ЕКОЛОГІЧНА ФІЗІОЛОГІЯ І БІОХІМІЯ ВОДНИХ ТВАРИН

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ВПЛИВ ТЕМПЕРАТУРИ НА РІСТ, ГЕМАТОЛОГІЧНІ ПОКАЗНИКИ І ВМІСТ ІФР-1 У ПЛАЗМІ КРОВІ СОМА *PANGASIANODON HYPOPHTHALMUS*

Проведено дослідження впливу можливого зростання температури у дельті річки Меконг на сома Pangasianodon hypophthalmus. Вплив температури було оцінено за ступенем виживання і фізіологічними процесами, пов'язаними з ростом. Гематологічні показники (кількість червоних кровяних тілець, вміст гемоглобіну і гематокрит) за 24 °С за більш високих температур (30, 32, 34 і 36 °С) були вищими, ніж за 27 °С, що може вважатись контрольною для культивування дослідженого виду. За 27 ° відмічені найвищі показники швидкості росту, добового приросту маси, питомої швидкості росту, при цьому ступінь виживання не змінювався. Зроблено припущення, що максимальні показники росту, зареєстровані при 34 °С, зумовлені найвищою інтенсивністю метаболізму, що уможливила більший рівень засвоюваності корму порівняно з іншими дослідженими температурними режимами. Більшість показників почали знижуватись починаючи з температури 36 °С. З огляду на те, що різкі зростання температури навколишнього середовища понад 34 °С малоймовірні, зроблено висновок про подальшу ефективність культивування сома Pangasianodon hypophthalmus.

Ключові слова: *зміна клімату, сом Pangasianodon hypophthalmus, температура, ІРФ-1, темпи росту.*

The environmental temperature range experienced by any animal can have a major impact on survival, performance and reproduction, and this is a particular problem for ectotherms that have limited capacity to regulate their own body temperature. For most species within their normal temperature range, a slight increase in temperature is likely to be beneficial to growth because it results in more energy which leads to higher reaction rates for growth. This is often due to how the molecular structure of mitochondria is affected by changes in temperature [14]. Within the normal tolerance range, the rate of biochemical processes roughly doubles for every 10 °C increase in temperature [5].

Raised temperature can often enhance metabolic activity and increase growth rates in fish, while lower temperatures generally reduce performance [22]. Most tropical fishes show optimal growth performance in temperatures that range from 25—32 °C [5], but the ways in which individual species adapt to new temperature ranges however, can vary significantly. Laboratory studies

frequently demonstrate that temperature can increase to a point where it becomes detrimental for growth, and eventually becomes lethal [37] and the inflection point, at which growth begins to deteriorate, tends to be species specific.

Tra catfish (*Pangasianodon hypophthalmus*) is now the most widely traded fish commodity around the world and currently, most production comes from a single region in the Mekong River Delta (MRD) in the south of Vietnam [9]. Total production of farmed tra in 2010 reached 1.14 million tonnes with fish exported to 136 countries on most continents, producing an estimated export income of US$ 1,4 billion [9]. Tra play a very important and significant role in the Vietnamese aquaculture sector and now accounts for more than 50 % of total aquaculture production [35]. The catfish culture industry also provides direct employment to nearly 200 000 people in Vietnam, with the great bulk of these being women, primarily engaged in the processing sector [9, 35].

The MRD, where most tra culture is based, is predicted to be one of the top three regions worldwide, likely to be impacted by potential sea level rises associated with global warming [18]. Forecasts of temperature increases in this area are in the order of 1—6 °C by the year 2100 [19]. Investigations to assess effects of temperature increase on tra catfish culture performance are needed therefore, to assist sustainable development of this industry in the future.

Increasing gill blood flow, plasma glucose, locomotory activity, gluconeogenesis and declining food intake, growth, reproduction, glycogen store and muscle proteins are major responses to stressful conditions and are controlled by the brain and endocrine system [46]. IGF-1, which promotes tissue growth and differentiation [8], and cortisol, the major hormone for responding to stress [21, 26] are appropriate indicators therefore, for assessing the effects of temperature change on growth performance. Temperature change is also directly correlated however, with dissolved oxygen concentration [5] and therefore, hematological parameters and plasma glucose levels are also useful indicators to assess the capacity of fish to perform under temperature change or other stresses. In the current study, we examined the response of replicated cohorts of juvenile tra to temperatures overlapping with the range predicted under current climate change models on mean water temperature across the MRD region of Vietnam over the next 50 to 100 years. The primary objective was to identify temperature conditions that provide optimum growth and survival in cultured tra. Furthermore, we wished to identify the specific temperature reached at which the stress response of individuals was to divert energy away from growth and direct it to dealing with the stress.

Materials and Methods [1]

Experimental system and animals. Pangasiodon hypophthalmus (Siluriformes: Pangasiidae) juveniles (10—20 g) were obtained from an artificial seed production centre located in Can Tho City in the Mekong Delta, Vietnam. Individuals were maintained in freshwater tanks at the College of Aquaculture and Fisheries, Can Tho University, Can Tho City. Test individuals were accli-

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mated to tank condition for two weeks prior to experimentation in 4000 L tanks equipped with a continuous supply of well aerated freshwater (approximately 500 individuals per tank) in freshwater (1.27±0.44 mOsm) at 27 °C. Juveniles were fed by commercial pellets (Aquafeed, 25 % protein, *d* = 2mm, Grobest & I-Mei Industrial) twice a day to satiation. After the acclimation period, cohorts were used in growout trials.

Short-term trial: 810 individuals were distributed randomly into six treatments with three replicate 500 L tanks (45 individuals per tank per treatment) which has been found to be the optimal density for tra catfish [20]. The six temperature treatments were based on a pilot study that recorded 100 % mortality at <21 °C and >39 °C. The treatments here were: 24 °C, 27 °, 30 °, 32 °, 34 ° and 36 °C. In some respects, the 27 °C treatment may be considered as a control as the ambient temperature during the experiment was 27—28 °C. For the 24 °C treatment, tanks were set up in a cold room using an air conditioner to maintain the desired temperature. Individuals assigned to higher temperature treatments (30, 32, 34, and 36 °C) were acclimated gradually to their individual treatment temperatures before the experiment commenced using thermostats (Mennekes System, Germany) in a stepwise fashion at 2 °C per day until all tanks had reached their target temperatures. Highest temperature treatment acclimations began first and in sequence such that all treatments achieved their respective experimental temperatures on the same day. When all tanks had reached their target temperatures, fish were sampled at 0 h, 24 h, 96 h, 7th 14th day. At each sample collection time, three individuals were sampled randomly for hormone samples of IGF-1, cortisol, glucose level, osmolality and hematological parameters.

Long-term trial: Temperature acclimation was conducted in an identical manner as for the first trial. Six individuals were sampled on 28th and 56th day to be analysed like in the first trial. The remaining 45 individuals were used to calculate survival rate and individual growth performance.

Individuals in each tank were fed twice daily at 8:00—9:00 and 15:00—16:00 by the commercial food as described above. Fish were allowed to feed for one hour after which any remaining feed pellets were retrieved and counted, so that the mean food intake (FI) per day per individual could be determined based on the number of fish remaining in each tank.

Water in all tanks was aerated and water replacement was conducted weekly by siphoning 20 % from the bottom (to prevent large changes in water temperature) and replacing with dechlorinated tap water. All acclimation and treatments were exposed to a 12 : 12 h photoperiod for the duration of experimentation.

Data and sample collections. Water quality was checked daily using the YSI Professional plus meter that assessed dissolved oxygen (DO), NH_3 concentration, pH and water temperature. 2 mL water samples from each tank were also collected and stored in 2 mL plastic tubes at the time of every fish sample collection to measure ambient water osmotic pressure.

Growth performance in trial 2 was estimated per treatment as: weight gain (WG), daily weight gain (DWG), specific growth rate (SGR) and food conversi-

on ratio (FCR) based on the following standard formulae: WG (g) = final mean weight — initial mean weight; LG (cm) = final length — initial length; DWG (g d^{-1}) = WG/56; SGR (% d^{-1}) = [ln(final weight) — ln(initial weight)] × 100/56; FCR (g/g) = daily food intake × 56/WG.

Individuals were sampled from tanks using a hand net. The head of each sampled individual was placed under a cold moist towel to reduce stress during handling [45]. According to [13], capture and handling time can have a significant effect on measurement of cortisol and glucose concentrations in the fish blood plasma, so blood samples from the caudal vein were taken immediately within 5 minutes of sampling with 1 mL heparin coated syringes [2], prepared under ice. Quantity of blood taken was approximately 400 μL per individual. Individual samples were then transferred to 1.5 mL labelled tubes that were then stored on ice prior to centrifugation. Fish were then euthanized by immersion in ice slurry.

Hematology and biochemical indices. Total red blood cell (RBC) count was determined manually in a 1:200 dilution of the blood sample in Natt-Herrick's solution as a diluent stain using a Neubauer hemacytometer [32]. Microhematocrit tubes were used to determine the hematocrit at 12000 rpm for 5 min (Hct %) [25]. Hemoglobin concentration (Hb g dL^{-1}) was determined using the cyanohemoglobin method. A 10 μL blood sample was mixed with 2.5 mL of Drackin reagent [15]. Hemoglobin concentration of samples was determined at 540 nm using a spectrophotometer (GENESYS™ 20, Thermo Scientific).

Plasma analysis. Blood samples were centrifuged for 10 mins at 4500 rpm at 4 °C. Following this, plasma was separated and frozen and stored at -20 °C for later analysis. Plasma IGF-1 and cortisol concentrations were determined using IGF-1 600 ELISA kit (EIA-4140 DRG Instruments GmbH; sensitivity 0—600 ng/ml) and Cortisol ELISA kit (EIA-1887, DRG Instruments GmbH; sensitivity 0—800 ng/ml), respectively. Assay procedures were as per the manufacturer's instructions. Glucose levels (g/L) in plasma samples were quantified using a standard glucose assay [17]. Osmolality levels (mOsm) were measured using a Fiske Associates Osmometer, Model 110.

Statistical analysis. To determine differences in growth performance, one way ANOVAs using IBM SPSS 21 Statistic were applied individually to each performance indicator. Additionally, a two-way ANOVA was used to evaluate whether there were any interactions between treatment and sampling period for glucose, IGF-1 levels, and also for osmotic pressure. Where significant differences were identified, comparisons among treatment means were made using a Duncan's *post hoc* test, applying a 95 % confidence level.

Results

Environmental conditions. There was no significant difference in pH value among treatment over the course of the experimental period while DO and NH_3 concentrations both tended to be higher in the 24 °C and 27 °C treatments (tabl. 1). Although NH_3 concentration in treatments varied, all records were lower than those seen under standard healthy pond conditions [4]. While the negative correlation between temperature and DO is well established, the variati-

on seen in DO levels among treatments in this study (i.e. lower DO in warmer temperatures) was well within the standard daily range of DO fluctuations observed in tra growout ponds in the MRD [27] (see tabl. 1).

Survival rate and growth performance. The survival rate observed in the 24 °C treatment was lower than for all other treatments (P<0.05). There was no significant difference in fish survival rate among all other treatments except that survival in the 36 °C treatment was lower as compared with 27 °C (tabl. 2). Fish WG rose quite rapidly in association with temperature increase, from the lowest level at 24 °C to the highest at 34 °C, then from 34 °C to 36 °C WG significantly decreased, by approximately 20—25 % (see tabl. 2). The same pattern was generally seen for DWG and SGR while the effect was less for LG.

Table 1

Environmental factors of experiment

Environmental factors	24 °C	27 °C	30 °C	32 °C	34 °C	36 °C	Mean	Normal pond condition [4]
pH	7.90± 1.22	8.04± 1.15	8.14± 1.05	8.06± 0.89	7.94± 0.68	8.06± 0.61	8.02± 0.96	—
DO (mg/L)	4.94± 1.32^{d}	4.72± 0.72^{cd}	4.54± 0.74^{bc}	4.34± 0.93^{b}	3.58± 0.92^{a}	3.37± 1.06^{a}	4.25± 1.12	6.4±2.0
NH_3 (mg/L)	0.11± 0.06^{d}	0.08± 0.05^{c}	0.07± 0.04^{bc}	0.04± 0.03^{a}	0.04± 0.03^{a}	0.06± 0.05^{b}	0.07± 0.05	0.21±0.39

Note. Here and in tables 2—5: means±*SD* in row do not share the same letter are significantly different (p<0.05).

Table 2

Mean growth performance indices of tra catfish under different temperature treatments over a 56-day experimental trial (N = 2)

T, °C	Initial *W* (g)	Final *W* (g)	Survival (%)	*WG* (g)	*LG* (cm)	*DWG* (g/day)	*SGR* (%/day)	*FI* (g/ind/day)	*FCR*
24	21.99± 0.88^{ab}	39.21± 2.54^{d}	70.37± 3.39^{a}	17.21± 2.53^{a}	0.83± 0.13^{a}	0.31± 0.05^{a}	1.03± 0.13^{a}	0.73± 0.07^{a}	2.40± 0.49^{a}
27	20.23± 1.76^{b}	51.09± 3.87^{bcd}	97.78± 2.22^{c}	30.86± 5.06^{b}	2.64± 0.68^{b}	0.55± 0.09^{b}	1.66± 0.25^{b}	0.62± 0.02^{a}	1.15± 0.19^{b}
30	20.88± 2.05^{ab}	50.68± 9.49^{cd}	91.85± 7.14^{bc}	29.79± 7.70^{b}	2.37± 0.40^{ab}	0.53± 0.14^{b}	1.57± 0.21^{b}	0.80± 0.27^{ab}	1.47± 0.13^{b}
32	24.22± 0.39^{a}	61.22± 4.95^{abc}	90.37± 5.13^{bc}	37.00± 4.73^{bc}	3.00± 0.85^{bc}	0.66± 0.08^{bc}	1.65± 0.13^{b}	1.05± 0.09^{b}	1.59± 0.16^{b}
34	22.09± 0.89^{ab}	75.52± 4.99^{a}	96.30± 3.40^{bc}	53.43± 4.29^{d}	4.73± 1.81^{c}	0.95± 0.08^{d}	2.19± 0.07^{c}	1.42± 0.19^{c}	1.49± 0.16^{b}
36	23.85± 0.30^{a}	65.17± 1.93^{ab}	88.89± 2.22^{b}	41.32± 1.81^{c}	3.44± 0.97^{bc}	0.74± 0.03^{c}	1.79± 0.05^{b}	1.01± 0.06^{b}	1.37± 0.12^{b}

As all treatments were sampled at 56 days, the statistical significance of differences among LG, DWG and SGR measures largely reflect that seen in the WG analysis. In general, 27 °C and higher temperatures facilitated increased performance across these indicators in comparison with the lowest treatment temperature (24 °C). Both DWG and SGR of tra maintained at 24 °C were poorer than in all other temperature treatments ($P<0.05$) while fish at 34 °C showed the highest DWG and SGR values ($P<0.05$). Daily food intake was significantly different among treatments, with the highest consumption at 34 °C, while FCR values among treatments did not differ from 27 °C to 36 °C, however all exceeded those at 24 °C treatment.

Hematological parameters. Estimates of the three hematological parameters (RBCs, hemoglobin and hematocrits) in fish sampled from the various thermal conditions showed significant differences among temperatures, sampling time and also their interaction (tabl. 3).Water temperature therefore had a clear effect on tra during both short term and long term exposure. Results show that RBC, Hct and Hb were significantly lower at 27 °C. There was no difference between mean values at any other temperature (tabl. 4).

Biochemical and hormonal changes. No significant effects were evident for temperature, time and their interaction on osmotic pressure in tra catfish (see tabl. 3) and there was no significant difference among treatment (see tabl. 4). Tra catfish plasma osmotic pressure was 267.18±31.09 mOsm (n = 331). Glucose concentrations however, were significantly affected by temperature and exposition although there was no apparent interaction between the two variables (see tabl. 3). Significant changes in fish plasma glucose concentrations among treatments occurred on 1st and 4th day of the experiment (fig. 1). The highest glucose concentration was seen at 34 and 36 °C, significantly higher than at 24 ° and 27 °C treatments on 1st day. Plasma glucose levels at 34 ° and 36 °C to the 4th day decreased. By 7th day, survivors in different thermal environments had probably acclimated to the temperature and were able to regulate their glucose levels in the range of 0.24—0.33 g/L. No significant differences were observed among treatments from 7th day to end of the experiment.

Although cortisol plays a key role in osmoregulation and stress response through it effect on stimulating gill NKA activity, modulates the tissue inflammatory response through inhibitory effects on cytokine production and a range of other immune system responses [34], no significant differences were observed in cortisol levels within either of the main effects or their interaction (data not supplied), probably due to the very large degree of variation among values within treatments (across 2 orders of magnitude within some treatments). We assume that this high variation was a natural phenomenon [40] or stress caused by long time handling [13] of 5 individuals per tank. While IGF-1 levels were not significantly different among treatments at the time of collection, fish at different growth stages showed highly significantly different IGF-1 levels, but there was no interaction between thermal treatment and sample time for levels of this hormone (see tabl. 3). In general, IGF-1 levels in warmer conditions (27—34 °C) increased gradually until they reached a peak on 14th

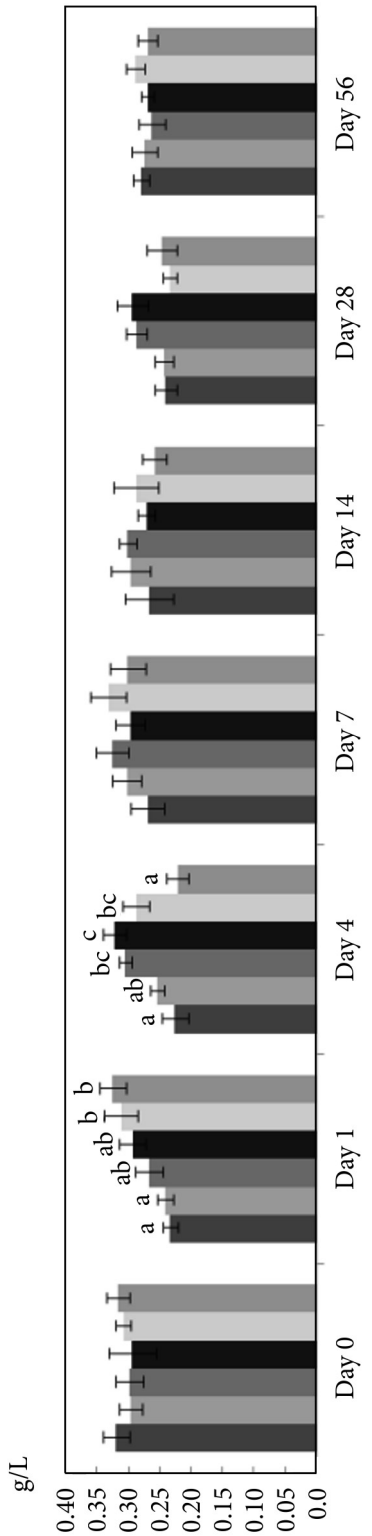

Figure. Changes of plasma glucose levels (g/L) relating to temperature and time of collection in tra catfish. Standard errors bars of the means (n=4-9) have different letter are significantly different (p<0.05).

Table 3

Results of ANOVA analyses for effects of temperature on hematological parameters and plasma indicators over experiment

Factors	df	Mean Square	F	P
Red blood cell				
Treatment	5	1.735	5.664	<0.001
Time of collection	6	2.141	6.988	<0.001
Treatment × Times of collection	30	0.950	3.101	<0.001
Error	311	0.347		
Hemoglobin				
Treatment	5	29.29	12.59	<0.001
Time of collection	6	54.09	23.25	<0.001
Treatment × Times of collection	30	7.086	3.05	<0.001
Error	314	2.327		
Hematocrit				
Treatment	5	208.022	4.74	<0.001
Time of collection	6	113.113	2.577	0.019
Treatment × Times of collection	30	93.919	2.14	0.001
Error	284	43.89		
Osm				
Treatment	5	444.454	0.468	0.800
Time of collection	6	918.607	0.967	0.448
Treatment × Times of collection	30	1090.18	1.148	0.278
Error	289	949.884		
Glucose				
Treatment	5	0.008	2.35	0.041
Time of collection	6	0.014	3.892	0.001
Treatment × Times of collection	30	0.005	1.41	0.081
Error	292	0.004		
IGF-1				
Treatment	5	6.785	0.416	0.837
Time of collection	6	229.29	14.059	<0.001
Treatment × Times of collection	30	9.522	0.584	0.961

day and then declined subsequently, whereas at 24 °C no change over time was evident (Table 5).

Discussion

The results of this study clearly indicate that a moderate increase in water temperature in the tanks improved performance of tra catfish cultivation, and this effect could be possibly translated to wild populations as well. However, it is well known that as temperature increases, we should see an associated decline in DO, with all other things being equal. This effect was evident in the current study with DO declining from almost 5 mg/L at 24 °C to approximately 3.4 mg/L at 36 °C. While these values were statistically different from each other, we believe that the observed variation in DO levels insignificantly affected growth of tra for a number of reasons: i) tra is an air breathing species [38] and can usually access at least 10 % of its oxygen requirements directly from the air [27]; ii) lower DO levels would normally result in some degree of stress and the-

Table 4

Mean hematological parameters and plasma osmotic pressure of tra catfish under different temperatures

Parameter	N/treat.	24 °C	27 °C	30 °C	32 °C	34 °C	36 °C
RBCs (10^6 cells/mm^3)	52—61	2.78± 0.10^{bc}	2.42± 0.07^{a}	2.79± 0.07^{c}	2.75±. 011^{bc}	2.87± 0.09^{c}	2.62± 0.08^{ab}
Hb (g/ dL)	55—62	7.99± 0.19^{bc}	6.68± 0.24^{a}	7.97± 0.20^{bc}	8.21± 0.27^{c}	8.53± 0.25^{c}	7.52± 0.31^{b}
Hct (%)	48—58	28.80± 0.95^{ab}	26.40± 0.99^{a}	29.41± 0.84^{b}	29.18± 1.29^{b}	32.23± 0.77^{c}	28.86± 0.91^{ab}
Osmotic pressure (mOsm)	49—61	271.98± 3.99	264.36± 3.80	264.35± 6.19	269.98± 3.65	268.02± 3.56	265.79± 3.57

Table 5

Changes of IGF-1 levels (ng/ml) relating to temperature and time of collection in tra catfish

Sampling time	24 °C	27 °C	30 °C	32 °C	34 °C	36 °C
Day 0	16.6±1.1^{Aa}	17.2±1.2^{Aa}	17.0±0.9^{Aa}	16.5±1.7^{Aa}	17.3±1.3^{Aa}	16.3±0.5^{Aa}
Day 1	16.7±0.9^{Aa}	17.8±0.6^{ABa}	17.1±0.9^{Aa}	19.8±1.9^{Aa}	18.3±1.0^{Aa}	20.7±2.1^{Ba}
Day 4	19.8±4.9^{Aa}	18.1±1.0^{ABa}	20.2±3.1^{ABa}	17.9±1.2^{Aa}	17.8±1.0^{Aa}	19.0±1.0^{ABa}
Day 7	18.7±0.6^{Aa}	20.3±3.8^{ABa}	19.1±1.3^{ABa}	19.8±1.1^{Aa}	20.7±3.0^{ABa}	20.5±2.0^{Ba}
Day 14	19.6±1.8^{Aa}	22.1±2.0^{Ba}	23.9±2.1^{Ba}	26.4±2.7^{Ba}	25.4±2.7^{Ba}	20.6±1.0^{Ba}
Day 28	20.3±0.8^{Ab}	19.3±0.4^{ABab}	19.7±0.5^{ABab}	20.6±0.5^{Ab}	19.1±0.4^{Aab}	18.5±0.3^{ABa}
Day 56	16.8±0.3^{Aa}	15.8±0.5^{Aa}	18.3±0.8^{Ab}	16.2±0.3^{Aa}	16.3±0.3^{Aa}	16.5±0.5^{Aa}

refore we would expect growth to decline at higher temperatures, which was not observed; iii) fish with the highest DO had the lowest survival and growth rate, while the reverse was evident for the lowest DO; and iv) DO levels in the current study remained in the acceptable range for all treatments [11], with hypoxic conditions considered to be below 2—3 ml/L.

Each fish species of has an optimal temperature range for growth performance [11]. For warm water fishes or fishes in tropical regions in general, optimal temperature for growth ranges generally from 20 to 32 °C [11, 29]. The relationship between temperature and growth is represented by the thermal growth coefficient effect [42], whereby metabolic rates increase in raised temperature producing faster growth rates at higher temperatures. In this study, low temperature affected growth of tra more significantly, as individuals under these conditions not only consumed approximately half the amount of food compared with those under (34 °C), but also showing much higher FCR value (see tabl. 2) than in all other treatments. Thus, at the lowest temperature (24 °C) apparently more food is required to gain a unit of the body weight at suboptimal water temperature. Furthermore, this was evident in a low relative growth rate and reduced length gain. As most fishes are true ectotherms, their body temperature (and hence metabolic rate) essentially follow the temperature of the surrounding water [47], as a tropical fish tra not only showed poorer survival rate at 24 °C, but also lower growth rate.

In this study, tra showed optimal response to temperatures ranging from 27 °C (current mean ambient temperature in MRD culture) to 36 °C, but 34 °C provided the best thermal conditions for its cultivation. At this temperature, fish approximately showed twice the weight gain in comparison with the 27 °C, at the same time had no difference in FCR value. At 34 °C individuals consumed approximately double the amount of daily food and the DGR in comparison with 27 °C, what might be considered as control conditions. Together, these results suggest that increased temperature to at least 34 °C did not result in a stress response and that the majority of energy derived from the increased FI was directed to growth rather than to dealing with stress.

Temperature clearly affects ectothermic animals by impact on their mitochondrial capacities for substrate oxidation and ADP re-phosphorylation [36]. Mitochondrial capacities fall at lower temperatures, following a simple Q_{10} relationship and, conversely, they increase at higher temperatures, so tropical fishes can increase their metabolic rates by activating their mitochondrial capacity. There is an evidence in this study that water temperature increased to 36 °C, as the beginning of the growth performance (WG, DWG, and SGR) decline in comparison with 34 °C, suggesting that thermal stress was becoming significant at this temperature and that some energy was now being diverted to coping with stress. However, it is impossible to determine whether the observed decline in growth rate from 34 °C to 36 °C was due to temperature stress *per se*, or to the associated DO level. It was reported [27] that hypoxic conditions can inhibit growth of tra catfish by: reducing appetite, reducing assimilation efficiency (i.e. increasing FCR), and a shift in energy balance due to the requirement for increased surfacing activity for air breathing. In this study, it was apparent

that at 36 °C fish had reduced appetite (compared with 34 °C) although the DO values did not significantly differ from those at 34 °C. Therefore it is difficult to differentiate between temperature and DO related hypotheses with respect to the observed decline in growth rate at this higher temperature.

Dealing with thermal stress in either lower (24 °C) or higher (30—3 °C), temperatures was reflected in sampled individuals showing significant increases in hematological parameters including RBC, Hb and Hct (see tabl. 4). Increased RBC, Hb and Hct are a common response to hypoxia or anoxia [16] and to dealing with stress [6]. When individuals were exposed to either low or elevated temperature, RBC, Hb and Hct levels were all significantly increased as compared with the ambient 27 °C (see tabl. 3). The study was carried out to assess the effect of diazinon and deltamethrin on tra [16] and indicated that under these stressful conditions, RBC, Hct and Hb were raised to increase oxygen-carrying capacity of the blood. Osmotic and thermic stress both can affect fish blood parameters including Hb, Hct and cortisol levels [39]. Temperatures can cause stress because of decreased oxygen solubility in water and hence its availability to fish [7]. The increase of the red blood cells quantity led to increases in Hb, Hct and MCH. The internal osmotic pressure was probably unchanged, however so red blood cell volume and relative quantity of Hb in each red cell was not essentially affected by changes in water temperature. Tra catfish possibly responded to thermal stress by increasing RBC number that in turn increased Hb, Hct and MCH to ensure they meet higher oxygen demands (see tabl. 4). Therefore tra appeared very suitable for high density culture [35], in particular, it possesses not only an air bladder for air breathing [38], it also can respond by changing hematological parameters to deal with thermal stress ensuring higher oxygen demands can be met efficiently. However, although mean Hb concentration at 36 °C was significantly higher than at 27 °C, RBC and Hct tended to decline after reaching a peak at 34 °C. These data show the limitations of hematological acclimation in thermal/oxygen demand stress response and these apparently reflect a tendency towards growth reduction at 36 °C.

Average internal osmotic pressure for tra individuals was 267.19 ± 31.09 mOsm (n = 331), which did not significantly differ from levels reported previous under normal freshwater conditions 269.03±16.70 mOsm (n = 30) (one sample t-test, P>0.05) [33]. Furthermore, plasma osmotic pressure in the experimental tra was not substantially different from other freshwater fishes, including bowfin (279 mOsm), carp (274 mOsm), or euryhaline steelhead trout (260 mOsm) [10]. In this study, temperature appeared to have no effect on plasma osmotic pressure of tra (see tabl. 3, 4), and this result provides similar conclusion to other studies on freshwater fishes including Mozambique tilapia *Oreochromis mossambicus* [12] and Mozambique tilapia hybrids *O. mossambicus*×*O. urolepis hornorum* [41] that temperature has little effect on fish osmotic pressure; osmolality levels however, can change when combined with different salinity levels [12, 41]. A single study observed a temperature-related impact on common carp's plasma osmolality [30], however the authors could provide no clear explanation for their observation.

When exposed to stress, fishes will use more energy from food or glycolysis for swimming, regulation and respiration instead of growth, reproduction, and storage [23], thereby leading to increases in plasma glucose concentration. In this study, on the 1st day, plasma glucose at high temperature was mobilised at significantly higher levels than at 24 or 27 °C, presumably to deal with thermal stress. Plasma glucose concentration has been reported to increase from hours to days under regulation of some stress response hormones including cortisol [1, 13, 21]. At 36 °C, 10 % of individuals died within the first week owing to low feed intake over early exposure days to tank environments and excessive expenditure of energy for swimming activity [28], attempting to escape from high temperatures, or surfacing for air oxygen [27]; glucose levels then declined under higher (34 and 36 °C) in 4 days. From the 7th day, physiological responses of changing hematological properties, apparently caught up with oxygen demand or energy mobilisation for swimming activity, were regulated and fish acclimated to their medium resulted in no significant differences in plasma glucose concentrations.

No studies have investigated IGF-1 level in tra to date. It is interesting to note that IGF-1 level reported here of 19.07±4.48 ng/ml (n = 283), was at least two to three times higher than reported in other fishes including channel catfish, *Ictalurus punctatus*, 4.19±0.36 ng/ml (at 21.7 °C) and 5.39±0.28 ng/ml (at 26.0 °C) [44], and coho salmon *Oncorhynchus kisutch*, <8 ng/ml [43]. A number of studies have reported the strong correlation between plasma GH and IGF-1 levels [31] and high plasma IGF-1 levels was observed in fast growing fish [3]. Data here show that temperature had no effects on IGF-1 levels in tra catfish juveniles (see tabl. 3) although there were differences in growth performance among different temperature treatments. However, IGF-1 levels at higher temperatures (32 and 34 °C) significantly increased in the long-term measurements on 14th and 28th day (tabl. 5). This finding is consistent with previous studies [3, 24], where IGF-1 levels increased over a few weeks before returning to base levels.

Conclusions

Temperature has a clear effect on the tra catfish survival rate, growth performance and short term stress responses. Water temperatures ≤24 °C significantly affect survival rate and growth performance, and temperature >34°C tends to inhibit fish growth rate. In this study, 34 °C appeared to be the optimum temperature for tra catfish with no significant difference on FCR values in comparison with lower temperature but producing higher growth rates. Temperatures across the thermal tolerance range in tra catfish do not have clear effect on fish osmoregulation or IGH-1 levels, but individuals respond to rapid changes in temperature by increasing plasma glucose concentration. If average water temperature across the Mekong Delta do not decline below current levels or increase above 34 °C, the tra catfish culture is not likely to experience any declines in productivity or efficiency.

It should be noted however, that while some increase in water temperature would appear to be beneficial to tra culture performance, it would be unwise to

rely on these data in isolation, particularly with respect to global warming. In association with temperature rise, significant inundation of the MRD through marine incursion (sea level rise) has also been predicted [33]. It would be pertinent therefore, to investigate the interaction between these environmental factors, particularly with respect to tra culture.

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Abstract

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EFFECTS OF TEMPERATURES ON GROWTH PERFORMANCE, HEMATOLOGICAL PARAMETERS AND PLASMA IGF-1 LEVEL OF TRA CATFISH (*PANGASIANODON HYPOPHTHALMUS*)

This study focuses on the impact of possible temperature increase on tra catfish (*Pangasianodon hypophthalmus*) in the Mekong River Delta. The effects of a range of different temperatures were assessed on survival rates, and physiological processes relating to growth. Two trials were conducted: (1) short-term (14 days) and (2) long-term (56 days) that investigated the impacts of temperature (24—36°C) on fish growth performance, physiological and hormonal responses. Hematological parameters including red blood cell counts, hemoglobin concentrations and hematocrits at 24°C and higher sublethal temperature (30, 32, 34 and 36°C) treatments were significantly higher than what may be considered the control temperature, 27°C. 34°C appeared to be the optimum temperature for tra catfish growth with the highest weight gain, daily weight gain, specific growth rate, with no observed decline in survival. It is suggested that maximal growth performance at 34°C was due to the more intensive metabolism, enabling greater food intake with no associated increase in food conversion ratio in comparison with other treatments as might be expected under stressful conditions. Most growth performance parameters started declining at 36°C. Providing culture environments do not rapidly increase above 34°C, cultivated tra catfish should continue to perform well.

Keywords: *climate change, Pangasianodon hypophthalmus, temperature, IGF-1, growth performance.*