

RESEARCH ARTICLE

Cost of transport is a repeatable trait but is not determined by mitochondrial efficiency in zebrafish (*Danio rerio*)

Miki Jahn and Frank Seebacher*

ABSTRACT

The energy used to move a given distance (cost of transport; CoT) varies significantly between individuals of the same species. A lower CoT allows animals to allocate more of their energy budget to growth and reproduction. A higher CoT may cause animals to adjust their movement across different environmental gradients to reduce energy allocated to movement. The aim of this project was to determine whether CoT is a repeatable trait within individuals, and to determine its physiological causes and ecological consequences. We found that CoT is a repeatable trait in zebrafish (*Danio rerio*). We rejected the hypothesis that mitochondrial efficiency (P/O ratios) predicted CoT. We also rejected the hypothesis that CoT is modulated by temperature acclimation, exercise training or their interaction, although CoT increased with increasing acute test temperature. There was a weak but significant negative correlation between CoT and dispersal, measured as the number of exploration decisions made by fish, and the distance travelled against the current in an artificial stream. However, CoT was not correlated with the voluntary speed of fish moving against the current. The implication of these results is that CoT reflects a fixed physiological phenotype of an individual, which is not plastic in response to persistent environmental changes. Consequently, individuals may have fundamentally different energy budgets as they move across environments, and may adjust movement patterns as a result of allocation trade-offs. It was surprising that mitochondrial efficiency did not explain differences in CoT, and our working hypothesis is that the energetics of muscle contraction and relaxation may determine CoT. The increase in CoT with increasing acute environmental temperature means that warming environments will increase the proportion of the energy budget allocated to locomotion unless individuals adjust their movement patterns.

KEY WORDS: Oxygen consumption, Temperature, Exercise, Locomotion, Acclimation, Allocation trade-off

INTRODUCTION

Locomotor performance is closely related to ecological success and fitness by influencing foraging, predator escape, dispersal and behavioural interactions (Halsey, 2016; Husak et al., 2006; Miles, 2004). Maximal locomotor capacities are important for high-intensity tasks such as aggressive interactions and predator escape (Grigaltchik et al., 2012; Husak and Fox, 2006). However, animals rarely travel at these maximal speeds, for example, assuming a slower speed when moving undisturbed through their environment

while foraging or during dispersal (Wilson et al., 2015). Locomotion is costly (Di Santo et al., 2017; Han et al., 2017), and the energetic cost (cost of transport, CoT) associated with moving at different speeds may impact the speed adopted by an individual (Claireaux et al., 2006; Halsey, 2016). Limited resources may lead to an allocation trade-off so that allocation of energy to locomotion can constrain energy available for other functions (Lailvaux and Husak, 2014). Increased CoT would therefore also increase the likelihood of an allocation trade-off (Halsey et al., 2018). Interestingly, CoT differs between individuals of the same species (Seebacher et al., 2016). Unless energy-production capacity varies proportionally with CoT, animals from the same population may experience different trade-offs, which can scale up to influence higher level functions such as social behaviour (Killen et al., 2017; Seebacher and Krause, 2017). Understanding the physiological mechanisms that underlie individual differences in CoT will permit predictions about animal behaviour and inform ecological models of animal movement.

At the most fundamental level of energy production, differences in CoT could be explained by differences in the efficiency of mitochondrial ATP production (Salin et al., 2015). Mitochondrial efficiency is determined by the amount of oxygen used to phosphorylate an ADP molecule to ATP (P/O ratio) (Brand, 2005). However, P/O ratios are not fixed and may vary as a result of slip reactions and proton leak, for example, which would lead to oxygen consumption in the absence of ATP production (Brand, 2005; Jastroch et al., 2010). Mitochondrial coupling (i.e. coupling of oxygen consumption to ATP production) is known to be affected by temperature and exercise, where exercise training (Conley, 2016) and decreasing temperatures (Abele et al., 2002; Pörtner et al., 1999) can decrease proton leak and increase coupling of mitochondrial respiration to ATP synthesis. Exercise training and thermal acclimation may therefore also affect CoT.

Our aims were to determine causes and consequences of differences in CoT between individuals. We tested whether: (i) lower mitochondrial P/O ratios are associated with increased CoT in individuals; (ii) acclimation to cooler temperatures and flowing water (exercise) lead to lower CoT, and (iii) greater CoT is associated with decreased movement and dispersal of individuals. Preliminary to our experiments, we determined whether CoT is a repeatable trait within individuals, thereby representing a consistent characteristic that may affect individual performance and fitness.

MATERIALS AND METHODS**Study animals and experimental design**

Experiments were performed with the approval of the University of Sydney Animal Ethics Committee (approval number 587). Adult zebrafish [*Danio rerio* (Hamilton 1822)] were obtained from a commercial supplier (Livefish, Bundaberg, Australia). Fish were kept in plastic tanks (0.6×0.4×0.35 m at a density of 1–2 fish per litre) in aged aerated water for 1 week before experiments began. All

School of Life and Environmental Sciences A08, University of Sydney, Sydney, NSW 2006, Australia.

*Author for correspondence (frank.seebacher@sydney.edu.au)

 F.S., 0000-0002-2281-9311

Received 6 February 2019; Accepted 2 April 2019

tanks were kept at 23°C ($\pm 0.5^\circ\text{C}$) with a submersible heater (200W; AquaWorld, Australia), and each tank contained a sponge filter attached to an air pump (AC-9908; Resun, China). The light cycle was 12 h:12 h light:dark, and fish were fed daily with commercial flake food (Wardley's Tropical Fish Flakes, The Hartz Mountain Cooperation, Secaucus, NJ, USA); fish were not fed for 24 h before experimentation.

Cost of transport

We determined aerobic CoT from measurements of oxygen consumption rate (\dot{M}_{O_2}) at different swimming speeds in a swimming flume respirometer (170 ml, Loligo Systems, Denmark) according to the manufacturer's instructions and as per previously published protocols (Seebacher et al., 2016). Before measurements, fish were rested in the flume for 60–90 min at a low flow speed (0.025 m s^{-1}) to recover from handling stress. \dot{M}_{O_2} was measured at a flow of 0.025 m s^{-1} and then at incrementally increased speeds of 0.1, 0.2, 0.3, 0.35 and 0.4 m s^{-1} or until fish became exhausted and could no longer hold their place in the flume (Seebacher et al., 2016). The flume was flushed with a submersible pump (Eheim, Germany) between measurements to ensure that water was fully saturated with oxygen at the start of each measurement. All CoT measurements were conducted at a water temperature of 23°C ($\pm 0.5^\circ\text{C}$), except during temperature acclimation experiments. The swimming flumes were drained, cleaned and dried daily to prevent confounding effects from microorganisms, and control trials without fish were conducted regularly to ensure this procedure of cleaning was working.

To test whether CoT is repeatable within individuals, we measured CoT twice, 10 days apart, in 15 fish. These fish were not used in subsequent experiments.

Mitochondrial bioenergetics

We measured CoT in 18 fish, and within 24 h following CoT measurements fish were anaesthetised with ethyl 3-aminobenzoate methanesulfonate (MS-222; 0.3 g l^{-1} buffered to pH 7; Sigma-Aldrich, Australia) and then euthanised by cervical dislocation. We dissected the skeletal tail muscle and divided it along the longitudinal axis of the fish in two equal halves, and tissue was used immediately for measurements of mitochondrial bioenergetics. Mitochondrial bioenergetics were measured according to published protocols (Ghanizadeh Kazerouni et al., 2015; Dos Santos et al., 2013) except where noted otherwise below. Skeletal muscle tissue ($0.13\text{--}0.26 \text{ g}$) was homogenised on ice in a Potter–Elvehjem glass tissue homogeniser in nine volumes (w/v) of isolation buffer (140 mmol l^{-1} KCl, 20 mmol l^{-1} HEPES, 5 mmol l^{-1} MgCl_2 , 2 mmol l^{-1} EGTA, 1 mmol l^{-1} ATP, pH 7.0). Homogenised tissue was centrifuged at 1400 g for 5 min at 4°C to remove larger cell debris. The supernatant was removed and centrifuged at 9000 g for 9 min at 4°C to suspend mitochondria. The mitochondrial pellet was resuspended in assay buffer containing 110 mmol l^{-1} sucrose, 60 mmol l^{-1} KCl, 0.5 mmol l^{-1} EGTA, 3 mmol l^{-1} MgCl_2 , 20 mmol l^{-1} taurine, 10 mmol l^{-1} KH_2PO_4 , 10 mmol l^{-1} HEPES and 1% fatty acid free bovine serum albumin, pH 7.1 ($200 \mu\text{l}$ buffer per 0.1 g of tissue).

Mitochondrial respiration was measured (at 23°C) in a respiration chamber (Mitocell MT200; Strathkelvin Instruments, North Lanarkshire, UK), with a microelectrode (model 1302; Strathkelvin Instruments) connected to an oxygen meter (model 782; Strathkelvin Instruments); the assay volume was $50 \mu\text{l}$ of suspended mitochondria. State II respiration was induced by addition of malate (final concentration= 5 mmol l^{-1}) and pyruvate (final concentration= 2.5 mmol l^{-1}). State III respiration was initiated with the addition of ADP (final concentration= 0.1 mmol l^{-1}) to measure the maximal

rate of substrate oxidation. We measured uncoupled state IV respiration when rates of oxygen decline stabilised at a low value after state III, which indicated that all ADP was used up; we confirmed state IV rates by the addition of oligomycin (final concentration= $2\text{--}4 \mu\text{g ml}^{-1}$). At the end of each assay, we added *p*-trifluoromethoxy carbonyl cyanide phenyl hydrazone (FCCP; final concentration= $0.5 \mu\text{mol l}^{-1}$) to verify mitochondrial integrity. The protein concentration of the isolated mitochondria solution was determined with a Bradford assay (Sigma-Aldrich, Castle Hill, Australia) using bovine serum albumin as the standard.

We calculated P/O ratios from the known amount of ADP added to the reaction and divided this by the oxygen consumed during state III respiration.

Acclimation treatments

Zebrafish were allocated randomly to one of two temperature treatments (18 or 28°C). After 1 week in the temperature treatments, fish were further subdivided into two different water flow treatments in a fully factorial design: half the fish from each temperature treatment were allocated to a flowing water treatment, while the other half remained in still water. Hence, there were four different treatments (two temperatures \times two exercise conditions). Fish were then acclimated to the temperature and exercise conditions for another 2 weeks; 3 weeks of thermal acclimation is sufficient to elicit phenotypic responses (Little and Seebacher, 2013), and 2 weeks of our exercise training protocol results in a positive training effect and elicits an increase in swimming performance (Simmonds and Seebacher, 2017). Each treatment was dispersed across four tanks ($0.6\times 0.4\times 0.35 \text{ m}$) containing six fish each.

The tank design followed Simmonds and Seebacher (2017), where fish were contained in a smaller section of the tank within which flow was linear. In exercise treatments, water flow was 0.08 m s^{-1} ($2\text{--}3$ body lengths s^{-1}) for the 2 week acclimation period (flow was generated by two small submersible pumps; SP-900; Resun, China). Short bursts of higher flow rates (0.21 m s^{-1}) were provided daily with an additional pump (24 W, JHQ-2000; Sunsun, China). In the first week of acclimation, bursts were provided three times daily for 1 h with 3 h breaks between intensive flow periods. In the second week, bursts occurred three times daily for 1.5 h with 2.5 h rest periods (Simmonds and Seebacher, 2017). Following the acclimation to temperature and exercise treatments, the CoT of fish was measured ($n=10$ fish per treatment) as above at 18 and 28°C acute test temperatures in alternating order with at least 24 h between measurements.

Effect of CoT on movement

We tested whether there is an association between CoT and movement by measuring locomotor behaviour of fish in an artificial stream (Le Roy and Seebacher, 2018). Firstly, we measured CoT ($n=25$ fish) as above. Then, 24–48 h after CoT measurements, fish were placed individually into the artificial stream to determine their movement patterns. The artificial stream consisted of five pools ($0.4\times 0.3\times 0.3 \text{ m}$, 10 l each) connected by narrow channels (each 1.4 m long, 9.5 cm wide and 4 cm deep), giving a total length of 8 m. The recirculating water flow (0.08 m s^{-1} in the channels) in the stream was provided by a submersible pump (140 W, Grech CTP-16000 Amphibious Eco Pump $16,000 \text{ l}^{-1} \text{ h}^{-1}$, Sunsun, China), which was inside a large sump tank ($1.25\times 0.65\times 0.65 \text{ m}$). Fish were introduced to the furthest downstream pool, where they stayed for 15 min. After 15 min, the mesh barrier blocking the exit from the first pool was removed remotely, permitting fish access to the stream. Once the fish left the first pool they were allowed 10 min to

explore the stream. We recorded the following response variables: (i) voluntary speed in the first channel (in m s^{-1}) after leaving the first pool; (ii) number of exploration decisions made and (iii) distance travelled against the current (in m). To determine voluntary speed, we filmed fish swimming in the first channel with a digital camera (HD910, Logitech, China, filming at 30 frames s^{-1}). We analysed videos in Tracker software (Open Source Physics, www.opensourcephysics.org) to determine swimming speed. An exploration decision was defined as a decision to move out of a pool and travel more than half of a channel length (either upstream or downstream).

Statistical analyses and calculations

We used non-linear regression (in GraphPad Prism 5.0 software) to fit power functions ($Y=aX^b$) to oxygen consumption (in $\mu\text{mol g}^{-1} \text{min}^{-1}$) data at different swimming speeds of individual fish. We extrapolated the curves to zero swimming speed to determine resting metabolic rates (Claireaux et al., 2006). We used oxygen consumption data to calculate the metabolic CoT (in $\mu\text{mol kg}^{-1} \text{m}^{-1}$) at different swimming speeds by dividing oxygen consumption by swimming speed (in m s^{-1} ; Claireaux et al., 2006). We determined the total CoT including resting metabolic rates and oxygen consumed during movement from the raw measurements of oxygen consumption at each swimming speed, and we determined net CoT (CoT_{net}), i.e. oxygen consumed for movement only, by subtracting resting metabolic rates from the oxygen consumption data before calculating CoT (Claireaux et al., 2006). As above, we used non-linear regression to fit power functions to the CoT data of each fish. We integrated the power functions of CoT across all speeds to estimate the aerobic metabolic cost of movement across all speeds for each fish ('integrated cost' $\int\text{CoT}$ and $\int\text{CoT}_{\text{net}}$ in W kg^{-1}). These metrics are useful in determining differences between individuals in the energetic cost of locomotion (Seebacher et al., 2016).

We tested the repeatability of $\int\text{CoT}$ and $\int\text{CoT}_{\text{net}}$ within individuals using the rptR package (Stoffel et al., 2017) in R, which uses mixed-effect models to test for the variance explained by within and between subject effects. The statistical significance of repeatability was tested by permutation tests (Stoffel et al., 2017), and we report the repeatability and permutational P -values.

We tested whether mitochondrial bioenergetics predicted $\int\text{CoT}$ and $\int\text{CoT}_{\text{net}}$ by conducting permutational linear model analyses in the lmPerm package in R (<https://cran.r-project.org/web/packages/lmPerm/>) with $\int\text{CoT}$ or $\int\text{CoT}_{\text{net}}$ as the dependent variable and either P/O ratio, state III respiration or state IV respiration as the independent variable, and Fulton's factor [$\text{mass (g)/length (cm)}^3$] as a covariate to account for possible effects of differences in fish dimensions. Similarly, we tested whether $\int\text{CoT}$ predicted movement in the artificial stream using a permutational linear model, with movement speed when leaving the first pool, total distance moved against the current during the trial and the number of exploration decisions made as dependent variables, and condition factor as a covariate. Data from the acclimation experiment were analysed with a factorial permutational analysis, with acclimation temperature (18 and 28°C), exercise training (exercise and control) and acute test temperature (18 and 28°C) as independent factors. We used fish ID as a random factor to account for repeated measures at the different test temperatures.

We chose permutational methods because they are free of assumptions about underlying distributions, and use the data per se for statistical inference, which is superior to frequentist approaches, particularly when sample sizes are small (relative to the total population) (Drummond and Vowler, 2012; Ludbrook and Dudley,

1998). Permutational analysis randomizes the data set while retaining the data structure (treatment groups and numbers of samples within treatments). Each randomized dataset is compared with the actual dataset to assess the proportion of randomized datasets for which the treatment effects are as great or greater than the experimental dataset. Probabilities are calculated as the number of random data sets that have the same or greater effect as the measured data set divided by the total number of permutations. The null hypothesis is that the treatment effects in the experimental dataset are no greater than in the randomized datasets (Ludbrook and Dudley, 1998). Hence, permutational analyses do not assume underlying distributions and therefore do not have a test statistic such as a t - or F -value (Drummond and Vowler, 2012). lmPerm uses type III sums of squares and implements ANOVA models, but calculates permutation probabilities (Wheeler and Torchiano, 2016).

RESULTS

CoT is a repeatable trait

Oxygen consumption increased non-linearly with increasing water flow rate (Fig. 1A), and CoT decreased with increasing flow rate (Fig. 1B), and responses were similar on both testing days (day 0 and day 10; Fig. 1A,B). There was a threefold difference in integrated CoT ($\int\text{CoT}$) between individuals, and $\int\text{CoT}$ was significantly repeatable within individuals over 10 days (repeatability=0.92, $P<0.001$; Fig. 1C). $\int\text{CoT}$ and $\int\text{CoT}_{\text{net}}$ were linearly related during both testing periods (day 0 and day 10; Fig. 1D).

Mitochondrial bioenergetics did not predict CoT

Mitochondrial P/O ratio ($R^2=0.056$, $P=0.65$), state III respiration ($R^2=0.046$, $P=0.70$) and state IV respiration ($R^2=0.067$, $P=0.59$) did not predict $\int\text{CoT}$ (Fig. 2). The results were similar for $\int\text{CoT}_{\text{net}}$, and there was no significant association between $\int\text{CoT}_{\text{net}}$ and any of the mitochondrial measures (all $R^2<0.05$, all $P>0.35$; Fig. 2).

Acclimation to flow and temperature did not affect CoT

$\int\text{CoT}$ and $\int\text{CoT}_{\text{net}}$ increased with increasing test temperature (both $P<0.0001$), but neither acclimation to different temperatures ($P=0.13$ and $P=0.59$, respectively), acclimation to flowing water ($P=0.84$ and $P=0.88$, respectively) nor their interaction ($P=0.98$ and $P=0.18$, respectively) had a significant effect (Fig. 3).

Exploration in an artificial stream was related to CoT

$\int\text{CoT}$ did not predict the speed at which fish left the first pool ($P=0.36$; Fig. 4A), but fish with greater $\int\text{CoT}$ moved shorter distances during the trial ($P=0.024$, $R^2=0.19$; Fig. 4B). Similarly, the number of decisions made by fish to leave a pool and enter a channel (exploration decisions) decreased with increasing $\int\text{CoT}$ ($P=0.042$; Fig. 4C).

DISCUSSION

We have shown that differences in CoT between individuals are consistent, and that these differences impact on how animals move through their environment. CoT increased significantly with temperature, so that warmer environments elicit greater movement costs. Furthermore, individuals with higher CoT explore less, possibly to reduce the allocation of energy to movement. These data imply that the reduced movement may be the result of an allocation trade-off to preserve energy for growth and reproduction (Angilletta et al., 2003).

We reject the hypothesis that differences in mitochondrial efficiency explain differences in CoT between individuals. During exercise, skeletal muscle uses 90% of oxygen consumed to fuel

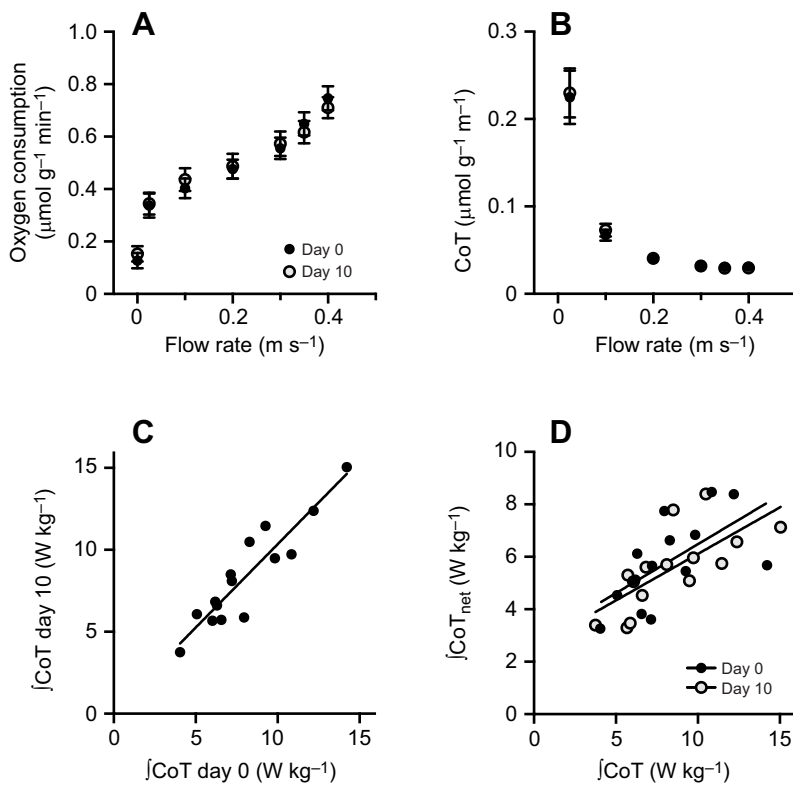


Fig. 1. Repeatability of cost of transport (CoT) within individuals. The mean (\pm s.e.) (A) increase of oxygen consumption and (B) decrease in CoT with increasing water flow rate was similar when measured twice in the same individuals, 10 days apart. (C) The integrated CoT, i.e. the integral of CoT across all speeds within individuals ($JCoT$), was significantly repeatable when measured 10 days apart in the same individuals. (D) There was a linear relationship between $JCoT$ and net integrated CoT ($JCoT_{net}$, calculated after resting metabolic rate was subtracted from the oxygen consumption data shown in A) when measured 10 days apart in the same individuals. Data were analysed with permutational analysis; means \pm s.e. are shown in A and B, data from individual fish are shown in C and D; $n=15$ fish.

contraction and relaxation, and the mitochondrial efficiency of coupling electron transport to ATP production can have a profound influence on energy expenditure (Conley, 2016). For example, experimentally induced uncoupling led to a decrease in exercise efficiency in rats (Schlagowski et al., 2014). When rats were treated with 2,4-dinitrophenol (DNP), which increases the rate of proton leak across the inner-mitochondrial membrane and decreases P/O ratios (Salin et al., 2012), oxygen uptake at rest and during exercise was greater and rats experienced reduced capacity to run at higher speeds (Schlagowski et al., 2014). These data show that mitochondrial uncoupling can directly affect exercise efficiency, and it is surprising that mitochondrial efficiency did not affect CoT in our zebrafish considering that both P/O ratios and CoT were highly variable between individuals.

It is possible that the differences in CoT between individuals arise from differences in ATP use by muscle for contraction and

relaxation. Muscle contraction is stimulated by Ca^{2+} release from the sarcoplasmic reticulum and it is powered by myosin ATPase activity (Gordon et al., 2000; Gundersen, 2011). Calcium cycling dynamics, myosin ATPase activity and cross-bridge dynamics determine the speed of muscle contraction, which can vary between muscles (Gundersen, 2011). Muscle relaxation is mediated by re-sequestering Ca^{2+} back into the sarcoplasmic reticulum, which is principally achieved by the activity of sarco(endo)plasmic reticulum ATPase (SERCA) (Berchtold et al., 2000). The efficiency of SERCA, that is, the amount of Ca^{2+} transported for a given amount of ATP used, differs between SERCA isoforms (Inesi and Tadini-Buoninsegni, 2014). Differences in ATPase activities during muscle contraction and relaxation can therefore change with muscle fibre types and ATPase isoforms, which differ between individuals (James et al., 2011; Simmonds and Seebacher, 2017). It is therefore possible that this variation underpins differences in the CoT between individuals in addition to, or interacting with, differences in mitochondrial efficiencies. Additionally, differences in swimming kinematics may change swimming efficiency. For example, female guppies (*Poecilia reticulata*) had greater swimming efficiency in response to harassment by males over a 5 month period (Killen et al., 2016). The greater efficiency was explained by changes in swimming mechanics because harassed females performed less pectoral fin-assisted swimming (Killen et al., 2016).

We rejected the hypothesis that temperature acclimation and exercise training affect CoT. Higher acclimation temperatures led to higher CoT in the fish *Brachymystax lenok tsinlingensis* (Xia et al., 2016). However, that study did not distinguish between acclimation and acute test temperatures, so the temperature effect may have been due to increased acute temperatures, which we also found and which is consistent with other species (Dickson et al., 2002; Lim and Ellerby, 2009). Thermal acclimation may have affected CoT because proton leak in mitochondria can decrease with decreasing temperatures so that mitochondria are more coupled at lower

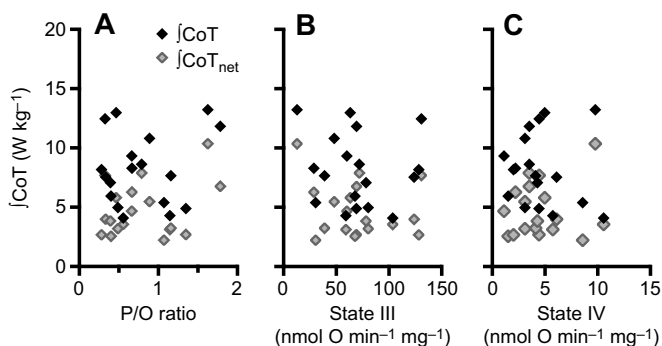


Fig. 2. Integrated CoT is not related to mitochondrial energetics. $JCoT$ or $JCoT_{net}$ were not related to (A) mitochondrial efficiency (ATP/O ratio), (B) maximal substrate oxidation rates or (C) uncoupled rates of mitochondrial oxygen consumption. Data were analysed with permutational analysis; data from individual fish are shown, $n=18$ fish.

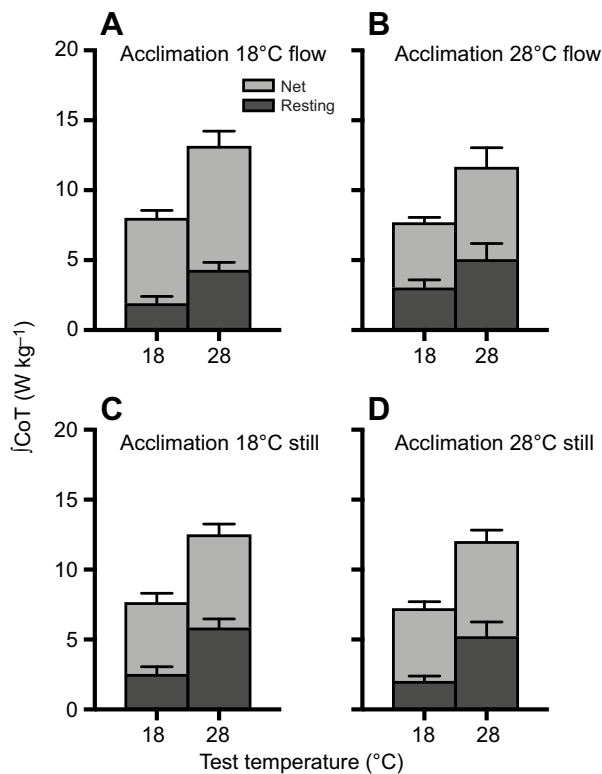


Fig. 3. Integrated CoT did not change with thermal acclimation or exercise training. Acclimation to (A,C) 18°C or (B,D) 28°C, exercise training [(A,B) flow or (C,D) still water control] or their interaction did not change \int CoT, but acute test temperature had a significant effect. \int CoT comprises resting rates of oxygen consumption (dark bars) and net CoT (light grey bars) used for locomotion. Data were analysed with a fully factorial permutational analysis; means \pm s.e. are shown; $n=10$ fish per treatment.

temperatures (Abele et al., 2002). Similarly, exercise training can increase mitochondrial coupling (Conley, 2016) and may thereby decrease CoT, as in the bream *Megalobrama pellegrini* (Li et al., 2017). However, our data show that mitochondrial efficiency is unrelated to CoT, and the lack of response to chronic temperature changes indicates that the efficiency of other mechanisms also does not respond to thermal acclimation (Barclay et al., 2010). The lack of effect of exercise training may be related to the training regime. Our training regime led to higher critical swimming speed (U_{crit}) in zebrafish (Simmonds and Seebacher, 2017). However, different modes of exercise such as sprint or endurance training can elicit different physiological responses (Coffey and Hawley, 2016). The effect of different types of exercise, which may result from different environmental demands for locomotor function, on potential underlying mechanisms determining CoT, such as ATPase efficiency, needs to be tested experimentally.

The absence of acclimation in CoT along with its high repeatability indicate that CoT is a remarkably stable trait within individuals. The stability of CoT has important implications for animals that experience changing environments such as fluctuations in water flow and temperature. For example, changes in water flow and temperature are particularly relevant for migrating fish, which move long distances against a current (Eliason et al., 2011; Martin et al., 2015; Thiem et al., 2016). The energetic cost of movement will be directly proportional to environmental conditions, and success in migration or any other movement objective may decrease as water becomes warmer. These effects may be exacerbated because cardiovascular and metabolic processes can become

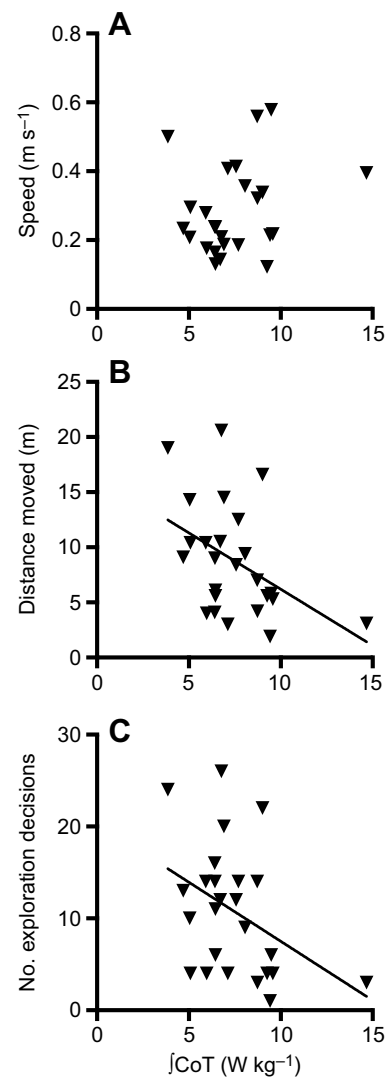


Fig. 4. Integrated CoT modified movement patterns. Movement patterns were measured in an artificial stream. (A) The speed at which fish moved along a narrow channel against a current after voluntarily leaving their home pool to enter a narrow channel did not change with \int CoT. Both (B) the total distance moved against the current and (C) the number of decisions made by fish to leave a pool and enter a channel (exploration decisions) were very variable, but there was a weak but significant trend showing that both decreased with increasing \int CoT. Data were analysed with permutational analysis and significant regression lines are shown; data from individual fish are shown; $n=25$ fish.

compromised at higher temperatures (Eliason et al., 2011). The pronounced differences in CoT between individuals mean that animals with relatively high CoT may be selected against so that warming can change the genetic structure of populations.

Animals may compensate behaviourally and we found that exploration and distances moved were lower in fish with higher CoT, which would be associated with lower energy expenditure. Note, however, that these data were very variable between individuals, and even though there was a significant negative relationship between CoT and dispersal, CoT explained only 15–20% of variability. Reduced exploration may represent an allocation trade-off, where fish reduce movement to maintain energy allocation to other traits such as growth, reproduction and immune function (Lailvaux and Husak, 2014). However, reduced movement by individuals with a higher CoT may compromise

fitness (Cattarino et al., 2015), and decreased exploration of novel environments in fish with high CoT may also mean that these individuals disperse less (Le Roy and Seebacher, 2018). If CoT is a genetically determined trait, as the high repeatability indicates, differences in CoT between individuals may contribute to genetic structuring of dispersing populations. Interestingly, the suggestion that CoT is genetically determined is in line with results from humans, which show that muscle plasticity in response to aerobic training is genetically determined (Bouchard, 2012). Differences in CoT between individuals may contribute to fitness differentials within moving groups, because individuals with a high CoT will have to trade off optimising energy efficiency with staying in the group and gaining the advantages of group living (Krause and Ruxton, 2002; Seebacher and Krause, 2017).

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: F.S.; Methodology: F.S.; Formal analysis: F.S.; Investigation: M.J.; Resources: F.S.; Data curation: F.S.; Writing - original draft: M.J.; Writing - review & editing: F.S.; Supervision: F.S.; Project administration: F.S.; Funding acquisition: F.S.

Funding

This work was supported by the Australian Research Council [Discovery Grant DP160102260 to F.S.].

Data availability

Data have been deposited in the Dryad Digital Repository (Jahn and Seebacher, 2019): doi:10.5061/dryad.6062s6t.

References

- Abele, D., Heise, K., Portner, 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.
- Angilletta, M. J., Jr., Wilson, R. S., Navas, C. A. and James, R. S. (2003). Tradeoffs and the evolution of thermal reaction norms. *Trends Ecol. Evol.* **18**, 234-240. doi:10.1016/S0169-5347(03)00087-9
- Barclay, C. J., Woledge, R. C. and Curtin, N. A. (2010). Is the efficiency of mammalian (mouse) skeletal muscle temperature dependent? *J. Physiol. (Lond)* **588**, 3819-3831. doi:10.1113/jphysiol.2010.192799
- Berchtold, M. W., Brinkmeier, H. and Müntener, M. (2000). Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease. *Physiol. Rev.* **80**, 1215-1265. doi:10.1152/physrev.2000.80.3.1215
- Bouchard, C. (2012). Genomic predictors of trainability. *Exp. Physiol.* **97**, 347-352. doi:10.1113/expphysiol.2011.058735
- Brand, M. D. (2005). The efficiency and plasticity of mitochondrial energy transduction. *Biochem. Soc. Trans.* **33**, 897-904. doi:10.1042/BST0330897
- Cattarino, L., McAlpine, C. A. and Rhodes, J. R. (2015). Spatial scale and movement behaviour traits control the impacts of habitat fragmentation on individual fitness. *J. Anim. Ecol.* **85**, 168-177. doi:10.1111/1365-2656.12427
- Claireaux, G., Couturier, C. and Groison, A.-L. (2006). Effect of temperature on maximum swimming speed and cost of transport in juvenile European sea bass (*Dicentrarchus labrax*). *J. Exp. Biol.* **209**, 3420-3428. doi:10.1242/jeb.02346
- Coffey, V. G. and Hawley, J. A. (2016). Concurrent exercise training: do opposites distract? *J. Physiol. (Lond)* **595**, 2883-2896. doi:10.1113/JP272270
- Conley, K. E. (2016). Mitochondria to motion: optimizing oxidative phosphorylation to improve exercise performance. *J. Exp. Biol.* **219**, 243-249. doi:10.1242/jeb.126623
- Dickson, K. A., Donley, J. M., Sepulveda, C. and Bhoopat, L. (2002). Effects of temperature on sustained swimming performance and swimming kinematics of the chub mackerel *Scomber japonicus*. *J. Exp. Biol.* **205**, 969-980.
- Di Santo, V., Kenaley, C. P. and Lauder, G. V. (2017). High postural costs and anaerobic metabolism during swimming support the hypothesis of a U-shaped metabolism-speed curve in fishes. *Proc. Natl Acad. Sci. USA* **114**, 13048-13053. doi:10.1073/pnas.1715141114
- Dos Santos, R. S., Galina, A. and Da-Silva, W. S. (2013). Cold acclimation increases mitochondrial oxidative capacity without inducing mitochondrial uncoupling in goldfish white skeletal muscle. *Biol. Open* **2**, 82-87. doi:10.1242/bio.20122295
- Drummond, G. B. and Vowler, S. L. (2012). Different tests for a difference: how do we do research? *J. Physiol. (Lond)* **590**, 235-238. doi:10.1113/jphysiol.2011.225235
- Eliason, E. J., Clark, T. D., Hague, M. J., Hanson, L. M., Gallagher, Z. S., Jeffries, K. M., Gale, M. K., Patterson, D. A., Hinch, S. G. and Farrell, A. P. (2011). Differences in thermal tolerance among sockeye salmon populations. *Science* **332**, 109-112. doi:10.1126/science.1199158
- Ghanizadeh Kazerouni, E., Franklin, C. E. and Seebacher, F. (2015). UV-B exposure reduces locomotor performance by impairing muscle function but not mitochondrial ATP production. *J. Exp. Biol.* **219**, 96-102. doi:10.1242/jeb.131615
- Gordon, A. M., Homsher, E. and Regnier, M. (2000). Regulation of contraction in striated muscle. *Physiol. Rev.* **80**, 853-924. doi:10.1152/physrev.2000.80.2.853
- Grigaltchik, V. S., Ward, A. J. W. and Seebacher, F. (2012). Thermal acclimation of interactions: differential responses to temperature change alter predator-prey relationship. *Proc. R. Soc. B* **279**, 4058-4064. doi:10.1098/rspb.2012.1277
- Gundersen, K. (2011). Excitation-transcription coupling in skeletal muscle: the molecular pathways of exercise. *Biol. Rev.* **86**, 564-600. doi:10.1111/j.1469-185X.2010.00161.x
- Halsey, L. G. (2016). Terrestrial movement energetics: current knowledge and its application to the optimising animal. *J. Exp. Biol.* **219**, 1424-1431. doi:10.1242/jeb.133256
- Halsey, L. G., Green, J. A., Twiss, S. D., Arnold, W., Burthe, S. J., Butler, P. J., Cooke, S. J., Grémillet, D., Ruf, T., Hicks, O. et al. (2018). Flexibility, variability and constraint in energy management patterns across vertebrate taxa revealed by long-term heart rate measurements. *Funct. Ecol.* **33**, 260-272. doi:10.1111/1365-2435.13264
- Han, A. X., Berlin, C. and Ellerby, D. J. (2017). Field swimming behavior in largemouth bass deviates from predictions based on economy and propulsive efficiency. *J. Exp. Biol.* **220**, 3204-3208. doi:10.1242/jeb.158345
- Husak, J. F. and Fox, S. F. (2006). Field use of maximal sprint speed by collared lizards (*Crotaphytus collaris*): compensation and sexual selection. *Evolution* **60**, 1888-1895. doi:10.1111/j.0014-3820.2006.tb00532.x
- Husak, J. F., Fox, S. F., Lovorn, M. B. and Van Den Bussche, R. A. (2006). Faster lizards sire more offspring: sexual selection on whole-animal performance. *Evolution* **60**, 2122-2130. doi:10.1111/j.0014-3820.2006.tb01849.x
- Inesi, G. and Tadini-Buoninsegni, F. (2014). Ca²⁺/H⁺ exchange, luminal Ca²⁺ release and Ca²⁺/ATP coupling ratios in the sarcoplasmic reticulum ATPase. *J. Cell Commun. Signal.* **8**, 5-11. doi:10.1007/s12079-013-0213-7
- Jahn, M. and Seebacher, F. (2019). Data from: Cost of transport is a repeatable trait but is not determined by mitochondrial efficiency in zebrafish (*Danio rerio*). Dryad Digital Repository. doi:10.5061/dryad.6062s6t
- James, R. S., Walter, I. and Seebacher, F. (2011). Variation in expression of calcium-handling proteins is associated with inter-individual differences in mechanical performance of rat (*Rattus norvegicus*) skeletal muscle. *J. Exp. Biol.* **214**, 3542-3548. doi:10.1242/jeb.058305
- Jastroch, M., Divakaruni, A. S., Mookerjee, S., Treberg, J. R. and Brand, M. D. (2010). Mitochondrial proton and electron leaks. *Essays Biochem.* **47**, 53-67. doi:10.1042/bse0470053
- Killen, S. S., Croft, D. P., Salin, K. and Darden, S. K. (2016). Male sexually coercive behaviour drives increased swimming efficiency in female guppies. *Funct. Ecol.* **30**, 576-583. doi:10.1111/1365-2435.12527
- Killen, S. S., Marras, S., Nadler, L. and Domenici, P. (2017). The role of physiological traits in assortment among and within fish shoals. *Phil. Trans. R. Soc. B* **372**, 20160233. doi:10.1098/rstb.2016.0233
- Krause, J. and Ruxton, G. D. (2002). *Living in Groups*. Oxford: Oxford University Press.
- Lailvaux, S. P. and Husak, J. F. (2014). The life history of whole-organism performance. *Q. Rev. Biol.* **89**, 285-318. doi:10.1086/678567
- Le Roy, A. and Seebacher, F. (2018). Transgenerational effects and acclimation affect dispersal in guppies. *Funct. Ecol.* **32**, 1819-1831. doi:10.1111/1365-2435.13105
- Li, X. M., Pang, X., Zheng, H., Li, X. J., Fu, S. J. and Zhang, Y. G. (2017). Effects of prolonged exercise training and exhaustive chasing training on the swimming performance of an endangered bream *Megalobrama pellegrini*. *Aquat. Biol.* **26**, 125-135. doi:10.3354/ab00681
- Lim, S. M. and Ellerby, D. J. (2009). The effects of acute temperature change on cost of transport at maximal labriform speed in bluegill *Lepomis macrochirus*. *J. Fish Biol.* **75**, 938-943. doi:10.1111/j.1095-8649.2009.02349.x
- Little, A. G. and Seebacher, F. (2013). Thyroid hormone regulates muscle function during cold acclimation in zebrafish (*Danio rerio*). *J. Exp. Biol.* **216**, 3514-3521. doi:10.1242/jeb.089136
- Ludbrook, J. and Dudley, H. (1998). Why permutation tests are superior to t and F tests in biomedical research. *Am. Stat.* **52**, 127-132. doi:10.2307/2685470
- Martin, B. T., Nisbet, R. M., Pike, A., Michel, C. J. and Danner, E. M. (2015). Sport science for salmon and other species: ecological consequences of metabolic power constraints. *Ecol. Lett.* **18**, 535-544. doi:10.1111/ele.12433
- Miles, D. B. (2004). The race goes to the swift: fitness consequences of variation in sprint performance in juvenile lizards. *Evol. Ecol. Res.* **6**, 63-75.
- Pörtner, H.-O., Hardewig, I. and Peck, L. S. (1999). Mitochondrial function and critical temperature in the Antarctic bivalve, *Laternula elliptica*. *Comp. Biochem. Physiol. A* **124**, 179-189. doi:10.1016/S1095-6433(99)00105-1

- Salin, K., Luquet, E., Rey, B., Roussel, D. and Voituron, Y.** (2012). Alteration of mitochondrial efficiency affects oxidative balance, development and growth in frog (*Rana temporaria*) tadpoles. *J. Exp. Biol.* **215**, 863-869. doi:10.1242/jeb.062745
- Salin, K., Auer, S. K., Rey, B., Selman, C. and Metcalfe, N. B.** (2015). Variation in the link between oxygen consumption and ATP production, and its relevance for animal performance. *Proc. R. Soc. B* **282**, 20151028-9. doi:10.1098/rspb.2015.1028
- Schlagowski, A. I., Singh, F., Charles, A. L., Gali Ramamoorthy, T., Favret, F., Piquard, F., Geny, B. and Zoll, J.** (2014). Mitochondrial uncoupling reduces exercise capacity despite several skeletal muscle metabolic adaptations. *J. Appl. Physiol.* **116**, 364-375. doi:10.1152/jappphysiol.01177.2013
- Seebacher, F. and Krause, J.** (2017). Physiological mechanisms underlying animal social behaviour. *Phil. Trans. R. Soc. B* **372**, 20160231. doi:10.1098/rstb.2016.0231
- Seebacher, F., Borg, J., Schlotfeldt, K. and Yan, Z.** (2016). Energetic cost determines voluntary movement speed only in familiar environments. *J. Exp. Biol.* **219**, 1625-1631. doi:10.1242/jeb.136689
- Simmonds, A. I. M. and Seebacher, F.** (2017). Histone deacetylase activity modulates exercise-induced skeletal muscle plasticity in zebrafish (*Danio rerio*). *Am. J. Physiol. Regul. Int. Comp. Physiol.* **313**, R35-R43. doi:10.1152/ajpregu.00378.2016
- Stoffel, M. A., Nakagawa, S. and Schielzeth, H.** (2017). rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods Ecol. Evol.* **67**, 1-6. doi:10.1111/2041-210X.12797
- Thiem, J. D., Dawson, J. W., Hatin, D., Danylchuk, A. J., Dumont, P., Gleiss, A. C., Wilson, R. P. and Cooke, S. J.** (2016). Swimming activity and energetic costs of adult lake sturgeon during fishway passage. *J. Exp. Biol.* **219**, 2534-2544. doi:10.1242/jeb.140087
- Wilson, R. S., Husak, J. F., Halsey, L. G. and Clemente, C. J.** (2015). Predicting the movement speeds of animals in natural environments. *Integr. Comp. Biol.* **55**, 1125-1141. doi:10.1093/icb/icv106
- Xia, J., Ma, Y., Fu, C., Fu, S. and Cooke, S. J.** (2016). Effects of temperature acclimation on the critical thermal limits and swimming performance of *Brachymystax lenok tsinlingensis*: a threatened fish in Qinling Mountain region of China. *Ecol. Res.* **32**, 61-70. doi:10.1007/s11284-016-1418-z