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Growth performance and body composition of *Oreochromis niloticus* reared at different water temperatures

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Summary — We evaluated the growth performance and body composition of *Oreochromis niloticus* reared at different water temperatures (23, 26, 29 and 32°C) and fed a standard diet twice daily of about 3% of their body weight for a period of 58 days. The total weight gain and specific growth rate of fish differed significantly ($P < 0.05$) at different water temperatures and increased with the increase in temperature up to 29°C, after which no significant increase was observed. The values for the feed conversion ratio (FCR) decreased with the increase in water temperature indicating a better utilization of feed per unit live weight gain. The differences were however non-significant at 29 and 32°C. Differences were also observed in the body composition of fish. Fat and ash contents decreased and moisture content increased as the water temperature increased, whereas the crude protein and gross energy contents of fish were not affected ($P > 0.05$). Our results suggest that the optimum temperature for *Oreochromis niloticus* lies somewhere near 29°C.

Oreochromis niloticus / growth / body composition / water temperature

Résumé — Performance de croissance et composition corporelle d'*Oreochromis niloticus* soumis à différentes températures d'élevage. Les performances de croissance et la composition corporelle d'*Oreochromis niloticus*, soumis à différentes températures d'élevage (23, 26, 29 et 32 °C), ont été évaluées. Les poissons ont reçu un aliment standard représentant environ 3 % de leurs poids corporels, deux fois par jour, pendant une période de 58 j. Le gain de poids total et le taux de croissance spécifique ont été significativement différents ($p < 0,05$), ils ont augmenté avec la température de l'eau jusqu'à 29 °C, température à partir de laquelle plus aucune augmentation significative n'a été observée. Les valeurs de taux de conversion alimentaire (FCR) ont diminué avec l'augmentation de la température indiquant une meilleure utilisation de l'aliment par unité de gain de poids vif ; les valeurs du FCR n'ont cependant pas été significativement différentes entre 29 et 32 °C. La composition

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corporelle des poissons a été modifiée par la température : la proportion d'eau corporelle est plus élevée et les contenus en lipides et en cendres plus faibles lorsque la température de l'eau est plus élevée. Le pourcentage de protéines brutes et l'énergie brute n'ont pas été affectés. Les résultats de l'étude suggèrent que la température optimale d'élevage pour *Oreochromis niloticus* se situe autour de 29 °C.

Oreochromis niloticus / croissance / composition corporelle / température de l'eau

INTRODUCTION

Temperature is one of the most influential factors affecting the physiological and biochemical functions of fish (Brett et al, 1969; Corey et al, 1983; Jobling, 1994). For most of the fish species studied so far, when the food supply is not limited, the specific growth rate of fish has been found to increase with increasing temperature, until the optimum temperature, above which it declines (Talbot, 1993). Although the increasing temperature produces a positive effect on the efficiency of transfer of food energy into net energy, it poses a negative effect on growth due to increment in energy cost for maintenance metabolism (Xie and Sun, 1992). Different fish species have got different temperature preferences for optimal growth. The temperature at which the total weight gain is the greatest may be considered as an optimal temperature, although there may be some exceptions to this rule (Jensen, 1985; Konstatinov et al, 1988; Stefens, 1989; Rasmussen and From, 1991; Jobling, 1994). Temperature can also affect the chemical composition of an organism, particularly the fatty acid composition (Shikata et al, 1995).

The regulatory mechanisms underlying the relationship between the growth rate and temperature are largely unknown but are likely related to the hormonal modulation of metabolic processes (Sumpter, 1992). The limits of temperature tolerance and the effects of temperature on growth rate are not fixed and many fish species demonstrate metabolic and biochemical adaptations to

different temperature regimes (Konstatinov et al, 1988; Johnston, 1993). Since the body temperature of fish varies with the water temperature, the rate of metabolic reactions is affected accordingly and a thermal compensation occurs to avoid the decrease or increase of metabolic activities at cold and warm acclimated water temperatures respectively. Thus, Gelman et al (1992) reported that *Tilapia* species have certain physiological limits for temperature adaptations.

Tilapias constitute a major group of fish species which may be cultured the whole year in the tropics with some variation in the gross fish yields, depending upon the seasonal differences related to the area (Green et al, 1990). In general, *Tilapias* are able to tolerate temperatures up to 42°C but do not grow below 16°C and can not survive for more than a few days below 10°C (Chervinski, 1982). Limited information is available about the optimum water temperature for the maximum growth of this fish. The present study was therefore conducted to evaluate the effect of different water temperatures on the growth performance, nutrient utilization and body composition of *Oreochromis niloticus*.

MATERIALS AND METHODS

Oreochromis niloticus with an average weight of 11.24 ± 0.23 g were collected from the fish hatchery of King Abdul Aziz City for Science and Technology (KACST) (Deerab, Riyadh, Saudi Arabia). To determine their initial body composition, 30 randomly selected fish were killed immediately and after recording their body

weight and length, were stored at -30°C for the proximate analysis at a later stage. One hundred and twenty fish were then randomly divided into four different groups with three replicates containing 10 fish in each replicate. Each group of fish was then transferred to glass aquariums ($100 \times 42 \times 50$ cm) containing well aerated tap water and having four different temperature regimes: 23, 26, 29 and 32°C . The temperature of water in different aquariums was maintained through a thermostatically controlled heating system to the nearest $\pm 0.5^{\circ}\text{C}$. All the aquariums were fitted with a waste filtration facility. Compressed air was used to maintain the oxygen supply. Regular monitoring of water quality was carried out for pH, dissolved oxygen, ammonia, nitrite and nitrate levels. These parameters were kept within the tolerance limits for *Oreochromis niloticus*. The fish were given a 10-day adaptation period to thermally acclimatize themselves before the actual start of the experiment.

The fish were fed a standard diet containing: casein 12.0%; fish meal 25.0%; soybean meal 15.0%; maize grain 25.0%; cod liver oil 2.0%; corn oil 5.0%; dextrin 11.0%; gelatin 2.0%; mineral mixture 2.0% and vitamin mixture 1.0%. The diet was prepared as already described by Al-Asgah and Ali (1994). The proximate chemical composition of the diet was: dry matter 92.2%; crude protein 39.2%; crude fiber 2.2%; total fat 11.7%; ash 11.1%; nitrogen free extract 36.3%; and gross energy 19.91 MJ kg^{-1} on dry matter basis. The diet was offered twice daily at about 3% of fish body weight. The uneaten portions of the diet were siphoned out and weighed after drying. The feed offered was adjusted fortnightly in relation to body weight and satiety of fish. The daily feed intake and 2-week weight gains were recorded. The experiment lasted for 58 days. At the end of the experimental period all the fish were killed and their body weight and length recorded.

To determine their carcass composition, the fish were cut into pieces and minced through a meat mincer. The homogenized samples were immediately frozen at -30°C for further analysis. The proximate chemical composition was determined according to the methods of Association of Official Analytical Chemists (1984). The gross energy content of fish was calculated from the fat and protein contents using the equivalents of 39.54 MJ kg^{-1} for crude fat and 23.64 MJ kg^{-1} for crude protein (Kleiber, 1961). Feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) and net protein

retention (NPR) values were calculated as follows:

Feed conversion ratio = g feed dry matter consumed per g live weight gain;

Specific growth rate (as percentage of body weight gain per day) = $100 \times [\ln \text{ final wt (g)} - \ln \text{ initial weight (g)}] / \text{time (days)}$;

Protein efficiency ratio = live weight gain (g)/protein consumed (g); and

Net protein retention = $[\text{increase in carcass protein} / \text{protein fed}] \times 100$.

The condition factor (k) was calculated according to the equation $k = [W(\text{g})/L(\text{cm})^3] \times 100$, where W is the wet weight of fish in grams and L is the length in centimeters. The data so collected were subjected to statistical analysis using the analysis of variance technique and the means were compared by Fisher's LSD test according to Snedecor and Cochran (1989).

RESULTS

The results on the growth performance of *Oreochromis niloticus* reared at different temperatures are presented in table I. The results indicated that the total weight gain and specific growth rate of fish differed significantly ($P < 0.05$) and increased with the increase in water temperature up to 29°C , after which no significant increase was observed. Figure 1 shows the growth performances in relation to temperatures; significant differences started appearing after 28 days and the same pattern continued till the end of the experiment. The highest condition factor was obtained at 29°C , but no significant differences were observed at 29 or 32°C . The fish reared at 23°C consumed significantly ($P < 0.05$) less feed as compared to those reared at 26, 29 or 32°C . The values for the feed conversion ratio decreased with the increase in rearing temperature. The differences in the FCR values for fish reared at 29 or 32°C were however, non-significant. The PER and NPR values increased with increase in water temperature. The body composition of fish changed with water temperatures (table II).

Table I. Growth performance of *O niloticus* reared at different water temperatures.

Parameters	Temperature				SE*
	23°C	26°C	29°C	32°C	
Initial weight (g/fish)	11.78	10.96	11.19	11.01	± 0.54 ^{NS}
Final weight (g/fish)	19.89 ^c	25.39 ^b	30.65 ^a	31.09 ^a	± 1.28
Total weight gain (g/fish)	8.11 ^c	14.43 ^b	19.46 ^a	20.08 ^a	± 0.89
Specific growth rate (SGR)	0.90 ^c	1.45 ^b	1.74 ^a	1.79 ^a	± 0.08
Condition factor (k)	2.87 ^c	2.95 ^{bc}	3.14 ^a	3.03 ^{ab}	± 0.21
Total feed consumed (g/fish)	18.37 ^b	24.68 ^a	26.07 ^a	25.70 ^a	± 1.05
Feed conversion ratio (FCR)	2.27 ^a	1.71 ^b	1.34 ^c	1.28 ^c	± 0.12
Protein efficiency ratio (PER)	1.22 ^c	1.62 ^b	2.06 ^a	2.16 ^a	± 1.13
Net protein retention (NPR)	21.87 ^c	28.00 ^b	33.51 ^a	32.62 ^a	± 1.76

*. pooled standard error; NS, non-significant.

a, b, c Different superscript letters in the same row mean significance at 5%.

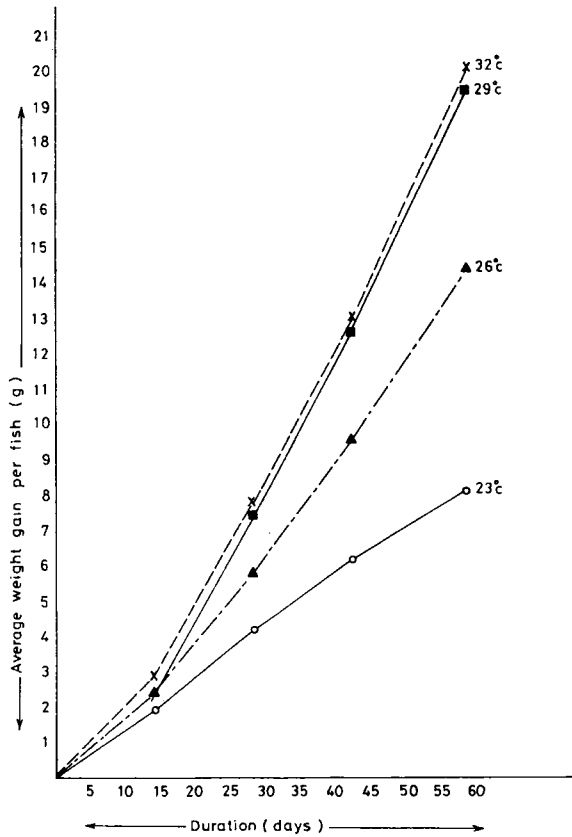


Fig 1. Effect of different temperatures on the growth performance of *Oreochromis niloticus*.

Table II. Data on the proximate chemical composition of *O niloticus* reared at different water temperatures (on percentage wet weight basis)¹.

Parameters	Temperature				SE*
	23°C	26°C	29°C	32°C	
Initial weight (g/fish)	11.78	10.96	11.19	11.01	± 0.54 ^{NS}
Moisture (%)	72.86 ^a	72.47 ^a	73.31 ^b	73.27 ^b	± 0.19
Crude protein	15.53	15.48	15.85	15.79	± 0.26 ^{NS}
Total fat	6.10 ^a	5.70 ^b	5.42 ^b	5.56 ^b	± 0.19
Ash	4.48 ^a	4.35 ^a	4.25 ^{ab}	4.08 ^b	± 0.11
Gross energy (MJ kg ⁻¹)	6.08	5.90	5.89	5.93	± 0.12 ^{NS}

¹ Proximate chemical composition of fish slaughtered at the beginning of the experiment (moisture, 73.56%; crude protein, 14.83%; total fat, 5.77%; ash, 4.51% and gross energy, 5.78 MJ kg⁻¹).

*. pooled standard error; NS, non-significant.

^{a, b} Different superscript letters in the same row mean significance at 5%.

The moisture content of fish increased with temperature. The differences in the moisture content of fish reared at 23 or 26°C and 29 or 32°C were however, non-significant. Change in temperature did not affect ($P > 0.05$) the crude protein content of fish. The highest fat content was observed in fish reared at 23°C. The body fat content of fish reared at 26, 29 or 32°C did not differ significantly ($P > 0.05$). Although the fish reared at 32°C showed the lowest body ash content, the differences between the ash content of fish reared at 29 or 32°C were non-significant. The differences in the body ash content of fish reared at 23, 26 or 29°C were also non-significant. The water temperature did not affect ($P > 0.05$) the gross energy content of fish.

DISCUSSION

The specific growth rate of most of the fish species increases with increasing temperature, provided the food supply is not limited (Corey et al, 1983; Heap and Thorpe, 1987; Talbot, 1993). The increase in growth rate reflects the increase in appetite, food

consumption and biochemical reaction rate within the thermal tolerance range of fish (Brett and Groves, 1979; Jobling, 1993). According to Jobling (1993), the appetite peaks at temperatures approaching the upper thermal tolerance limit of the species but falls dramatically towards both extremes of the tolerated temperature range. At higher water temperatures, the fishes feed more vigorously and their digestive processes are accelerated (Cossins and Bowler, 1987). Heap and Thorpe (1987) observed that the O-group malpigmented and normally pigmented turbot, *Scophthalmus maximus* (L) and turbot-brill hybrids, *S maximus* × *rhombus* (L) grew faster at higher environmental temperatures, mainly because of much improved appetite but also perhaps due to an increase in food conversion efficiency. Cai and Curtis (1990) however reported that the growth rate and food consumption, but not the assimilation efficiency in triploid grass carp (*Ctenopharyngodon idella*) increased with environmental temperature. Likongwe (1995) observed that the temperature, salinity and their interaction affected the growth of Nile tilapia (*Oreochromis niloticus*), which was higher ($P < 0.05$) at 28 and 32°C

than at 24°C. The results of the present study are in line with these findings. They are also in line with the work of Iwata et al (1994), who reported that the daily growth rate of Japanese flounder (*Paralichthys Olivaceus*) increased with temperatures up to 20 or 25°C after which it decreased. They concluded that the high energy expenditure for catabolism appears to reduce the energy available for growth of larger flounder at 30°C.

The effects of environmental temperature on the enzyme activities in fish occur even at molecular level and are species-specific. The proteolytic, amylolytic and lipolytic activities in the pyloric caeca of rainbow trout have been reported to depend upon the temperature and food quality and fall on lowering the temperature (Platnikow, 1982). The temperature also affects the rate of protein synthesis and degradation in fish (Watt et al, 1988). The protein requirement of young fishes changes with water temperature (NRC, 1981). In fed fry, the rate of protein turnover decreased with increasing temperature (Fauconneau et al, 1986; Mather et al, 1993). Mather et al (1993) reported that as water temperature was raised from 5 to 15°C, higher rates of protein growth were brought about by an increase in the rate of protein synthesis and also by increased efficiency of retention of synthesized protein (reduced protein turnover). Likongwe (1995) reported that the feed conversion efficiencies and protein efficiency ratios in *Oreochromis niloticus* increased with temperature. The higher PER and NPR values observed in this study at 29 and 32°C are in close agreement with these findings and indicated a better utilization of protein at higher temperatures. No significant interaction has however been reported between water temperature and feeding rates (Hung et al, 1993; Foster et al, 1992, 1993; Hasan and Macintosh, 1993). Foster et al (1992, 1993) demonstrated that fish fed similar ration sizes exhibited the same rates of growth and protein synthesis regardless of temperature.

They concluded that it is necessary to consider the compounding effect of ration level and temperature in order to separate the effects of temperature alone from temperature related to feed consumption.

The body composition of fish is affected by both the endogenous and exogenous factors (Haard, 1992). Brett et al (1969) observed that ration or temperature did not affect the body protein content of juvenile sockeye salmon that received a sufficient amount of ration to gain weight. Elliot (1975) reported that temperature and ration size affected the body composition of brown trout (*Salmo trutta* L) but did not change the protein content. Hung et al (1993) reported that temperature and feeding rate did not affect the body moisture and protein contents of white sturgeon but significantly affected the body ash content. The results of the present study are in line with these findings. The results however, do not agree with the work of Gill and Weatherley (1984), who reported that bluntnose minnow (*Pimephales notatus*) raised at low temperatures had significantly higher levels of body protein. The variability in the reported results may be because of the species differences. Our results indicated that the body composition of *Oreochromis niloticus* was significantly affected with the change in temperature. The body moisture content increased whereas the lipid and ash contents decreased with the increase in water temperature. The higher body lipid content observed in this study at 23°C may be because of the activated synthesis of fatty acids at lower temperatures (Shikata et al, 1995). The differences in the ash content are in accordance with the growth rate of fish and can be explained as the higher growth rate means higher proportion of soft tissues and thus less ash content. The results of the present study suggest that the optimum temperature for *Oreochromis niloticus* lies somewhere near 29°C.

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REFERENCES

- Al-Asghar NA, Ali A (1994) Feeding of various carbohydrate sources on the growth performance and nutrient utilization in *Oreochromis niloticus*. *Agric Biological Research* 47, 1–12
- Association of Official Analytical Chemists (1984) *Official Methods of Analysis of The Association of Official Analytical Chemists*. Washington, DC, USA
- Brett JR, Groves TDD (1979) Physiological energetics. In: *Fish Physiology*. VIII. (WS Hoar, DJ Randall, JR Brett, eds), Academic Press, New York, NY, 280–352
- Brett JR, Shelbourn JE, Shoop CT (1969) Growth rate and body composition of fingerling sockeye salmon (*Oncorhynchus nerka*) in relation to temperature and ration size. *J Fish Res Board Canad* 26, 2363–2394
- Cai Z, Curtis LR (1990) Effects of diet and temperature on food consumption, growth rate and tissue fatty acid composition of triploid grass carp. *Aquaculture* 88, 313–327
- Chervinski J (1982) Environmental Physiology of Tilapias. In: *The Biology and Culture of Tilapias*. (RSV Pullin, RH Lowe-McConnell, eds), Proceedings of the International Conference on the Biology and Culture of Tilapias, 2–5 September 1980 at the Study and Conference Centre of the Rockefeller Foundation, Bellagio, Italy, sponsored by the ICLARM, Manila, 119–128
- Corey PD, Leith DA, English MI (1983) A growth model for coho salmon including effects of various ration allotments and temperature. *Aquaculture* 30, 125–143
- Cossins AR, Bowler K (1987) *Temperature Biology of Animals*. Chapman & Hall, London, 399 p
- Elliot JM (1975) Body composition of brown trout (*Salmo trutta* L) in relation to temperature and ration size. *J Anim Ecol* 45, 273–289
- Fauconneau B, Aguirre P, Bergot P (1986) Protein synthesis in early life of coregonids: Influence of temperature and feeding. *Archive für Hydrobiologie Beihefte* 22, 171–188
- Foster AR, Houlihan DF, Hall SJ, Burren LJ (1992) The effects of temperature acclimation on protein synthesis rates and nucleic acid content of juvenile cod (*Gadus morhua* L). *Can J Zool* 70, 2095–2102
- Foster AR, Hall SJ, Houlihan DF (1993) The effects of seasonal acclimatization in cod. *J Fish Biol* 42, 461–464
- Gelman A, Cogan U, Mokady S (1992) The thermal properties of fish enzymes as a possible indicator of the temperature adaptation potential of the fish. *Comp Biochem Physiol* 101B, 205–208
- Gill HS, Weatherley AH (1984) Protein, lipid and caloric contents of bluenose minnow, *Pimphales notatus* Rafinesque, during growth at different temperatures. *J Fish Biol* 25, 491–500
- Green BW, Teichert-Coddington DR, Phelps PR (1990) Response of tilapia yield and economics to varying rates of organic fertilization and season in two Central American countries. *Aquaculture* 90, 279–290
- Haard NF (1992) Control of chemical composition and food quality attributes of cultured fish. *Food Res Int* 25, 289–307
- Hasan MR, Macintosh DJ (1993) Effect of environmental temperature and feeding rate on the growth, food utilization and body composition of common carps (*Cyprinus carpio* L) fry. In: *Fish Nutrition in Practice* (SJ Kaushik, P Luquet, eds), Institut National de la Recherche Agronomique, Paris, France, No 61, 767–778
- Heap SP, Thorpe JP (1987) A preliminary study of comparative growth rates in O-group malpigmented and normally pigmented turbot, *Scophthalmus maximus* (L), and turbot-brill hybrids, *S. maximus* × *rhombus* (L), at two temperatures. *Aquaculture* 60, 251–264
- Hung SSO, Lutes PB, Shqueir AA, Conte FS (1993) Effect of feeding rate and water temperature on growth of juvenile white sturgeon (*Acipenser transmontanus*). *Aquaculture* 115, 297–303
- Iwata N, Kikuchi K, Honda H, Kiyono M, Kurokora H (1994) Effect of temperature on the growth of Japanese Flounder. *Fisheries Sci* 60, 527–531
- Jensen JW (1985) The potential growth of salmonids. *Aquaculture* 48, 223–234
- Jobling M (1993) Bioenergetics: feed intake and energy partitioning. In: *Fish Ecophysiology* (Rankin JC, Jensen FB, eds), Chapman & Hall, London, 1–44
- Jobling M (1994) *Fish Bioenergetics*. Chapman & Hall, London, 328 p
- Johnston IA (1993) Phenotypic plasticity of fish muscle to temperature change. In: *Fish Ecophysiology* (JC Rankin, FB Jensen, eds), Chapman & Hall, London, 322–340
- Kleiber M (1961) *The Fire of Life. An introduction to animal energetics*. John Wiley, New York, USA
- Konstatinov AS, Zdanovich VV, Kalashnikov YuN (1988) Effect of temperature variation on the growth of eurythermous and stenothermous fishes. *J Ichthyol* 28, 61–67
- Likongwe JS (1995) *Effects of temperature, salinity, dietary protein and energy on juvenile Nile tilapia (Oreochromis niloticus* Linnaeus). Ph D Disserta-

- tion, The Pennsylvania State University, USA, 128 p
- Mather EM, Houlihan DF, McCarthy ID, Burren LJ (1993) Rates of growth and protein synthesis correlated with nucleic acid content in fry of rainbow trout, *Oncorhynchus mykiss*: effects of age and temperature. *J Fish Biol* 43, 245–263
- National Research Council - National Academy of Sciences (1981) *Nutrient requirements of coldwater fishes*. No. 16. National Academy Press, Washington, DC, USA, 63 p
- Platnikow H (1982) Einfluß der Milieutemperatur und des Protein- / Fettgehaltes in der Diät auf die Proteaseaktivität in den Pylorasanhängen der Regenbogenforelle (*Salmo gairdneri* R). *Wiss Z Wilhelm-Pieck Univ Rostock, math-naturwiss R* 31, 45–50
- Rasmussen G, From J (1991) Improved estimates of growth model and body composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792) as function of feeding level, temperature and body size. *Dana* 9, 15–30
- Shikata T, Iwanaga S, Shimeno S (1995) Metabolic response to acclimation temperature in carp. *Fisheries Sci* 61, 512–516
- Snedecor GW, Cochran WG (1989) *Statistical Methods. 8th Edition*. The Iowa State University Press, Ames, Iowa, USA
- Steffens W (1989) *Principles of Fish Nutrition*. Ellis Horwood Limited, Publishers, Chichester, West Sussex, UK, 383 p
- Sumpter JP (1992) Control of growth of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 100, 299–320
- Talbot C (1993) Some aspects of the biology of feeding and growth in fish. *Proc Nutr Soc* 52, 403–416
- Watt PW, Marshall PA, Heap SP, Loughna PT, Goldspink G (1988) Protein synthesis in tissue of fed and starved carp acclimated to different temperatures. *Fish Physiol Biochem* 4, 165–173
- Xie XJ, Sun R (1992) The bioenergetics of southern catfish (*Silurus meridionalis* Chen): Growth rate as a function of ration level, body weight and temperature. *J Fish Biol* 40, 719–730