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A little goes long way: Improved growth in Atlantic cod (*Gadus morhua*) fed small amounts of wild zooplankton

T. Katan [a,1], G.W. Nash [a], M.L. Rise [a], J.R. Hall [b], J.M.O. Fernandes [c], D. Boyce [a], C.A. Johnsen [c], A.K. Gamperl [a,*]

[a] Department of Ocean Sciences, Memorial University of Newfoundland, St. John's, NL A1C 5S7, Canada
[b] Aquatic Research Cluster, CREAIT Network, Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, NL A1C 5S7, Canada
[c] Faculty of Biosciences and Aquaculture, University of Norland, P.O. Box 1490, Bodø, Norway

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ABSTRACT

We examined the effects of partial dietary supplementation with wild zooplankton or fish protein hydrolysate on cod production traits, and how they related to the expression of growth and appetite regulating transcripts and muscle cellularity. Atlantic cod larvae were fed three different diets: enriched rotifers and *Artemia* (RA); RA + fish protein hydrolysate (RA-PH); and RA supplemented with 5–10% wild zooplankton (RA-Zoo). Partial supplementation with zooplankton significantly improved cod dry weight at 60 days post-hatch (by approximately 4-fold), specific growth rate (by 2.5% day^{-1}) and the general development of cod larvae. Although the zooplankton fed cod were still larger at approximately 1.5 years of age, the growth advantage of this group decreased with age (the difference in wet mass decreasing from approximately 30% at 0.5 years old to 11% at 1.5 years old). In contrast, the protein hydrolysate enrichment did not improve growth, had a negative effect on survival, and increased the incidence of external deformities in 1.5 year old fish. The growth enhancement observed in the RA-Zoo larvae was largely unrelated to differences in the transcript levels of several important growth [Insulin-Like Growth Factor 1 (*igf-1*); *igf-2*; Growth Hormone (*gh*); GH Receptor-1 (*ghr-1*); *ghr-2*; and myostatin (*mstn*)] or appetite regulating genes [Cocaine and Amphetamine Regulated Transcript (*cart*) and Neuropeptide Y (*npy*)], but was associated with an increase in the number and size of skeletal muscle fibers. Our findings suggest that incorporating small amounts of wild zooplankton into larval feeding regimes may significantly enhance the production of marine fishes, but that the transcript levels of the above hormones and hormone receptors are not valuable biomarkers of growth in cod larvae.

Statement of relevance: This research shows that small amounts of zooplankton significantly improve the growth of Atlantic cod larvae, and thus, that cultured zooplankton may be a viable and cost-effective strategy for increasing the growth (and potentially health) of intensively cultured marine finfishes.

1. Introduction

Slow growth rates, and a high incidence of mortalities and deformities, have been major impediments to the successful commercialization of Atlantic cod aquaculture (Rosenlund and Halldorsson, 2007; Rosenlund and Skretting, 2006). Thus, many in the cod aquaculture industry believe diets must be further optimized, and have identified diet development as a research priority (Rosenlund and Halldorsson, 2007).

Recent studies show that feeding intensively cultured Atlantic cod (*Gadus morhua*) with wild zooplankton (copepods), as opposed to enriched rotifers for even a short period, can provide a better scope for growth and general development (i.e., a significantly lower incidence of skeletal deformities) during the larval period (Busch et al., 2010; Imsland et al., 2006; Karlsen et al., 2015; Koedijk et al., 2010). Further, this improved larval growth has been shown to translate into significantly larger juveniles by 20–25% (Busch et al., 2010; Imsland et al., 2006; Koedijk et al., 2010). While the critical window for feeding zooplankton appears to have been identified (from ~20–35 dph; Koedijk et al., 2010), it is still not known whether feeding small amounts of zooplankton will achieve similar results (with regards to larval growth, skeletal deformities and survival) to those seen in earlier studies (Imsland et al., 2006; Karlsen et al., 2015; Koedijk et al., 2010). Such information is important as exclusively feeding zooplankton (copepods) to marine larvae is not currently economically viable on a commercial scale.

The addition of fish protein hydrolysates to larval diets has also been examined as an approach for overcoming the limited digestive capacity and slow growth of fish larvae (Dabrowski, 1984; Govoni et al., 1986; Kaushik, 1995) as these compounds have higher absorption efficiencies as compared to intact protein (Tonheim et al., 2005) and result in improved larval growth, survival (Cahu et al., 1999; Carvalho et al., 2004; Kotzamanis et al., 2007; Zambonino Infante et al., 1997) and skeletal

* Corresponding author.
[1] Present Address: Golden Eagle Sable Fish. 335 Walkers Hook Rd, Salt Spring Island, BC V8K 1N7, Canada.

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development (Cahu et al., 2003; Zambonino Infante et al., 1997). Further, cod fed with pollock (*Pollachius virens*) protein hydrolysate enriched live feeds (rotifers and *Artemia*) are reported to have significantly improved growth, survival (Bjornsdottir et al., 2013) and morphological quality (i.e., reduced deformities) (Johannsdottir et al., 2013) as compared to those provided with a standard rotifer/*Artemia* diet.

Nutritional status (i.e., the quantity and quality of food) can regulate fish growth through direct and indirect effects on the hormones of the GH-IGF system (Ayson et al., 2007; Beckman et al., 2004; Fox et al., 2009; Valente et al., 2013), and subsequently the complex process of myogenesis (Galloway et al., 1999; Ostaszewska et al., 2008; Salze et al., 2014; Valente et al., 2013). It has been shown previously that the improved growth of copepod-fed yellowtail clownfish (*Amphiprion clarkii*) was associated with higher transcript levels of insulin-like growth factors (IGF) I and II and lower levels of myostatin as compared to enriched rotifer-fed clownfish (Olivotto et al., 2008a,b). However, very few studies have investigated the effects of these dietary regimes (i.e., supplementation with wild zooplankton and protein hydrolysate) on the expression of growth and appetite regulating genes, and attempted to develop suitable molecular markers for growth in cod (Kortner et al., 2011; Picha et al., 2008). This knowledge is essential, in order to effectively modify the current commercial enrichments to achieve the growth rates required by the industry.

Consequently, the aim of the present study was to: 1) examine whether partial dietary supplementation with wild zooplankton (5–10% of total prey items) or protein hydrolysate improves Atlantic cod production traits (i.e., growth, survival and the degree / types of deformities) as compared to a traditional rotifer / *Artemia* based diet; and 2) investigate whether any of the observed effects of these diets are associated with changes in the transcript levels of several growth [Insulin-Like Growth Factor 1 (*igf*-1); *igf-2*; Growth Hormone (*gh*); GH Receptor-1 (*ghr-1*); *ghr-2*; and myostatin (*mstn*)] and appetite [(Cocaine and Amphetamine Regulated Transcript (cart) and Neuropeptide Y (*npy*)] regulating genes, and muscle cellularity (growth).

2. Materials and methods

2.1. Live feed

Rotifers (*Brachionus plicatilis*) were supplemented with Ori-Culture (ORI-GO, Skretting, Vervins, France) (0.25–0.35 g million^{-1} rotifers) for 4 days, then further enriched with Ori-Green (ORI-GO, Skretting, Vervins, France) at a concentration of 0.15–0.25 g million^{-1} rotifers for two hours before being fed to the larvae. Eight hours after hatching, the *Artemia* were fed Ori-Culture (Skretting) for 8 h. Then, they were enriched with Ori-Green for 12 h before being fed to the cod larvae. The fish (Pollock; *P. virens*) protein hydrolysate was purchased from IceProtein Ltd. (Iceland) and fed to the rotifers or *Artemia* at a concentration of 0.1 g liter^{-1} for two hours prior to these live feeds being offered to the cod larvae.

The zooplankton was collected from Conception Bay, Newfoundland, using a 100 μm mesh plankton net (1 m in diameter), then passed through a 400 μm mesh filter and counted. The zooplankton collected consisted primarily (>90%) of copepods (*Temora* sp., *Oithona* sp. and *Pseudocalanus* sp.). However, the phytoplankton *Ceratium* was also collected, and could not be separated from the zooplankton.

2.2. Experimental design and larval rearing

All experiments were carried out in accordance with the guidelines of the Canadian Council on Animal Care, and approved by the Institutional Animal Care Committee of Memorial University of Newfoundland (protocol # 11-30-KG).

Eggs for this study were collected from communally spawning broodstock, and had an average diameter of 1.6 mm, a 95% fertilization rate and 91% of them had symmetrical cleavage. At 93.4 degree days (October 19th, 2011) the larvae were transferred to 16, 400 liter flow-through tanks at a density of 50 larvae l^{-1}. These tanks were then divided randomly into three different treatments based on the following feeding regimes / diets (Fig. 1);

2.2.1. Treatment 1 (6 replicate tanks)

Rotifers and *Artemia* that were enriched with Ori-Green (RA); three feedings per day (9:00 AM, 3:00 PM and 9:00 PM). Initial rotifer and *Artemia* prey densities during feeding ranged from 800–9000 and 1200–5400 l^{-1} (depending on larval age), respectively.

2.2.2. Treatment 2 (4 replicate tanks)

Rotifers/*Artemia* with Ori-Green enrichment, supplemented with 5–10% wild caught zooplankton (RA-Zoo). The larval diet was supplemented with zooplankton until 30 days post-hatch (dph) (Fig. 1), and the numbers of rotifers/*Artemia* fed to each tank were reduced according to the amount of zooplankton that was added (~250,000 per feeding). This ensured that the number of prey items was consistent between tanks. The feedings were at 9:00 AM during the first week and at 9:00 AM and 3:00 PM thereafter. At all feedings, the larvae were allowed to feed exclusively on the zooplankton for one hour prior to adding the required number of enriched rotifers or *Artemia*. Gut squashes were conducted periodically, and these confirmed that the larvae were feeding on the zooplankton.

2.2.3. Treatment 3 (6 replicate tanks)

Rotifers/*Artemia* with Ori-Green enrichment four days per week and Rotifers/*Artemia* fed Protein Hydrolysate (RA-PH) three days per week.

All tanks were fed rotifers from 2 days post-hatch (dph) until they reached 9–10 mm in length, *Artemia* from this size to 13 mm, and then weaned onto a commercial micro-diet (Gemma micro W 0.2, Skretting, Vervins, France) over a co-feeding period of 10 days (Fig. 1). Potters clay (400 ml) was added to all tanks, twice a day, to increase water turbidity and reduce bacterial numbers within the tanks (Prickett et al., 2010). Rearing temperature was increased from 6–7 °C (incubation temperature) to 10.5 °C over a period of 10 days, and the flow of oxygenated seawater into the tanks was gradually increased from 0.8 l min^{-1} at 0 dph to 4.5 l min^{-1} at 35 dph.

2.3. Larval sampling

Cod larvae were randomly collected from each tank at 0, 10, 20, 30, 40 and 60 dph, anesthetized in MS-222 (tricaine methane-sulphonate; 0.05 g liter^{-1}; Syndel Laboratories, B.C., Canada), and rinsed in UV sterilized seawater before being processed further.

For measurements of standard length (SL), at least 20 larvae per treatment were individually photographed using a photomicroscope (Wild M420, ON, Canada) connected to a camera (Infinity 2-2c, ON, Canada), and measured from the tip of the snout to the end of the hypurals using a calibrated ocular micrometer. For dry weight (DW) 8–12 samples of between 5 and 20 larvae per treatment were counted into a Millipore glass filter holder apparatus (Fisher Scientific) containing a previously dried and weighed Whatman GF/C glass microfiber filter (VWR International), and the larvae were then rinsed down onto the filter with a small volume of seawater under slight vacuum to remove the liquid and finally rinsed with 5–10 mL isotonic (3%) ammonium formate under slight vacuum. The vacuum dried larvae and filter paper were then transferred to pre-weighed aluminum weigh boats and dried at 80 °C for a minimum of 24 h before being weighed on an analytical balance (Denver Instrument APX-60, Arvada, Co, USA). Percent survival was calculated by subtracting the total number of sampled larvae from the initial number (i.e., at 0 dph) and dividing by the number of fish remaining at the time of grading.