

Full Length Research Paper

# Evaluation of the effect of temperature on egg development in an attempt to improve hatching success and fry production in *Oreochromis karongae*

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A study was conducted to assess the effect of temperature on egg development in an attempt to improve hatching success and fry production in *Oreochromis karongae*. Temperature-dependent development rates and hatching period for fertilized eggs stripped from *O. karongae*, a mouth brooder, were determined in a recirculating system set up in a hatchery at the National Aquaculture Center, Domasi, Malawi. Three treatments namely; 25, 27 and 29°C, were replicated thrice in 2-L Macdonald type incubation jars stocked with 265 fertilized stage 1 eggs of *O. karongae*, at water flow rate of 0.15 L/s. There was a curvilinear relationship between temperature and egg development, which was best described by a logarithm regression function. Hatching period decreased with increase in incubation temperature. The shortest hatching period was 7.3 days, which was observed at the highest incubation temperature (29°C) while the longest hatching period was 14.7 days, observed at the lowest temperature (25°C). Hatchability and fry survival were higher at higher temperatures. The study has, for the first time, ably described *O. karongae* egg development rates which suggest that increasing incubation temperature holds the potential to increase fry production, which is currently a bottleneck.

**Key words:** *Oreochromis karongae*, temperature-dependent, egg development rates, hatchability, survival, hatching period.

## INTRODUCTION

*Oreochromis karongae* (Trewavas, 1983), locally known as “Chambo”, is one of the indigenous mouth brooding *Tilapia* species endemic to Lake Malawi. The tilapine species are preferred for aquaculture because they exhibit tolerance to harsh conditions such as temperature changes, high salinity and low water quality (Maluwa and Brooks, 1996). They can also feed on locally available feeds since they are herbivorous and omnivorous, and are resistant to diseases (Beveridge and McAndrew, 2000). Studies have proved that of the three species that constitute 93% of aquaculture production in Malawi, *O.*

*karongae* exhibits superior growth characteristics over *Oreochromis shiranus* and *Tilapia rendalli* in earthen ponds, which is the most common aquaculture system (Msiska and Costa-Pierce, 1996). The culture of *O. karongae* in Malawi dates back to the 1970s. The species is cultured extensively by some small holder farmers and only a few commercial intensive operators. The species, is favored by consumers for its good flavor, shiny appearance and bigger size (Kaunda et al., 2005), and contributes to tourist attraction in Malawi. For this reason, the species has been overfished from the major water

**Table 1.** Criteria for egg staging based on Ahmed et al. (2007), Geffen et al. (2006) and the NAC Tilapia Hatchery Manual (n.d.).

Stage	Criteria
I	Fertilization to blastodisc formation (no evident organ formation)
II	Blastula to germ or signet ring formation-organ formation, that is, eye is evident with the naked eye III Gastrula-start of gastrula to closure of blastophore-eye further developed and tailbud formation
IV	Embryo- further development/straightened tail formation to hatching; tail seen flapping V At least 50% hatched and swimming out of jar into tray

quantities is necessary to meet both market demand for consumption and restocking purposes. However, scarcity of fingerlings remains the key constraint to the desired aquaculture production of *O. karongae* in Malawi. While other species such as *O. shiranus* can easily produce large quantities of eggs even in ponds as they are prolific breeders, *O. karongae*, despite its ability to breed several times a year, has low fecundity (Msiska, 1998) producing fewer (200 to 600 per clutch) but larger eggs. While success has been registered in fry production from the slow growing *O. shiranus*; the production of *O. karongae* fry has remained limited and challenging, even in a closed system hatchery, as hatchability of the already few eggs is very low. The constraining factors are not yet well understood. Theoretically, apart from biological factors, physical and chemical parameters are known to affect egg development. For example, temperature is known to be the main environmental factor governing fish egg development (Blaxter, 1992). Temperature affects certain morphological features, hatching rate and larval behavior. In earlier studies, temperature influenced egg development and hatching in *O. niloticus* (Bhujel et al, 2000), *Tilapia zillii* (Omotosho, 1988) common carp, *Cyprinus carpio*, (El-Gamal, 2009), and cod, *Gadus morhua* L (Page and Frank, 1989; Geffen et al., 2006). The effect of temperature on hatching of *O. karongae* eggs has not been studied in Malawi. Such information can be necessary in: (i) The process of adapting a hatchery system for increased production of *O. karongae*, and (ii) When setting the basis for developing egg development schemes that are required for management of aquaculture production schedules. In fisheries, predictive relationships relating egg incubation time with environmental factors are necessary for developing individual-based models (IBMs) that link fecundity of adult populations to recruitment via planktonic egg development stages. Temperature is also known to explain most of the variance in planktonic egg development stages (Pauly and Pullin, 1988). Temperature-dependent egg development relationships are instrumental to the estimation of spawning stock biomass using egg production methods (Lo et al., 1992; Armstrong et al., 2001). The main objective of the present study was to establish temperature- dependent egg development rates hatchability and survival rate for *O.*

*karongae*, in a recirculating system hatchery.

## MATERIALS AND METHODS

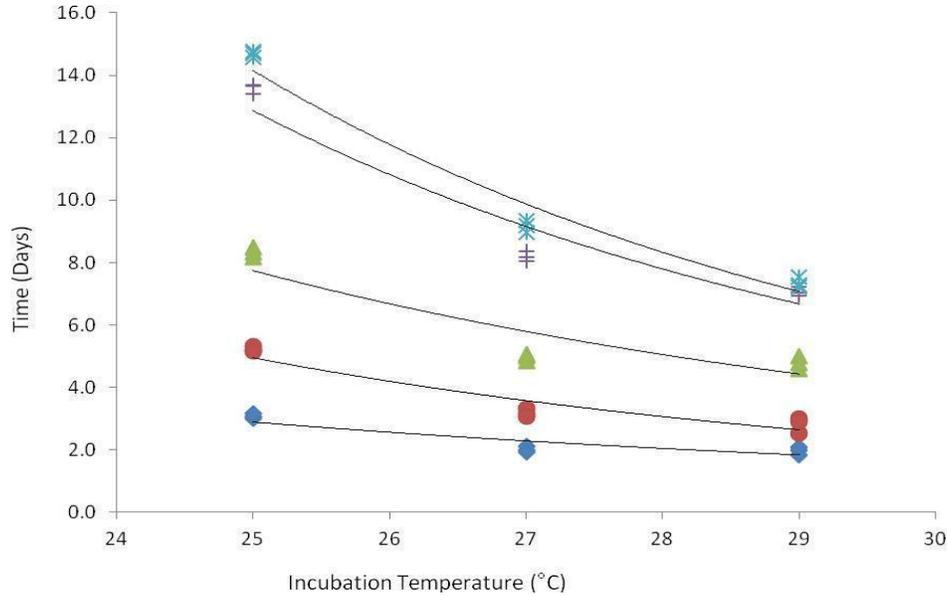
An experiment was conducted at the National Aquaculture Centre, Domasi, Malawi for a period of 15 days. Broodstock were conditioned in earthen ponds 30 days prior to the start of the experiment. The female broodstock were monitored for fertilized eggs. Applying the clutch removal method as used by Ahmed et al. (2007), fertilized eggs were collected directly from the mouth of incubating female broodstock, and were checked using the egg staging criteria described in Table 1. Only stage I eggs were cleaned and stocked in 2 L McDonald type incubations jars. Each incubation jar was stocked with 265 eggs. The egg population in the jars was limited by availability of stage 1 eggs. The eggs were not

1 treated prior to incubation. Three temperature treatments: 25, 27 and 29°C were used, each replicated thrice. The two higher water temperature levels were attained by placing one heater (RL-200N, Nippe, China) in each of the two sumps to achieve 27 and 29°C, respectively. The third sump used unheated water which was at an average of 25°C. A submersible pump (DAB pump Nova 180, Mastrino, Italy) was installed in each of the three 2,800 L sumps which recirculated water at 0.15L/s. Each temperature treatment had a separate recirculating system. Daily, 10% of the water was replaced using fresh water from the main system to maintain good water quality. Hatched, unhatched and dead eggs were enumerated at the end of each stage by pouring out all eggs from the jar into a plastic basin (100% sampling) and close observations were made under clear water. The end of each stage was defined as the time when at least 50% of the eggs from each jar had passed into the next stage. The stage period (days) was computed as the difference between the end time of one stage and the end of the previous stage. Hatching period was the total number of days from fertilization to where 50% of the eggs had hatched and the hatchlings or fry had swam out of the jar into the tray. Criteria for age staging used in this study came from synchronization of stages used by various authors in Tilapia and other related fishes. Thus the five stages are assigned to broad, common embryonic periods (Table 1).

Dissolved oxygen, pH, and temperature were measured twice daily at 10:00 and 14:00 h, while ammonia was measured twice during the experimental period.

A regression function, in SPSS 16.0, was used to relate the cumulative time up to the end of each developmental stage, and stage duration with incubation temperature. The best model was then selected. Adequacy and suitability of model fit was assessed using standard procedures by comparing p-values, sums of squares and regression coefficient ( $R^2$ ). Hatching period was compared among treatments using Analysis of Variance (ANOVA) and means were separated using the Least Squares Difference

<sup>1</sup> Other studies recommended 27°C for hatching of Tilapia. Therefore we added 2 levels below and above 27°C.



**Figure 1.** *Oreochromis karongae* development times to the end of stages I (◆), II (●), III(▲), IV(+) and V(\*). Lines are the predicted relationships for regression.

(LSD) method.

## RESULTS

Mean hatching period was statistically different among the three treatments ( $p=0.000$ ). The longest hatching period was  $14.70\pm0.10$  days after fertilization (DAF) at  $25^{\circ}\text{C}$ , followed by  $9.20\pm0.20$  DAF at  $27^{\circ}\text{C}$ . The shortest hatching period was  $7.33\pm0.15$  DAF at  $29^{\circ}\text{C}$ . Hatchability at high temperatures was higher (51.9% at  $29^{\circ}\text{C}$ , and 52.2% at  $27^{\circ}\text{C}$ ), than (41.2%) at lower temperature. High mortalities were observed at the beginning of the experiment at  $29^{\circ}\text{C}$  due to temperature shock. By the end of the study, there was 100% fry mortality in the  $25^{\circ}\text{C}$  treatment. Fry survival rate was higher at high temperatures (99% at  $27^{\circ}\text{C}$ ) and 93% at  $29^{\circ}\text{C}$ ), than for low temperature (50% at  $25^{\circ}\text{C}$ ). There was a curvilinear relationship between egg development and temperature (Figure 1). The following model (1) was selected and fitted using the logarithm regression function of SPSS:

$$D = \alpha + \beta \ln(T) \quad (1)$$

where  $D$  is cumulative time (days) to the end of development stage,  $T$  is mean incubation temperature ( $^{\circ}\text{C}$ ), residuals are normally distributed and  $\alpha$  and  $\beta$  are estimated parameters (Table 2). The stage duration data were similarly adequately fitted using model (1) and model parameter estimates,  $\alpha$  and  $\beta$  are presented in Table 2.

Mean temperatures were different;  $25.3\pm0.1$ ,  $27.0\pm0.1$  and  $29.0\pm0.1^{\circ}\text{C}$  ( $p=0.000$ ). Mean dissolved oxygen was

not statistically different ( $p=0.221$ ) across the treatments:  $7.44\pm0.06$ ,  $6.75\pm0.6$  and  $6.64\pm0.06$ , for  $25$ ,  $27$  and  $29^{\circ}\text{C}$  treatments, respectively. Similarly, pH;  $7.73\pm0.05$ ,  $7.70\pm0.05$  and  $7.78\pm0.05$  was not statistically different ( $p=0.485$ ) across the treatments, respectively. Very low levels of ammonia were detected but they were statistically different ( $p=0.000$ );  $0.006\pm0.000$ ,  $0.007\pm0.000$  and  $0.009\pm0.000$  mg/L, respectively.

## DISCUSSION

The longest hatching period was 14.7 days and was recorded at  $25^{\circ}\text{C}$  while the shortest hatching period was 7.3 days and it was in the highest temperature treatment ( $29^{\circ}\text{C}$ ). Similar results were observed in Common carp, *C. carpio*, (El-Gamal, 2009), *Oreochromis niloticus* (Bhujel et al., 2000), and in cod, *G. morhua*, (Geffen et al., 2006) where hatching period decreased with increase in incubation temperature. In another study on *O. niloticus*, hatching period decreased from 7 to 3.3 days with increase in temperature from  $24$  to  $27^{\circ}\text{C}$  (El-Naggar et al., 1998). While the trend is the same as in the present study, the difference in extent of reduction in hatching period may be attributed to differences in species performance. For example, *O. niloticus* generally grows faster and bigger than *O. karongae* (Msiska, 1998; Kapute et al., 2007) used in this study. Since dissolved oxygen and pH were uniform, ammonia despite being different was lower than  $0.01$  mg/L; they were within tolerable limits for good growth of the Tilapia eggs (Beveridge and McAndrew, 2000). Therefore, dissolved oxygen, pH and ammonia may not have significantly

**Table 2.** Parameter estimates for regression model fit to *Oreochromis karongae* egg development data (A) cumulative days to end of stage and (B) stage duration.

Stage	Parameter	Estimate	S.E.	T value	F (d.f.)	R <sub>2</sub>	Overall P
<b>(A) Cumulative days to end of stage</b>							
I	A	24.791	4.662	5.317	23.211(1.7)	0.735	0.002
	B	-6.818	1.415	-4.818			
II	A	48.539	7.388	6.57	36.796(1.7)	0.817	0.001
	B	-13.602	2.242	-6.066			
III	A	73.373	13.987	5.246	23.211(1.7)	0.735	0.002
	B	-20.453	4.245	-4.818			
IV	A	143.732	18.29	7.859	53.854(1.7)	0.869	0.000
	B	-40.74	5.552	-7.339			
V	A	159.835	14.775	10.818	102.465(1.7)	0.927	0.000
	B	-45.396	4.485	-10.123			
<b>(B) Stage duration in days</b>							
I	A	24.791	4.662	5.317	23.211(1.7)	0.735	0.002
	B	-6.818	1.415	-4.818			
II	A	23.748	2.726	8.713	67.256(1.7)	0.892	0.000
	B	-6.784	0.827	-8.201			
III	A	24.834	6.599	3.763	11.700(1.7)	0.572	0.011
	B	-6.851	2.003	-3.42			
IV	A	70.358	4.303	16.35	241.233(1.7)	0.968	0.000
	B	-20.287	1.306	-15.532			
V	A	16.103	3.515	4.582	19.046(1.7)	0.693	0.003
	B	-4.656	1.067	-4.364			

influenced hatching period and the decrease in hatching period is highly attributed to temperature increase. Similar results were observed in marine species whereby egg development was enhanced by increased temperature in horse mackerel, *Trachurus trachurus*, (Cunha et al., 2008), largemouth bass, *Micropterus salmoides*, and smallmouth bass, *Micropterus dolomieu*, (Landsman et al., 2011). These findings confirm earlier studies suggesting that temperature is an important factor determining egg and larval development as it influences metabolic rate (Blaxter, 1992; Kamler, 2008) and cellular function (Somero and Hofmann, 1997). Increase in temperature is known to speed up metabolism through biochemical activity stimulated by heat energy (Beveridge and McAndrew, 2000) which results in enhanced development of the fish eggs. This phenomenon also resulted in higher hatchability rate at higher temperatures and low hatchability at low temperatures as also observed between 27 and 30°C in *C. carpio* by El-Gamal (2009).

Hatchability was generally low even at 29°C than 27°C due to high egg mortalities at the beginning of the experiment which were as a result of temperature shock that was not adequately managed when introducing eggs in the jars. Nevertheless, the hatchability for higher temperatures was still higher than for low temperature. The highest fry mortality (100%) was observed at the lowest temperature (25°C). El-Gamal (2009) also recorded high fry mortalities at low temperatures (62% at 24°C and 100% at 20°C, respect-

ively) in *C. carpio*. However, these results suggest that temperature affects the tolerance level of eggs and fry. Low temperatures are likely to impose a cold shock which may increase in intensity with decrease in temperature resulting in increased stress and egg and fry mortality.

Determining temperature-dependent development rates for *O. karongae*, in Malawi, is a rather novel idea. Thus the current findings have formed the basis for future comprehensive work on temperature-dependent development schemes for eggs of *O. karongae* and other important related fish species for aquaculture and fisheries management. For this reason, it is important to understand the rationale for adopting the methodologies used in this kind of study. The staging and ageing scheme used in this study is based on other schemes developed and refined over three decades for similar fish such as *O. niloticus*, *T. zilli* and other species such as *G. morhua*. The scheme adopted in this study is however, simplified to make it more practical for easy application by small scale hatchery operators and researchers. It combines both microscopic and apparent stage and development features that make it a conventional scheme. Similar attempts have been made by Geffen et al. (2006), in the development of *G. morhua* egg development schemes and rates. These authors have argued that there is always undeniable inherent variability in temperature and egg development relationships because more than one parent female brood fish is used.

Furthermore, there is no clear instantaneous end of egg development stage. Therefore, it would not be appropriate to wait until all eggs advance past a certain stage to designate stage duration. In this regard, days after fertilization refer to the period starting from clutch removal after fertilization to where 50% of eggs reach the stage marker. It is justifiable to adopt the criterion of 50% of sampled individuals passing the particular stage marker, as was used in the present study where all eggs were sampled. By definition, stage duration is the time that an egg is in a stage or the time from the point when 50% of the eggs were in stage  $n - 1$  to when 50% of the eggs are in stage  $n + 1$  (Geffen et al., 2006). Some authors, however, refer to stage duration as the cumulative time from egg fertilization to the end of stage (Page and Frank, 1989).

## Conclusions

The logarithm temperature-dependent development model was selected, in this study, as it was easier to describe than the power model. The model described the curvilinear relationship between temperature and cumulative days after fertilization more adequately than the log-linear regression function used in other studies. The logarithm model was however close enough to the power model. There is potential for increased temperature between 25 and 29°C to significantly reduce hatching period and increase hatchability and fry survival. This being the first research of its kind in *O. karongae*, it is advisable to apply the model between 25 and 29°C.

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