Group movement dynamics improves aerobic performance and conserves anaerobic energy in schooling fish

Short title: Energy conservation by schooling fish

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Abstract:

Schooling fish move in a viscous fluid environment and are a model system for understanding how group movement dynamics enables energy conservation¹. But how group movement in the water reduces locomotor cost remains elusive. We measured both aerobic and anaerobic metabolic contributions to energy use in schools of giant danio, and show an energetic reduction per tail beat of up to 126% compared to solitary fish. Fish in schools used a smaller proportion of their aerobic capacity to swim and reduced the anaerobic cost by 2.8 fold, which lowered the total energy expenditure and cost of transport by up to 112% at higher speeds. Fish schools also exhibit a U-shaped metabolism–speed curve, with a minimum metabolic cost at 1 body length s⁻¹, the migratory speed of diverse fish species²,³. This finding suggests that physical constraints on movement through water may be a key driver for the evolution of active directional schooling behaviour in fishes.

One-Sentence Summary: Fish schools reduce the metabolic cost of swimming by up to 112% and the energy used per tail beat of up to 126% compared to solitary fish.
Main Text: Newton’s laws of motion underpin animal locomotion, from fine-scale maneuvers to long-distance migrations. Since fluid drag scales as velocity squared, movement at higher speeds places a premium on mechanisms to conserve energy. As speed increases or during migration involving sustained movement over long distances, animals often move in groups: e.g., migratory birds in V-formations, cyclists in a peloton, elite marathon runners and fish schools. To overcome gravitational or fluid dynamic resistance to forward motion, animals use metabolism to generate energy and sustain movement in the air, on land, and in the water. The total energy cost of locomotion is met by the amount of energy generated by two metabolic energy sources in vertebrates. Energy use at lower speeds is primarily aerobic, while for high-speed movement anaerobic metabolic pathways are activated to supply the additional (largely shorter-term) energy needs. While whole-animal aerobic metabolism is measured by oxygen (O$_2$) uptake ($\dot{V}O_2$), the non-aerobic O$_2$ cost (mostly substrate-level phosphorylation) is measured as excess post-exercise O$_2$ consumption (EPOC). Both metabolic energy sources provide the total energy expenditure (TEE) required for movement, and the partitioning of aerobic and anaerobic energy contribution changes as the speed of locomotion increases.

In water, a fluid that is 50-times more viscous than air, the need to reduce hydrodynamic drag for energy conservation is even greater than for aerial or terrestrial locomotion. Here, we use fish schooling as a model system to explore how the hydrodynamics of group movement can enable energetic savings compared to locomotion by solitary individuals. Hydrodynamic models, kinematic studies, and experimental robotic analyses of fish schools indicate that the cost of swimming can be reduced when fish swim beside neighbouring fish, directly behind or in front of another fish, or in-between leading individuals (Fig. S1). But direct measurements of the metabolic cost of locomotion in fish schools compared to solitary individuals are lacking, and thus how fish schooling behaviour could reduce both the aerobic and anaerobic cost of
locomotion is unknown. No previous study has directly measured the energy use of fish schools over speeds that range from low-speed group locomotion to high-speed directional swimming where the anaerobic metabolic cost of locomotion can be substantial.

We hypothesized that schooling dynamics reduces the TEE in fishes exhibiting active directional schooling (Table S1) over a range of speeds compared to the energy used by solitary fish. We predicted that if collective energy expenditure can be minimized, then fish schools should exhibit a J-shaped metabolism-speed curve, and that at high speeds schooling dynamics should enable fish to reduce the anaerobic contribution to TEE and maintain swimming speed compared to anaerobic energy use by solitary fish. A reduction in the anaerobic metabolic contribution at high swimming speeds as a result of schooling dynamics also would reduce the recovery time needed to restore the resting metabolic state compared to locomotion by solitary fish. Furthermore, we expected that fish within schools could become more energetically efficient by reducing the amount of energy expended per tail beat.

To evaluate these hypotheses, we directly measured aerobic and anaerobic energy used by schooling fishes over a wide range of swimming speeds (0.3–8.0 body lengths s⁻¹; BL s⁻¹), and then compared the swimming cost to that of solitary fish. We equipped a high-resolution swim-tunnel respirometer with two orthogonal high-speed cameras mounted on the respirometry system. We simultaneously measured $\dot{M}O_2$, the three-dimensional dynamics of fish schools (n=5) and the kinematics of solitary fish (n=5) (Suppl. Material). This enabled us to focus on the energetics and schooling dynamics of a model species, giant danio (*Devario aequipinnatus*), that exhibits active directional group swimming from minimal speed to maximum sustained speeds (equivalent to a Reynolds number range of $6.4 \times 10^3$ to $1.8 \times 10^5$; Fig. S2), using a critical swimming speed test ($U_{crit}$).
We discovered that both fish schools and solitary fish have a U-shaped metabolism-speed curve over the lower portion of their speed range (0.3–3 BL s\(^{-1}\), Fig. 1A, D). Danio schools have a minimum aerobic cost ($\dot{M}O_{2\text{min}} = 212.9$ mg O\(_2\) kg\(^{-1}\) h\(^{-1}\)) at 1.25 BL s\(^{-1}\). $\dot{M}O_{2\text{min}}$ at this optimal speed ($U_{\text{opt}}$) was lower than both the $\dot{M}O_2$ of aggregating behaviours exhibited before $U_{\text{crit}}$ test (Table S1, Fig. S3) and for the group at rest in still water $\dot{M}O_2$ (by 54% and 26% respectively) ($F = 16.7$, $p \leq 0.02$, Fig. 1A,B). Swimming at $U_{\text{opt}}$ reduces the energetic cost below that of swimming at either slower or higher speeds. Indeed, the Strouhal number of fish schools decreased from 2.1 at the lowest speed to 0.3 at the energetic minimum $U_{\text{opt}}$, and then increased to 0.4 at the higher $U_{\text{crit}}$. We also characterized the kinematic features that result in elevated $\dot{M}O_2$ at low speeds. Danio swimming in low-speed aggregations had a higher tail beat frequency ($f_{TB}$) than solitary fish ($F \geq 9.8$, $p \leq 0.002$). However, at $U_{\text{opt}}$ and higher speeds when group directional schooling began, fish in schools had a lower $f_{TB}$ ($F \geq 4.6$, $p \leq 0.035$) than solitary fish (Fig. 3B). The angular heading of schooling fish transitioned from omnidirectional to pointing against water flow when speed is above 0.75 BL s\(^{-1}\), and individual fish orient into the flow starting at 0.3 BL s\(^{-1}\) (Fig. 3C). Although body angle and turning frequency of solitary fish and schools decreased with speed, schooling fish had a higher turning frequency ($F \geq 15.6$, $p < 0.002$) below 0.75 BL s\(^{-1}\) than solitary fish (Fig. 3D,E).

We discovered that, across the entire 0.3–8 BL s\(^{-1}\) range, $\dot{M}O_2$-speed curves of fish schools are J-shaped (reached 1053.5 mg O\(_2\) kg\(^{-1}\) h\(^{-1}\) at 8 BL s\(^{-1}\)) whereas solitary fish showed an S-shaped curve (reaching a plateau of 760.2 mg O\(_2\) kg\(^{-1}\) h\(^{-1}\) & ~10% CV) in 6–8 BL s\(^{-1}\) (Fig. 1C), demonstrating that schooling dynamics results in a 39% higher aerobic performance ($F = 30.0$, $p < 0.001$). At high swimming speeds, aerobic limits are reached, and anaerobic metabolism is engaged to permit continued movement\(^{13}\). Often vertebrates at higher speeds use more fast twitch fibers which generate high-frequency contractile force in part through anaerobic glycolysis\(^{14,15}\).
Given that fish schools have a lower $f_{TB}$ than the solitary fish at 8 BL s$^{-1}$ ($F = 15.1, p < 0.001$), we predicted that fish schools, compared with solitary fish, use less anaerobic energy to supplement aerobic energy during exercise. Hence, we measured post-exercise O$_2$ utilization which is used to restore glycolytically-induced metabolic perturbations (EPOC). We discovered that fish schools have a 2.8-fold lower EPOC ($t = 4.5, p = 0.0021$), and recover 1.7-times faster than solitary fish (8 vs 14 h; $t = 2.8, p = 0.025$; Fig. 1E,F).

Physiological studies of exercise metabolism and locomotion suggest that a higher proportional use of aerobic scope during locomotion relates to a larger accumulation of anaerobic end-products$^9$. We show that schooling dynamics results in a lower use of aerobic capacity compared to solitary fish over 0.3–8 BL s$^{-1}$. Collectively, fish schools used a 36% lower proportion of their aerobic scope than solitary fish (Wilcoxon test: $p = 0.0002$, Fig. 1H). Solitary fish used 38% higher proportion of aerobic scope at 4 BL s$^{-1}$ (50% $U_{crit}$ & > 50% aerobic scope onward), and consistently used ~25% higher proportion of their aerobic scope at 6 and 7 BL s$^{-1}$ than fish schools ($F \geq 4.8, p \leq 0.03$, Fig. 1G). Fish often engage glycolysis for swimming above 50% $U_{crit}$ and ~50% aerobic scope is a commonly observed inflection point of faster anaerobic end-product accumulation$^9$. Collectively, reduced aerobic capacity for swimming above 50% $U_{crit}$ in fish schools likely played a key role in reducing the use of glycolysis for high-speed swimming$^{10,16}$.

To overcome hydrodynamic drag during swimming, both aerobic and anaerobic metabolic pathways simultaneously fuel muscles to generate locomotor thrust. To estimate the relative proportions of aerobic and anaerobic energy contributions at each swimming speed, we modeled EPOC in addition to $\dot{M}_O_2$ (> 50% $U_{crit}$ & aerobic scope, Fig. 2A) (Table S2). We also estimated a total O$_2$ cost during the entire swimming process for each school and individual and calculated total energy expenditure (TEE)$^{10}$. The TEE of fish schools was 62–112% lower than...
that of the solitary fish between 5–8 BL s\(^{-1}\) \((F \geq 7.4, p \leq 0.008; \text{Fig. 2D})\). TEE of fish schools was only 42–143 % higher than the aerobic metabolic rate at 5–8 BL s\(^{-1}\) \((F \geq 3.5, p \leq 0.001)\) and anaerobic metabolic energy only accounted for 29–58 % of the TEE depending on speed (Fig. 2B). In contrast, TEE of solitary fish was 131–465 % higher than the aerobic metabolic rate between 5–8 BL s\(^{-1}\) \((F \geq 10.7, p \leq 0.001)\), where anaerobic energy accounted for 62–81 % of the TEE depending on speed (Fig. 2C).

Schooling dynamics reduced the total cost (aerobic plus anaerobic) of transport (TCOT) by an average of 43 % compared to swimming alone \((F = 6.9, p = 0.01)\), and most of this energy conservation happens at higher speeds. Schooling dynamics in danio enables an extremely shallow rate of increase in TCOT with speed compared to that of an individual (Fig. 2E). The TCOT of solitary fish increased by 490 % \((6.5 \text{ kJ km}^{-1} \text{ kg}^{-1})\) at 8 BL s\(^{-1}\), whereas the schooling TCOT increased by only 200 % \((3.6 \text{ kJ km}^{-1} \text{ kg}^{-1})\) at 8 BL s\(^{-1}\). Notably, the TCOT of fish schools at a swim speed of 3 BL s\(^{-1}\) \((-1.2 \text{ kJ km}^{-1} \text{ kg}^{-1})\) is less than the TCOT \((1.5 \text{ kJ km}^{-1} \text{ kg}^{-1})\) of jellyfish moving at <2 BL s\(^{-1}\) \((4)\), one of the most energetically efficient low-speed swimmers.

To answer the question of how schooling dynamics reduce TEE, we combine video analysis of fish tail beat kinematics with simultaneous aerobic and anaerobic measurements to compute energy expended \(\textit{per tail beat (beat}^{-1})\), and compared values for fish in schools to those for solitary fish. Schooling fish reduced TEE\(\text{beat}^{-1}\) by 44–126 % at higher speeds compared to solitary fish, a substantial reduction in TEE\(\text{beat}^{-1}\) consumed both by the school, and by fish in a school (Fig. 3A). Notably, the energetic benefits during active directional schooling occur when fish schools become more streamlined as school length increased with speed and plateaued beyond 2 BL s\(^{-1}\) (Fig. 3F), while the three-dimensional distance among individuals stayed relatively constant at ~1.2 BL (Fig 3G). These results directly demonstrate schooling dynamics.
benefits the swimming kinematics of individual fish and results in a net outcome of up to 112% TEE reduction in fish schools compared to solitary fish.

Although the benefits of reducing the energetic cost of locomotion is likely not a major factor underlying the behaviours of low-speed milling\textsuperscript{18} and aggregation\textsuperscript{19} in fishes, energy conservation is critical for active directional swimming occurring at speeds above 3 BL s\textsuperscript{-1} that are particularly important for evolutionary fitness, e.g., fish swimming in predator-avoidance bait balls\textsuperscript{20}, during an escape from predators, and food searching group motion\textsuperscript{21}. In nature, fish routinely exhibit active directional group locomotion above ~6 BL s\textsuperscript{-1}(Fig. S4, Table S3)\textsuperscript{22}, a speed that engages anaerobic metabolism. Yet no previous measurement of the anaerobic costs of schooling energetics is available and only one direct measurement of aerobic swimming cost has been reported for a group of four eels\textsuperscript{23} at low (aerobic) speeds. Hence, one of the unrecognized but key benefits of active directional group swimming is increased aerobic performance, a reduced need for aerobic capacity and anaerobic energy, and a resultant faster recovery from the associated metabolic perturbations and costs.

Quantifying the energetic cost of movement over a wide speed range is directly relevant to understanding fish migratory speeds. The energy use of fish schools shows a U-shaped curve (< 3 BL s\textsuperscript{-1}) where swimming at ~1.0 BL s\textsuperscript{-1} consumes less energy than at slower speeds while swimming at 3 BL s\textsuperscript{-1} consumes a similar amount of energy to movement at 0.3 BL s\textsuperscript{-1} (Fig. 1D). Although \textit{Devario aequipinnatus} is not a migratory species, this species does exhibit active directional schooling behaviour and has been a model species for analyses of fish schooling behaviour. These results demonstrate that fish moving in schools can collectively manifest a minimal absolute energetic cost at a mean group swimming speed of ~1.0 BL s\textsuperscript{-1}. Indeed, long-distance swimming commonly occurs in the speed range of 0.5 to 1.5 BL s\textsuperscript{-1} as recorded by tags on migrating fish\textsuperscript{2} (Fig. S4; Tables S1, S3.).
The substantial energy conservation resulting from schooling dynamics is critical to understanding rapid collective movement in animals. Fish schooling in a viscous fluid, such as water where drag forces are high, serves as a model for understanding how group movement by animals can be a more energy-efficient biological system than movement by isolated individuals. Individuals in groups gain hydrodynamic benefits to achieving energy conservation, especially when moving at high speeds where there is a premium on energy savings. When a lower proportion of metabolic capacity is devoted to locomotion, animals can apportion more energy to other fitness-related activities, such as digestion, growth and reproduction. More broadly, understanding how collective dynamics of animal movements in the water, land, and air can modify the energy use profiles of individuals will allow us to better understand the ecological, evolutionary and robotic implications of group locomotion.

Methods

Experimental animals

The experiments were performed on giant danio (*Devario aequipinnatus*) that were acquired from a local commercial supplier near Boston, Massachusetts USA (Table S4). Five schooling groups are randomly distributed and housed separately in five 37.9 l aquaria (n=8 per tank). The five solitary individuals are housed separately in five 9.5 l aquaria (n=1 per tank). All aquaria have self-contained thermal control (28 °C), an aeration system (>95 % air saturation, % sat.) and a filtration system. Water changes (up to 50% exchange ratio) were carried out weekly. Fish were fed *ad libitum* daily (TetraMin, Germany). Animal holding and experimental procedures were approved by the Harvard Animal Care IACUC Committee (protocol number 20-03-3).
Integrated Biomechanics & Bioenergetic Assessment Platform (IBBAP)

The core of our experimental system is a 9.35-l (respirometry volume plus tubing) customized Loligo® swim-tunnel respirometer (Tjele, Denmark). The respirometer has an electric motor, and a sealed shaft attached to a propeller located inside the respirometer. By regulating the revolutions per minute (RPM) of the motor, the water velocity of the motor can be controlled. The linear regression equation between RPM and water velocity (V) is established (V = 0.06169*RPM – 5.128, R² = 0.9988, p < 0.0001) by velocity field measured by particle image velocimetry (PIV).

The swim-tunnel respirometer is oval-shaped. The central hollow space of the respirometry increases the turning radius of the water current. As a result, the water velocity passing the cross-section of the swimming section (80 × 80 × 225 mm) is more homogenous (validated by PIV). Moreover, a honeycomb flow straightener (80 × 80 × 145 mm) is installed in the upstream of the swimming section to create laminar flow (validated by PIV).

To increase the signal-to-noise ratio for the measurement of water dissolved O₂, a water homogenous loop is installed 95 cm downstream of the propeller and the water is returned to the respirometer 240 cm before the swimming section. The flow in the water homogenous loop moves (designated in-line circulation pump, Universal 600, EHEIM GmbH & Co KG, Deizisau, Germany) in the same direction as the water flow in the swimming tunnel. A high-resolution fibre optic O₂ probe (Robust oxygen probe OXROB2, PyroScience GmbH, Aachen, Germany) is sealed in the homogenous loop at the downstream of the circulation pump (better mixing) to continuously measure the dissolved O₂ level in the water (recording frequency ~1 Hz, response time < 15s). The oxygen probe was calibrated to anoxic (0 % sat., a solution created by super-saturated sodium sulphite and bubbling nitrogen gas) and fully aerated water (100 % sat.). The background \( \dot{M}_O_2 \) in the swim-tunnel respirometer was measured for 20 min before and after each
trial. The average background $\dot{M}O_2$ (< 6% of fish $\dot{M}O_2$) was used to correct for the $\dot{M}O_2$ of fish.

The pre-filtered water (laboratory grade filtration system) is constantly disinfected by UV light (JUP-01, SunSun, China) located in an external water reservoir to suppress the growth of microbials. Water changes of 60% total volume occurred every other day and a complete disinfection by sodium hypochlorite is conducted weekly (Performance bleach, Clorox & 1000 ppm).

To simultaneously measure schooling dynamics and swimming kinematics, the customized oval-shaped swim-tunnel respirometer is located on a platform with an open window beneath the swimming section. The platform is elevated 243 mm above the base to allow a front surface mirror to be installed at a 45° angle. This mirror allows a high-speed camera (FASTCAM Mini AX50 type 170K-M-16GB, Phontron Inc., United States, lens: Nikon 50mm F1.2, Japan) to record the ventral view. The second camera (FASTCAM Mini AX50 type 170K-M-16GB, Phontron Inc., United States, lens: Nikon 50mm F1.2, Japan) is positioned 515 mm to the side of the swimming section to record a lateral view. Synchronized lateral and ventral video recordings were made at 125 fps, and each frame was 1024 by 1024 pixels. To avoid light refraction passing through the water and distorting the video recordings, the swim-tunnel respirometry is not submerged in the water bath. Temperature regulation of the respirometer is achieved by regulating room temperature, installing thermal insulation layers on the respirometry and replenishing the water inside the respirometer from a thermally regulated (28 °C, heater: ETH 300, Hydor, United States & chiller: AL-160, Baoshishan, China) water reservoir (insulated 37.9-l aquarium) located externally.

The aerated (100% sat., air pump: whisper AP 300, Tetra, China) reservoir water is flushed (pump: Universal 2400, EHEIM GmbH & Co KG, Deizisau, Germany) to the respirometer through an in-line computer-controlled motorized ball valve (U.S. Solid) installed at the in-flow...
tube. The other in-line one-way valve is installed at the out-flow tube. The out-flow tube is also equipped with a valve. The value is shut during the measurement period, a precautionary practice to eliminate the exchange of water between the respirometer and the external reservoir when the water moves at a high velocity inside the respirometer. This flushing was manually controlled to maintain DO above 80% sat. Every time the respirometer was closed to measure \( \dot{M}O_2 \), the water temperature fluctuates no more than 0.2 °C. The water temperature inside the respirometer is measured by a needle temperature probe (Shielded dipping probe, PyroScience GmbH, Aachen, Germany) sealed through a tight rubber port of the respirometer.

To allow fish to reach the undisturbed quiescent state during the trial, the entire Integrated Biomechanics & Bioenergetic Assessment Platform (IBBAP) is covered by laser blackout sheet (Nylon Fabric with Polyurethane Coating; Thorlabs Inc, New Jersey, United States). The room lights are shut off and foot traffic around the experimental rig is restrained to the absolute minimum. Fish are orientated by dual small anterior spots of white light (lowest light intensity, Model 1177, Cambridge Instruments Inc, New York, United States) for orientation (one to the top and the other to the side) of the swimming section. The test section is illuminated by infrared light arrays.

**Experimental Protocol**

We studied five replicate schools and five replicate individuals drawn from within each school. Swimming performance test trials were conducted with *Devario aequipinnatus* fasted for 24 hours, a sufficient period for a small species at 28 °C (*i.e.* high resting \( \dot{M}O_2 \)) to reach an absorptive state. In fact, we observed no specific dynamic action, the amount of oxygen consumed for digestion during the first diurnal cycle (Fig. S3). Prior to the swimming performance test, testing fish were gently weighted and placed in the swim-tunnel respirometer.
The fish swam at 35% \( U_{\text{crit}} \) for 30 mins to help oxidize the inevitable but minor lactate accumulation during the prior handling and help fish become accustomed to the flow conditions in the swim-tunnel respirometer \(^{24}\). After this time, the fish to be tested were habituated (>20 hours) to the respirometer environment under quiescent and undisturbed conditions. During this time, we used an automatic system to measure the resting \( \dot{M}O_2 \) for at least 19 hours. Relays (Cleware GmbH, Schleswig, Germany) and software (AquaResp v.3, Denmark) were used to control the intermittent flushing of the respirometer with fresh water throughout the trial to ensure \( O_2 \) saturation of the respirometer water. \( \dot{M}O_2 \) was calculated from the continuously recorded dissolved \( O_2 \) level (at 1 Hz) inside the respirometer chamber. The intermittent flow of water into the respirometer occurred over 930 s cycles with 30 s where water was flushed into the respirometer and 900 s where the pumps were off and the respirometer was a closed system. The first 240 s after each time the flushing pump was turned off were not used to measure \( \dot{M}O_2 \) to allow \( O_2 \) levels inside the respirometer to stabilize. The remaining 660 s when the pumps were off during the cycle were used to measure \( \dot{M}O_2 \). The in-line circulation pump for water in the \( O_2 \) measurement loop stayed on throughout the trial.

We characterize the swimming performance of fish using an established incremental step-wise critical swimming speed (\( U_{\text{crit}} \)) test\(^{10}\). The first preliminary trial determined the \( U_{\text{crit}} \) of this population of *Devario aequipinnatus* as 8 BL s\(^{-1}\). Characterizing the swimming performance curve required a second preliminary trial to strategically select 10 water velocities (0.3, 0.5, 0.8, 1.0, 1.3, 1.5, 1.8, 2.3, 2.8 BL s\(^{-1}\)) to bracket the hypothesized \( U \)-shaped metabolism-speed curve at the lower speed (< 40% \( U_{\text{crit}} \)). Additional five water velocities (3.8, 4.9, 5.9, 6.9, 8.0 BL s\(^{-1}\)) are used to characterize the exponentially increasing curve to the maximum and sustained swimming speed, \( U_{\text{crit}} \) (see Fig. S5). Altogether, 14 points provide a reliable resolution to characterize the swimming performance curve. At each water velocity, fish swam for 10 mins\(^{25}\).
to reach a steady state in $\dot{M}O_2$ at low speeds (see Fig. S6). Above 40% $U_{\text{crit}}$, $\dot{M}O_2$ can become more variable\textsuperscript{26}. Hence, in this protocol, we focus on measuring the sustained aerobic energy expenditure by calculating the average $\dot{M}O_2$ for each 10-min velocity step using Eqn 1. The respirometry system reaches a stable signal-to-noise ratio once the sampling window is longer than 1.67 mins (see Fig. S7), well within the duration of the velocity step to obtain a stable signal-to-noise ratio for calculating $\dot{M}O_2$\textsuperscript{26}. At the 5th min of each velocity step, both ventral and lateral-view cameras are triggered simultaneously to record 10-sec footage at 125 frames per second, at 1/1000 shutter speed and 1024×1024 pixel resolution. Thus, both data streams of $\dot{M}O_2$ and high-speed videos are recorded simultaneously. The $U_{\text{crit}}$ test is terminated when 12.5% of fish in the school or a solitary individual touches the back grid of the swimming section for more than 20 secs\textsuperscript{24}. The $U_{\text{crit}}$ test lasted ~140 mins and estimates the aerobic portion of energy expenditure over the entire range of swimming performance.

To measure the contribution of non-aerobic O\textsubscript{2} cost, where the majority of the cost is related to substrate-level phosphorylation, and to calculate the total energy expenditure for swimming over the entire speed range, we measured excess post-exercise oxygen consumption (EPOC) after the $U_{\text{crit}}$ test for the ensuing 19 hours, recorded by an automatic system. Most previous measurements of EPOC have used a duration of ~5 hours, but our extended measurement period ensured that longer duration recovery O\textsubscript{2} consumption during EPOC was measured completely as fish were exercised to $U_{\text{crit}}$ (see summary table in 16). The intermittent flow of water into the respirometer occurred over 30 s to replenish the dissolved O\textsubscript{2} level to ~95% sat. For the following 900 s the flushing pump remained closed, and the respirometer becomes a closed system, with the first 240 s to allow O\textsubscript{2} saturation inside the respirometer to stabilize. The remaining 660 s when the flushing pump was off during the cycle were used to measure $\dot{M}O_2$ (see Eqn 1). The cycle is automated by computer software (AquaResp v.3) and provided 74
measurements of $\dot{M}O_2$ to compute EPOC. Upon the completion of the three-day protocol, the school or individual fish are returned to the home aquarium for recovery. The fish condition was closely monitored during the first 48 hours after the experiment, during which no mortality was observed.

**Bioenergetic measurement and modeling**

To estimate the steady-rate whole-animal aerobic metabolic rate, $\dot{M}O_2$ values were calculated from the sequential interval regression algorithm (Eqn. 1) using the dissolved O$_2$ (DO) points continuously sampled (~1 Hz) from the respirometer.

$$\dot{M}O_2 = \frac{[d_{DO}(l,i+a)]}{d_{i}(l,i+a)} \cdot \left( V_r - V_f \right) \cdot S_o \left/ (t \cdot M_f \right) \quad \text{(Eqn. 1)}$$

Where $d_{DO}/d_i$ is the change in O$_2$ saturation with time, $V_r$ is the respirometer volume, $V_f$ is the fish volume (1 g body mass = 1 ml water), $S_o$ is the water solubility of O$_2$ (calculated by AquaResp v.3 software) at the experimental temperature, salinity and atmospheric pressure, $t$ is a time constant of 3600 s h$^{-1}$, $M_f$ is fish mass, and $a$ is the sampling window duration, $i$ is the next PO$_2$ sample after the preceding sampling window.

To account for allometric scaling, the $\dot{M}O_2$ values of solitary fish were transformed to match the size of the individual fish in the school (see Table S4) using an allometric scaling exponent ($b = 0.7546$). The calculation of scaling relationship \[\log_{10}(\dot{M}O_2) = b \cdot \log_{10}(M) + \log_{10}(a),\] where $M$ is the body mass & $a$ is a constant] were performed by least squares linear regression analysis ($y = 0.7546 \cdot x + 0.2046; R^2 = 0.6727, p < 0.0001$) on the 180 data points of metabolic rate and body mass from a closely related species (the best available dataset to our knowledge$^{28}$. The allometrically scaled $\dot{M}O_2$ values were used to derive other energetic metrics (listed below)
for the solitary fish. The energetic metrics of fish schools are calculated from the mass-specific 
\( \dot{M}O_2 \).

The resting oxygen uptake (\( \dot{M}O_{2\text{rest}} \)), the minimum resting metabolic demands of a group of fish or a solitary individual, is calculated from a quantile 20\% algorithm\(^9\) using the \( \dot{M}O_2 \) estimated between the 10\(^{th}\)–18\(^{th}\) hour and beyond the 32\(^{nd}\) hour of the trial. These are the periods of quiescent state when fish completed the EPOC from handling and swimming test.

The \( \dot{M}O_2 \) for the aggregation (Fig. 1B, \( \dot{M}O_{2\text{aggregate}} \)) is calculated as the average value using the \( \dot{M}O_2 \) estimated between the 10\(^{th}\)–18\(^{th}\) hour without the effects of any tests.

Minimum oxygen uptake (\( \dot{M}O_{2\text{min}} \)) is the lowest \( \dot{M}O_2 \) value recorded in the entire trial, which always occurred at the optimal speed when a school of fish collectively reached the lowest \( \dot{M}O_2 \) value.

Active oxygen uptake (\( \dot{M}O_{2\text{active}} \)) is the highest average \( \dot{M}O_2 \) when fish are actively swimming\(^{10}\).

The aerobic scope is the numerical difference between \( \dot{M}O_{2\text{active}} \) and \( \dot{M}O_{2\text{min}} \) (i.e. \( \dot{M}O_{2\text{active}} - \dot{M}O_{2\text{min}} \))\(^{10}\).

The excess post-exercise oxygen consumption (EPOC) is an integral area of \( \dot{M}O_2 \) measured during post-exercise recovery, from the end of \( U_{\text{crit}} \) until reached \( \dot{M}O_{2\text{rest}} \) plus 10\%\(^{27}\). This approach reduces the likelihood of overestimating EPOC due to spontaneous activities\(^{27}\). To account for the allometric scaling effect, we used the total amount of \( O_2 \) consumed (mg \( O_2 \)) by the standardized body mass of fish (1.66 g) for fish schools and solitary fish.
We model EPOC (i.e. non-aerobic O$_2$ cost) to estimate a total O$_2$ cost over the duration of the swimming performance test. Our conceptual approach was pioneered by Brett$^{10}$ in fish and is also used in sports science$^9$. Mathematical modeling was applied to study the effects of temperature on the cost of swimming for migratory salmon$^{24}$. We improved the mathematical modeling by applying the following physiological and physics criteria. The first criterion is that significant accumulation of glycolytic end-product occurred when fish swimming above 50% $U_{\text{crit}}$ which corresponds to > 40% $\dot{M}O_{\text{max}}$ (or ~ 50% aerobic scope)$^9$. This is also when fish start unsteady-state burst-&-glide swimming gait$^{16}$. The second criterion is that the integral area for the non-aerobic O$_2$ cost during swimming can only differ by ≤ 0.09% when compared to EPOC. The non-aerobic O$_2$ cost during swimming is the area bounded by modeled $\dot{M}O_2$ and measured $\dot{M}O_2$ as a function of time when fish swim > 50% $U_{\text{crit}}$ (see Fig. 2A & Table S2). The third criterion is that total energy expenditure is expected to increase exponentially with swimming speed (Fig. S8). Specifically, these curves were fitted by power series or polynomial models, the same models that describe the relationship between water velocity and total power and energy cost of transport (Fig. S8). Following these criteria, the non-aerobic O$_2$ cost at each swimming speed is computed by a percentage (%) modifier based on the aerobic O$_2$ cost (Table S2). The exponential curve of total O$_2$ cost as swimming speed of each fish school or solitary individual was derived by an iterative process until the difference between non-aerobic O$_2$ cost and EPOC met the 2nd criterion. The sum of non-aerobic O$_2$ cost and aerobic cost gives the total O$_2$ cost.

Total energy expenditure (TEE) is calculated by converting total O$_2$ cost to kJ × kg$^{-1}$ using an oxy-calorific equivalent of 3.25 cal per 1 mg O$_2$ $^{30}$.

Cost of transport (COT), in kJ × km$^{-1}$ × kg$^{-1}$ is calculated by dividing TEE by speed (in km × h$^{-1}$)$^{25}$. 


Three-dimensional kinematic data extraction from high-speed videography

We used two synchronized 10-sec high-speed videos (lateral and ventral views, at each speed) for kinematic analyses. We calibrated the field of view of the high-speed cameras using a direct linear transformation for three-dimensional kinematic reconstruction (DLTdv8)\textsuperscript{31} by applying a stereo calibration to the swimming section of the respirometer (see Fig. S9). We digitized the anatomical landmarks of fish (see Fig. S10) to obtain the X, Y, Z coordinates for each marker at the 1\textsuperscript{st} sec, 5\textsuperscript{th} sec and 10\textsuperscript{th} sec for videos recorded at each speed. These coordinates are used to calculate the following kinematic parameters. All the calculations are validated on the known length and angle of test objects inserted into the tank working section.

The three-dimensional distance between the tip of the nose of each fish in the school per frame is calculated in vector Eqn. 2

$$d = \sqrt{(X_a - X_b)^2 + (Y_a - Y_b)^2 + (Z_a - Z_b)^2}$$ \hspace{1cm} \text{(Eqn. 2)}

Where spatial coordinates of two neighbouring fish are $(X_a, Y_a, Z_a)$ and $(X_b, Y_b, Z_b)$ respectively.

The three-dimensional angle of each fish in the school to the frontal plane per frame is calculated by Eqn. 3

$$\theta = \arccos \left( \frac{(X_2-X_1)(X_3-X_1)+(Y_2-Y_1)(Y_3-Y_1)+(Z_2-Z_1)(Z_3-Z_1)}{\sqrt{(X_2-X_1)^2+(Y_2-Y_1)^2+(Z_2-Z_1)^2}\sqrt{(X_3-X_1)^2+(Y_3-Y_1)^2+(Z_3-Z_1)^2}} \right) \hspace{1cm} \text{(Eqn. 3)}$$

where the spatial coordinates of the caudal peduncle, nose of fish and right angle crosshair between the peduncle and the nose are $(X_1, Y_1, Z_1)$, $(X_3, Y_3, Z_3)$ and $(X_2, Y_2, Z_2)$ respectively (see Fig. S10).
The fish’s angle to water flow per frame is calculated using the arctangent function in Excel (Microsoft, United States) using the spatial coordinates of the caudal peduncle and nose of the fish.

The school length (in $X$ axis) is calculated as a three-dimensional distance (Eqn. 2) between the nose of the first fish of the school to the caudal peduncle of the last fish in the school.

Tail beat frequency (TBF), in Hz, is calculated as the number of tail beats observed within one second. We sampled 10 tail beats from fish exhibiting steady-state swimming to obtain an average and representative TBF for each solitary fish at each speed. We sampled 10 tail beats of steady-state swimming for three individuals in the center of the fish school for an average and representative TBF for each fish school.

The values calculated above were averaged among the three frames at each speed as a representative kinematic feature of the schools (or solitary fish) for each speed. The standard deviation of the angle to water flow in fish schools is calculated from the angular values of the individuals.

Turning frequency is the total number of events when fish made a $90^\circ$ turn in the 10-sec video. To make the total number of turns per individual and fish schools comparable (multiple individuals inevitably have a larger absolute number of turns), we derived the average turning frequency per schooling fish by dividing the total number of turns of fish school by the number of fish in the school.

Stride length (BL) is calculated as the relative swimming speed (BL s$^{-1}$) of a fish over one cycle of the tail beat ($U \cdot TBF^{-1}$)$^{32}$.

Total energy expenditure per tail beat (kJ kg$^{-1}$ beat$^{-1}$) is calculated by $TEE \cdot TBF^{-1}$ to estimate the total metabolic energy spent to achieve one stride length. Additional calculations done were:
Strouhal number = (TBF • tail beat amplitude) • U^{-1.5}. Tail beat amplitude is measured as the average distance of five peak-to-peak oscillation amplitudes of the tip of the fish’s tail. The measurement is conducted on the calibrated high-speed video in video analysis software (Phontron FASTCAM Viewer 4, Photron USA, Inc.).

Reynolds number = (water density • U • fish fork length) • water dynamic viscosity^{-1.5}.

Water density and dynamic viscosity are given at 28 °C.

Statistical analyses

Measurement points are presented as mean ± s.e.m. For the metrics that failed normality tests, logarithm transformations were applied to meet the assumptions of normality of residuals, homoscedasticity of the residuals, and no trend in the explanatory variables. The majority of statistical comparisons used two-way (solitary fish vs. fish schools & swimming speed) ANOVA with Holm–Šídák post-hoc tests. The few comparisons that used other statistical models are listed below. The comparison of \( \dot{M}O_{2\text{min}} \) at optimal speed, \( \dot{M}O_2 \) of aggregating behaviours exhibited at the lowest speed (\( \dot{M}O_{2\text{aggregate}} \)) and resting \( \dot{M}O_2 \) (\( \dot{M}O_{2\text{rest}} \)) in fish schools used one-way ANOVA with Holm–Šídák post-hoc tests. The comparison of EPOC between solitary fish and fish schools used a two-tailed Student’s t-test. The comparison of the duration of EPOC between solitary fish and fish schools used a two-tailed Student’s t-test. The overall difference in percentage aerobic scope (% aerobic scope) between fish schools and solitary individuals is compared by the Wilcoxon signed-rank test over the entire range of 14 swimming speeds. The statistical analyses were conducted in SPSS v.28 (SPSS Inc. Chicago, IL, USA). The best-fitting regression analyses were conducted using Prism v.9.4.1 (GraphPad Software, San Diego, CA, USA). 95% C.I. values were presented for all regression models as shaded areas around the
regression or data points. Statistical significance is denoted by *, **, ***, **** for p-values of ≤ 0.05, ≤ 0.01, ≤ 0.001, ≤ 0.0001 respectively.

References and Notes


Acknowledgments: Many thanks to members of Lauder Laboratory for numerous discussions about fish schooling behaviour, for comments on the manuscript, and to Cory Hahn for fish care.

Funding: Funding provided by the National Science Foundation grant 1830881 (GVL), the Office of Naval Research grants N00014-21-1-2661 (GVL), N00014-16-1-2515 (GVL), 00014-22-1-2616 (GVL), and a Postdoctoral Fellowship of Natural Sciences and Engineering Research Council of Canada PDF - 557785 – 2021 (YZ).

Author contributions: Y.Z. and G.L. conceptualized the study. Y.Z. performed experiments and data analyses and wrote the manuscript. Y.Z. and G.L. provided manuscript edits and comments and approved the final version.

Competing interests: Authors declare that they have no competing interests.

Data and materials availability: All data generated or analysed during this study are included in this published article (and its supplementary information files).

Supplementary Materials:
Introductory text
Figs. S1 to S10
Tables S1 to S4
Captions for Movies S1 to S3

Other Supplementary Materials for this manuscript include the following:
Movies S1 to S3
FIGURES & FIGURE CAPTIONS
To compensate for reduced aerobic performance, individuals use more anaerobic energy.

\[
\text{To compensate for reduced aerobic performance, individuals use more anaerobic energy. See (E) (F)}
\]
Fig. 1. Measurements of aerobic and anaerobic locomotor cost of fish schools and solitary fish. (A) Average traces of metabolic rate (Ṁ\textsubscript{O}_2) of fish schools over a 40-hour experiment. Following the first 18-h quiescent state, a critical swimming speed (U\textsubscript{crit}) test quantifies the aerobic cost of active swimming. The ensuing 18-h measurement of excess post-exercise oxygen consumption (EPOC) quantifies the anaerobic cost. (B) Comparison of Ṁ\textsubscript{O}_2 for conditions of aggregating behaviour, minimum demand speed, and resting condition with the minimal flow (Ṁ\textsubscript{O}_2\textsubscript{aggregate}, Ṁ\textsubscript{O}_2\textsubscript{min}, Ṁ\textsubscript{O}_2\textsubscript{rest}). (C) Comparisons of J-shaped Ṁ\textsubscript{O}_2-speed curve over the entire range (0.3–8 body length s\textsuperscript{-1}, BL s\textsuperscript{-1}) and (D) U-shaped Ṁ\textsubscript{O}_2-speed curve at the lower speeds (0.3–3 BL s\textsuperscript{-1}). (E, F) Comparisons of EPOC and EPOC durations between fish schools and solitary fish. (G, H) Percentage (%) aerobic scope used by fish schools and solitary fish during the U\textsubscript{crit} test. Statistical significance is denoted by asterisk(s). Green color = school data (n=5); blue color = solitary fish data (n=5); shading indicates the 95% confidence interval. Statistical details are available in the statistical analyses section.
**Aerobic contribution:**

- School: 18 29 39 51 58%
- Individual: 82 71 61 49 42%

**Anaerobic contribution:**

- School: 45 62 71 77 81%
- Individual: 55 38 29 23 19%

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**Model**

- !$\hat{\mathbf{M}}$!
- !$\mathbf{O}_2$!
- !$\text{rest}$!
- EPOC
- !$5.25 \text{ mg O}_2$!
- !$U_{\text{crit}}$!
- !$\mathbf{M}_\text{O}_2 \text{ (mg O}_2 \text{ kg}^{-1} \text{ h}^{-1})$!
- !$\mathbf{M}_\text{O}_2 \text{rest}$!
- !$\mathbf{M}_\text{O}_2 \text{ (mg O}_2 \text{ kg}^{-1} \text{ h}^{-1})$!

**Measurement**

- !$\mathbf{M}_\text{O}_2 \text{ (mg O}_2 \text{ kg}^{-1} \text{ h}^{-1})$!
- !$\mathbf{M}_\text{O}_2 \text{ (mg O}_2 \text{ kg}^{-1} \text{ h}^{-1})$!
- !$\mathbf{M}_\text{O}_2 \text{ (mg O}_2 \text{ kg}^{-1} \text{ h}^{-1})$!

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**Speed (BL s$^{-1}$)**

- **B** School
- **C** Individual
- **D** School
- **E** School

**Total energy expenditure (kJ kg$^{-1}$)**

- **B**
- **C**
- **D**
- **E**

**Total cost of transport (kJ km$^{-1}$ kg$^{-1}$)**

- **B**
- **C**
- **D**
- **E**
Fig. 2. Modeling of simultaneous aerobic and anaerobic costs of fish schools and solitary fish for a critical swimming speed ($U_{crit}$) test. (A) Modeling the O$_2$ cost of the metabolic rate ($\dot{M}O_2$)-speed curve and the ensuing recovery cost (excess post-exercise oxygen consumption, EPOC) as a function of speed. After $U_{crit}$, fish returned to the same resting $\dot{M}O_2$ ($\dot{M}O_{2rest}$) as a pre-test. (B, C) In addition to $\dot{M}O_2$ (solid line & filled symbols), we modeled the total O$_2$ cost (dash line & half-filled symbols) for fish schools and solitary fish and when performing the $U_{crit}$ swimming test. The estimated partitioning of aerobic and anaerobic contributions to swimming are denoted (red-&-bold) with respect to speed for 4–8 BL s$^{-1}$ is shown below each graph. (D, E) Using total O$_2$ cost, we computed total energy expenditure and the total cost of transport (including both aerobic and anaerobic metabolism) for both fish schools and solitary fish. Statistical significance is denoted by asterisk(s). Green color = school data (n=5); blue color = solitary fish data (n=5); shading indicates the 95% confidence interval. See methods for modeling and statistical details.
### A. Total energy expenditure per tail beat (kJ kg\(^{-1}\) beat\(^{-1}\))

- **Individual**
  - \(R^2 = 0.7650\), AIC = 41.41
- **School**
  - \(R^2 = 0.8555\), AIC = 82.49

### B. Tail beat frequency (Hz)

- **Individual**
  - \(R^2 = 0.7073\), AIC = 52.25
- **School**
  - \(R^2 = 0.5627\), AIC = 39.61

### C. Angle to water flow (°)

- **Individual mean**
- **School mean**
- **School S.D.**

### D. 3D angle to frontal plane (°)

- **Individual**
- **School**
  - \(R^2 = 0.7839\), AIC = 57.11

### E. Turning frequency (turns 10-sec\(^{-1}\) individual-1)

- **Individual**
- **School**
  - \(R^2 = 0.8684\)
  - \(R^2 = 0.5274\)

### F. School length (mm)

- **Individual**
- **School**
  - \(R^2 = 0.2365\), AIC = -81.15

### G. 3D distance (BL)

- **Individual**
- **School**
  - \(R^2 = 0.8674\)
  - \(R^2 = 0.5274\)
Fig. 3. Three-dimensional characterization of swimming kinematics and fish schooling dynamics as a function of speed. (A) Total energy expenditure per tail beat, (B) Tail beat frequency, (C) The angle of fish to free-stream water flow, measured as the mean and the S.D. of the angles of the individuals within the school. (D) Three-dimensional angle of fish to the frontal plane. (E) Turning frequency, (F) Three-dimensional school length. (G) Three-dimensional distances among all individuals in the school and the S.D. of the distance. The visual illustration of the upper and lower boundaries of the metrics are indicated. Statistical significance is denoted by asterisk(s). Green color = school data (n=3-4); blue color = solitary fish data (n=3-4); shading indicates the 95% confidence interval. See methods for details of three-dimensional reconstruction and statistics.