

Size-dependent effects of daily thermal fluctuations on the growth and size heterogeneity of Nile tilapia *Oreochromis niloticus*

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The growth of Nile tilapia *Oreochromis niloticus* (0.02–20.00 g) was measured when fed to excess during the hours of light, following their exposure to five thermal regimes fluctuating around the thermal optimum for growth ($T_{opt} = 30^{\circ}\text{C}$) over the diel cycle of day (light, L) and night (dark, N), i.e. $27^{\circ}\text{C(L)}:33^{\circ}\text{C(N)}$, $28.5^{\circ}\text{C(L)}:31.5^{\circ}\text{C(N)}$, $30^{\circ}\text{C(L)}:30^{\circ}\text{C(N)}$, $31.5^{\circ}\text{C(L)}:28.5^{\circ}\text{C(N)}$ and $33^{\circ}\text{C(L)}:27^{\circ}\text{C(N)}$ (two replicates per treatment, six weeks' rearing, growth measurements at weekly intervals). A model constructed with a stepwise multiple-regression analysis accounted for 87.4% of the variation of the specific growth rate (G , % $M \text{ day}^{-1}$) from the variations of wet mass (M), the extent of the thermal fluctuation (F_T) and their interactions, i.e. $\log_{10} G = 1.7686 - 0.2136 \log_{10} M + 0.0806 [\log_{10} M \times \log_{10} (1 + F_T)] - 0.0394 [\log_{10} M \times \log_{10} (1 + F_T)]^2$. Based on this model, the thermal fluctuation that produces the fastest growth ($F_{T_{opt}} (^{\circ}\text{C})$) decreases in a curvilinear way, from 5.1°C at 20 mg to c. 0.7°C at 20 g. Thermal regimes that produce the slowest growth also produce the highest size heterogeneity. Functional hypotheses behind the size-dependent effects of thermal fluctuations are discussed, together with their implications in natural habitats and aquaculture systems with in different contexts of food availability.

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Key words: cycling thermal regimes; growth dispersion; optimum temperature.

INTRODUCTION

Temperature is probably the most pervasive environmental factor (Fry, 1971) in poikilotherms as it governs their physiology, growth, distribution, habitat use and behaviour. Aquatic animals can be more affected by changes in temperature than their terrestrial counterparts, because water has a greater specific heat than air. The majority of animal species have evolved under temperatures that fluctuate among years and seasons, but also during a diel cycle. The extent of daily thermal fluctuations vary between latitudes, climates and seasons, but also between habitats, as they are inversely proportional to depth and water current. The majority of aquaculture takes place in waters that are not thermoregulated and undergo more or less pronounced daily variations. Although thermal fluctuations prevail in the wild and in most culture systems, the bulk of knowledge on the thermal biology of aquatic poikilotherms

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originates from laboratory studies under constant or almost constant temperatures. Without proper validation, it is uncertain whether results obtained at constant temperatures can be extrapolated to fluctuating environments (Crawshaw, 1977).

Previous comparisons between the effects of stable and fluctuating thermal regimes on fishes have produced contrasting conclusions. Some studies demonstrated that exposure to fluctuating thermal regimes enhanced the tolerance of fishes to high temperatures [mosquitofish *Gambusia affinis* (Baird & Girard), Otto, 1974; sheepshead minnow *Cyprinodon variegatus variegatus* Lacépède, Bennett & Beitinger, 1997; cutthroat trout *Oncorhynchus clarkii clarkii* (Richardson), Johnstone & Rahel, 2003; spinedace *Meda fulgida* Girard, Carveth *et al.*, 2007], whereas no difference was found in others (Currie, 1995). Daily fluctuations in temperature boosted growth [rainbow trout *Oncorhynchus mykiss* (Walbaum), Hokanson *et al.*, 1977; striped bass *Morone saxatilis* (Walbaum), Cox & Coutant, 1981; brown trout *Salmo trutta* L., Spigarelli *et al.*, 1982; coho salmon *Oncorhynchus kisutch* (Walbaum) and goldfish *Carassius auratus* (L.), Konstantinov *et al.*, 1990; spinedace *Meda fulgida* Girard, Carveth *et al.*, 2007]. In other studies, fluctuating thermal regimes produced slower growth or lower growth efficiencies than constant temperatures [Tahoe sucker *Catostomus tahoensis* Gill & Jordan, Vondracek *et al.*, 1982; Arctic charr *Salvelinus alpinus* (L.), Lyytikäinen & Jobling, 1998, 1999; *S. trutta*, Flodmark *et al.*, 2004]. In other species, no significant effect was documented [Lahontan cutthroat trout *Oncorhynchus clarkii henshawi* (Gill & Jordan), Dickerson & Vinyard, 1999; Japanese medaka *Oryzias latipes* (Temminck & Schlegel), Dhillon & Fox, 2007; blue tilapia *Oreochromis aureus* Steindachner, Baras *et al.*, 2000].

The interactions between fish growth and temperature and the thermal preferenda of fishes generally vary between ontogenetic intervals (Jobling, 1994). This is, at least in part, because the ratio between gill surface area and body volume varies allometrically during the ontogeny. In general, small fishes prefer (McCauley & Huggins, 1979; Baras & Nindaba, 1999; Hernández *et al.*, 2002) and perform better at warmer temperatures than larger conspecifics (Pedersen & Jobling, 1989; Imsland *et al.*, 1996, 2006). Cichlids are no exception to this general rule [Mozambique tilapia *Oreochromis mossambicus* Peters, Mironova, 1976; Nile tilapia *Oreochromis niloticus* (L.), Mélard, 1986; *O. aureus*, Baras *et al.*, 2002]. The breadth (*i.e.* the kurtosis) of the growth-to-temperature response also varies during ontogeny [*e.g.* stone loach *Barbatula barbatula* (L.), Elliott *et al.*, 1996; *O. aureus*, Baras *et al.*, 2002]. Hence, the effect of fluctuating temperatures on growth is also expected to vary during the ontogeny of fishes, but evidence for this is lacking.

This study tested the effects of constant and fluctuating thermal regimes on the survival, growth and size heterogeneity of *O. niloticus*, and investigated whether these effects were size dependent (size range of 0.02 to 20 g). The pivot temperature for the fluctuating regimes was set as close as possible to the optimum temperature for growth (T_{opt}).

MATERIALS AND METHODS

FISH

The fish were *O. niloticus* from a captive population (Maryut), which originated from the Marine Centre of Tajoura (Libya), and was transferred in 1999 to the Aquaculture Research

Station of the Tunisian National Institute for Marine Sciences and Technologies at Bechima Gabès (Turki & Kraïem, 2002). In this study, the fish were full siblings that were collected as hatchlings from the mouth of a mouth-brooding female. Water temperature in the broodfish tank and rearing facilities prior to the experiments was 30° C, range $\pm 1^\circ$ C.

EXPERIMENTAL DESIGN

Two daily thermal fluctuations (3 and 6° C) were evaluated and compared with a control, in which temperature was maintained as constant as technically possible. In two fluctuating thermal regimes, temperature was warmer during the day than during the night, as normal for the wild. In addition to these natural regimes, two mirror treatments were also evaluated, with the same daily fluctuations (3 and 6° C), but in which water temperature was warmer during the night than the day (Fig. 1). This design allowed a test of whether the effect of daily thermal fluctuation was dependent on the coupling or decoupling of light and temperature. It also allowed a test of whether the variables under scrutiny were dependent on the temperature at the time of feeding, since fish were fed exclusively during the day. The absence of night-time feeding also aimed at preserving water quality, the degradation of which might have affected fish growth to a greater extent than the variable under study, especially in a context in which fish were fed in slight excess. For all five thermal regimes, two replicate groups were used. In the text, each thermal treatment is designated as $X^\circ\text{C(L)}:Y^\circ\text{C(N)}$, where $X^\circ\text{C}$ and $Y^\circ\text{C}$ are the temperatures during the hours of day (light, L) and night (dark, N), respectively.

The mean daily temperature in all groups was set as 30° C, which corresponds to the thermal optimum for growth (T_{opt}) of young *O. niloticus* (Azaza *et al.*, 2008). The maximum thermal fluctuation that was evaluated was 6° C (*i.e.* 27–33° C), a value that is frequently observed in natural environments, as well as in culture ponds in tropical regions. No fluctuation $>6^\circ\text{C}$ was evaluated, because it was important that the maximum daily temperature did not exceed the temperature at which food intake is maximal (a few degrees above T_{opt} ; Brett, 1979; Jobling, 1997). The temperature at which food intake is maximal is currently unknown

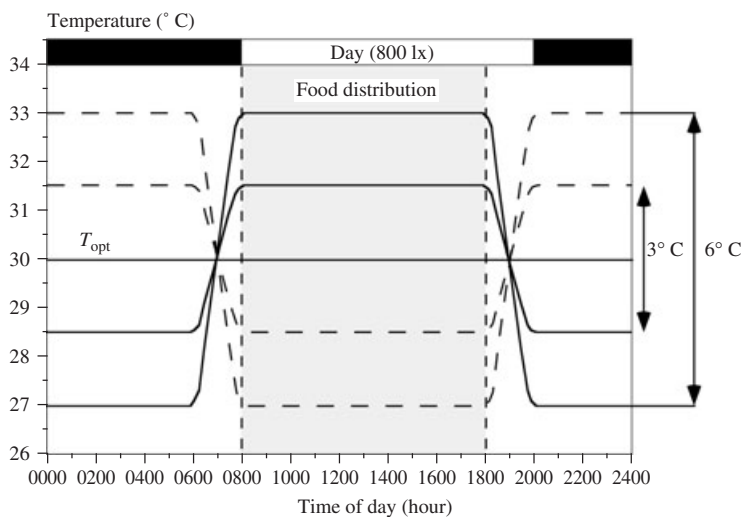


FIG. 1. Illustration of the experimental protocol that was used to test for the effect of the daily thermal fluctuations on the survival, growth and size heterogeneity in *Oreochromis niloticus* (indoor recirculating systems, 12L:12D). The pivot temperature is the thermal optimum for growth (T_{opt}). The continuous lines show the fluctuating regimes where days are (3 or 6° C) warmer than nights, whereas the mirror anomalous regimes, with nights warmer than days, are illustrated by the broken lines. Both types of fluctuating regimes are compared with a control reared at T_{opt} throughout.

for the Maryut population, but in view of previous studies on the thermal biology of this population, it is certainly not exceeded at 33°C for fish ranging from 0.02 to 20.00 g (Azaza *et al.*, 2008).

The periods of thermal transition were synchronized with the light cycle and were programmed to last for 2 h each (at 0600–0800 and 1800–2000 hours). This schedule permitted maintaining water temperature as constant as possible during the 10 h feeding period (0800–1800 hours). Thermal transitions as steep as 3°C h⁻¹ do not exceed the rapidity of thermal transitions of natural nursery habitats on sunny days (Baras & Nindaba, 1999; Finlay *et al.*, 2000).

REARING CONDITIONS

The experimental design was implemented in two experiments (duration: 3 weeks each) with fish of identical origins but of different sizes and ages. The first experiment started in the middle of the first week of exogenous feeding, after fish had fully exhausted their yolk [mean \pm s.d. wet mass (M) = 22.3 \pm 2.3 mg, mean skewness of -0.337, groups of 100 fish each]. The second experiment started with fish about 100 times larger (mean \pm s.d. M = 2.27 \pm 0.17 g, mean skewness of -0.046, groups of 30 fish each), which were about the same size as at the end of the first experiment. These two experiments allowed testing the effects of fluctuating thermal regimes over three orders of magnitude for M (0.02–20 g), which represents *c.* 60% of the species' size range when plotting M on a logarithmic scale (*i.e.* *O. niloticus* rarely grows beyond 2 kg). In both experiments, fish were sedated with tricaine methanesulphonate (50 mg l⁻¹), weighed individually to produce groups in which mean size and size heterogeneity were as similar as possible. Thereafter, the groups of fish were randomly allocated to the different thermal regimes.

The five thermal regimes were evaluated in five indoor water recirculating systems in the rearing facilities of the Aquaculture Research Station of Bechima Gabès (Tunisia). Each system comprised two 85 l rearing aquaria [350 \times 800 \times 450 (h) mm], a 180 l reservoir tank for filtration and thermoregulation (with a 2 kW thermostatic immersion heater) and a 20 l biofilter with UV-sterilization lamp (Oase, model Filtoclear UWC 9/11W; www.pondsupplies.com.au). The water flow in the aquaria was 1.5–2.0 and 2.0–4.5 l min⁻¹ (start to end of experiment), in the first and second experiments, respectively. Supplemental aeration was provided by individual air stones to maintain the oxygen level as close as possible to saturation. Water pH and alkalinity averaged 7.5 and 105 mg CaCO₃ l⁻¹, respectively. Day length was maintained at 12L:12D, with lights on from 0800 to 2000 hours [light intensity of 800 lx at the surface of the water (Digital Instrument LX-101; www.instrumentchoice.com.au)].

In the systems with a fluctuating thermal regime, the setting of the thermostat was changed at 0600 and 1800 hours to attain the dedicated temperatures at 0800 and 2000 hours, and thus to fit the thermal regime to the light cycle (Fig. 1). The experiments were conducted during winter (November 2007 and January 2008), when the air temperature inside the rearing facility was cool enough to enable the return of the temperature to the daily minimum within 2 h. Every day, however, it was verified that the fluctuations followed the desired patterns, and small amount of cool water was added to cool down the system on slightly warmer days.

Fish were fed formulated feed, which was distributed with automatic feeders from 0800 to 1800 hours, during the period when lights were on and water temperature was constant. The restriction of the feeding period to the hours of light was based on the hypothesis that *O. niloticus* is a diurnal species, which feeds during the day (even though juvenile *O. niloticus* can grow as fast when fed during the night; Baras *et al.*, 1995). This restriction was further motivated by reducing the risk of water degradation during the night if fish consumed less food than expected in a particular treatment. Food composition was 42% protein, 8% fat and 18.9 kJ g⁻¹ gross energy for the first experiment on small juveniles, and 36% protein, 6% fat and 17.7 kJ g⁻¹ gross energy for the second experiment on larger fish. These compositions produced fast growth in Maryut *O. niloticus* (Azaza *et al.*, 2005). The diameter of pressed pellets was <0.25 mm and 1.0–1.5 mm for the first and second experiments, respectively. Food rations were calculated after Mélard (1986) so fish were fed at slight excess. Food rations were incremented every day, partly based from calculations, partly from the observation of the amount of the uneaten food at the end of each feeding period. Uneaten food was removed

by siphoning during the hour following the end of food distribution, always with the objective of preventing any degradation of water quality that might have interfered with the effect of daily thermal fluctuations.

Aquaria were searched for dead fish twice a day, before and after the period of food distribution. Water temperature in the reservoir tank of each recirculating system was recorded automatically every hour, and dissolved oxygen twice a day (at 0800 and 1800 hours) with a digital thermo-oxymeter [WTW, MIQ/C184 (www.memecosales.com), accuracy of 0.1°C and $0.1\text{ mg O}_2\text{ l}^{-1}$]. Total ammonium and nitrite concentrations were measured with a spectrophotometer on the days of fish measurement [days 8 (D8), 15 (D15) and 22 (D22)]. Thereafter, water was added to compensate for evaporation during the previous rearing week, so the values of water quality that were measured here are slightly pessimistic since they were measured on the day when the water volume in the recirculating system was lowest.

Each experiment lasted for 3 weeks, with weekly measurements (D8, D15 and D22). On the morning of the measurement days, fish were captured with a dip-net, sedated with 50 ppm tricaine methanesulphonate, weighed (nearest 0.1 mg and nearest 0.01 g for the first and second experiment, respectively) and returned to their aquarium. The sole difference between the two experiments was that all survivors ($n \leq 30$) were measured in the second experiment, whereas in the first experiment, 30 fish were randomly sampled from each aquarium. Food distribution on the measurement day was suspended during the morning, and resumed in the early afternoon, at least 3 h after the last fish were measured, to minimize the effects of handling on food intake.

DATA ANALYSIS

Survival rates were compared between groups with contingency table analysis. Factorial ANOVA and Scheffe F -tests were used to test for differences between groups at the start of the experiments. The effect of the thermal regime on growth was tested with repeated measures ANOVA, using tanks as units of replication. Kruskal–Wallis and Mann–Whitney U -tests were used wherever parametric analyses could not be applied [between-treatment comparisons of coefficient of variation (c.v.) of M , skewness coefficients, minimum and maximum M within each group of fish]. For each rearing week, the average specific growth rate (G , $\% M\text{ day}^{-1}$) was calculated as $G = 100 (\ln M_2 - \ln M_1) (t_2 - t_1)^{-1}$, where M_2 and M_1 are M (g) at times t_2 and t_1 , respectively. Here, time was expressed by reference to the number of feeding days, thus 7 days for the first week of each experiment, and 6.5 days for the next 2 weeks of each experiment.

A stepwise multiple-regression analysis was used to test for the influence of the thermal regimes on the growth of fish of different sizes, using the 60 data points (*i.e.* 3 weeks \times five thermal regimes \times two experiments \times two replicate per thermal regime). The explicative variables were fish size (M), the extent of the thermal fluctuation (F_T , from 0 to 6°C), and the interaction of the variables (*i.e.* their product). All variables were tested in linear and \log_{10} form. Null hypotheses were rejected at $P < 0.05$.

RESULTS

Fish size at the start of the experiments did not differ significantly between groups (mean M of $22.1\text{--}22.4\text{ mg}$ and $2.25\text{--}2.30\text{ g}$; ANOVA, d.f. = 299, $P > 0.05$, for both experiments 1 and 2), nor did the c.v. of the distributions of M ($10.0\text{--}11.3\%$ and $6.1\text{--}8.4\%$, Kruskal–Wallis test, d.f. = 9, $P > 0.05$ for both) and the skewness coefficients (from -0.60 to -0.06 and -0.38 to $+0.41$; Kruskal–Wallis test, d.f. = 9, $P > 0.05$ for both). Temperature never deviated from the experimental targets by $>0.05^{\circ}\text{C}$ on average, and by $>0.3^{\circ}\text{C}$ at a maximum, and thermal transitions always took place within 2.0 h, range $\pm 0.2\text{ h}$ (Table I). Oxygen levels never dropped below 70% saturation, and the concentration of nitrogenous compounds remained low in

all treatments, during each week of the two experiments (Table I). In both experiments, survival on D22 was high and did not differ significantly between replicates or treatments (*i.e.* 92–95 and 90.0–93.3% in the first and second experiments; χ^2 , $P > 0.05$ for both).

TABLE I. Environmental characteristics during two experiments on the effects of daily thermal fluctuations on Maryut *Oreochromis niloticus*. Unless specified otherwise, values are the mean \pm S.D. of the variable under study in each recirculating system over 3 weeks

Experiment 1					
Variables	Thermal regimes				
	33° C(L) 27° C(N)	31.5° C(L) 28.5 N	30° C(L) 30° C(N)	28.5° C(L) 31.5° C(N)	27° C(L) 33° C(N)
Temperature (° C)					
Day	33.0 \pm 0.1	31.5 \pm 0.0	30.0 \pm 0.0	28.6 \pm 0.1	27.0 \pm 0.1
Min–max	32.9–33.1	31.4–31.6	30.0–30.1	28.5–28.8	26.9–27.1
Night	27.0 \pm 0.0	28.5 \pm 0.1	30.0 \pm 0.0	31.50 \pm 0.03	33.0 \pm 0.1
Min–max	26.9–27.1	28.4–28.6	30.0–30.1	28.4–28.6	32.9–33.3
Slope of thermal transitions (° C h ⁻¹)	3.0 \pm 0.2	1.5 \pm 0.1	—	1.5 \pm 0.1	3.0 \pm 0.1
O ₂ saturation (%)					
0800 hours	102.1 \pm 8.9	99.6 \pm 5.8	98.5 \pm 6.3	93.4 \pm 8.7	91.5 \pm 7.1
1800 hours	74.0 \pm 12.6	84.4 \pm 7.2	84.8 \pm 8.7	80.8 \pm 13.4	87.6 \pm 10.6
NO ₃ ⁻ (mg l ⁻¹)	4.7 \pm 0.2	4.8 \pm 0.7	4.3 \pm 0.4	4.7 \pm 0.4	4.5 \pm 0.6
NO ₂ ⁻ (mg l ⁻¹)	0.03 \pm 0.00	0.03 \pm 0.00	0.04 \pm 0.01	0.04 \pm 0.01	0.03 \pm 0.01
NH ₃ -N and NH ₄ ⁺ -N	0.04 \pm 0.01	0.04 \pm 0.01	0.05 \pm 0.01	0.04 \pm 0.01	0.05 \pm 0.01
Experiment 2					
Variables	Thermal regimes				
	33° C(L) 27° C(D)	31.5° C(L) 28.5 D	30° C(L) 30° C(D)	28.5° C(L) 31.5° C(D)	27° C(L) 33° C(D)
Temperature (° C)					
Day	33.0 \pm 0.1	31.5 \pm 0.1	30.2 \pm 0.1	28.5 \pm 0.1	27.0 \pm 0.1
Min–max	32.7–33.2	31.3–31.8	29.7–30.4	28.3–28.8	26.7–27.3
Night	27.0 \pm 0.0	28.5 \pm 0.1	30.0 \pm 0.1	31.5 \pm 0.0	33.0 \pm 0.1
Min–max	26.9–27.1	28.3–28.7	29.7–30.3	28.4–28.6	32.7–33.3
Slope of thermal transitions (° C h ⁻¹)	3.0 \pm 0.1	1.5 \pm 0.1	—	1.5 \pm 0.1	3.0 \pm 0.1
O ₂ saturation (%)					
0800 hours	91.9 \pm 11.9	98.7 \pm 10.1	85.3 \pm 10.7	86.9 \pm 6.6	99.5 \pm 10.0
1800 hours	86.9 \pm 15.2	81.4 \pm 10.4	83.1 \pm 12.2	88.2 \pm 13.4	83.6 \pm 11.3
NO ₃ ⁻ (mg l ⁻¹)	4.9 \pm 0.7	4.7 \pm 0.5	4.4 \pm 0.2	4.6 \pm 0.3	4.6 \pm 0.2
NO ₂ ⁻ (mg l ⁻¹)	0.05 \pm 0.00	0.05 \pm 0.00	0.04 \pm 0.00	0.05 \pm 0.01	0.04 \pm 0.00
NH ₃ -N and NH ₄ ⁺ -N	0.04 \pm 0.01	0.05 \pm 0.01	0.05 \pm 0.01	0.05 \pm 0.01	0.05 \pm 0.01

Min–max, absolute minima and maxima of the measures (measures at hourly intervals for temperature, bidaily intervals for oxygen and weekly intervals for nitrogenous compounds).

L, light (day); N, dark (night).

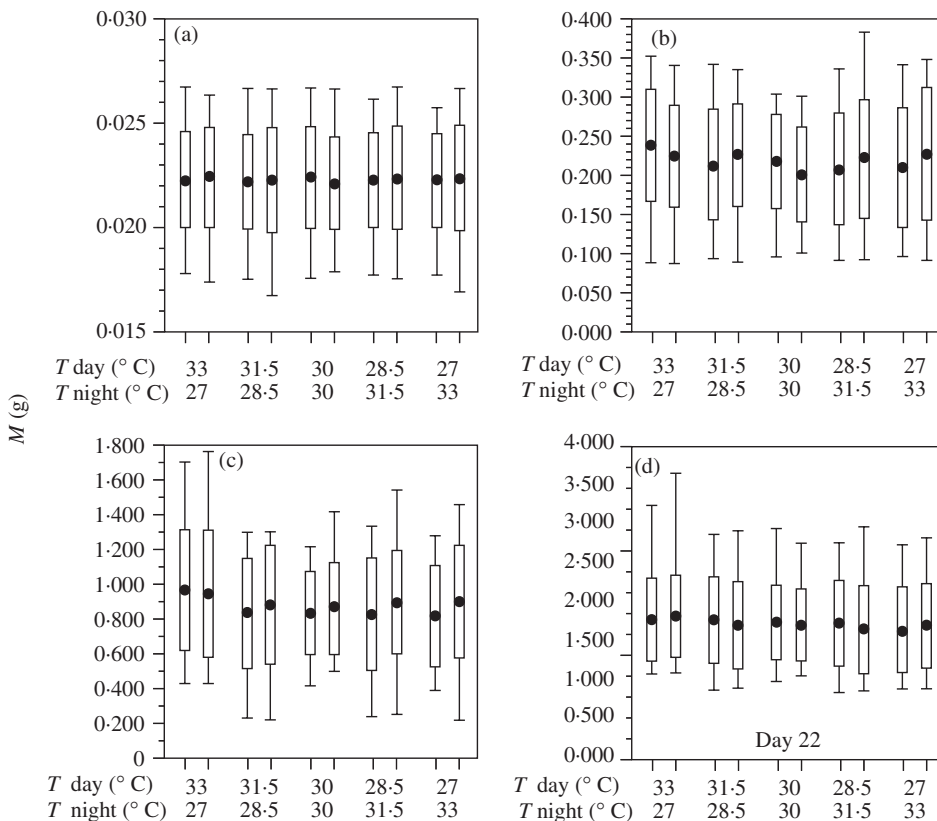


FIG. 2. Wet mass (M) of young juveniles of Maryut *Oreochromis niloticus* (mean initial M of 0.0223 g; 100 fish per group), depending on the sense and extent of daily thermal fluctuations day (light) and night (dark) around T_{opt} (see Fig. 1) on days (a) 1, (b) 8, (c) 15 and (d) 22. Values are mean (●), \pm s.d. (□) and the smallest and largest individuals in each group (I).

At the end of the first experiment, the mean M ranged between 1.637 and 1.802 g and all groups were strongly heterogeneous in size (c.v. M of 31.1%; Fig. 2). The repeated measures ANOVA revealed that growth was not significantly influenced by the thermal regime ($P > 0.05$) and that the interaction between age and the thermal regime was not significant either ($P > 0.05$). Kruskal–Wallis tests revealed no significant ($P > 0.05$) treatment effect of the extent of the thermal fluctuation on the c.v. of M or skewness coefficients and on the sizes of the smallest (M_{min}) and largest individuals (M_{max}). No single significant difference was observed between replicates or treatments for any of the variables tested on D8 and D15 either. This was partly because the c.v. of M increased rapidly in all groups during the first rearing week (on average from 10.6 to 32.3%) and remained high thereafter. Skewness also increased in all groups during the experiment, but mostly during the second and third rearing weeks (mean skewness of -0.35 , -0.24 , -0.03 and $+0.55$, on D1, D8, D15 and D22, respectively).

By contrast, during the second experiment, fish growth was significantly dependent on the extent of the thermal fluctuation (repeated measures ANOVA, $P < 0.01$). The

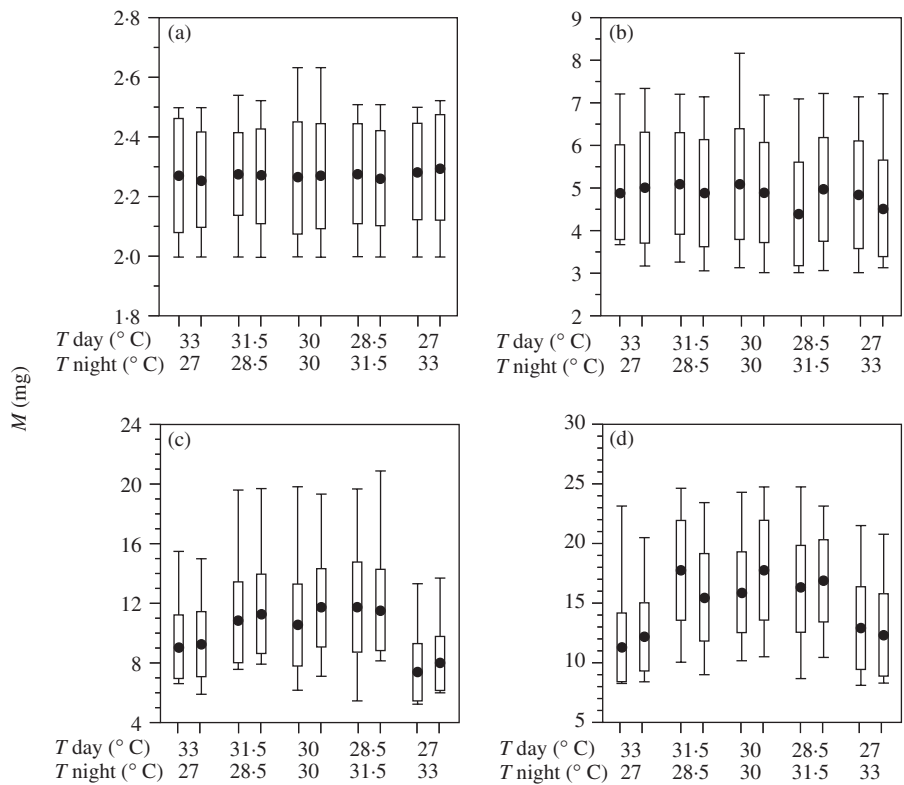


FIG. 3. Wet mass (M) of juveniles of Maryut *Oreochromis niloticus* (mean initial M of 2.27 g; 30 fish per group), depending on the sense and extent of daily thermal fluctuations around T_{opt} (see Fig. 1) on days (a) 1, (b) 8, (c) 15 and (d) 22. Values are means (●), \pm s.d. (□) and the smallest and largest individuals in each group (I).

TABLE II. Multiple-regression model of growth (G , % M day $^{-1}$), wet mass (M , g) and extent of the daily thermal fluctuation (F_T , °C) in juvenile Maryut *Oreochromis niloticus*. Predictor variables are presented in order of entrance in a stepwise multiple-regression analysis. Growth is calculated over 1 week, and M is the wet mass at the start of the week.

Dependent variable: $\log_{10} G$, $F_{2,57} = 124.30$, d.f. = 59, $P < 0.001$, $r^2 = 0.874$				
Predictor variables	Coefficient	S.E.	F_T	P
Intercept	1.769	0.054	1082.5	<0.001
$\log_{10} M$	-0.214	0.018	148.6	<0.001
$[\log_{10} M \times \log_{10} (1 + F_T)]^2$	-0.039	0.013	9.0	<0.01
$\log_{10} M \times \log_{10} (1 + F_T)$	0.081	0.039	4.3	<0.05

significant interaction between time and the extent of the fluctuation ($P < 0.001$) also indicated that the growth trajectories during this experiment were treatment dependent. On D22, fish raised under 27° C(L):33° C(N) and 33° C(L):27° C(N)

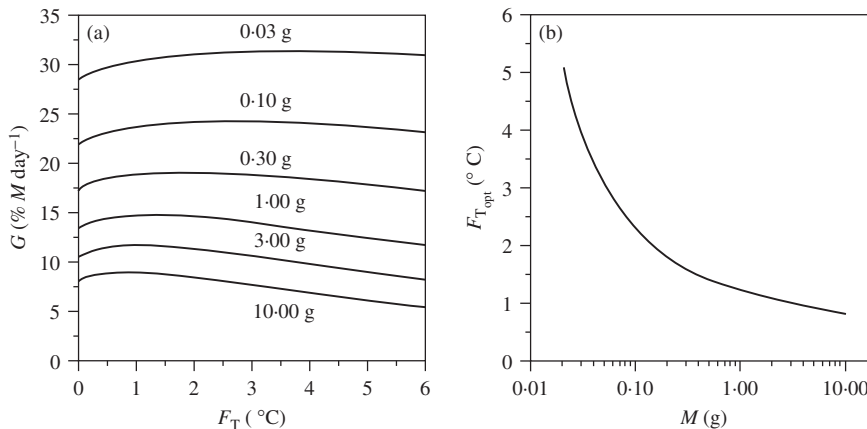


FIG. 4. (a) Specific growth rate (G) of juvenile Maryut *Oreochromis niloticus* depending on the extent of daily thermal fluctuations (F_T), as predicted by the multiple regression model in Table II. (b) Ontogenetic variation of the optimum extent of daily thermal fluctuation [$F_{T_{opt}}$, deduced from this model, i.e. $\log_{10} F_{T_{opt}} = 1.439 - 0.678 \log_{10} M + 0.074 (\log_{10} M)^2$, where M is wet mass].

averaged not more than 11.1 and 12.8 g, whereas the others averaged 15.4–17.7 g (Fig. 3). Between-treatment differences on D22 were also conspicuous for the smallest and largest individuals within each group, thereby indicating that all size classes were negatively affected by marked thermal fluctuations (i.e. M_{min} of 8.00–8.31 g v. 8.62–10.43 g; Kruskal–Wallis test, d.f. = 9 $P < 0.05$; M_{max} of 20.30–23.08 v. 23.08–24.70 g; Kruskal–Wallis test, d.f. = 9 $P < 0.05$). The c.v. of M and skewness of size distributions on D22 were significantly higher when fish were raised under strongly fluctuating temperatures than under more stable thermal regimes (c.v. of M of 24.0–28.1% v. 20.5–23.6%; Kruskal–Wallis test, d.f. = 9 $P < 0.05$; skewness of 0.55 to +2.25 v. –0.29 to +0.62; Kruskal–Wallis test, d.f. = 9, $P < 0.05$).

The stepwise multiple-regression analysis over all 60 data points produced a three-component model accounting for 87.4% of the variation of G (Table II). The influence of the thermal fluctuation was significant ($P < 0.05$), but in a polynomial and interactive form. By contrast, the sense of the thermal fluctuation did not influence G . The growth rates predicted by this model are shown in Fig. 4. Small fish gain a slight growth advantage when exposed to strong daily fluctuations, whereas large fish incur a major penalty and perform much better at constant or slightly fluctuating temperatures.

The model in Table II enables the calculation of the value of F_T that produces the fastest growth ($F_{T_{opt}}$) for fish of a particular M . This operation was repeated for a series of values of M , to describe the ontogenetic variation of $F_{T_{opt}}$ in *O. niloticus* [Fig. 4(b)]. $F_{T_{opt}}$ decreases in a curvilinear way in fish of increasing size (Fig. 4): $F_{T_{opt}}$ is 5.1° C at 20 mg, 2.3° C at 100 mg, 1.2° C at 1 g and c. 0.8° C at 10 g.

DISCUSSION

This study demonstrated that the effects of daily thermal fluctuations on the growth of *O. niloticus* are size dependent: small juveniles grow slightly faster when raised

under fluctuating temperatures than under constant temperatures, but strong thermal fluctuations become increasingly unsuitable in fish of increasing size. It also demonstrated that slower growth is accompanied by greater size dispersal. The study also provided evidence that the growth rates under mirror regimes were similar in fish of all sizes (Figs 2 and 3). This suggests that the coupling between light and temperature has no major importance on the growth of *O. niloticus*. A similar conclusion was drawn in a study on *O. aureus* in which mirror regimes fluctuating between 28 and 35° C were evaluated (Baras *et al.*, 2000), indicating that this feature is shared by several fish species, at least among the tilapiines, but not generalizable to all fishes [see the contrasting responses of Alaska pollock *Theragra chalcogramma* (Pallas) and sablefish *Anoplopoma fimbria* (Pallas); Sogard & Olla, 1998].

METHODOLOGICAL ASPECTS

The experimental settings were well maintained throughout the study, fish were fed in excess throughout, and water quality remained good according to the recommendations for rearing *O. niloticus* (Ballarin & Hatton, 1979). Hence, it can be reasonably assumed that the results reflect the genuine effects of thermal fluctuations on the growth of *O. niloticus*, rather than discrepancies between experimental tanks, or side effects resulting from treatment-dependent alterations of water quality. Yet, the fish used in this study had been raised at almost constant temperatures around T_{opt} prior to the experiments. It cannot be ruled out that the absence of acclimation to fluctuating temperatures might have been responsible for the slower growth under strong daily thermal fluctuations. If the lack of acclimation had been the key to the differences observed in this study, however, then the differences would have been expected to increase during the first rearing week of the experiment, which was not the case in either of the two experiments of this study. It cannot be strictly ruled out that the lack of acclimation to fluctuating temperatures impacted on fish growth, but if it did, the effect was insignificant or extremely transient.

The use of T_{opt} as the pivotal temperature is probably the best context for testing whether temperature fluctuations have a genuine negative, positive or neutral effect, for two complementary reasons. T_{opt} is not only the temperature at which fishes grow fastest, but is deemed to correspond to the final thermal preferendum of fishes, *i.e.* the temperature at which fishes will ultimately congregate if given time (Jobling, 1981). Second, any gain in growth under a regime fluctuating around T_{opt} unequivocally indicates a benefit from living under the fluctuating thermal regime, since T_{opt} is by definition the (constant) temperature at which fishes grow fastest. Similarly, if fishes raised under the fluctuating regime grow at the same rate as under constant T_{opt} , this strongly suggests that the effect of the temperature fluctuation is not neutral but beneficial (otherwise a growth penalty would be incurred, based on the parabolic nature of the relationship between fish growth and water temperature). Yet, meaningful conclusions have been drawn in other experimental contexts (Konstantinov & Zdanovich, 1986; Meeuwig *et al.*, 2004), but in other circumstances such contexts lead to ambiguous conclusions. For example, Baras *et al.* (2000) identified a neutral effect of thermal fluctuations between 28 and 35° C in *O. aureus* (12–1500 mg), by comparison to the growth observed at 35° C. It was later found, however, that the T_{opt} over this ontogenetic interval was precisely 31.5° C, *i.e.* the pivot temperature of the

fluctuating regimes (Baras *et al.*, 2002). If the effect of the daily thermal fluctuations in this study had been neutral, fish raised under thermal regimes fluctuating around 31.5° C should have grown faster than at constant 35° C, whereas their growth was similar.

SIZE-DEPENDENT EFFECTS OF THERMAL FLUCTUATIONS

The present study demonstrated that the effects of thermal fluctuations around T_{opt} were size dependent. The absence of significant between-treatment differences during the first experiment of this study suggests that fish exposed to strong thermal fluctuations initially gained a benefit from this thermal regime, but then incurred a cost as they grew larger and their $F_{T_{\text{opt}}}$ progressively shifted to lower values (Fig. 4). In the second experiment, fish were large (>2 g) and $F_{T_{\text{opt}}}$ was <1° C, so strong daily thermal amplitudes were detrimental throughout. No between-treatment difference was observed at the end of the first rearing week of the second experiment. It is suggested that the effect of fluctuating temperatures during this period was, at least in part, masked by the establishment of dominance hierarchies. Dominance hierarchies often re-establish soon after fishes have been rearranged into groups, especially when group size is low and fishes are homogenous in size, as was the case in this study. The establishment of a dominance hierarchy is generally accompanied by heterogeneous food intake and growth, generally to the detriment of the smallest fishes, thereby resulting in an overall slower growth rate, greater size dispersal and increased skewness. All three characteristics were observed at the end of the first week of the second experiment. Thereafter, size heterogeneity remained almost stable, skewness increased slightly and growth rebounded, as it is frequently the case after a hierarchy has been established. Nevertheless, size heterogeneity and skewness were higher, and growth was slower among the groups that were raised under strongly fluctuating temperatures than in other treatments. The exact reasons behind this difference are uncertain. It is possible that not all fishes tolerate fluctuating regimes equally well. Any formal test of this hypothesis would require the study of individual growth trajectories, and thus individually tagged fishes or growth reconstruction from otoliths. It cannot be excluded that fluctuating temperatures either affect the behavioural repertoire or exacerbate dominance hierarchies through enhanced aggressiveness. Recent experiments on two tilapia species (*O. niloticus* and the black chinned tilapia *Sarotherodon melanotheron* Rüppel) indeed provided evidence that aggressiveness was higher and could result in higher mortality when fishes were raised at warm temperatures (E. Baras & M. S. Azaza, unpubl. data).

The reasons why the effect of thermal fluctuations shifted from a benefit to a disadvantage during the ontogeny of *O. niloticus* remain obscure. In view of the kurtosis of the predicted growth rates shown in Fig. 4, it cannot be excluded that fluctuations of identical amplitudes became increasingly unsuitable and stressful to fish of increasing size. Abrupt thermal shocks are known to induce stress in fishes (Reaves *et al.*, 1968; Bevelhimer & Bennett, 2000). Dong *et al.* (2008) recently documented that sea cucumbers *Apostichopus japonicus* exposed to strongly fluctuating temperatures suffered from an oxidative stress and exhibited a higher level of heat-shock proteins. These results suggest a greater level of protein damage that might have accounted for their slower growth. There is currently little equivalent information on fishes raised under fluctuating temperatures (salmonids: Thomas *et al.*, 1986;

Shrimpton *et al.*, 2007), so it can only be speculated that these mechanisms might have intervened here.

Parallels can be drawn between this experimental study and field studies on the ontogenetic variations of habitat use by fishes of different sizes. Young, small fishes with low swimming capacities but high T_{opt} frequently live in shallow habitats with slow water velocity, which undergo strong daily thermal fluctuations on sunny days. By contrast, larger fishes tend to occupy deeper or faster flowing habitats, which are thermally more stable. Several factors, including predation pressure, ontogenetic variations of oxygen transport and thermal preferenda have been invoked to account for these size-dependent variations in habitat use and daily migrations (Baras & Nindaba, 1999). In view of the results of this study, which show that small fish tolerate thermal fluctuations better than larger and older conspecifics, it cannot be excluded that the search for environments that are more thermally stable be an additional factor that fosters the shift towards deeper and faster flowing habitats.

This study provided evidence that the life in thermally stable or fluctuating environments can have a cost or bring a benefit, depending on fish size and age. The models that were developed in this study probably apply to a number of fish species, at least as regards their general nature (*i.e.* young fishes being more tolerant than large fishes to marked daily thermal fluctuations). Data on how fluctuating temperatures affect fishes of different sizes, however, are still lacking for other fish species, from other taxa or latitudes.

As regards aquaculture, the information provided in this study indicates clearly that the growth observed under almost constant temperatures cannot be extrapolated to situations in which fishes experience marked daily thermal fluctuations. The differences observed in this study referred to a context in which fish were fed in slight excess. If life under fluctuating temperatures is more energy demanding than under more stable thermal regimes (Lyytikäinen & Jobling, 1998), the growth penalty might be more severe in rearing conditions, in which the feeding level generally lies in between the optimum and the maximum food rations to avoid food wastage. Future studies should aim at investigating this issue in the contexts of different food availability. In addition to their interest for aquaculture, these studies would also contribute to document the respective advantages or drawbacks of living in fluctuating thermal environments.

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