

# Effect of developmental temperature on swimming performance of zebrafish (*Danio rerio*) juveniles

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**Abstract** It is widely known that water temperature affects the swimming capacity of fish. But the effect of the rearing temperature on the swimming ability of the fish at later stages, has not had similar attention. In this study, four populations of zebrafish, were reared in different water temperatures (22, 25, 28 and 31°C) and after being acclimatized in a common temperature (26.5°C) for over a month, they were subjected to swimming trials in order to evaluate the maximum relative critical velocity ( $RU_{crit}$ ) in each case. Fish that were reared in 22°C showed statistically significant lower performance than the ones reared in 31°C ( $7.72 \pm 0.17$  vs.  $8.79 \pm 0.28$ , means  $\pm$  S.E.). Possible explanations for the observed differentiation could be the effect of early life temperature on fish muscle ontogeny or on body shape.

**Keywords** Critical velocity ·  $U_{crit}$  ·  $RU_{crit}$  · Rearing · Plasticity

## Introduction

Swimming performance is a biological characteristic that has a very important role on fish survival

(Jones et al. 1974), as it can strongly affect food capture, mating success and the avoidance of unfavourable situations (Drücker 1996). Moreover, it has been stated that the ability of locomotion is a main trait that defines to a great extent the Darwinian fitness (Reidy et al. 2000). Most of the environmental factors that affect swimming capacity have already been studied thoroughly and in a wide range of species. Among them, temperature (Fuiman and Batty 1997; Beamish 1978; Wieser and Kaufmann 1998; Ojanguren and Brana 2000; Dickson et al. 2002; Koumoundouros et al. 2002a, b; Wilson et al. 2002; Franklin et al. 2003), oxygen (Beamish 1978; Hammer 1995; Korsmeyer et al. 1996; Steffensen and Farrell 1998) and carbon dioxide concentration (Dahlberg et al. 1968; Beamish 1978), pollutants (Howard 1975; Beamish 1978; Beaumont et al. 2000; Randall and Tsui 2002; Wicks et al. 2002), salinity (Beamish 1978), light and photoperiod (Beamish 1978; Hammer 1995; Young et al. 2004) seem to play a particularly important role.

Temperature itself seems to be the most important environmental factor that affects the life of fish. Besides swimming performance, it has been shown to affect growth, metabolism, time of hatching (Herzig and Winkler 1986), development (Herzig and Winkler 1986; Fukuhara 1990; Polo et al. 1991; Gibson and Johnston 1995; Lein et al. 1997), yolk absorption (Fukuhara 1990), muscle ontogeny and development (Johnston 1981, 1993, 2006; Wilkes et al. 2001; Johnston et al. 2009),

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ontogeny of internal organs (Fukuhara 1990; Gibson and Johnston 1995), external morphology (Lindsey 1988; Wimberger 1992; Tudela 1999; Koumoundouros et al. 2001a; Pakkasmaa and Piironen 2001; Cabral et al. 2003; Silva 2003; Turan 2004), meristic characters (Turan 2004), appearance of skeletal deformities (Polo et al. 1991; Koumoundouros et al. 2001b), sex determination (Baroiller et al. 1999; Pavlidis et al. 2000; Koumoundouros et al. 2002a) and the overall survival (Fukuhara 1990; Lein et al. 1997) and lifespan of fish (Malek et al. 2004). Although the effect of swimming temperature on the performance of fish has been studied thoroughly and in a wide range of species, there are only a few studies focusing on the impact of rearing temperature on the swimming performance of adult fish (i.e. Koumoundouros et al. 2009).

Zebrafish is a small, colourful, tropical fish inhabiting small ponds and slow moving streams in Southeast Asia (Talwar and Jhingran 1991). For the past few decades, it has been a very important model-organism in research fields such as Genetics, Neurophysiology, Developmental Biology and Biomedicine (Amsterdam and Hopkins 2006). Numerous laboratories worldwide are using zebrafish for fundamental or applied research and there is an increasing interest for its use in new research fields (Gerlai 2003). In spite of its popularity as a research tool, only recently did some integrated studies about its biological and ecological characteristics emerge (Engeszer et al. 2007; Spence et al. 2008).

Concerning the temperature range in which zebrafish live and reproduce in the environment, more than one suggestion exists. Froese and Pauly (2010) argue that zebrafish's natural habitat temperature ranges from 18°C to 24°C, whereas Engeszer et al. (2007) report that the observed temperature range for zebrafish in the wild lies between 24.6°C and 38.6°C. Spence et al. (2008) on the other hand, mention that temperature in *D. rerio*'s habitats ranges from 6°C in the winter to 38°C in the summer. Our personal observations throughout many rearing trials showed that the rearing temperatures of 22°C and 31°C are the lower and upper limit values for successful rearing in the laboratory. At lower or higher – respectively – temperatures, the risk of unsuccessful hatching, mass larvae mortality and severe skeletal malformations throughout the populations, is extremely high.

The purpose of this article is to study the effect of four different rearing temperatures (ranging from 22°C to 31°C) on the swimming performance of adult zebrafish.

## Materials and methods

A single batch of eggs of *D. rerio* was obtained from wild type broodstock (ZF WT2 F5, Wageningen Agricultural University, The Netherlands) that was kept in a 30 l tank at 28±0.5°C and fed three times per day with industrial dry food in flakes (Sera Vipan, Germany) and three times per week with *Artemia* sp. nauplii (Instar I). The eggs were collected approximately 2 h after fertilization, examined under the stereoscope (Olympus, SZX9), selected in terms of quality and submerged for 12–15 min in hydrogen peroxide (3 ml l<sup>-1</sup>) for antifungal protection. Afterwards (approximately 3 h after fertilization), batches of 200 eggs were transferred into hatching devices that were placed in four rearing tanks of 130 l of different water temperatures (22, 25, 28 and 31°C). The regulation of temperature was achieved with the use of electrical thermostat heaters (Aquarium Systems, Visi-Therm, 100–150 W) and coolant device, when needed. The rearing was performed in duplicate (A and B).

Hatching occurred at 2 (28 and 31°C), 3 (25°C), or 6 days (22°C) after spawning. Larvae were fed four times per day, initially (at 2 days post hatching, dph) with *Paramecium* sp. (Blades Biological CO, UK) and later (at 33 dph) with newly hatched *Artemia* sp. nauplii (Instar I) (Westerfield 1995). Approximately at 73 dph, industrial dry food in flakes was introduced, which consisted the main diet of the fish throughout the acclimation and experimental periods. With every food-type change, a transitional period of three days was applied, at which both food types were provided. At approximately 143 dph, the acclimation – to the common temperature of 26.5°C – period started and lasted 1.5 month, until the start of the swimming exercises.

Swimming performance trials were carried out in an apparatus described by Koumoundouros et al. (2002b). Water velocity was calibrated by means of an electromagnetic flow-meter (Valeport, Model 801). Fish forward escape was prevented using a mesh screen located at the upstream end of the swimming

channel. A homogenous current velocity throughout the whole depth and length of the channel was verified by means of dye injected into the swimming channel. Temperature was maintained constant at 26.5°C using a thermostat heater, while the oxygen saturation was >99%.

For the critical velocity ( $U_{crit}$ ) measurements to be valid, the speed tests should be conducted based on the Total Length (TL) of the specimens used (Brett 1964). Therefore, the swimming performance trials were carried out not in one but at 3 different swimming speeds in order to accurately cover different fish sizes. The swimming speeds (and the velocity increments) used to test the fish were - according to their size - the following: 30, 32.5 and 35 mm sec<sup>-1</sup>. The TL of the fish was assessed visually before the trial to avoid excess stress from the unnecessary fish handling (Ramsay et al. 2009). If the actual TL (as measured at the end of the experiments on the digital photographs of the anaesthetized fish) differed from the estimated one and therefore from the applied speed and the velocity increment more than 1.25 mm, then the data concerning that fish were discarded. Only male fish were used for the trials, so as to avoid possible sex-dependent differences (Williams and Brett 1987). Fish were starved for 24 h prior to the swimming trials. Fish were also macroscopically examined prior to the trials for any visible morpho-anatomical malformations. Before the trial, fish were acclimated for 15 min to the holding tank's conditions and then one fish was placed in the swimming tunnel for 10 min at a water velocity of 2 TL sec<sup>-1</sup>. Water velocity increased every 10 min at a rate of 1 TL sec<sup>-1</sup> until the fish fatigued. Fatigue was reached when a fish was carried away by the current, without been able to hold its position in the swimming tunnel or react to visual and acoustic stimuli.

Since body mass can affect metabolism (Hochachka 1987) and body length can affect swimming power output, we assessed both in all groups after treatments (McClelland et al. 2006). To do so, fish were anaesthetized (ethylenglycol-monophenylether, Merck, 0.2–0.3 ml l<sup>-1</sup>), individually photographed using a digital camera (Olympus, Camedia C3030 Zoom) and weighed (M, 0.001 g), while the maximum body width (BW) was measured under a stereoscope (0.01 mm). Landmarks were also placed to the obtained pictures using an image processing software (tpsDig2, Rohlf, version 5.0.3.32), for the accurate measurement of the

TL, maximum body depth (BD<sub>max</sub>), body depth at the anus (BD) and caudal peduncle depth (CpD). Moreover, selective double staining of cartilage and bone tissue was performed on the specimens after the trials and the stained samples were examined under a stereoscope for skeletal deformities (Divanach et al. 1997; Koumoundouros et al. 1997a, b, 2001b). The abnormal fish were discarded from the analysis.

Critical swimming speed was calculated using the equation introduced by Brett (1964):

$$U_{crit} = U_i + U_{ii} \left( \frac{T_i}{T_{ii}} \right)$$

where  $U_i$  is the highest velocity maintained for the whole 10 min (mm sec<sup>-1</sup>),  $U_{ii}$  is the velocity increment (30, 32.5 and 35 mm sec<sup>-1</sup>),  $T_i$  is the time elapsed at fatigue velocity and  $T_{ii}$  is the time between velocity increments (10 min).

To compare the swimming performance of many individuals that varied in length, the  $U_{crit}$  had to be transformed to a new value that would be independent of the factor TL. So, after calculating the  $U_{crit}$ , the relative critical swimming speed ( $RU_{crit}$ ) for every fish was calculated using this equation:

$$RU_{crit} = \frac{U_{crit}}{TL} \quad (\text{Beamish 1978})$$

As the assumption of homogeneity of variances was not fulfilled, the Mann-Whitney U-test was used for the comparison of swimming performance between the four different temperatures and the 2 replicates, after the appropriate correction for tied measurements (Sokal and Rohlf 1995). The non parametric Kruskal-Wallis was used to test the effect of temperature on  $RU_{crit}$  (Sokal and Rohlf 1995).

All experimental procedures were in accordance with the European Communities Council directive (86/609/EEC) for the care and use of laboratory animals and approved by the University of Crete research committee.

## Results

The relative critical velocities measured for the individuals of each duplicate of the four temperature groups are presented in Table 1. Statistical analysis showed a significant influence of the factor, rearing temperature, on the swimming performance of the

**Table 1**  $RU_{crit}$  (SE, standard error) for each duplicate (A and B) and for each temperature group (22, 25, 28 and 31°C)

Temperature	Dup.	$RU_{crit}$ (TL sec <sup>-1</sup> )	SE	N	Average $RU_{crit}$	SE	<i>p</i>
22°C	A	7.68	0.17	10	7.72	0.17	a
	B	7.76	0.30	11			
25°C	A	8.05	0.42	8	8.13	0.27	–
	B	8.20	0.36	9			
28°C	A	8.23	0.33	9	8.36	0.28	–
	B	8.46	0.44	11			
31°C	A	8.69	0.37	10	8.79	0.28	a
	B	8.89	0.44	10			

<sup>a</sup> $p < 0.05$  (Mann-Whitney U-test)

juveniles ( $p < 0.05$ , Kruskal-Wallis). At all temperature regimes, by means of Mann-Whitney U-test, no significant differences were revealed between the two duplicates.

Fish raised at 31°C have achieved the higher swimming performances in terms of  $RU_{crit}$  while the ones reared in 22°C had the worst performance ( $p < 0.05$ , Mann-Whitney). Fish cultured in 25 and 28°C achieved intermediate critical swimming speeds although not significantly different from the others (Table 1).

The measurements of the morphometric characters performed after the swimming trials as well as the ones that derived from the morphometric analysis of the landmarks placed on the specimen photographs are presented in Table 2.

For those characters, the performed Mann-Whitney U-tests revealed no statistical significant differences between the fish raised at the different temperature regimes ( $p > 0.05$ ).

## Discussion

Fish are known to react to their environment's fluctuations in all possible ways and it is an evolutionary imperative to try and adjust to these changes. Zebrafish, although considered to be a

tropical fish, inhabits ecosystems with “monsoon climate”, that present an extraordinary seasonal variation of temperatures that can range from as low as 6°C in winter to over 38°C in summer (Spence et al. 2008). With the phenomenon of global warming being more and more imminent, the effects that such a wide range of temperatures can have on many important processes and aspects of the fish life are certainly worth of extensive study.

Developmental temperature plays an important role on the subsequent life of fish. On the present study the role of early rearing temperature on the swimming capacity of zebrafish juveniles was examined. The results showed that fish reared at 31°C have a higher swimming ability (8.79 TL sec<sup>-1</sup>) than fish reared at 22°C (7.72 TL sec<sup>-1</sup>). Fish raised at the two intermediate temperatures (25 and 28°C), although not statistically different from either one of the extreme temperatures, they seem to perform better than the fish of 22°C and worse than the fish of 31°C (8.13 and 8.36 TL sec<sup>-1</sup> respectively).

Swimming ability of zebrafish has been studied before in the case of larval (i.e. Budick and O'Malley 2000; Levin et al. 2004) and adult specimens (Plaut and Gordon 1994; Plaut 2000; McClelland et al. 2006; Widmer et al. 2006) but never in respect to developmental temperature. Plaut (2000) studied the effect of the fin size on the swimming performance of

**Table 2** Morphometric characters (SD standard deviation). TL total length, W weight, BW body width, BD<sub>max</sub> maximum body depth, BD body depth (at anus), CpD caudal peduncle depth

Temp.	TL (mm)	SD	W (g)	SD	BW (mm)	SD	BD <sub>max</sub> (mm)	SD	BD (mm)	SD	Cpd (mm)	SD
22°C	32.66	1.60	0.349	0.04	4.773	0.32	6.736	0.36	5.695	0.37	3.011	0.19
25°C	32.29	2.51	0.335	0.07	4.744	0.43	6.493	0.57	5.554	0.58	3.004	0.30
28°C	32.71	1.82	0.347	0.05	4.882	0.24	6.770	0.45	5.789	0.44	2.961	0.23
31°C	33.11	2.06	0.357	0.07	4.816	0.48	6.663	0.54	5.666	0.47	2.933	0.24

zebrafish, Widmer et al. (2006) studied the effect of hypoxia versus normoxia and Plaut and Gordon (1994) compared wild-type and cloned zebrafish. In the present study, the maximum relative critical swimming speed was estimated from 7.7 to 8.8 TL  $\text{sec}^{-1}$  which is in agreement with the results of Widmer et al. (2006), who reported it between 8.5 and 9.5 SL  $\text{sec}^{-1}$ , as this value is lowered if converted to TL  $\text{sec}^{-1}$ . Plaut (2000) measured  $RU_{crit}$  values around 12.6 TL  $\text{sec}^{-1}$  (after the appropriate adjustment from SL to TL), and Plaut and Gordon (1994) were unable to exhaust their wild-type specimens, but conclude that they swam more than 13 SL  $\text{sec}^{-1}$ . Similar results were described also by McClelland et al. (2006). The presented differences in  $RU_{crit}$  values between these studies and also the present one, can be explained by either the different measuring techniques and apparatus used, or the higher swimming temperature maintained by the authors during the trials (28°C).

There have been of course other studies on the effect of rearing temperature on swimming performance of other – mostly farmed marine – species. For example, Koumoundouros et al. (2009) studied the effect of two rearing temperatures on the swimming performance of European sea bass juveniles. In this study, the authors argued that fish raised in lower temperature (15°C) performed better than the fish raised in higher temperature (20°C). This is in contrast with our present results (where fish raised in higher temperature ultimately performed better than the others) but both studies suggest that the rearing temperature had a profound effect on the swimming capacity of fish at later stages.

There are two possible explanations for the observed effect. The influence of developmental temperature on the muscle ontogeny of the fish which has long been established (Johnston 1981, 1993, 2006; Johnston et al. 2009; Koumoundouros et al. 2009) appears to have a substantial effect on swimming performance. Different temperatures lead to variation in body muscle mass (in terms of size and number of muscle fibres) that directly affect swimming ability. Koumoundouros et al. (2009), showed that in the case of European sea bass, the best swimmers had relatively higher red muscle to white muscle ratio and more red muscle fibres than the others. In the case of zebrafish, Johnston et al. (2009) showed that the optimal embryonic temperature for

fast muscle fibre recruitment (hyperplasia) is 26°C, as in that temperature there were 18.8% more fast fibers than at 22°C and 13.7% more fibers than at 31°C. After this phase, myotube formation stops and a new phase begins (hypertrophy) that consists of nuclear accretion and increase in fibre length and diameter (Johnston et al. 2004). Even though higher developmental temperature does not lead to increased number of fast muscle fibres, it is still possible that it affects the second phase of muscle formation, resulting in longer and thicker muscle fibres.

The second is that temperature in early life affects body shape (Georgakopoulou et al. 2007; personal unpublished results) and meristic count (Blaxter 1991; Georgakopoulou et al. 2007; personal unpublished results). Georgakopoulou et al. (2007), concluded that European sea bass juveniles reared in 15°C had more slender bodies than the ones reared in 20°C, a fact that could explain the higher swimming performance of the former as observed by Koumoundouros et al. (2009).

In the present study, we tried to investigate whether there is a direct correlation between a single morphometric character and  $RU_{crit}$ . The fact that the examined morphometric characters do not significantly vary among the four temperature groups leads us to conclude that there is no such correlation between these size-related values and the observed differentiation of swimming performance. On the other hand, unpublished results of our team indicate that zebrafish grown in different temperatures tend to differ in both body shape and meristic character count, which is in agreement with the results of Georgakopoulou et al. (2007). It is logical to assume therefore that it is a combination of those characters (body shape in general) and its variation among the different temperature regimes rather than the effect of a single one that eventually influences the swimming ability of a fish.

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