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BONE DEVELOPMENT IN ATLANTIC BLUEFIN TUNA (Thunnus thynnus) AND SKELETAL EFFECTS OF FIRST FEEDING WITH COPEPODS (Acartia tonsa) OR ROTIFERS (Brachionus ibericus)

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Introduction

Juvenile production of Atlantic bluefin tuna (*Thunnus thynnus*) is characterized by high mortalities and low growth rates during the larval stage. Startfeeding of Bluefin tuna larvae in hatcheries depends on the traditional food organisms rotifers and *Artemia* nauplii (Biswas et al., 2006), and skeletal malformations have been observed in 70% of larvae and juveniles (Libert et al., 2013). When copepods are used as live food, the results are generally improved compared to the use of traditional live feed organisms (Evjemo et al., 2003), with higher survival, increased growth, normal development, earlier onset of ossification and less skeletal anomalies compared to larvae fed rotifers and *Artemia* (Imsland et al., 2006). The aims of this study were to describe the bone development in the Atlantic Bluefin tuna fed copepods, and to evaluate the effects of start-feeding with enriched rotifers or with cultivated copepods on skeletal deformities.

Material and Methods

Atlantic bluefin tuna eggs were supplied by Fortuna Mare SL in Spain, and two larval startfeeding experiments were performed. The first was carried out in SINTEF Fisheries and Aquaculture's laboratories, where cultivated copepods (*Acartia tonsa*) of progressing stages were used as live feed from 3 days post hatch (dph), in a 3000 litre tank (23 - 25 °C, 35 ppt, 24 h light). The second experiment was performed by Fortuna Mare SL at the Instituto Espanol De Oceanografi (IEO)in Spain, where larvae were fed either cultivated copepods or enriched rotifers and *Artemia* in commercial larval rearing tanks (temp, salinity, size of tanks…). Larval development and skeletal ossification were studied from larvae sampled through the experiments, fixed in 4% formaldehyde in phosphate buffered saline (pH 7.4, Apotekproduksjon AS; Norway), 0 - 20 dph in experiment 1 (n= 5 - 10 per day)

and 8 - 23 dph in experiment 2. In the second experiment, larvae between 18-23 dph were analysed for bone anomalies (N=33 - 39 larvae per group). Analyses of larval bone development were performed at NTNU Centre of Fisheries and Aquaculture, by staining with Alizarine Red according to our standard procedures (Kjørsvik et al., 2009).

Results and Discussion

In experiment 1, feeding with intensively produced copepods resulted in growth rates of 39 % day-1 between day 7 and day 10 dph, decreasing to 30 and 15 % at day 12 and 16, respectively. Atlantic bluefin tuna larval skeletal development first started with the ossification of the feeding apparatus (mouthparts and teeth, from 7 dph), followed by the swimming structures (caudal fin, dorsal fins, from 10 dph), then cranium, vertebral column (from 13 dph), other fin rays (anal fin, pectoral fins, pelvic fins), and finally the pterygiophores. Ossification of neural arches and vertebrae were first seen when the larva reached 6.6 mm SL (from 11-12 dph). At the end of the experiment (20 dph), most of the vertebrae were compact in the largest larvae. The total number of larval vertebrae observed was 36 - 38.

There was no significant difference in size between the Atlantic Bluefin tuna larvae from both experiments at 18 dph (mean SL 9.5 - 10.0 mm SL). At the end of experiment 2 (23 dph), the standard length (SL) of the larvae reared in the commercial hatchery were very variable, ranging between 13.9 - 33.9 mm SL, with no significant difference between the groups. However, sampling of larvae were probably biased at this time, due to very high avoidance speed and deeper distribution of the larger larvae. Ossification of the neural arches in the anterior part of vertebral column was first observed by 10 dph, and all 39 - 40 vertebrae were ossified by 23 dph. The rotifer fed tuna larvae had a significantly higher incident of skeletal anomalies than the copepod fed group, and a significantly higher number of larvae were affected (39 vs 68%), observed at 18 - 23 dph.

Ossification as found in experiment 1 was observed much earlier than in larvae studied in the SELFDOTT project (larvae fed rotifers/Artemia), where ossification of neural arches and vertebrae were observed from 16.6 mm SL (Libert et al., 2013). The earlier ossification of the spinal column in our study is probably due to faster growth of larvae fed the copepod *A. tonsa*.

Although the number of larvae analysed for skeletal anomalies in both experiments were low (n = 23-31, for 18-23 dph), the larvae from experiment 1 had a relatively low incidence of skeletal anomalies (6.5 %), compared to experiment 2, and compared to the 60 % of the larvae with anomalies (15 - 26 dph) observed by Libert et al. (2013). A high percentage of larvae with skeletal anomalies (61.5 %) were also observed in Pacific Bluefin tuna larvae (Shimizu & Takeuchi, 2002) fed rotifers, *Artemia*, and live fish larvae. The difference between larval skeletal anomalies found in our two experiments suggests that other factors than larval diet also are of importance.

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