**Supplementary material for:**

“Energetic costs of activity in wild Lake Trout: a calibration study using acceleration transmitters and positional telemetry”

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**1. Details of procedures followed**

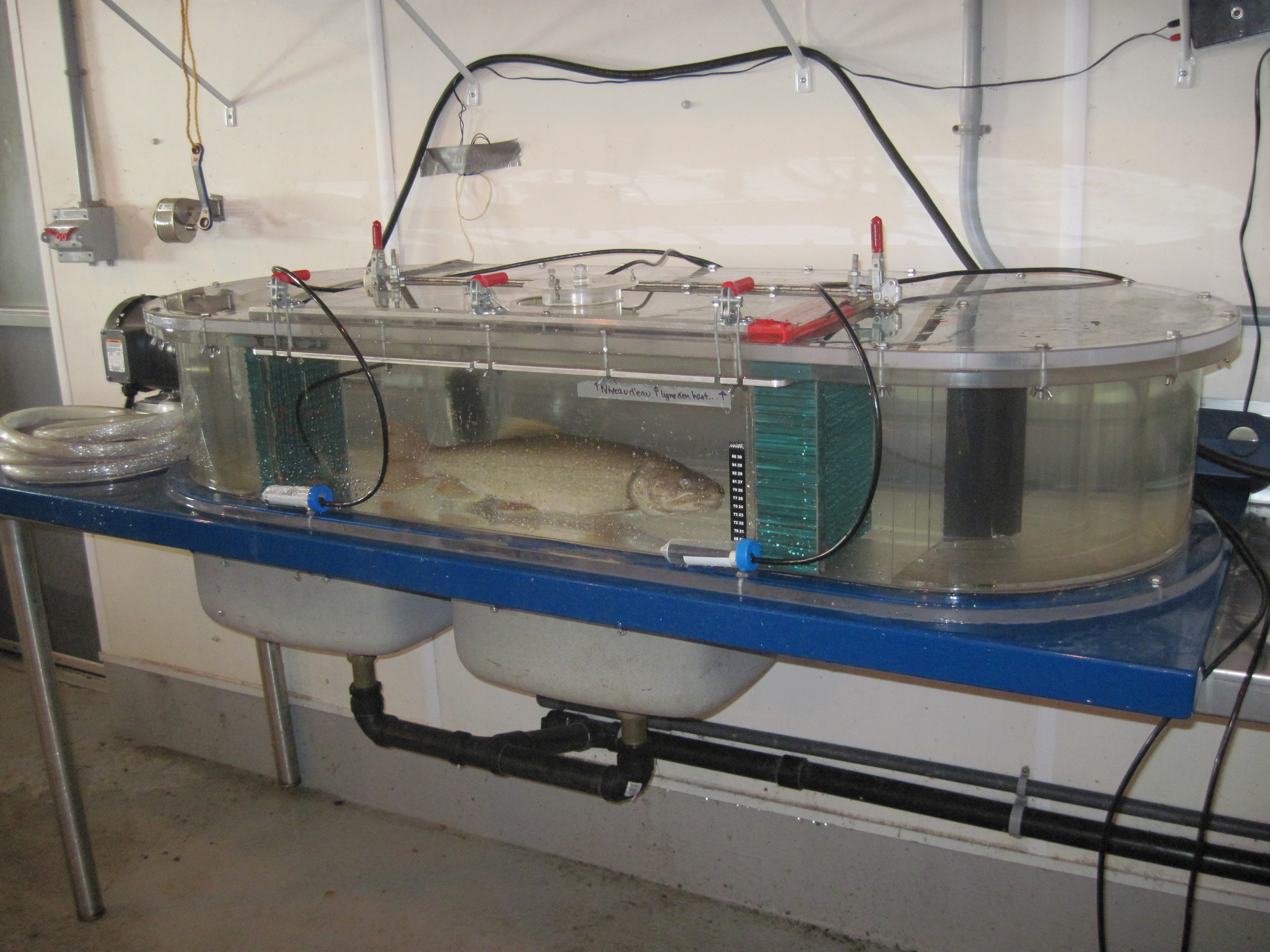
***S1. Surgical Procedures:***

Laboratory and field procedures followed similar transmitter implantation protocols. Each fish selected for implantation was anaesthetized in a bath of 60 mg·L-1 of tricaine methanesulphonate (MS-222, AquaLife TMS, Syndel Laboratories, British Columbia). Fork and total length (mm), and weight (g) were recorded before transmitter implantation. To minimize post surgery effects on behaviour (Wagner and Stevens 2000), a shallow, 2 cm long incision was made through the body wall along the midline of the ventral surface of the fish, just anterior to the pelvic fin girdle, taking care to avoid puncturing internal organs. A disinfected (using ethanol 70%) tail beat frequency (TBF) transmitter was inserted into the peritoneal cavity through this incision and sterile stainless steel tweezers were used to push the transmitter towards the end of the body cavity, until significant resistance was met. This ensured proper placement of the transmitter near the tail of the fish. The incision was closed with three simple interrupted stitches using absorbable monofilament suture (Monocryl Polyglecaprone 25, Ethicon Inc.). The transmitters used in wild fish were tethered to ‘secure’ the transmitter in place (Dale Webber, VEMCO, Nova Scotia, Canada, personal communication, 2009). For this procedure, a segment of monofilament is attached to one end of the transmitter, opposite to where the accelerometer sensor is, and then the monofilament is sutured to the incision. In the laboratory, transmitters were not tethered, except for three fish used to test the effects of the transmitter position inside the body cavity of fish. In the wild, fish were kept oxygenated during surgery by streaming a maintenance solution of oxygenated anesthetic (30 mg·L-1 MS-222) over the gills (Blanchfield *et al*. 2005). This extra animal care procedure was not followed in the laboratory, where surgeries were completed in a controlled environment and at a faster rate. After surgery, fish recovered in an aerated bath of freshwater. Recovery generally occurred within the first five minutes after surgery; however, fish were left in the recovery bath for an extra ten to fifteen minutes.

***S2. Swim tunnel design***

The swim tunnel used (Loligo Systems, Denmark) was an oval, water-filled, clear Plexiglas tank with an impeller controlled by a variable speed, direct current electrical motor that created a recirculating flow of water around the tank. Built into the tank was a rectangular holding compartment (dimensions: 70 cm length, 25 cm width, and 24 cm height) to contain the experimental animal. A wall made of a honeycomb plastic grid at each end of the holding compartment produced a laminar flow of water within the compartment itself. The rate of water flow through this section was regulated using an electrical frequency converter that controlled the impeller motor. The holding compartment was accessible through a door located at the top of this compartment. When the door was closed, five clamps tightened it and maintained the holding compartment closed, which was then only accessible though a circular aperture (97 mm diameter) on the center of the door. During all the experiments performed, the door was maintained closed, and when necessary, the holding compartment was accessed through the top circular aperture.

During the transmitter calibration measurements, fish had to swim for periods of time of more than one hour. It was important to maintain the dissolved oxygen concentration in the water at values higher than 70%. Therefore, the top circular aperture during these experiments was left open, allowing access to the fish. For the oxygen consumption experiments the tunnel needed to be hermetically sealed, while still allowing for the dissolved oxygen probe to measure water oxygen levels. To achieve this, a custom-made plastic cap with a center hole of 25 mm was built and fixed to the tunnel door. The swim tunnel was then able to function as a respirometer chamber (Fig. *S1*).



**Fig.** ***S1*.** Photo of the swim tunnel taken while a laboratory fish was being acclimated.

***S3. Tail Beat Frequency (TBF) estimation***

Video recordings were visually analyzed to obtain estimates of the TBF at increasing values of swimming speed. We selected portions of the video corresponding to swimming speeds of around 0.5 BL·s-1, 1 BL·s-1, and 1.5 BL·s-1 to simplify the procedure. At each of those speeds, we identified periods of time of one minute or less in which fish were actively swimming and were not showing spontaneous movements such as bursts and gliding behaviours. The number of tail beat cycles completed, from one side of the fish to the other side and back, within each time period were counted. Then, the counts per period of time were standardized to one minute of activity.

***S4. Swimming speed calculation in the field***

To calculate the distance swum by the fish over the time interval (SS*T*) characterized by sequential detections of depth, acceleration and depth, we added the linear distances between the three successive positions detections. The Pythagorean Theorem was used to estimate distances swum, where the vertices of the triangle represented the latitude (*Y*), longitude (*X*), and depth (*Z*) reported by the VRAP system (eq. *S4*). We applied a correction to eq. *S4* because the change in depth within that time interval is given by the two depth positions, which are the first and third transmission within the interval: we assumed no change in depth between the first two position transmissions (*Z*2 – *Z*1 = 0), then assumed that the observed change in depth occurred between the second and third position transmissions:

[eq. *S4*]

where:

SS*T* – swimming speed of an individual fish (in cm·s-1) over an interval *T*

[*t*3 – *t*1] – time interval *T* between sequential depth transmissions; assumes no missing data between depth-acceleration-depth transmission (in s)

*X* – longitude coordinate (converted to meters using the Universal Transverse Mercator (UTM) coordinate system)

*Y* – latitude coordinates (converted to meters using the UTM system)

*Z* – depth as reported by the telemetry transmitter (in m)

***S5. Translating transmitter-reported acceleration to metabolic costs in the field***

***S5.1. Metabolic rates equations***

We started with the general equation that describes the total metabolic rate (Mtot) based on two main processes: standard metabolic rate (Mstd), and active metabolic rate (Brett 1972; Brett and Groves 1979; Boisclair and Tang 1993). Swimming costs (Mswim) are usually a large component of the active metabolic rate (Boisclair and Leggett 1989), so we can express the total metabolic rate in terms of the standard and swimming metabolic rates:

Mtot = Mstd + Mswim [eq. *S5.1*]

where all measures are in units: mg O2·hr-1 per fish.

In general, metabolic processes are related to temperature, particularly in ectothermic organisms like fish. For example, Evans (2007) provided the following equations for the temperature-dependence of standard and maximum-active metabolic costs for Lake Trout:

(i) Mstd per kg of a 0.1 kg Lake Trout held at a temperature T is given by 46.072·*e*0.0607·T

(ii) maximum active metabolic rate per kg of a 0.1 kg Lake Trout held at a temperature T is given by 105.31·*e*0.0847·T

The size dependence of metabolism has been intensively studied (e.g. Peters 1983) and described with an allometric relationship with body size (W), with M = *a·*W*b*. For Lake Trout, several authors have used the exponent *b* = 0.85 (Beamish *et al.* 1989; Evans 2007). We compared our laboratory estimates of total metabolic rate at different levels of activity with the values reported by Evans (2007) using the above exponent. Our values fell within the expected range of values for Lake Trout, validating our methodology. Therefore, in this study, the Mstd was estimated by the equation reported by Evans (2007) and adjusted for fish weight differences using the 0.85 exponent.

***S5.2. Swimming costs***

Variations in the cost of swimming have been mostly explained by the influence of body mass and speed on active metabolism (Boisclair and Tang 1993; Ohlberger *et al.* 2005; Claireaux *et al.* 2006; Hein and Keirsted 2012). Additionally, Wilson *et al.* (2013) tested the relationship between oxygen consumption and transmitter-sampled acceleration of similar size fish at different temperatures. They found that the slope of this relationship was relatively constant for temperatures between 12 and 18 °C, suggesting that the additional metabolic cost associated with an increase in activity (increased swimming speed) is independent of temperature. For temperatures over 18 °C, Wilson *et al.* (2013) observed increasing metabolic costs for tagged fish swimming at fixed levels of acceleration, which suggests elevated metabolic costs for fish that have to endure temperatures outside their preferred range.

From a kinematics point of view, the energy needed to move a body through water can be described by the power loss due to drag (Fish 2010):

PD = 0.5·*ρ*·S*·*CD·U3 [eq. *S5.2*]

where *ρ*: density of the water (freshwater, 1000 kg·m-3; salt water, 1025 kg·m-3); S: wetted surface area of the body; U: velocity; and CD: dimensionless drag coefficient. This equation describes the energy loss in locomotion associated with the density of water, the surface area of the fish body, and the speed at which the fish is swimming. The rate of change in water density from 10 to 15 °C is small (~ 0.06 % from 999.7 kg⋅m-3 at 10 °C to 999.1 kg⋅m-3 at 15 °C, Webb 1975), therefore, for the temperatures experienced by Lake Trout in the wild and in the laboratory, changes in the cost of swimming caused by changes in water density alone would be minimal. So, the cost of swimming would be related to the surface area of the body of the fish, and to the swimming speed. The surface area dependence of power loss due to drag can be expressed as ~ *c*⋅W2/3 since the surface area of a body that changes in size but maintains its basic shape and density will be roughly proportional to W2/3 (e.g. Peters 1983).

***S5.3. Obtaining metabolic rates for Lake Trout***

All these results support the hypothesis that swimming costs are independent of water temperature, so by estimating the relationships that link swimming speed and associated swimming costs with measured acceleration values at a single temperature in the laboratory (l), we can estimate swimming costs in the field (f) over a range of temperatures using these laboratory-derived relationships. In the laboratory, we measured oxygen consumption of fish at several swimming speeds while the temperature was held relatively constant (Tl = 11.76 + 1.26 °C). Also, we described the relationship between transmitter-acceleration and swimming speed. Therefore, we can express the total metabolism observed in the laboratory at the swimming speed associated with an acceleration value (Ai) for a fish of weight Wl as:

Mtot(Ai, Wl, 11.76) = Mstd(Wl, 11.76) + Mswim(Ai, Wl)

Rearranging this equation, we estimate the costs of swimming at a rate associated with an acceleration value of Ai as:

Mswim(Ai, Wl) = Mtot(Ai, Wl, 11.76) - Mstd(Wl, 11.76) [eq. *S5.3*]

We then assume that, in the field, the cost of swimming associated with an activity value of Ai will equal Mswim (Ai) independent of the temperature experienced in the field. We base this assumption on the fact that the drag forces that determine swimming costs are relatively independent of temperature and that observed costs from Sockeye Salmon (Wilson *et al.* 2013) over the temperature range of 12-18 °C were consistent with this assumption. For temperature values exceeding the preferred temperature for Lake Trout (> 15 – 18 °C) we can expect an increase in the metabolic cost (as observed by Wilson *et al.* 2013): we will note when our field data suggest that this effect might be occurring but we will not attempt to characterize it quantitatively.

In the field, we tagged fish of various weights (Wf). In order to estimate the metabolic rates of these fish in the field, it was necessary to allow for how the differences in weight between laboratory fish and field fish would affect both the Mstd and the Mswim. To allow for the effects of size differences on Mstd, we followed Evans (2007) and assumed an allometric relationship linking Mstd to body mass, with an exponent of 0.85 (Mstd = *a*·W*0.85*). To allow for the effects of size differences on swimming costs, we assumed that swimming costs would follow the relationship that links increasing body size to power losses due to increased drag forces – this is consistent with an allometric relationship linking swimming costs to body mass with an exponent of 0.667 (Mswim = *c*·M0.667). The sets of equations used for each of these adjustments are given below.

A. Evans (2007) gives the following equation for the standard metabolic rate per kg of a 0.1 kg fish held at temperature T:

Mstd = 46.072·*e*0.0607·T

Given an allometric dependence on body weight with exponent of 0.85, then the following equation holds for fish of weight Wf exposed to a temperature of Tf:

[eq. *S5.4*]

B. Given that the energy required to move the fish body, and the fish tail, at a certain value of acceleration (Ai) varies with the surface area of the fish body, which in turn is an allometric function of fish body weight with an exponent equal to 2/3, then the field swimming cost of a fish with weight Wf experiencing a temperature of Tf and exhibiting an acceleration of Ai can then be estimated as:

[eq. *S5.5*]

The procedure outlined above is summarized in the following equation for estimating the total metabolism of a fish of weight Wf observed in the field to be exhibiting an acceleration of Ai while experiencing a temperature of Tf:

[eq. *S5.6*]

These formulae provide direct estimates of raw metabolic costs incurred by fish living in field situations, from which we derived a cost index based on the ‘activity multiplier’ parameter used in the Wisconsin bioenergetics model (Kitchell *et al.* 1977; Hanson *et al.* 1997 <http://limnology.wisc.edu/research/bioenergetics/bioenergetics.html>):

[eq. *S5.7*]

which simply measures activity costs in terms of multiples of standard metabolic costs. **2. Additional Tables and Figures**

**Table S1**. Parameters of the regression lines obtained for each individual laboratory-fish used in the transmitter placement study to describe the relationship between acceleration (dependent variable) and swimming speed. Three fish (numbers 755, 612, and 127) were implanted with transmitters in the mid-ventral position of the body cavity (labeled MID). Three other fish (numbers 4, 69, and 724) were implanted with transmitters at the posterior of the body cavity (labeled BACK). ‘Lake’ represents the source population (LO: Louisa, OP: Opeongo) from which laboratory fish were obtained. The sample size indicates the number of data-pairs (acceleration-speed) used in the analyses.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Fish No. | Lake | | Fork Length (mm) | Weight (g) | Transmitter placement | Sample size | Regression parameters | | | |
| intercept | slope | *R2* | *p* |
| **Laboratory- trials – transmitter placement** | | | | | | | | | | |
| 4 | LO | 507 | | 1768 | MID | 1375 | 0.62 | 0.02 | 0.89 | <0.001 |
| 729 | OP | 510 | | 1510 | MID | 750 | 0.75 | 0.02 | 0.84 | <0.001 |
| 69 | LO | 554 | | 2137 | MID | 1201 | 0.60 | 0.02 | 0.94 | <0.001 |
| 755 | LO | 479 | | 1319 | MID | 549 | 0.74 | 0.02 | 0.85 | <0.001 |
| 612 | OP | 492 | | 1375 | MID | 420 | 0.56 | 0.02 | 0.92 | <0.001 |
| 127 | OP | 532 | | 1834 | MID | 563 | 0.74 | 0.01 | 0.80 | <0.001 |
| 4 | LO | 507 | | 1768 | BACK | 1375 | 0.84 | 0.02 | 0.94 | <0.001 |
| 729 | OP | 510 | | 1510 | BACK | 750 | 0.93 | 0.02 | 0.89 | <0.001 |
| 69 | LO | 554 | | 2137 | BACK | 1201 | 0.85 | 0.02 | 0.97 | <0.001 |

**Table S2**. Parameters of the regression lines that describe the relationship between log10-transformed swimming speed (SS, dependent variable) and acceleration for laboratory and wild fish. ‘Lake’ represents the source population (LO: Louisa, OP: Opeongo, 373: Lake 373, and 626: Lake 626). The sample size indicates the number of successful estimations of SS obtained.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Fish No. | Lake | Fork Length (mm) | Weight (g) | Transmitter type and delay | Sample size | Regression parameters | | | | |
| intercept | slope | *R2* | *p* | |
| **Laboratory- trial – transmitter calibrations** | | | | | | | | | | |
| 242 | LO | 437 | 1010 | V9A  Fixed delay of 25 sec | 357 | 0.95 | 0.43 | 0.91 | <0.001 |
| 266 | LO | 438 | 956 | 409 | 0.61 | 0.61 | 0.92 | <0.001 |
| 265 | LO | 442 | 1032 | 409 | 0.89 | 0.45 | 0.92 | <0.001 |
| 494 | OP | 448 | 980 | 284 | 0.90 | 0.47 | 0.87 | <0.001 |
| 444 | LO | 449 | 938 | 356 | 1.06 | 0.38 | 0.88 | <0.001 |
| 178 | LO | 457 | 1115 | 280 | 0.84 | 0.48 | 0.93 | <0.001 |
| 51 | LO | 464 | 1147 | 296 | 0.89 | 0.45 | 0.86 | <0.001 |
| 772 | LO | 493 | 1548 | 287 | 0.89 | 0.46 | 0.91 | <0.001 |
| 526 | LO | 496 | 1395 | 518 | 1.00 | 0.47 | 0.87 | <0.001 |
| 193 | LO | 497 | 1404 | 275 | 0.95 | 0.42 | 0.83 | <0.001 |
| 145 | LO | 505 | 1661 | 356 | 1.04 | 0.42 | 0.89 | <0.001 |
| 711 | OP | 505 | 1739 | 425 | 0.86 | 0.48 | 0.88 | <0.001 |
| 414 | OP | 516 | 1599 | 358 | 0.85 | 0.50 | 0.91 | <0.001 |
| 244 | LO | 518 | 1770 | 374 | 0.95 | 0.44 | 0.93 | <0.001 |
| 759 | LO | 534 | 1823 | 288 | 1.00 | 0.41 | 0.88 | <0.001 |
| **Field- trial – Experimental Lakes Area** | | | | | | | | | | | |
| 3334 | 373 | 454 | 762 | V9AP  Random delay (100 – 140 sec) | 220 | 0.119 | 0.589 | 0.33 | <0.001 |
| 3536 | 373 | 413 | 785 | 9 |  |  |  |  |
| 3738 | 373 | 396 | 666 | 92 | 0.281 | 0.364 | 0.16 | <0.001 |
| 3940 | 373 | 380 | 536 | 4 |  |  |  |  |
| 6162 | 373 | 401 | 659 | 2 |  |  |  |  |
| 6364 | 373 | 390 | 729 | 41 | 0.393 | 0.215 | 0.03 | <0.001 |
| 6566 | 373 | 413 | 658 | 80 | 0.259 | 0.468 | 0.23 | <0.001 |
| 6768 | 373 | 390 | 626 | 128 | 0.687 | 0.331 | 0.06 | <0.001 |
| 6970 | 373 | 423 | 728 | 29 | 0.069 | 0.678 | 0.25 | <0.001 |
| 7172 | 373 | 354 | 499 | 28 | 0.413 | 0.350 | 0.35 | <0.001 |
|  |  |  |  |  |  |  |  |  |  |
| 4142 | 626 | 407 | 716 | V9AP  Random delay (100 – 140 sec) | 274 | 0.034 | 0.590 | 0.26 | <0.001 |
| 4344 | 626 | 416 | 720 | 41 | -0.026 | 0.660 | 0.52 | <0.001 |
| 4546 | 626 | 356 | 487 | 104 | -0.078 | 0.732 | 0.53 | <0.001 |
| 4748 | 626 | 413 | 785 | 18 | 0.095 | 0.606 | 0.23 | <0.001 |
| 4950 | 626 | 430 | 926 | 78 | 0.039 | 0.596 | 0.38 | <0.001 |
| 5152 | 626 | 423 | 846 | 36 | -0.085 | 0.628 | 0.28 | <0.001 |
| 5354 | 626 | 540 | 1848 | 4 |  |  |  |  |
| 5556 | 626 | 381 | 601 | 49 | 0.198 | 0.497 | 0.21 | <0.001 |
| 5758 | 626 | 403 | 611 | 90 | 0.148 | 0.495 | 0.17 | <0.001 |
| 5960 | 626 | 437 | 850 | 116 | -0.106 | 0.783 | 0.43 | <0.001 |

|  |  |
| --- | --- |
| Log10 SS (cm·s-1) |  |
|  | Log10 [acceleration (a.u.)] |

**Figure S2**. Linear models fitted to swimming speed (SS) and acceleration data from laboratory fish implanted with short-delay acceleration transmitters (V9A) and long-delay acceleration-depth transmitters (V9AP). The lines were parallel, indicating a similar relationship between the variables. However, short-delay transmitters predicted higher swimming speeds.

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