EFFECTS OF REARING CONDITIONS ON LOW-TEMPERATURE TOLERANCE OF NILE TILAPIA, *Oreochromis niloticus,* JUVENILES

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Abstract

This paper summarizes the results of two experiments in which the effects of genotype, age, size, condition factor and diet (natural phytoplankton versus formulated protein pellets) on low-temperature tolerance of juvenile *Oreochromis niloticus* were studied. The experiments were conducted at the WFC experimental facilities in Abbassa, Egypt. In the first experiment, 775 juveniles from 43 sires and 80 dams were reared under mid-summer conditions for 41-91 days. In the second experiment, 393 juveniles were produced by single-pair mating of 20 dams and 20 sires from the same brooders as in the first experiment. These fish were reared for 42 days under autumn conditions with either high protein (40%) pellets or natural tilapia diet. At the end of the growth period fish from each experiment were tagged and exposed to gradually lowered temperatures. Cold tolerance was expressed as "temperature at death" (TAD and cumulative degree hours (CDH). Cold tolerance was significantly affected by genotype, size, aquarium, and condition factor (P= 0.0001). In both experiments, smaller fish were more vulnerable to cold stress. Diet and age did not significantly affect cold tolerance. Fish reared under mid-summer conditions died between 13.6 ºC and 8.6 ºC while those reared under autumn conditions died between 11.7 ºC and 7.5 ºC. This suggests that acclimatization to lower temperatures before cold stress can improve the cold tolerance ability of *O. niloticus.*

Introduction

One major constraint to the global expansion of tilapia farming is their sensitivity to low ambient temperatures. Of the tilapiine species, Nile tilapia, *Oreochromis niloticus (L.)* and its hybrids are the most important, constituting 90% of all tilapia cultured outside Africa. Exposure to extreme cold temperatures leads to mass mortality (Chervinski and Lahav, 1976). This makes over-wintering a serious economic challenge in major tilapia producing countries such as China and Egypt. In fish, the degree of tolerance to lethal temperatures is

dependent upon environmental effects, history of the fish and genetic effects (Cnaani *et al.*, 2000) as well as fish health and nutrition status. It has been reported for many ectotherms that animals can extend their thermal tolerance range through acclimatization and acclimation (Cossins and Bowler, 1987). In tilapia, prior acclimation temperature and rate of temperature reduction are considered important factors in determining mortality at a given temperature (Stauffer, 1986; Stauffer *et al*., 1988). It is thought that the ability of fish to adapt to different temperatures is closely linked to the lipid composition in their muscles (Hazel, 1984, Greene and Selivonchick, 1987). Fatty acid composition is in turn influenced by the fish's diet (Henderson and Tocher, 1987). Kelly and Kohler (1999), working with bass reported that fish fed their natural diet suffered no mortality when exposed to simulated cold fronts while those fed on a prepared diet had 50-90% mortality.

In this paper we present the results of two experiments in which the effects of genotype, age, size, condition factor, and diet (natural phytoplankton versus formulated protein pellets) on low-temperature tolerance of juvenile *Oreochromis niloticus* were studied. The experiments were conducted in two different periods of the year at the World Fish Centre experimental facilities at Abbassa, Egypt.

Materials and Methods

Experiment 1

This experiment was carried out during warmer temperatures at the beginning of the summer (June-July 2003). Fish were produced in a full-sib/half-sib mating design in which each sire was mated to two dams and each dam mated to only one sire. A total of 80 full-sib families were produced from 43 sires and 80 dams. 60 fry from each full sib family were reared in 80 separate 2 x 3 m hapas until they were tagged. The hapas were fixed in two 1000 m^2 ponds. Fry were 41-91 days old at the beginning of the cold tolerance challenge. 10 healthy individuals from each full-sib family were tagged with Floy tags between the dorsal fin and lateral line and used in the cold tolerance challenge. Individual body weights and standard lengths were recorded.

Experiment 2

This experiment was carried out in the fall (September-October) of 2003. Twenty full-sib families were produced by single-pair matings of 20 dams and 20 sires chosen randomly from the brooders used in the first experiment. Experimental fish were reared in separate 6 m^2 hapas up to swim-up. Two groups of 30 swim-up fry each were obtained from each family and randomly assigned to two treatments described below. The growth experiment was carried out in a 4000 m^2 pond. The pond was fertilized with chicken manure at the rate of 50 kg $ha^{-1}day^{-1}$. Two rows of 20 (2 m X 1 m) hapas were placed in opposite ends of the pond. Fish in one row could feed only on naturally available food (Bowen, 1982; Spataru *et al*., 1983) and phytoplankton induced by the chicken manure application. In the other row, fish were in addition fed twice daily (9.00 and 13.00 hrs) with 40% formulated protein pellets at 30 % of their body weight. Fish were sampled on day 14, 21, 28, 35 and 42. In each sampling day, fry were counted, bulk weighed and average (family) weight recorded. At day 42, individual body weights and standard length measurements were also

taken. Next, 20 randomly chosen individual fry from each full-sib family (10 per treatment) were tagged and used for the cold tolerance challenge.

Fish condition and growth

Fulton's condition factor was computed for each individual by the formula: CF = $100W/L^3$ (Ricker, 1975), where W= body weight and L= body length. Specific growth rate (SGR; Experiment 2 only) was calculated according to Cho and Kaushik (1985): (ln final weight – ln initial weight)/time (days).

Cold tolerance challenge

After one day in ceramic tanks at ambient temperature, individuals from each family were randomly assigned to any of five 450 L glass aquaria set in a cold room. The room was served by a thermostatically controlled chilling unit. 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 compressor settings of the chiller. Fish were acclimatized to these aquarium conditions for 48 hours at 20 ºC prior to initiating the challenge. Fish were not fed during the cold challenge.

Following acclimatization, the temperature was first lowered to 16 ºC within 48 hours, and then to 11 ºC within the next 48 hours. From then on, water temperature was reduced at the rate of 1°C per day. 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. Dead fish were removed from the tanks at the end of each hour with a scoop net, and their tag and aquarium numbers recorded. Cold tolerance was quantified as cooling degree hours (CDH) (Behrends *et al*., 1996) and temperature at death (TAD). CDH represents the sum of hours the fish survived multiplied by the difference between the hourly and initial temperature for each fish. As in earlier studies (Behrends *et al*., 1996; Cnaani *et al*., 2000, Caani, 2003), the initial temperature for calculation of CDH was 16 ºC.

Aquarium water temperature was monitored hourly from beginning to end of the experiment. DO, temperature and pH were measured once a day with WTW® multi 340i meter. To maintain water quality within acceptable levels, total ammonia, nitrate and nitrite, were measured daily with HACH kits. Aquaria were cleaned twice daily by suction to remove faeces. Water that was removed during aquarium cleaning was replaced with clean water that had been pre-cooled with ice cubes.

Data analysis

All analyses were carried out using SAS software (SAS, Institute, Cary, NC, USA). Factors affecting cold tolerance in the first experiment were analysed by analysis of variance with the generalised linear model (GLM) including sire, dam, aquarium, age, and size effect using the following model.

$$Y_{ijkl} = \mu + a_i + \beta_1 * AGE_{ijk\,l} + \beta_2 * ln(w)_{\,ijkl} + s_j + d_k(s_j) + e_{ijkl} \qquad \text{(Model 1)}$$

Where Y_{ijkl} = cooling degree hours for the lth individual; μ = overall mean; a_i = fixed effect of aquarium (i = 1, 2, 3, 4, 5); β_1 = regression coefficient of age; AGE_{ijkl} = a co-variable of age of the lth individual; β_2 = regression coefficient of natural logarithm of body weight; $ln(w)_{ijkl}$ = a co-variable of the natural logarithm of body weight of the lth individual; s_j = effect of the jth sire; $d_k(s_j)$ = effect of the kth dam nested within the jth sire; and e_{ijkl} = random residual effect associated with the lth individual.

In the second experiment the effects of diet, genotype, aquarium, body weight, standard length, specific growth rate, and condition factor were analyzed. Specific growth rate did not affect cold tolerance in the presence of body weight and was therefore removed from the model. As in Model 1, the natural logarithm of body weight was used instead of body weight. To investigate presence of genotype by environment interaction for cold tolerance, an interaction of family and treatment (diet) was included in the model. The following model was finally fitted

$$Y_{ijkl} = \mu + a_i + g_j + t_k + \beta_1 * lnw_{ijkl} + \beta_2 * c_{ijkl} + g_j * t_k + e_{ijkl} \qquad \text{(Model 2)}$$

Where Y_{ijkl} = cooling degree hours for the lth individual; μ = overall mean; a_i = fixed effect of aquarium (i = 1, 2, 3, 4, 5); g_j = effect of the jth family; t_k = effect of diet $(k = 1, 2)$; β_1 and β_2 = regression coefficients of body weight and condition factor respectively; lnw_{ijkl} = a co-variable of natural log of body weight of the lth individual; c_{ijkl} = a co-variable of condition factor of the lth individual; and e_{ijkl} = random residual effect associated with the lth individual. Comparison of the two diet groups was done using Student's t-test.

Results

Experiment 1

Means and standard deviation of body weight (BW) and length (SL), condition factor (CF), temperature at death (TAD) and cooling degree hours (CDH) are shown in Table 1. Size of fish ranged from 1 to 20.6 g body weight and 29.6 to 78 mm standard length. Fish died due to cold from 13.6 ºC to 8.6 ºC and had a mean cooling degree hours of 298. The GLM analysis from Model 1 showed significant effects ($P < 0.0001$) of body weight, and genotype (sire and dam) on cold tolerance. Age did not significantly affect cold tolerance. There was a tendency for smaller fish to have lower CDH values suggesting that size affects the ability of fingerlings to survive low temperatures.

The correlation coefficient of CDH on body weight was low but significant (0.58, P = 0.0001) with a standard error of 0.92 and R^2 of 0.34. The relationship between body weight and CDH was logarithmic with an inflection point around 5g.

Table 1. Overall means and standard deviations of body weight, standard length, age and cold tolerance responses of *Oreochromis niloticus* juveniles exposed to experimentally lowered temperatures.

Trait	Mean	Std. deviation	Minimum	Maximum
Body weight (g)	5.10	2.35	1.0	20.6
Standard length (mm)	50.61	7.60	29.6	78.0
Age (days post-hatch)	79.00	8.62	41.0	91.0
Temperature at death (ºC)	10.10	0.56	8.6	13.6
Cooling degree hours	298.07	67.86	6.4	440.3
*LT_{50} (full-sib) (ºC)	10.10	0.37	9.3	11.5
LT_{50} (half-sib) (ºC)	10.10	0.24	9.4	11.1

***Experiment* 2**

Means and standard deviation of body weight (BW) and length (SL), condition factor (CF), specific growth rate (SGR), TAD and CDH within diet treatments are shown in Table 2. Mortality of fish from the two treatments with lowering of temperature is shown in Figure 1. The natural-fed fish started dying at 11.7 °C while the pellet-fed group began dying at 11.5 °C. The lowest TAD at which all fish died was 7.5 °C and 7.6 °C for the pellet-fed and natural-fed fish respectively. Fish from the two treatments differed significantly with respect to CF (P= 0.0002) and cold tolerance (when expressed as TAD (P= 0.0348) or CDH (P= 0.0414)). There were no significant differences in SGR (P = 0.8659), BW (P = 0.4771) or SL (P = 0.2239) between the two diet groups although these values were slightly higher for the pellet-fed fish. Size (BW), condition factor, family, and aquarium significantly affected cold tolerance. Cold tolerance was not significantly affected by diet (P = 0.3255) or specific growth rate. There was a significant interaction between family and diet on cold tolerance (p= 0.011). Pellet-fed fish had generally higher CDH values, but in some families natural-fed fish had higher CDH values (Figure 2).

Table 2. Means and standard deviations of body weight, standard lengths, specific growth rate, condition factor, and subsequent temperature at death and cooling degree hours of juvenile *O. niloticus* reared for 42 days on different diets.

Parameter	Diet		
	Pellet-fed	Natural-fed	P-value
Initial weight (g)	0.045(0.03)	0.045 (0.03)	-
Final weight (g)	1.97 (0.65)	1.92 (0.61)	0.4771
Standard length (mm)	38.05 (3.99)	37.58 (3.81)	0.2239
Specific growth rate (%/day)	9.37 (1.21)	9.34 (1.29)	0.8659
Condition factor	3.86 (0.40)	3.71 (0.37)	0.0002
Temperature at death (ºC)	8.9 (0.67)	9.0 (0.64)	0.0348
Cooling degree hours	551.66 (104.53)	530.56 (99.80)	0.0414

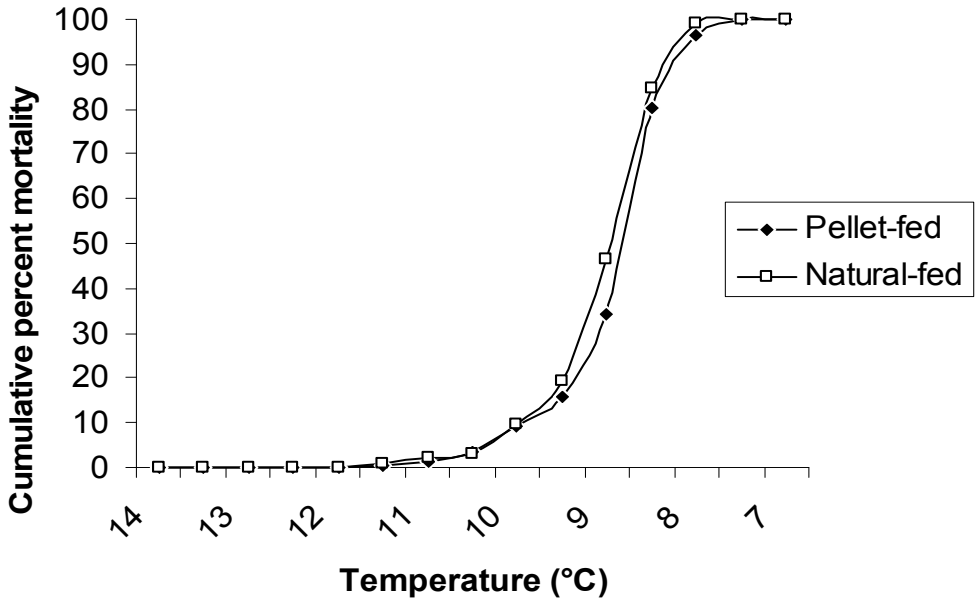

Figure 1. Mortality rate of *Oreochromis niloticus* juveniles exposed to temperature reduced at the rate of 1°C per day following acclimation at between 16 °C and 11°C. Fish had been grown under either pellet-fed (40% protein formulated pellets) or natural-fed (chicken manure only) conditions.

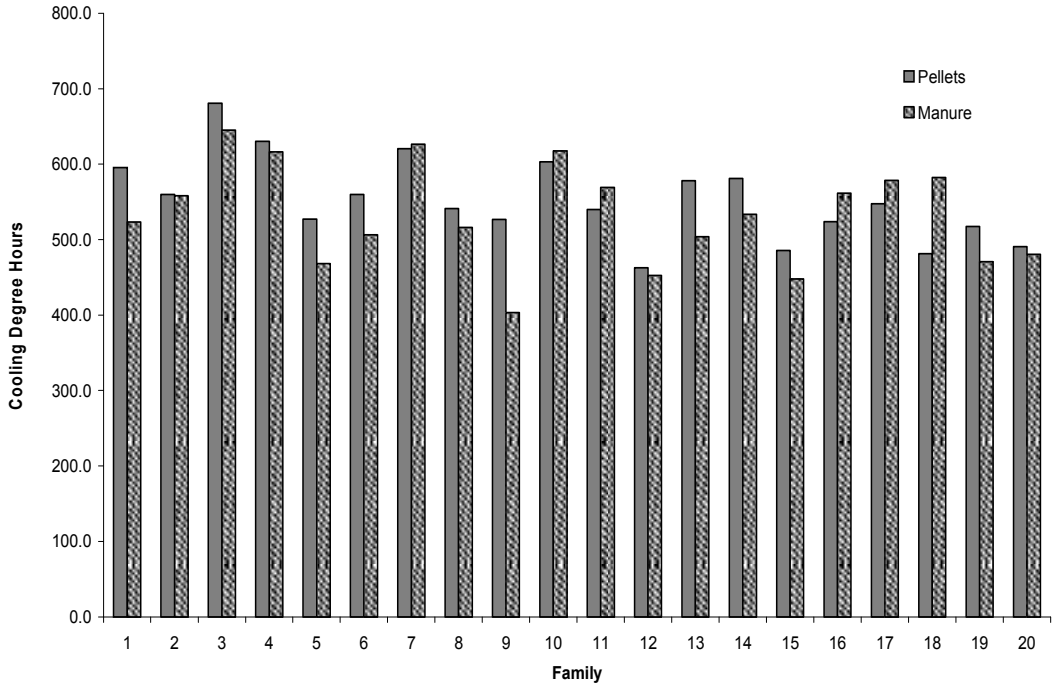

Figure 2. Least square means of CDH among families of *Oreochromis niloticus* reared in two treatments: fertilisation only and 40% protein pellets.

Comparison of Experiments 1 and 2

The temperature profiles during Experiments 1 and 2 are shown in Figure 3. Temperature ranged from 21.4 – 28.4 ºC (minimum readings) and 24.3 – 33.1 ºC (maximum readings) in the summer period and from 20.3 – 24.1 ºC (minimum) and 23.6 – 29.1 ºC (maximum) in the autumn period. The combined results of TAD, fish size and CDH for the two experiments are shown in Table 3. To produce the Experiment 2 values in Table 3, measurements of fish from the two dietary treatments in Experiment 2 were pooled. Fish began to die from 13.6 ºC to 8.6 ºC in Experiment 1 and from 11.7 °C to 7.5 °C in Experiment 2 (Figure 4). In Experiment 1, mean TAD and CDH were 10.1 ºC and 298.07, respectively while in Experiment 2, TAD and CDH were 9°C and 541.1, respectively. This indicates that fish in Experiment 2 were more cold tolerant.

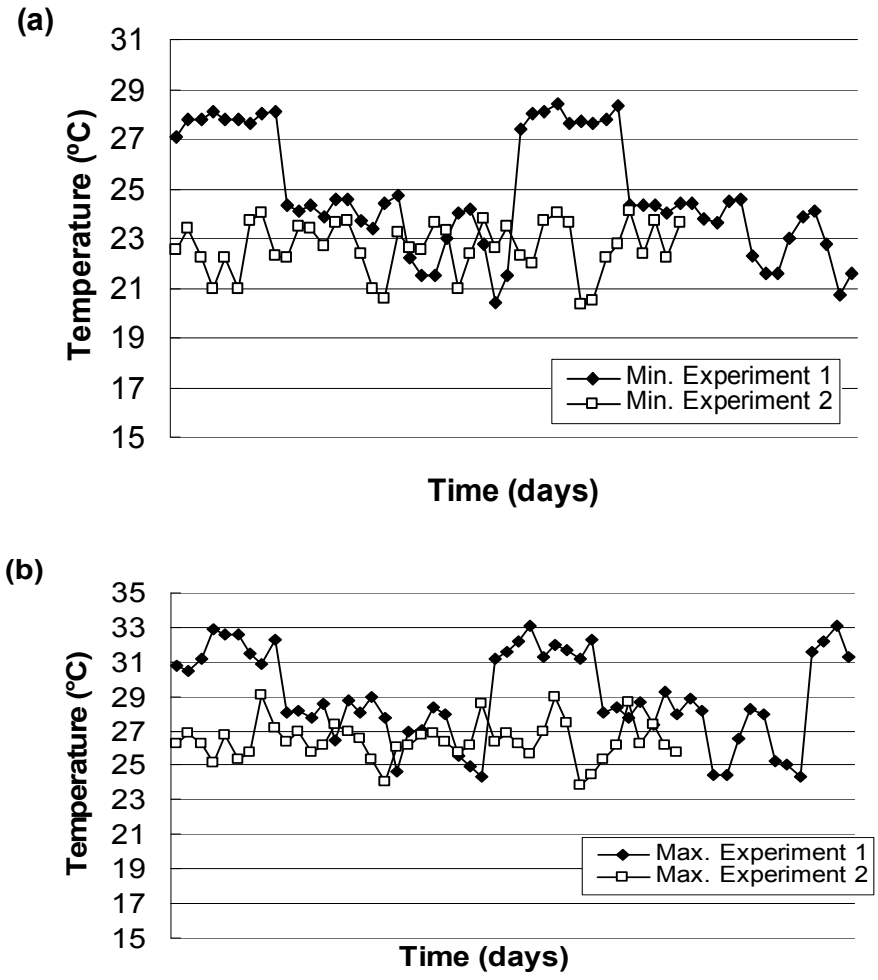

Figure 3. Temperature profiles during the rearing period of two groups (Experiment 1 and 2) of *Oreochromis niloticus* juveniles before exposure to lowered temperatures. Chart (a) and (b) show morning and afternoon temperature readings respectively. Experiment 1 was carried out under summer conditions and Experiment 2 in autumn.

Table 3. Means and standard deviations of body weight, standard lengths, temperature at death and cooling degree hours of juvenile *O. niloticus* in Experiments 1 and 2.

	Experiment 1		Experiment 2	
Parameter	Mean (SD)	Range	Mean (SD)	Range
Body weight (g)	5.1 (2.33)	1.0 - 20.6	2.0 (0.63)	0.8 - 4.7
Standard length	50.8 (7.35)	29.9 – 78.0	37.8 (3.90)	27.4 - 51.2
Temperature at death	10.7 (0.56)	8.6 - 13.6	9.0 (0.66)	7.5 - 11.7
Cooling degree hours	298.1 (67.86)	6.4 - 440.3	541.1 (102.6)	181.8 - 763

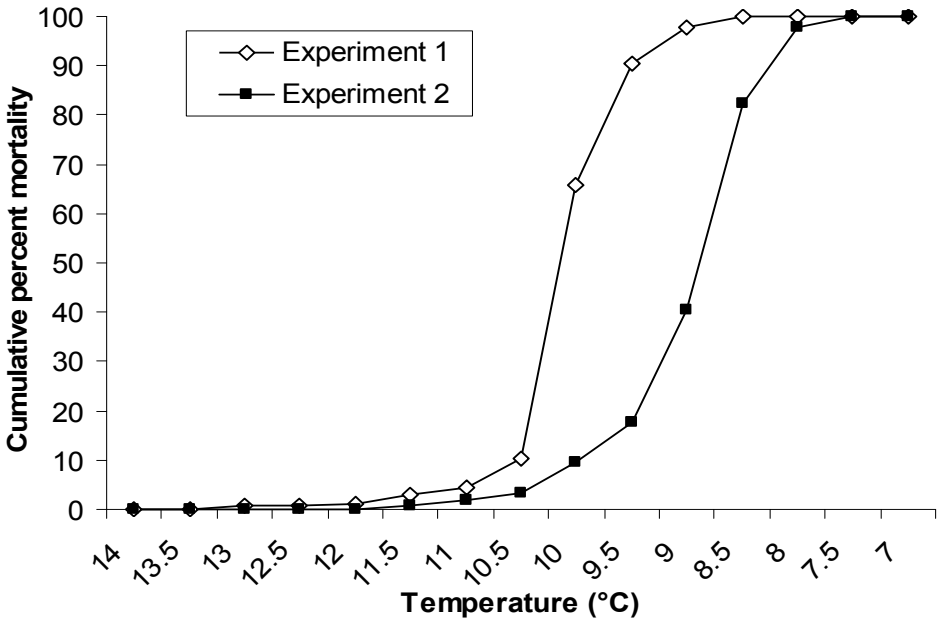

Figure 4. Mortality rate of *Oreochromis niloticus* juveniles under reduced temperatures. Fish reared under high (Experiment 1) and low (Experiment 2) ambient temperature regimes.

Discussion

Temperature at death values of between 13.6 ºC to 8.6 ºC in Experiment 1 and 11.7 °C to 7.5 °C in Experiment 2 are comparable with earlier findings on cold tolerance of *O. niloticus*. The Egyptian strain has been reported to experience mortality from 13 ºC to 10 ºC (Lahav and Raanan, 1998) and from 11 ºC to 9 ºC (Khater and Smitherman, 1998). Slightly better cold tolerance of between 11 ºC and 7.4 ºC have been reported for the Egyptian strain of *O. niloticus* used in China (Sifa *et al*., 2002).

The existence of size-dependent over-winter mortality has been reported for many

freshwater and marine fishes, with smaller individuals being in most cases more susceptible than larger con-specifics (Sogard, 1997). The effect of size on cold tolerance in tilapia has been reported as either significant or insignificant by different authors (Behrends *et al.,* 1990; Cnaani *et al.,* 2000; Cnaani *et al.*, 2003). Atwood *et al*. (2003), working with larger fish indicated that size significantly affected cold tolerance in *O. niloticus*. Similarly, Hofer and Watts (2002) suggested that small fingerlings (average 5.8 g) are more susceptible to cold stress than larger fingerlings (average 9.6 g). Our study indicated that size affected cold tolerance with smaller fish (<5 g) being more susceptible. This confirms the observation by farmers that smaller fish are more vulnerable during winter months. For better over-winter survival, juveniles of Nile tilapia should be at least 5g in size.

Diet has been known to improve the ability of fish to tolerate low temperatures. In striped and white bass for example, Kelly and Kohler (1999) reported that fish fed their natural diet suffered no mortality and had higher levels of unsaturated lipids than those artificially fed. Similarly, dietary supplementation of L-carnitine at different levels led to higher cold tolerance in an ornamental cichlid, *Pelvicachromis pulcher* (Harpaz *et al*., 1999). It has been shown that the level of dietary protein affects lipid content of muscles and liver in Nile tilapia (Ogunji and Wirth, 2002) and its hybrids (*O. niloticus* x *O. aureus*; Chou *et al*., 2001; Huang *et al.*, 1998). Atwood *et al*. (2003) found that when *O. niloticus* were fed either menhaden oil or coconut oil diets, they incorporated differing levels of saturated (n-6) or unsaturated (n-3) fatty acids into their muscles. However, this did not significantly affect cold tolerance of the two fish groups (Atwood *et al*., 2003). In this study, fish fed high protein pellets had significantly higher mean cold tolerance than natural (phytoplankton) fed fish.

Our study indicated a significant effect of condition factor on cold tolerance. Despite the higher mean cold tolerance of the pellet-fed group, the effect of diet was not significant. Since the pellet-fed fish also had higher condition factor, their higher cold tolerance may be directly attributed to their having had better condition. Morphometric indices which assume that heavier fish of a given length are in better condition are simple indicators of energy storage (Lloret *et al.*, 2002). Fish condition or well-being has a large influence on growth, reproduction and survival of fish populations (Lambert and Dutil, 2000). It appears that by improving fish condition factor, one can increase winter survival of *O. niloticus*.

We observed significant family and family x diet interaction effects. Some families had higher cold tolerance ability after growth with either natural or formulated pellet diets. This within family variation with respect to diet indicates the presence of a genotype x environment interaction for cold tolerance in Nile tilapia. Significant genotype by diet interaction may point to the need for particular genotype-diet combinations for better low temperature tolerance. The presence of genotype by diet interaction for cold tolerance in tilapia should therefore be further studied.

In this study, fish grown under lower autumn temperature showed better tolerance to exposure to lower temperatures as shown in Figure 4. This confirms the hypothesis (Stauffer, 1986; Stauffer *et al*., 1988) that prior acclimatization to lower temperature conditions can lead to better tolerance of low temperatures in tilapia. Temperature in

conjunction with photoperiod has been shown to affect fish physiology. Survival, growth rate and feed utilization of *O. niloticus* fry are affected by changes in photoperiod (El-Sayed and Kawanna, 2004). Atwood *et al*. (2003) showed that for Nile tilapia, the effect of experimentally altering photoperiod alone has some effect on cold tolerance when the trait was expressed as cooling degree hours (CDH) but not when expressed as temperature at death (TAD).

In this study, acclimatization to lower temperature not only improved CDH values but also led to substantial decrease in TAD values indicating better cold tolerance. While it is not possible, based on this study, to point out the exact factors that led to the improved cold tolerance in the second experiment, prior low temperature acclimatization coupled with photoperiod may have played a key role. The large differences in cold tolerance in the two experiments point to a possibility of using lowered temperature acclimatization conditions before fish over-wintering as a tool for improvement of cold tolerance in Nile tilapia.

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