Maternal swimming exercise training improves survival and the heritability of thermal tolerance and length in brown trout offspring.

Pettinau Luca (1,4), Tytti-Maria Uurasmaa (1), Eila Seppänen (2), Asko Sikanen (2), Miika Raitakivi (2), Jenni M. Prokkola (1, 3), Amelie Crespel (1), Katja Anttila (1)

(1) Department of Biology, University of Turku, FI-20014 Turku, Finland;

(2) Natural Resources Institute Finland (Luke), Laasalantie 9, FI-58175 Enonkoski, Finland

(3) Natural Resources Institute Finland (Luke), Paavo Havaksen tie 3, FI-90570 Oulu, Finland

(4) Department of Animal Health and Welfare, Wageningen University and Research, 6708 WD Wageningen, Netherlands

Corresponding authors: Luca Pettinau; email: luca.pettinau@wur.nl; ORCID: 0000-0001-6597-9908. Department of Animal Health and Welfare, Wageningen University and Research, 6708 WD Wageningen, Netherlands

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Abstract

Hatchery-reared fish released to sustain wild populations exhibit low survival in the wild. Possible reasons are rearing conditions that preclude fish from displaying their natural swimming behaviour, which negatively affect their cardiorespiratory physiology. This might also make them vulnerable to heatwaves, reducing their survival. Here, we investigated if the negative effects of captive rearing on cardiac performance and fitness-related traits could be mitigated by maternal exercise in brown trout (*Salmo trutta*). We reared the mothers with two different exercise training programmes: control (0.12 $m*s^{-1}$) and exercise (0.41 $m*s^{-1}$). Exercise training not only improved cardiac thermal performance and reproductive success, but also enhanced the survival of embryos via transgenerational plasticity.

Maternal exercise did not, however, affect thermal tolerance variables in the offspring. Nevertheless, the heritability and thereby evolutionary potential of body length and thermal tolerance were increased in the offspring from trained mothers. Therefore, exercise training represents a valuable tool to improve the physiological performance of hatchery-reared fish, having cascading beneficial effects on the next generation. This study strengthens the idea that transgenerational plasticity and Genotype-by-Parental-Environment interactions can be harnessed to improve conservation and restoration programs by producing more resilient offspring.

INTRODUCTION

Human-induced habitat degradation and global warming contribute to the decline of natural populations, leading to the necessary release of captive-bred animals to sustain wild populations (Aprahamian et al., 2003). However, several studies have shown that the survival of captive-bred animals is lower in nature compared to wild conspecifics (Araki et al., 2008). This phenomenon has been widely studied in fishes, where hatchery-reared salmonids are commonly used as supplementation of wild populations (Schwinn et al., 2017). Reasons for the declined survival of hatchery-reared fish are multiple; they possess a reduced physiological performance, e.g. lower cardiac performance (Twardek et al., 2021), thermal tolerance (Carline & Machung, 2001; Hirakawa & Salinas, 2020), physiological plasticity (Morgan et al., 2022; Zhang et al., 2016), anti-predator behaviour (Houde et al., 2010) and swimming capacity (Claireaux et al., 2005). Cardiac deformities are also common in hatchery-reared fish (Brijs et al., 2020) leading to poor cardiac performance, which, in turn, could be linked with lower organismal thermal tolerance (Carline & Machung, 2001; Hirakawa & Salinas, 2020) a key trait influencing fish fitness (Diamond, 2017). With an increasing frequency of heatwaves (Pörtner et al., 2022; Smalås et al., 2020), the released hatchery-reared fish, with low cardiac performance and thermal tolerance, might not be able to acclimate fast enough, compromising conservation programs. Therefore, new strategies are needed to improve the physiological performance of the restocked fish and to increase the success of restocking programs.

The hatchery fish are typically reared in barren environments, possibly causing behavioural and physiological differences compared to their wild counterparts. Hatchery fish are usually also reared at low water flows, stable temperatures, on an artificial diet, and in the absence of predator-prey interactions, and are, therefore, under reduced natural selection. These conditions do not prepare the fish for the complexity of stimuli and stressors of the natural environment. This is especially relevant in actively swimming fish, such as salmonid species, which are adapted to perform arduous upstream migration before spawning. Exercise training program, i.e., increasing the water flow in the rearing environment, has been proposed as a useful enrichment strategy to improve the physiological performance of hatchery-reared fish (McKenzie et al., 2021). Several studies have shown that swimming exercise training improves swimming performance (Lu et al., 2020), cardiac performance (Farrell et al., 1990, 1991; Gallaugher et al., 2001), growth rate and muscle composition (Anttila et al., 2008; Nilsen et al., 2019; Palstra et al., 2010), robustness against diseases (Castro et al., 2011), reproductive success and the number of sexually mature females (Patterson et al., 2004), as well as survival in nature (Franssen et al., 2021; Zhou et al., 2019). Among these benefits, the exercise-induced improvements on cardiorespiratory performance are particularly relevant for the current global warming scenario (Eliason & Anttila, 2017). These include improved blood-oxygen delivery (Castro et al., 2011; Farrell et al., 1990; Gallaugher et al., 2001; Papadopoulou et al., 2022) and efficiency in ATP production and utilization (Farrell et al., 1991; Pettinau, Seppänen, et al., 2022), which may contribute to enhance oxygenation capacity, thus making aerobic exercise training a strong candidate for improving thermal tolerance. Recent findings showed that optimal exercise training can improve thermal performance for maximal heart rate ($f\mathrm{H}_{\mathrm{MAX}}$) and delay the onset of temperature-induced cardiac arrhythmias in rainbow trout by enhancing aerobic ATP production in the ventricle(Pettinau, Seppänen, et al., 2022). Despite these results, it is unclear if exercise training improves organismal thermal tolerance, measured as critical thermal maximum ($\mathrm{CT}_{\mathrm{MAX}}$) (Gomez Isaza & Rodgers, 2022). Furthermore, investigating the effect of exercise training in adult fish is highly relevant for conservation since adults might be more sensitive to thermal stress in their reproductive state (Dahlke et al., 2020), although this has rarely been tested empirically, possibly negatively affecting their fitness and reproductive success.

The environmental conditions experienced by reproductive adults can also have cascading effects on offspring through transgenerational plasticity (TGP) (Donelson et al., 2018; Jonsson & Jonsson, 2016; Salinas & Munch, 2012; Shama et al., 2014). Here, we consider the TGP in a broad sense, including intergenerational and transgenerational plasticity. Depending on the intensity of the stressor experienced by the parents, TGP can be adaptive, helping the population to cope with environmental stressors across generations (Donelson et al., 2018). Doing so, TGP can precondition the offspring to the new environment and gain time for genetic evolution to occur (Fox et al., 2019). Harnessing of TGP could be used as a conservation tool for endangered species to enhance the tolerance of offspring to climate change (Chakravarti et al., 2016). In fish, TGP has been shown to reshape the offspring phenotype, such as growth and metabolic rate to better cope with the novel environment, especially higher temperature or hypoxia (Ho & Burggren, 2012; Shama et al., 2014). However, so far only three studies investigated the role of TGP to high temperature on offspring thermal tolerance showing that the effects are highly variable, depending on the species and the traits measure (Butzge et al., 2021; Penney et al., 2021, 2022). Like temperature, waterflow is a key factor in aquatic environment that is strongly related to fitness of fish, deeply affecting their morphology and physiology (Domenici & Kapoor, 2010). Previous evidence suggested that a variable water flow might induce TGP in the survival or growth of offspring when exposing fish to natural river environments (Evans et al., 2014) or different water flow (Cortese et al., 2022). It is therefore possible that maternal exercise, induced by increasing water flow in the tank, might affect the phenotypes of offspring (Gienapp et al., 2008). However, the TGP effect of maternal exercise has not been thoroughly studied in fish.

In addition to TGP, genetic inheritance is another mechanism that can regulate the expression of a phenotype in the next generation. Plasticity and TGP are considered relatively short-term adjustments to help populations to cope with climate change, while genetic inheritance of advantageous traits (such as thermal tolerance) can lead to a long-term adaptation that can ensure population survival in a new environment (Gienapp et al., 2008). For phenotypic adaptation through genetic inheritance to occur, traits must display variation, possess heritability, and be under selection (Falconer & Mackay, 1996). The magnitude of heritability can thus affect the rate and trajectory of the evolution of a population, e.g. lower heritability will reduce the rate of evolution. Previous studies have shown that thermal tolerance traits, such as cardiac thermal performance and CT_{MAX}, are heritable (Morgan et al., 2020; Muñoz et al., 2014) and global warming could lead to a natural selective pressure toward higher thermal tolerance phenotypes (Gilbert & Miles, 2017). Similarly, a recent study demonstrated that reduced water flow in a spawning river induced the selection and evolution towards smaller body size in a population of Atlantic salmon (Merilä, 2022). Environmental conditions are key factors that shape genetic inheritance, not only by imposing selective pressure, but also affecting the magnitude of a trait heritability via Genotype-by-Environment interactions (GxE). Indeed, GxE can change the underlying set of genes expressed for a phenotypic trait, thereby potentially modifying its heritability (Charmantier

& Garant, 2005). Changes in heritability via GxE have been observed in fish through acclimation to high temperature (Debes et al., 2021; Shama et al., 2014). However, most studies on heritability have only investigated the influence of the environmental condition within the same generation (F0 exposure - F0 heritability) but it could be possible that TGP, via Genotype-by-Parental Environment interaction (GxPE), could also affect heritability in a similar way. Since unfavourable environment may decrease heritability (Charmantier & Garant, 2005), it is important to consider how the effects of GxE or GxPE on captive animals could affect their released and potential adaptation to the natural environment.

Harnessing TGP and GxPE may improve the resilience of salmonid populations that are bred for restocking programs. This approach could help create stress-resilient populations that are better equipped to withstand global warming (Chakravarti et al., 2016; Van Oppen et al., 2015). To assess whether exercise training program can be used to improve the resilience of hatchery-reared salmonid to global warming (within and across generations), we performed an intergenerational experiment (Fig. 1) using brown trout (*Salmo trutta*). We evaluated the impact of exercise training at three levels of response, i.e., within-generation phenotypic plasticity, TGP and GxPE. We investigated if an exercise training program could improve cardiac thermal performance ($f\mathrm{H}_{\mathrm{MAX}}$) and reproductive success in hatchery-reared adult female brown trout. Then, we evaluated effect of maternal exercise training on thermal tolerance ($\mathrm{CT}_{\mathrm{MAX}}$ and $f\mathrm{H}_{\mathrm{MAX}}$), survival, and body mass and length in the offspring via TGP. Finally, we tested if the maternal exercise training affected the heritability of $\mathrm{CT}_{\mathrm{MAX}}$, body mass and length to assess whether exercise training could affect the evolutionary potential of these traits.

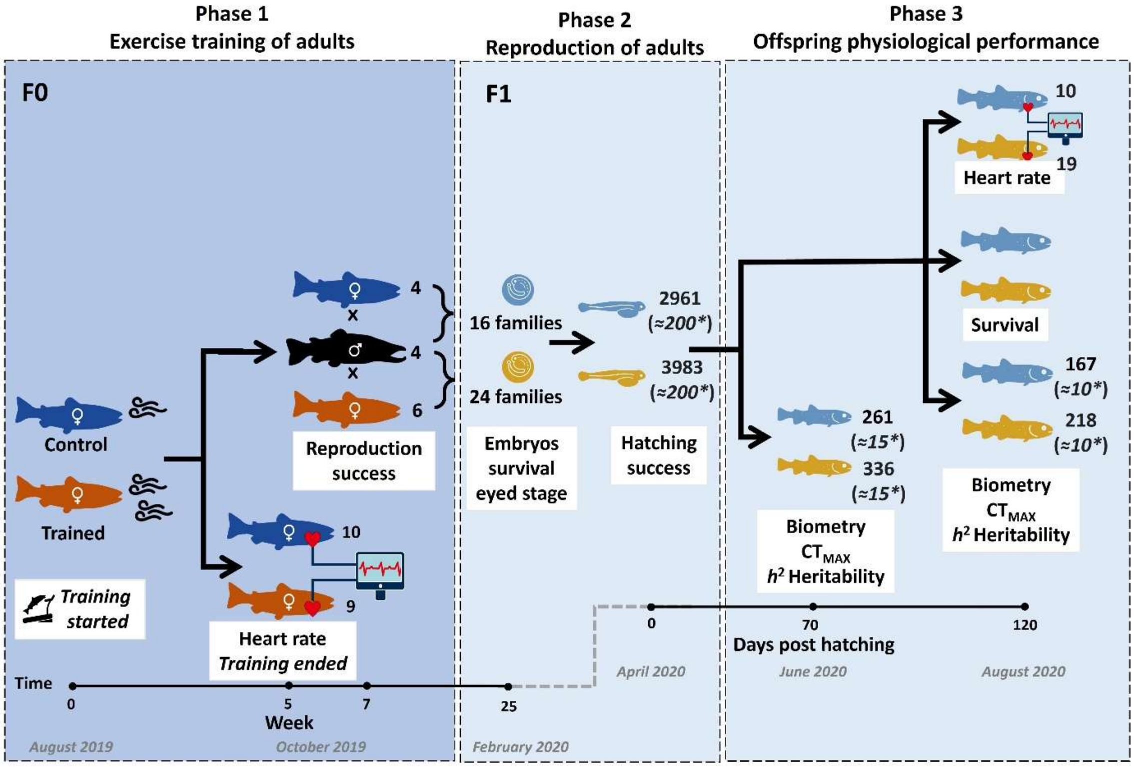

Figure 1: Experimental design: The experiment (12 months) was divided into three phases to evaluate the effects of maternal exercise training (F0: dark blue = control; dark orange = training, F1: light blue = control mother; light orange = trained mother). **Phase 1**: Training broodstock and evaluating the training effect on the cardiac thermal performance. **Phase 2**: Evaluating the effect of training on the reproductive success of the adults. **Phase 3**: Evaluating the effect of maternal exercise training on the survival, CT_{MAX} and cardiac thermal performance (only at 120 days-post-hatching, dph) of the offspring, and the heritability of body mass, fork length, and CT_{MAX}. The sample sizes of each group are indicated for each measurement, italic numbers between brackets represent the sample size for each family. Statistical units are: Phase 1 = mother; Phase 2 = family; Phase 3 = offspring individuals.

MATERIAL AND METHODS

Exercise training in adults

The training was conducted at the facility of the XXX (*omitted name for double blind peer-review*) with adult brown trout (*Salmo trutta*) (N=58; body mass=2.73 kg; length=57.33 cm) between August – October to mimic the timing of natural spawning migration of brown trout. These fish were offspring of wild brown trout from river Mustajoki, the first generation that was reared in captivity. The fish (n= 29 per treatment) were equally distributed into two tanks (12 m diameter) differing from each other with water flow velocity: 1) untrained control with normal water flow velocity used in the facility ($0.12m*s^{-1}$; 0.2 body length*seconds^{-1} [bl*s^{-1}]) and 2) training group. The fish in the training group were swimming at 0.41 m*s^{-1} (0.7 bl*s^{-1}) water flow velocity for 6h per day, between 8am and 2pm, five days per week, and experienced the same water velocity as the control fish for the rest of the time. The fish in both groups were restricted to a specific area of the tanks (length 5.8 m, width 0.9 m x 1.5 m) to make sure that they were always exposed to the desired water flow velocity (Fig. S1). Water velocities were measured three times per week from four different points of the area (Fig. S1 & Table S1). The training program continued for five weeks. The temperature and oxygen level followed the seasonal natural fluctuations of the incoming lake water (Table S1).

Measurement of $f\mathrm{H}_{\mathrm{MAX}}$ during acute warming in adults

After five weeks of training (early October), the temperature-specific, pharmacologically induced maximum heart rate ($f\mathrm{H}_{\mathrm{MAX}}$) was measured according to Casselman method (Casselman et al., 2012; Gilbert et al., 2024) (details in the supplementary material, SM). The capacity to increase of maximal heart rate as temperature rises represents the upper limit of the cardiac pacemaker to support oxygen delivery as metabolic demand rises.(Eliason & Anttila, 2017; Muñoz et al., 2014). This makes thermal performance curve (TPC) of $f\mathrm{H}_{\mathrm{MAX}}$ an ideal proxy to measure thermal tolerance. The capacity to reach a higher $f\mathrm{H}_{\mathrm{MAX}}$ indicates enhanced cardiac thermal tolerance and a greater ability to maintain oxygen transport during challenging situations. The strengths and the ecological relevance of this methodology have been detailed in previous studies (Anttila et al., 2013; Eliason et al., 2013; Gilbert et al., 2024; Pettinau, Lancien, et al., 2022). Briefly, we were especially interested in the $f\mathrm{H}_{\mathrm{MAX}}$ since its plateauing and decline during acute warming have been linked to the reduction in aerobic scope when fish are exposed to high temperatures in salmonids and could, thus, be related to their thermal tolerance (Eliason et al., 2011). The $f\mathrm{H}_{\mathrm{MAX}}$ experimental setup consisted of four chambers connected in parallel to a sump tank with aerated and temperature-controlled water (see SM).

The $f\mathrm{H}_{\mathrm{MAX}}$ was recorded from each individual during an acute warming challenge with a stepwise warming rate of 1°C 15 min^{-1}. This warming rate was selected to equilibrate core body temperature with water temperature according to Gilbert et al. (2022) (Arctic char = mass 3245±726 g, mean±sd).

Each $f\mathrm{H}_{\mathrm{MAX}}$ measurement started at 10°C, corresponding to tank water temperature, and continued until cardiac arrhythmias were detected and the temperature associated to the arrhythmia was recorded ($\mathrm{T}_{\mathrm{ARR}}$). The cardiac arrhythmia was defined as abrupt skipping of ventricular beat (absence of QRS complex in the ECG signal; see Fig. S2), after which the fish was considered to have reached the experimental endpoint. Since the vagal regulation of the heart rate was blocked by atropine and the maximal heart rate was induced by isoproterenol, the onset of sudden missing of QRS complex was due to intrinsic impairments of the heart contraction or action potential conduction. Therefore, the $\mathrm{T}_{\mathrm{ARR}}$ was considered the cardiac thermal limit, above which the heart cannot efficiently pump blood to the gills.

Immediately upon the heart rate becoming arrhythmic, fish were removed from the $f\mathrm{H}_{\mathrm{MAX}}$ setup. The adult fish were tagged with passive integrated transponder (PIT) tags and returned to the original tank to recover, without exercise. Based on the ECG traces, $f\mathrm{H}_{\mathrm{MAX}}$ was calculated by measuring the duration for 15 continuous R-R peaks at the end of each temperature exposure. We also calculated several transition temperatures for the cardiac TPC: the Arrhenius breakpoint temperature for $f\mathrm{H}_{\mathrm{MAX}}$ ($\mathrm{T}_{\mathrm{ABP}}$), considered a proxy for the thermal optimum for aerobic scope of an organism (Anttila et al., 2013; Casselman et al., 2012) and the temperature where the highest $f\mathrm{H}_{\mathrm{MAX}}$ was achieved ($\mathrm{T}_{\mathrm{peak}}$).

Breeding design and adult sampling

The adult fish were allowed to recover for two weeks after the training and $f\mathrm{H}_{\mathrm{MAX}}$ measurements, simulating the condition when they reach natural spawning grounds and therefore the end of the migration (Fig 1). This recovery also allowed the fish to reach the correct maturity for spawning. For the artificial reproduction, gametes (30mL of eggs) were collected from six randomly picked trained females and four untrained females and crossed with four trained males using 20µL of milt under a full factorial design, obtaining 40 families. This design produced four families per mother, providing quadruplicates of the maternal exercise effect in the offspring. After fertilization, unfertilized eggs were removed, and fertilized eggs were incubated in duplicate racks, each family in its own slot. After collection of gametes, the fish were euthanised and several morphological parameters were collected: body mass, fork length, condition factor, ventricle mass, relative ventricle mass (RVM), the dry mass of the spongy and the compact myocardium, spongy/compact myocardium ratio, liver mass, hepatosomatic index (HSI), clutch mass (defined as the total mass of ovulated eggs (in g) that was possible to strip out manually), relative fecundity and egg diameter (see formulas in SM).

Offspring rearing

From fertilisation up to 120 days-post-hatching (dph), eggs and offspring from trained and untrained mothers were reared under the same conditions. Each family was maintained in a separate tank supplied from the same water system and managed identically, so that maternal exercise was the only experimental treatment.

The fertilised eggs were incubated in duplicate racks with each family in its own slot. The water flow in the incubator tanks was 2L per minute. The water temperature throughout the incubation period was 1.9 ± 0.2°C. The eggs were checked each day and the dead eggs were counted and removed until the eggs had reached the eyed stage. Next, after survival to the eyed stage was assessed, 200 eggs were randomly selected from each family, and each family was placed into a circular tank (diameter 80 cm) in floating baskets. After hatching, the alevins were released from the baskets to swim freely in the rearing tanks. Upon entry into the exogenous feeding stage (210 days post-fertilization), the fish were fed daily to satiation using organic fish pellets (0.5 mm), the size of pellets varying according to the growth of the fish. The temperature and oxygen level followed seasonal natural fluctuations.

Offspring survival, body mass and length

The survival to eyed stage, hatching success and survival at 120 dph were calculated for each family (see formulas in SM). The body mass and fork length of the offspring from trained and untrained mothers were measured at two different time points: 70 dph (n control=261; n trained=336), 120 dph (n control=167; n trained=218).

Offspring thermal tolerance

The CT_{MAX} of a subsample of fish was measured at two time points from each family, in June (70 dph) and in August (120 dph) (Fig 1). Details about the CT_{MAX} setup are shown in the SM. These two time points allowed us to evaluate if the tolerance changed during development and if the real heatwave that occurred during July influenced the thermal tolerance of the fish with trained and untrained maternal background differently.

Fish were acclimated to the measuring boxes at 16±1°C for 1h. Then the temperature was increased for the initial 30 min at the rate of 0.3°C min^{-1}. Thereafter, the heating rate was decreased to 0.1°C min^{-1} for a more precise evaluation of the loss of equilibrium (LOE), corresponding to the thermal limit (CT_{MAX}). The LOE was defined as the temperature where the fish displayed a loss of equilibrium and inability to locomotor activity for over 3s [46]. When the LOE was observed, the fish was removed from the experimental tank and placed in an individual recovery container. Then, the fish were euthanized with an overdose of MS-222 (200ppm), and their body mass (0.1 g accuracy) and fork length (in mm) were measured. To test 25 fish (around 15 fish per family in June and around 10 fish per family in August) from all the 40 families, the CT_{MAX} measurements were carried over one week. A total of 982 fish were tested over the two time points (70 dph: control n=261, trained n=336; 120 dph: control n=218, trained n=167, see Table S4 for CT_{MAX} among families).

Measurement of fH_{MAX} during acute warming of the offspring

At the age of 120 dph, the thermal performance for fH_{MAX} and cardiac transition temperatures (T_{ABP}, T_{PEAK} and T_{ARR}) of the offspring were measured from a random subsample of the trained (n=24) and untrained groups (n=13). The fH_{MAX} measurements followed similar protocols as in adults, with some different optimizations due to the differences in body mass between adults and offspring. The offspring were subjected to a stepwise acute warming rate of 0.16°C min^{-1}, and each fish was laying on two silver electrodes placed in contact with the skin close to the heart, ensured in position by the prone position of the fish. The fH_{MAX} of the offspring was induced with an intraperitoneal injection of atropine (dose 2.5 µg/g, Alfa Aesar) and followed by an intraperitoneal injection of isoproterenol (dose 8 ng/g, Sigma-Aldrich) as in the adult protocol. The measurements started at 13°C for the offspring and lasted until the onset of the cardiac arrhythmias. Once the arrhythmias were observed, the juvenile fish were removed from the system and euthanised by cranial concussion.

STATISTICAL ANALYSES

The values provided in the study are presented as mean with standard error of the mean (s.e.m.). We chose $p < 0.05$ to indicate statistical significance. Variables were log-transformed if needed for the normal distribution of residuals. Using an Akaike Information Criteria (AIC) backward step-wise model selection, we compared different models including several random factors and the best model according on the lowest AIC was selected (see SM for model selection). Statistical analyses were performed using R v. 4.1.2 (R Core Team, 2022) and SPSS ver. 27 (IBM Corp., Version 27.0. Armonk, NY, USA) (see Table S2:summary of the statistical analyses).

Adults

The effect of exercise training on fH_{MAX} was tested using a repeated measure linear mixed model (rLMM) in SPSS with training group and temperature (10-24°C) as main factors with their interaction. The final model included day of the measurement and chamber of the setup as random factors (see SM for AIC model selection). Bonferroni post hoc tests were used to the test the differences between the groups within each temperature. One fish had to be excluded from the analysis of fH_{MAX} and cardiac variables due to issues in the measurements (final N number for control group n=10 and for trained group n=9). The effect of exercise training on the cardiac transition temperatures for cardiac performance (T_{ARR}, $T_{ABP,}$ T_{peak}) were tested using a linear model (see SM for AIC model selection). Five fish were excluded from the T_{ABP} analysis since it was not possible to fit two linear regressions lines to their data to calculate the transition temperature of the heart rate (T_{ABP}) (final N number for control group n=8 and for trained group n=7).

The effect of exercise training on the morphological variables (body mass, fork length, condition factor, ventricle mass, RVM, compact myocardium dry mass, spongy myocardium dry mass, spongy/compact myocardium ratio, compact myocardium percentage, liver mass, and HSI) and reproductive variables (clutch mass, relative fecundity, and eggs diameter) were tested using independent-samples T-test with training group as factor. If the parametric model assumptions were not respected, non-parametric independent-samples Mann-Whitney U-tests were used.

Offspring

To evaluate the effect of maternal exercise training on the survival to eyed stage, hatching success and parr stage survival (120 dph), Mann–Whitney U tests were used.

The effect of maternal exercise training on fH_{MAX} was tested using rLMM in SPSS with training background and temperature (10-23°C) as main factors with their interaction. The final model included day of the measurement, chamber of the setup, and mother ID as random factors (see SM for AIC model selection). Bonferroni post hoc tests were used to the test the differences between the groups. The effect of maternal exercise training on $T_{ABP,}$ T_{PEAK} and T_{ARR} was tested using linear models with maternal training type as factor (see SM for AIC model selection). Nine fish had to be excluded from the fH_{MAX} analysis and cardiac variables due to the noisy ECG signal impeding any clear determination of the R-R peaks (final N number for control group n=9 and for trained group n=19).

A linear mixed model (LMM) (*lme4* package (Bates et al., 2015)) was used to test the effect of maternal exercise training on the CT_{MAX}, body mass, body length and condition factor of the offspring (SM - model selection). The maternal training group and time point (70 dph and 120 dph) of the measurement were used as fixed factors, while box ID, day, and mother and father IDs, and family number as random factors.

Heritability analyses

The factorial breeding design permitted us to quantify the narrow-sense heritability (h^2) using an animal model with the ASReml software (Version 4.2; www.vsni.co.uk; (Gilmour et al., 2015)). Univariate analyses were used to decompose the total phenotypic variance (VP) of the CT_{MAX}, body mass and length for the trained and untrained sub-population into additive genetic (VA) and residual (VR) variances. The model was: $y = \mu + C + A + e$, where y is the phenotypic observation, μ is the overall mean, C is the random effect of the common environment (family effect, including parental effects and tank effects), A is the individual random additive genetic effect (linked to the pedigree structure) and e is the residual effect. Narrow-sense heritability (h^2) for each trait was then estimated as the ratio of the additive genetic variance (VA) to the total phenotypic variance (VP): $h^2 = VA/VP$. The significance of the additive genetic variances within offspring from control mothers and from trained mothers was tested by comparing the full model to a model where the additive genetic variance was removed (i.e., no random additive genetic effects included in the model), using a likelihood ratio test. A likelihood ratio test was used to compare the differences between the genetic variance estimates for the same traits between the control and the trained group. The differences were evaluated by comparing the full model with a constrained model (i.e., in which the additive variance estimates were set to be equal).

RESULTS

Cardiac thermal performance in adults

Training increased fH_{MAX} at all the temperature steps (main effect training: df=1; F=26.5; $p<0.001$) compared to the control untrained fish (Fig. 2 & Fig. S3). As expected, the fH_{MAX} of both groups increased significantly during the acute warming (main effect temperature: df=14; F=55.3; $p<0.001$). No significant effect of interaction was detected (df=14; F=2.1; p=0.14). Furthermore, exercise training improved the upper thermal tolerance for cardiac function (T_{ARR}) by 3.5°C (df=1; F=5.9; p=0.026). Trained fish achieved peak fH_{MAX} at 3.9°C higher than the control fish (df=1; F=9.9; p=0.005). Additionally, trained fish displayed a significantly higher the T_{ABP}, the estimate for thermal optimum, compared to the control fish (df=1; F=6.4; p=0.026).

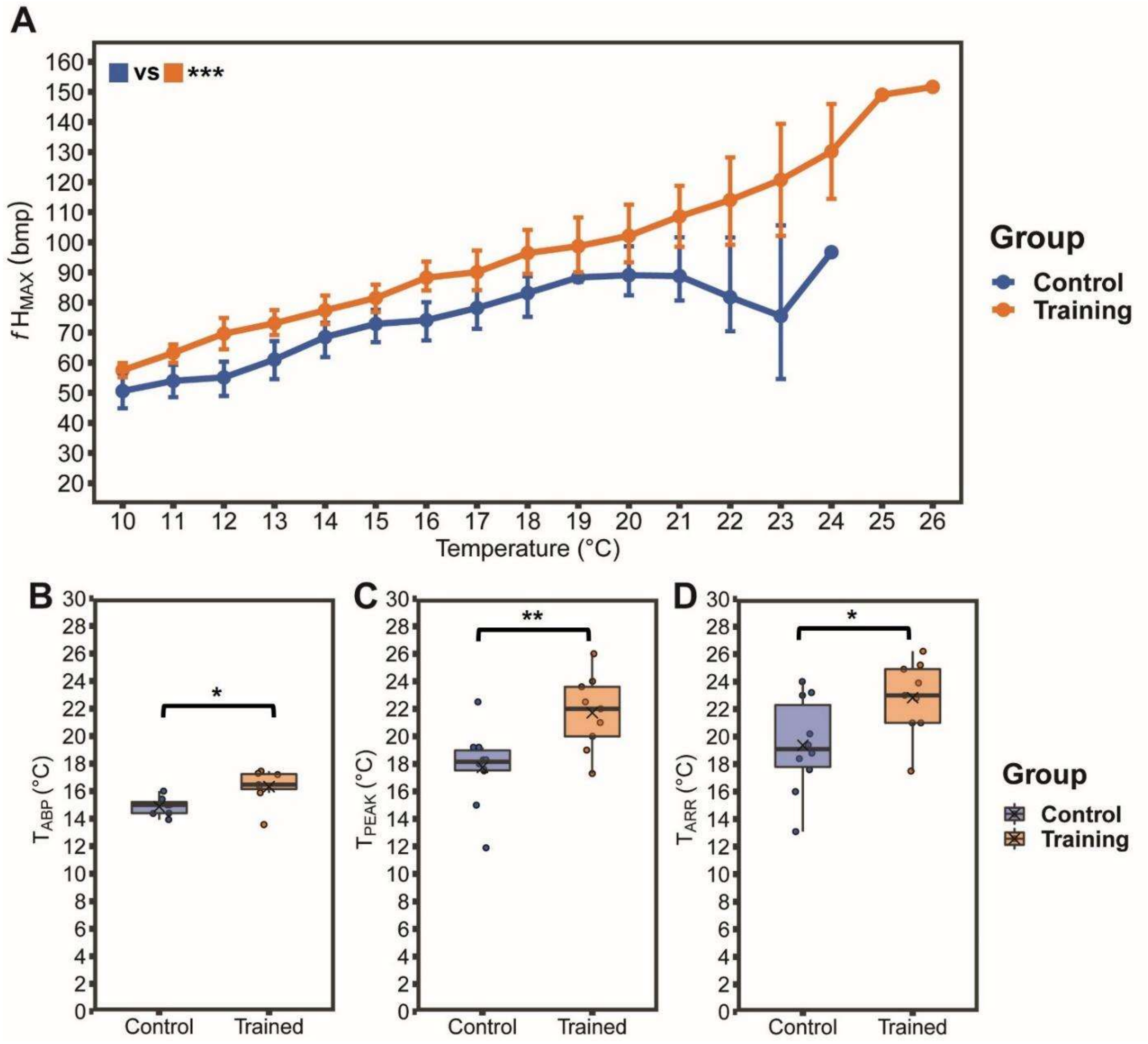

Figure 2: Effect of training program on the cardiac thermal performance of adult female brown trout. A) The average of maximal heart rate (fH_{MAX}) ± s.e.m. during acute warming of the trained (orange, n=9) and control mothers (blue; n=10). B) The Arrhenius break point temperature (T_{ABP}) for the fH_{MAX}. C) The temperature where the fish achieved the peak of the fH_{MAX} (T_{PEAK}). D) The temperature where cardiac arrhythmias (missing QRS complex from the ECG signal) were observed (T_{ARR}). Significant difference between the training groups is represent as **p≤0.01, *p≤0.05.

Adult morphology and reproductive success

Exercise training decreased liver mass (t=6.1; p<0.001) and hepatosomatic index of adults (t=6.8; p<0.001) (Table S3). Moreover, exercise trained mothers had higher matured clutch mass at stripping (t=2.4; p=0.028; Mean ± sem control group=314.17 ±44.80 vs Mean ± sem trained group=418.64±20.85 and marginally higher relative fecundity (t=2.0; p=0.082) (Table S3). There were no significant differences between trained and control groups in body mass, length, condition factor, ventricle mass, RVM, spongy and compact myocardium dry mass, and egg diameter (Table S3).

Offspring survival

The families obtained from exercise trained mothers had a 61% survival to eyed egg stage, significantly higher as compared to the survival to eyed stage from control group, which was 45% (U=266; p<0.013) (Table S4). Furthermore, after removing the outlier mother (survival to eyed stage from the outlier mother: family 17=4%, family 18=1.5%, family 19=2%, family 20=1.3%), families from exercise trained mothers had a 73% survival to eyed egg stage, significantly higher compared to the 45% obtained from control group (U=266; p<0.001). After randomly selecting 200 eggs from each family to follow up studies, there were no differences in hatching success (U=161; p=0.94) and survival rate until 120 dph (U=151; p=0.77) between the offspring of trained and control mothers (Table S4).

Cardiac thermal performance of offspring

We did not find any differences in the cardiac thermal performance of the offspring depending on the training experience of their mothers (Fig S4). The fH_{MAX} was not significantly different between the offspring of trained and control mothers (main effect training: df=1; F=0.007; p=0.94; main effect temperature: df=10; F=137.1, p<0.001; interaction training*temperature: df=10; F = 0.55; p=0.84) (Fig. S4). Maternal training did not affect the transition temperatures for cardiac function in the offspring, i.e. T_{ABP} (df=1; F=0.23; p=0.64), T_{PEAK} (df=1; F=1.2; p=0.28) and T_{ARR} (df=1; F=0.19; p=0.67) (Fig S4). Likewise, ventricle mass was similar between the two groups (t=1.2; p=0.24) as well as the RVM (t=1.4; p = 0.19).

Offspring CT_{MAX} and morphology

There were no differences in CT_{MAX} between the offspring of trained and untrained mothers (df=1; F=2.5; p=0.19). There was a significant difference between months (June=70 dph vs. August=120 dph) (df=1; F=8.1; p=0.013) (Fig. S4 & Table S5). The interaction between maternal exercise background and dph was not significantly different (df=1; F=0.28; p=0.59).

Body mass (df=1; F=1.98; p=0.21), length (df=1; F=2.61; p=0.15) and condition factor (df=1; F=0.03; p=0.87) did not differ between the fish with trained and untrained family background. The month of the sampling had an expected significant effect on morphological variables (body mass: df=1; F=176.28; p<0.001; body length: df=1; F=182.33; p<0.001; Condition factor: df=1; F=79.39; p<0.001) (Table S6).

Heritability

In the offspring from control mothers, CT_{MAX} (X^2=0.53, p=0.47), body mass (X^2=0.46, p=0.50), length (X^2=0.21, p=0.65) and condition factor (X^2=2.07, p=0.15) did not show significant genetic variance and heritability (Table 1). On the other hand, in the offspring from exercise trained mother, CT_{MAX} (X^2=4.27, p=0.038) and length (X^2=6.7, p=0.009) displayed significant genetic variance and heritability, but body mass and condition factor did not (X^2=1.19, p=0.28; X^2=1.95, p=0.16, respectively) (Table 1). The genetic variance and the heritability of CT_{MAX} and body length were higher in the offspring from trained mothers (CT_{MAX} h^2=0.18; length h^2=0.29) compared to the offspring from control mothers (CT_{MAX} h^2=0.06; length h^2=0.07) (Table 1), although the difference was only marginally (CT_{MAX} p=0.07) or not (length p=0.42) significant. The residual variance was not different for all variables (Table 1).

Table 1: Heritability of the different traits within maternal exercise training program. Estimates of heritability [h^2 (SE)], genetic variance [VA (SE)], and family effect [VF (SE)] in the offspring from untrained and trained mothers. The p values for offspring from untrained and trained mothers were obtained by likelihood ratio tests between a full model and a constrained model where the additive variance was removed. Bold text and * represent significant heritability values (**p≤0.01, *p≤0.05).

	Untrained					Trained				
	h^2	VA	VF	residuals	p	h^2	VA	VF	residuals	p
Mass (g)	0.1 (0.16)	0.015 (0.025)	0.019 (0.012)	0.119 (0.015)	0.499	0.06 (0.07)	0.008 (0.010)	0.005 (0.004)	0.132 (0.010)	0.275
Length (cm)	0.07 (0.16)	0.015 (0.037)	0.032 (0.018)	0.156 (0.021)	0.472	**0.29**** (0.17)	0.058 (0.039)	0.005 (0.005)	0.137 (0.022)	**0.009**
CT_{MAX} (°C)	0.06 (0.09)	0.005 (0.008)	0.004 (0.004)	0.085 (0.007)	0.648	**0.18*** (0.13)	0.022 (0.017)	0.007 (0.004)	0.089 (0.010)	**0.038**

DISCUSSION

This study provides evidence that aerobic exercise training not only reshapes the phenotype of the trained mothers but can improve the phenotype of their offspring via transgenerational plasticity (TGP). Five weeks of aerobic exercise training enhanced the thermal performance curve (TPC) of the fH_{MAX} of adult fish and increased all the transition temperatures that define the cardiac thermal performance (T_{ABP}, T_{PEAK} and T_{ARR}). Importantly maternal exercise training improved life-history traits of the offspring by enhancing the survival of the embryos to the eyed stage by 16%. By training the brood-stock we can obtain more offspring, having a potentially relevant impact on restocking for conservational purposes. Interestingly, the exercise training experienced by the mothers seemed to increase the heritability (h^2) of CT_{MAX} and body length in offspring, by especially increasing the genetic variance of CT_{MAX}. To our knowledge, this is the first study to show that the experience of the parental generation not only influences the offspring survival but also affects the heritability of traits. These findings may have important implications in the context of evolutionary and conservation biology, since CT_{MAX} and body length of trained fish can potentially evolve faster when under natural or artificial selection compared to hatchery-reared fish farmed in normal conditions.

Aerobic exercise training improves cardiac thermal performance in adults

These results demonstrate that aerobic exercise training can, when used as environmental enrichment, improve the cardiac thermal performance curve and cardiac transition temperatures (T_{ABP}, T_{PEAK} and T_{ARR}) in adult fish. These findings are consistent with Pettinau et al. (2022), showing that exercise training in rainbow trout improved cardiac transition temperatures. Especially, increasing the cardiac thermal tolerance (T_{ARR}) by 3.5 °C of spawning adults means that we are potentially able to increase the thermal tolerance and robustness of the brood-stock (Gilbert et al., 2020). Migration and spawning are critical periods, especially now as global warming extends the duration of summers and increases the magnitude and frequency of heatwaves, which might occur during migration or even spawning (Eliason et al., 2011). Recently, a significant amount of en-route migration mortality has been reported for salmonids during warm summer and/or fall (Hinch et al., 2021). Our results indicate that exercise training can shift the thermal performance curve to higher temperatures, possibly mitigating the negative effect of high temperatures during the spawning period.

Exercise training improved reproductive success and embryo survival

In the current study trained mothers not only produced more eggs but also their eggs had a higher survival from fertilisation to the eyed stage, independently of the mother's body size. Therefore, trained families resulted in a significantly higher number of live embryos. Due to logistical reasons, we randomly sub-sampled the eggs that reached the eyed stage, retaining approximately 200 embryos per family for later assessments. In the later stages, we found no significant difference in hatching success or survival rate of the offspring between the groups. If we had the possibility to keep all the eyed eggs, we would expect a significantly higher number of live offspring from trained mothers, with clear implications for restocking programs. These results are consistent with those in mature female sockeye salmon (*Oncorhynchus nerka*)(Patterson et al., 2004), where exercise increased the ratio of sexually mature females, egg deposition rate, and egg survival to eyed stage. In our study, the enhanced survival to eyed stage might be related to egg nutritional quality rather than nutritional quantity, since egg diameter did not differ between the training groups. However, the mechanisms underpinning the higher offspring survival after maternal exercise are not entirely clear.

Maternal exercise training increased thermal performance in mothers without affecting thermal performance of offspring

Although there was direct evidence of phenotypic improvement of the cardiac thermal performance (fH_{MAX}, T_{ABP}, T_{PEAK}, T_{ARR}) of adults in response to exercise training, this did not translate to TGP-mediated improvements in either the cardiac thermal performance, CT_{MAX}, or body size of the offspring. The lack of TGP in thermal tolerance suggests that TGP induced by maternal exercise may not necessarily translate into higher resilience to global warming in future generations. To the best of our knowledge, no previous studies have investigated the TGP effect of maternal exercise on thermal tolerance. Few studies have investigated TGP in CT_{MAX} in fish, e.g. in *Danio rerio* following parental hypoxia acclimation (Ho & Burggren, 2012) and in *Salvelinus namaycush* following parental warm acclimation (Penney et al., 2021, 2022). However, none of these studies found significant transgenerational effects on CT_{MAX}. Rainbow trout showed significant TGP following paternal warm acclimation, using offspring survival at high temperature (28 °C), as a measure of thermal tolerance (Butzge et al., 2021). Although acclimation to hypoxia or high temperature are different acclimation stimuli than acclimation to exercise training, they might share an analogous limiting effect on aerobic scope and cardiac performance (Gamperl & Farrell, 2004). Consistently, our results showed limited TGP for CT_{MAX}. Therefore, TGP induced by this maternal exercise programme may not provide an advantage for conservation purposes to produce offspring with higher thermal tolerance and to help the restocked population to cope with global warming. Nevertheless, it must be noted that while maternal exercise training did not alter thermal tolerance, the overall outcome of maternal exercise training was beneficial, since the offspring of the trained mother exhibited greater survival, and never impaired performance. Although not the primary focus of this study, it is notable that the cardiac thermal tolerance of offspring was very similar to that of trained mothers [T_{ARR} (mean±s.e.m.): control mother = 19.4±1 °C; offspring of control mother =23.2± 0.2 °C; trained mother = 22.8±0.9°C°C; offspring of trained mother = 22.9±0.4 °C]. Although the T_{ARR} of control mothers was lower, the flexibility in performance observed in the mothers is not indicating a general decline in thermal performance of reproductive stages, as hypothesized by Dahlke et al(Dahlke et al., 2022, 2020) based on variable and sometimes inconsistent metrics (Pottier et al., 2022).

Higher potential for evolution in offspring from trained mothers

Interestingly, maternal exercise training increased the heritability of CT_{MAX} and fork length, but not of body mass. To our knowledge, this study is the first study to show that maternal experience can affect the heritability of offspring CT_{MAX} and body length. These results suggest that parental environment can interact with heritable variation across generations as shown in previous studies within generation (Debes et al., 2021) . To our knowledge, only Shama et al. (2014) have showed that the effect of parental thermal acclimation can affect the heritability of growth rate through Genotype-by-Parental-Environment interaction (GxPE). Training, through GxPE, has the potential to increase h^{2} and genetic variance, which could accelerate the rate of evolution of CT_{MAX} in a trained population. This could have important implications for conservation as we could proactively accelerate the evolutionary response of a restocked population, especially if CT_{MAX} is under selection in a new, warmer environment. This concern is increasingly relevant due to documented cases of heat stress in wild populations of salmonids (Eliason et al., 2013; Mottola et al., 2020; O'Sullivan et al., 2023; Sullivan et al., 2025). These results highlight the necessity of exercise in active animals kept in captivity, such as salmonid species, since smaller h^2 values are often reported in unfavourable and stressful environments (Charmantier & Garant, 2005). The evolutionary implication of water flow in salmonids has been recently confirmed by a field-based study on Atlantic salmon population that evolved to smaller body size in response to reduced water flow (Jensen et al., 2022). As the difference in length h^2 in our study was not linked to a difference in genetic variance, the rate of evolution of this trait might not accelerate as fast. Overall Here, maternal exercise training may therefore still favour the evolution of higher CT_{MAX} and length if these traits are under positive selection. Despite the promising results, the mechanisms underlying these changes remain to be elucidated. A recent study suggests that changes in heritability may happen through epigenetic mechanisms (Venney et al., 2022), which would be a relevant direction for future studies.

Exploratory follow-up monitoring of morphology during grow-out

Similarly to thermal tolerance variables, maternal exercise did not have transgenerational effects on body mass and length at 70 and 120 dph. However, after 120 dph, we conducted an exploratory follow-up study to monitor morphology over one year, from 150 to 400 dph (see details in SM). Families were combined within each maternal training group and placed in two separate tanks (one tank per training group). During this period, the offspring of trained mothers had higher body mass (lm; df=1; F=11.9; $p<0.001$) and body length (lm; df=1; F=15.6; $p<0.001$) as compared to those of untrained mothers (supplementary Fig. S6 & Table S6). For example, at 239 dph (December), offspring from trained mothers were larger than those of control mothers: body mass 18.11 ± 1.16 vs 14.86 ± 0.92 g (+21.9%) and body length 115.23 ± 2.51 vs 107.50 ± 2.16 mm (+7.2%). Similarly, at 400 dph (May), mass and lengths of trained and control offspring were larger: body mass 24.09 ± 1.22 vs 21.57 ± 1.29 g (+11.7%) and body length 130.19 ± 2.00 vs 123.73 ± 2.18 mm (+5.2%). Nevertheless, for this exploratory study, we were not able to keep replicate tanks due to logistical reasons and therefore these results should be taken with caution. Further studies are needed to determine if maternal training could potentially improve the growth rate of the offspring and, therefore, be beneficial for restocking purposes.

Conclusion

In conclusion, this study demonstrated that aerobic exercise training represents a valuable environmental enrichment strategy to improve the physiological performance of hatchery-reared fish, having also cascading beneficial effects on the fitness of the next generation. We showed not only that exercise training can directly (within the same generation) improve cardiac thermal performance and reproductive success of adult brown trout but also that it can increase early survival via TGP. However, maternal exercise training did not directly improve the thermal tolerance of offspring. Nonetheless, this study demonstrated for the first time that the maternal environmental experience, i.e., increased aerobic exercise resulting from increased water flow, can also increase the heritability of key traits of offspring, such as CT_{MAX}, and therefore increase their evolutionary potential. Overall, this study strengthens the idea that TGP and GxPE can be harnessed to improve conservation and restoration programmes to produce offspring that are potentially more tolerant to global warming. Despite the promising results, several questions would need further investigation. For example, future studies should evaluate the benefit of exercise training in offspring after release into a natural environment.

Authors contribution:

1. **Pettinau Luca:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing
2. **Tytti-Maria Uurasmaa:** Investigation, Writing – review & editing
3. **Eila Seppänen :** Investigation, Project administration, Resources, Writing – review & editing.
4. **Asko Sikanen:** Investigation, Methodology,
5. **Miika Raitakivi:** Investigation
6. **Jenni M. Prokkola:** Investigation, Supervision, Writing – review & editing
7. **Amelie Crespel:** Data curation, Formal analysis, Investigation, Software, Supervision, Writing – review & editing
8. **Katja Anttila:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, *Methodology,* Project administration, Resources, Supervision, Writing – review & editing

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Ethics: All the experiments were conducted under animal care licence ESAVI/11880/2019.

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Supplementary material 1:

Material and methods:

Measurement of $f\mathrm{H}_{\mathrm{MAX}}$ of adult fish during acute warming

To measure maximal heart rate ($f\mathrm{H}_{\mathrm{MAX}}$) each fish was first anesthetised with 100ppm buffered MS-222, to safely measure the body mass and the length of the fish. Then, the fish was let to briefly recover from the high dose of MS-222 in untreated water. Thereafter, the fish were positioned supine in the heart rate measurement chamber. The fish were fully immersed in the chamber for the duration of the fHmax measurement. The water in the chamber contained a lower dose of buffered MS-222 (60ppm), which was essential to avoid excessive movements of the skeletal muscle that create electrical artefacts in the ECG signal. The dose was, however, so low that fish were spontaneously breathing during the measurements. Nevertheless, to ensure oxygenation, a customized mouthpiece was placed within the fish jaws to flush the gills with aerated water.

Two needle-electrodes were placed in the proximity of the heart along the sagittal plane. The analogic ECG signal was amplified by Grass P122 AC/ DC Strain Gage Amplifier (Grass Technologies, Warwick, USA), digitalized and sent to the computer through a BIOPAC Data Acquisition Unit (MP100, BioPac Inc, Goleta, CA, USA). The ECG was recorded using Acknowledge software ver. 3.8.1. (BioPac). After placing the electrodes, the anesthetised fish was let to rest for 30 min to stabilize the heart rate. Then, the maximal heart rate was induced by blocking the muscarinic receptors of the parasympathetic nervous system with an intravenous injection in the caudal vein of atropine (1.25 μg/g, Alfa Aesar by ThermoFisher Scientific, Karlsruhe, Germany) diluted to 5 μg/μL in Salmonid Saline solution (150mM NaCl; 310 mM KCl, 0.4 mM HEPES, 0.34 mM CaCl2, 0.1 mM MgCl2, 0.03 mM MgSO4, pH 7.85 at 10°C). After 15 minutes, a second intravenous injection was given in the caudal vein containing isoproterenol (dose 4 ng/g, Sigma-Aldrich Chemie GmbH, Munich, Germany) diluted at 16 ng/μL with Salmonid saline solution to induce the maximal stimulation of the β1-receptors of the sympathetic nervous system, lasting several hours.

Breeding design and Adult sampling

Based on these parameters, several indexes were calculated: condition factor as (body mass/ fork length)3*100), relative ventricle mass as (RVM) [(Ventricle mass/body mass) * 100], hepatosomatic index (HSI) as [(Liver mass/body mass) * 100]. Additional reproductive parameters were also measured: clutch mass (defined as the total mass of ovulated eggs (in g) that was possible to strip out manually), relative fecundity (calculated as [(clutch mass/ body mass) * 100]), and egg diameter. To measure egg diameter, an average of 13 eggs (min= 8 eggs, max= 24 eggs) per female were place into a petri dish with millimetre paper and a picture was taken for later image analysis with ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA). The ventricle was divided along the sagittal plane and fixed in 70% ethanol in order to determine the dry mass of the spongy and the compact myocardium and spongy/compact myocardium ratio, as well as the compact myocardium percentage ((dry mass compact myocardium/ dry mass of dry ventricle) * 100) as described by (Farrell et al., 2007).

[1]

Farrell AP, Simonot DL, Seymour RS, Clark TD. 2007 A novel technique for estimating the compact myocardium in fishes reveals surprising results for an athletic air-breathing fish, the Pacific tarpon. *J Fish Biol* 71, 389–398. (doi:10.1111/J.1095-8649.2007.01496.X)

Offspring rearing

After fertilization, the unfertilized eggs were carefully removed. The fertilized eggs were incubated in duplicate racks with each family in its own slot. The water flow in the incubator tanks was 2L per minute. The water temperature throughout incubation period was 1.9 ± 0.2°C. The eggs were checked each day and the dead eggs were counted and removed until the eggs had reached the eyed stage. Next, after survival to the eyed stage was assessed, 200 eggs were randomly selected from each family and each family was placed into a rearing tank (80 cm) in floating baskets. After hatching, the alevins were released from the baskets to swim freely in the rearing tanks. Upon entry into the exogenous feeding stage (210 days post-fertilization), the fish were fed daily to satiation using organic fish pellets (0.5 mm), the size of pellets varying according to the growth of the fish. The temperature and oxygen level followed seasonal natural fluctuations.

Survival and growth of offspring

The survival to eyed stage was calculated for each family as [(number of eyed eggs/total number of fertilized eggs) * 100]. The hatching success was calculated for each family as [(number of hatched eggs/number of eggs moved to rearing tanks) * 100]. To do so, the number dead eggs were counted from the fertilization until hatching. The survival until 120 dph was calculated for each family as: [(number of fish survived at 120 dph/ total number of hatched fish) * 100].

CT_{MAX} setup for offspring.

The CT_{MAX} setup consisted of a thermal-insulated plastic tank (78 x 56 x 43 cm, 60-liter) with 3 metal coils at the bottom of the tank. The coils were connected to a chiller-heater (Lauda RE 1050 GN, Lauda). To maintain a homogenous water conditions in the tank, the water was constantly aerated with air-pumps and mixed with small water-pumps carefully avoiding any direct current to the fish. To recognize the family of each fish during the measurement, each family was placed in a separate mesh box (11x6x9 cm for 1st CT_{MAX}; 18.5x11.5x10.5 cm for 2nd CT_{MAX} measurement). A total of eight boxes were placed hanging in a frame on the top of the tank so that the boxes were merged to top layer of the water. Each box contained five fish from the same family, therefore a total of 40 fish were tested during each measurement. We ensured the repeatability of the CT_{MAX} protocol by programming the automatic heating ramp in the chiller/heater and by recording the water temperature of each CT_{MAX} ramp with Fibox 3 Set (PreSens Precission Sensing GmbH, Regensburg, Germany).

Figures and tables

Figure S1: Top view of the tanks. Coloured area represents the cage that constrained the fish to swim at the desired speed. Number 1 & 2 represent the two points were the speed of water were measured at the surface and at the bottom. Letter A correspond to the water inflow. Letter B represent the central column with the outflow. B) representative picture of a fish tank.

Table S1: The average water speeds, temperatures and oxygen levels during the training session during the five weeks of the experiment in each group.

	Control	Training
	Mean ±sem	Mean ±sem
Front surface (m*s^{-1})	0.13 ±0.01	0.39 ±0.01
Front bottom (m*s^{-1})	0.11 ±0.01	0.38 ±0.01
Back surface (m*s^{-1})	0.12 ±0.01	0.39 ±0.01
Back bottom (m*s^{-1})	0.11 ±0.01	0.45 ±0.02
Temperature (°C)	13.2 ±0.8	13.6 ±0.8
O2 (mg*l^{-1})	9.95 ±0.18	9.87 ±0.15

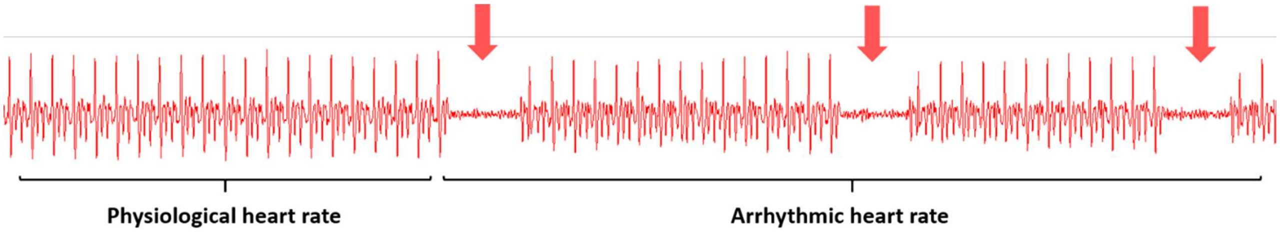

Figure S2: Physiological rhythmic heart rate vs arrhythmic heart rate. Real ECG recording section with representative cardiac arrhythmias (red arrows) where the QRS complexes are missing.

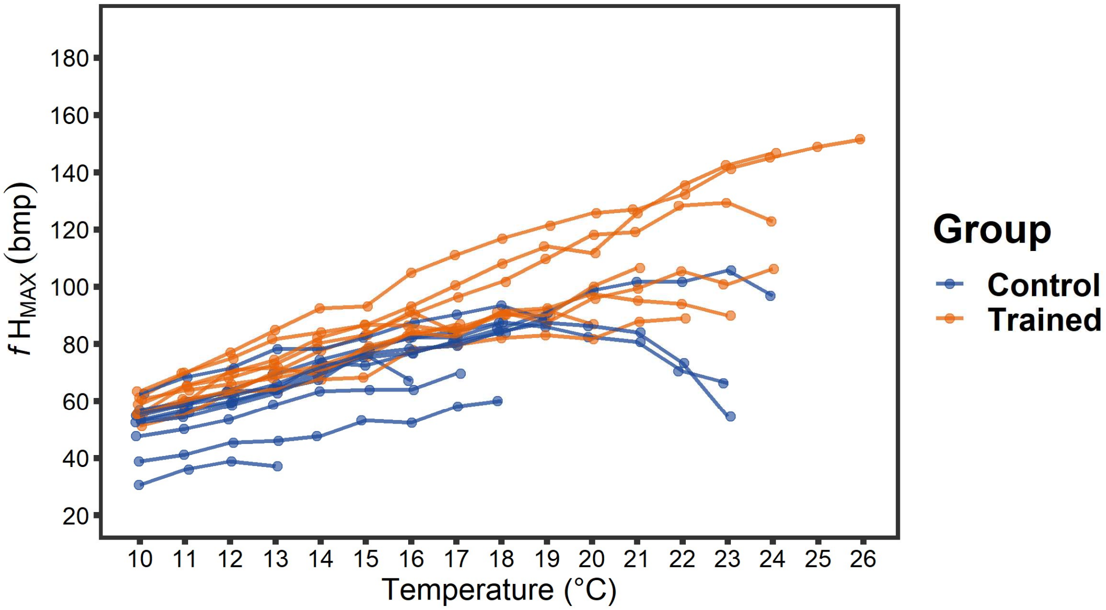

Fig. S3: Effect of exercise training program on the individual $f\mathrm{H}_{\mathrm{MAX}}$ of the adults during the acute warming ramp.

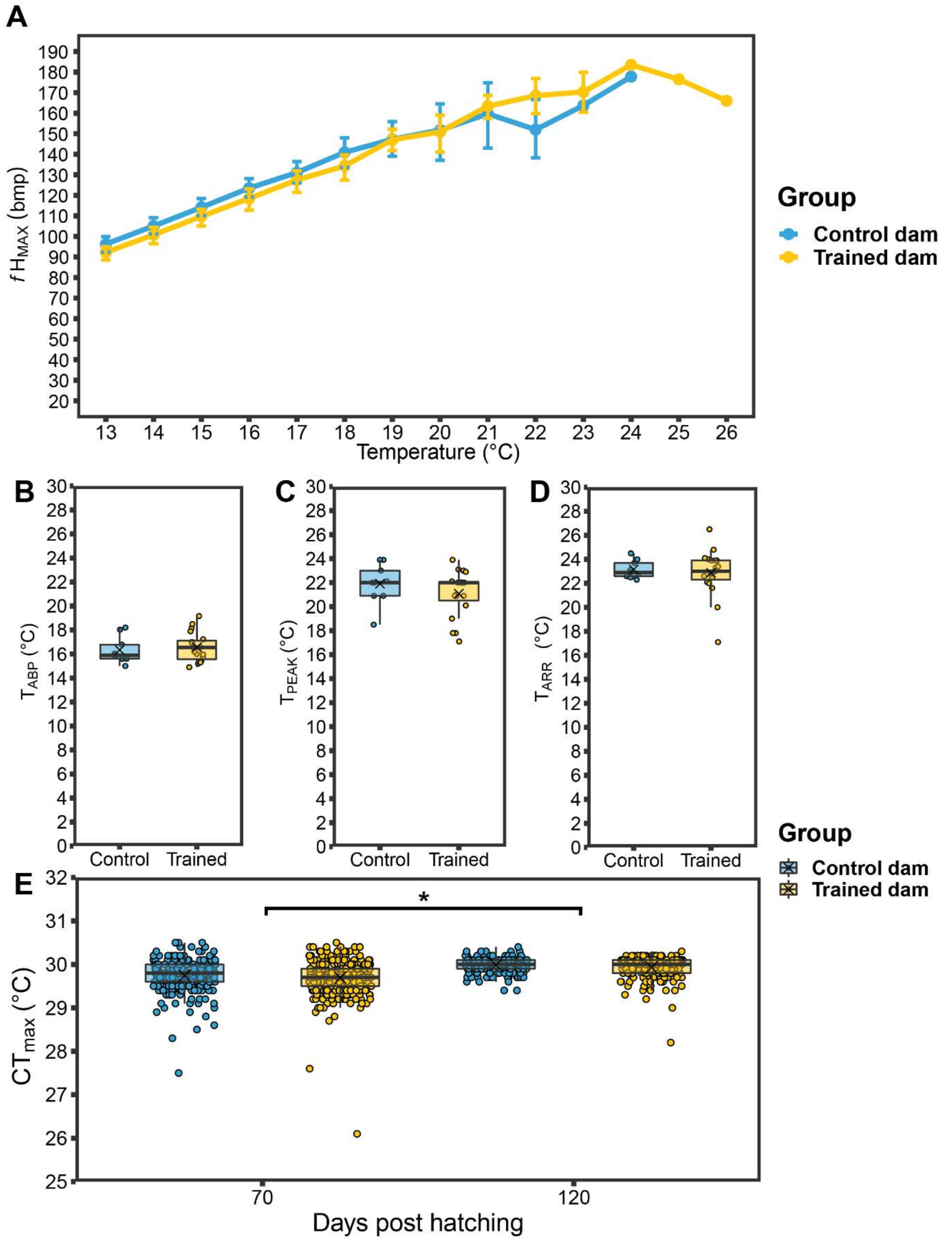

Figure S4: Effect of maternal exercise training on the cardiac thermal performance variables and critical thermal maximum of the offspring at 120 dph. A) average of maximal heart rate fH_{MAX}(± s.e.m.) during acute warming of the fish from trained mothers (yellow; n= 19) and from control mothers (light blue, n=9). B) Boxplots of the Arrhenius break point temperature (T_{ABP}) for the fH_{MAX}. C) Boxplot of the temperature where the fish achieved the peak of the fH_{MAX} (T_{PEAK}). D) Boxplot of the temperature where cardiac arrhythmias (missing QRS complex from the ECG signal) were observed (T_{ARR}). No significant differences were observed between the groups. E) Effect of maternal exercise training on the CT_{MAX} of the offspring at two time points. Seventy-day post hatching represents the early summer (June), while 120 dph represents late summer (August). No significant differences between the maternal training group were detected. * Represents significant difference between the time points (*$p \leq 0.05$).

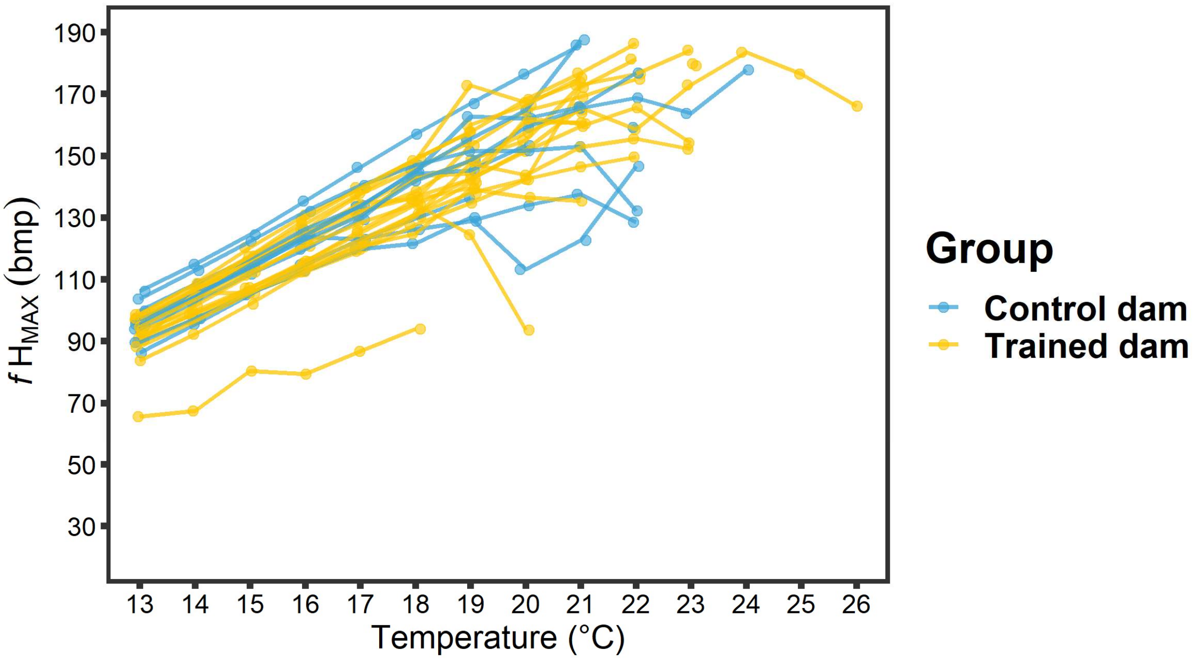

Fig S5: Effect of maternal exercise training program on the individual fH_{MAX} of the offspring from trained mothers (yellow) and from control mothers (light blue) during acute warming ramp.

Table S2: Summary of final statistical analyses for adult (F0) and offspring (F1).

Life stage	topic	statistical software	Test	Dependent Variable	statistical unit	Main Factor	Repeated measure	Random factors
Adult	Cardiac thermal performance	Spss 27	rLmm	fH_{MAX}	individual	Training / Temperature	Temperature	Day / Chamber
Adult	Cardiac thermal performance	R	lm	T_{ABP}	individual	Training	-	-
Adult	Cardiac thermal performance	R	lm	T_{PEAK}	individual	Training	-	-
Adult	Cardiac thermal performance	R	lm	T_{ARR}	individual	Training	-	-
Adult	Morphology	Spss 27	t test	Body mass	individual	Training	-	-
Adult	Morphology	Spss 27	t test	Body length	individual	Training	-	-
Adult	Morphology	Spss 27	t test	Condition factor	individual	Training	-	-
Adult	Morphology	Spss 27	t test	Liver mass	individual	Training	-	-
Adult	Morphology	Spss 27	t test	HSI	individual	Training	-	-
Adult	Cardiac morphology	Spss 27	t test	Ventricle mass	individual	Training	-	-
Adult	Cardiac morphology	Spss 27	t test	RVM	individual	Training	-	-
Adult	Cardiac morphology	Spss 27	t test	Compact myocardium dry mass	individual	Training	-	-
Adult	Cardiac morphology	Spss 27	t test	Spongy myocardium dry mass	individual	Training	-	-
Adult	Cardiac morphology	Spss 27	t test	Spongy/compact myocardium ratio	individual	Training	-	-
Adult	Cardiac morphology	Spss 27	t test	Compact myocardium percentage	individual	Training	-	-
Adult	Reproductive success	Spss 27	t test	Clutch mass	individual	Training	-	-
Adult	Reproductive success	Spss 27	t test	Relative fecundity	individual	Training	-	-
Adult	Reproductive success	Spss 27	t test	Egg diameter	individual	Training	-	-
Embryo	Survival offspring	Spss 27	Mann-Whitney U-test	Survival to eyed stage	Family	Training	-	-
Offspring	Survival offspring	Spss 27	Mann-Whitney U-test	Hatching success	Family	Training	-	-
Offspring to 120 dph	Survival offspring	Spss 27	Mann-Whitney U-test	Survival	Family	Training	-	-
Offspring at 70 & 120 dph	Thermal tolerance	R	Lmm	CT_{MAX}	individual	Training / Dph	-	Mother / Father / Day / Box
Offspring at 70 & 120 dph	Morphology	R	Lmm	Body mass	individual	Training / Dph	-	Mother / Father / Day / Box
Offspring at 70 & 120 dph	Morphology	R	Lmm	Body length	individual	Training / Dph	-	Mother / Father / Day / Box

Offspring at 70 & 120 dph	Morphology	R	Lmm	Body condition factor	individual	Training / Dph	-	Mother / Father / Day / Box
Offspring at 70 & 120 dph	Heritability	ASReml	Animal model	h2 CT_{MAX}	individual	Dph	-	Family / ID
Offspring at 70 & 120 dph	Heritability	ASReml	Animal model	h2 Body mass	individual	Dph	-	Family / ID
Offspring at 70 & 120 dph	Heritability	ASReml	Animal model	h2 Body length	individual	Dph	-	Family / ID
Offspring at 120 dph	Cardiac thermal performance	Spss 27	rLmm	fH_{MAX}	individual	Training / Temperature	Temperature	Mother / Father / Day / Chamber
Offspring at 120 dph	Cardiac thermal performance	R	lm	T_{ABP}	individual	Training	-	-
Offspring at 120 dph	Cardiac thermal performance	R	lm	T_{PEAK}	individual	Training	-	-
Offspring at 120 dph	Cardiac thermal performance	R	lm	T_{ARR}	individual	Training	-	-
Offspring from 150 to 400 dph	*Exploratory study Long-term Morphology*	*R*	*Lm*	*Body mass*	*individual*	*Training / Dph*	-	-
Offspring from 150 to 400 dph	*Exploratory study Long-term Morphology*	*R*	*Lm*	*Body length*	*individual*	*Training / Dph*	-	-
Offspring from 150 to 400 dph	*Exploratory study Long-term Morphology*	*R*	*Lm*	*Condition factor*	*individual*	*Training / Dph*	-	-

Table S3: Morphology and reproductive parameters of the control and trained adult females. * indicates a significant difference between the training groups (***p≤0.001,**p>0.001, *p<0.05 and >0.01).

	Control Mother			**Trained Mother**		
	Mean	**S.e.m.**	**N**	**Mean**	**S.e.m.**	**N**
Body mass (kg)	2.60	0.24	8	2.86	0.16	13
Body length (cm)	56.19	1.42	8	58.47	1.14	13
Condition factor	1.43	0.05	8	1.42	0.03	13
Liver mass (g)	**30.61*** **	**1.70**	**7**	**16.45*** **	**1.51**	**11**
Hepato-somatic index (HSI)	**1.00** **	**0.16**	**7**	**0.48** **	**0.06**	**11**
Mature clutch mass (g)	**314.17* **	**44.80**	**6**	**418.64* **	**20.85**	**11**
Relative fecundity	0.13	0.01	6	0.15	0.01	11
Egg diameter (mm)	5.67	0.04	5	5.63	0.05	11
Ventricle mass (g)	2.76	0.26	7	2.79	0.20	12
RVM	0.09	0.01	8	0.09	0.01	13
Spongy myocardium dry mass (mg)	135.12	8.97	6	153.54	14.89	12
Compact myocardium dry mass (mg)	49.42	3.71	6	56.01	5.91	12
ratio Compact/spongy	0.37	0.02	6	0.37	0.02	12
Compact % over all ventricle	26.79	1.10	6	26.76	1.19	12

Table S4: Effect of the maternal exercise training between control and trained families on the survival to eyed stage, hatching success and survival rate. N refers to the number of families. Bold text and * represent significant difference between the groups *p≤0.05).

	control				**trained**			
	Mean ± sem	N	95.0% Lower CL for Mean	95.0% Upper CL for Mean	Mean ± sem	N	95.0% Lower CL for Mean	95.0% Upper CL for Mean
survival to eyeing	**45.1 ± 3.6 ***	15	37.3	52.9	**61.3 ± 6.5 ***	24	47.9	74.6
hatching success %	99.8 ± 0.1	16	99.7	100.0	99.2 ± 0.1	24	98.0	100.5
survival rate	43.1 ± 5.8	16	30.7	55.5	34.9 ± 4.7	24	25.1	44.7

Table S5 : CT_{MAX} of the offspring divided by family

			Control mother						**Trained mother**					
			70 dph			**120 dph**			**70 dph**			**120 dph**		
Family	**Father**	**Mother**	**Mean**	**S.e.m**	**N**	**Mean**	**S.e.m**	**N**	**Mean**	**S.e.m**	**N**	**Mean**	**S.e.m**	**N**
total			**29.7**	**0.02**	**261**	**30.0**	**0.01**	**167**	**29.7**	**0.02**	**336**	**29.9**	**0.01**	**218**
1	a	37827	29.9	0.0	15	30.0	0.1	10			0			0
2	b	37827	29.9	0.2	2	0	0	0			0			0
3	c	37827	29.7	0.1	20	29.8	0.0	15			0			0
4	d	37827	29.5	0.1	15	30.0	0.0	10			0			0
5	a	71988	29.9	0.1	23	30.2	0.0	14			0			0
6	b	71988	29.4	0.2	15	30.2	0.0	9			0			0
7	c	71988	29.7	0.1	27	30.0	0.0	15			0			0
8	d	71988	29.8	0.1	15	30.0	0.0	10			0			0
9	a	CF4	29.8	0.1	15	30.0	0.0	14			0			0
10	b	CF4	29.8	0.1	15	30.1	0.0	10			0			0
11	c	CF4	29.7	0.1	15	29.9	0.1	10			0			0
12	d	CF4	29.9	0.1	15	29.9	0.1	10			0			0
13	a	73446	29.9	0.0	15	30.0	0.1	10			0			0
14	b	73446	29.7	0.1	15	30.0	0.0	10			0			0
15	c	73446	29.9	0.0	20	29.9	0.0	10			0			0
16	d	73446	29.7	0.0	19	29.9	0.0	10			0			0
17	a	71410			0			0	29.9	0.1	10			0
18	b	71410			0			0			0			0
19 & 20	c & d	71410			0			0	30.0	0.0	4			0
21	a	74999			0			0	29.8	0.1	15	30.0	0.1	10
22	b	74999			0			0	29.4	0.2	15	29.6	0.2	9

23	c	74999			0			0	29.7	0.1	15	29.8	0.1	10
24	d	74999			0			0	29.5	0.1	15	29.7	0.1	10
25	a	73958			0			0	29.9	0.1	25	30.0	0.0	10
26	b	73958			0			0	29.6	0.1	15	29.8	0.1	11
27	c	73958			0			0	29.8	0.0	15	30.0	0.0	10
28	d	73958			0			0	29.6	0.1	15	30.0	0.0	10
29	a	73666			0			0	29.8	0.1	20	30.0	0.0	15
30	b	73666			0			0	29.9	0.1	16	30.1	0.0	10
31	c	73666			0			0	29.6	0.1	15	30.1	0.0	10
32	d	73666			0			0	29.6	0.1	21	30.1	0.0	15
33	a	74915			0			0	29.7	0.1	15	30.0	0.0	10
34	b	74915			0			0	29.6	0.1	15	29.9	0.1	10
35	c	74915			0			0	29.9	0.1	15	30.1	0.0	14
36	d	74915			0			0	29.6	0.1	15	29.7	0.1	10
37	a	73839			0			0	29.7	0.1	15	30.1	0.0	14
38	b	73839			0			0	29.6	0.1	15	29.9	0.0	10
39	c	73839			0			0	29.6	0.1	15	29.7	0.0	10
40	d	73839			0			0	29.4	0.2	15	29.9	0.0	10

Exploratory study: long-term morphology

After 120 dph, the families were combined within each training group (trained vs. untrained mothers) and transferred to two circular tanks (2.06 m, one tank for each training group) to follow up the morphology of the juveniles during the following year (from 150 to 400 dph).

To assess the changes in morphology in the long-term exploratory study, linear models (stats package, R) were used for log-transformed body mass, log-transformed length, and condition factor, respectively. The body mass and the length were log transformed to meet the normal distribution of the residuals. The maternal training background and month of the measurements were used as fixed effects.

The morphological changes between 150 and 400 dph revealed that offspring of trained mothers had significantly higher body mass (df=1; F=11.9; p<0.001) and body length (df=1; F=15.6; p<0.001) as compared to the offspring of untrained mothers (Fig. S6 & Table S6). On the other hand, maternal exercise training did not affect the condition factor of their offspring (df=1; F=1.5; p=0.225). However, these results must be interpreted with caution because of the lack of duplicate tanks that do not fully allow us to detangle the potential confounding factor of tank effect.

Table S6 : Effect of maternal exercise training on the A) body mass B) fork length and C) condition factor of the offspring from 70 to 400 dph.

	Dph	**Control mother**			**Trained mother**		
		Mean	S.e.m	N	Mean	S.e.m	N
Body mass (g)	*70*	0.49	0.01	258	0.52	0.01	336
	120	1.63	0.05	167	1.70	0.04	218
	150	3.69	0.18	34	3.81	0.15	38
	174	6.84	0.25	46	6.99	0.34	41
	203	11.52	0.72	30	13.09	0.74	28
	239	14.86	0.92	34	18.11	1.16	31
	350	16.07	0.80	32	19.57	1.09	33
	400	21.57	1.29	33	24.09	1.22	31
Body length (mm)	*70*	38.41	0.21	259	39.15	0.20	336
	120	53.78	0.45	167	54.77	0.34	218
	150	69.85	1.06	34	70.79	0.87	38
	174	86.28	0.94	46	86.63	1.32	41
	203	101.07	1.91	30	106.11	1.89	28
	239	107.50	2.16	34	115.23	2.51	31
	350	113.41	1.74	32	120.18	2.09	33
	400	123.73	2.18	33	130.19	2.00	31
	70	0.83	0.01	258	0.83	0.01	336

Condition factor	*120*	1.01	0.01	167	1.00	0.01	218
	150	1.05	0.01	34	1.05	0.01	38
	174	1.04	0.01	46	1.04	0.01	41
	203	1.08	0.01	30	1.06	0.01	28
	239	1.14	0.01	34	1.13	0.01	31
	350	1.07	0.01	32	1.09	0.01	33
	400	1.10	0.01	33	1.06	0.01	31

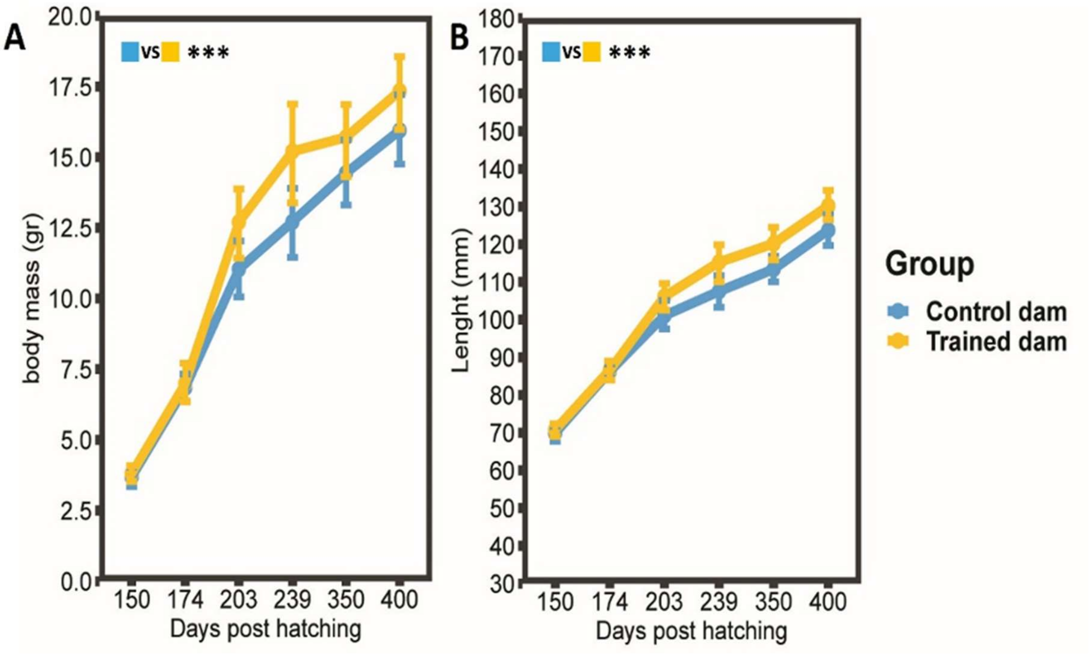

Figure S6: Effect of maternal exercise training on A) body mass B) fork length of the offspring from 150 to 400 dph. * Represents significant main effect of the maternal training (***p≤0.001).

Supplementary material 2 – Models selection

Table S7: Model selection for the $f\mathrm{H}_{\mathrm{MAX}}$ of the Adult using SPSS. List of Linear Mixed Models (LMMs) that were performed in SPSS on the $f\mathrm{H}_{\mathrm{MAX}}$, along with their corresponding Akaike Information Criterion (AIC) values. The model with lowest AIC was chosen as final model. $f\mathrm{H}_{\mathrm{MAX}}$ is the dependent variable and it is the maximal heart rate measured ad given temperature during acute warming. Training is treatment type (control vs trained). Temperature is the given temperature at which the heart rate was measured. The following factor are use as random factors in the models. Chamber is the chamber of the heart rate setup where each individual was placed to measure the heart rate. Fungus is the presence or assent information of skin damage due to mycotic infection. Day is the day when the heart rate measurement was performed.

$f\mathrm{H}_{\mathrm{MAX}}$						
	Main factor		Repeated measure	Random Factors		
Model	**Training**	**Temperature**	**Temperature**	**Day**	**Chamber**	**AIC**
1	**x**	**x**	**x**	**x**	**x**	**1386**
2	x	x	x	x		1452.9
3	x	x	x		x	1433.8
4	x	x	x			1485.2

Table S8: Model selection cardiac transition temperatures in adults using R. List of Linear Mixed Models (LMMs) that were performed in R for each cardiac transition temperature ($\mathrm{T}_{\mathrm{ABP}}$; $\mathrm{T}_{\mathrm{PEAK}}$, $\mathrm{T}_{\mathrm{ARR}}$), along with their corresponding Akaike Information Criterion (AIC) values. The model with lowest AIC was chosen as final model. $\mathrm{T}_{\mathrm{ABP}}$; $\mathrm{T}_{\mathrm{PEAK}}$, $\mathrm{T}_{\mathrm{ARR}}$ are the dependent variables in each sub-tables. Training is treatment type (control vs trained). The following factor are use as random factors in the models. Chamber is the chamber of the heart rate setup where each individual was placed to measure the heart rate. Day is the day when the heart rate measurement was performed.

$\mathrm{T}_{\mathrm{PEAK}}$				
	Main factor	Random factors		
Model	**Training**	**Day**	**Chamber**	**AIC**
1	x	x	x	99.3
2	x	x		97.6
3	x		x	98.2
4	**x**			**96.1**
$\mathrm{T}_{\mathrm{ARR}}$				
	Main factor	Random factors		
Model	**Training**	**Day**	**Chamber**	**AIC**
1	x	x	x	105
2	x	x		103
3	x		x	102.7

4	**x**			**100.7**
T_{ABP}				
	Main factor	Random factors		
Model	**Training**	**Day**	**Chamber**	**AIC**
1	x	x	x	49.5
2	x	x		47.5
3	x		x	47.5
4	**x**			**45.5**

Table S9: Model selection for the $f\mathrm{H}_{\mathrm{MAX}}$ of the offspring using SPSS. List of Linear Mixed Models (LMMs) that were performed in SPSS on the $f\mathrm{H}_{\mathrm{MAX}}$, along with their corresponding Akaike Information Criterion (AIC) values. The model with lowest AIC was chosen as final model. Fhmax is the dependent variable, and it is the maximal heart rate measured ad given temperature during acute warming. Training is treatment type of the mothers (control vs trained). Temperature is the given temperature at which the heart rate was measured during acute warming. The following factor are use as random factors in the models. Mother is the ID of the mother where the offspring belong. Father is the ID of the father were the offspring belong. Chamber is the chamber of the heart rate setup where each individual was placed to measure the heart rate. Day is the day when the heart rate measurement was performed.

$f\mathrm{H}_{\mathrm{MAX}}$								
	Main factor		Repeated measure	Random Factors				
Model	**Training**	**Temperature**	**Temperature**	**Day**	**Chamber**	**Mother**	**Father**	**AIC**
1	x	x	x	x	x	x	x	1892.30
2	**x**	**x**	**x**	**x**	**x**	**x**		**1891.70**
3	x	x	x	x	x		x	1914.27
4	x	x	x	x		x	x	1895.49
5	x	x	x		x	x	x	1906.70
6	x	x	x	x	x			1914.20
7	x	x	x	x		x		1902.16
8	x	x	x	x			x	1915.17
9	x	x	x		x	x		1904.89
10	x	x	x		x		x	1935.78
11	x	x	x			x	x	1919.46
12	x	x	x				x	1953.11
13	x	x	x			x		1920.08
14	x	x	x		x			1933.79
15	x	x	x	x				1922.63
16	x	x	x					1955.31

Table S10: Model selection cardiac transition temperatures in adults using R. List of Linear Mixed Models (LMMs) that were performed in R for each cardiac transition temperature (T_{ABP}; T_{PEAK}, T_{ARR}), along with their corresponding Akaike Information Criterion (AIC) values. The model with lowest AIC was chosen as final model. T_{ABP}; T_{PEAK}, T_{ARR} are the dependent variables in each sub-tables. Training is treatment type of the mother (control vs trained). The following factor are use as random factors in the models. Mother is the ID of the mother where the offspring belong. Father is the ID of the father were the offspring belong. Chamber is the chamber of the heart rate setup where each individual was placed to measure the heart rate. Day is the day when the heart rate measurement was performed.

T_{PEAK}							
	Main factor	**Random Factors**					
Model	**Training**	**Day**	**Chamber**	**Mother**	**Father**	**Family**	**AIC**
1	x	x	x	x	x	x	127.7
2	x	x	x	x	x		125.7
3	x	x	x	x		x	126.2
4	x	x	x		x	x	125.7
5	x		x	x	x	x	125.7
6	x	x		x	x	x	125.7
7	x	x	x	x			124.2
8	x	x	x		x		123.7
9	x	x	x			x	124.2
10	x		x	x			123.7
11	x		x	x		x	124.3
12	x		x		x	x	123.7
13	x	x		x	x		123.7
14	x	x		x		x	124.2
15	x	x			x	x	123.7
16	x				x	x	123.7
17	x	x	x				122.2
18	x		x	x			122.3
19	x		x		x		121.7
20	x		x			x	122.2
21	x	x		x			122.2
22	x	x			x		121.7
23	x	x				x	122.2
24	x			x			121.7
25	x			x	x	x	122.3
26	x					x	121.7
27	x	x	x				120.1
28	x	x					120.2
29	x			x			120.3
30	x				x		119.7
31	x					x	120.2
32	x						**118.1**

TABP							
	Main factor	**Random Factors**					
Model	**Training**	**Day**	**Chamber**	**Mother**	**Father**	**Family**	**AIC**
1	x	x	x	x	x	x	100.8
2	x	x	x	x	x		98.8
3	x	x	x	x		x	100.5
4	x	x	x		x	x	98.8
5	x		x	x	x	x	98.7
6	x	x		x	x	x	98.8
7	x	x	x	x			98.5
8	x	x	x		x		96.8
9	x	x	x			x	98.5
10	x		x	x			96.7
11	x		x	x		x	98.5
12	x		x		x	x	96.7
13	x	x		x	x		96.8
14	x	x		x		x	98.4
15	x	x			x	x	96.8
16	x				x	x	96.7
17	x	x	x				96.5
18	x		x	x			96.5
19	x		x		x		94.7
20	x		x			x	96.5
21	x	x		x			96.4
22	x	x			x		94.8
23	x	x				x	96.4
24	x			x			94.7
25	x			x	x	x	96.4
26	x					x	94.7
27	x	x	x				94.5
28	x	x					94.4
29	x			x			94.4
30	x				x		92.7
31	x					x	94.4
32	x						**92.4**

TARR							
	Main factor	**Random Factors**					
Model	**Training**	**Day**	**Chamber**	**Mother**	**Father**	**Family**	**AIC**
1	x	x	x	x	x	x	122.3
2	x	x	x	x	x		120.3
3	x		x			x	120.1
4	x					x	120.3
5	x	x	x	x	x	x	120.3

6	x	x		x	x	x	120.3
7	x	x	x	x			118.1
8	x	x	x		x		118.3
9	x	x	x			x	118.1
10	x		x	x			118.3
11	x		x	x		x	118.1
12	x		x		x	x	118.3
13	x	x		x	x		118.3
14	x	x		x		x	118.1
15	x	x			x	x	118.3
16	x				x	x	118.3
17	x	x	x				116.1
18	x		x	x			116.1
19	x		x		x		116.3
20	x		x			x	116.1
21	x	x		x			116.1
22	x	x			x		116.3
23	x	x				x	116.1
24	x			x			116.3
25	x			x	x	x	116.1
26	x					x	116.3
27	x	x	x				114.1
28	x	x					114.1
29	x			x			114.1
30	x				x		114.3
31	x					x	114.1
32	x						**112.1**

Table S11: statistical model using R for $\mathrm{CT}_{\mathrm{MAX}}$ of the offspring between 70 and 120 dph. List of Linear Mixed Models (LMMs) that were performed in R for the rank-transformed **$\mathrm{CT}_{\mathrm{MAX}}$** along with their corresponding Akaike Information Criterion (AIC) values. The model with lowest AIC was chosen as final model. Training is treatment type of the mother (control vs trained). The following factor are use as random factors in the models. Mother is the ID of the mother of the offspring. Father is the ID of the father of the offspring. Chamber is the chamber of the heart rate setup where each individual was placed to measure the heart rate. Day is the day when the heart rate measurement was performed.

Both m 1 and m 4 are equally good models based on several model metrics, including AIC, BIC, RMSE, and R^2, with only minor differences in their performance. Model m1 (AIC = 13386.4, RMSE = 199.255, $R^2 = 0.158$) incorporates Background * month as fixed effects and includes random intercepts for date, box, mother, father, and family, while m4 (AIC = 13384.4, RMSE = 199.324, $R^2 = 0.160$) excludes mother as a random effect. Although m4 is slightly simpler, so more parsimonious, m1 was chosen because it captures the inter-individual maternal variation that is not explained by only the experimental exercise program. Given that maternal identity can influence offspring traits, including mother as a random effect in m1 allows for a more biologically comprehensive model, while still maintaining a similar level of model fit (ICC = 0.432 for m1 vs. 0.429 for m4).

$\mathrm{CT_{MAX}}$									
	Main factor			Random factor					
Model	**Training**	**Dph**	**Training* Dph**	**Day**	**Box**	**Mother**	**Father**	**Family**	**AIC**
m1	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**13386.4**
m2	x	x	x		x	x	x	x	13639.72
m3	x	x	x	x		x	x	x	13390.77
m4	x	x	x	x	x		x	x	13384.43
m5	x	x	x	x	x	x		x	13392.34
m6	x	x	x	x	x	x	x		13415.04
m7	x	x	x	x	x		x		13422.97
m8	x	x	x	x	x	x			13470.13
m9	x	x	x			x	x	x	13640.18
m10	x	x	x		x		x	x	13637.68
m11	x	x	x	x			x	x	13388.89
m12	x	x	x	x		x		x	13396.67
m13	x	x	x	x	x			x	13390.25
m14	x	x	x		x	x	x		13662.41
m15	x	x	x	x		x	x		13424.13
m16	x	x	x				x	x	13638.1
m17	x	x	x			x	x		13665.26
m18	x	x	x		x			x	13642.88
m19	x	x	x	x				x	13394.52
m20	x	x	x		x		x		13665.39
m21	x	x	x	x			x		13437.07
m22	x	x	x		x	x			13706.13
m23	x	x	x	x		x			13487.37
m24	x	x	x	x	x				13479.2
m25	x	x	x					x	13643.08
m26	x	x	x				x		13671.51
m27	x	x	x			x			13713.33
m28	x	x	x		x				13709.32
m29	x	x	x	x					13501.7
m30	x	x		x	x	x	x	x	13384.65
m31	x	x			x	x	x	x	13637.74
m32	x	x		x		x	x	x	13389.26
m33	x	x		x	x		x	x	13382.66
m34	x	x		x	x	x		x	13390.59
m35	x	x		x	x	x	x		13413.54
m36	x	x		x	x		x		13421.13
m37	x	x		x	x	x			13468.8

m38	x	x				x	x	x	13638.18
m39	x	x			x		x	x	13635.69
m40	x	x		x			x	x	13387.34
m41	x	x		x		x		x	13395.09
m42	x	x		x	x			x	13388.52
m43	x	x			x	x	x		13660.43
m44	x	x		x		x	x		13423.34
m45	x	x					x	x	13636.1
m46	x	x				x	x		13663.28
m47	x	x			x			x	13640.9
m48	x	x		x				x	13392.96
m49	x	x			x		x		13663.46
m50	x	x		x			x		13435.73
m51	x	x			x	x			13704.22
m52	x	x		x		x			13486.49
m53	x	x		x	x				13477.35
m54	x	x						x	13641.08
m55	x	x					x		13669.52
m56	x	x				x			13711.34
m57	x	x			x				13707.47
m58	x	x		x					13500.16
m59	x	x							13717.53
m60	x	x							13719.51

Table S12: statistical model using R for Body mass of the offspring between 70 and 120 dph. List of Linear Mixed Models (LMMs) that were performed in R for the **Body mass** along with their corresponding Akaike Information Criterion (AIC) values. The model with lowest AIC was chosen as final model. Training is treatment type of the mother (control vs trained). The following factor are use as random factors in the models. Mother is the ID of the mother of the offspring. Father is the ID of the father of the offspring. Chamber is the chamber of the heart rate setup where each individual was placed to measure the heart rate. Day is the day when the measurement was performed

Body mass									
	Fixed factors			Random factors					
Model	**Training**	**Dph**	**Training* Dph**	**Day**	**Box**	**Mother**	**Father**	**Family**	**AIC**
m1	x	x	x	x	x	x	x	x	12594.33
m2	x	x	x		x	x	x	x	12674.81
m3	x	x	x	x		x	x	x	12603.01
m4	x	x	x	x	x		x	x	12594.83

m5	x	x	x	x	x	x		x	12597.01
m6	x	x	x	x	x	x	x		12610.84
m7	x	x	x	x	x		x		12635.98
m8	x	x	x	x	x	x			12629.31
m9	x	x	x			x	x	x	12680.81
m10	x	x	x		x		x	x	12674.57
m11	x	x	x	x			x	x	12602.64
m12	x	x	x	x		x		x	12605.55
m13	x	x	x	x	x			x	12596.44
m14	x	x	x		x	x	x		12707.63
m15	x	x	x	x		x	x		12628.51
m16	x	x	x				x	x	12680.13
m17	x	x	x			x	x		12718.31
m18	x	x	x		x			x	12673.91
m19	x	x	x	x				x	12604.34
m20	x	x	x		x		x		12731.05
m21	x	x	x	x			x		12652.22
m22	x	x	x		x	x			12720.67
m23	x	x	x	x		x			12650.53
m24	x	x	x	x	x				12657.74
m25	x	x	x					x	12679.55
m26	x	x	x				x		12740.32
m27	x	x	x			x			12735.01
m28	x	x	x		x				12746.8
m29	x	x	x	x					12678.88
m30	**x**	**x**		**x**	**x**	**x**	**x**	**x**	**12592.4**
m31	x	x			x	x	x	x	12672.81
m32	x	x		x		x	x	x	12601.04
m33	x	x		x	x		x	x	12592.89
m34	x	x		x	x	x		x	12595.05
m35	x	x		x	x	x	x		12608.99
m36	x	x		x	x		x		12634.05
m37	x	x		x	x	x			12627.36
m38	x	x				x	x	x	12678.82
m39	x	x			x		x	x	12672.57
m40	x	x		x			x	x	12600.67
m41	x	x		x		x		x	12603.57
m42	x	x		x	x			x	12594.48
m43	x	x			x	x	x		12705.64
m44	x	x		x		x	x		12626.53
m45	x	x					x	x	12678.14
m46	x	x				x	x		12716.32
m47	x	x			x			x	12671.91
m48	x	x		x				x	12602.37
m49	x	x			x		x		12729.1
m50	x	x		x			x		12650.24

m51	x	x			x	x			12718.67
m52	x	x		x		x			12648.56
m53	x	x		x	x				12655.75
m54	x	x						x	12677.55
m55	x	x					x		12738.35
m56	x	x				x			12733.01
m57	x	x			x				12744.83
m58	x	x		x					12676.9
m59	x	x							12757.47
m60	x	x							12759.41

Table S13: statistical model using R for Body length of the offspring between 70 and 120 dph. List of Linear Mixed Models (LMMs) that were performed in R for **Body length** along with their corresponding Akaike Information Criterion (AIC) values. The model with lowest AIC was chosen as final model. Training is treatment type of the mother (control vs trained). The following factor are use as random factors in the models. Mother is the ID of the mother of the offspring. Father is the ID of the father of the offspring. Chamber is the chamber of the heart rate setup where each individual was placed to measure the heart rate. Day is the day when the measurement was performed.

Body lenght									
	Fixed factors			**Random factors**					
Model	Training	Dph	Training* Dph	Day	box	Mother	Father	Family	AIC
m1	x	x	x	x	x	x	x	x	12645.9
m2	x	x	x		x	x	x	x	12714.54
m3	x	x	x	x		x	x	x	12656.04
m4	x	x	x	x	x		x	x	12646.7
m5	x	x	x	x	x	x		x	12647.36
m6	x	x	x	x	x	x	x		12662.92
m7	x	x	x	x	x		x		12689.31
m8	x	x	x	x	x	x			12679.67
m9	x	x	x			x	x	x	12722.16
m10	x	x	x		x		x	x	12715.1
m11	x	x	x	x			x	x	12655.93
m12	x	x	x	x		x		x	12657.34
m13	x	x	x	x	x			x	12646.91
m14	x	x	x		x	x	x		12740.35
m15	x	x	x	x		x	x		12682.24
m16	x	x	x				x	x	12722.24
m17	x	x	x			x	x		12752.6
m18	x	x	x		x			x	12713.8
m19	x	x	x	x				x	12656.28
m20	x	x	x		x		x		12769.28

m21	x	x	x	x			x		12707.25
m22	x	x	x		x	x			12750.9
m23	x	x	x	x		x			12702.18
m24	x	x	x	x	x				12706.79
m25	x	x	x					x	12721.01
m26	x	x	x				x		12780.61
m27	x	x	x			x			12766.46
m28	x	x	x		x				12780.57
m29	x	x	x	x					12729.4
m30	**x**	**x**		**x**	**x**	**x**	**x**	**x**	**12644.11**
m31	x	x			x	x	x	x	12712.67
m32	x	x		x		x	x	x	12654.07
m33	x	x		x	x		x	x	12644.87
m34	x	x		x	x	x		x	12645.53
m35	x	x		x	x	x	x		12661.28
m36	x	x		x	x		x		12687.51
m37	x	x		x	x	x			12677.94
m38	x	x				x	x	x	12720.18
m39	x	x			x		x	x	12713.25
m40	x	x		x			x	x	12653.96
m41	x	x		x		x		x	12655.37
m42	x	x		x	x			x	12645.07
m43	x	x			x	x	x		12738.6
m44	x	x		x		x	x		12680.27
m45	x	x					x	x	12720.27
m46	x	x				x	x		12750.64
m47	x	x			x			x	12711.93
m48	x	x		x				x	12654.31
m49	x	x			x		x		12767.55
m50	x	x		x			x		12705.26
m51	x	x			x	x			12749.11
m52	x	x		x		x			12700.23
m53	x	x		x	x				12704.94
m54	x	x						x	12719.05
m55	x	x					x		12778.69
m56	x	x				x			12764.52
m57	x	x			x				12778.81
m58	x	x		x					12727.44
m59	x	x							12793.25
m60	x	x							12795.13

Table S14: statistical model using R for condition factor of the offspring between 70 and 120 dph. List of Linear Mixed Models (LMMs) that were performed in R for **condition factor** along with their corresponding Akaike Information Criterion (AIC) values. The model with lowest AIC was chosen as final model. Training is treatment type of the mother (control vs trained). The following factor are use as random factors in the models. Mother is the ID of the mother of the offspring. Father is the ID of the father of the offspring. Chamber is the chamber of the heart rate setup where each individual was placed to measure the heart rate. Day is the day when the measurement was performed

condition factor									
	Main factor			**Random Factors**					
Model	Training	Dph	Training* Dph	Day	box	Mother	Father	Family	AIC
m1	x	x	x	x	x	x	x	x	12966.9
m2	x	x	x		x	x	x	x	13076.02
m3	x	x	x	x		x	x	x	12969.32
m4	x	x	x	x	x		x	x	12971.11
m5	x	x	x	x	x	x		x	12971.67
m6	x	x	x	x	x	x	x		12971.45
m7	x	x	x	x	x		x		13017.68
m8	x	x	x	x	x	x			12986.98
m9	x	x	x			x	x	x	13076.73
m10	x	x	x		x		x	x	13077.44
m11	x	x	x	x			x	x	12971.99
m12	x	x	x	x		x		x	12973.81
m13	x	x	x	x	x			x	12973.69
m14	x	x	x		x	x	x		13099.94
m15	x	x	x	x		x	x		12978.57
m16	x	x	x				x	x	13077.66
m17	x	x	x			x	x		13102.42
m18	x	x	x		x			x	13078.35
m19	x	x	x	x				x	12974.67
m20	x	x	x		x		x		13126.12
m21	x	x	x	x			x		13021.3
m22	x	x	x		x	x			13121.06
m23	x	x	x	x		x			12998.03
m24	x	x	x	x	x				13040.58
m25	x	x	x					x	13078.74
m26	x	x	x				x		13126.22
m27	x	x	x			x			13127
m28	x	x	x		x				13152.99
m29	x	x	x	x					13048.94
m30	**x**	**x**		**x**	**x**	**x**	**x**	**x**	**12965**
m31	x	x			x	x	x	x	13074.37
m32	x	x		x		x	x	x	12967.32
m33	x	x		x	x		x	x	12969.14
m34	x	x		x	x	x		x	12969.8
m35	x	x		x	x	x	x		12969.63

m36	x	x		x	x		x		13015.76
m37	x	x		x	x	x			12985.42
m38	x	x				x	x	x	13074.84
m39	x	x			x		x	x	13075.71
m40	x	x		x			x	x	12969.99
m41	x	x		x		x		x	12971.81
m42	x	x		x	x			x	12971.73
m43	x	x			x	x	x		13098.29
m44	x	x		x		x	x		12976.58
m45	x	x					x	x	13075.74
m46	x	x				x	x		13100.48
m47	x	x			x			x	13076.64
m48	x	x		x				x	12972.67
m49	x	x			x		x		13124.17
m50	x	x		x			x		13019.31
m51	x	x			x	x			13119.68
m52	x	x		x		x			12996.04
m53	x	x		x	x				13038.88
m54	x	x						x	13076.83
m55	x	x					x		13124.23
m56	x	x				x			13125.08
m57	x	x			x				13151.11
m58	x	x		x					13046.94
m59	x	x							13153.54
m60	x	x							13155.53