water within each tank was  
159 completely exchanged every 2 h, 16 min. The water renewal of the total system was 7.2% daily.  
160 Water temperature (range = 21–24 °C) and dissolved oxygen ( $\geq 7$  mg/L) were monitored daily in  
161 each tank throughout the experiment. The photoperiod applied was constant (16 h light: 8 h dark),  
162 and the light intensity at the surface of the rearing tanks was 630 lx (artificial blue light). Fish were  
163 fed daily using an automatic belt feeder that supplied a constant feed ration over two 8-h periods  
164 (i.e., from about 0700 to 1500 hours and from 1500 to 2300 hours). Fish were fed to satiation, as  
165 indicated by the presence of excess feed in tanks after the two feeding cycles. Tanks were cleaned  
166 daily, and dead fish were removed and weighed each day.

167

## 168 **Experimental feeds**

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170 After the 3-week acclimation period, two dietary treatments were tested: an experimental  
171 (EXP) diet and a commercial (COM) diet. The EXP diet was formulated to meet or exceed all  
172 known nutrient requirements for Walleye juveniles (Summerfelt and Johnson, 2015) and was  
173 prepared at the Département des Sciences Animales at Université Laval, Quebec, Canada,  
174 following the recipe of Bharadwaj et al. (2002) for Walleye juveniles (Walleye grower pellet) but  
175 substituting the fish meal (menhaden) with White Sucker fish meal. To make the fish meal, filets  
176 were removed from White Suckers (20-22 adults weighing  $0.894 \pm 0.086$  kg) captured in the

177 Philomène River during late April 2016. The heads, skin, and viscera were discarded. Briefly,  
178 fillets were washed, autoclaved for 1.5 h at 120 °C, dried for 48 h at 65 °C, and ground to make  
179 the fish meal. Ingredients for the EXP pellets (size = 1.0–1.2 mm) are listed in Table 1. The dry  
180 ingredients were mixed and steam pelleted; the pellets were then dried in a forced-air oven (30 °C,  
181 24 h), sieved, coated with mackerel oil, and stored at -20 °C until used. The ingredients of the COM  
182 pellets (EWOS micro, 1.0–1.2 mm) are listed in Table 1. Both pellets were slightly pulverized  
183 during the first days of feeding to ensure that small individuals had access to the feed.

184

### 185 **Sampling and data collection procedures**

186

187 At the beginning and at the end of the experiment, all fish from each tank were individually  
188 weighed and their TLs were recorded. From each tank (6 tanks total; 3 tanks/treatment), five  
189 replicate samples of five juveniles were frozen in liquid nitrogen and stored at -80 °C for further  
190 biochemical analysis.

191 The following indices were calculated:

192

- 193 • Weight gain,  $WG = \text{final weight (g)} - \text{initial weight (g)}$
- 194 • Feed conversion ratio,  $FCR = \frac{\text{Weight of feed consumed (g)}}{\text{Weight gain (g)}}$
- 195 • Specific growth rate,  $SGR = 100 \times \frac{\ln \text{final body weight (g)} - \ln \text{initial body weight (g)}}{\text{rearing time (days)}}$
- 196 • Survival (%),  $S = 100 \times \frac{\text{Starting N individuals}}{\text{Final N individuals}}$
- 197 • Condition factor,  $K = \frac{\text{Mass}}{\text{Length}^3} \times 100$

198

199 To obtain an indicator of nutritional quality of the two diets, we used the ratio of the polar  
200 fraction of fatty acids (phospholipids constituting cell membranes) in juveniles to the polar  
201 fraction of fatty acids present in the diets. This ratio indicates the juveniles' selective  
202 incorporation or elimination of a given dietary fatty acid in polar lipids in the cell  
203 membranes.

204

## 205 **Chemical analysis**

206

207 Walleye body water content was determined by drying the samples to a constant weight for  
208 24 h at 70 °C. Proximate analyses were performed in accordance with standard methods (AOAC  
209 International 2012; ash [Mthod 942.05], crude protein [Method 990.03], fat [Method 954.02], and  
210 moisture [Method 930.15] by New Jersey Feed Laboratory (Trenton, New Jersey).

211 Lipids were extracted using the Folch method (Folch et al., 1957). Lipids were separated  
212 into neutral and polar lipid fractions using silica gel (30 × 5 mm internal diameter, packed with  
213 Kieselgel 60, 70–230 mesh; Merck, Darmstadt, Germany) hydrated with 6% water and were eluted  
214 with 10 mL of chloroform : methanol (98:2 volume/volume) for neutral lipids followed by 20 mL  
215 of methanol for polar lipids (Marty et al., 1992). The neutral lipid fraction was further eluted on an  
216 activated silica gel with 3 mL of hexane and diethyl ether to eliminate free sterols. All fatty acid  
217 methyl esters (FAMES) were prepared as described by Lepage and Roy (1984) and analyzed in  
218 MSMS scan mode (ionic range = 60–650 m/z) on a Polaris Q ion trap coupled to a Trace GC  
219 (Thermo Finnigan, Mississauga, Otario) equipped with a Valcobond VB-5 capillary column (Valco  
220 Instruments Co. Inc., Broakville, Ontario). The FAMES were identified by comparison of retention  
221 times with known standards (37-Component FAME Mix, PUFA-3, BAME, and menhaden oil;  
222 Supelco Bellefonte, Pennsylvania) and quantified with tricosanoic acid (23:0; i.e. 23 carbon atoms

223 and zero double bonds) and nonadecanoic acid (19:0) as internal standards. Chromatograms were  
224 analyzed using the Xcalibur version 1.3 (Thermo Scientific, Mississauga, Ontario).

225 For amino acid (AA) analysis, samples of either whole body tissues of juveniles or pellets  
226 were diluted with 2 mL distilled water and hydrolyzed with equal parts of 12-N HCl plus 0.1%  
227 phenol at 110 °C for 24 h. After HCl removal by evaporation under vacuum, determination of o-  
228 phtalaldehyde derivatives of AAs were made by high-performance liquid chromatography  
229 separation, as detailed in Kaushik et al. (1994).

230 Total energy (cal/g) was analyzed using a bomb calorimeter (Parr 6200; Preiser Scientific,  
231 Inc., St. Albans, West Virginia), employing benzoic acid as a standard with a known weight and  
232 energy content.

233

## 234 **Statistical analysis**

235

236 Weight, weight gain, length, FCR, SGR, survival, condition factor, and moisture and ash  
237 content were tested with one-way analysis of variance (ANOVA) followed by Hsu's a posteriori  
238 multiple comparisons tests after assumptions of homoscedasticity and normality had been verified  
239 by Levene and Shapiro-Wilk tests, respectively. These analyses were performed with the JMP Pr  
240 12 package (SAS Institute Inc., Cary, NC). Permutational multivariate analysis of variance  
241 (PERMANOVA with 9999 permutations), including a posteriori pair-wise comparisons, was  
242 performed on fatty acid and amino acid profiles in experimental and commercial pellets. Fatty acids  
243 from neutral and polar lipid fractions and AA profiles in walleye juveniles were tested with one-  
244 factor PERMANOVA (dietary treatment, EXP and COM). Assumptions of homoscedasticity were  
245 verified with a PERMDISP test and data were transformed (arcsine square root) when necessary.  
246 To analyze the similarity between the profiles, non-metric multi-dimensional scaling (n-MDS) and

247 SIMPER analyses were performed using a Bray-Curtis similarity matrix with PRIMER 6 (v.  
248 6.1.12) and PERMANOVA+ (v. 1.0.2) (Anderson, 2001).

249

## 250 **Results**

251

### 252 **Diets**

253

254 The dry weight percentage from White sucker filets was  $20.8 \pm 0.5\%$ . (mean  $\pm$  SD).

255 Proximate composition of ECP and COM pellets is presented in Table 1.

256

### 257 **Growth and fish quality parameters**

258

259 At the end of the 6-week feeding period, no significant difference was observed between

260 the two dietary treatments for Walleye TL, *S*, moisture content, or and ash content (Table 2).

261 Survival percentages decreased significantly 4 weeks after the start of the experiment (Fig. 1). No

262 significant difference was seen for FCR or SGR values. However, *K*, final weight, and WG were

263 significantly higher in fish fed that were fed the EXP diet (hereafter “EXP fish”). Similarly, the

264 neutral lipid content (energetic lipids) of EXP fish (mean  $\pm$  SD =  $5 \pm 0.5$  g/kg) was also found to

265 be higher than that of fish receiving the COM diet (hereafter, “COM” fish”;  $3.3 \pm 0.5$  g/ kg), while

266 the polar lipid content (lipids in cell tissues) was similar between fish from the two treatments.

267 Total protein content was not different between the two dietary treatments (EX fish; mean  $\pm$  SD =

268  $115 \pm 10$  g/kg; COM fish:  $107 \pm 11$  g/kg).

269

270 **Nutrient composition of the diet**

271  
272 Percentages of different fatty acids in the EXP and COM diets are presented in Table 3.  
273 The AA percentages of COM and EXP pellets are presented in Table 4. Threonine (THR) was the  
274 most prominent of the essential AAs (EAAS; Table 3). Serine (SER), glycine (GLY), proline,  
275 and taurine quantitatively dominated the nonessential AAs (NEAAs; Table 4).

276  
277 **Nutrient composition of juveniles**

278  
279 Neutral lipids

280  
281 At the start of the experiment (day 0), the fatty acid composition of the neutral lipids was  
282 similar in all Walleye juveniles ( $p = 0.69$ ). However, this composition varied according to diet at  
283 the end of the experiment (pseudo- $F_{\text{diet}[1,4]} = 16.34$ ,  $P = 0.005$ ; Table 5). The fatty acid profiles of  
284 COM fish did not resemble those of the EXP fish, with both profiles resembling those of the diets.  
285 Oleic acid (18:1[n-9], where the number to the left of the colon is the number of carbon atoms, the  
286 number to the right of the hyphen is the position of the first double bound from the methyl end),  
287 docosahexaenoic acid (DHA; 22:6[n-3]), and total polyunsaturated fatty acids (PUFAs) were  
288 highest in COM juveniles. However, 20:1(n-9), 22:1(n-9), 20:4(n-6), and total monounsaturated  
289 fatty acids (MUFAs) were significantly higher in EXP fish than in COM fish.

290  
291 Polar lipids

292

293           The same tendency was observed in the polar lipid fraction as in the neutral lipid fraction:  
294 there was no difference in fatty acid composition between juveniles at the start of the experiment  
295 ( $P = 0.56$ ), whereas the fatty acid composition in juveniles at the end of the experiment varied  
296 according to the diet they were fed (pseudo- $F_{\text{diet}[1, 4]} = 17.71$ ,  $P = 0.003$ ; Table 5). For polar lipids,  
297 20:1(n-9), 22:1(n-9), MUFA, and 20:4(n-6) were significantly higher in EXP juveniles, while DHA  
298 was higher in COM juveniles.

299

300 Nutritional ratio

301

302           For COM juveniles, the observed juvenile : diet ratio was above 1.5 for ARA and DHA  
303 (Fig. 2). indicating selective incorporation of these fatty acids. For EXP fish, the was around 2 for  
304 oleic acid, indicating potential selective retention of this fatty acid by Walleye juveniles and  
305 suggesting its importance for cell membranes integrity under the rearing conditions. Results for  
306 18:2(n-6) and 18:3(n-3) showed no difference between diets, with the ratio less than 0.5 for both  
307 diets, indicating elimination of this fatty acid in the cell membrane.

308

309 Amino acids

310

311           Both at the start and at the end of the experiment, the AA composition of juvenile tissues  
312 did not vary with diet (start:  $P = 0.329$ ; end:  $P = 0.196$ ). Threonine, an EAA, represented more than  
313 18% of the total AAs. Serine and GLY (NEAAs) constituted over 31 and 8% of total AAs,  
314 respectively (Table 6).

315

## 316 **Discussion**

317

318           Because of the continuous expansion and development of aquaculture worldwide, it is  
319 urgent that sustainable alternatives for pelagic fish meal be investigated, developed, and introduced  
320 for use in feed formulations. The use of an undesirable and/or invasive fish species as feed for the  
321 culture of the economically important Walleye is a very interesting sustainable approach. The  
322 White Sucker is a regionally feasible alternative to traditional fish meal sources. The results from  
323 this study indicate that the White Sucker, which is considered an undesirable species in Quebec  
324 lakes, has the potential to be used as such. Pellets incorporating White sucker as the fish meal were  
325 readily accepted by Walleye juveniles, who showed better WG, higher K, and significantly higher  
326 energy lipid accumulation compared to those that received the COM diet. Our results suggest that  
327 the EXP pellet generally was nutritionally balanced but could have been improved with additional  
328 oleic acid. In contrast, the COM diet showed some potential imbalanced proportions of DHA and  
329 ARA, and COM juveniles had lower WG than EXP fish..

330

## 331 **Growth**

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333           Growth was better in the EXP diet, indicating that White Sucker fish meal was readily  
334 accepted by Walleye juveniles. Bharadwaj et al. (2002) indicated that salmon and trout grower  
335 diets could be used satisfactorily for Walleye juveniles pending more information on their  
336 nutritional needs. In our study, final weight, WG, and K were higher in juveniles that were fed the  
337 EXP diet compared to those receiving the COM diet formulated for salmonids diet. The SGR was  
338 not different between the two dietary treatments, ranging between 8.6% and 8.9% per day, and was

339 similar to observations reported for other percid juveniles (Hilge and Steffens, 1996; Kestemont  
340 and Mélard, 2000; Mandiki et al., 2004; Nyina-Wamwiza et al., 2005; Schulz et al., 2006; Schulz  
341 et al., 2007; Lepič et al., 2017). However, in contrast to our results, previous investigations on  
342 percid fish have shown relatively low SGRs. Indeed, SGR values ranged from 2.1% to 3.5% per  
343 day for Zander *Sander lucioperca* juveniles that either were given commercial diets formulated for  
344 Rainbow trout or were fed natural diets (Zakes and Demska-Zakes, 1996; Schulz et al., 2006). High  
345 FCR may be related to the territorial behavior of dominant individuals, therefore a high daily  
346 feeding ratio (25% of total biomass per 24 h) was chosen to ensure adequate feed intake for all fish.  
347 However, this resulted in excessive amounts of uneaten feed and probably led to the overestimation  
348 of FCR values. A negative impact on FCR by possible aggressive feeding behavior of dominant  
349 individuals has also been described for other percid fish, such as the Eurasian Perch *Perca*  
350 *fluviatilis* (Schulz et al., 2007). Survival rates of Walleye juveniles in this study were less than  
351 50%. Such high mortality was most likely caused by the small initial size of the juveniles (15.7  
352 mm; 0.03 g). In agreement with our results, Summerfelt and Johnson (2015) observed low survival  
353 rates (ranging from 26 to 69.7%) in Walleye juveniles. Malison and Held (1996) also reported that  
354 35- 46 mm (0.3-0.5 g) fish had a survival rate of 33.9%.

355

### 356 **Fatty acid and nutritional ratio**

357

358 The body fatty acid profiles reflected those of the diets (Tables 3.). Although the  
359 percentages of ARA were relatively low in both treatments, tissue levels were influenced by levels  
360 supplied in the diet. A higher proportion of ARA in the EXP diet was reflected in a higher  
361 percentage of ARA in the polar and neutral lipids of the whole fish. Furthermore, we observed the  
362 selective incorporation of ARA in COM juveniles, suggesting that this fatty acid is essential for

363 juvenile Walleye growth. In comparison, the EXP diet showed no selective incorporation of ARA,  
364 indicating that this diet meets the physiological ARA needs of juvenile Walleyes. Several studies  
365 have indicated the importance of ARA in fish metabolism, and it is known to be the main fatty acid  
366 precursor of eicosanoids in fish (Bell et al., 1983; Henderson and Tocher, 1987; Bell and Dick,  
367 1990; Bell et al., 1994). In addition, research has been conducted on dietary ARA in several fish  
368 species, and these studies have confirmed that elevated ARA can improve growth and survival  
369 (Bessonart et al., 1999; Bae et al., 2010) as well as resistance to handling stress (Koven et al.,  
370 2001). In addition to the selective incorporation of ARA in COM Walleye juveniles, we also  
371 observed a higher selective incorporation of DHA in COM fish compared to EXP fish. These  
372 results suggest that ARA and DHA levels may be imbalanced in the COM diet. In comparison,  
373 juveniles receiving the EXP diet showed a weak selective incorporation of DHA and EPA but a  
374 marked selective retention of oleic acid. Thus the use of White Sucker fish meal in the EXP diet  
375 seems so support the physiological essential fatty acid needs, but oleic acid could be increased as  
376 a potential energy source for Walleyes. Previous studies have demonstrated that oleic acid is a good  
377 energy substrate for *Totoaba macdonaldi* juveniles (Zapata et al. 2016) and Walleye larvae  
378 (Mejri et al. 2014). Thus, the EXP diet, as prepared in our study, should be adjusted to contain  
379 higher proportion of oleic acid.

380  
381 Most PUFAs were found in the polar lipids. The preferential conservation of PUFAs in the  
382 polar portion indicated a strong metabolic response of Walleye juveniles. Polyunsaturated fatty  
383 acids are the major constituents of structural lipids and are therefore important in maintaining the  
384 structural integrity of biological membranes. Previous studies have shown that when PUFAs are  
385 insufficient or limited, they are removed from the neutral lipids and conserved in the polar lipids  
386 (D'Abramo and Sheen, 1993; Mejri et al., 2014).

387 Our results clearly demonstrate that feeding juvenile Walleyes the EXP pellets resulted in  
388 significantly higher energy lipid (neutral lipid) accumulation, better *K*, and modified fatty acid  
389 composition in the juveniles. The higher energy reserves of EXP juveniles indicates that these fish  
390 have a higher nutritional condition compared to those fed the COM diet.

391

### 392 **Amino acids**

393

394 The AA composition, especially EAA, is another excellent indicator of how the nutritional  
395 needs are satisfied. Amino acids have essential roles in fish growth and diet costs (Wilson and Poe,  
396 1985; Conceição et al., 2003). High percentages of THR, SER, and GLY in the EXP and COM  
397 pellets were reflected in juvenile AA profiles. Studies conducted on early stages of Walleye have  
398 determined the importance of methionine (MET) and SER during embryogenesis, suggesting the  
399 need for minimal levels of 0.1% MET and 6% SER in the total AAs (Mejri et al., 2014; Mejri et  
400 al., 2017). Compared to the other AAs, SER levels were the highest in Walleye juveniles from both  
401 treatments, suggesting its potential role as an energy source. The MET levels were over 2% of total  
402 AAs, which should meet the nutritional requirement for Walleyes (Mejri et al., 2017). After lysine  
403 and MET, THR is generally one of the most limiting EAAs in feed ingredients (Saldana et al.,  
404 1994; Yu et al., 2015). In our study, THR was the most prominent EAA (> 18% of total AAs),  
405 showing no significant difference between the COM and EXP groups. Threonine deficiency  
406 resulted in retarded growth and poor feed efficiency values in Red Drum *Sciaenops ocellatus*  
407 juveniles (Boren and Gatlin, 1995), Olive Flounder, *Paralichthys olivaceus* (Alam et al., 2003),  
408 and Rainbow Trout (Rodehutschord et al., 1997). Feng et al. (2013) showed that THR enhanced  
409 growth and increased the digestive and absorptive capacities in Jian Carp (a variant of the Common  
410 Carp *Cyprinus carpio*).

411

## 412 **Conclusions**

413

414           This study has demonstrated the beneficial effects of using White Sucker as the main fish  
415 meal in feed pellets. Reducing feed costs by the substitution of marine fish meal (expensive  
416 ingredient) with more cost-effective White Sucker meal can also assist the growth of the  
417 aquaculture industry, mainly on a regional scale. The EXP feed resulted in improved growth of  
418 Walleye juveniles, and its use may contribute to reducing costs related to the elimination of this  
419 undesirable fish species, thus contributing to environment protection. Additional work should be  
420 done to optimize the fatty acid composition of pellets made using White Sucker fish meal by  
421 supplementing with oleic acid.

422

423

424

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426

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598

599 **Figure captions**

600 **Fig. 1** Survival of walleye juveniles fed an experimental diet based on the use of white sucker  
601 (*Catostomus commersoni*) as fish meal or a commercial diet (EWOS micro; Ewos Canada Ltd)  
602 during a six weeks feeding trial.

603

604

605 **Fig. 2** Ratio of polar fatty acids in walleye (*Sander vitreus*) juveniles to dietary fatty acids.  
606 Measurements were made six weeks after juveniles were fed an experimental diet based on the use  
607 of white sucker (*Catostomus commersoni*) as fish meal or a commercial diet (EWOS micro; Ewos  
608 Canada Ltd). The dashed line indicates equal amounts of fatty acids in the juveniles and in the diet.

609 PUFA: polyunsaturated fatty acid.

610

611

1 Table 1. Composition (%) of experimental (EXP; size = 1.0–1.2 mm) and commercial (COM;  
 2 EWOS Micro, 1.0–1.2 mm) diets fed to juvenile Walleyes.

Ingredient or component	EXP diet	COM diet	6
			7
White Sucker fish meal	49.5	-	8
Fish meal (mix of anchovies, menhaden, pollock, white fish, and herring trimmings from US and Canadian Pacific Coast fisheries)	-	58.8	9
Blood meal	4.8	6.7	10
Soybean meal*	12		11
Corn gluten meal*	8.3		
Wheat flour*	12.1	11.6	12
Wheat gluten	-	2.5	
Fish oil	11.8	10	13
Vitamin mix*	0.8	-	
Ascorbic acid	0.2	-	14
Choline chloride	0.5	-	
Yeast-amino acid premixes-vitamin and mineral premix	-	10.4	15

16 <sup>a</sup>soybean meal in the EXP diet was obtained from Jefo, St-Hyacinthe, Quebec.

17 <sup>b</sup>Corn gluten meal in the EXP diet was obtained from Meunerie Gérard Soucy, Inc., Ste-Croix,  
 18 Québec.

19 <sup>c</sup>Wheat flour in the EXP diet was obtained from la Seigneurie des Aulnaies, Inc., Saint-Roch-des-  
 20 Aulnaies, Québec.

21 <sup>d</sup>Vitamin mix in the EXP diet was obtained from Corey Feed Mills Ltd., Fredericton, New-  
 22 Brunswick (Vitamin Mix 30): vitamin A, 650; vitamin D3, 1,000; vitamin E, 50 %, vitamin K,  
 23 MSBC50%, 16.5; vitamin B12, 10%; biotin, 2%, folic acid 20%, niacin 50%; vitamin B6,  
 24 pyridoxine hydrochloride, 99%; vitamin B2, 96%, BHT-KI, 76%; FeSO<sub>4</sub> 7H<sub>2</sub>O (20% Fe); MnSO<sub>4</sub>  
 25 7 H<sub>2</sub>O (36% Mn); ZnSO<sub>4</sub> 7 H<sub>2</sub>O (40% Zn); CuSO<sub>4</sub> 5H<sub>2</sub>O (25% Cu); Na<sub>2</sub>SeO<sub>3</sub> (45.6% Se); CoSO<sub>4</sub>  
 26 (21% Co) and starch or cellulose.

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 28  
 29

30 Table 2. Growth and condition indices (mean  $\pm$  nSD) of Walleye juveniles fed a commercial  
 31 (COM) diet (EWOS Micro; EWOS Canada Ltd.) or an experimental (EXP) diet (containing White  
 32 Sucker as fish meal) over a six-week study period. Within a given row, means with different letters  
 33 are significantly different (ANOVA:  $P < 0.05$ )

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 35

<b>Growth and condition indices</b>	<b>EXP diet</b>	<b>COM diet</b>	<b>One-way ANOVA (<math>P</math>-value)</b>
Initial weight (g)	0.03 $\pm$ 0.008	0.03 $\pm$ 0.009	0.787
Final weight (g)	1.12 $\pm$ 0.36 <sup>z</sup>	0.87 $\pm$ 0.35 <sup>y</sup>	< 0.0001
Weight gain (g)	1.09 $\pm$ 0.06 <sup>z</sup>	0.86 $\pm$ 0.15 <sup>y</sup>	0.042
Final TL (mm)	50.92 $\pm$ 5.99	49.81 $\pm$ 6.72	0.082
Feed conversion ratio	2.85 $\pm$ 1.12	2.21 $\pm$ 1.06	0.145
Specific growth rate	8.87 $\pm$ 0.16	8.58 $\pm$ 0.43	0.413
Survival (%)	43.11 $\pm$ 10.96	46.23 $\pm$ 9.34	0.727
Condition factor	0.83 <sup>z</sup>	0.67 <sup>y</sup>	< 0.0001
Moisture (g/kg)	208.61 $\pm$ 2.73	203.38 $\pm$ 1.75	0.150
Ash (g/kg <sup>1</sup> )	20.27 $\pm$ 1.65	24.97 $\pm$ 2.21	0.138
Neutral fatty acids (g/kg)	5.01 $\pm$ 0.45 <sup>z</sup>	3.30 $\pm$ 0.53 <sup>y</sup>	0.013
Polar fatty acids (g/kg)	2.02 $\pm$ 0.47	1.27 $\pm$ 0.43	0.116
Proteins (g/kg)	115.23 $\pm$ 9.99	107.17 $\pm$ 10.53	0.227

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 37

38 Table 3. Fatty acid composition (percent of total fatty acids; mean  $\pm$  SD) of the experimental (EXP)  
 39 diet (containing White Sucker as fish meal) and the commercial (COM) diet (EWOS Micro; EWOS  
 40 Canada Ltd) fed to juvenile Walleyes.

41  
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43	<b>Fatty acid</b>	<b>EXP diet</b>	<b>COM diet</b>
44	14:0	2.14 $\pm$ 0.40	3.84 $\pm$ 0.38
45	16:0	16.82 $\pm$ 1.79	22.55 $\pm$ 1.15
46	18:0	3.97 $\pm$ 0.67	5.70 $\pm$ 0.90
47	$\Sigma$ SFA $\alpha$	25.39 $\pm$ 4.46	34.56 $\pm$ 6.02
48	16:1	4.80 $\pm$ 0.57	3.98 $\pm$ 0.43
49	18:1 n-9	8.44 $\pm$ 0.29 <sup>y</sup>	15.64 $\pm$ 0.85 <sup>z</sup>
50	20:1 n-9	8.82 $\pm$ 2.04 <sup>z</sup>	4.76 $\pm$ 1.58 <sup>y</sup>
51	22:1 n-9	14.62 $\pm$ 3.68 <sup>z</sup>	4.52 $\pm$ 1.29 <sup>y</sup>
52	24:1 n-9	0.49 $\pm$ 0.09	1.02 $\pm$ 0.13
53	$\Sigma$ MUFA $\beta$	37.48 $\pm$ 5.48	30.43 $\pm$ 5.20
54	18:2 n-6	10.01 $\pm$ 0.72	7.53 $\pm$ 0.31
55	18:3 n-3	1.64 $\pm$ 0.12	1.36 $\pm$ 0.08
56	18:4 n-3	0.44 $\pm$ 0.03	1.53 $\pm$ 0.18
57	20:4 n-6	4.94 $\pm$ 0.67 <sup>z</sup>	1.11 $\pm$ 0.32 <sup>y</sup>
58	20:5 n-3	5.98 $\pm$ 0.21	9.11 $\pm$ 0.72
59	22:6 n-3	13.21 $\pm$ 2.10	13.65 $\pm$ 1.21
60	$\Sigma$ PUFA $\delta$	37.13 $\pm$ 4.61	35.01 $\pm$ 4.12
61	$\Sigma$ n-3	21.33 $\pm$ 2.71	25.68 $\pm$ 4.12
62	$\Sigma$ n-6	15.48 $\pm$ 4.65	9.13 $\pm$ 3.52

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66  <sup>$\alpha$</sup> Sum of saturated fatty acids (SFA) includes 11:0, 12:0, 13:0, 15:0, 17:0, 20:0, 21:0, 22:0, 23:0,  
 67 24:0, for which the combined percentages are  $\leq$  0.5% of total fatty acids

68  <sup>$\beta$</sup> Sum of monounsaturated fatty acids (MUFA) includes 14:1 and 17:1, for which the combined  
 69 percentages are  $\leq$  0.5% of total fatty acids

70  <sup>$\delta$</sup> Sum of polyunsaturated fatty acids (PUFA) includes 20:2, 18:3(n-6), 20:3(n-6), 20:3(n-3), for  
 71 which the combined percentages are  $\leq$  0.5% of total fatty acids.

72

73 Table 4. Amino acid composition (percent of total amino acids; mean  $\pm$  SD) in the experimental  
 74 (EXP) diet containing White Sucker as fish meal) and the commercial (COM) diet (EWOS  
 75 Micro; EWOS Canada Ltd) fed to juvenile Walleyes. Amino acids constituting less than 1% are  
 76 not listed

<b>Amino acid</b>	<b>EXP diet</b>	<b>COM diet</b>
<i>Essential amino acids</i>		
Threonine, THR	15.04 $\pm$ 2.05	15.54 $\pm$ 1.94
Methionine, MET	3.30 $\pm$ 0.19	2.79 $\pm$ 0.35
All-Leucine type, AILE	3.49 $\pm$ 0.60	5.29 $\pm$ 0.43
Phenylalanine, PHE	4.11 $\pm$ 0.20	3.30 $\pm$ 0.26
<i>Non-essential amino acids</i>		
Taurine, TAU	6.41 $\pm$ 4.32	9.64 $\pm$ 7.00
Serine, SER	21.80 $\pm$ 7.84	21.04 $\pm$ 12.49
Tyrosine, TYR	4.34 $\pm$ 0.27	2.01 $\pm$ 0.89
Alanine, ALA	8.59 $\pm$ 3.41	8.68 $\pm$ 5.00
Sarcosine, SAR	3.64 $\pm$ 4.28	3.20 $\pm$ 3.31
Glycine, GLY	15.46 $\pm$ 4.89	14.24 $\pm$ 2.41
Hydroxyproline, HYP	1.04 $\pm$ 0.52	1.02 $\pm$ 0.29
Proline, PRO	7.71 $\pm$ 1.12	7.80 $\pm$ 1.36
Trimethylglycine, TMG	3.89 $\pm$ 0.36	4.52 $\pm$ 1.40

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80 Table 5. Fatty acid composition of neutral and polar lipids in Walleye juveniles (percent weight of  
81 weight of total neutral and polar lipids; mean  $\pm$  SD) after 6 weeks of feeding with either the  
82 experimental (EXP) diet (containing White Sucker as fish meal) or the commercial (COM) diet  
83 (EWOS Micro; EWOS Canada Ltd). Different letters indicate significant differences between  
84 treatments (ANOVA:  $P < 0.05$ ), with polar and neutral fatty acids being tested separately.

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Fatty acid	Neutral fraction		Polar fraction	
	EXP diet	COM diet	EXP diet	COM diet
14:0	4.11 $\pm$ 0.13	4.04 $\pm$ 0.96	1.92 $\pm$ 0.09	1.66 $\pm$ 0.31
16:0	14.86 $\pm$ 2.23	15.02 $\pm$ 0.65	23.08 $\pm$ 0.22	25.45 $\pm$ 0.73
18:0	2.15 $\pm$ 0.36	3.13 $\pm$ 0.79	5.66 $\pm$ 0.63 <sup>y</sup>	7.96 $\pm$ 0.60 <sup>z</sup>
$\Sigma$ SFA $\alpha$	22.65 $\pm$ 3.98	24.14 $\pm$ 4.02	32.92 $\pm$ 6.15	38.32 $\pm$ 6.85
16:1	10.61 $\pm$ 0.42	7.73 $\pm$ 0.53	4.78 $\pm$ 0.34 <sup>z</sup>	2.76 $\pm$ 0.25 <sup>y</sup>
18:1 n-9	16.76 $\pm$ 0.92 <sup>y</sup>	24.19 $\pm$ 2.19 <sup>z</sup>	16.52 $\pm$ 1.13	14.97 $\pm$ 0.33
20:1 n-9	12.62 $\pm$ 0.29 <sup>z</sup>	5.62 $\pm$ 2.06 <sup>y</sup>	6.27 $\pm$ 0.35 <sup>z</sup>	2.19 $\pm$ 0.50 <sup>y</sup>
22:1 n-9	16.05 $\pm$ 0.60 <sup>z</sup>	4.19 $\pm$ 1.65 <sup>y</sup>	3.91 $\pm$ 0.26 <sup>z</sup>	1.14 $\pm$ 0.22 <sup>y</sup>
24:1 n-9	0.63 $\pm$ 0.05	0.94 $\pm$ 0.14	0.79 $\pm$ 0.10	0.97 $\pm$ 0.20
$\Sigma$ MUFA $\beta$	56.84 $\pm$ 7.62 <sup>z</sup>	43.23 $\pm$ 8.11 <sup>y</sup>	33.01 $\pm$ 5.54 <sup>z</sup>	23.15 $\pm$ 4.96 <sup>y</sup>
18:2 n-6	5.97 $\pm$ 1.98	7.34 $\pm$ 1.40	3.06 $\pm$ 0.29	2.79 $\pm$ 0.39
18:3 n-3	1.04 $\pm$ 0.11	1.50 $\pm$ 0.18	0.53 $\pm$ 0.04	0.69 $\pm$ 0.08
18:4 n-3	0.69 $\pm$ 0.06	1.42 $\pm$ 0.14	0.37 $\pm$ 0.04	0.59 $\pm$ 0.05
20:4 n-6	1.33 $\pm$ 0.12 <sup>z</sup>	0.73 $\pm$ 0.24 <sup>y</sup>	3.64 $\pm$ 0.24 <sup>z</sup>	1.76 $\pm$ 0.22 <sup>y</sup>
20:5 n-3	5.17 $\pm$ 0.39	7.12 $\pm$ 1.66	7.39 $\pm$ 0.37	8.80 $\pm$ 0.89
22:6 n-3	5.57 $\pm$ 0.87 <sup>y</sup>	13.39 $\pm$ 1.53 <sup>z</sup>	18.09 $\pm$ 1.00 <sup>y</sup>	22.78 $\pm$ 1.28 <sup>z</sup>
$\Sigma$ PUFA $\delta$	20.50 $\pm$ 2.42 <sup>y</sup>	32.62 $\pm$ 4.39 <sup>z</sup>	34.06 $\pm$ 5.48	38.53 $\pm$ 6.88
$\Sigma$ n-3	12.47 $\pm$ 2.34 <sup>y</sup>	23.48 $\pm$ 3.14 <sup>z</sup>	26.40 $\pm$ 3.55	32.87 $\pm$ 4.20
$\Sigma$ n-6	7.82 $\pm$ 2.73	8.82 $\pm$ 3.43	7.40 $\pm$ 1.75 <sup>z</sup>	5.26 $\pm$ 1.20 <sup>y</sup>

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89  <sup>$\alpha$</sup> Sum of saturated fatty acids (SFA) includes 11:0, 12:0, 13:0, 15:0, 17:0, 20:0, 22:0, 24:0, for  
90 which the combined percentages are  $\leq 0.5\%$  of total fatty acids.

91  <sup>$\beta$</sup> Sum of monounsaturated fatty acids (MUFA) includes 14:1 and 17:1, for which the combined  
92 percentages are  $\leq 0.5\%$  of total fatty acids.

93  <sup>$\delta$</sup> Sum of polyunsaturated fatty acids (PUFA) includes 20:2, 18:3(n-6), and 20:3(n-6), for which  
94 the combined percentages are  $\leq 0.5\%$  of total fatty acids

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96 Table 6. Amino acid composition (percent of total amino acids; mean  $\pm$  SD) of Walleye juveniles  
 97 at the end of the 6-week experiment during which they were fed the experimental (EXP) diet  
 98 (containing White Sucker as fish meal) or the commercial (COM) diet (EWOS Micro; EWOS  
 99 Canada Ltd). Amino acids constituting less than 1% are not listed. Amino acid codes are defined  
 100 in Table 4.  
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Amino acid	EXP diet	COM diet
	<i>Essential amino acids</i>	
THR	18.97 $\pm$ 2.49	19.76 $\pm$ 2.52
MET	2.41 $\pm$ 0.60	3.18 $\pm$ 0.21
AILE	7.17 $\pm$ 1.12	5.55 $\pm$ 0.94
PHE	7.14 $\pm$ 0.82	4.18 $\pm$ 0.28
	<i>Non-essential amino acids</i>	
TAU	4.82 $\pm$ 3.39	11.07 $\pm$ 3.81
SER	34.08 $\pm$ 9.82	31.94 $\pm$ 4.46
TYR	4.25 $\pm$ 0.85	2.18 $\pm$ 2.09
ALA	1.95 $\pm$ 0.25	3.04 $\pm$ 0.23
SAR	2.11 $\pm$ 0.27	3.28 $\pm$ 0.25
GLY	9.30 $\pm$ 4.48	8.55 $\pm$ 2.03
PRO	4.35 $\pm$ 2.96	3.21 $\pm$ 1.16
TMG	2.02 $\pm$ 0.26	2.57 $\pm$ 0.36

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