Effect of alternative lipids and temperature on growth and growth factor gene expression in yellowtail kingfish (*Seriola lalandi*)

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i. **Preface**

This thesis has been written in manuscript format for the international journal Aquaculture. Formatting, including layout, section numbers, figures, tables and referencing style has been written in accordance with this journal's guidelines for authors (see page 33).

We, the undersigned, hereby acknowledge that Honours student, Geoffrey M Collins, conducted this research project and wrote the following manuscript in fulfilment for the degree of Bachelor of Technology (Aquaculture), Honours, at Flinders University of South Australia.

Professor Andy Ball

Associate Professor Jian Qin

Dr. David Stone

Ms. Jenna Bowyer

ii. **Declaration**

I certify that this thesis does not contain, without acknowledgement, any previously submitted material for a degree or diploma at any university, and that to the best of my knowledge does not include any material previously published or written by another person where due reference is not made in the text.

Geoffrey M Collins

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ABSTRACT

In this study the suitability of canola oil (CO) and poultry fat (PO) as alternatives to fish oil (FO) was assessed. Furthermore, a real-time RT-qPCR assay to detect hepatic insulin-like growth factor-I (IGF-I) and insulin-like growth factor binding protein-1 (IGFBP-1) was developed. Four isoproteic (450 g kg^{-1}), isolipidic (250 g kg^{-1}) dry extruded diets were formulated to contain PO or CO at 50 or 100% inclusion, as a substitute for FO. Yellowtail kingfish growth and growth factor gene expression was compared with fish fed a diet containing 100% FO. Two experiments were conducted separately at optimal (22 ^{o}C; 33 d) and sub-optimal (18 ^{o}C; 34 d) temperatures with mean initial fish weights of 95.6 ± 0.1 g and 101 ± 0.1 g respectively. At 22 ^{o}C yellowtail kingfish fed the 50% PO diet grew significantly larger (281.2 ± 1.2 g) than individuals fed the 100% FO diet (266.9 ± 5.9 g). Inclusion of CO at 50% produced equivalent weight gain to the 100% FO diet, however, 100% inclusion of CO produced poor performance in both experiments. Trends in gene expression reflected the response in weight gain to alternative lipids. A positive relationship between hepatic IGF-I mRNA levels and weight gain and also hepatic IGFBP-1 mRNA and weight gain was found. A positive correlation between hepatic IGF-I and IGFBP-1 mRNA levels was also found. Irrespective of diet, fish grew larger at 22 ^{o}C than at 18 ^{o}C. The findings of this research indicate that improved diet formulations for yellowtail kingfish may be achieved through the inclusion of alternative lipid sources and that IGF-I mRNA may be used as a rapid indicator of growth in this species.

Keywords

yellowtail kingfish, alternative lipids, IGF-I, IGFBP-1, growth factor, temperature

1. Introduction

The yellowtail kingfish (*Seriola lalandi*; Valenciennes, 1833) is a marine, pelagic, carnivorous fish in the family Carangidae that occurs circumglobally in sub-tropical and temperate waters (PIRSA, 2002; Kolkovski and Sakakura, 2004). This species is considered to have excellent attributes for aquaculture including fast growth, good taste and market acceptance. Yellowtail kingfish and related sub-species are currently cultured in Australia, New Zealand, Japan, China, USA and Chile (Chai, et al., 2009). In Australia, established locations for the grow-out of yellowtail kingfish exist in the near-shore waters of the Spencer Gulf, South Australia, where commercial culture of this species has been undertaken since 1998. In the financial year of 2007/2008, 2 900 tonnes of yellowtail kingfish was produced in South Australian waters, compared with just 45 tonnes in 1999/2000 (Fowler, et al., 2003; Chai, et al., 2009).

The major lipid component in manufactured fish feeds (aquafeeds) has traditionally been fish oil which is derived from marine capture fisheries (Leaver, et al., 2008; Tacon and Metian, 2008). Marine capture fisheries are currently at their maximum sustainable limit with global production of around 90 million tonnes per annum in the last decade (Leaver, et al., 2008; FAO, 2009; Perón, et al., 2010). Partial and total replacement of fish oil (FO) by

alternative sources has received major research focus over the past two decades as farmers seek to minimise production costs. Both terrestrial animal fats and plant oils have the economical and practical potential to substitute fish oil (Raso and Anderson, 2003; Benedito-Palos, et al., 2007; Lewis and Kohler, 2008). Canola oil (CO) and poultry fat (PO) are two alternatives to fish oil that have been successfully included in diets for a variety of species, including sunshine bass (Wonnacott, et al., 2004), Atlantic salmon (Higgs, et al., 2006; Deslauriers and Rideout, 2008), red seabream (Glencross, et al., 2003), Chinook salmon (Grant, et al., 2008; Huang, et al., 2008), Japanese seabass (Xue, et al., 2006), rainbow trout (Liu, et al., 2004), and Murray cod (Francis, et al., 2006; Francis, et al., 2009). In a recent study by Seno-O *et al*., (2008), the inclusion of olive oil at up to 100% was shown to have no negative effects on growth or proximate composition in the congeneric Japanese yellowtail (*Seriola quinqueradiata*) cultured for 40 days. Seno-O *et al*., (2008) also reported that replacement of dietary FO with olive oil prevented muscle discolouration post-mortem when fillets were stored at 4 ^{o}C.

Molecular tools are increasingly being utilised in the aquaculture industry to complement existing husbandry techniques and improve production by providing insight into responses to altered environmental conditions (Cancela, et al., 2010; Panserat and Kaushik, 2010). Potential commercial applications of this technology include genetic improvement through marker assisted selection and improved husbandry through understanding metabolic pathways involved in nutrition and reproduction (De-Santis and Jerry, 2007; Panserat and Kaushik, 2010). Additionally, recent advances in molecular techniques used to measure gene expression such as quantitative real-time RT-PCR

(RT-qPCR), may assist in reducing costs involved in lengthy feeding experiments, whereby a suitable gene may give rapid indication of fish performance in as little as two weeks (Pérez-Sánchez and Bail, 1999; Cruz, et al., 2006; Picha, et al., 2008). Therefore, the use of molecular technology will shorten the time taken to evaluate the efficacy of diets for commercial application.

Growth pathways appear to be highly conserved in vertebrate evolution and genomic research into teleost fish has been aided by molecular studies involving terrestrial livestock (De-Santis and Jerry, 2007). Much of the genomic research involving terrestrial livestock has also focussed on genes that influence traits that are of equal interest in aquaculture such as growth rate, carcass yield, flesh quality and disease resistance (De-Santis and Jerry, 2007). A number of candidate genes have been identified as having potential for use as markers of somatic growth in teleost fish, including components of the somatotropic axis, myogenic regulatory factors and the transforming growth factor superfamily (De-Santis and Jerry, 2007; Panserat and Kaushik, 2010). The somatotropic axis hormone system, which includes growth hormone (GH), insulin-like growth factors-I and -II (IGF-I and IGF-II) and their respective receptors and binding proteins, exerts a profound effect on metabolism and development (Duan, 1997; Duan, 1998; Wood, et al., 2005). The liver is the primary site of IGF-I production (Duan, 1998; Caelers, et al., 2004), although a number of other tissues including the heart, kidneys and muscle produce IGF-I locally, where the mature IGF-I ligand acts in an autocrine/paracrine manner (Duan, 1998; Moriyama, et al., 2000). The availability of IGF-I is regulated by IGF binding proteins (IGFBP's) which

prevent their destruction by proteolytic degradation and prevent IGF-I from binding to cell-surface receptors (Duan and Xu, 2005; Wood, et al., 2005; Bower, et al., 2008). Over 99% of circulating IGF-I is bound to IGFBP's in salmonids and the specific role that these peptides play in regulating IGF-I is yet to be fully elucidated (Reinecke, et al., 2005).

Both IGF-I and IGFBP-1 have been positively correlated with growth in nutritional studies involving teleost fish (Moriyama, et al., 2000; Dyer, et al., 2004; Cruz, et al., 2006; Picha, et al., 2008). The aims of this project were to: (1) evaluate the growth performance of yellowtail kingfish fed on diets partially or completely composed of alternative lipid sources, (2) develop a sensitive RT-qPCR assay for the detection of genes associated with somatic growth in yellowtail kingfish and (3) evaluate the potential of these genes as rapid indicators of growth in this species. This study tested the hypothesis that dietary compounds can alter gene expression in regulating fish growth performance. Specifically, the focus of this study was on the expression of genes involved in somatic growth in response to changing dietary compounds.

2. Materials and Methods

Two separate feeding trials were conducted at the South Australian Research and Development Institute (SARDI) Aquatic Sciences Centre, West Beach, South Australia (34^{o}57.2'S, 138^{o}30.4'E). A total of 233 juvenile yellowtail kingfish were used for each study. Yellowtail kingfish used in the high (22^{o}C) and low (18^{o}C) temperature experiments were the same cohort of fish and originated from CleanSeas Tuna Ltd., Arno Bay, South Australia.

126 Prior to stocking in experimental tanks fish were maintained in 5 000 L
127 fibreglass tanks under ambient temperature and photoperiod and were fed a
128 commercial pelleted feed (Ridley's Aquafeeds; 450 g kg^{-1} crude protein, 200 g
129 kg^{-1} crude lipid). The two experiments were conducted separately in
130 March/April (22 ^{o}C) and August/September (18 ^{o}C).
131
132 *2.1 Diets, Feeding Trials and Sample Storage*
133
134 Each of the experimental diets was formulated and manufactured at the
135 SARDI Australasian Experimental Stockfeed Extrusion Centre (AESEC),
136 Roseworthy, South Australia. Diets consisted of 250 g kg^{-1} crude lipid, 450 g
137 kg^{-1} crude protein and 24 MJ kg^{-1} gross energy. Approximately 50 g kg^{-1} of the
138 lipid component of the diet consisted of residual fish oil from the fish meal
139 used for the protein component. The remaining 200 g kg^{-1} of lipid in the diets
140 was manipulated to formulate five different dry, extruded diets: 100% fish oil
141 (FO; control diet), 50% poultry fat and 50% fish oil (50% PO), 100% poultry fat
142 (100% PO), 50% refined canola oil and 50% fish oil (50% CO) and 100%
143 refined canola oil (100% CO). Prior to stocking in experimental systems a sub-
144 sample of fish (n=9) were weighed and measured and liver samples taken
145 and stored in RNAlater (Ambion, Applied Biosystems, Foster City, CA, USA).
146 These samples were labelled Time 0 and were taken to compare gene
147 expression with fish fed different diets at the completion of each experiment.
148 Yellowtail kingfish juveniles (95.6 ± 0.1 g [22 ^{o}C] and 101.1 ± 0.1 g [18 ^{o}C])
149 were stocked at random into 15 × 700 L fibreglass tanks (n=14 fish $tank^{-1}$).
150 Experimental tanks were situated in a temperature and photoperiod (14h light :

151 10h dark) controlled room. For the duration of the experiments, animals were
152 hand-fed to apparent satiation twice daily for 34 d (22 ^{o}C) and 33 d (18 ^{o}C) at
153 0900 and 1530 h.
154 At the completion of the feeding trials, three fish from each tank were
155 removed and immediately euthanased by a spike to the brain. Duplicate liver
156 samples were obtained from each of the three fish and placed in four volumes
157 of RNAlater. Samples were then stored at -20 ^{o}C until analysis in the
158 laboratory.
159 Weight gain (g) was assessed by group means (n=3) after weighing fish to
160 the nearest 0.1 g. Hepatosomatic index (HSI) was calculated as liver weight (g)
161 / total weight (g) × 100.
162
163 *2.2 RNA isolation and cDNA synthesis*
164
165 Total RNA was extracted from 50 mg of liver sample. Samples were
166 homogenised for approximately 20 s using a tissue homogeniser (Multipro
167 395; Dremel Corporation, Racine, WI) into 300 µL of TRIzol (Invitrogen,
168 Newcastle, NSW, Australia) according to the manufacturer's instructions.
169 Additionally, 16 samples from the higher temperature experiment (22 ^{o}C) were
170 extracted using RNeasy mini-kit (QIAGEN, Hilden, Germany) according to the
171 manufacturer's instructions. All RNA was re-suspended in 200 µL RNase-free
172 water. RNA quality and quantity was determined using a Nanodrop-8000
173 spectrophotometer (Nanodrop Technologies) and denaturing gel
174 electrophoresis. RNA used for cDNA synthesis was treated with RNase-free
175 DNase (Promega, Madison, WI, USA) to remove any genomic DNA

contamination in 12 µL reactions using 2 U DNase, 1 × DNase buffer and 2 µg RNA. First-strand cDNA was synthesised from 1 µg total RNA in 30 µL reactions containing 60 U M-MLV reverse transcriptase (RT; Promega), 1 × RT-buffer, 0.5 mM dNTPs, 250 ng oligo $d(T)_{15}$ primers and 250 ng random hexamers. Cycling conditions for reverse transcription were: 40^0C for 10 min, 55^0C for 50 min and 75^0C for 15 min.

2.3 RT-qPCR

RT-qPCR was performed using an iQ5 qPCR thermocycler (Bio-Rad, Gladesville, NSW, Australia) with SYBR Green mastermix (Bio-Rad). Amplification of each sample was performed in triplicate in 96-well plates (Bio-Rad) with each individual reaction containing 2 µL template cDNA, 12.5 µL SYBR Green mastermix, 12.5 pmol each of sense and anti-sense primers and RNase-free water to a total volume of 25 µL. The sequences and source of primers are displayed in Table 1. Thermal cycling conditions for IGF-I and IGFBP-1 were identical to those described by Pedroso *et al*. (2009). Thermal cycling conditions for the reference gene (18S rRNA) were as follows: initial denaturation at 50 ^{o}C for 2 min followed by 35 cycles of 10 s at 95 ^{o}C, 30 s at 55 ^{o}C and 30 s at 72 ^{o}C. A temperature gradient from 55 ^{o}C to 95 ^{o}C and subsequent dissociation curve was used to confirm the specificity of each RT-qPCR. No template and minus reverse transcriptase controls were also run to confirm there were no contaminants present in reactions and to confirm the efficiency of the DNase reactions.

201 *2.4 Normalisation and Statistical Analysis*

202

203 A serial dilution of cDNA was run for each set of primers to generate a
204 standard curve. The amplification efficiency (E) of the RT-qPCR was
205 calculated using the formula ($E = 10^{-1 / slope}$). Threshold cycle (Ct) values for
206 both IGF-I and IGFBP-1 were normalised to 18S rRNA using the Q gene
207 method (Simon, 2003). Statistical tests were performed using PASW v. 18.0.1
208 (SPSS Inc., Chicago, IL, USA). Final fish weight and HSI were analysed using
209 a one-way ANOVA. After a natural logarithm transformation of normalised
210 gene expression data from the higher temperature experiment (22 ^{o}C) and a
211 cube-root transformation of normalised gene expression data from the lower
212 temperature experiment (18 ^{o}C), all variables met the requirements for a one-
213 way ANOVA. Significant ANOVA's were followed by *a posteriori* Student
214 Newman-Keuls test. Linear regression was used to assess the relationship
215 between weight and hepatic gene expression. Pearson's correlation co-
216 efficient was used to assess the relationship of IGF-I to IGFBP-1. Results
217 were considered statistically significant at $P \leq 0.05$.

218

219 **3. Results**

220

221 *3.1 Effect of alternative lipids on growth and gene expression*

222

223 The inclusion of alternative lipids in diets had a significant effect on
224 growth at both 22 ^{o}C and 18 ^{o}C (Figure 1, A and B; $P < 0.05$). Fish fed the
225 50% PO diet at 22 ^{o}C obtained the highest weight, growing from 95.6 ± 0.01 g

226 to 281.2 ± 1.2 g in 30 d, compared with the 100% FO (266.9 ± 5.9 g; Figure
227 1A). The 100% CO diet resulted in significantly poorer growth at both 22 ^{o}C
228 and 18 ^{o}C (Figure 1, A and B). Mortalities were negligible for the duration of
229 both experiments.
230 Inclusion of alternative lipids also had a significant effect on mean HSI
231 (data not shown). Fish fed on diets containing no added fish oil (100% PO and
232 100% CO) had significantly greater mean HSI at both 22 ^{o}C and 18 ^{o}C (P <
233 0.05; data not shown).
234 Expression of hepatic IGF-I in yellowtail kingfish at 22 ^{o}C showed an
235 increasing trend in fish fed the 100% FO, 50% PO and 100% PO diets after
236 30 d when compared with Time 0 (Figure 2A; P = 0.16). Similarly for IGFBP-1
237 there was a trend towards increased expression in the 50%PO and 100% PO
238 diets at 22 ^{o}C (Figure 3A; P = 0.42). However, no significant differences were
239 found between treatments for either IGF-I or IGFBP-1 at 22 ^{o}C.
240 Hepatic IGF-I in fish cultured at 18 ^{o}C was significantly greater in fish fed
241 the 100% FO and 50% CO diets compared with Time 0 (Figure 2B; P < 0.05).
242 There was an increasing trend in expression of hepatic IGF-I in all
243 experimental fish grown at 18 ^{o}C when compared with IGF-I expression at
244 Time 0 (Figure 2B). Similarly, expression of hepatic IGFBP-1 showed a trend
245 towards increasing in all dietary treatments compared with Time 0 at 18 ^{o}C.
246 However, there were no significant differences between diets (Figure 3B; P =
247 0.12).
248 A positive linear relationship between expression of IGF-I and final
249 weight (g) was found (Figure 4A; P < 0.05, r^2 = 0.514). Similarly, a positive
250 linear relationship was found between IGFBP-1 and final weight (g) (Figure 4B;

251 $P < 0.05$, $r^2 = 0.398$). A positive correlation was found between hepatic
252 IGFBP-1 and IGF-I expression (Figure 5; $P < 0.05$, r = 0.821).
253
254 *3.2 Effect of temperature on growth and gene expression*
255
256 Irrespective of diet, fish grew larger at 22 ^{o}C than at 18 ^{o}C (Figure 1).
257 Fish fed the 50% PO diet grew significantly larger than all other dietary
258 treatments when cultured at 22 ^{o}C but not at 18 ^{o}C. HSI was lower for all
259 dietary treatments at 18 ^{o}C than at 22 ^{o}C after 33 d and 34 d respectively
260 (data not shown).
261 Temperature, while having a profound effect on growth also had an
262 effect on hepatic gene expression. This was particularly evident in the 18^{o}C
263 trial, where hepatic gene expression for all dietary treatments was elevated
264 compared to Time 0. Significant differences for hepatic IGF-I were found at 18
265 ^{o}C ($p < 0.05$). Elevated IGF-I and IGFBP-1 expression for the 100% FO, 50%
266 PO and 100% PO diets at 22 ^{o}C were observed (Figures 2A and 3A). Similar
267 to weight gain at both 18 ^{o}C and 22 ^{o}C (Figure 1), hepatic IGF-I and IGFBP-1
268 for the 100% CO diet had the lowest expression levels of all the dietary
269 treatments. Contrary to other trends in gene expression and growth, both IGF-
270 I and IGFBP-1 were found to be more highly expressed at 18 ^{o}C than at 22 ^{o}C
271 for the 50% CO diet.
272

4. Discussion

The results obtained from this study indicate that up to 100% of added FO may be substituted with PO in diets for yellowtail kingfish without negatively affecting growth. Furthermore, growth was enhanced at 22 ^{o}C when 50% of the dietary lipid source was PO (Figure 1). Several other studies have also reported no negative effects on growth using partial or complete substitution of FO with plant or animal sources for marine, carnivorous fish, including the closely related Japanese yellowtail (Mugrditchian, et al., 1981; Raso and Anderson, 2003; Liu, et al., 2004; Wonnacott, et al., 2004; Higgs, et al., 2006; Xue, et al., 2006; Benedito-Palos, et al., 2007; Piedecausa, et al., 2007; Deslauriers and Rideout, 2008; Seno-O, et al., 2008; Salze, et al., 2010; Welch, et al., 2010). However, this is the first time that a positive effect on growth by partial substitution with an alternative lipid source (PO) has been reported for a marine, carnivorous teleost.

This study also demonstrates that up to 50% of included FO may be substituted with 50% refined CO without negatively affecting growth. At 100% inclusion, however, CO negatively affected growth at both 18 ^{o}C and 22 ^{o}C. Glencross *et al*., (2003) reported a negative effect on growth for total FO substitution with crude CO for red seabream (*Pagrus auratus*). However, total inclusion of refined CO in this species produced comparable weight gain to fish fed the 100% FO diet in this study. Huang *et al*., (2008) also report no negative effects on growth when refined CO was used to substitute fish oil for juvenile Chinook salmon (*Oncorhynchus tschawytscha*) at up to 72% of total dietary lipids. Therefore, while partial inclusion of refined CO produced

298 comparable growth to a FO diet for yellowtail kingfish, this species could not
299 tolerate total inclusion of this lipid source.
300 Irrespective of diet fish grew larger at 22 ^{o}C than at 18 ^{o}C. Pirozzi and
301 Booth, (2009), demonstrated that the optimal temperature for growth of
302 yellowtail kingfish is close to 22 ^{o}C. Masumoto (2002) reports that below 17 ^{o}C,
303 Japanese yellowtail reduce their feed intake, which results in reduced growth
304 performance. The results obtained for gene expression and growth from this
305 study demonstrate that temperature is a critical factor in the ability of fish to
306 metabolise nutrients. The influence of temperature, therefore, needs to be
307 taken into account when formulating practical diets for marine finfish.
308 It has been suggested that IGF-I is the most promising molecular
309 marker to date as a rapid indicator of growth in teleost fish (Picha, et al., 2008).
310 More recently, the use of RT-qPCR to detect IGF-I mRNA has taken
311 precedence over previous technologies used, such as radioimmunoassay
312 (RIA). The vast majority of research investigating hepatic IGF-I in relation to
313 growth in cultured finfish has centred around studies on feed deprivation or
314 feed restriction, rather than the manipulation of a selected dietary component
315 such as protein or lipid (Picha, et al., 2006; Bower, et al., 2008; Hagen, et al.,
316 2009). This may be due to a number of factors, including the expense and
317 resources involved in formulating experimental diets and running large-scale,
318 commercially relevant feeding trials. Nevertheless, the field of nutrigenomics
319 in aquaculture is incorporating an increasing number of species and is
320 assisting the aquafeed industry to achieve optimal dietary formulations
321 through an improved understanding of nutrient metabolism.

Investigating the potential of IGF-I and IGFBP-1 to detect changes in growth using alternative lipids is a unique approach using a familiar and highly-studied growth pathway. The highest expression of hepatic IGF-I was found in fish fed the 50% PO diet at 22 ^{o}C. Although the result was not significant due to variations in individual fish, this trend reflects the mean final weight obtained for fish from this same treatment. This trend towards increasing gene expression at 22 ^{o}C was seen for 50% and 100% PO diets and for both IGF-I and IGFBP-1. Hepatic IGF-I and IGFBP-1 were least expressed in fish fed diets containing 100% CO at 22 ^{o}C, also reflecting trends in final weight gain. The effect of lipid source on hepatic IGF-I and IGFBP-1 production appears to be less pronounced than the effect seen in feed deprivation studies (Picha, et al., 2006; Terova, et al., 2007; Bower, et al., 2008; Hagen, et al., 2009; Pedroso, et al., 2009). Although the effect of lipid source on hepatic IGF-I production is subtle, this study has shown that there is a positive relationship between IGF-I and growth. The relationship between individual macro-nutrients and gene expression has recently been incorporated into an entirely new field of research entitled nutrigenomics. Future research on the influence of different macro-nutrients on components of the somatotropic axis hormone system may lead to the development of more effective dietary formulations for yellowtail kingfish.

Linear regression revealed a positive relationship between both hepatic gene expression and weight gain in response to altered dietary lipid composition. Furthermore, we demonstrated a positive correlation between IGF-I and IGFBP-1. Cruz *et al*., (2006) demonstrated that hepatic IGF-I mRNA was significantly correlated with growth rate for Nile tilapia (*Oreochromis*

niloticus) cultured at different temperatures and using different feeding regimes. Pedroso *et al*., (2009) also demonstrated that both IGF-I and IGFBP-1 mRNA levels were indicative of the nutritional status in the congeneric Japanese yellowtail. It can therefore be concluded that IGF-I and IGFBP-1 would be suitable molecular markers for giving a rapid indication of growth in nutritional studies involving yellowtail kingfish.

Future research in nutritional studies on yellowtail kingfish will help to further elucidate the subtle effects of macronutrients on genes involved in growth. With the decreasing price of sequencing, more sophisticated molecular tools such as microarray and sequence tag-based technology, which are already used in more established species such as salmonids, may soon become available to researchers investigating less established species, including yellowtail kingfish. The use of these technologies and validation with RT-qPCR will ultimately lead to a more thorough understanding of both the IGF system and of fish nutrition.

5. Conclusions

In conclusion, this study has demonstrated the efficacy of including PO and CO in extruded diets for yellowtail kingfish. When fed a diet containing 50% added PO at 22 ^{o}C, yellowtail kingfish grew significantly larger than fish fed the 100% FO diet, demonstrating that alternative lipids may enhance growth for a marine, carnivorous teleost. At 100% inclusion, CO produced poor performance at both 22 ^{o}C and 18 ^{o}C. Irrespective of diet, yellowtail kingfish grew larger at 22 ^{o}C than at 18 ^{o}C. Using linear regression, a positive

372 relationship between hepatic IGF-I and final weight was established, indicating
373 that this gene would be a suitable molecular marker for rapid indication of
374 growth in yellowtail kingfish.
375

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Figure Captions

Figure 1: Mean initial (Time 0) and final weight of yellowtail kingfish fed 100% fish oil (FO) and fish oil replacement with 50% poultry fat (PO), 100% PO, 50% canola oil (CO) and 100% CO for 34 d at 22 ^{o}C (A) and 33 d at 18 ^{o}C (B) . Values represent mean ± S.E.M (n=3). Significantly different values are labelled a, b and c ($P < 0.05$).

Figure 2: Mean initial (Time 0) and final IGF-I expression normalised to 18S rRNA of yellowtail kingfish fed 100% fish oil (FO) and fish oil replacement with 50% poultry fat (PO), 100% PO, 50% canola oil (CO) and 100% CO for 34 d at 22^{o}C (A) and 33 d at 18 ^{o}C (B). Values represent mean ± S.E.M (n=3) and each sample was assayed in triplicate.

Figure 3: Mean initial (Time 0) and final IGFBP-1 expression normalised to 18S rRNA of yellowtail kingfish fed 100% fish oil (FO) and fish oil replacement with 50% poultry fat (PO), 100% PO, 50% canola oil (CO) and 100% CO for 34 d at 22^{o}C (A) and 33 d at 18 ^{o}C (B). Values represent mean ± S.E.M (n=3) and each sample was assayed in triplicate.

Figure 4: Positive linear relationship between yellowtail kingfish weight (g) and IGF-I expression (A: $r^2 = 0.51$, $P < 0.05$) and IGFBP-1 expression (B: $r^2 = 0.40$, $P < 0.05$). Gene expression was normalised to 18S rRNA and each sample was assayed in triplicate. The equations of the linear regression are $y = 3\times10^{-5} x – 0.0015$ (A) and $y = 3\times10^{-5} x – 0.0020$ (B).

Figure 5: Positive linear relationship between IGFBP-1 and IGF-I expression in yellowtail kingfish fed alternative lipids twice daily to satiation (r^2 = 0.67, P < 0.05). Data is derived from means collected during both 22 ^{o}C and 18 ^{o}C trials. The equation of the line is y = 0.8561x – 0.0004.

603 **Figures**

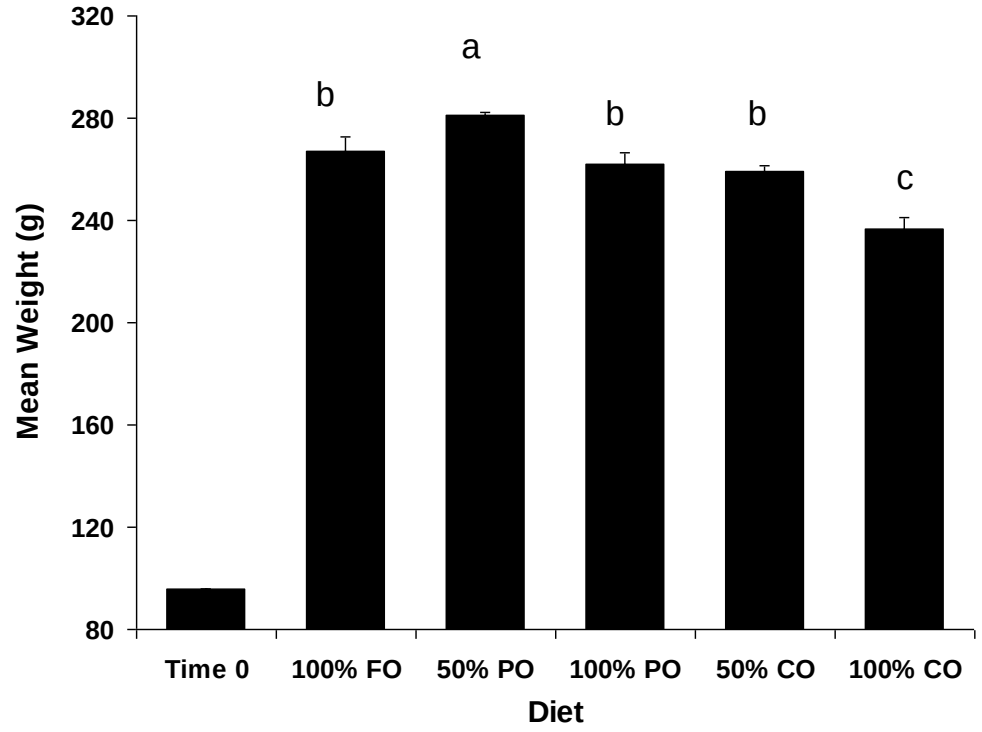


Fig. 1A.

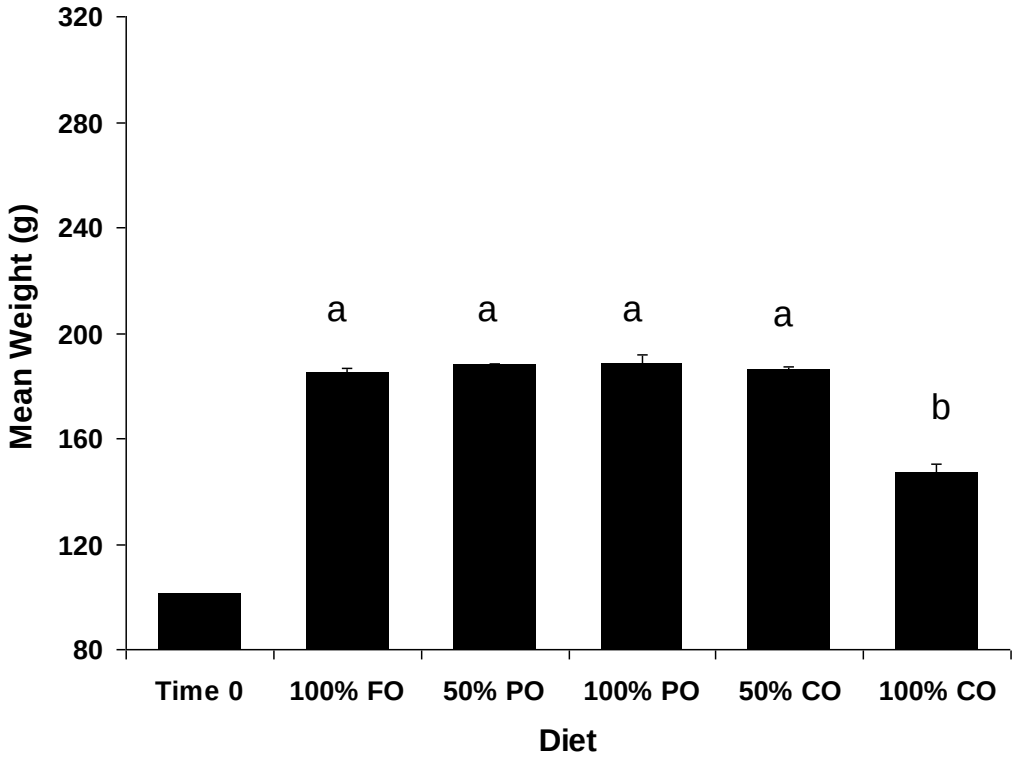
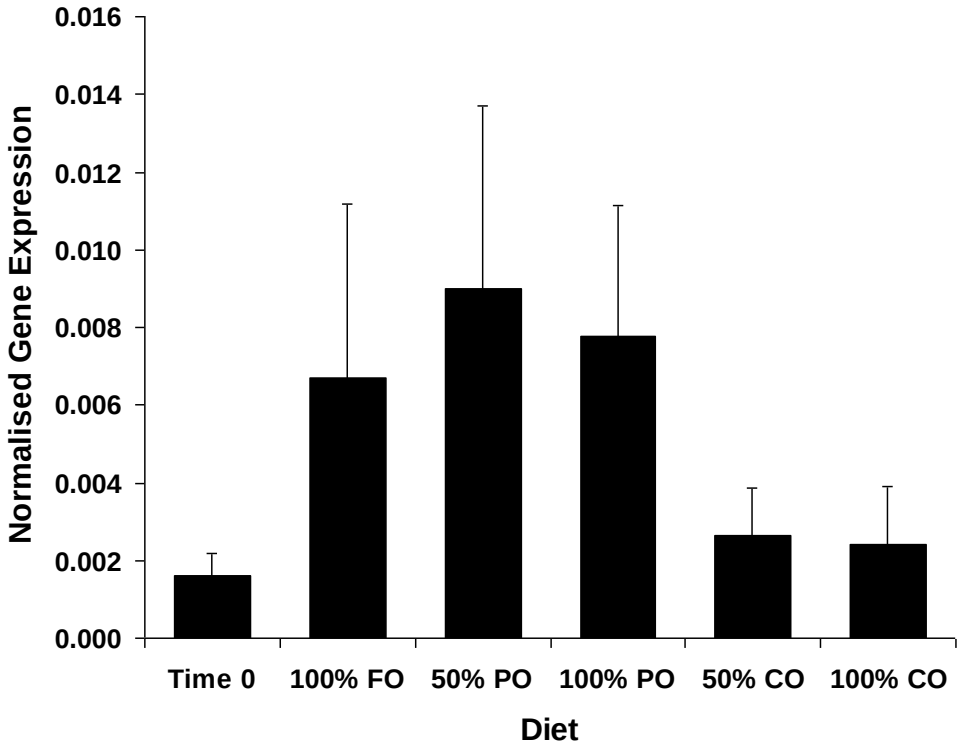


Fig. 1B.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Normalised Gene Expression
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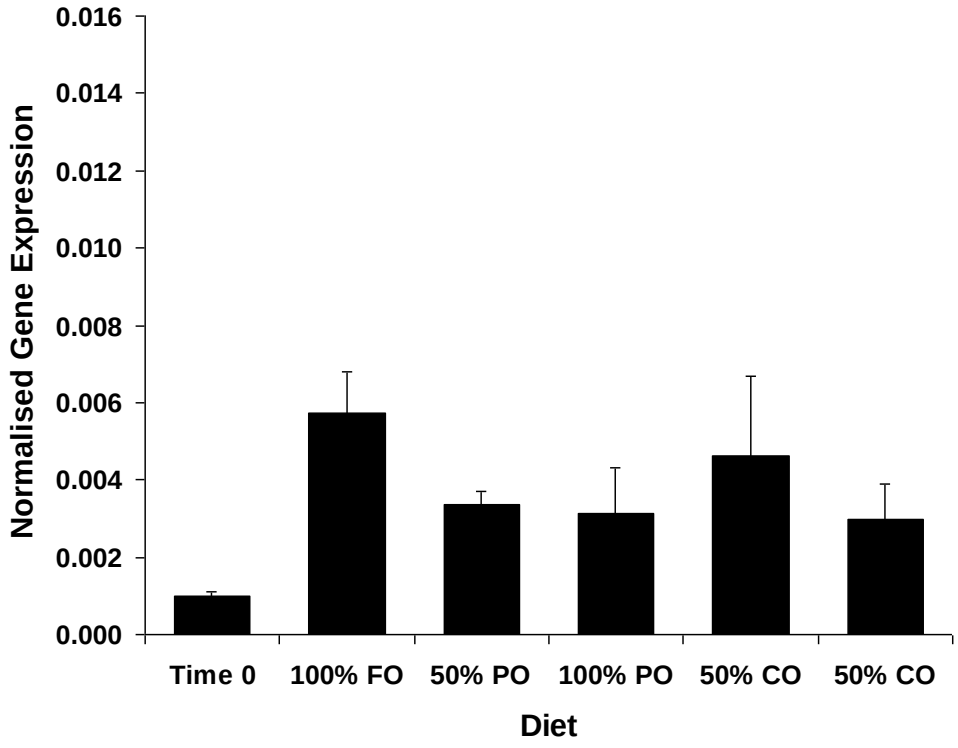
50% PO
100% PO
50% CO
100% CO
Diet

FIG. 2A.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50% CO
50% CO
Diet

FIG. 2B.

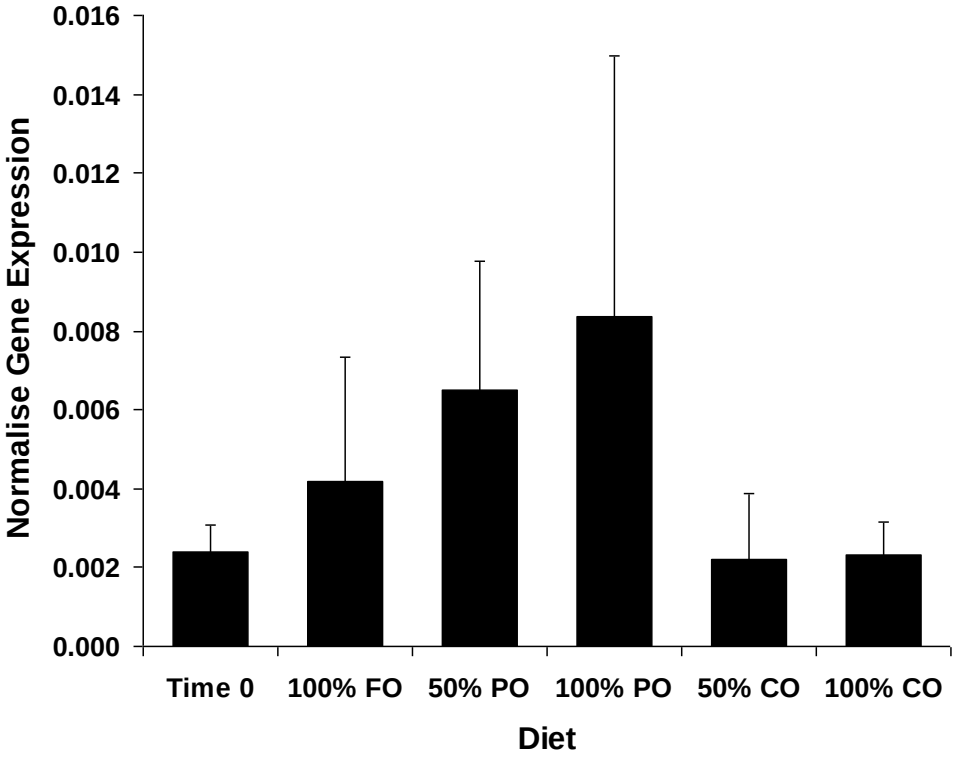
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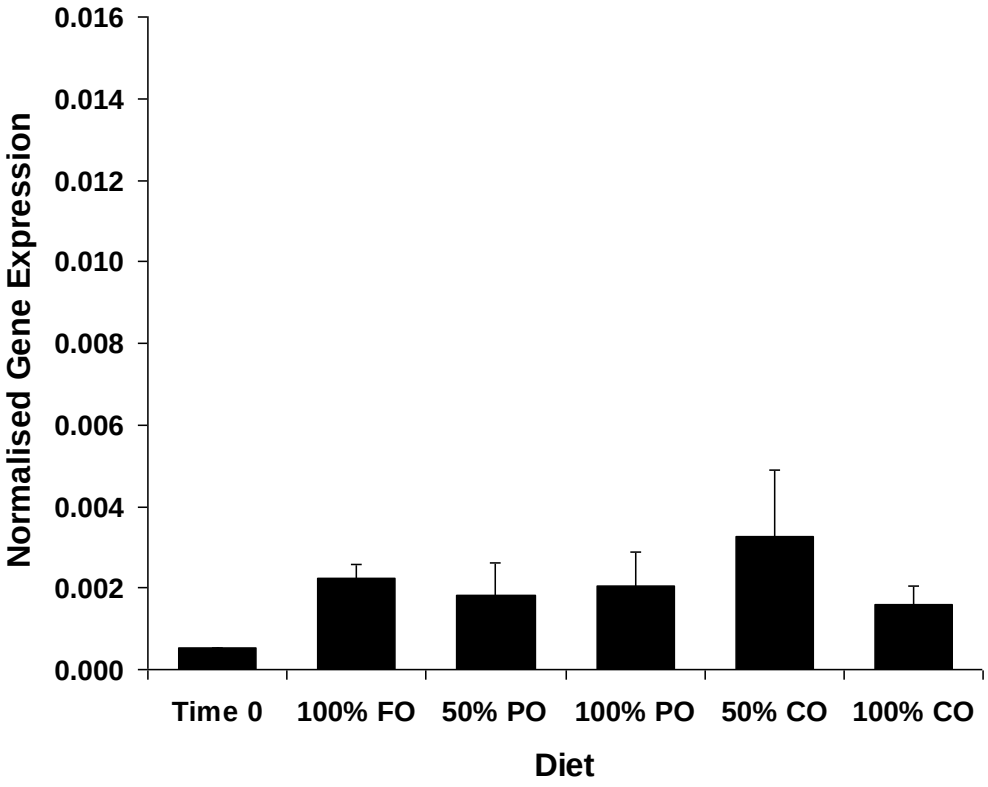
FIG. 3A.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50% CO
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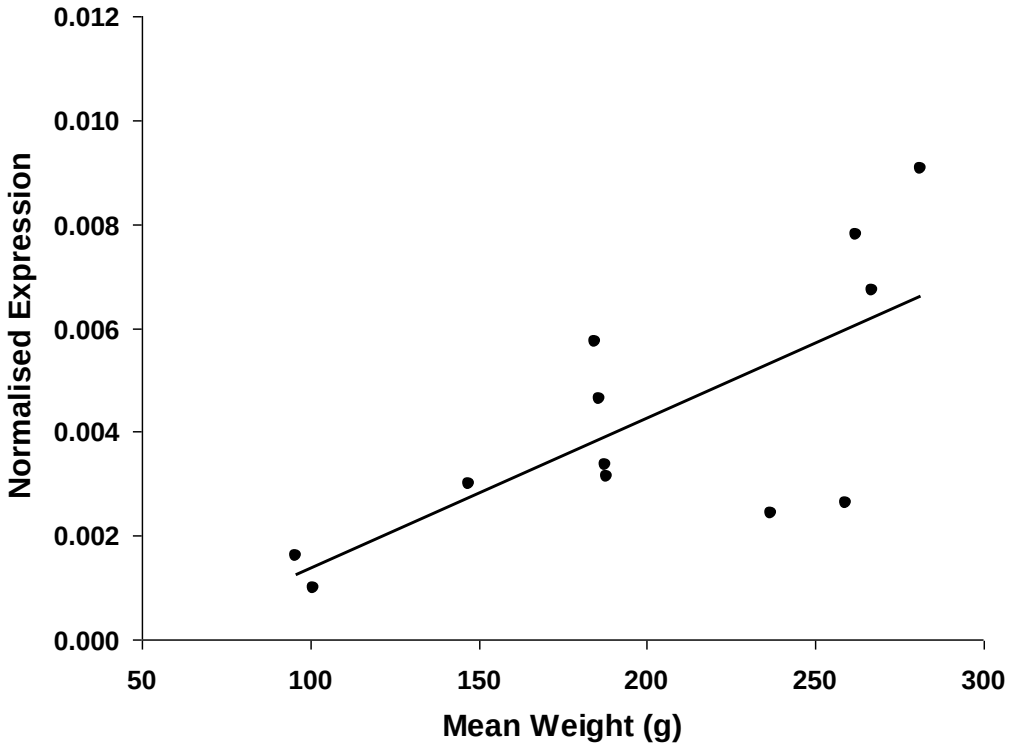
Diet

FIG. 3B.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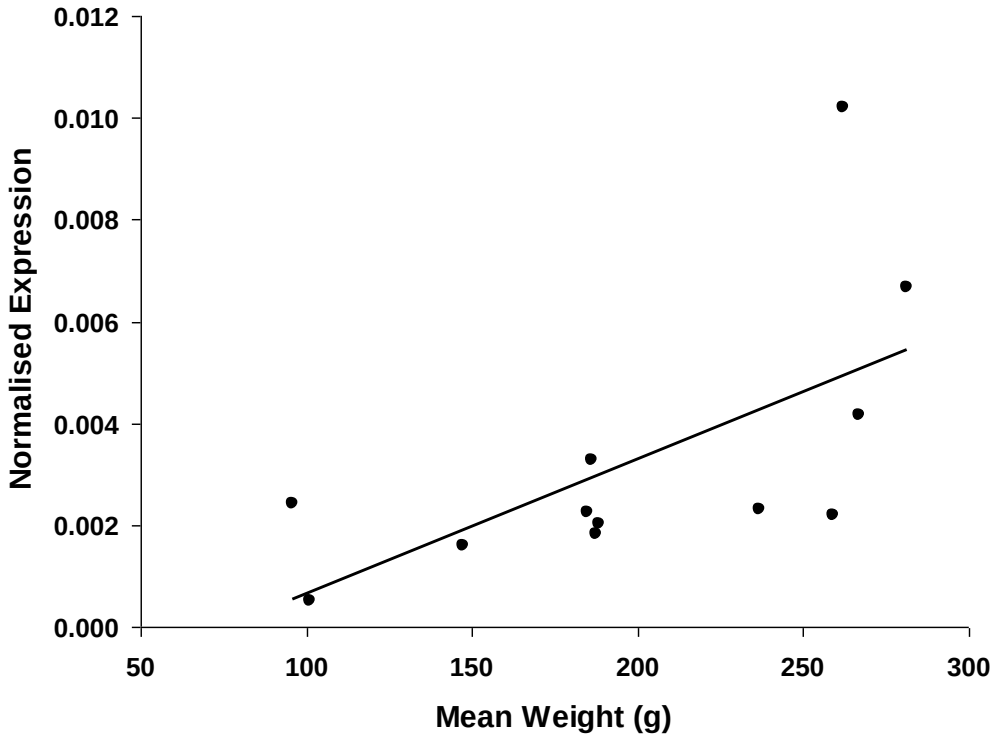
Mean Weight (g)

FIG. 4A.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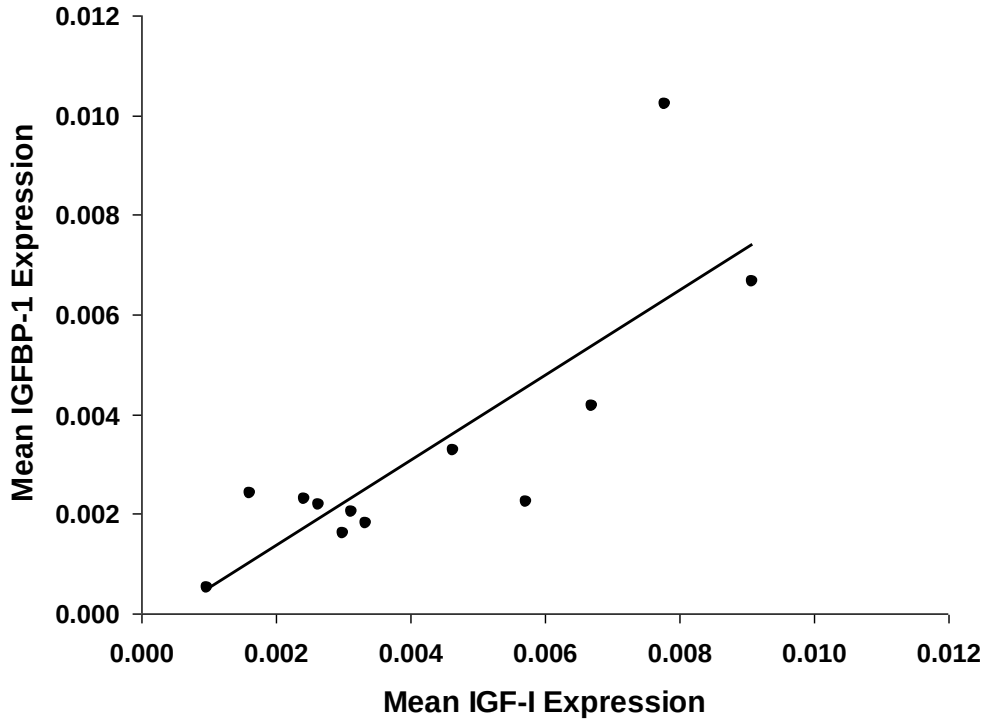
Mean Weight (g)

FIG. 4B.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Mean IGF-I Expression

FIG. 5.

Tables

Table 1: Forward (F) and reverse (R) primers used for real-time quantitative RT-PCR

Gene	Primer Sequence: 5' - 3'	Genbank Accession Number	Reference
IGF-I F	GATGTCTTCAAGAGTGCGATGTG	AB439208	Pedroso *et al*. (2009)
IGF-I R	CCGTCGGAGTCAGGGTGAGG	AB439208	Pedroso *et al*. (2009)
IGFBP-1 F	CCCTTTGACCACCATGACACT	EU650626	Pedroso *et al*. (2009)
IGFBP-1 R	GGGTCCCTGTTGTTCCAGTTT	EU650626	Pedroso *et al*. (2009)
18S rRNA F	TACCACATCCAAAGAAGGCA		Tom *et al.*(2004)
18S rRNA R	TCGATCCCGAGATCCAACTA		Tom *et al.*(2004)

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Aquaculture

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