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RESEARCH ARTICLE

Non-stressful temperature effect on oxidative balance and life history traits in adult fish (*Oryzias latipes*)

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ABSTRACT

Temperature is well known to affect many biological and ecological traits, especially in ectotherms. From a physiological point of view, temperature is also positively correlated to metabolism and is often associated with an increase in reactive oxygen species (ROS) production. It has recently been suggested that ROS play a role in lifespan and resource allocation. However, only a few authors have attempted to explore the relationships between temperature, resource allocation and oxidative balance in ectotherms. Here, we measured the effect of temperature on growth, reproductive effort, offspring quantity and quality, hatching and survival rates, and the associated proximal costs, which were evaluated through the quantification of oxidative balance elements. We reared adult fish (*Oryzias latipes*) at two non-stressful temperatures (20 and 30°C) during a relatively long period (4 months, approximately the entire adult life). The results show a trade-off between reproduction and maintenance because investment toward growth could be neglected at the adult stage (confirmed by our results). Intriguingly, ROS-dependent damages did not differ between the two groups, probably because of the higher rate of activation of the antioxidant enzyme superoxide dismutase for warm-acclimated fish. The allocation toward antioxidant defences is associated with an earlier reproduction and a lower quality of offspring. These interesting results bring new perspectives in terms of the prediction of the impact of global warming on biota through the use of ecological theories based on oxidative balance and metabolism.

KEY WORDS: Maintenance, Reproduction, Oxidative metabolism, Ectotherms

INTRODUCTION

Global warming is now considered a major threat for terrestrial and aquatic ecosystems. The two most well-known ecological impacts of climate change on biota are: (1) shifts in the distribution areas of species towards higher latitudes and altitudes, and (2) shifts in phenology, with spring events occurring earlier under warmer conditions (Wood and McDonald, 1997; Parmesan and Yohe, 2003; Root et al., 2003). Recently, Daufresne et al. (Daufresne et al., 2009) highlighted that the size of aquatic ectotherms also tends to decrease with global warming. Changes in phenology and organism size are necessarily related to physiological changes, but such physiological changes are not yet understood. Of course, the thermal sensitivity of the physiology of ectothermic organisms has been widely studied, but most of these studies have investigated thermal stress and not a slow and slight deviation from thermal optima [i.e. above the 80% performance breadth ('B80') reviewed by Angilletta et al. (Angilletta et al., 2002)].

Temperature has a direct influence on metabolism (Gillooly et al., 2001; Pörtner et al., 2006), playing a role in enzymatic kinetics (Rao and Bullock, 1954) and, in turn, increasing oxygen demand (Fry and Hart, 1946) and respiratory activity (Clarke, 2004; Gillooly et al., 2006). The core of metabolism is oxidative phosphorylation [i.e. consumption of oxygen in order to generate energy as ATP (=ATP)], which mainly occurs in mitochondria. These organelles are involved in a range of other processes [e.g. cellular differentiation, cell death and cell growth (Lane, 2006)] and probably play a role in ageing (Kirkwood and Austad, 2000). Rubner (Rubner, 1906) was the first to suggest that higher mass-specific metabolism could lead to a shorter lifespan, and later Metchnikoff (Metchnikoff, 1908) proposed that ageing and death could be the consequences of toxic by-products of metabolism. Harman (Harman, 1956) developed the idea and stated that these toxic by-products are the reactive oxygen species (ROS), which derive from oxygen affecting lipids, proteins and DNA integrity. According to Harman's 'free radical theory of ageing' (FRTA), the increase in ROS production or change in oxidative balance (e.g. increased production or fewer antioxidant defences) leads to a decrease in lifespan (Finkel and Holbrook, 2000). Recently, the idea of oxidative balance led some authors to integrate the FRTA into the concepts of life history trade-offs and resource allocation, especially regarding growth and reproduction (Alonso-Alvarez et al., 2004; Alonso-Alvarez et al., 2006; Alonso-Alvarez et al., 2007; Dowling and Simmons, 2009; Monaghan et al., 2009; Metcalfe and Alonso-Alvarez, 2010). The main idea is that enzymatic defences require energy that may not be allocated to other functions ['physiological cost' described by Zera and Harshman (Zera and Harshman, 2001)]. Most of these studies have focused on endotherms and few have dealt with ectotherms (Salin et al., 2012). Regarding ectotherms, increasing environmental temperature leads to an increase in oxidative metabolism and thus to an increase in ROS production [1–4% of oxygen is transformed into ROS (Abele et al., 2002; Speakman, 2005)], especially because mitochondria exposed to high temperatures are less efficient [the ADP/O ratio is lower in warmer conditions (Abele et al., 2002)].

Here, we predict that the thermal stimulation of aerobic metabolism in ectotherms will lead to an increase in ROS production, which may, in turn, induce damage to macromolecules. This damage may influence the trade-off in resource allocation between growth, reproduction and maintenance (mechanisms against the ROS damage). Considering that, at the adult stage, allocation of energy toward growth could be neglected (von Bertalanffy, 1957), the trade-off should be between the two other important traits: reproduction and maintenance. From this, there are two non-mutually exclusive ways that could be used by individuals to deal with this extra ROS production. First, fish living in warm conditions may upregulate their antioxidant activities in order to

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List of symbols and abbreviations

FRTA	free radical theory of aging
GPx	glutathione peroxidase
H	hatching rate
H_2O_2	hydrogen peroxide
j_0	first day that a female laid
j_f	last day that a female laid
L_{egg}	egg size
L_f	final body size
L_{female}	female size
L_i	initial body size
MDA	malondialdehyde
M_{F1}	survival rate until maturity of the offspring
NADPH	nicotinamide adenine dinucleotide phosphate
N_e	total number of eggs from a given tank
N_{eji}	number of eggs laid at a given date
N_{fji}	number of females that laid eggs at a given date
N_j	number of days where females laid eggs
N_l	total number of larvae from a given tank
N_m	total number of individuals that achieved maturity from a given tank
$O_2^{\cdot -}$	superoxide anion
$OH^{\cdot -}$	radical hydroxyl
Q_{10}	thermal coefficient
ROS	reactive oxygen species
SOD	superoxide dismutase
TBARs	thiobarbituric acid reactive substances
$\bar{x}$	mean number of eggs laid per female per day

limit oxidative damage. However, because of the amount of energy invested in defences, reproductive effort could be reduced. The second scenario may result from a no-energy allocation to antioxidant defences in order to sustain the ATP pool necessary for reproduction. As a consequence, an increase in oxidative damage and, in turn, mortality rate, may result. In this paper, we used an experimental approach to test these hypotheses. Adult fish were reared at two non-stressful temperatures. We used *Oryzias latipes* Temminck & Schlegel 1846 because this species can live in a wide range of temperatures without exhibiting any stress. We observed responses throughout the duration of the adult stage (from 4 to 8 months old). This duration is relatively long for this species [mean lifespan in controlled conditions at 27°C is 1 yr (Shima and Mitani, 2004), confirmed in Leaf et al. (Leaf et al., 2011)] and corresponds to almost the entire reproductive period (Hirshfield, 1980). This study was conduced over this duration because the physiological responses observed after short-term acclimation (<1 month) could be different than the responses seen after a long-term exposure [>1 month (Sidell et al., 1973; Greaney et al., 1980; Sellner and Hazel, 1982)]. To our knowledge, no study has focused on such a long-term temperature exposure (relative to lifespan) in controlled conditions for fish species at the adult stage. The results of our study provide evidence that increasing temperature leads to changes in oxidative balance and life history traits in fish.

RESULTS

Initial versus final size after 4 months of acclimation

In the warm treatment (30°C), the mean initial size of fish (L_i) was 34.9±2.3 mm and the mean final size (L_f) was 35.0±2.3 mm. In the cold treatment (20°C), the mean initial size was 35.4±2.0 mm and the mean final size was 35.5±2.6 mm. We observed that all individuals grew during the experiment because the initial size was significantly smaller than the final size for the two thermal groups (Table 1). However, no interaction between final size and temperature was found (P>0.05; we removed this effect from the model presented in Table 1), suggesting that individuals from the warm group grew similarly to individuals from the cold group. The gain in size was extremely limited (*ca.* 1 mm), presumably because individuals were already mature at the beginning of the experiment. Finally, there was also no significant effect of sex on growth of adult *O. latipes*.

Reproduction

Clutches: quantity and quality

There was a significant difference in the mean clutch sizes (=number of eggs) per day and per female ($\bar{x}$) between the two temperature

Table 1. Details of the different linear mixed models

			Random effects		Fixed effects		
Property	N (cold; warm)	Dependent variable	Effect	s.d.	Effect	Estimate	P
Growth	76 (38; 38)	L_f	Replicate	0.031	Intercept	0.755	0.437$^{n.s.}$
					L_i	1.022	0.004**
					Temperature	–0.023	0.401$^{n.s.}$
					Sex	0.007	0.947$^{n.s.}$
Reproduction	131 (67; 64)	$\bar{x}$	Replicate	0.279	Intercept	10.382	0.000***
			Date	1.249	Temperature	–3.105	0.000***
	279 (142; 137)	L_{egg}	Replicate	0.029	Intercept	0.706	0.003**
			FemaleID	0.028	L_{female}	0.030	0.000***
					Temperature	–0.112	0.000***
Damage	43 (23; 20)	TBARs	Replicate	0.844	Intercept	18.208	0.000***
					Temperature	1.833	0.195$^{n.s.}$
Defence	43 (23; 20)	SOD	Replicate	0.410	Intercept	386.541	0.000***
					Temperature	258.170	0.008**
	42 (23; 19)	GPx	Replicate	0.168	Intercept	374.300	0.000***
					Temperature	14.500	0.823$^{n.s.}$
	42 (22; 20)	Catalase	Replicate	0.660	Intercept	2.792	0.000***
					Temperature	–0.136	0.842$^{n.s.}$
Oxidative status	42 (23; 19)	TBARs	Replicate	0.256	Intercept	13.956	0.000***
					SOD/GPx	1.168	0.000***
					Temperature	–0.829	0.006**
					SOD/GPx × Temperature	0.725	0.001**

GPx, glutathione peroxidase; L_{egg}, egg size; L_f, final body size; L_{female}, female size; L_i, initial body size; SOD, superoxide dismutase; TBARs, thiobarbituric acid reactive substances; $\bar{x}$, mean clutch size.
**P<0.01; ***P<0.001; n.s., not significant.

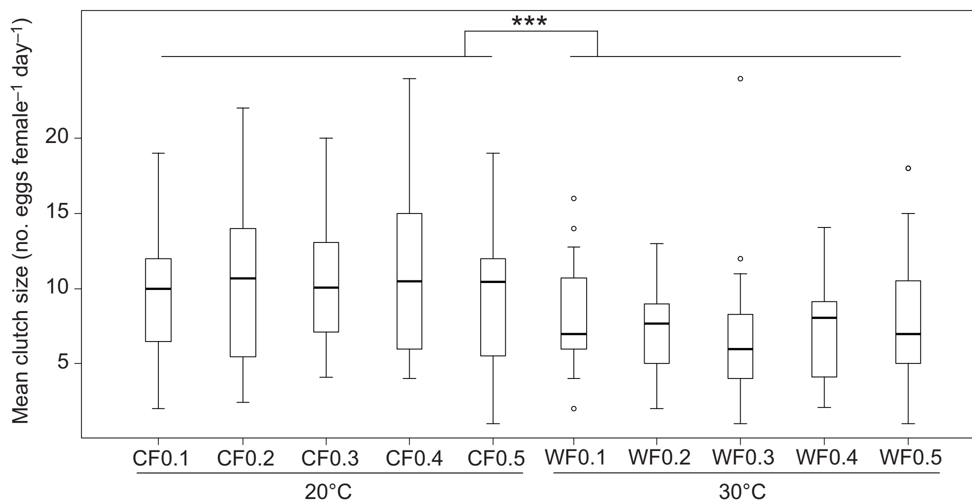

Fig. 1. Eggs laid by female *Oryzias latipes* at 20°C (cold group) and 30°C (warm group). Boxplots represent the first and the third quantiles and the medians of the mean size of clutch per day observed in each tank (WF0.1 to WF0.5 correspond to the tanks from the warm group and CF0.1 to CF0.5 correspond to the tanks from the cold group). The whiskers extend to the most extreme data point, which is no more than 1.5 times the interquartile range from the box. Open circles (outliers) are defined as data points that are located outside the whiskers of the boxplot. Asterisks indicate a significant difference between thermal treatments (***P<0.001).

treatments (Table 1), with fewer eggs laid by the individuals of the warm group (Fig. 1). The hatching rate (H) was significantly lower in the warm group (Z=3.90, P<0.001; Table 2), but survival until maturity (M_{F1}) seemed to be the same in both groups (Z=1.77, P=0.083; Table 2).

Phenology

Temporal distributions of the egg production over the experiment were significantly different between the two temperature treatments (χ^2=1544.63, d.f.=226, P<0.001). Indeed, spawning started immediately in the warm group whereas the first clutch was observed 35 days after the beginning of the experiment in the cold group.

Egg size

Egg sizes were positively correlated to female size (Fig. 2). The linear model showed a similar slope for the two temperature treatments, the interaction term being non-significant. Temperature decreased the intercept of the egg size–female size relationship and eggs were, for a given female size, on average 0.11 mm bigger in the cold group compared with the warm group (Table 1).

Table 2. Reproductive effort parameters determined for both temperature treatments

Temperature	Replicate	N_c	N_e	N_l	N_m	H	M_{F1}
20°C	CF0.1	30	309	154	67	49.8	21.7
	CF0.2	31	327	179	53	54.7	16.2
	CF0.3	28	299	176	50	58.9	16.7
	CF0.4	33	320	164	55	51.3	17.2
	CF0.5	32	350	201	65	57.4	18.6
	Total	154	1605	874	290	54.5***	18.1$^{n.s.}$
30°C	WF0.1	30	273	140	52	51.3	19.0
	WF0.2	31	294	136	50	56.3	17.0
	WF0.3	38	315	133	39	42.2	12.4
	WF0.4	34	306	148	44	48.4	14.4
	WF0.5	40	341	169	55	49.6	16.1
	Total	173	1529	726	240	47.5***	15.7$^{n.s.}$

Results are detailed by tank and means per treatment are determined. The differences were determined only between the rate of hatching (H) and the rate of survival until maturity (M_{F1}) because these parameters take into account the numbers of eggs, larvae and adults.

N_c, number of clutches observed; N_e, number of eggs; N_l, number of larva at the end of the experiment; N_m, number of offspring that achieve maturity.

***P<0.001; n.s., not significant.

Oxidative balance

Quantification of oxidative damage

The mean values concerning the membrane damage were 16.36±1.75 nmol MDA mg^{-1} fresh tissue for the cold group and 16.43±1.20 nmol MDA mg^{-1} fresh tissue for the warm group. No significant differences between the two groups were observed. Similarly, no significant thermal effect was observed for carbonylated proteins despite a mean that was two times lower for fish from the cold group compared with fish from the warm group (4.6±2.6 vs 8.3±3.6 nmol mg^{-1} fresh tissue for 20 vs 30°C, respectively; W=16, P=0.19).

Quantification of antioxidant defences

Our linear model highlighted a significant difference between the two temperature treatments regarding the activity of superoxide dismutase (SOD) (Table 1), with a higher activity in the warm group (Fig. 3). Conversely, glutathione peroxidase (GPx) activity did not differ between the two groups (Table 1, Fig. 4). In the same way, we showed no significant differences for catalase (Table 1, Fig. 4). Finally, there was more membrane damages when the SOD/GPx ratio was low (Fig. 4, Table 1). The interaction between temperature treatment and SOD/GPx ratio was significant, indicating that the increase in damage on membranes is more efficiently controlled by the increase in SOD/GPX ratio for fish from the cold group than for fish from the warm group.

DISCUSSION

Throughout this 4-month experiment, very little energy was allocated to growth for adult *O. latipes* for both temperatures. This result contrasts with that observed by Leaf et al. (Leaf et al., 2011), probably because of their acknowledged overestimation of adult growth and the differences in experimental design. Because no differences in growth were observed, we have especially focused on

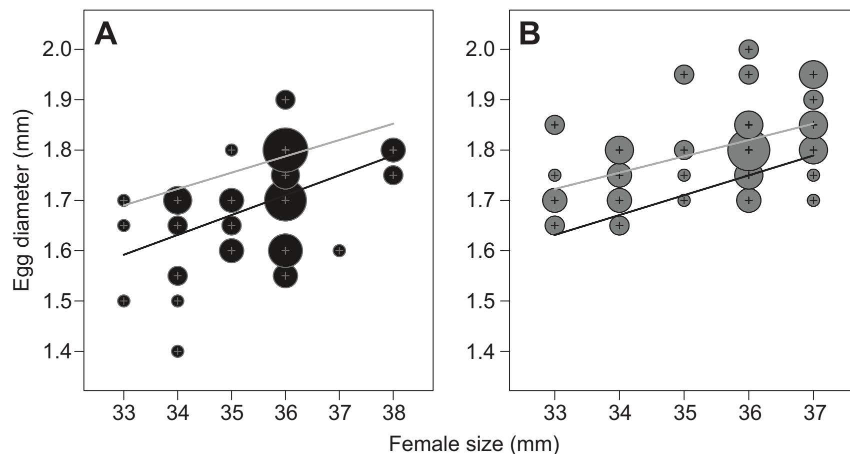

Fig. 2. Relationship between female size and egg size of *Oryzias latipes* at 30°C (A; warm group) and 20°C (B; cold group). Black and grey linear regression lines correspond to the warm and cold groups, respectively. The size of the circles is proportional to the number of females that present the same body size and the same egg size. The centre of each circle is indicated by a '+' symbol to increase readability.

the trade-off between reproduction and maintenance (here assessed through antioxidant processes). Concerning reproduction, we observed: (1) differences in phenology and in reproductive effort between females exposed to 20 and 30°C, and that females from the cold group laid (2) more eggs per day and (3) larger eggs than fish from the warm group, and (4) their hatching rates were higher, suggesting a better quality of offspring.

From a physiological point of view, adults seemed to be similarly impacted by ROS despite the higher aerobic metabolism theoretically observed under higher temperature (Rao and Bullock, 1954). The present study demonstrates that long-term (relative to lifespan) exposure to high (but non-stressful) temperature has an impact on oxidative metabolism without affecting oxidative damage. However, the lack of significant differences in oxidative damage between temperature treatments raises some questions regarding oxidative markers as sensors of trade-offs.

Concerning the antioxidant defence (SOD activity), we observed higher activity in individuals in the warm group. More surprisingly, temperature seemed to have a potential effect on the antioxidant capacity (evaluated through the SOD/GPx ratio). This effect could thus become a key element regarding the sensitivity of individuals to temperature.

Our study show no direct negative correlation between reproduction and maintenance [as previously observed (Monaghan et al., 2009; McGraw et al., 2010; Metcalfe and Alonso-Alvarez, 2010)], but the increase in SOD activity associated with the small clutch size observed under warm treatment suggests a possible trade-off between maintenance and reproduction.

Thermal effect on oxidative damage and antioxidant defences

The thermal sensitivity of the metabolism of ectothermic species is well known and well documented (Rao and Bullock, 1954). Thus, we expected a higher metabolism for fish exposed to the warm treatment and higher associated ROS damage. However, the results of our study showed no significant differences concerning damages to membranes or proteins.

First of all, it is important to note that the lack of a significant effect of temperature on oxidative damage could be due to the limited number of samples used to measure damage, especially to proteins (i.e. the type II statistical error is high). In addition, the absence of observed differences could be related to the age of the fish. Indeed, the individuals tested were 8 months old, which does not exactly correspond to the end of life for *O. latipes* [mean lifespan at 27°C: 1 yr (Shima and Mitani, 2004)]. According to the FRTA, older individuals exhibit greater damage. In this context, we cannot exclude that older fish could exhibit higher MDA contents in the warm group towards the end of their life. Thus, complementary analysis on damage to proteins or DNA over a much longer duration could help to achieve a more accurate picture of the relationship between temperature and oxidative damage.

In addition, with increasing temperature, the degree of unsaturation of the fatty acids of the membranes is known to change (Hazel and Williams, 1990; Cossins, 1994). This change, commonly referred to as 'homeoviscous adaptation' (Hazel and Williams, 1990), has been interpreted as a mechanism for compensation of membrane fluidity and/or order, a physical property (Cossins, 1994). This also makes the membranes of highly acclimated fish probably less sensitive to oxidative stress (Bielski et al., 1983). We cannot exclude that such a phenomenon occurred in our experiment, and this could explain a stronger between-temperature difference in damage to proteins than to membranes. Overall, even if our results for damage have to be analysed cautiously, our experiment reveals that if a sharp increase in temperature induces an oxidative stress (Lushchak and Bagnyukova, 2006), this effect is subtler when organisms are submitted to slight changes in temperature over the long term.

Regarding antioxidant defence, we confirmed higher antioxidant activities for fish from the warm group than from the cold group. Indeed, SOD tested at the same temperature (25°C) for the two groups showed higher activities for the individuals that were exposed to 30°C. The higher SOD activity for fish from warm conditions has been shown previously in a shrimp species (*Macrobrachium nipponense*) (Wang et al., 2006). Surprisingly, the other enzymes involved in oxidative defence (GPx and catalase) presented the same activity in both thermal groups. The higher activity for SOD under warm conditions may thus have induced an increase in H_2O_2 concentration in the cell. We indeed observed that the positive correlation between the SOD/GPx ratio and the membrane damage indicated that the more incomplete the antioxidant defence chain, the more individuals are affected by ROS. This correlation is thermally dependent and the different slopes suggest once again higher ROS production at 30°C. In addition, de Haan et al. (de Haan et al., 1995) proposed the existence of a biological optimum for the ratio between SOD and GPx + catalase activities, which might be more relevant than the absolute activities of the enzymes themselves. The accumulation of H_2O_2 could also induce another deleterious effect via the Fenton reaction (Mao et al., 1993), which leads to the production of the radical hydroxyl ($OH^{-\cdot}$), which is the most dangerous oxidative molecule. In other words, accumulation of H_2O_2 because of higher SOD

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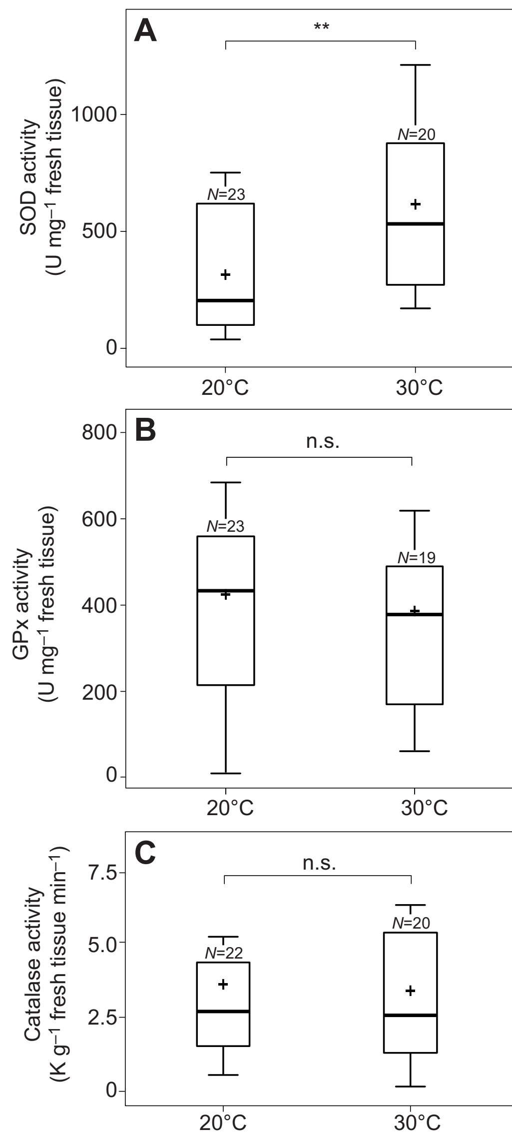

Fig. 3. Antioxidant enzyme activities measured in *Oryzias latipes* at 20°C (cold group) and 30°C (warm group). Boxplots represents the mean (cross) the median (middle line) and the first and third quantiles of the results obtained for (A) superoxide dismutase (SOD), (B) glutathione peroxidase (GPx) and (C) catalase. The whiskers extend to the most extreme data point, which is no more than 1.5 times the interquartile range from the box. Asterisks indicate significant differences between thermal treatments (***P*<0.01; n.s., *P*>0.05). The sample sizes (*N*) are given.

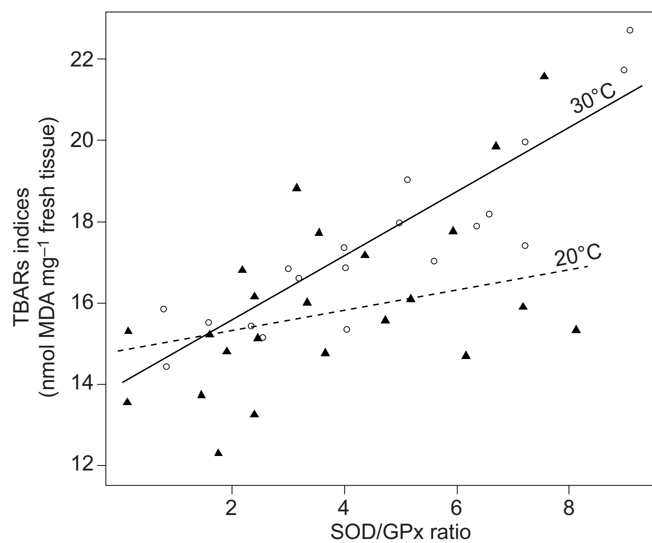

Fig. 4. Log–log relationship between SOD/GPx ratio and oxidative membrane damage of *Oryzias latipes* individuals at 20°C (cold group) and 30°C (warm group). The solid linear regression line and open circles correspond to results obtained for the warm group, and the dashed linear regression line and filled triangles are for fish from the cold group. TBARs, thiobarbituric acid reactive substances.

activity under warm conditions could have induced an increase in $OH^{\cdot-}$ concentration with harmful effects, even if we failed to detect them here. Further studies focusing on other cell components (e.g. genetic material) and including more individuals would help generate a more complete picture of oxidative damage to cells under warm conditions. Nevertheless, the higher antioxidant activities for fish from the warm group is a crucial point within our resource allocation context.

Mortality and reproductive effort

Quality versus quantity of offspring is one of the trade-offs often studied by evolutionary biologists (Charnov and Gillooly, 2004). According to Hirshfield (Hirshfield, 1980), offspring mortality increases with temperature for *O. latipes* and our results partially validate this relationship. Indeed, the hatching rate was significantly higher for eggs from the cold group. However, no significant differences in survival until maturity were observed.

Besides the mortality rate of eggs and offspring, we observed a delay in the occurrence of the reproductive pattern in the cold group. This delay can probably be explained by the time necessary to produce sexual cells at a low temperature (Robinson and Rugh, 1943). In addition to this temperature effect on the timing of the reproduction pattern, we observed a change in the clutch size. Indeed, clutches from the warm group were significantly smaller than those from the cold group. Regarding medakas, this is dissimilar to what has been suggested by Hirshfield (Hirshfield, 1980), but is in accordance with results from Leaf et al. (Leaf et al., 2011). Interestingly, because females from the warm group laid eggs more often than the females from the cold group, there was almost the same total number of eggs laid in both temperature treatments. Thus, despite a change in the phenology, one could consider that the investment in reproduction was similar between the two thermal groups. It is important to note, however, that for a given length, a warm-acclimated female produced eggs that were significantly smaller in diameter than those from cold-acclimated females, as previously shown by Hirshfield (Hirshfield, 1980) and Leaf et al. (Leaf et al., 2011). These authors stated that it is not the size of the egg that changes with thermal treatment, but its quality. We cannot exclude that size and quality of the eggs were related in our experiment. Actually, the hatching rate was lower under warmer conditions (and thus when eggs were smaller). Considering the absence of growth during the 4 months of the experiment and the higher investment in antioxidant defences, we can also hypothesize that eggs were smaller under higher temperatures because of a lower investment in reproduction per clutch event.

The clutch size ($\bar{x}$) given by Eqn 1 (see Materials and methods) did not take into account the sex ratio, which was different between the two groups and changed during the experiment. However, it is commonly observed that the number of males is not limiting for reproduction, notably in fish species (Andersson, 1994).

These results have several ecological implications. It is generally assumed that warmer temperatures could allow the reproductive period to increase for species that are not submitted to thermal stress. In addition, an earlier reproduction is an advantage in stressful environments (Stearns, 1992). From this viewpoint, the strategy of the fish from the warm group, which actually invested relatively steadily and quickly in reproduction, could be advantageous. However, the lower quality of the eggs (observed here with the lower hatching rate) could balance the benefit of the increase in the length of the reproductive period. Indeed, it is interesting to note that, at the end of the experiment, the total number of mature individuals of the generation F1 (a proxy of the fitness of the F0 individuals) was quite similar for the two thermal groups (240 versus 290 for the warm and the cold groups, respectively). Thus, the changes in oxidative metabolism and life history traits could help to maintain similar population dynamics in different thermal environments. Further studies focusing on the whole ontogeny and over several generations could validate such hypothesis.

MATERIALS AND METHODS

Biological material and rearing conditions

The Japanese medaka (*Oryzias latipes*) is a small (20–40 mm, 250–500 mg) and iteroparous (Robinson and Rugh, 1943; Hirshfield, 1980) freshwater fish native to East Asia. This eurythermic fish [living in 5–35°C environments, optimal temperature: 25°C (Dhillon, 2007)] requires only 10–12 weeks to reach sexual maturity at 25°C. The experimental design is detailed in Fig. 5. Here, we used 76 individuals belonging to the CAB strain from Carolina Biological Supply Company (Burlington, NC, USA; from AMAGEN, Gif-sur-Yvette, France) and WatchFrog (Evry, France). Fish of this standard strain present differences in the colour of eyes and other tissues (Loosli et al., 2000; Furutani-Seiki and Wittbrodt, 2004; Furutani-Seiki et al., 2004; Sasado et al., 2010; Kinoshita et al., 2009), but can be used in ecophysiological studies because their development characteristics are similar to those of the wildtype (Sasado et al., 2010; Lawrence et al., 2012). They were studied from 4 months old (mature) to 8 months old [almost the end of life at 27°C (Shima and Mitani, 2004; Leaf et al., 2011)]. These individuals were reared under two contrasted temperatures: 20 and 30°C. These temperatures were chosen because of the wide range of thermal conditions supported by *O. latipes* (Shima and Mitani, 2004). Because of the possible effect of food resources, fish from each thermal group were fed *ad libitum* every day at 18:00 h with TetraMin, as in Hirshfield (Hirshfield, 1980). Individuals were kept for 5 days at the same rearing temperature as the supplier (25°C). After these 5 days, the temperature was increased or decreased by 1°C every 2 days, reaching 20°C for the 'cold' group and 30°C for the 'warm' group within 10 days. During this 15 day period, the photoperiod was 12 h:12 h light:dark.

Thirty-eight individuals (generation F0) were randomly placed in the cold group and 38 in the warm group at a ratio of 1.33 to 1.66 females for one male to maximize the reproductive effort and to avoid overtaxing females [this density, far below 2–3 fish l^{-1}, causes no stress and no agonistic behaviour (Denny et al., 1991)]. In order to avoid the loss of all fish because of a common disease (e.g. *Ichthyophthirius multifilis*), fish were placed in groups of seven or eight in five 20 l tanks (see details in Fig. 5; WF0.1 to WF0.5 corresponding to the tanks of the warm group and CF0.1 to CF0.5 corresponding to the tanks of the cold group). After the 'settling' phase (5 days of animal housing and 10 days for the change in temperature), the photoperiod changed to 16 h:8 h light:dark to trigger reproduction (Hirshfield, 1980).

Eggs were collected every day and were placed in a small nursery (2.5 l) in the same tank as their parents (maximum density reported: 1 larva per

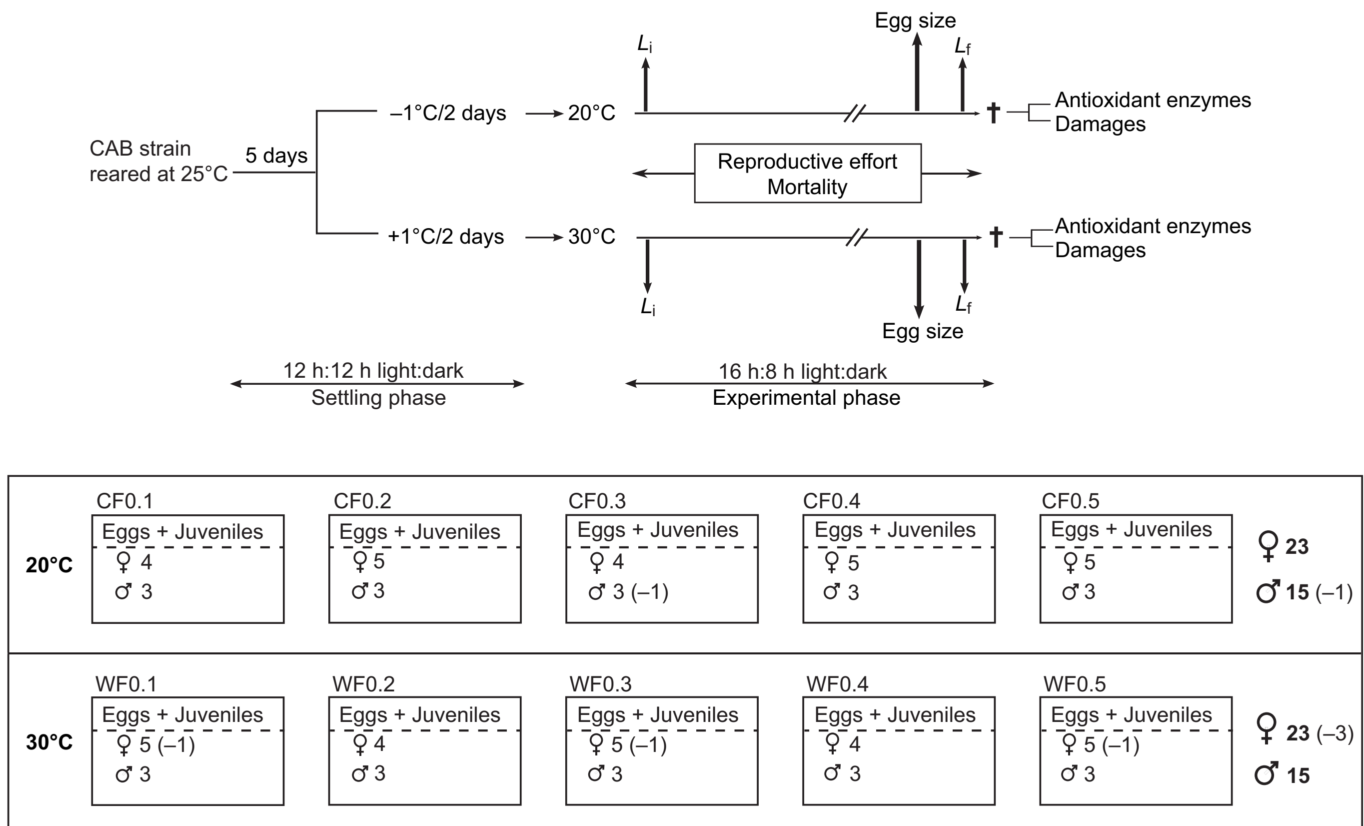

Fig. 5. Experimental design. WF0.1 to WF0.5 correspond to the tanks from the warm group (30°C) and CF0.1 to CF0.5 correspond to the tanks from the cold group (20°C). Daggers correspond to the end of the experiment, when individuals were euthanised prior to measurement of oxidative elements (TBARs and SOD/GPx). Sample sizes are indicated next to the male and female symbols. Numbers in parentheses indicate the number of dead individuals during the experiment (i.e. before euthanasia). Eggs and juveniles were placed in a nursery in the same tank as their parents.

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50 ml of water). Larvae were fed *ad libitum* every day at the same time as the parents. According to legislation, our laboratory obtained an agreement for running experiments on *O. latipes* for this study (Direction départementale de la protection des populations, no. A 13-510).

Measurement of growth

In order to confirm that adult fish did not grow during the experiment, we measured the initial and the final total lengths (in mm) of each individual. At the beginning of the experiment, non-anaesthetised fish were placed in a small Petri dish with water. The sex was determined before individuals were assigned to each tank [females present a smaller anal fin than males (Kinoshita et al., 2009)] and the initial sizes (L_i) were measured with millimetre graph paper with a precision of ±0.5 mm. Four months later (at the end of experiment), the final sizes (L_f) were determined following the same protocol.

Reproductive effort

Reproductive effort (phenology, quantity of offspring, egg size and quality of offspring) was only determined in females.

Phenology

Clutches were collected every day directly from the female [females keep eggs after fertilisation (Shima and Mitani, 2004)] with a brush early in the morning (08:00 h). The 10 groups (five tanks per temperature treatment) were followed during the entire experiment in order to determine the phenology of the reproduction processes. The timing of the first clutches was noted in each tank to determine if there was a delay between the two thermal groups.

Quantity of offspring

The clutch sizes (number of eggs) were measured every day. Because the number of females was not exactly the same at the beginning of the experiment (see Fig. 5) and because it changed slightly during the 4 months (due to the loss of three females in the warm group), we determined the mean number of eggs produced per female that laid and per day ($\bar{x}$) as:

$$\bar{x} = \frac{1}{N_j} \sum_{j_0}^{j_f} \left(\frac{N_{eji}}{N_{fji}} \right), \quad (1)$$

where j_0 corresponds to the first day where females from a tank laid eggs and j_f is the last day, N_j corresponds to the number of days where females from a given tank laid eggs (not every day), N_{eji} corresponds to the number of eggs laid at a given date (i) and N_{fji} is the number of females that laid eggs on the same date (i).

Egg size

A clutch was taken from each of 15 females (with no significant differences in mean female size between the two groups: *t*-test: d.f.=67.5, P=0.16) on a single day (27 November 2011, 101 days from the beginning of the experiment). The diameters of 137 and 142 eggs collected from the warm and cold groups, respectively, were measured with a microscope system (Leitz DIALUX 20 EB, Midland, ON, Canada) with ±0.01 mm precision.

Quality of offspring

In order to determine the quality of offspring (i.e. the individuals of the F1 generation), the number of larva were observed at the end of the experiment, which allowed us to determine the hatching rate (H) following the equation:

$$H = \frac{N_l}{N_e} \times 100, \quad (2)$$

where N_l is the total number of larva observed in a given tank and N_e is the total number of eggs laid in this tank.

We also determined the rate of survival until maturity of the offspring generation (M_{F1}) with the equation:

$$M_{F1} = \frac{N_m}{N_e} \times 100, \quad (3)$$

where N_m corresponds to the total number of offspring (corresponding to the F1 generation) that lived until maturity in a given tank.

Oxidative stress

To avoid any bias in the results due to the possible sex effect in resource allocation associated with oxidative balance, we measured physiological properties only on females, as suggested by Heiss and Schoech (Heiss and Schoech, 2012).

After the 4 months of the experiment, fish were euthanised (by de-medulation) and placed in an Eppendorf tube (2 ml). They were then placed in liquid nitrogen in order to fix enzymatic reactions and for later tissue analysis. The 23 females from the cold group and the 20 (less three individuals that died during the experiment) from the warm group were used for physiological measurements of oxidative balance. After removing the head and the fins, fish were first homogenised twice with ball milling for 4 min at 50 Hz in a monopotassium phosphate solution (KH_2PO_4 100 mmol l^{-1}). These homogenates were then divided into two equal parts, one used for damage determinations and the other for quantification of antioxidant activities.

Quantification of ROS damage

Under oxidative stress, membrane lipids are peroxidised. The product of this reaction is malondialdehyde (MDA). The thiobarbituric acid reactive substances (TBARs) method is based on the measurement of the presence of MDA in the sample following the protocol described by Yagi (Yagi, 1976). The homogenates obtained above were homogenised again with a buffer solution (100 mmol l^{-1} KH_2PO_4, with the addition of 0.05% bovine serum albumin, 10 mmol l^{-1} EDTA, 0.13 mmol l^{-1} butylated hydroxytoluene and 0.13 mmol l^{-1} desferoxamine). These new homogenates were then centrifuged at 1100 ***g*** (4°C 10 min^{-1}) and supernatants were collected. These extracts were conserved at −80°C and analysed later. Two hundred microlitres of supernatant was added to 30 µl of 8.1% SDS, 225 µl of 20% acetic solution and 225 µl of 0.8% thiobarbituric acid. These samples were incubated and shaken at 95°C for 1 h. Then, after addition of 600 µl of butanol/pyridine (15/1), the samples were shaken again for 10 min and centrifuged once more for 10 min at 1600 ***g*** (ambient temperature). The upper phases were removed and dosed at 532 nm at 25°C and compared with a standard range with TEP (1,1,3,3-tetraethoxypropane). The results were expressed as nanomoles of MDA per milligramme of fresh tissue.

Even if the TBARs method is commonly used to assess ROS damage [examples in ectotherm species (Wilhelm-Filho et al., 2001; Sukhotin et al., 2002; Mila-Kierzenkowska et al., 2005)], its use is criticised (Almroth et al., 2005). Therefore, in order to better evaluate damages caused by ROS, we also measured carbonylated proteins on a subsample of individuals already tested for TBARs (N=5 for the cold group and N=4 for the warm group). The remains of homogenates were centrifuged at 10,000 ***g*** for 15 min at 4°C. The supernatants collected were then tested with the Cayman Protein Carbonyl Assay Kit using DNPH (2,4-dinitrophenylhydrazine) as indicative of carbonylated proteins (item N.10005020, Cayman Chemical, Ann Arbor. MI, USA). The results were expressed as nanomoles of carbonylated proteins per milligramme of total protein content (compared with a BSAe standard at 280 nm and 25°C).

Quantification of antioxidant defences: enzyme activities of SOD, GPx and catalase

The extraction method was the same for the three enzymes and quantification of enzyme activities followed the protocols described in Voituron et al. (Voituron et al., 2006). We homogenised the samples in a buffer solution (1 mmol l^{-1} dithiothreitol, 2 mmol l^{-1} EDTA, pH adjusted to 7.4 at 4°C) during 4 min at 50 Hz with ball milling. Homogenates were then centrifuged for 5 min at 4°C and 2380 ***g*** and supernatants were used for measurements.

The enzyme superoxide dismutase (SOD) catalysed the dismutation of $O_2^{\cdot -}$ (superoxide anion) to produce H_2O_2 (hydrogen peroxide). This enzyme is very unstable; thus, the samples were measured directly after extraction. Homogenates were frozen (−80°C) and analysed later for glutathione peroxidase (GPx) and catalase activities. Measurements of SOD activities were indirect. We used the xanthine/xanthine-oxidase system as a producer of $O_2^{\cdot -}$ and the oxidation of cytochrome *c* was followed to determine the activity at 550 nm and 25°C. The results were expressed as units per milligramme of fresh tissue. Regarding GPx, its function consists of the

conversion of free H_2O_2 produced by SOD in water. To evaluate GPx activities, measurements were based on tracking, using spectrophotometry, and the NADPH disappearance at 340 nm and 37°C. We also measured the catalase activities. This enzyme also allows the decomposition of H_2O_2 to water and oxygen (like the GPx enzyme but not located in the same cellular compartment). The decrease in H_2O_2 was measured in the sample by spectrophotometry at 240 nm at 25°C in quartz cuvettes in order to quantify catalase activities.

Statistical analysis

Values are given as the means ± s.e.m. For all tests, the level of significance was fixed at 5%. Growth, reproductive effort and physiological properties were analysed by linear mixed models because residuals followed a Gaussian law. We took into account the pseudoreplication effect in these linear mixed models considering the tank as a random effect ('replicates'). All tests were performed using R version 2.14.2 (R Development Core Team, 2012). The linear mixed models were performed with the package 'lme4' (Bates et al., 2012). The significance of the fixed effects was evaluated by Markov chain Monte Carlo tests (Baayen, 2011).

Growth

Growth was analysed by a linear mixed model with the final length (L_f) as a dependent variable and initial size (L_i), temperature (°C) and the interaction between L_i and temperature as fixed variables. To check for a potential difference in growth of male versus female individuals, sex was also considered as a fixed effect. The replicates were considered as random effects. The interaction term was removed from the model if it was not significant.

Reproduction

Differences in mean clutch size ($\bar{x}$) between the two thermal groups were estimated by a linear mixed model with temperature as a fixed effect and date and replicates as random effects. Then, in order to study the differences in the phenology of reproductive events, a χ^2 test of homogeneity was carried out. This test allowed us to observe differences regarding the distribution of clutch events over time between the two thermal groups.

To estimate differences regarding egg size (L_{egg}), a linear mixed model was used with female size (L_{female}), temperature and their interaction as fixed effects, and replicate and female identity ('FemaleID') as random effects.

Finally, the hatching rate (H) and survival rate until maturity for the offspring (M_{F1}) were compared between the two thermal groups using homogeneity tests to compare two proportions.

Oxidative balance

All females from a given temperature were pooled together at the end of experiment in order to have more statistical power when analysing the differences in the physiological parameters (damage and defence). SOD, GPx and catalase activities and MDA contents were analysed by linear mixed models with temperature as a fixed effect and replicate as a random effect. Because of the small sample sizes, the differences between the rate of proteins carbonylated in the two thermal groups were analysed by a non-parametric test (Wilcoxon Mann–Whitney).

In order to determine the efficiency of antioxidant enzymes, we also calculated the mean ratio between SOD and GPx activities (Sánchez-Rodríguez et al., 2007). This ratio allowed us to observe, using a linear mixed model, the relationship between damage to membranes (TBARs) and antioxidant capacities (SOD/GPx ratio), temperature and their interaction. Again, the replicates were considered as a random effect. The interaction term was removed from the model if it was non-significant.

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Competing interests
The authors declare no competing financial interests.

Author contributions
C.H.-B. and M.D. contributed to the conception of the study. C.H.-B., M.D. and Y.V. designed the experiment. C.H.-B., L.R. and C.R. performed the experiments. C.H.-B. performed the statistical analyses. C.H.-B. and M.D. wrote the paper. All authors contributed to the interpretation of the results and critically reviewed the early versions of the manuscript.

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References

Abele, D., Heise, K., Pörtner, H. O. and Puntarulo, S. (2002). Temperature-dependence of mitochondrial function and production of reactive oxygen species in the intertidal mud clam *Mya arenaria*. *J. Exp. Biol.* **205**, 1831-1841.
Almroth, B. C., Sturve, J., Berglund, A. and Förlin, L. (2005). Oxidative damage in eelpout (*Zoarces viviparus*), measured as protein carbonyls and TBARS, as biomarkers. *Aquat. Toxicol.* **73**, 171-180.
Alonso-Alvarez, C., Bertrand, S., Devevey, G., Prost, J., Faivre, B. and Sorci, G. (2004). Increased susceptibility to oxidative stress as a proximate cost of reproduction. *Ecol. Lett.* **7**, 363-368.
Alonso-Alvarez, C., Bertrand, S., Devevey, G., Prost, J., Faivre, B., Chastel, O. and Sorci, G. (2006). An experimental manipulation of life-history trajectories and resistance to oxidative stress. *Evolution* **60**, 1913-1924.
Alonso-Alvarez, C., Bertrand, S., Faivre, B. and Sorci, G. (2007). Increased susceptibility to oxidative damage as a cost of accelerated somatic growth in zebra finches. *Funct. Ecol.* **21**, 873-879.
Andersson, M. (1994). *Sexual Selection*. Princeton, NJ: Princeton University Press.
Angilletta, M., Niewiarowski, P. and Navas, C. (2002). The evolution of thermal physiology in ectotherms. *J. Therm. Biol.* **27**, 249-268.
Baayen, R. (2011). *Analyzing Linguistic Data: A Practical Introduction to Statistics*. Cambridge: Cambridge University Press.
Bates, D., Maechler, M. and Bolker, B. (2012). lme4: Linear mixed-effects models using S4 classes. R package version 0.999999-0.
Bielski, B. H., Arudi, R. L. and Sutherland, M. W. (1983). A study of the reactivity of HO_2/O_2 with unsaturated fatty acids. *J. Biol. Chem.* **258**, 4759-4761.
Charnov, E. L. and Gillooly, J. F. (2004). Size and temperature in the evolution of fish life histories. *Integr. Comp. Biol.* **44**, 494-497.
Clarke, A. (2004). Why does metabolism scale with temperature? *Funct. Ecol.* **18**, 243–251.
Cossins, A. (1994). *Temperature Adaptation of Biological Membranes*. Chapel Hill, NC: Portland Press.
Daufresne, M., Lengfellner, K. and Sommer, U. (2009). Global warming benefits the small in aquatic ecosystems. *Proc. Natl. Acad. Sci. USA* **106**, 12788-12793.
de Haan, J. B., Cristiano, F., Iannello, R. C. and Kola, I. (1995). Cu/Zn-superoxide dismutase and glutathione peroxidase during aging. *Biochem. Mol. Biol. Int.* **35**, 1281-1297.
Denny, J., Spehar, R., Mead, K. and Shirin, C. (1991). *Guidelines for Culturing the Japanese Medaka, Oryzais latipes*. Washington, DC: US Environmental Protection Agency.
Dhillon, R. (2007). Growth-independent effects of a fluctuating thermal regime on the life-history traits of the Japanese medaka (*Oryzias latipes*). *Ecol. Freshw.* **16**, 425-431.
Dowling, D. K. and Simmons, L. W. (2009). Reactive oxygen species as universal constraints in life-history evolution. *Proc. Biol. Sci.* **276**, 1737-1745.
Finkel, T. and Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature* **408**, 239-247.
Fry, F. E. and Hart, J. S. (1946). The relation of temperature to oxygen consumption in the goldfish. *Anat. Rec.* **96**, 586.
Furutani-Seiki, M. and Wittbrodt, J. (2004). Medaka and zebrafish, an evolutionary twin study. *Mech. Dev.* **121**, 629-637.
Furutani-Seiki, M., Sasado, T., Morinaga, C., Suwa, H., Niwa, K., Yoda, H., Deguchi, T., Hirose, Y., Yasuoka, A., Henrich, T. et al. (2004). A systematic genome-wide screen for mutations affecting organogenesis in medaka, *Oryzias latipes*. *Mech. Dev.* **121**, 647-658.
Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M. and Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science* **293**, 2248-2251.
Gillooly, J., Allen, A., Savage, V., Charnov, E., West, G. and Brown, J. (2006). Response to Clarke and Fraser: effects of temperature on metabolic rate. *Funct. Ecol.* **20**, 400-404.
Greaney, G., Place, A., Cashon, R., Smith, G. and Powers, D. (1980). Time course of changes in enzyme activities and blood respiratory properties of killifish during long-term acclimation to hypoxia. *Physiol. Zool.* **53**, 136-144.
Harman, D. (1956). Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* **11**, 298-300.
Hazel, J. R. and Williams, E. E. (1990). The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. *Prog. Lipid Res.* **29**, 167-227.
Heiss, R. S. and Schoech, S. J. (2012). Oxidative cost of reproduction is sex specific and correlated with reproductive effort in a cooperatively breeding bird, the Florida scrub jay. *Physiol. Biochem. Zool.* **85**, 499-503.

Hirshfield, M. (1980). An experimental analysis of reproductive effort and cost in the Japanese medaka, *Oryzias latipes*. *Ecology* **61**, 282-292.
Kinoshita, M., Murata, K., Naruse, K. and Tanaka, M. (2009). *Medaka: Biology, Management, and Experimental Protocols*. Ames, IA: Wiley-Blackwell.
Kirkwood, T. B. and Austad, S. N. (2000). Why do we age? *Nature* **408**, 233-238.
Lane, N. (2006). *Power, Sex, Suicide: Mitochondria and the Meaning of Life*. Oxford: Oxford University Press.
Lawrence, C., Adatto, I., Best, J., James, A. and Maloney, K. (2012). Generation time of zebrafish (*Danio rerio*) and medakas (*Oryzias latipes*) housed in the same aquaculture facility. *Lab Anim. (NY)* **41**, 158-165.
Leaf, R., Jiao, Y., Murphy, B., Kramer, J., Sorensen, K. and Wooten, V. (2011). Life-history characteristics of Japanese medaka *Oryzias latipes*. *Copeia* **2011**, 559-565.
Loosli, F., Köster, R. W., Carl, M., Kühnlein, R., Henrich, T., Mücke, M., Krone, A. and Wittbrodt, J. (2000). A genetic screen for mutations affecting embryonic development in medaka fish (*Oryzias latipes*). *Mech. Dev.* **97**, 133-139.
Lushchak, V. I. and Bagnyukova, T. V. (2006). Temperature increase results in oxidative stress in goldfish tissues. 1. Indices of oxidative stress. *Comp. Biochem. Physiol.* **143C**, 30-35.
Mao, G. D., Thomas, P. D., Lopaschuk, G. D. and Poznansky, M. J. (1993). Superoxide dismutase (SOD)-catalase conjugates. Role of hydrogen peroxide and the Fenton reaction in SOD toxicity. *J. Biol. Chem.* **268**, 416-420.
McGraw, K., Cohen, A., Costantini, D. and Hõrak, P. (2010). The ecological significance of antioxidants and oxidative stress: a marriage between mechanistic and functional perspectives. *Funct. Ecol.* **24**, 947-949.
Metcalfe, N. and Alonso-Alvarez, C. (2010). Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. *Funct. Ecol.* **24**, 984-996.
Metchnikoff, E. (1908). *The Prolongation of Life: Optimistic Studies.* Classics in Longevity and Aging. New York: Springer.
Mila-Kierzenkowska, C., Woźniak, A., Woźniak, B., Drewa, G., Chesy, B., Drewa, T., Krzyzyńska-Malinowska, E. and Cerafícki, R. (2005). Activity of superoxide dismutase (SOD) and concentration of thiobarbituric acid reactive substances (TBARS) in liver and muscles of some fish. *Acta Biol. Hung.* **56**, 399-401.
Monaghan, P., Metcalfe, N. B. and Torres, R. (2009). Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecol. Lett.* **12**, 75-92.
Parmesan, C. and Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**, 37-42.
Pörtner, H. O., Bennett, A. F., Bozinovic, F., Clarke, A., Lardies, M. A., Lucassen, M., Pelster, B., Schiemer, F. and Stillman, J. H. (2006). Trade-offs in thermal adaptation: the need for a molecular to ecological integration. *Physiol. Biochem. Zool.* **79**, 295-313.
R Development Core Team (2012). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. Available at: http://www.R-project.org/.
Rao, K. and Bullock, T. (1954). Q10 as a function of size and habitat temperature in poikilotherms. *Am. Nat.* **88**, 33-44.
Robinson, E. and Rugh, R. (1943). The reproductive processes of the fish, *Oryzias latipes*. *Biol. Bull.* **84**, 115-125.
Root, T. L., Price, J. T., Hall, K. R., Schneider, S. H., Rosenzweig, C. and Pounds, J. A. (2003). Fingerprints of global warming on wild animals and plants. *Nature* **421**, 57-60.
Rubner, M. (1906). Energieumsatz im leben einiger spaltpilze. *Arch. Hyg.* **57**, 192-243.
Salin, K., Roussel, D., Rey, B. and Voituron, Y. (2012). David and Goliath: A mitochondrial coupling problem? *J. Exp. Zool.* **317**, 283-293.
Sánchez-Rodríguez, M. A., Ruiz-Ramos, M., Correa-Muñoz, E. and Mendoza-Núñez, V. M. (2007). Oxidative stress as a risk factor for osteoporosis in elderly Mexicans as characterized by antioxidant enzymes. *BMC Musculoskelet. Disord.* **8**, 124-130.
Sasado, T., Tanaka, M., Kobayashi, K., Sato, T., Sakaizumi, M. and Naruse, K. (2010). The National BioResource Project Medaka (NBRP Medaka): an integrated bioresource for biological and biomedical sciences. *Exp. Anim.* **59**, 13-23.
Sellner, P. A. and Hazel, J. (1982). Time course of change in fatty acid composition of gills and liver from rainbow trout (*Salmo gairdneri*) during thermal acclimation. *J. Exp. Zool.* **221**, 159-168.
Shima, A. and Mitani, H. (2004). Medaka as a research organism: past, present and future. *Mech. Dev.* **121**, 599-604.
Sidell, B., Wilson, F., Hazel, J. and Prosser, C. (1973). Time course of thermal acclimation in goldfish. *J. Comp. Physiol.* **84**, 119-127.
Speakman, J. R. (2005). Body size, energy metabolism and lifespan. *J. Exp. Biol.* **208**, 1717-1730.
Stearns, S. (1992). *The Evolution of Life Histories*. Oxford: Oxford University Press.
Sukhotin, A., Abele, D. and Pörtner, H.-O. (2002). Growth, metabolism and lipid peroxidation in *Mytilus edulis*: age and size effects. *Mar. Ecol. Prog. Ser.* **226**, 223-234.
Voituron, Y., Servais, S., Romestaing, C., Douki, T. and Barré, H. (2006). Oxidative DNA damage and antioxidant defenses in the European common lizard (*Lacerta vivipara*) in supercooled and frozen states. *Cryobiology* **52**, 74-82.
Von Bertalanffy, L. (1957). Quantitative laws in metabolism and growth. *Q. Rev. Biol.* **32**, 217-231.
Wang, W., Wang, A., Liu, Y., Xiu, J., Liu, Z. and Sun, R. (2006). Effects of temperature on growth, adenosine phosphates, ATPase and cellular defense response of juvenile shrimp *Macrobrachium nipponense*. *Aquaculture* **256**, 624-630.
Wilhelm Filho, D., Torres, M. A., Tribess, T. B., Pedrosa, R. C. and Soares, C. H. (2001). Influence of season and pollution on the antioxidant defenses of the cichlid fish acará (*Geophagus brasiliensis*). *Braz. J. Med. Biol. Res.* **34**, 719-726.
Wood, C. and McDonald, D. (1997). *Global Warming: Implications for Freshwater and Marine Fish*. Cambridge: Cambridge University Press.
Yagi, K. (1976). A simple fluorometric assay for lipoperoxide in blood plasma. *Biochem. Med.* **15**, 212-216.
Zera, A. and Harshman, L. (2001). The physiology of life history trade-offs in animals. *Annu. Rev. Ecol. Syst.* **32**, 95-126.