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Effects of growth hormone transgenesis on metabolic rate, exercise performance and hypoxia tolerance in tilapia hybrids

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Swimming respirometry was employed to compare inactive metabolic rate (R_r), maximum metabolic rate (R_{max}), resultant aerobic scope and maximum sustainable (critical) swimming speed (U_{crit}), in growth hormone transgenic (GHT) and wild-type (W) tilapia *Oreochromis* sp. hybrids. Although the R_r of GHT tilapia was significantly (58%) higher than their W conspecifics, there were no significant differences in their net aerobic scope because GHT tilapia exhibited a compensatory increase in R_{max} that was equal to their net increase in R_r. As a consequence, the two groups had the same U_{crit}. The GHT and W tilapia also exhibited the same capacity to regulate oxygen uptake during progressive hypoxia, despite the fact that the GHT fish were defending a higher demand for O_2. The results indicate that ectopic expression of GH raises metabolic rate in tilapia, but the fish compensate for this metabolic load and preserve such physiological determinants of fitness as aerobic scope, swimming performance and tolerance of hypoxia.

Key words: aerobic scope; citrate synthase; critical swimming speed; lactate; maximum metabolic rate; oxygen uptake.

INTRODUCTION

There has been much recent interest in the potential for enhancement of fish growth in aquaculture through genetic manipulation (Du *et al.*, 1992; Devlin *et al.*, 1994; Martinez *et al.*, 1996, 1999). A strain of growth hormone transgenic (GHT) tilapia *Oreochromis* sp. hybrids has been created that expresses homologous tilapia growth hormone (GH) at low levels in its tissues and exhibits increased growth relative to wild-type (W) conspecifics (de la Fuente *et al.*, 1995; Martinez *et al.*, 1996, 1999).

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Tilapia are important in warm-water aquaculture throughout the world, but one major risk perceived in the exploitation of GHT fishes is that escaped animals could pose a serious threat to the future of wild populations if they have a mating advantage due to larger size coupled with a reduced fitness (Muir & Howard, 1999; Reichhardt, 2000; Hedrick, 2001). It is, therefore, important to investigate how GH transgenesis might influence the fitness of fishes.

The current study investigated the effects of GH transgenesis on some physiological traits which are believed to contribute to fitness by determining the ecological niches that a fish can occupy successfully (Prosser, 1950). These are metabolic rate, aerobic scope, the capacity for sustained aerobic exercise and tolerance of hypoxia (Fry, 1971; Priede, 1985; Randall & Brauner, 1991; Wikelski & Ricklefs, 2001). The increased growth elicited by GH transgenesis in tilapia (Martinez *et al*., 1996, 1999) may entail an increase in metabolic rate and consequent demand for O_2. (Stevens *et al*., 1998; Cook *et al*., 2000*a*). This increased metabolic load may reduce aerobic scope and the capacity to allocate O_2 for sustained aerobic exercise in transgenic fishes, and also impair their ability to defend aerobic metabolism in the face of a reduced O_2 supply (Herreid, 1980).

Swimming respirometry was employed to measure rates of oxygen uptake over a range of swimming speeds, and to derive inactive metabolic rate (R_r), aerobic scope, and maximum sustainable aerobic exercise performance, in individual fish. The same individuals were then exposed to a progressive decline in water oxygen tension, to assess their tolerance of hypoxia. These traits were then compared in size-matched GHT *v*. W tilapia.

MATERIALS AND METHODS

ANIMALS

Transgenic tilapia were created by micro-injection of early embryos with a gene construct containing homologous tilapia GH cDNA under the regulatory sequences of a human cytomegalovirus, as described by de la Fuente *et al*. (1995). The GHT fishes used in this study were hybrids of the Java tilapia *Oreochromis mossambicus* Peters and the Wami tilapia *Oreochromis hornorum* (Trewavas), the F4 generation of the transgenic IG91-03/F70 germline described in detail by Martinez *et al*. (1999, 2000). The GHT fishes were compared with non-transgenic W hybrids descended from the same stock of *O. mossambicus* and *O. hornorum*, maintained at the Centro de Ingeniería Genética y Biotecnologia (CIGB) aquaculture unit in Camaguey, Cuba.

Based on previous analyses of relative growth (Martinez *et al*., 1999), the W fishes were spawned *c*. 3 months prior to the GHT animals, in order to provide size-matched animals for this study. The fishes were spawned by placing sexually mature adults (two males, four females) in glass aquaria (500 l) over a sandy bottom at a constant temperature of 28° C. Eggs were incubated in the parents' mouths and, when larvae hatched, these were collected and transferred to glass aquaria (500 l) provided with a flow of dechlorinated and aerated Camaguey tapwater at 25–27° C. They were fed daily at 0800 hours, to satiation with a crumbled or pelleted feed (CENPALAB, Havana, Cuba) of suitable size until the juveniles were large enough (5 cm fork length, L_F) to transfer to the CIGB in Havana. In Havana the fishes were maintained at the CIGB headquarters in circular fibreglass tanks (500 l) provided with a flow of dechlorinated and aerated Havana tapwater at 25° C. Fishes were fed daily at 0800 hours, to satiation with a pelleted feed (CENPALAB, Havana, Cuba). The animals were used when they had reached *c*. 15 cm L_F. The age of the GHT fishes was *c*. 20 weeks, whereas the age of the W fishes was *c*. 31

weeks, indicating that the GHT fishes grew at >1·5 times that of the W conspecifics (Martinez *et al.*, 1999).

RESPIRATORY METABOLISM AND EXERCISE PERFORMANCE

All tilapia were starved for 24 h prior to respirometry, to avoid any confounding effects of digestion on metabolic rate or exercise performance (Ross *et al.*, 1992; Alsop & Wood, 1997). Swimming respirometry was performed with a Plexiglas Brett-type swim-tunnel respirometer (8·3 l) designed to exercise individual fish in a non-turbulent water flow with a uniform velocity profile (Steffensen *et al.*, 1984). The water flow was generated by a propeller attached to a variable speed DC permanent magnet motor. Motor speed was controlled by a PC and Labtech Notebook software. Water velocities were measured as $cm\,s^{-1}$ and swimming speeds as bodylengths s^{-1} ($BL\,s^{-1}$) corrected for the solid blocking effect of the fish as described by Bell & Terhune (1970). The temperature of the respirometer was controlled by immersion in a large outer Plexiglas tank that received a flow of dechlorinated aerated tapwater.

Instantaneous O_2 uptake (M_{O_2}) was measured by intermittent flow-through respirometry (Steffensen, 1989) over 10 min cycles, with an automated system described in Steffensen *et al.* (1994). Briefly, this system alternated 6 min of closed recirculation of the sealed respirometer with 4 min when the activation of a pump submerged in the outer tank flushed the respirometer with fresh aerated water. An O_2 electrode and meter recorded the linear decline in O_2 partial pressure (P_{O_2}) during the 6 min period of closed recirculation, the variations in P_{O_2} then being acquired and stored by a PC and Labtech Notebook, transferred into a spread-sheet programme (Lotus 1-2-3), and M_{O_2} calculated automatically for the last 5 min of recirculation. Blank tests run at the end of each experiment revealed that the proportion of total M_{O_2} attributable to bacterial metabolism was negligible and, accordingly, no corrections were applied.

Individual tilapia were rapidly dip-netted from their holding tank, blotted dry, weighed (to 0·1 g), measured (L_F to 1 mm), then placed in the respirometer at *c.* 1800 hours and allowed to recover and acclimate overnight (at least 14 h) while swimming gently at $1{\cdot}0\,BL\,s^{-1}$. This swimming speed was chosen because, at lower speeds, the tilapia did not swim but rested on the floor of the respirometer chamber. The following day, starting between 0800 and 0900 hours, the fishes were exposed to progressive increments in swimming speed of $0{\cdot}5\,BL\,s^{-1}$ every 30 min until fatigue. Maximum sustainable aerobic (critical) swimming speed (U_{crit}) was calculated in $BL\,s^{-1}$ as described by Brett (1964).

Oxygen uptake at each swimming speed was calculated as the average of three respirometry cycles. For each individual fish, a least-squares exponential regression was applied to the relationship between swimming speed and M_{O_2}. Extrapolation back to the *y*-intercept, a notional swimming speed of zero, was employed to correct for the contribution to M_{O_2} of locomotor muscle activity (Brett, 1964; Fry, 1971). The value thus derived was termed inactive metabolic rate (R_r) and it was assumed that any differences in mean R_r between GHT and W fishes could be ascribed to metabolic costs of GH transgenesis. Maximum metabolic rate (R_{max}) was estimated as the highest M_{O_2} measured during the swim protocol (Fry, 1971; Beamish, 1978; Thorarensen *et al.*, 1993). Net aerobic scope was estimated from $R_{max} - R_r$ and factorial scope as $R_{max}\,R_r^{-1}$ (Fry, 1971). Net metabolic cost of swimming was calculated at each swimming speed from mean $M_{O_2} - R_r$ (Beamish, 1978). Counts of tailbeat frequency were made at each swimming speed, as an index of propulsive muscular power.

TOLERANCE OF HYPOXIA

Following a 2 h recovery from exercise, each tilapia was exposed to progressive hypoxia while swimming gently at a speed of $1\,BL\,s^{-1}$. Water P_{O_2} was reduced from normoxia ($P_{O_2} = c.$ 18 kPa or 140 mmHg) to <2 kPa (15 mmHg) in eight steps over a 2 h period, by bubbling 100% nitrogen into the outer respirometer tank. Two 10 min measures of instantaneous M_{O_2} were collected at each step.

The critical P_{O_2} below which the tilapia could no longer regulate M_{O_2} (P_{crit}), was calculated for each fish by plotting M_{O_2} against P_{O_2}, then drawing a line parallel to the abscissa at R_r. A least-squares linear regression was applied to those data points lying below each line, and the resultant equations used to calculate the P_{crit} at the appropriate M_{O_2}. A similar procedure was followed to determine P_{crit} for the M_{O_2} measured at $1\,BL\,s^{-1}$ (the value measured following overnight recovery) as this was the swimming speed of the tilapia during the hypoxia test.

SUPPLEMENTAL ANALYSES

Following at least 48 h recovery from the exercise and hypoxia protocol, a series of supplemental variables were measured in support of the respirometric analyses. Plasma lactate accumulation was measured in hypoxia, to gain insight into rates of anaerobic metabolism; cardio-somatic and hepato-somatic indices were calculated to compare relative organ sizes (Bishop, 1999), and activity of citrate synthase (CS), a key enzyme for aerobic metabolism, was measured in the heart and liver.

Tilapia were exposed for 1 h to a hypoxic P_{O_2} of 2·63 kPa (20 mmHg) in open aquaria, then rapidly anaesthetized ($0{\cdot}5\,g\,l^{-1}$ MS-222) and a blood sample taken by caudal puncture. The sample was centrifuged, plasma decanted and lactate concentration measured (Sigma Lactate Kit method 735). The anaesthetized animals were killed with a blow to the head, blotted dry, weighed (to 0·1 g), and the heart and liver dissected out and weighed to an accuracy of ±0·01 g. Heart and liver indices were calculated as a percentage of total body mass. Activity of CS in the tissues was measured as described by Hansen & Sidell (1983).

RESULTS

Data are presented for six animals from each of the GHT and W groups, on which the entire protocol (exercise, hypoxia, supplemental analyses) was completed. These had statistically identical mean mass and L_F (Table I).

RESPIRATORY METABOLISM AND EXERCISE PERFORMANCE

When first placed in the respirometer, the GHT and W tilapia all exhibited an immediate rheotactic response and thereafter exhibited no spontaneous locomotory behaviours other than swimming against the water current. During the

TABLE I. Mean ± S.E. mass, fork length, maximum sustainable swimming speed (U_{crit}), inactive metabolic rate (R_r), maximum metabolic rate (R_{max}), net aerobic scope and factorial aerobic scope, in growth hormone transgenic (GHT) or wild-type (W) tilapia. $n = 6$

	GHT	W
Mass (g)	60·2 ± 9·9	75·8 ± 16·0
L_F (cm)	14·7 ± 0·9	15·7 ± 1·0
U_{crit} ($BL\,s^{-1}$)	5·22 ± 0·49	4·94 ± 0·45
R_r ($mmol\,O_2\,kg^{-1}\,h^{-1}$)	8·63 ± 0·86*	5·39 ± 0·27
R_{max} ($mmol\,O_2\,kg^{-1}\,h^{-1}$)	36·03 ± 2·04	32·16 ± 1·84
Net aerobic scope ($mmol\,O_2\,kg^{-1}\,h^{-1}$)	27·90 ± 1·64	26·77 ± 1·84
Factorial scope ($R_{max}\,R_r^{-1}$)	4·33 ± 0·29*	6·02 ± 0·41

*, significantly different from wild-type ($P < 0{\cdot}05$ two-tailed t-test).

exercise protocol, all tilapia swam vigorously until they collapsed completely against the back screen, from which they could not be persuaded to resume swimming by such stimuli as rapid increases in current velocity or gentle prodding. Maximum sustainable swimming speed (U_{crit}) was not significantly different, at *c.* 5 BL s^{-1}, in both groups (Table I). There was a negative relationship between L_F and U_{crit} in the tilapia, which was linear over the small size-range studied, and the individuals of both the GHT and the W tilapia were distributed along the same line (unpubl. data).

After the overnight acclimation to the respirometer, while exercising gently at a speed of 1 BL s^{-1}, GHT fishes had a significantly higher mean M_{O_2} than their W conspecifics, $10{\cdot}80 \pm 1{\cdot}00$ *v.* $8{\cdot}07 \pm 0{\cdot}16$ mmol kg^{-1} h^{-1}, (mean ± S.E., $P < 0{\cdot}02$ by two-tailed *t*-test). The derivation of R_r involves extrapolation beyond measured points (Brett, 1964), such that a reliable estimation is critically dependent upon a high correlation coefficient (r^2) for the least-squares regression of the relationship between swimming speed and M_{O_2}. All individual GHT and W fishes exhibited a clear exponential increase in M_{O_2} with increased swimming speed (Fig. 1), there was no evidence of a plateau in M_{O_2} prior to U_{crit}, and R_{max} was always measured at the velocity which the fishes achieved immediately prior to fatigue. The clear exponential relationships provided a high correlation coefficient in all individual fishes ($r = 0{\cdot}92 - 0{\cdot}989$ in the six GHT and

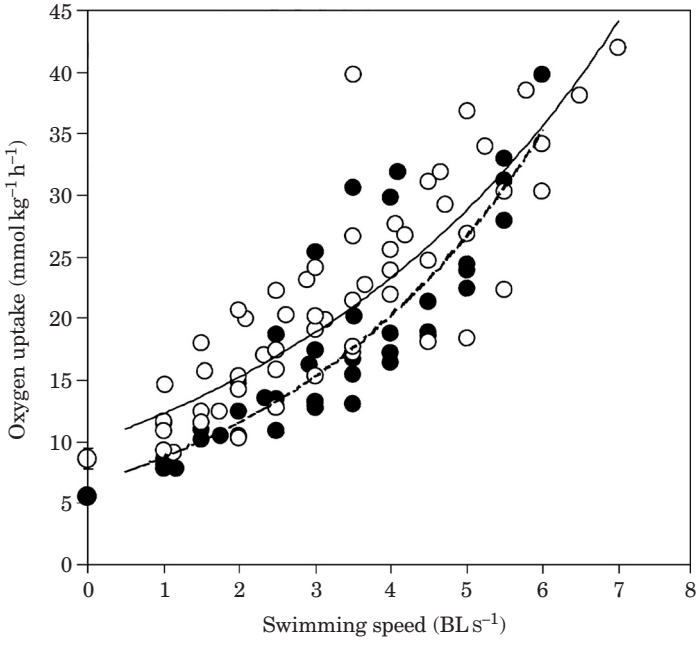

FIG. 1. The exponential relationships between swimming speed and O_2 uptake in growth hormone transgenic (○) or wild-type (●) tilapia. For the total of 54 observations on six transgenic fishes, the overall exponential relationship was described as $y = 9{\cdot}95e^{0{\cdot}212x}$ ($r^2 = 0{\cdot}729$) (—); for the 44 observations on six wild-types, the exponential relationship was $y = 6{\cdot}64e^{0{\cdot}278x}$ ($r^2 = 0{\cdot}800$) (---). The symbols on the *y*-axis show mean ± S.E. intercepts of the exponential relationships for individual transgenics (○) or wild-types (●), which were taken as an estimate of mean inactive metabolic rate (R_{min}). $n = 6$ for both groups.

$r = 0{\cdot}926 - 0{\cdot}997$ in the six W tilapia). The derived mean R_r was significantly higher in the GHT than in the W tilapia, *c.* 58% (Table I).

The power functions (Beamish, 1978), which described the relationships between swimming speed and net metabolic cost of swimming were indistinguishable amongst the GHT and W fishes (Fig. 2). Therefore, the elevated M_{O_2} measured in the transgenics at a speed of 1 BL s^{-1} could be attributed to factors other than costs of exercise and presumably, therefore, to their elevated R_r. Mean R_{max} was also higher in the GHT relative to the W fishes (Table I) although the difference was not statistically significant. The net difference in mean R_r, however, was similar to the net difference in mean R_{max}, 3·2 mmol kg^{-1} h^{-1} in the former and 4·4 mmol kg^{-1} h^{-1} in the latter. As a result, net aerobic scope was statistically equal in the two groups, although factorial scope was significantly lower in the GHT tilapia as a direct consequence of their high R_r (Table I).

At velocities <2 BL s^{-1}, locomotion was partially labriform (*i.e.* by sculling with pectoral fins), but exclusively sub-carangiform (*i.e.* by tailbeating) at higher velocites. Tailbeat frequency determined swimming speed in a very similar manner for both groups, being described by the logarithmic equations frequency, 3·060 ln(speed) − 1·836 for the GHT animals ($r^2 = 0{\cdot}802$, $n = 40$ observations on six fish) and frequency, 2·651 ln(speed) − 1·263 for W animals ($r^2 = 0{\cdot}746$, $n = 35$ observations on six fish), with speed in BL s^{-1} and frequency in Hz. Sub-carangiform locomotion usually provides a linear relationship between tailbeat frequency and swimming speed (Beamish, 1978), the logarithmic

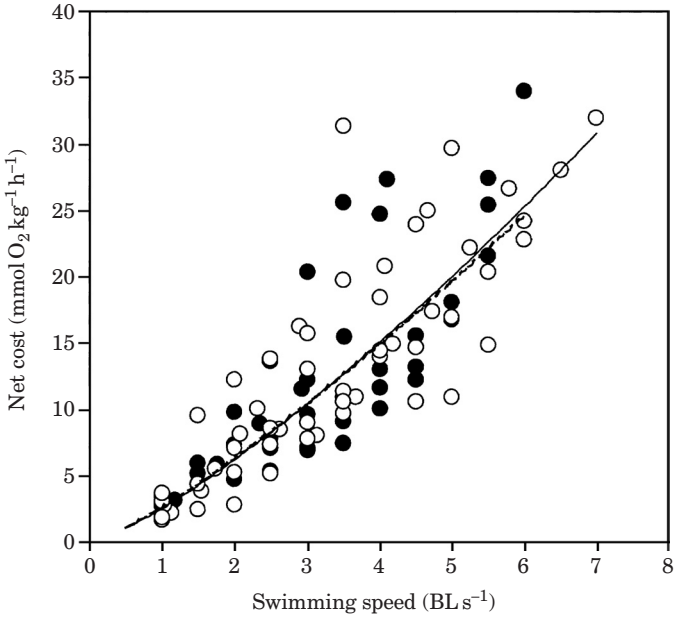

FIG. 2. The power relationship between swimming speed and net metabolic cost of swimming (as O_2 uptake) in growth hormone transgenic (○) or wild-type (●) tilapia. For the total of 48 observations on six transgenics, the power relationship was described as $y = 2{\cdot}56x^{1{\cdot}28}$ ($r^2 = 0{\cdot}800$) (—); for the 38 observations on six wild-types, the power relationship was $y = 2{\cdot}68x^{1{\cdot}24}$ ($r^2 = 0{\cdot}813$) (---).

equations presumably reflect the fact that pectoral sculling contributed to locomotion at lower speeds.

HYPOXIA TOLERANCE

At 2 h after exercise to fatigue, both GHT and W groups exhibited an exercise-related elevation of $\dot{M}O_2$, to rates that were significantly higher than those measured at 1 BL s^{-1} before the exercise protocol (*i.e.* after the overnight acclimation period in the respirometer). When exposed to progressive hypoxia, both GHT and W tilapia exhibited regulation of $\dot{M}O_2$ at the normoxic rate down to critical PO_2, beyond which an abrupt decline in aerobic metabolism, to below R_r, was observed (Fig. 3). Rates of $\dot{M}O_2$ were significantly higher in GHT *v.* W fishes throughout the progressive hypoxia protocol, down to a PO_2 of <2 kPa (Fig. 3). There were no differences in P_{crit}, however, between the two groups, which was 2·18 ± 0·21 kPa in GHT *v.* 2·47 ± 0·61 kPa in W fishes (Fig. 3). Furthermore, when a P_{crit} value was derived relative to the metabolic rate of the animals swimming at 1 BL s^{-1}, it proved to be 2·75 ± 0·29 kPa in GHT *v.* 4·16 ± 1·53 kPa in W fishes.

SUPPLEMENTAL MEASUREMENTS

Plasma lactate levels were significantly higher in GHT as compared with W tilapia following exposure to a hypoxic PO_2 of 2·63 kPa (20 mmHg) for 1 h (Table II). There were no significant differences in cardio-somatic or hepato-somatic indices between the GHT and W fishes (Table II). Both GHT and W

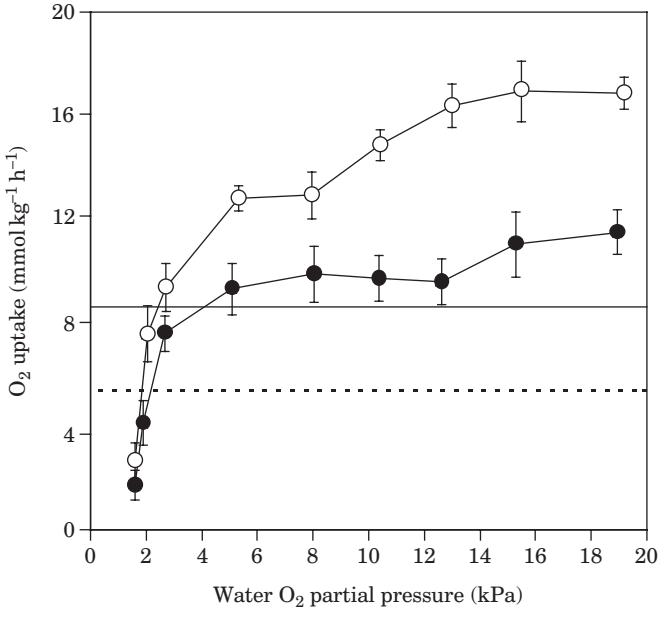

FIG. 3. The effects of progressive hypoxia on mean ± S.E. oxygen uptake in growth hormone transgenic (○) or wild-type (●) tilapia. Note that the fishes were swimming at a speed equivalent to 1 bodylength s^{-1}. —, mean inactive metabolic rate (R_r) of transgenics; ---, R_r of wild-types.

TABLE II. Mean ± S.E. plasma lactate concentration following 1 h of hypoxia at $Po_2 = 20$ mmHg; somatic indices, and activity of citrate synthase (CS) in the heart and liver of growth hormone transgenic (GHT) or wild-type (W) tilapia. $n = 6$

	GHT	W
Plasma lactate (mmol l^{-1})	1·62 ± 0·48*	0·60 ± 0·19
Cardio-somatic index (% body mass)	0·112 ± 0·012	0·094 ± 0·017
Hepato-somatic index (% body mass)	1·167 ± 0·036	1·244 ± 0·074
Heart CS (μmol g^{-1} wet tissue h^{-1})	102·3 ± 9·05*	63·7 ± 6·4
Liver CS (μmol g^{-1} wet tissue h^{-1})	24·4 ± 2·8*	15·0 ± 1·8

*, significantly different from W ($P < 0·05$, two-tailed t-test).

tilapia had higher CS activity in their hearts as compared to their livers. In both tissues, the GHT tilapia had significantly higher CS activity than their W conspecifics (Table II).

DISCUSSION

The results show that the increased growth rates of the IG91-03/F70 population of GHT tilapia relative to wild-types (Martinez *et al.*, 1996, 1999) are associated with increased rates of aerobic metabolism. Stevens *et al.* (1998) and Cook *et al.* (2000*a*) reported that groups of GHT Atlantic salmon *Salmo salar* L. have increased metabolic rate under routine culture conditions.

The term R_r is taken to indicate the metabolic rate of the post-absorptive growing animal. It is unknown whether the GHT tilapia also have an elevated standard metabolic rate (R_s), the minimum energy expenditure required to sustain life processes (Fry, 1971). Standard metabolic rate is measured on starved animals and does not, by definition, derive any contribution from growth (Fry, 1971). Groups of starved GHT Atlantic salmon have the same rate of O_2 uptake as their W conspecifics (Cook *et al.*, 2000*b*). Bishop (1999) suggested that the primary determinant of mass-specific R_s in birds and mammals is the relative size of metabolically expensive organs such as the liver, brain and heart. The GHT tilapia did not differ from the W conspecifics in cardio-somatic or hepato-somatic indices. For GHT tilapia, whether they are in culture or have escaped into the wild, it will be the ability of the feeding and growing animal to compensate for increased metabolic rate that will determine the constraints imposed on them by their respiratory milieu. The present data indicate that there is compensation in the transgenic animals, with precise matching of aerobic scope, maximum sustainable swimming speed and tolerance of hypoxia, in the GHT and W fishes.

It could be argued that although the increase in R_{max} in the GHT fishes was not statistically significant, it was physiologically significant in maintaining net aerobic scope. As a consequence, the GHT fishes achieved the same U_{crit} as their W conspecifics. The ability of the GHT fishes to achieve the same U_{crit} can be ascribed exclusively to their higher R_{max}, because the analyses of the metabolic costs of swimming, and of tailbeat frequencies, both indicate that swimming

efficiency was equal between the two groups. An increase in R_{max} implies an increased capacity for O_2 uptake and internal convection. Therefore, the GHT tilapia appeared to have a greater capacity for gas-exchange, which was also indicated by the same P_{crit} for regulation of M_{O_2} in progressive hypoxia in both GHT and W fishes, even though GHT animals were 'defending' higher rates of metabolism. It must be acknowledged that P_{crit} was determined on fishes that still exhibited a post-exercise elevation of M_{O_2}, and studies under more carefully controlled conditions might derive a lower P_{crit} for both groups. This, however, should not influence the validity of the comparison between the GHT and W fishes. In animals that regulate O_2 uptake during hypoxia, P_{crit} will depend on the ratio between M_{O_2} and the conductance of O_2 to the tissues. If M_{O_2} is higher in the GHT fishes, but P_{crit} does not change, then the capacity for conductance of O_2 to the tissues must be greater (Herreid, 1980).

These results reveal that the teleost respiratory transport chain is plastic in response to internal O_2 demand during development. Any number of the factors comprising the respiratory transport chain could contribute to the compensation. In birds and mammals, relative heart size is the most important single determinant of interspecific differences in R_{max} (Bishop, 1999), but the GHT and W tilapia did not differ in this respect. Stevens & Sutterlin (1999) have shown that GHT Atlantic salmon have increased gill surface area compared with W conspecifics, and this may also be true of the GHT tilapia. The pressure for compensation may have been the chronic elevation of routine O_2 demand. For birds and mammals it has, however, been argued that the maximal capacity of the cardiorespiratory system should reflect maximal aerobic function (Bishop, 1999). Indeed, the compensation by GHT tilapia of aerobic scope, in defence of maximum sustainable swimming speed, and their compensation of P_{crit} relative to R_r, were so exact in comparison to those of their W conspecifics as to argue against them being fortuitous. Thus, the pressure for compensation may have been exerted by the need to perform sustained exercise or regulate metabolism in hypoxia. The daily costs of digestion ('specific dynamic action' SDA) also cause large increases in O_2 uptake in tilapia (Ross *et al.*, 1992) that may have required compensation by the cardiorespiratory system. Interestingly, the GHT animals do not eat more than their W conspecifics but, rather, grow more efficiently (Martinez *et al.*, 2000).

The increased plasma lactate concentration following exposure to hypoxia in the GHT tilapia relative to their W conspecifics indicates that rates of anaerobic metabolism may also be higher in the transgenic animals. Furthermore, these physiological differences in aerobic and anaerobic metabolism were associated with metabolic differences at a cellular level, the GHT fishes exhibited increased activity of CS, a key enzyme for aerobic metabolism, in their heart and liver, and Martinez *et al.* (1999) reported increased tissue activity of phosphofructokinase (PFK), a key enzyme for anaerobic metabolism. Hill *et al.* (2000) found increased activity of cytochrome oxidase, an indicator of aerobic ATP production, and of PFK, in tissues of GHT coho salmon *Oncorhynchus kisutch* (Walbaum).

The current study is the first to explore the links between increased R_r, aerobic scope, swimming performance and hypoxia tolerance in individual GHT animals (rather than on groups of fishes under routine culture; Stevens

et al., 1998; Cook *et al.*, 2000*a*), and also the first on a non-salmonid. The results indicate that the impaired exercise performance (Farrell *et al.*, 1997) and reduced tolerance of hypoxia (Stevens *et al.*, 1998) measured in GHT salmonids may be due to an incomplete compensation for the O_2 demands of accelerated growth. Such incomplete compensation in salmonids (Farrell *et al.*, 1997; Stevens *et al.*, 1998) may be a consequence of higher degrees of growth hormone transgenesis and growth acceleration (Devlin *et al.*, 1994). It may also indicate that the salmonid cardiorespiratory system is less plastic, such that increased costs of accelerated growth place greater constraints on the capacity for internal O_2 allocation. There is, however, good evidence that the salmonid cardiorespiratory system is indeed plastic in response to a consistent increase in oxygen demand (Gallaugher *et al.*, 2001) and, furthermore, W salmonids can defer the payment of metabolic loads in order to sustain their maximum exercise performance (Farrell *et al.*, 1998). Investigating differences in cardiorespiratory compensation for GH transgenesis amongst different GHT lines, and different GHT species, may well provide insight into the physiological factors that limit maximum metabolic rate, aerobic scope and exercise performance in teleosts, and how these differ amongst species with different ecologies (Priede, 1985).

The introduction of any GHT fish into commercial aquaculture requires a thorough understanding of all factors that might influence the fitness of any escapes (Reichhardt, 2000), as natural populations may be damaged if GHT fishes have a reduced fitness but a mating advantage due to their larger size (Muir & Howard, 1999; Hedrick, 2001). The uncertainties in techniques for transgenesis are such that each GHT fish line will be unique (Reichhardt, 2000), but the current study indicates that the IG91-03/F70 line of GHT tilapia have compensated for the potential decline in fitness that might have derived from the effects of accelerated growth on their respiratory metabolism.

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