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Prey quality impact on the feeding behavior and lipid composition of winter flounder (*Pseudopleuronectes americanus*) larvae

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ABSTRACT

Copepods are the main natural food of many marine fish larvae. However, enriched rotifers are the most commonly used live prey in larval rearing. Impacts on the feeding behavior, growth, survival, and fatty acid (FA) composition of winter flounder larvae fed with copepods and rotifers were determined and compared to the FA composition of the two live prey. Nauplii of *Eurytemora* spp. and *Acartia* sp., two of the main species of copepods present in the St. Lawrence estuary, showed no significant differences in their essential fatty acid profiles, suggesting similar nutritional quality. Thus, only *Eurytemora herdmani* was compared to enriched rotifers in this study. Copepod nauplii were characterized by higher levels of essential fatty acids, particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The selective incorporation of essential fatty acids from diets in larval tissues (polar lipids) indicated that nauplii might better fulfill larval nutritional requirements for DHA than rotifers. Furthermore, larval behavior was modified according to the diet: larvae fed with nauplii spent more time swimming with no changes in the occurrence of hunting events.

1. Introduction

In nature, it has been demonstrated that marine fish larvae mainly feed on zooplankton, especially copepod nauplii and copepodites (Llopiz, 2013; Robert, Murphy, Jenkins, & Fortier, 2014). However, in marine larval fish rearing, which is the case with winter flounder (*Pseudopleuronectes americanus*), the most common live prey used as a proxy for copepods are rotifers (Fraboulet, Lambert, Tremblay, & Audet, 2011, 2010). Rotifers are easy to produce in high abundance year-round, they are an edible size for many species of fish larvae, and their slow swimming speeds make them easy for fish larvae to capture (Øie, Reitan, Evjermo, Stottrup, & Olsen, 2011). However, the nutritional value of rotifers is generally considered poor for marine fish larvae because of their low levels of essential fatty acids (EFA; Castell et al., 2003).

EFA include eicosapentaenoic acid (20:5n-3, EPA), docosahexaenoic acid (22:6n-3, DHA), and arachidonic acid (20:4n-6, AA), which have been reported to be essential for the optimal growth of several marine fish species whose biosynthetic production is insufficient to meet their nutritional requirements (Glencross, 2009). EFA functions can be divided into two broad areas, with EPA and DHA involved in maintenance of the structural and functional integrity of biological membranes (Hazel, 1995), while AA and EPA act as precursors of eicosanoids, a group of highly biologically active hormones (Howard & Stanley, 1999). Several studies have evaluated different ways to enrich rotifers to obtain the best proportions of essential fatty acids (Castell et al., 2003; Haché & Plante, 2011; Haché, Plante, Forward, & Pernet, 2016; Vagner, de Montgolfier, Sévigny, Tremblay, & Audet, 2014).

The optimization of EFA composition in marine fish feed is complicated by the competitive interaction between EPA and DHA for phospholipid biosynthesis and between AA and EPA for the eicosanoid response (Sargent et al., 1999). Thus, the correct balance of AA, EPA, and DHA with total fatty acid content is important to avoid nutritional deficiency (Glencross, 2009). Seychelles, Audet, Tremblay, Fournier, and Pernet (2009; 2011) and Vagner, de Montgolfier, Sevigny, Tremblay, and Audet (2013; 2014) used different rotifer enrichments with specific proportions of EFA to rear winter flounder larvae. They found that while low levels of

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highly unsaturated fatty acids (HUFA) did not affect growth performance or lipid reserves, an essential combination of EPA, AA, and DHA is required to sustain the up-regulation of growth hormone gene expression throughout larval development and metamorphosis in winter flounder. These authors found that the best combination was an enrichment containing an EPA/DHA/AA ratio of around 4/3/1.

Despite fast growth in winter flounder reared with enriched rotifers, a high larval mortality rate persists at metamorphosis (Audet & Tremblay, 2011). Mercier et al. (2004) suggested that the use of another live prey more representative of the natural environment of larvae could be an interesting hypothesis to test for optimizing larval rearing of winter flounder. Therefore, a new alternative in aquaculture is the use of copepods as live feed, since they are natural prey (Ajiboye, Yakubu, Adams, Olaji, & Nwogu, 2011; Hernandez-Molejon & Alvarez-Lajonchere, 2003; Støttrup, 2003; van der Meeren, Olsen, Hamre, & Fyhn, 2008) and some copepods are exploited successfully in extensive systems in Taiwan and Denmark (Hansen, 2017).

Several studies have established that copepods are naturally richer in EFA than rotifers (Avella, Olivotto, Gioacchini, Maradonna, & Carnevali, 2007; Barroso, de Carvalho, Antoniassi, & Cerqueira, 2013; Støttrup & Norsker, 1997), with higher levels of phospholipids (>50%) (McEvoy, Naess, Bell, & Lie, 1998); this could improve rearing success because this lipid class seems to be more easily assimilated by various fish species (Gisbert, Villeneuve, Zambonino-Infante, Quazuguel, & Cahu, 2005; Tocher, Bendiksen, Campbell, & Bell, 2008). Another important advantage of copepods is that their size varies according to their ontogeny, providing prey of different size ranges for marine fish larvae and juveniles (Lee, O'Bryen, & Marcus, 2005). Shaheen et al. (2001) evaluated the preference of winter flounder larvae for two copepod species (*E. affinis* and *A. hudsonica*) and demonstrated that behavior and morphology of the prey are key factors in selection by fish. However, the relationship between prey preferences and their nutritional value in the early stages of winter flounder remains unknown. Furthermore, knowledge of the biochemical and nutritional value of copepods for early stages of marine fish is fragmentary.

The objectives of this current study were (1) to characterize the composition, abundance, and fatty acid content of adult copepods from a natural environment in order to know their availability and their nutritional value as live prey for winter flounder rearing; (2) to compare the nutritional value of copepod nauplii and enriched rotifers with the larval rearing success of winter flounder; and (3) to analyze and compare the feeding behavior (swimming, resting, and hunting) of larvae fed with copepod nauplii or rotifers. We hypothesized that copepod nauplii would be more preyed upon and more appropriate to meet nutritional needs. This study highlights new knowledge of the nutritional needs and feeding behavior of winter flounder larvae.

2. Material and methods

2.1. Copepod sampling

Three stations were sampled in the St. Lawrence estuary (Rivière-du-Loup: 47°84′N, 69°57′W; Kamouraska: 47°56′N, 69°87′W; Rivière-Ouelle: 47°48′N, 70°02′W) on five occasions (1, 16 June; 1, 29 July; 14 August 2015). Seawater salinity and temperature were measured at the surface at the beginning and end of sampling at each site with a YSI professional plus probe. Zooplankton was obtained by 10 min horizontal surface tows from the dock using a ring net (0.5 m diameter, 245 μm mesh size) equipped with a flux meter in the middle of the net. For each station, one sample was preserved in ethanol (95%), and placed in the freezer (−20 °C) until laboratory identification; after 24 h, ethanol was totally replaced. Zooplankton from the second sample were preserved in seawater at −80 °C for lipid analyses. Three 10 ml subsamples from a 1 L sample were taken for zooplankton identification (minimum of 300 individuals per subsample) and abundance determination using a counting chamber under a stereomicroscope.

2.2. Copepod nauplii production

Adult copepods (*Eurytemora herdmani*) were collected in June 2016 at the Rivière-du-Loup site and females with egg sacks were placed in ovitraps (100 ⌈m mesh size) within 10 L tanks filled with 1 μm filtered seawater at 18 °C and salinity 27. Hatched nauplii were collected and counted daily, then placed in a 1 L bottle at 10 °C with 1 μm filtered seawater at 18 °C, salinity 27, and low aeration until use for larval feeding experiments.

2.3. Winter flounder larval production

All experiments were conducted at the aquaculture station of the Institute des Sciences de la Mer de Rimouski, Québec, Canada (48°27′N, 68°32′W) from April to August 2016. Female winter flounder broodstock were collected in the Baie-des-Chaleurs, Québec, Canada (48°10′N, 67°30′W) and larvae were obtained using the fertilization protocol described by Ben Khemis, de la Noue, and Audet (2000). The rearing protocol was similar to the one used by Vagner et al. (2014). Newly hatched larvae were briefly reared in 55 L cylindroconical polyethylene tanks at a rearing density of 1 larva·ml^{-1}, supplied with filtered sea water (10 μm), and maintained at 10 °C with a 12 L:12D photoperiod. Permanent upwelling was maintained in each tank by an aeration system. From the mouth-opening stage (4 days post-hatch [dph]) until the beginning of experiments, larvae were fed daily with the rotifer *Brachionus plicatilis* enriched with Sparkle (Selco, INVE aquaculture Ltd., Thailand) at a concentration of 12 individuals ml^{-1}. In larval rearing tanks, the water supply was stopped each day for 12 h while a green water preparation (*Nannochloropsis oculata* at 1.6×10^6 cells·L^{-1}) was added to each tank. At the end of the day, water circulation resumed, allowing complete renewal of the tank water during the night. Tank bottoms were cleaned weekly.

2.4. Larval feeding experiments

E. herdmani nauplii and enriched rotifers used for larval feeding experiments were maintained in 0.2 μm filtered and aerated seawater (salinity 27) and fed daily with a mix of microalgae (*Nannochloropsis oculata*, *Isochrysis galbana*, and *Pavlova lutheri,* 1:1:1). Two similar experiments were carried out with two different batches of winter flounder larvae of the same age (16 dph). Larval growth, survival, and fatty acid composition were evaluated in six 20 ml well plates after feeding with *E. herdmani* nauplii or *Brachionus plicatilis* for six days. Two winter flounder larvae were placed in each well (total of 72 larvae, for 36 replicates) filled with 20 ml of filtered (1 μm) green seawater (as already described) at 10 °C and salinity 27. Larvae were transferred every day to a new well containing 24 prey (nauplii or rotifers). After six days, the surviving larvae were preserved at −80 °C for lipid analyses.

Specific growth rate (SGR) was calculated according to the following equation (where wt. is weight and the time interval used was six days; Cook, Mcniven, Richardson, & Sutterlin, 2000):