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Note

Effect of temperature on yolk sac utilisation and growth of newly hatched larvae of cobia *Rachycentron canadum* (Linnaeus, 1766)

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ABSTRACT

The effect of temperature on the utilisation of yolk-sac and growth in terms of length of the larvae of cobia *Rachycentron canadum* (Linnaeus, 1766) was investigated. Five temperature levels *viz*., 29, 30, 31, 32 and 33°C were experimented with the newly hatched larvae for a total duration of 52 hours post-hatch (hph). A clear-cut trend of increase in larval length with increase in temperature was noted. The maximum length recorded was 4.41±0.11 mm at 33°C by the end of the experiment. The yolk-sac volume decreased proportionately with rise in temperature. At the end of 52 hph, the lowest yolk-sac volume was recorded at temperature range of 31 to 33°C. Results of the present study suggest that temperature plays a vital role in the yolk-sac utilisation as well as on growth of cobia larvae.

Keywords: Cobia, Larval growth, *Rachycentron canadum,* Temperature, Yolk-sac

Water temperature is a critical factor that affects fish embryogenesis and larval development. The yolk-sac larvae of most marine teleosts have low functional capacity for swimming, vision and olfaction. The efficiency of yolk utilszation at this stage is influenced by the water temperature to a large extent (Polo *et al.*, 1991; Koumoundouros *et al.*, 2001). The egg and larval distribution as well as mechanisms of adapting to the variable marine environment can be understood by analysing the role of temperature in the early fish ontogeny. Currently, issues related to climate change such as ocean temperature rise have become a threat to fish eggs and larvae of many commercially important species. But, the temperature can also be effectively manipulated for marine larviculture and it is also possible to accelerate yolk utilisation and larval growth within the temperature ranges that are tolerable to the species. A good deal of investigations has been carried out on the temperature effects in the early larval development and metamorphosis of fish and crustacean embryos and larvae (Ye *et al.*, 1998; Zhang *et al.*, 2006; Roberts *et al.*, 2012). In recent years, cobia *Rachycentron canadum* (Linnaeus, 1766) is gaining momentum internationally as a lucrative species for sea cage farming due to its fast growth rate and high meat quality. In India, the breeding and seed production of cobia was first achieved by the ICAR-Central Marine Fisheries Research Institute (ICAR-CMFRI) (Gopakumar *et al.*, 2011; 2012). In the present communication, the results of investigations on the effect of temperature on the yolk-sac utilisation and growth in terms of length of cobia larvae are presented and discussed. The acceleration of larval growth by manipulating the temperature can be effectively applied in the commercial larviculture of cobia.

Newly hatched larvae obtained from the same brood of *R. canadum* spawned in the hatchery at Mandpam Regional Centre of ICAR-CMFRI were used in the study. The experiments were conducted in rectangular glass aquarium tanks (60x30x45 cm). Five different temperatures *viz.,* 29, 30, 31, 32 and 33°C were set and controlled using fully submersible aquarium heaters with built-in thermostats with a temperature accuracy of ± 0.1°C. Mild aeration was provided in all the tanks for uniform distribution of temperature. The ambient temperature was kept as the control which ranged from 27.0 to 28.5°C, during the period of study. For each temperature exposure, there were three replicates. One hundred newly hatched larvae from hatching tank were transferred to each experimental tank. The experiment was conducted up to 52 hours post-hatch (hph) by which time the yolk utilisation was almost complete.

A random sample of 5 larvae was collected from each tank (15 larvae per temperature treatment), anaesthetised with MS-222 (0.2±0.3 g l^{-1}) and observed under a stereoscopic microscope equipped with an eyepiece micrometer and the required measurements were recorded. The larvae were not returned to their original tanks to avoid interference from handling related mortality.

Length of the larvae was measured (to nearest 0.1 mm) at every 4 h from all the experimental groups. Total length of larvae (mm) was measured parallel to the longitudinal axis of the body and the depth of the yolk-sac, perpendicular to this axis. Total length (TL), pre-yolk-sac length (prYsc), post-yolk-sac length (psYsc) and yolk-sac depth (YsD) were measured directly while yolk-sac length (YsL) and yolk-sac volume (YsV) were estimated from the aforementioned measurements. Volume (mm^3) of the ellipsoidal larval yolk-sac was calculated using the formula:

$YsV = (\pi/6)\, YsL * YsD^2$ (Blaxter and Hempel,1963).

The data on larval length and yolk-sac volume were analysed with one-way ANOVA. Data were also analysed for correlation among larval length and yolk-sac volume. The statistical analyses were carried out using SPSS 20.0 statistical package (SPSS Inc., USA). Significance level of $p<0.05$ was chosen.

The larval length (mm) of *R. canadum* at different temperatures tested is given in Table 1. The trend of larval length at different temperatures is presented in Fig.1 and it was noted that the length increased proportionately with the rise in temperature. The larval length at the beginning of the experiment was 2.77±0.06 mm in all the temperature treatments. At the end of the experiment, the length (mm) ranges recorded were 4.20±0.07, 4.31±0.07, 4.32±0.07, 4.31±0.07, 4.40±0.08 and 4.41±0.11 in the control and at 29, 30, 31, 32, and 33°C, respectively. A clear trend of increase in larval length with increase in temperature was noted (Fig.1). The analysis of variance between different test temperature revealed that the effect of temperature was significant at 4, 36 and 40 hph ($p<0.01$) and at 16, 20 and 32 hph ($p<0.05$).

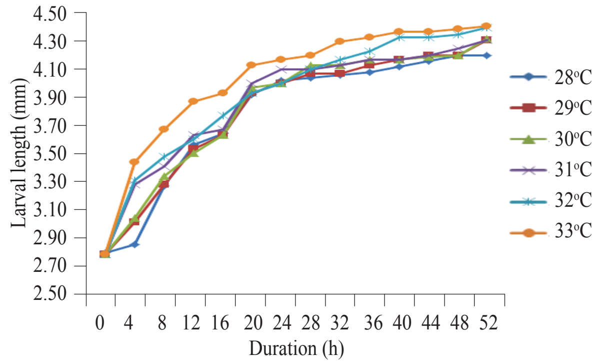

Fig. 1. Larval length (mm) of *R. canadum* recorded during different sampling intervals at different temperatures

However, no significant effect of temperature was seen at 8, 24 and 28 hph.

The yolk-sac volume (mm^3) of *R. canadum* at different temperature levels is given in Table 2. It was noted that the yolk-sac volume decreased proportionately with rise in temperature. At the beginning of the experiment, the yolk-sac volume (mm^3) was 0.936±0.030 in all the temperatures tested. At 40 hph, the same were 0.020±0.009, 0.008±0.00, 0.008±0.00, 0.004±0.00, 0.004±0.00 and 0.004 ±0.00, respectively. At the end of the experiment, almost complete exhaustion of yolk-sac was noted at all the test temperature except in the control. A clear decreasing trend of yolk-sac volume at all the time durations proportionate to the increasing temperature was evident (Fig. 2.) The analysis of variance between various temperature treatment groups also revealed that the effect of temperature was highly significant ($p<0.01$) at all the time durations of sampling.

A highly significant ($p<0.01$) correlation between the larval length and the yolk-sac volume was noted and the

Table 1. Length of cobia larvae (mm) (mean ± SE.) measured during different sampling intervals at different test temperatures

Hours post-hatch (hph)	Control	29°C	30°C	31°C	32°C	33°C	p value* (ANOVA)
0	2.78 ± 0.08	2.77 ± 0.06	2.77 ± 0.06	2.77 ± 0.06	2.77 ± 0.06	2.77 ± 0.06	NS
4	2.84^{a} ± 0.15	3.00^{a} ± 0.10	3.03^{ab} ± 0.12	3.27^{bc} ± 0.06	3.30^{c} ± 0.17	3.43^{c} ± 0.12	<0.01
8	3.26 ± 0.19	3.27 ± 0.06	3.33 ± 0.06	3.40 ± 0.10	3.47 ± 0.12	3.67 ± 0.23	NS
12	3.56^{a} ± 0.13	3.53^{a} ± 0.12	3.50^{a} ± 0.10	3.63^{a} ± 0.06	3.60^{a} ± 0.00	3.87^{b} ± 0.12	<0.01
16	3.64^{a} ± 0.18	3.63^{a} ± 0.06	3.63^{a} ± 0.06	3.67^{a} ± 0.06	3.77^{ab} ± 0.12	3.93^{b} ± 0.23	<0.05
20	3.92^{a} ± 0.04	3.93^{a} ± 0.06	3.97^{a} ± 0.06	4.00^{a} ± 0.10	3.93^{a} ±0.06	4.13^{b} ± 0.06	<0.05
24	4.02 ± 0.16	4.00 ± 0.10	4.00 ± 0.10	4.10 ± 0.10	4.00 ± 0.10	4.17 ± 0.06	NS
28	4.04 ± 0.18	4.07 ± 0.06	4.13 ± 0.06	4.10 ± 0.10	4.10 ± 0.10	4.20 ± 0.10	NS
32	4.06^{a} ± 0.15	4.07^{a} ± 0.06	4.13^{ab} ± 0.06	4.13^{ab} ± 0.06	4.17^{ab} ± 0.06	4.30^{b} ± 0.10	<0.05
36	4.08^{a} ± 0.13	4.13^{b} ± 0.06	4.17^{b} ± 0.06	4.17^{b} ± 0.06	4.23^{bc} ± 0.06	4.33^{c} ± 0.06	<0.01
40	4.12^{a} ± 0.13	4.17^{a} ± 0.06	4.17^{a} ± 0.06	4.17^{a} ± 0.06	4.33^{b} ± 0.06	4.37^{b} ± 0.06	<0.01
44	4.16^{a} ± 0.06	4.20^{b} ± 0.06	4.19^{b} ± 0.06	4.20^{b} ± 0.07	4.33^{c} ± 0.08	4.37^{c} ± 0.07	<0.01
48	4.20^{a} ± 0.07	4.20^{a} ± 0.07	4.20^{a} ± 0.06	4.25^{b} ± 0.07	4.35^{c} ± 0.08	4.39^{c} ± 0.10	<0.01
52	4.20^{a} ± 0.07	4.31^{b} ± 0.07	4.32^{b} ± 0.07	4.31^{b} ± 0.07	4.40^{c} ± 0.08	4.41^{c} ± 0.11	<0.01

*Means in a row with same superscripts are not significantly different (Duncan's, p value). NS = non-significant (p>0.05)

Table 2. Yolk sac volume of cobia larvae (mm^3) (mean ± SE) estimated during different sampling intervals at different test temperatures

Hours post-hatch (hph)	Control	29°C	30°C	31°C	32°C	33°C	p value* (ANOVA)
0	0.936 ±0.028	0.936 ±0.030	0.936 ±0.030	0.936 ±0.030	0.936 ±0.030	0.936 ±0.030	<0.01
4	0.673^{a} ±0.146	0.374^{b} ±0.022	0.283^{bc} ±0.103	0.197^{cd} ±0.051	0.160^{cd} ±0.041	0.119^{d} ±0.031	<0.01
8	0.151^{a} ±0.020	0.096^{b} ±0.030	0.065^{bc} ±0.024	0.069^{bc} ±0.005	0.041^{c} ±0.003	0.064^{bc} ±0.046	<0.01
12	0.107^{a} ±0.033	0.056^{b} ±0.024	0.041^{b} ±0.003	0.044^{b} ±0.012	0.036^{b} ±0.003	0.036^{b} ±0.003	<0.01
16	0.040^{a} ±0.003	0.034^{b} ±0.003	0.033^{b} ±0.005	0.012^{c} ±0.000	0.012^{c} ±0.000	0.013^{c} ±0.001	<0.01
20	0.040^{a} ±0.003	0.019^{b} ±0.012	0.011^{b} ±0.002	0.014^{b} ±0.008	0.010^{b} ±0.000	0.010^{b} ±0.000	<0.01
24	0.033^{a} ±0.009	0.011^{b} ±0.001	0.009^{b} ±0.001	0.008^{b} ±0.000	0.008^{b} ±0.000	0.008^{b} ±0.000	<0.01
28	0.034^{a} ±0.002	0.010^{b} ±0.001	0.009^{b} ±0.001	0.008^{b} ±0.000	0.008^{b} ±0.000	0.008^{b} ±0.000	<0.01
32	0.031^{a} ±0.003	0.008^{b} ± 0.000	0.008^{b} ±0.000	0.008^{b} ±0.000	0.004^{c} ±0.000	0.004^{c} ±0.000	<0.01
36	0.022^{a} ±0.009	0.008^{b} ± 0.000	0.008^{b} ±0.000	0.004^{b} ±0.000	0.004^{b} ±0.000	0.004^{b} ±0.000	<0.01
40	0.020^{a} ±0.009	0.008^{b} ± 0.000	0.008^{b} ±0.000	0.004^{b} ±0.000	0.004^{b} ±0.000	0.004^{b} ±0.000	<0.01
44	0.019^{a} ±0.009	0.004^{b} ±0.000	0.004^{b} ±0.000	N	N	N	<0.01
48	0.008 ±0.000	N	N	N	N	N	--
52	0.008 ±0.000	N	N	N	N	N	--

*Means in a row with same superscripts are not significantly different (Duncan's, p value). NS = non-significant (p>0.05). N = negligible

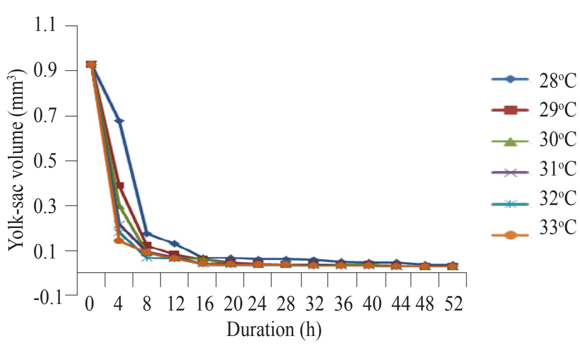

Fig. 2. Yolk-sac volume (mm^3) of *R. canadum* larvae estimated during different sampling intervals at different temperatures

coefficient of correlation was estimated as 0.825 which indicates that one unit decrease in yolk-sac volume resulted in 0.825 units of increase in larval length.

It is well understood that poikilothermic aquatic animals are directly affected by the variations in environmental parameters of the surrounding water medium. Among the different environmental parameters, water temperature is most vital. The impact of temperature on fish ontogeny affecting the growth and development rates (Batty *et al.*, 1986; Polo *et al.*, 1991), mortality (Watanabe *et al.*, 1995), deformities (Bolla and Holmefjord, 1988), size at feeding onset, notochord flexion or metamorphosis (Polo *et al.*, 1991; Overnell, 1997; Fuiman *et al.*, 1998), stage duration (Camus and Koutsikopoulos, 1984; Koumoundouros *et al.*, 2001), muscle ontogeny (Stickland *et al.*, 1988; Galloway *et al.*, 1999), sex determination (Conover and Kynard, 1981; Baroiller *et al.*, 1999; Koumoundouros *et al.*, 2002), hatching (Rana, 1990), survival rates (Marangos *et al.*, 1986; Polo *et al.*, 1991), total length at feeding onset (Polo *et al.*, 1991; Koumoundouros *et al.*, 2001) and efficiency of yolk utilisation (Polo *et al.*, 1991) have been used as the criteria for estimation of optimum temperatures for fish larvae.

In the present paper, the total length of the larvae and the yolk utilisation was studied for a total duration of 52 hph from hatching. It is well understood that the total length is directly related to the mouth opening and thus the prey size that larvae are able to consume. The total length of *R. canadum* larvae increased as the temperature increased. Statistical analysis showed significance at most of the time duration tested (Table 1). However, it could be seen that at 8, 24 and 28 hph, no significance was noted.. At the end of 52 hph, the maximum larval length of 4.41±0.11 mm was recorded at 33°C. The yolk-sac utilisation was also higher as the temperature increased. At the end of 52 hph, the lowest yolk-sac volume was recorded at a temperature range from 31 to 33°C. In all the temperatures tested, the yolk-sac utilisation was found to significantly increase (p<0.01) with increase in temperature.

The impact of temperature on embryogenesis, total length and yolk-sac utilisation varies with the species. In *Pagellus erythrinus*, within the temperature range of 18-21°C, higher efficiency of yolk utilisation was noted at 18°C and the total length of yolk-sac larval stage decreased with rise in temperature (Klimogianni *et al.*, 2004). Slower embryo development was reported at lower temperatures by Small and Bates (2001) and Lin *et al.* (2006), while higher temperature resulted in faster embryo development (Das *et al.*, 2006). However, sub-optimal conditions can increase the occurrence of fatal deformities and mortalities (Laurence and Rogers, 1976; Linden *et al.*, 1979; Das *et al.*, 2006). Results of the present study suggest that temperature plays a vital role in yolk-sac utilisation and growth of cobia larvae. Even though, better utilisation of yolk and maximum total length was recorded at 33°C, further investigations are required to confirm the optimal temperature with respect to yolk utilisation and growth of cobia larvae

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