DIGITAL PHOTO-MICROSCOPY

OF SABLEFISH (*ANOPLOPOMA FIMBRIA*)

EMBRYONIC DEVELOPMENT

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Abstract

Sablefish (*Anoplopoma fimbria*) eggs were incubated at temperatures ranging from 4 to 8 °C. Digital photo-microscopy was employed to obtain an accurate determination of embryonic development rates of surviving embryos and larvae at the various temperatures. Representative pictures of key developmental stages are presented.

Introduction

Research on sablefish (*Anoplopoma fimbria*) eggs and larvae has been ongoing at the Pacific Biological Station, Nanaimo, BC, Canada, since the late 1980s (Alderdice et al., 1988a, 1988b; Bowden et al., 1990; Jensen and Groot, 1990; Jensen et al., 1992; Whyte et al., 1994; Clarke and Jensen, 2001). In their paper on preliminary trials on incubation of sablefish eggs, Alderdice et al. (1988a) documented embryonic development prior to hatch at several temperatures (i.e. 3.6 to 7.9 °C) and included photo-microscopy images for some stages. We incubated sablefish eggs at similar temperatures (i.e. 4 to 8 °C) to obtain more information on early embryonic development rates in relation to temperature. In addition, we present new digital photo-microscopy images of both pre- and post-hatch embryonic development.

Materials and Methods

Small batches of fertilised sablefish eggs were obtained from broodstock held at the Pacific Biological Station and placed in 4-litre beakers filled with 34 ppt filtered (0.2 μ) seawater. The beakers were held in temperature-controlled (± 0.1 °C) water baths (i.e. 4, 5, 6, 7, and 8 °C). We attempted to replace the water periodically to prevent fouling in the static water. This was not completely successful and eggs only survived to day 4 in 8 °C, and to day 5 in 7 °C. For the 6 °C treatment, eggs and larvae were collected periodically every few days from a flow-through incubator to ensure that samples were not adversely influenced by poor water quality. In the lower temperatures eggs survived longer since bacterial growth was slower. Hence eggs survived in the static water temperatures of 6,5, and 4 °C for 7 days post-fertilization. Developing eggs were sampled at various intervals, initially at 4-hr intervals, from each temperature, and were photographed using a Nikon Coolpix 990, 3.1 megapixel, digital camera mounted on a Nikon SMZ1000 stereo microscope equipped with a diascopic stand that utilises Nikon's Oblique Coherent Contrast illumination system. This system was ideal for photographing the transparent eggs and developing embryos.

Results

Pictures of embryonic development were obtained from early cell division to almost complete yolk absorption in hatched larvae. A series of pictures of developmental stages of eggs and larvae from fertilization to near yolk absorption (38 days post fertilization or 228 °C-days at 6°C) have been compiled from the hundreds of digital pictures obtained, and some of the stages are illustrated in Figs 1 to 8.

To aid researchers and fish culturists, the development time for the above stages have been mathematically modelled, based on the 5.3 °C trial from Alderdice et al. (1988a) for eggs and from Clarke and Jensen (2001) for larval yolk absorption, to allow quick and accurate predictions of when these stages will occur at temperatures ranging from 4 to 8 °C. These predictions are tabulated as development time in hours and accumulated temperature units (ATUs; °C-days) in Table 1.

Figure 1. Sablefish egg at the 2-cell stage (i.e. first cell division has occurred). This occurs at 1.3 °C-days.

Figure 2. Sablefish egg at the 8-cell stage. This occurs at 2.9 °C-days.

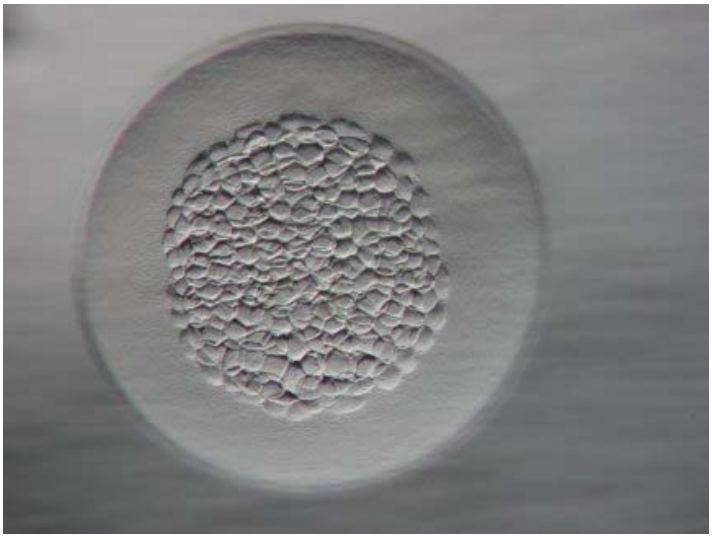

Figure 3. Sablefish egg at the late morula stage. This occurs at 7.6 °C-days.

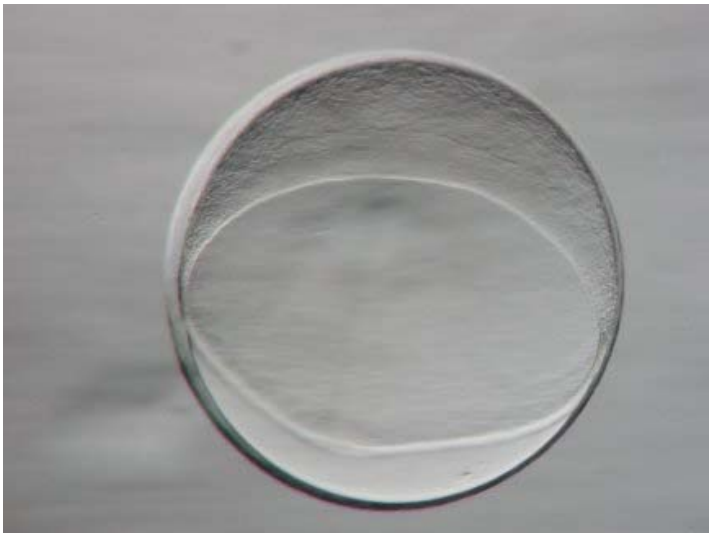

Figure 4. Sablefish egg at ½ to ¾ epiboly or yolk overgrowth. This occurs at 27.1 °C-days.

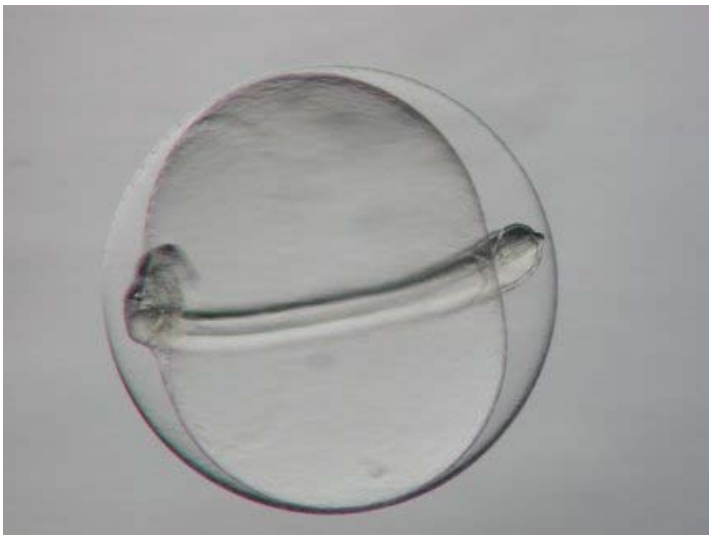

Figure 5. Sablefish egg with tailbud lifted off yolk. This occurs at 47.8 °C-days.

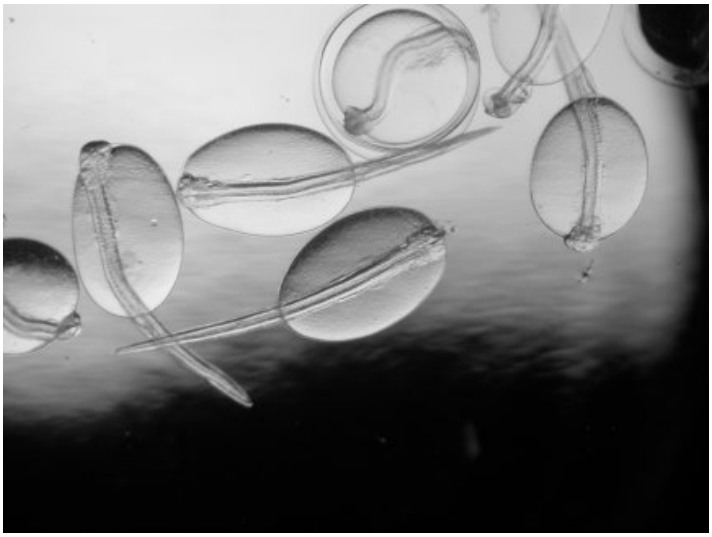

Figure 6. Hatched sablefish larvae. This occurs at 74.4 °C-days.

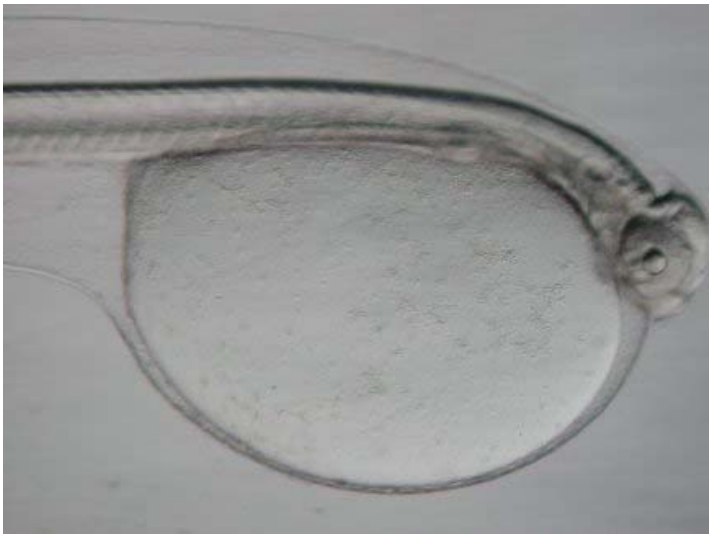

Figure 7. Sablefish larva with 50% yolk remaining. This occurs at 96.5 °C-days.

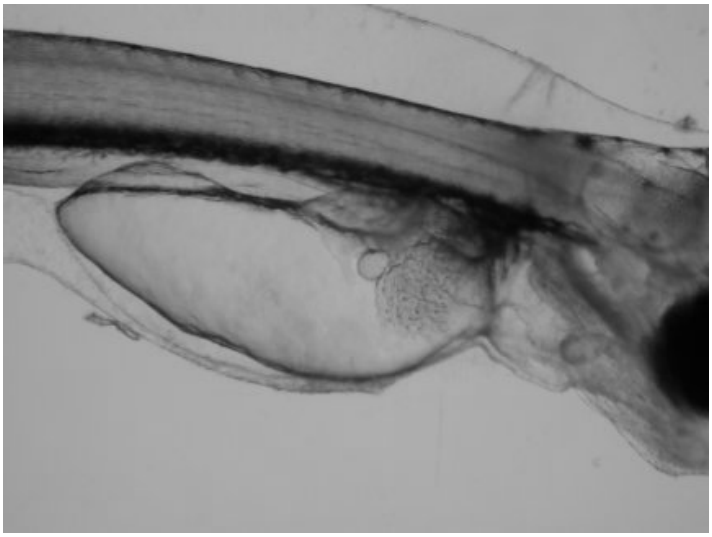

Figure 8. Sablefish larva near complete yolk absorption (about 2.5% yolk remaining). This occurs at 226 °C-days.

Table 1. Time (hr) and ATUs to various developmental stages of sablefish at temperatures ranging from 4 to 8 °C.
* Based on development at 6°C

Developmental stage	Time to stage (hr) 4°C	5°C	6°C	7°C	8°C	ATUs (°C-days)*
2-cell	7.1	5.9	5.3	4.8	4.6	1.3
4-cell	11.2	9.4	8.3	7.7	7.3	2.1
8-cell	15.7	13.1	11.6	10.7	10.2	2.9
16-cell	19.1	16.1	14.2	13.1	12.5	3.5
32-cell	23.6	19.8	17.5	16.2	15.4	4.4
64-cell	28.8	24.2	21.4	19.7	18.8	5.3
Late morula	41.0	34.4	30.4	28.1	26.7	7.6
Germ ring formation	84.5	70.9	62.7	57.9	55.1	15.7
Germ ring overgrowth beginning	108.3	91.0	80.4	74.2	70.6	20.1
1/4-1/2 epiboly	121.0	101.6	89.8	82.9	78.9	22.4
1/2-3/4 epiboly	146.0	122.6	108.3	100.0	95.2	27.1
Near yolk plug closure	181.3	152.2	134.5	124.1	118.2	33.6
Yolk plug closed	187.2	157.2	138.9	128.2	122.1	34.7
1-15 somites, kupffer's vesicle present	203.8	171.1	151.2	139.5	132.9	37.8
15-25 somites	222.8	187.0	165.3	152.5	145.3	41.3
>25 somites, optic cups developing	232.7	195.4	172.7	159.4	151.7	43.2
Tailbud lifted off yolk, otic vesicle, lens	257.6	216.2	191.1	176.4	167.9	47.8
Olfactory pits present	270.9	227.4	201.0	185.5	176.6	50.2
Tailbud extended, embryo near 1/2 circle	270.9	227.4	201.0	185.5	176.6	50.2
Embryo near 2/3-3/4 circle	308.7	259.2	229.1	211.4	201.3	57.3
Heart beating	332.6	279.2	246.8	227.8	216.9	61.7
Hatch	400.9	336.5	297.5	274.5	261.4	74.4
3.6 mm^3 ~75% yolk volume remaining	444.9	355.9	296.6	254.2	222.5	74.2
2.4 mm^3 ~50% yolk volume remaining	579.0	463.2	386.0	330.9	289.5	96.5
1.2 mm^3 ~25% yolk volume remaining	828.6	662.9	552.4	473.5	414.3	138.1
0.12 mm^3 ~2.5% yolk volume remaining	1356.0	1084.8	904.0	774.9	678.0	226.0

Concluding comment

The new pictures presented in this paper are an improvement in clarity over earlier pictures (Alderdice et al., 1988a). They are presented here to illustrate this improvement. In addition the time to the various developmental stages in Table 1 should prove useful to researchers and fish culturists alike.

Acknowledgements

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