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#### Short communication

# Optimal dietary ration for juvenile pigfish, *Orthopristis chrysoptera*, grow-out



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#### ABSTRACT

Pigfish (*Orthopristis chrysoptera*) have been identified as a good candidate for marine baitfish aquaculture. Initial research on the species has focused on captive spawning and larval rearing, but optimizing juvenile grow-out is also essential for economical production. We conducted an experiment to determine the optimal ration ( $R_{\rm opt}$ ) for maximizing growth rate while minimizing size variability and overfeeding. We measured total length (TL), wet weight (WW), specific growth rate (SGR), gross feed conversion efficiency (GFCE), and survival of juvenile pigfish (initial size:  $2.6 \pm 0.4$  cm TL) using six ration levels ( $4.8, 12, 16, 20, \text{ or } 24\% \text{ WW d}^{-1}$ ) for four weeks at  $24.7 \pm 0.2$  °C. Final size (TL and WW) increased with increasing ration at lower rations, reaching a plateau at intermediate levels ( $8-16\% \text{ WW d}^{-1}$ ). Survival increased with ration from 74% at the lowest ration to a plateau of 96.0% at rations > 10.7% WW d $^{-1}$ . GFCE decreased with increasing ration from 149% to 47%. To identify  $R_{\rm opt}$  and its change with fish size, we modeled SGR as a function of WW, ration, and their interaction and found that  $R_{\rm opt} = 11.19 \cdot \text{WW}^{-0.26}$  ( $R^2 = 0.70, P < 0.05$ ). This equation provides a guide for producing pigfish quickly and efficiently and, with further research on culture requirements, can be used to establish an efficient pigfish grow-out protocol.

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#### 1. Introduction

Pigfish (Orthopristis chrysoptera) is a popular marine baitfish throughout its range from the Atlantic Coast of Massachusetts to Florida and throughout the Gulf of Mexico (Darcy, 1983). Currently, pigfish are supplied to the baitfish industry by trapping from the wild and availability varies seasonally (Darcy, 1983). During times of high demand or seasons when pigfish cannot be trapped, supply is poor and the demand for inshore and offshore live bait is not met (Adams et al., 1998). In Florida and Texas, retail prices for live pigfish are typically \$6 to \$8 per dozen, but can be as much as \$15 per dozen (Adams et al., 1998; DiMaggio et al., 2013). Preferred bait size ranges from 6 to 11 cm, which corresponds to young-of-the-year that are available from May-August in Texas (Darcy, 1983). Demand for the fish, its market value, and relatively small length at market, as well as the economic pressure pushing U.S. aquaculture to expand into new markets, have led some to advocate for pigfish aquaculture (Cassiano et al., 2010; DiMaggio et al., 2013; Oesterling et al., 2004).

Pigfish have been spawned in captivity through either photoperiod and temperature control or hormone induction (DiMaggio et al., 2013;

Ohs et al., 2011). DiMaggio et al. (2013) hatched pigfish from captive spawned eggs and raised the larvae through metamorphosis to 30 d posthatch (1.5 cm total length [TL]) using a feeding regime that included copepods (*Pseudodiaptomus pelagicus*), rotifers (*Brachionus plicatilis*), and *Artemia*. DiMaggio et al. (2014) examined the effects of stocking density on juvenile pigfish. But, a feeding regime to grow pigfish from early juveniles to market size (6–11 cm TL) has not been established.

Determining optimal ration ( $R_{\rm opt}$ ) for juvenile grow-out is the next step in developing pigfish aquaculture. Identifying  $R_{\rm opt}$  enables producers to grow healthy fish as quickly and/or efficiently as desired and using  $R_{\rm opt}$  avoids overfeeding, which is costly and reduces water quality (Puvanendran et al., 2003; Sumagaysay, 1998). In this study, we used a range of daily rations (% wet weight [WW] d<sup>-1</sup>) of a dry pelleted feed to grow pigfish from early juvenile (2.6 cm TL) for four weeks to determine  $R_{\rm opt}$  by evaluating growth rate, survival, size variability, and gross feed conversion efficiency. Optimal ration is expected to be proportional to mass-specific metabolic rate, so we expected that the optimum ration would decrease with size according to a power function with an exponent of -0.25.

#### 2. Materials and methods

Pigfish were raised from eggs spawned by broodstock (n = 15) that were induced to spawn by photoperiod and temperature manipulation. Larvae were reared under controlled salinity (36.1  $\pm$  0.6 ppt),

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temperature (20.0  $\pm$  0.7 °C), and photoperiod (10 h light: 14 h dark) conditions at the Fisheries and Mariculture Laboratory of the University of Texas Marine Science Institute. Eggs were collected on mornings following evening spawning events and placed (5–10 eggs ml $^{-1}$ ) into a recirculating aquaculture system consisting of six 265-l light blue round tanks partially submerged in a 4500-l rectangular tank. The recirculating system was configured so that water was pumped from the outer rectangular tank through a bio-filter, protein skimmer, sand filter, and heat pump into the round tanks from which water flowed back into the rectangular tank through a standpipe in the center of each round tank.

Hatched larvae were reared in the round tanks using green water culture (with Isochrysis galbana, 60,000 cells ml<sup>-1</sup>) and feedings of rotifers (B. plicatilis, 7 ml<sup>-1</sup>) from 3 to 21 days posthatch (dph). To maintain live algae and rotifer concentrations, water flow was suspended throughout the rotifer feeding period. From 15 to 19 dph, newly hatched Artemia nauplii (Great Salt Lake-origin, Brine Shrimp Direct, Ogden, UT, USA) were added to the tanks once daily  $(0.1 \text{ ml}^{-1})$ . From 19 to 30 dph, enriched Artemia (Algamac 3050, Aqua-fauna Bio-Marine, Hawthorn, CA, USA) were fed and water flow was gradually increased from 1 l min<sup>-1</sup> to 3 l min<sup>-1</sup>. Feeding levels were increased as larvae grew and consumed more prey, eventually reaching 0.4 ml<sup>-1</sup> of enriched Artemia fed three times daily. This feeding regime insured a constant presence of enriched live food. At 30 dph, fish were weaned onto a 250-µm microdiet (52% crude protein; Otohime, Reed Mariculture, Campbell, CA, USA); granule size was gradually increased to 840-1410 µm as the fish grew. Temperature, salinity, and dissolved oxygen were recorded throughout larval rearing (YSI Inc., Yellow Springs, OH, USA) and maintained at 20.7  $\pm$  0.3 °C, 36.0  $\pm$  0.7 ppt, and >6.0 mg l<sup>-1</sup>, respectively. Photoperiod was 10 h light and 14 h dark throughout larval rearing. Ammonia, nitrite, and nitrate levels were monitored using API and Hach test kits (Pentair AES, Apopka, FL, USA) and remained below detectable limits throughout larval rearing.

#### 2.1. Ration study

The study began when individuals were approximately 2.6 cm TL and weighed 0.4 g WW. At that time, 150 randomly selected fish were placed into each of six dark blue round tanks (250 l) partially submerged within a 4500 l rectangular tank (as described above for larval rearing). Each tank of juvenile pigfish was fed one of six daily rations: 4, 8, 12, 16, 20, or 24% WW  $d^{-1}$ . Automatic feeders (Lifegard Aquatics, Cerritos, CA, USA) dispensed daily rations over the 14 h that lights were on. The initial diet contained 52% crude protein and 11% crude fat in 840–1410-um granules (Otohime, Reed Mariculture, Campbell, CA, USA). When mean WW exceeded 1.5 g, a larger (1.7-mm) extruded pellet composed of 48% crude protein and 14% crude fat (Otohime) was used. The experiment lasted 4 weeks and was replicated three times. Each replicate consisted of juveniles from a different spawn. Temperature, salinity, and photoperiod were 24.7  $\pm$  0.2 °C, 35.3  $\pm$  0.6 ppt, and 14 h light and 10 h dark, respectively, with water flowing into each round tank at 3 l min $^{-1}$ . Temperature (HOBO Pendant® temperature loggers; Onset Computer Corporation, Bourne, MA, USA), dissolved oxygen (5.7  $\pm$  0.02 mg l<sup>-1</sup>), and salinity were monitored daily; nitrogen compounds (nitrate, nitrite, and ammonia; <10, 0.3  $\pm$  0.1, and 0.4  $\pm$ 0.0 ppm, respectively) twice weekly; and pH (7.7  $\pm$  0.1) once weekly. Uneaten food was siphoned from the tanks daily or as needed.

Fish were randomly selected and weighed on a balance (MXX-612; Denver Instruments, Bohemia, NY, USA) in a beaker of sea water immediately before starting each replicate and weekly from each tank thereafter. After weighing, fish were placed onto a mesh screen with a ruler and a digital photograph was taken for subsequent measurement of TL using image analysis (ImageJ, National Institute of Health, Bethesda, MD, USA). Fish were then returned to their tanks. Feed amounts were adjusted weekly to maintain the nominal ration levels, using the new mean individual WW. After four weeks, the experiment was terminated

and the number of fish remaining in each tank and their final TL and WW were recorded. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Texas at Austin.

#### 2.2. Data analysis

One-way analysis of variance (ANOVA) with six treatment levels was performed, followed by post-hoc Tukey's HSD test for comparisons of final sizes (TL and WW) among rations. The relationship between SGR and WW was modeled as an exponential function for each ration, so daily specific growth rate (SGR, % d<sup>-1</sup>) was calculated weekly for each ration as:  $SGR = (ln(WW_t) - ln(WW_{t-1})) / 7 \cdot 100$ , where ln is the natural logarithm, WWt is the average WW for a given week, WW<sub>t-1</sub> is the average WW for the preceding week, and 7 is the number of days between t and t-1 (Ricker, 1979). Gross feed conversion efficiency (GFCE) over the entire experiment was calculated for each ration as the change in WW divided by total dry weight of feed delivered to the tank (Stickney, 2005). Since GFCE increases with ration to a point, then decreases as ration exceeds maximum intake, a second order polynomial (quadratic function) was used to model the relationship between GFCE and ration (Khan and Abidi, 2010; Zeitoun et al., 1976). Survival was high for most rations, but decreased linearly with decreasing ration when levels became limiting. Therefore, a piecewise regression was used to relate survival to ration and to determine the minimum ration for maximal survival. The effect of ration on size variability was examined using the standard deviation of the logtransformed final TL and log-transformed final WW for each treatment group as a scale-independent measure of variability (Jobling, 1983; Lewontin, 1966). In all analyses P < 0.05 was considered statistically

To derive an expression that defines  $R_{\rm opt}$  as the minimum ration that maximizes SGR for a given WW while accounting for a change in  $R_{\rm opt}$  with WW, SGR was modeled as a function of WW, ration, and their product (an interaction term):

$$\label{eq:SGR} \begin{aligned} \text{SGR} = \begin{cases} \left(k_1 \cdot e^{k_2 \cdot WW}\right) + (k_3 + k_4 \cdot R) + \left(\left(k_1 \cdot e^{k_2 \cdot WW}\right) \cdot (k_3 + k_4 \cdot R)\right), & \text{for } R < k_5 \cdot WW^{k_6} \\ \left(k_1 \cdot e^{k_2 \cdot WW}\right) + \left(k_3 + k_4 \cdot k_5 \cdot WW^{k_6}\right) + \left(\left(k_1 \cdot e^{k_2 \cdot WW}\right) \cdot \left(k_3 + k_4 \cdot k_5 \cdot WW^{k_6}\right)\right), & \text{for } R \geq k_5 \cdot WW^{k_6} \end{cases} \end{aligned}$$

where,  $k_1$ ,  $k_2$ ,  $k_3$ ,  $k_4$ ,  $k_5$ , and  $k_6$  are empirical constants solved iteratively by nonlinear regression, R is ration, and e is the base of the natural logarithm. This model is the embodiment of the complex interaction among growth rate, size, and ration alluded to by Brett and Shelbourn (1975). The model defines SGR as the sum of the exponential relationship between SGR and WW  $\left(k_1 \cdot e^{k_2 \cdot \text{WW}}\right)$ , plus the linear relationship between SGR and R  $\left(k_3 + k_4 \cdot \text{R}\right)$ , plus the interaction (product) of these two relationships for cases where R < R<sub>opt</sub>. When R exceeds the R<sub>opt</sub>, excess feed goes uneaten and SGR remains constant and equal to the value of SGR at R<sub>opt</sub>. This model allows R<sub>opt</sub> to vary with WW according to a power function:

$$R_{opt} = k_5 \cdot WW^{k_6} \tag{2}$$

as scaling and bioenergetics theory predicts, assuming R<sub>opt</sub> is proportional to mass-specific metabolic rate (Jobling, 1983; Kitchell et al., 1977; Kleiber, 1947). Statistical analyses were completed using R software version 3.0.0 (R Development Core Team, 2014).

#### 3. Results

Pigfish increased in length by 2.0–2.5 times and in weight by 6–14 times over the 28-day experiment (Table 1). Final TL and WW in the 4% ration were statistically different from all other rations, but there

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