

SWIMMING METABOLISM OF WILD-TYPE AND CLONED ZEBRAFISH *BRACHYDANIO RERIO*

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Summary

The availability of a gynogenetic isogenic homozygous diploid clonal strain (C) of the zebrafish (*Brachydanio rerio*), combined with the small adult body size of the species, made possible a study of the following two questions. (1) Is the genetic uniformity of a group of fish reflected in decreased variability of features of organismic performance physiology? (2) Is the metabolic cost of subcarangiform swimming significantly different in small fishes compared with large ones?

Wild-type (WT) and C strain zebrafish maintained at 28 °C can all swim very rapidly [up to relative swimming speeds of 13 body lengths s^{-1} ($BL s^{-1}$)] for extended periods (at least 2 h) without visibly tiring. Oxygen consumption rates were measured for both types at swimming speeds of 1.5–13 $BL s^{-1}$. Whole-body lactate concentrations were also measured during routine activity and after prolonged exercise for both fish types. The slopes of the linear regressions between the logarithm of mass-specific oxygen consumption rates and relative swimming speeds for WT zebrafish were low (0.010–0.024) and were not significantly different from zero. Regression slopes were also low (0.009–0.026), but different from zero, for C zebrafish. Standard metabolic rates were 0.60–1.54 and 0.40–0.85 $ml O_2 g^{-1} h^{-1}$ for WT and C zebrafish respectively. Variances of slopes were significantly larger for WT than for C fish. Whole-body lactate concentrations and their variances were not significantly different between types and between rested and exercised fishes. The results demonstrate unusual swimming performance capacities, a remarkably low cost of swimming and some reductions in variability of C fish. Several possible explanations for the results are discussed.

Introduction

This paper deals with two principal questions. (1) In the context of both performance and energetics of actively swimming fishes, are genetic variability and whole-animal variability correlated? (2) With respect to species of teleost fishes that use the subcarangiform mode of locomotion and are physically small as adults, are there important features of swimming performance and energetics that differ significantly from the patterns characteristic of much larger species using the same locomotor mode?

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The large and diverse literature on the swimming of teleost fishes that use the subcarangiform mode of locomotion (Beamish, 1978; Videler and Wardle, 1991; Daniel *et al.* 1992) contains almost no information relating either to swimming energetics in species that are small [approximately 3 cm standard length (*SL*) or less] as adults or to possible effects, at the level of the whole organism, on swimming performance of genetic uniformity as manifested in cloned strains. The availability of an isogenic homozygous diploid (IHD) cloned (C) strain of the zebrafish (*Brachydanio rerio*; Streisinger *et al.* 1981) makes it possible to investigate both questions simultaneously.

The zebrafish is a common, small (adults <3 cm *SL*), relatively easily maintained freshwater aquarium fish that had its original geographic and ecological home in rapid streams in the mountains of south Asia (Laale, 1977). In addition to its popularity in the aquarium trade, in recent times it has become widely used both as a bioassay organism in a range of situations relating to environmental water quality (Fogels and Sprague, 1977; Bresch, 1986) and as the subject of many research investigations in such areas as development and neurobiology (Westerfield, 1989; Davidson, 1990; Ho and Kimmel, 1993; Strehlow and Gilbert, 1993). The fact that it is one of the few vertebrates that can be relatively easily cloned, and has a generation time short enough to permit multi-generational genetic manipulations within reasonable periods, is an important part of the basis for its research uses.

We have studied aspects of swimming performance (estimates of maximum swimming speeds and endurances at different swimming speeds) and the energetic costs of swimming (rates of oxygen consumption and amounts of whole-body lactic acid accumulation, both at different swimming speeds) in normal diploid wild-type (WT) and C zebrafish. We found that the zebrafish is capable of unusually high levels of swimming performance compared with almost all other species studied previously. The metabolic costs of these high performance levels appear to be unusually low. Genetic uniformity of the fish studied dramatically reduced variability in rates of oxygen consumption, but not in lactic acid accumulation.

Materials and methods

Fish and their maintenance

WT zebrafish *Brachydanio rerio* (Hamilton-Buchanan) with a mean *SL* of 3.39 ± 0.27 cm (range 3.05–4.01 cm) and a mean body mass (*BM*) of 0.84 ± 0.36 g (range 0.44–1.44 g), in visually good physical condition, were purchased from local dealers in Los Angeles, CA. C zebrafish with a mean *SL* of 3.08 ± 0.17 cm (range 2.86–3.70 cm) and a mean *BM* of 0.59 ± 0.17 g (range 0.27–1.03 g) were obtained from an established line (strain C32; Streisinger *et al.* 1981, for cloning technique) from the Institute of Neuroscience, University of Oregon. These size differences were not significant. The fish were kept in groups of 4–8 individuals in 30 l aquaria with dechlorinated, continuously filtered fresh water at constant temperature (28 °C) and with a photoperiod of 10 h:14 h L:D. They were fed 1–2 times a day with dried brine shrimp and Tetramin flakes. Prior to experiments, fish were starved for 24 h. Only fish with no visible physical or behavioral

abnormalities were used. Great care was taken in handling fish to prevent injury and to minimize time spent out of the water.

Since approximately 12 years had passed between the time that Streisinger *et al.* (1981) first isolated strain C32 zebrafish and the time the present experiments were carried out, we tested the hypothesis that this strain had remained IHD. Starch-gel electrophoretic surveys were made of tissue (muscle, liver, central nervous system) homogenates from both WT and C fish for enzyme activities representing 32 independent genetic loci (Murphy *et al.* 1990); of these, six were found to be multiallelic in the WT fish. All six were isogenic and homozygous in the C fish.

Respirometry

Oxygen consumption rates of fish swimming at controlled speeds at constant temperatures of 28.0 ± 0.2 °C were measured in closed, recirculating Blazka-type respirometers filled with dechlorinated fresh water. We used two identical respirometers simultaneously. The system is described by Gordon *et al.* (1989). Speed calibrations were made with dye and small neutrally buoyant particles in the respirometers. Speeds were $5\text{--}40\text{ cm s}^{-1}$.

Dissolved oxygen measurements

Concentrations of dissolved oxygen in the water in the respirometers were continuously measured by a YSI model 5300 two-channel biological oxygen monitor and YSI model 5331 oxygen electrodes. Water from each respirometer was separately pumped through a closed external circuit of tubes to the electrode cells and back to the respirometer by a peristaltic pump. The electrode cells, made of transparent Perspex, were immersed in a water bath with the respirometers to maintain a constant, equal temperature in the whole system. A precision-thermoregulated circulating water bath was used to control the temperature in the experimental water bath. Data from the two channels of the oxygen meter were transferred through Sable System connector box model CB661 to a computer and collected by DATACAN III v.1.00 data acquisition package. The collected data were analyzed by DATACAN III v.1.00 data analysis package to calculate the rates of dissolved oxygen concentration decrease. The two channels of the oxygen monitor were separately calibrated using water aerated with air (100% O₂ saturation) and with nitrogen (0% saturation). The value used to calculate oxygen concentration was 5.46 ml l^{-1} at 28 °C under 101.3 kPa (1 atmosphere) pressure at 100% O₂ saturation (Green and Carritt, 1967). Control experiments demonstrated that no measurable oxygen movements occurred into or out of the experimental system in the presence of oxygen partial pressure gradients of substantial magnitude (>70% saturation).

Rates of oxygen consumption at different swimming speeds

Groups of 5–7 zebrafish were placed in the working sections of each respirometer. The respirometers were filled with aerated fresh water at 28 °C. Respirometers were immersed in the aerated experimental water bath, debubbled, and the fish allowed to become acclimated to the respirometers overnight for 16–18 h. After the acclimation period, the oxygen monitor and electrodes were calibrated, the respirometers were closed without

disturbing the fish and water velocities were adjusted to 5 cm s^{-1} . During experiments, the respirometers were visually shielded so the fish could not see human activity.

Experiments at each speed lasted for 30 min. Oxygen measurements started 10 min after fixing the velocities and lasted 20 min. Water velocities then increased by 5 cm s^{-1} .

When O_2 saturation approached 85 % (usually after 2–4 measurement periods), the respirometers were gently opened for about 5 min to allow 100 % O_2 -saturated water from the bath to replace the water inside, closed again, and the next measurements begun. This procedure caused no visible disturbance to the fish. During the intervals between measurement periods, the electrodes were recalibrated for 100 % O_2 saturation. Measurements continued until water velocities of 40 cm s^{-1} were reached.

Thus, each experimental series lasted approximately 4 h. During measurements, minimum disturbance occurred in the room and the fishes were photographed by video camera for further behavioral analysis. No fatigue occurred during experiments.

Each of four C fish groups (5–7 specimens) was used for one series of metabolic measurements. Four out of five groups of WT fish were used twice, with recovery periods of more than 1 month between experiments.

Blank runs were made after each experimental series. No significant rates of oxygen uptake attributable to bacterial action were measured.

Measurements of total length and body mass

Two weeks after being used in swimming metabolism experiments, the fish were anaesthetized in 0.25 ml l^{-1} 2-phenoxyethanol for 10 min (McFarland and Klontz, 1969), taken out of the water, and their total length (*TL*) and standard length (*SL*) were measured by mechanical caliper to $\pm 1 \text{ mm}$. Fish were then blotted lightly, weighed to the nearest 0.01 g and replaced into aerated dechlorinated fresh water. Great care was taken to minimize time spent out of the water (<30 s). Mortality associated with measuring and weighing of the fish was less than 5 %.

Behavioral analysis of fish swimming at different speeds

Typically, fish swam steadily upstream in the respirometers. Zebrafish, at low current velocities, sometimes tended to turn and swim downstream for short periods. To evaluate the effect of water velocity on this behavior, video pictures of the swimming fish were analyzed, and the number of turns fish made per minute at each swimming speed was counted for three groups of C and four groups of WT fish, for 5–8 min at each speed. The video camera used was a Minolta Master, series-8 80.

Body lactate concentrations at rest and after exercise

Lactate concentrations in the whole body were measured in C and WT zebrafish at rest (routine activity in their aquaria) and after exercise. The fish in the exercised groups were forced to swim at 40 cm s^{-1} for 2 h. No fatigue occurred during the exercise. The fish were taken, one at a time, from the aquarium (rest) or from the respirometer (exercise), killed immediately by a blow to the head, weighed and homogenized in 5 ml of 0.6 mol l^{-1} perchloric acid. Homogenates were chilled on ice and centrifuged for 10 min at $6000 \text{ revs min}^{-1}$. Supernatants were removed and lactic acid concentrations were

determined spectrophotometrically at 540 nm, using a lactate analysis kit (Sigma, catalog number 745-10).

Statistical analyses

Relationships between rates of oxygen consumption and swimming speeds were analyzed using the equation: $\log \dot{V}_{O_2} = bU_{rel} + \log a$, where mass-specific oxygen consumption, \dot{V}_{O_2} , is in $\text{ml O}_2 \text{g}^{-1} \text{h}^{-1}$ and relative swimming speed, U_{rel} , is in BL s^{-1} . For each group of fish, least-squares linear regression coefficients were calculated and each coefficient was tested for difference from zero. Tests for the homogeneity of slopes were performed pairwise among groups in each fish type and, for cases when slopes were not significantly different, analyses of covariance (ANCOVAs) were performed to detect differences in the intercepts (standard metabolic rate, SMR).

To test for differences in variances among groups and types, Bartlett's test of homogeneity of variances and variance ratio tests (Sokal and Rohlf, 1981) were conducted, using variances calculated from the residuals on the y-axes.

The results of body lactate measurements were tested by two-way analysis of variance (ANOVA) and for homogeneity of variance (Sokal and Rohlf, 1981).

Results

Endurance and maximum swimming speeds

Data relating to endurance and maximum swimming speeds of both zebrafish types were obtained in the course of the experiments carried out for other purposes. We were unable to estimate critical swimming speeds for either type since the maximum absolute and relative swimming speeds that were possible in the water tunnel respirometers used were not high enough to cause fatigue in any of the fish in experimental series lasting 4 h. The maximum speeds were 40 cm s^{-1} absolute, approximately 13 BL s^{-1} relative. In an experiment performed to estimate lactic acid production and accumulation, groups of fish of both types swam continuously at these maximum speeds for more than 120 min. Once again, no fish fatigued visibly.

Relationships between oxygen consumption and swimming speeds

For both WT and C groups, oxygen consumption increased in relation to increasing swimming speed (Fig. 1). However, the slopes of the regressions for all groups were remarkably low (ranges 0.010–0.024 for WT groups and 0.009–0.026 for C groups). Analyzing each group separately, we found that in all C groups the slopes were significantly different from zero, while in all WT groups the slopes were not significantly different from zero (Table 1).

Tests for homogeneity of slopes among groups (pairwise test) within each type gave different results for WT and C types. For WT groups, there were no significant differences. One of the WT groups had a significantly different intercept from the others (this group contained significantly larger fish), and two other groups also had significantly different intercepts (Table 2). Between groups of C fish, in some combinations, regression coefficients were significantly different. For combinations in

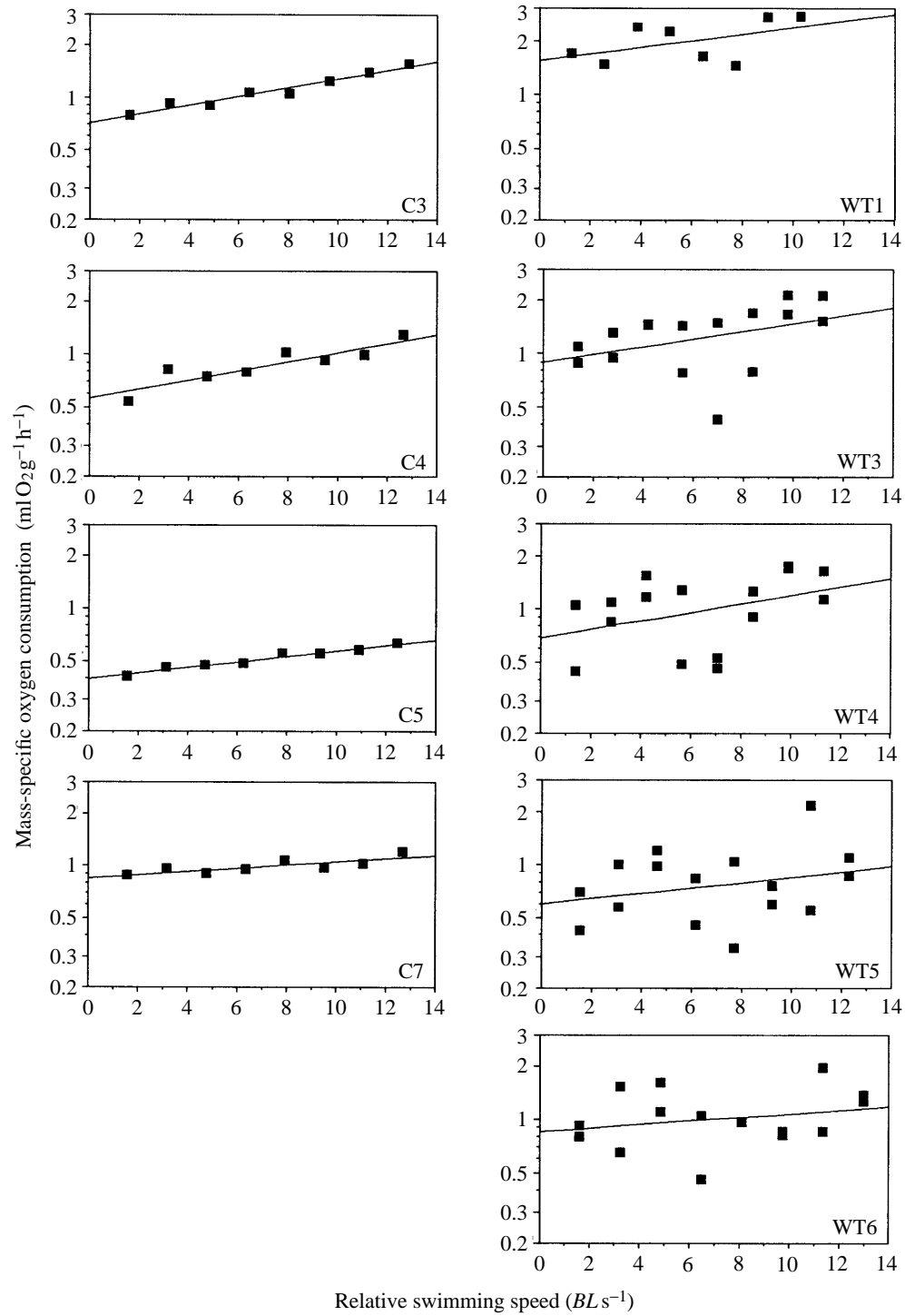


Fig. 1

Fig. 1. Mass-specific oxygen consumption rates of five groups of WT zebrafish and four groups of C zebrafish, swimming at different speeds at 28 °C. See Table 1 for regression values.

which the slopes were not significantly different, the intercepts were significantly different (Table 2).

Data variances for each WT group are visibly larger than for each C group (Fig. 1). Tests of the homogeneity of variances showed that the variances of all the groups were not homogeneous ($\chi^2_{\text{adj}}=82.44$, d.f.=8, $P<0.001$). Within types, the variances of WT groups were homogeneous ($\chi^2_{\text{adj}}=4.38$, d.f.=4, $P>0.05$), but for the C groups the variances were not homogeneous ($\chi^2_{\text{adj}}=10.34$, d.f.=3, $0.05>P>0.01$). This lack of

Table 1. Regression analyses of rates of mass-specific oxygen consumption versus relative swimming speeds in WT and C zebrafish, *Brachydanio rerio*, measured at 28 °C

Group	N	Slope, <i>b</i>	S.E.M.	<i>P</i>	Intercept, log <i>a</i>	S.E.M.	<i>r</i> ²
WT1	8	0.018	0.013	0.208	0.188	0.084	0.249
WT3	16	0.022	0.014	0.134	-0.049	0.096	0.153
WT4	16	0.024	0.015	0.136	-0.165	0.108	0.152
WT5	16	0.015	0.014	0.310	-0.222	0.110	0.071
WT6	16	0.010	0.011	0.358	-0.071	0.087	0.061
C3	8	0.025	0.002	0.000*	-0.145	0.017	0.958
C4	8	0.026	0.005	0.002*	-0.251	0.041	0.807
C5	8	0.016	0.001	0.000*	-0.402	0.010	0.960
C7	8	0.009	0.006	0.011*	-0.073	0.021	0.682

Calculations are based on the equation: $\log \dot{V}_{\text{O}_2} (\text{ml O}_2 \text{ g}^{-1} \text{ h}^{-1}) = bU_{\text{rel}} + \log a$.

P-values give the results of *F*-tests of the null hypothesis that slope=0; *slope significantly different from zero.

Table 2. Probability tables for homogeneity of slopes and intercepts between groups of WT and C zebrafish

	WT1	WT3	WT4	WT5	WT6		C3	C4	C5	C7
WT1		NS	NS	NS	NS		C3	NS	**	**
		**	**	**	**			**	-	-
WT3	0.878		NS	NS	NS		C4	0.088	NS	*
	0.004		NS	**	NS			0.000	**	-
WT4	0.820	0.913		NS	NS		C5	0.003	0.082	NS
	0.000	0.127		NS	NS			-	0.000	**
WT5	0.897	0.737	0.669		NS		C7	0.001	0.015	0.052
	0.000	0.003	0.105		NS			-	-	0.000
WT6	0.688	0.505	0.454	0.785						
	0.000	0.101	0.999	0.075						

Upper numbers in each pair refer to slope, lower numbers to intercept.

NS, not significant; * $P<0.05$; ** $P<0.005$.

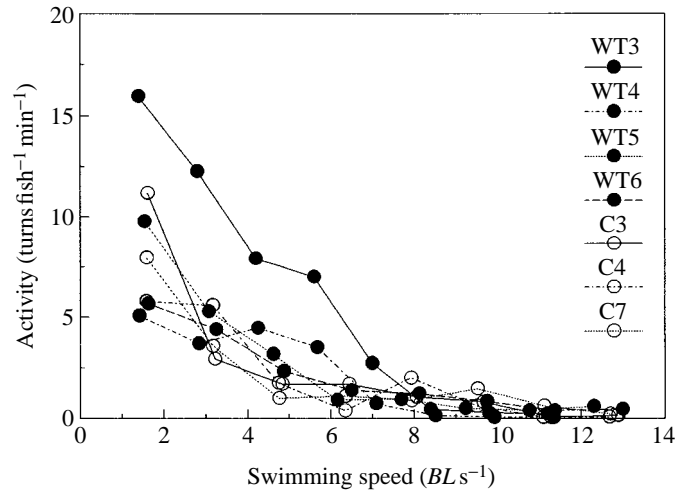


Fig. 2. Spontaneous turning activity in four groups of WT and three groups of C zebrafish measured as number of turns per fish per minute at different swimming speeds.

homogeneity is presumably due to the low values of the variances within C groups and has no biological significance. Pairwise comparisons of variances (variance ratio test) between WT and C groups showed that all the variances of WT groups were significantly larger than all the variances of C groups ($P < 0.001$).

Fish behavior in the swimming chamber

Zebrafish are very active animals. In the respirometers, when currents were imposed, they swam most of the time with their heads directed upstream. Frequently, however, primarily at lower speeds, the fish turned suddenly downstream, drifted for short distances, and then turned back and returned to their previous position. There was no noticeable synchronization in turns between the fish in each group. To evaluate the relationship between this pattern of behavior and swimming speed, we measured the frequency of turns per fish for each speed.

The frequency of turns (Fig. 2) at the slowest speed (5 cm s^{-1}) was $9.2 \pm 4.6 \text{ turns fish}^{-1} \text{ min}^{-1}$; this decreased to $2.5 \pm 2.6 \text{ turns fish}^{-1} \text{ min}^{-1}$ at 20 cm s^{-1} and became less than $1 \text{ turn fish}^{-1} \text{ min}^{-1}$ at higher swimming speeds. No consistent differences were found between WT and C fish.

No differences were found in the grouping pattern (school shape, distances between fish) between relative swimming speeds of $6\text{--}7 \text{ BL s}^{-1}$ and $12\text{--}13 \text{ BL s}^{-1}$.

Lactate concentrations

After swimming at 40 cm s^{-1} ($12\text{--}13 \text{ BL s}^{-1}$) for 2 h, both WT and C zebrafish had slightly higher body lactate concentrations (0.45 ± 0.22 and $0.42 \pm 0.05 \text{ mg g}^{-1}$ respectively) than resting zebrafish (0.37 ± 0.17 and $0.28 \pm 0.12 \text{ mg g}^{-1}$ respectively). These differences were not significant (Fig. 3). There were no significant differences in

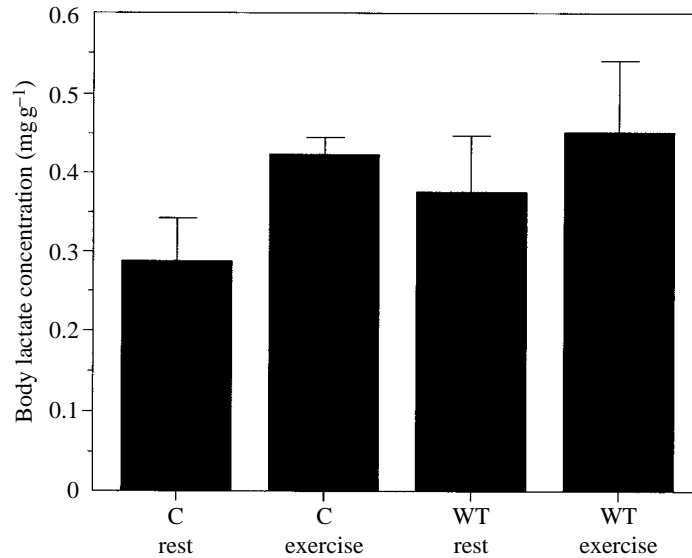


Fig. 3. Body lactate concentrations of WT and C zebrafish after routine activity (rest) and after 120 min of continuous swimming at 40 cm s^{-1} . Values are mean + S.E.M., $N=5$.

Table 3. Two-way ANOVA for body lactate concentrations WT versus C and for rested versus exercised zebrafish

Comparison	Sum of squares	d.f.	Mean square	F-ratio	P
(A) Exercised versus rested	0.056	1	0.056	1.855	0.192
(B) WT versus C	0.017	1	0.017	0.562	0.464
A versus B	0.004	1	0.004	0.144	0.709
Error	0.484	16	0.030		

body lactate concentrations between WT and C zebrafish (two-way ANOVA, Table 3). The variances were also not significantly different between WT and C zebrafish (test for homogeneity of variance, $\chi^2=1.87$, d.f.=3).

Discussion

These results demonstrate (1) that the zebrafish is capable of extraordinarily rapid swimming for unusually long periods, without significant fatigue; (2) that this high performance level is maintained aerobically at apparently low cost; and (3) that C zebrafish perform these feats with substantially less variability than do normal diploid WT zebrafish.

Swimming performance of zebrafish

Both WT and C zebrafish swam in sustained swimming modes at speeds up to

$13 BL s^{-1}$ (40 cm s^{-1}) for 2 h with no visible signs of fatigue. Unfortunately, owing to system limitations, we could not force the fish to swim at higher speeds to evaluate their critical swimming speeds or their endurance limits.

Beamish (1978) listed 57 critical swimming speeds for 26 species of fishes ranging in size from 4 to 62 cm. All critical speeds listed were from 0.7 to $8.6 BL s^{-1}$ except for *Stenodus leucichthys*. *Stenodus*, with body lengths of 8–41 cm, showed critical speeds of 12 – $18 BL s^{-1}$ in experiments that used velocity increments of 10 cm s^{-1} and times between increments of 10 min (Jones *et al.* 1974). Beamish (1978) also listed 117 values of maximum prolonged swimming speeds for 41 species of fishes that ranged from 0.7 to $13.6 BL s^{-1}$. Later studies (Dorn *et al.* 1979; Brett, 1982; Turnpenny and Bamber, 1983; Beamish, 1984; Bernatchez and Dodson, 1985; Williams and Brett, 1987; He and Wardle, 1988; Wardle and He, 1988) reported additional values for critical speeds of 1.4 – $7.1 BL s^{-1}$ (reviewed by Videler and Wardle, 1991). Exceptionally high critical speeds of 10 – $12 BL s^{-1}$ were reported by Turnpenny (1983) for juvenile sprat (*Sprattus sprattus*) and herring (*Clupea harengus*), with *SL* values of 2.9–4.8 cm and 4.6–5.8 cm, respectively. Fewer than 5 % of these critical speeds are higher than or comparable to the maximum sustained swimming speeds we have measured for zebrafish. Moreover, in our experiments the durations of swimming bouts were longer (30 min) and velocity increments were smaller (5 cm s^{-1}) than in most of these other studies.

The ability of zebrafish to swim continuously at high speeds is unusual; their critical swimming speeds must be well above previously measured values. Fuiman and Webb (1988) found that average and maximum routine swimming speeds for 3.5 cm zebrafish were $4.2 BL s^{-1}$ and $6.5 BL s^{-1}$ respectively. These speeds are in the range in which most zebrafish swimming in the respirometers changed their behavior and stopped turning downstream.

Generally, scaled maximum relative swimming speeds for small fishes are higher than those for large fishes. This generalization is based on intraspecific comparisons (Beamish, 1978; Videler and Wardle, 1991). It is partly explained by the fact that muscle contraction times are shorter for small fishes (Wardle, 1975), permitting higher tail-beat frequencies.

The literature includes few comparative data on swimming performances of adult small fishes belonging to different species (Fuiman and Webb, 1988). Data for larvae and juveniles are usually not comparable because of differences in morphology and physiology of early life history stages (Wieser *et al.* 1985, 1988; Dabrowski, 1986; Webb and Weihs, 1986; Wieser and Forstner, 1986; Kaufmann and Wieser, 1992). Goolish (1991), using calculations of power requirements to overcome drag for different body sizes of fishes, argued that the relationships for burst power requirement and aerobic muscle power may converge at small body sizes. As a result, energy demands at maximum swimming speeds may be met by small fishes without anaerobic metabolism. He supported this argument by comparing data from different species. The issue involved is a complex one. Interested readers should consult Goolish (1991) directly.

Our findings demonstrate no significant increases in body lactate concentrations of zebrafish after 2 h of fast swimming. Thus, anaerobic metabolism was not significantly involved in power generation. These observations are probably consistent with the

natural ecology of zebrafish. *B. rerio* is found in rivers in south Asia, probably under high current conditions (Laale, 1977). This kind of environment requires high swimming performance by midwater fishes if they are to avoid being swept downstream.

We note, however, that Lucas and Priede (1992) postulated that the high induced maximum metabolic rates (approximately 3.4 times standard metabolic rate, SMR) that they measured in stressed zebrafish were associated with oxygen debt repayment. These authors did not measure body lactate concentrations.

Aerobic metabolism in relation to swimming speed

Both WT and C zebrafish increased oxygen consumption rates by small amounts at higher swimming speeds. Zebrafish are subcarangiform swimmers, so we compare these results with those for other subcarangiform swimmers.

Beamish (1978) noted that, despite obvious variations between previous studies in methodology, fish size and temperature, measured rates of increase of oxygen consumption with higher relative swimming speeds are surprisingly similar among different species. A slope of 0.36 (for base 10 semilogarithmic plots) represents the relationship reasonably well.

There are, however, a number of other studies that have yielded much lower slopes (e.g. 0.13 for 4.0–6.0 cm *SL* juvenile coho salmon, *Oncorhynchus kisutch*, Puckett and Dill, 1984; 0.05–0.08 for 7.8–9.7 cm *SL* juvenile rainbow trout, *Oncorhynchus mykiss*, and 0.09 for 6.3–6.7 cm *SL* rosyside dace, *Clinostomus funduloides*, varying with the season, Facey and Grossman, 1990; and 0.12 and 0.21 for 26.0–29.0 cm *SL* juvenile cod, *Gadus morhua*, at 10 and 15 °C, respectively, Soofiani and Priede, 1985). Facey and Grossman (1990) actually found a few cases of negative slopes in rosyside dace (these coefficients were not significantly different from zero and were explained by high rates of routine activity at the lowest swimming speeds). Our slopes ranged from 0.009 to 0.026. For WT groups, these slopes were not significantly different from zero.

Undetectable to minimal increases in oxygen consumption rates with increased swimming speeds may indicate that the energetic cost of swimming in zebrafish is very low in comparison with that of other subcarangiform swimmers. The lack of published information about other fishes that are small as adults prevents comparison with other species. We suggest several possible explanations for the apparently low cost of swimming in zebrafish. (1) The power needed for swimming may be small in relation to other energetic demands. Whole-animal oxygen consumption rates indicate only total demands for oxygen. It is not possible to determine how much of the consumed oxygen is used to power swimming (Gordon *et al.* 1989). (2) It is possible that, while energetic demands for swimming increase with increasing speed, oxygen demands for other activities may decrease. The standard metabolic rate (SMR) might be reduced during swimming activity. If this were to occur, the total increase in oxygen consumption would represent an underestimation of the real cost of swimming. (3) Zebrafish may be able to reduce drag at high swimming speeds. We never observed fish in the respirometers to be taking advantage of boundary layer effects to reduce the cost of swimming at high speeds. Such reductions may also be produced by layers of hydrophobic mucus on the skin produced by the fish or by bacteria (Sar and Rosenberg,

1989). (4) Scaling effects may be involved. Schmidt-Nielsen (1984), while discussing data of Brett (1965) about the relationship between oxygen consumption rates and body masses for different swimming speeds in sockeye salmon ranging from 3 to 1400 g, stated that 'the larger fish must have a much greater factorial metabolic scope than small fish'. He showed that the factorial scope was four times higher than the SMR for the smallest fish and 16 times higher for the largest fish. This phenomenon is thus intraspecific, but it may also be interspecific. The low cost of swimming may be a result of small body size. (5) Group effects may have been important in our experiments. Metabolic rates of fishes may be modified when they are in groups. In nonterritorial fishes, metabolic rates often decrease when they are in groups (Smatresk and Herreid, 1980). In schooling fishes, performance also may become better in groups (Weihs, 1973), and the endurance of such fishes can increase 2–6 times when they are in schools (Belyayev and Zuyev, 1969). However, Lucas and Priede (1992) report that routine metabolic rates (RMRs) for zebrafish in groups of six were significantly (approximately 30%) higher than RMRs for individual fish under comparable conditions. To our knowledge there are no published data relating to possible group effects on active metabolic rates of fishes swimming at different speeds.

We can only speculate about possible bases for the difference existing between the present results and those of Lucas and Priede (1992) with respect to aerobic metabolic scopes (they report factorial scope of 3.4 times SMR for individual fish). There are many differences between their methods and ours, the most important of which may relate to group effects.

The small, but significant, differences we found among C groups, either in slopes or in intercepts, are probably due to small differences in the physical conditions of the different groups with respect to factors such as nutrition, age, body mass, etc. Similar differences were not found between most WT groups, probably because of the higher variability within these groups. Group WT1 did differ from others, probably because its members were larger in size.

Standard and routine metabolic rates

Since the active metabolic rates of zebrafish increased little, if at all, at higher swimming speeds, we assume that the SMR and RMR of zebrafish are virtually equal. Thus, we may compare our results with published RMR values for fish species with small body sizes (Cech *et al.* 1985; Jordan *et al.* 1993). These published values were 0.23–0.82 ml O₂ g⁻¹ h⁻¹ for seven species ranging in body mass from 0.2 to 1.0 g, measured at temperatures of 10–31 °C.

Zebrafish SMRs reported here were 0.6–1.5 and 0.4–0.8 ml O₂ g⁻¹ h⁻¹ for WT and C zebrafish, respectively, all at 28 °C. Lucas and Priede (1992), working with groups of six WT zebrafish maintained at 24 °C, reported mean RMRs of 0.56±0.08 mg O₂ g⁻¹ h⁻¹ (0.39±0.06 ml O₂ g⁻¹ h⁻¹). Allowing for Q₁₀ effects, our RMRs are comparable. The only other published data concerning oxygen uptake of adult zebrafish are those of Skidmore (1967), who also reported oxygen consumption rates for single fish of 0.4 ml O₂ g⁻¹ h⁻¹. Skidmore's fish were confined in Warburg respirometer chambers in only 15 ml water. We consider these animals to have been highly stressed.

Differences between WT and C zebrafish

We found substantial differences in the variabilities of oxygen consumption rates between the groups of WT and C zebrafish. The groups of C zebrafish had significantly lower variances in comparison with the groups of WT zebrafish. This difference probably derives from differences in metabolic variability within individuals. C fish were all isogenic and homozygous; thus, their physiological traits were probably less variable than those of the WT fish. The latter were both multiply allelic at many loci and heterozygous.

The present results, plus those of L. Adams (in preparation) describing the responses of early developmental stages of WT and C zebrafish to environmental pH variations, are some of the first data available on the whole-animal performance effects of cloning in fishes. One of our initial expectations in undertaking this work was that IHD C fish would be more consistent and less variable in performance in all respects than normal diploid WT fish. Our results are partly consistent with that expectation, but the results of L. Adams (in preparation) are not. Adams found similar levels of variance in both types in all features studied.

Another reason for carrying out this study was to determine whether IHD C strain zebrafish might be useful substitutes for WT fish in some of the many bioassay situations for which the species is used (Fogels and Sprague, 1977; Bresch, 1986). The lack of consistently reduced variability just mentioned is one reason why they may not be useful. Greater consistency and reproducibility of results will not necessarily follow from their use. A second reason for possible limited utility is that we found that the C strain fish were harder to maintain in healthy condition than the WT fish. Reduced hardiness and greater maintenance expense are not desirable features of organisms to be used in bioassay applications. However, this question needs further attention.

Finally, we note that there are several naturally occurring gynogenetically clonal species of freshwater fishes in the families Cyprinidae and Poeciliidae (Goddard and Schultz, 1993; Quattro *et al.* 1992*a,b*). These species, however, are not isogenic. Studies of these forms, similar to those presented here, might be of interest.

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