

Cold tolerance of selective breeding of *Oreochromis niloticus* and *oreochromis aureus*

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ABSTRACT

Selected fish for higher growth rate from both *O. niloticus* and *O. aureus* were collected and subjected to challenge test for cold. In addition to, fish of both species collected from production ponds of Abbassa were used to compare the cold tolerance of fish. Fry of similar age were grown under identical conditions. *O. niloticus* with an average weight of 1.29 ± 0.73 g and 0.95 ± 0.11 g, for *O. aureus* were used in this study. The selected *O. niloticus* revealed lower cold tolerance than the non-selected fish. The cooling degree hours were significantly different between the selected and non-selected *O. niloticus* ($P < 0.005$). The selected *O. aureus* exhibited greater cold tolerance than the non-selected and death began at 14.1°C , while non-selected occurred at 15.2°C .

The lowest lethal temperature of 50% individuals was 8.7°C for selected *O. aureus* at 198 cooling degree hours (CDH) and 9.3°C for non-selected fish at 147 CDH. Both selected and non-selected fish gave lowest lethal temperature of 60% individuals at 8.5°C . But in different degree hours which was longer in selected (220 CDH) and shorter in non-selected (182CDH). The selected fish with stood 330 cooling hours which was higher than that for the non-selected fish (311 CDH).

There was strong relationship between the cold and both temperature at death and cumulative degree hours represented by negative correlation coefficient for all tested fish (-0.928 ± 0.014). There was no correlation between cold tolerance and fish size for all tested fish.

INTRODUCTION

In aquaculture industry in tropical and subtropical regions of the world, tilapia plays an important role in fish production. The ability of tilapia to tolerate wide ranges of water quality, diets and farming system help in transferring and distributing tilapia from Africa to many countries.

Tilapia is not able to grow and survive in cold waters outside its

environment; before it is adapted (Gjedrem, 2005). Since different tilapia species and strains have been introduced to many different geographical regions of the world, their response to water temperature in their new habitats requires prime attention (El-Sayed, 2006). Egyptian tilapia is the best populations in growth during genetic improved tilapia (GIFT) program in Asia (Eknath *et al.*, 1993).

Tilapia generally survived constant at minimum temperatures over 12 °C but survival rate was mediated by the rate of decline, mass–length ratio and strain. For every 1°C increase in minimum temperature, tilapia was 2.76 times more likely to survive. Fish exposed to rapid temperature decline were less likely to survive than those exposed to slowly decreasing temperature (Wilson *et al.*, 2009). The survival rates of *Oreochromis aureus* juveniles, in concrete ponds and hapas suspended in earthen ponds, during the hard winter conditions in Egypt were recorded by Kamel *et al.* (2008).

Cold tolerance is a trait of great economic importance in tilapia, as severe mortalities occur during the winter in temperate climate countries (Tave *et al.*, 1990). The variation of cold tolerance among and within species is correlated to their geographical distribution (Khater & Smitherman, 1988). Also, the inability of tilapia to tolerate low temperature is of major economic concern as it reduces their growing season and leads to over winter mortality (Charo-Karisa *et al.*, 2005).

It is well documented that during winter, water temperature may drop to levels that cause severe growth inhibition and mortality. The fluctuation by increasing and decreasing of temperature during autumn and spring, affect the fish reproductive physiology and fry production.

The tolerance of tilapia species and their hybrid to cold was recorded (El Gamal, 1988; Khater & Smitherman, 1988; Behrends *et al.*, 1990; Tave *et al.*, 1990; Cnaani *et al.*, 2000; Sifa *et al.*, 2002).

Several studies have been carried out on interspecific hybrids of tilapia under ambient winter temperatures and concluded that cold tolerance behaved as a dominant trait (Wohlfarth *et al.*, 1983). On the other hand, studies by Behrends *et al.* (1990, 1996) and Tave *et al.* (1990) with *O. aureus* and *O. niloticus* under ambient winter temperatures concluded that cold tolerance was inherited as an additive trait.

Selection breeding can enhance the cold tolerance of tilapia (Behrends & Smitherman, 1984). The tolerance of selective GIFT tilapia to cold was reported (Sifa *et al.*, 2002). Cold tolerance as a trait has been variously described. It has been described as low lethal temperature or temperature at death (TAD) (Khater and Smitherman, 1988; Behrends *et al.*, 1990; Atwood *et al.*, 2003). It has also been described as cooling degree days (CDD) or cumulative degree hours (CDH) (Behrends *et al.*, 1990; Cnaani *et al.*, 2000; Atwood *et al.*, 2003) or simply as by number of days until death (Tave *et al.*, 1989).

The aim of this investigation was to study the impact of selection for higher body weight on improving cold tolerance in *O. niloticus* and *O. aureus*.

MATERIALS AND METHODS

Stock fish:

The original stock of *Oreochromis niloticus* fry fish used in this study was from Maruit hatchery and adapted to Abbassa environmental condition at 1999 (Kamel, 1999), while *Oreochromis aureus* from the production ponds at Abbassa was studied by Rezk *et al.* (2002). Fry of the same age from the fifth generation

of mass selection, of *O. niloticus* and *O. aureus* from the World Fish Center, Abbassa, Egypt were used, in addition to unselected fish groups for both, used as control groups. All groups for selected and unselected fry of similar age and grown under identical conditions *O. niloticus* were with an average weight of 1.29 ± 0.73 g, while, it was 0.95 ± 0.11 g, for *O. aureus*. The fry was transferred from hapas suspended in earthen ponds to glass aquaria (450 l). Two replicates aquaria for each group, random sample of one hundred fry per aquarium in addition to, one aquarium with water only. All fry were acclimated for one week to aquaria condition before starting the experiment and the aeration in the aquaria by air stone from air pump.

Challenge test for cold

Nine aquaria were placed in cold room operated by a thermostatically controlled chilling unit. The fry were acclimated at ambient temperature (20 °C). Each aquarium was constantly aerated using three air-stones connected to an air-pump.

The temperature of the aquarium water was adjusted to the desired level by adjusting the thermostat controlled chilling unit.

The acclimatizing the fry was done at 20 °C for 48 hours. After that aquarium water temperature was monitored each hour from the beginning to the end of the experiment.

The temperature measurements were done hourly, while the dissolved oxygen (DO), pH, total ammonia, nitrate and nitrite were measured once a day, using a WTWR multi 340i meter and HACH kits. Dissolved oxygen ranged between 6.1 and 10.3 mg/l; pH, 7.9–8.3; ammonia, 0.01–0.1 mg/l; nitrate, 0.5–2 mg/l; and nitrite,

0.01–0.02 mg/l. Aquaria were cleaned twice daily by suction to remove faeces. Water that was removed during aquarium cleaning was replaced with clean water pre-cooled to the same temperature with ice cubes.

Fish were not fed during the experiment. Temperature was first lowered to 16 °C within 48 h, and then water temperature was reduced at a rate of 1 °C daily till the end of the experiment. Aquaria were observed once each hour for any fish mortality. Death was defined as the point at which fish lost balance, fell on their side and ceased fin, body and opercula movements and lost response to external stimuli. Throughout the experiment, dead fish were removed from the tanks at the end of each hour with a scoop net, and their tag and aquarium numbers recorded. Mortality was recorded hourly for each fish, which enabled us to quantify cooling degree hours (CDH) Temperature at death (TAD), recorded hourly, was used in this study to measure the cold tolerance.

Data Analysis

The mean cooling-degree hours regression and correlation were calculated by SAS program. Individual cold tolerance was quantified using a cooling-degree-hours (CDH) statistic (after Cnaani *et al.*, 2000), calculated as

$$CDH = \sum_{i=1}^k (t_0 - t_i)$$

Where: i = days, t_0 = 16 °C, the initial temperature, t_i = temperature at the temperature measured at check-point i , and k is the check-point when mortality of the fish was recorded. For example, the score for a fish that survived 1 hour in 15.5 °C and 1 hour

in 15°C, is $(16 - 15.5) + (16 - 15) = 1.5$ hour.

RESULTS

The cold tolerance among the tested fish varied as shown in Table (1). The mortality was started for selected *O. niloticus* at 14.8 °C, while the non-selected fish began to die at 13.6 °C.

The lowest lethal temperature of 50% individuals was 9.9 °C for selected *O. niloticus* fish and complete mortality occurred at 8.5 °C. At the same time the lowest lethal temperature of 50% individuals was 8.9 °C for non-selected fish (Table 1). The cooling degree hours were significantly different between the selected and non-selected *O. niloticus* ($P < 0.005$). The selected fish survived up to 260 cooling hours, which was lower than that for the non-selected fish (294 CDH) as recorded in Table (1).

The selected *O. aureus* exhibited greater cold tolerance than the non-selected and started to die at 14.1 °C, against unselected death at 15.2 °C (Figure 1A,B). The lowest lethal temperature of 50% individuals was 8.7 °C for selected *O. aureus* and 9.3 °C for non-selected fish. The cooling degree hours of 50% individuals was 198 hrs for selected *O. aureus* and 147 hrs for non-selected fish (Table 2).

Both selected and non-selected fish gave lowest lethal temperature of 60% individuals at 8.5 °C. But in different cooling degree hours which was longer in selected (220 CDH) and shorter in non-selected (182CDH). From lowest lethal temperature of 70 individuals, the non-selected exhibited more tolerance to cold. Complete mortality occurred at 7.8 °C for selected and 7.4 °C for non-selected fish.

The selected fish survived up to 330 cooling hours which was higher than that for the non-selected fish (311 CDH) as recorded in Table (1)

There was no correlation between cold tolerance and fish size for all tested fish.

DISCUSSION

The results of the experiment indicated that the selected fish of *O. niloticus* (Abbassa) was less tolerate to cold which agreed with Sifa *et al.* (2002) they recorded higher tolerance to cold for Egypt 88 than the genetic improved farmed tilapia (GIFT). But during study of the growth of Abbassa fish was the lowest as recorded by (Kamel, 1999), this means that there was no correlation between growth and cold tolerance.

The selected fish was introduced to Abbassa at 1995 as fingerlings from Maryout hatchery and gave the highest growth rate as recorded By Kamel (1999) and Rezk *et al.* (2002); newly introduced tilapia have lower cold tolerance than those which have been introduced and adapted to local cold temperature for a longer time (El-Sayed, 2006). They had not previously been exposed to the selective pressures of low temperature, while, fish from Abbassa station were introduced from Ismailia Canal and had acclimatized during the winter condition in ponds to tolerate the decreasing temperature; which agreed with Cnaani and Hulata (2000) who reported that better fish survival rate during winter due to longer period of time as the fish were exposed to low temperatures on the farms. Temperature at death incidences detected in this study were in full agreement with those reported in earlier studies on *O. niloticus*. Many records for the mortality of the

Egyptian strain has been reported at 11 °C to 9 °C (Khater and Smitherman, 1988), 13 °C to 10 °C (Lahav and Ra'anani, 1998) and 10.1°C to 8.6°C (Charo-Karisa *et al.*, 2005). Better cold tolerance was recorded with the first mortality at 11 °C and total mortality at 7.4 °C for the Egyptian strain of *O. niloticus* used in China with fish size ranged from 60-120 g, (Sifa *et al.*, 2002). El Gamal, 1988 mentioned lower lethal temperature (LC50) for *T. nilotica* (8.28°C) and *T. aurea* (7.33 °C). The differences among all earlier experiments for cold and this study were due to the difference in fish size which was 1.29 ±0.73 g in this study. The same observation was recorded by Hofer and Watts (2002), who illustrated that smaller fingerlings, below 5 g, were more susceptible to acute exposure to lower temperatures stress. No correlation was recorded for fish weight and cold tolerance for all tested fish which agreed with Cnaani *et al.* (2000). Also body weight within populations was not significant factors affecting cold tolerance (Behrends, 1990). Size significantly affected mortality, with smaller fish being less tolerant to low temperatures than larger fish (Atwood *et al.*, 2003). In contrast, strong impact of weight on cold tolerance was reported by (Charo-Karisa *et al.*, 2005). There was strong relationship between the cold and both temperature at death and cumulative degree hours as indicated by negative correlation coefficient for all tested fish (-0.928± 0.014). The same observation was recorded with *O. niloticus* by Charo-Karisa *et al.* (2005), who recorded negative correlation (-0.88 ±0.019). The lowest lethal temperature of 50% individuals was

8.7 °C (198 CDH) for selected *O. aureus* and 9.3 °C (147 CDH) for non-selected fish. Both selected and non-selected fish gave the lowest lethal temperature of 60% individuals at 8.5 °C; but in different cooling degree hours which was longer in selected (220 CDH) and shorter in non-selected (182CDH). The previous results indicated that the cooling degree hours was more accurate measure of the genetic variation in cold tolerance than the temperature at death which agreed with Charo-Karisa *et al.* (2005). The results indicated positive genetic correlation response to selection for higher growth rate and cold tolerance in *O. aureus*, while it was negative for *O. niloticus* which agreed with Dunham (2006) who concluded that correlated responses to selection affected several other traits, both positively and negatively. Gjedrem and Baranski (2009) reported that selection for one trait will influence other traits that are genetically correlated and Fjalestad (2005) who found that farmed Atlantic salmon fish from seven generations of selection for increased growth rate seems to be less sensitive to environmental stress than genetically wild fish.

CONCLUSION

The selected *O. niloticus* fish was less tolerate to cold which mean that the selection for higher growth rate had negative effect to cold. While the selected *O. aureus* showed that tolerance to cold was positively correlated with selection. The cooling degree hours were more accurate measure of the genetic variation in cold tolerance than the temperature at death. No correlation was recorded for fish weight and cold tolerance for all tested fish

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Table (1): Mortality percentage, cooling degree hours (CDH) and temperature at death (TAD) for selected and non-selected *O. niloticus*

Mortality %	Selected <i>O. niloticus</i>		Non-selected <i>O. niloticus</i>	
	CDH	TAD	CDH	TAD
10	12	14.8	29	11.3
20	40	12.1	72	10.4
30	63	11.0	114	9.8
40	89	10.5	167	9.1
50	119	9.9	195	8.9
60	143	9.6	210	8.7
70	150	9.5	225	8.5
80	176	9.1	240	8.4
90	210	9.3	270	8.3
100	260	8.5	294	8.2

Table (2): Mortality percentage, cooling degree hours (CDH) and temperature at death (TAD) for selected and non-selected *O. aureus*

Mortality %	Selected <i>O. aureus</i>		Non-selected <i>O. aureus</i>	
	CDH	TAD	CDH	TAD
10	35	11.2	6	14
20	73	10.2	20	12.7
30	110	9.7	51	11.8
40	157	9.2	98	10.4
50	198	8.7	147	9.3
60	220	8.5	182	8.5
70	236	8.4	237	8.1
80	259	8.3	245	8.1
90	282	8.1	277	7.7
100	330	7.8	311	7.4

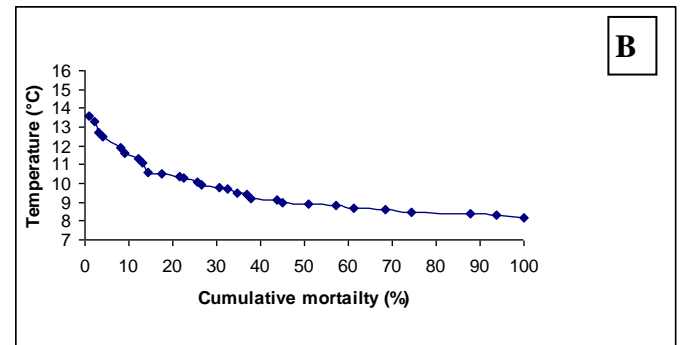
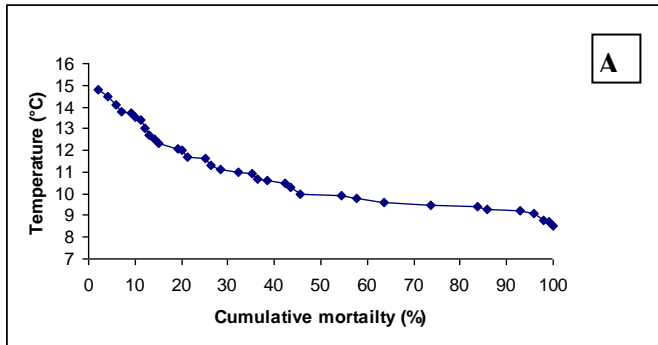


Figure (1). Cumulative mortality curves in cold-challenged for *O. niloticus*. (A) selected (B) non-selected

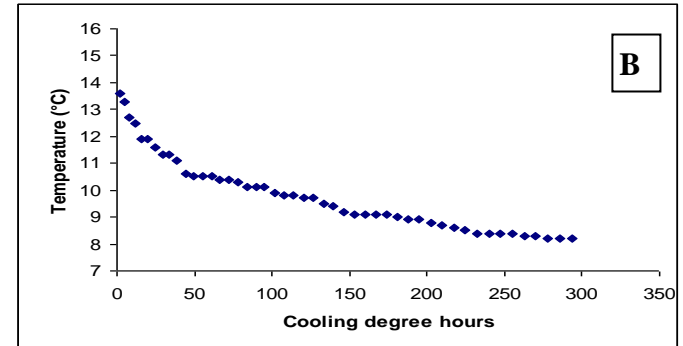
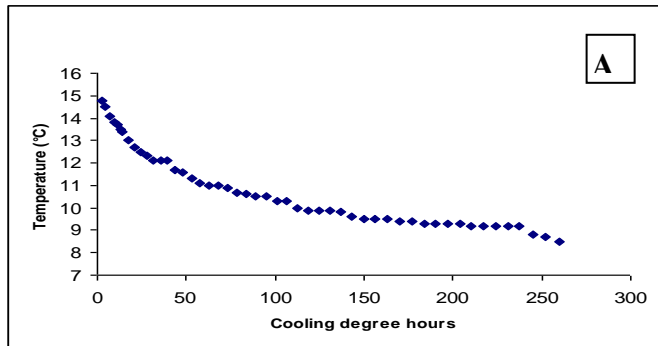


Figure (2). The relationship between the cooling degree hours and temperature for *O. niloticus* (A) selected (B) non-selected

Cold tolerance of selective breeding of *oreochromis niloticus* and *oreochromis aureus*

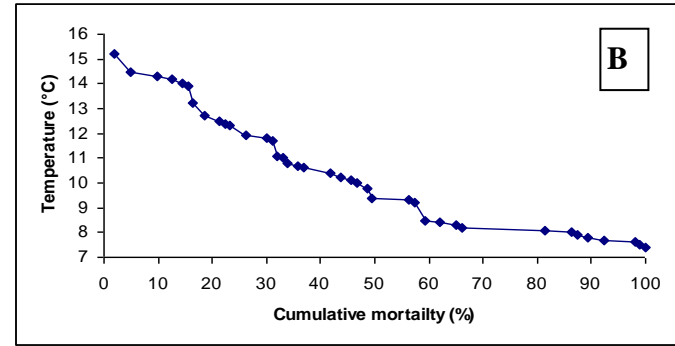
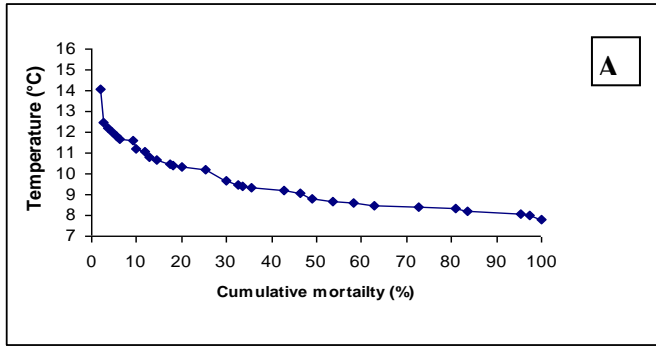


Figure (3). Cumulative mortality curves in cold-challenged for *O. aureus* (A) selected (B) non-selected

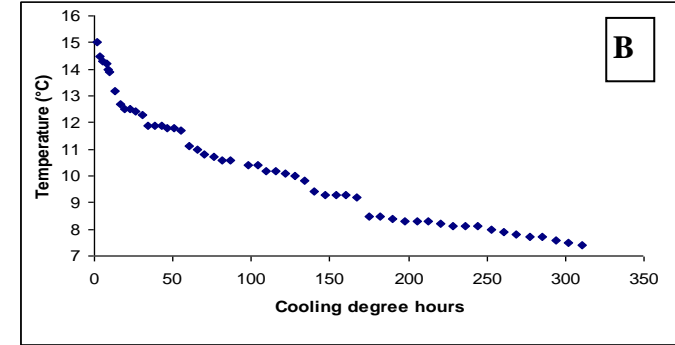
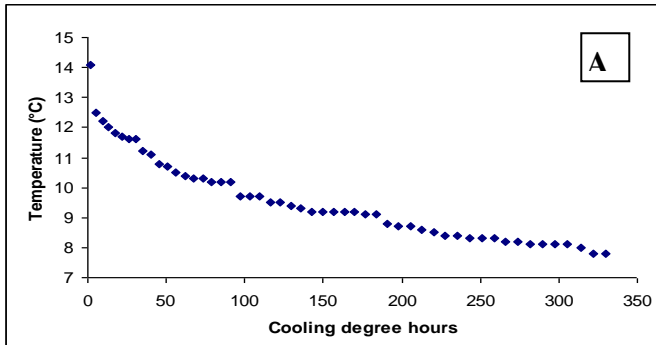


Figure (4). The relationship between the cooling degree hours and temperature for *O. aureus* (A) selected (B) non-selected

تحمل البرودة لأسماك منتخبة وراثيا من البلطي النيلي و البلطي الاوربا

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الملخص العربي

تم تعريف أسماك البلطي النيلي والبلطي الاوربا المنتخبة لأعلى معدلات و أسماك مجمعة عشوائيا من الأحواض الانتاجية بالعباسة لنفس الانواع للبرودة وذلك لدراسة مقارنة لتحمل البرودة في أسماك البلطي النيلي والبلطي الاوربا.

تم استخدام زريعة بمتوسط وزن 0.73 ± 1.29 جرام للبلطي النيلي و 0.11 ± 0.95 جرام للبلطي الاوربا وتعرضها لانخفاض في درجات الحرارة بدأ من 16 درجة مئوية حتى نفوق جميع الأسماك المستخدمة في الدراسة. تسجيل البيانات كان يتم كل ساعة لتجميع النافق و قياس درجات الحرارة.

وقد اظهرت النتائج ان هناك فروق معنوية بين أسماك البلطي النيلي المنتخبة و غير المنتخبة. بينما كانت أسماك البلطي النيلي المنتخبة أقل تحملاً للبرودة عن الأسماك غير المنتخبة.

بالنسبة لأسماك البلطي الاوربا المنتخبة كانت الاكثر تحملاً لدرجات البرودة عن الأسماك غير المنتخبة، وكان بداية النفوق في الأسماك المنتخبة عند 14,1 درجة مئوية بينما الأسماك غير المنتخبة عند 15,2 درجة مئوية.

كانت درجة البرودة المميتة لنسبة 50% من أسماك البلطي الاوربا المنتخبة 8,7 درجة مئوية بعد 198 ساعة من التعرض لها. بينما كانت 9,3 درجة مئوية بعد 147 ساعة للأسماك غير المنتخبة.

اظهرت كل من الأسماك المنتخبة والأسماك غير المنتخبة من أسماك البلطي الاوربا نسبة نفوق 60% عند درجة حرارة 8,5 درجة مئوية بينما كان هناك فروق معنوية في ساعات التعرض للبرودة والتي كانت 220 ساعة في الأسماك المنتخبة و 182 ساعة من التعرض للبرودة في الأسماك غير المنتخبة.

ثبت وجود علاقة قوية بين ساعات التعرض للبرودة ودرجة البرودة المميتة تمثلت بمعامل ارتباط سالب قوى ($-0,928 \pm 0,14$) لجميع الأسماك تحت الدراسة.

لم يتم تسجيل اى ارتباط بين تحمل البرودة وحجم الأسماك المستخدمة في هذه التجربة

يتضح من هذه الدراسة ان الانتخاب لأعلى معدلات للنمو في أسماك البلطي النيلي كان له تأثير سالب على تحمل البرودة بينما كان الانتخاب لأعلى معدلات نمو له تأثير موجب على تحمل أسماك البلطي الاوربا للبرودة.