Available online at www.sciencedirect.com
ScienceDirect
Aquaculture 270 (2007) 535–540
Aquaculture
www.elsevier.com/locate/aqua-online

Effects of temperature and lipid droplet adherence on mortality of hatchery-reared southern hake *Merluccius australis* larvae

Claudia A. Bustos [a,b,*], Mauricio F. Landaeta [a], Enrique Bay-Schmith [c], Rodrigo Lewis [d], Ximena Moraga [d]

[a] *CIEN Austral, Centro de Investigación en Nutrición, Tecnología de Alimentos y Sustentabilidad, Universidad Austral de Chile, Campus Puerto Montt, Av. Los Pinos s/n Balneario Pelluco, Casilla 1327, Puerto Montt, Región de Los Lagos, Chile*
[b] *Programa de Magíster en Ciencias mención Zoología, Universidad de Concepción, Chile, Barrio Universitario s/n, Concepción, Chile*
[c] *Departamento de Zoología, Universidad de Concepción, Chile*
[d] *Centro Experimental Quillaipe, Fundación Chile, Chile*

Received 23 December 2006; received in revised form 30 March 2007; accepted 30 March 2007

Abstract

Effects of exogenous (water temperature) and endogenous (lipid droplet adherence) factors were experimentally tested on early survival of southern hake *Merluccius australis* reared under controlled conditions. Experiments to determine the effect of temperature (10, 12 and 14 °C) on larval growth rates and yolk-sac absorption rates of unfed southern hake were carried out under laboratory conditions. There was no significant differences in growth rates at the temperature range tested (ANCOVA, $F=0.164$, $p>0.25$), but yolk-sac absorption rates and mortality increased with temperature (ANCOVA, $F=53.84$, $p<0.001$). A high percentage (between 31 and 81%) of hake eggs showed a lipid droplet not adhered (i.e., freely moving in the yolk, and not located in the posteriormost portion of the yolk-sac). In a second experiment, fed southern hake larvae with the lipid droplet not adhered during embryonic development did not survive after yolk-sac absorption. This study provides the first data on the influence of the lipid droplet absorption on larval survival of cultured hake, and can be used as an early indication of the quality of the batch.

Keywords: Southern hake; Survival; Temperature; Lipid droplet

1. Introduction

Southern hake, *Merluccius australis* is a gadoid fish that sustain an important industrial and artisan demersal fishery in southern Chile and Argentina (Payá and Ehrhardt, 2005). In recent years efforts have been carried out in Chile to develop a commercial culture of this species. A pilot scale hatchery produced 15,000 juveniles in 2004, and early development of eggs and yolk-sac larvae of southern hake under laboratory conditions have been described (Bustos and Landaeta, 2005); however, critical processes in early life history in captive rearing conditions are unknown.

Temperature is one of the most important regulating factors in fish growth and survival in culture conditions (Blaxter, 1992; Hart et al., 1996; Fielder et al., 2005;

* Corresponding author. CIEN Austral, Centro de Investigación en Nutrición, Tecnología de Alimentos y Sustentabilidad, Universidad Austral de Chile, campus Puerto Montt, Av. Los Pinos s/n Balenario Pelluco, Puerto Montt, Chile. Tel.: +56 65 277120; fax: +56 65 255583.
E-mail address: clausbustos@gmail.com (C.A. Bustos).

0044-8486/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2007.03.032

Olivotto et al., 2006). Broodstock age and nutrition may affect egg and larval quality, through variation in the amount of essential fatty acids in the yolk (Furuita et al., 2000, 2002) and/or the lipid droplet. The lipid droplet is likely the major source of energy sustaining larvae during the transition to exogenous feeding, is an important source of triacylglycerol (TAG) and affects larval survival (Norton et al., 2001; Berkeley et al., 2004). However, stress or nutritional status of broodstock may affect the mechanical absorption of the lipid droplet and the fatty acids available in it, through abnormalities in the intestinal mucosa of larvae or the formation of the yolk syncytial layer (Deplano et al., 1991; Poupard et al., 2000). The present study was conducted to investigate the effects of temperature and lipid droplet adherence (i.e., localization of the lipid droplet in the posteriormost portion of the yolk-sac) on the survival of larval southern hake after the total exhaustion of the endogenous reserves, and represents the first efforts to study this gadoid species in incipient culture.

2. Methods

Captured wild southern hakes were kept in captivity in large tanks (30 m^3) in the Quillaipe Experimental Center of Fundación Chile, Puerto Montt, Chile from May 2002, under natural photoperiod and temperature. One male (M1: 3.3 kg body weight (BW), 71.5 cm total length (TL)) and two females (female 1, F1: 3.0 kg BW, 79 cm TL; female 2, F2: 4.5 kg BW, 77 cm TL) were utilized for the experiments carried out in September 2004. Fishes were induced to spawn with gonadotropin releasing hormone (GnRH, Ovaplant®, Syndel Laboratories Inc., Canada), by intramuscular injection in the dorsal region. Fishes were kept in well-aerated tanks, with open water circulation.

When females were ripe, a gentle pressure on the abdomen from head to tail was applied to collect the spawned eggs. Eggs were fertilized by the dry method using milt obtained from the male. All batches were artificially fertilized. The eggs were transferred to a beaker to measure the percentage of buoyant eggs, as normal eggs float while poor quality or abnormal eggs sink. Buoyant eggs were stocked in 3001 cylindroconical incubation tanks. Hatching success ranged between 82 and 86% for the batches utilized in the experiments.

2.1. Experiment I

The buoyant eggs were stocked at a density of ~150 eggs l^{-1} and incubated at 11 °C in three tanks just after fertilization and the temperature of the tanks was thereafter gradually changed to 10, 12 and 14 °C, respectively. Since the spawning of hake occurs below surface waters, the incubators were kept in darkness at these temperatures for the remaining part of the experiment to reduce environmental stress. In each tank, hatched larvae were kept in three glass jars at a density of 20 larvae l^{-1}, and maintained in UV-sterilized and filtered seawater (0.5 μm). One third of the water contained in the jars was replaced daily throughout the duration of the experiment. For each temperature treatment (10, 12 and 14 °C), larvae were randomly collected each day from day 1 post-hatch (dph) to yolk-sac exhaustion, and were preserved in buffered 10% formalin. Larvae were maintained without food until death. Dead larvae were removed and counted daily from the bottom of the tank by siphoning. From this data, instantaneous mortality rates were calculated using the following formula: $Z = (\ln(N_t) - \ln(N_0)/t)\ 100$, where Z = instantaneous mortality rate (d^{-1}), N_t is the number of larvae alive at time t, N_0 is the number of larvae alive at time 0, and t is the duration in days. Notochord length (NL) of preserved larvae was measured from the tip of the mouth to the tip of the notochord. Yolk-sac volume was estimated considering the yolk-sac as an ellipsoid ($V = 4/3\pi a * b^2$, where a is half of the yolk-sac length, and b is half of the yolk-sac height). All measurements were carried out using a Sony CCD-IRIS video camera attached to a stereomicroscope connected to a PC with Optimas 6.1® software. No corrections for shrinkage in larval length were carried out.

2.2. Experiment II

Eggs with the lipid droplet not adhered (i.e., freely moving in the yolk, and not located in the posteriormost portion of the yolk-sac) and adhered were visually selected from F1 and F2 fertilizations and were placed in separate treatments (three treatments: F1 eggs with lipid droplet not adhered, F2 eggs with lipid droplet not adhered, and F1–F2 eggs with lipid droplet adhered). Early stages of *M. australis* were kept in three glass jars at a density of 20 larvae l^{-1} by treatment and maintained in UV-sterilized and filtered seawater (0.5 μm) at 10 °C, in darkness throughout the experiment (up to 17 dph). Prior to yolk-sac exhaustion, larvae were fed twice a day with *Isochrysis galbana* (~6×10^{10} cells l^{-1}) and rotifers (5000 prey l^{-1}). After 10 dph, *Artemia* were added twice per day at a density of 3000 prey l^{-1}. Dead larvae were removed and counted daily from the bottom of the tank by siphoning, and instantaneous mortality rates were calculated.