

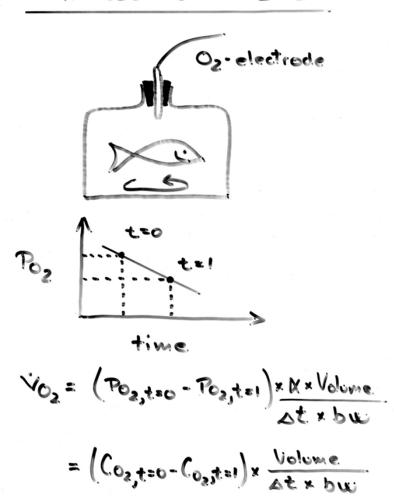
#### Faculty of Science

### Design and setup of an intermittent-flow respirometry system for aquatic organisms

JF Steffensen<sup>1</sup>

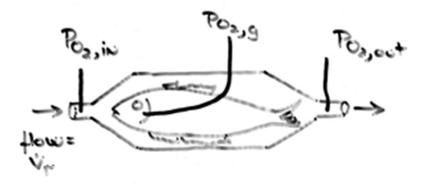
Marine Biological Section, Department of Biology, University of Copenhagen, Helsingør, Denmark

### RESPIROMETRY IN CLOSED SYSTEMS:





### RESPIROMETRY IN OPEN OR FLOW-THROUGH SYSTEMS:



V= oxygen solubility
Vs= gill water flow
bw = weight



# WASH-OUT IN OPEN SYSTEMS: Vol.=1L Flow= o.1 L/min Vol = 1/.1 = 10 min 1 - e (- How/volume x time) 100% 20 30 40 10 time, min



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0300-9629/84 \$3.00 + 0.00 © 1984 Pergamon Press Ltd

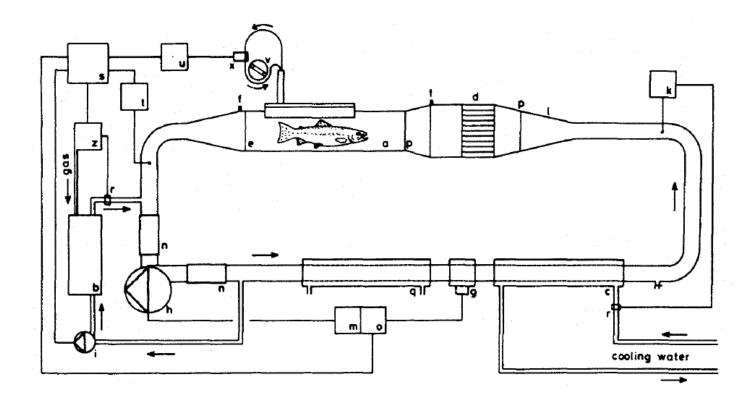
#### AN AUTOMATED SWIMMING RESPIROMETER

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(Received 22 February 1984)

Abstract—An automated respirometer is described that can be used for computerized respirometry of trout and sharks.





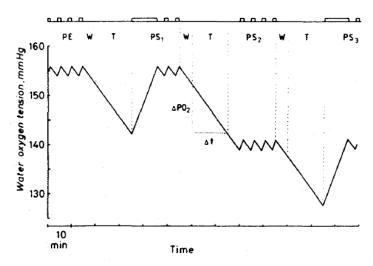


Fig. 2. Schematic diagram which illustrates the application of the respirometer. PE = pre-experiment period (hr, min),  $PS_n = preset$  water oxygen tension (mm Hg), W = wait period (min), T = test period (min). The open squares at the top indicate when a supply of  $air/O_2$  mixture was directed through the

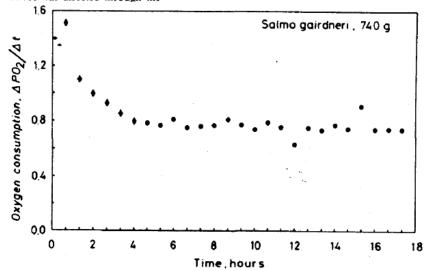
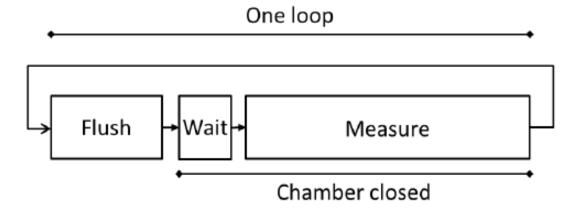


Fig. 3. Time course of oxygen consumption ( $\Delta PO_2/\Delta t$ ) in a rainbow trout swimming 25 cm · sec<sup>-1</sup>. There was an adjustment period of 5 hr after the fish was transferred to the respirometer, during which oxygen consumption declined ( $\spadesuit$ ), before stable readings were obtained ( $\spadesuit$ ).





**Figure 1:** During intermittent-flow respirometry one complete measurement cycle (loop) consists of three timing periods; the flush period where the chamber is open (i.e. water is flowing through it) followed by two closed periods, wait and measure, when the flush pump is off. A short wait period is needed before the measurement period to allow all the water in the chamber to mix and the oxygen content to begin declining in in a linear fashion. Data from measurement period is used for determining the oxygen consumption of the organism.



#### Resting:

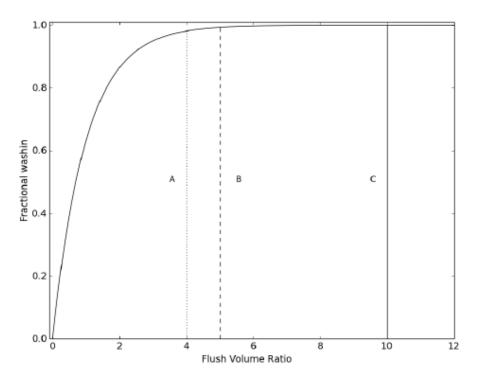


Figure 8: During the flush period, "clean" flush water mixes with "dirty" respirometer water in an exponential manner which depends on the ratio of flush water volume to respirometer volume (Flush volume ratio). The vertical lines represent examples of specific flush volume ratios and the fractional washin achieved. For example, once the respirometer has been flushed with 4x its volume (A) 98.2% of the water in the respirometer will have been replaced (fractional washin). Extending the flush volume ratio to 5x (B) or 10x (C) increases the fractional washin to 99.3% and 99.9%, respectively.



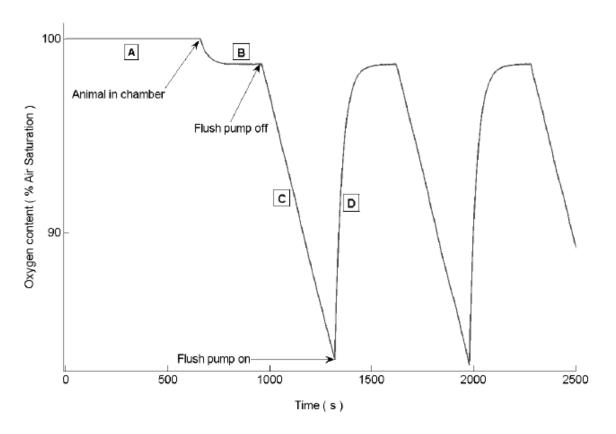


Figure 2: Ideally, an intermittent-flow respirometry experiment should consist of a series of linear declines in oxygen content during the measurement period interrupted by exponential increases during the flush period. Before an organism is placed in the chamber oxygen levels should be near air-saturation (a) and will decline slightly and reach a new equilibrium after the animal is introduced (b). Once the flush pump is turned, off a linear decline in oxygen levels should occur (c), the slope of which is used to calculate oxygen consumption. Turning the flush pump back on should cause oxygen levels in the chamber to increase in an exponential fashion (d).



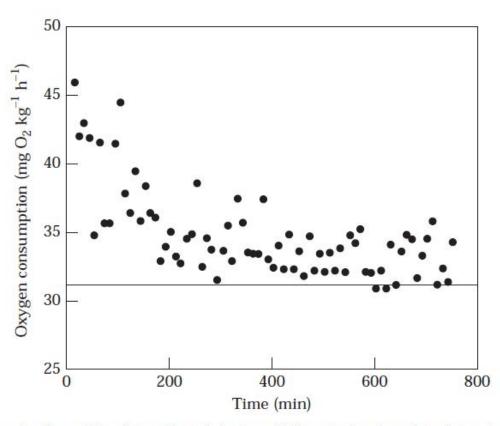


Fig. 2. An example of raw  $M_{\rm O_2}$  data collected during a 12-h period and used to determine the SMR and  $S_{crit}$  for one cod at 5° C. The elevated  $M_{\rm O_2}$  initially was caused by handling stress. The horizontal line indicates SMR.

Schurmann, H. & Steffensen, J. F. (1997) Effects of temperature, hypoxia and activity on the metabolism of Atlantic cod, Gadus morhua. J. Fish Biology. 50; 1166-1180.



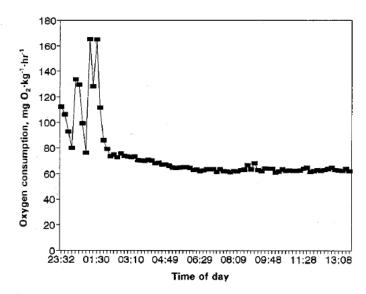


Fig. 1 An example of measurement of oxygen consumption of an uvak, *Gadus ogac*, starved for three days. Initially, after transfer to the respirometer, oxygen consumption was elevated due to handling stress. But after 2–3 h, and for the following 6 hours, VO<sub>2</sub> stabilized at a level considered to represent standard VO<sub>2</sub>

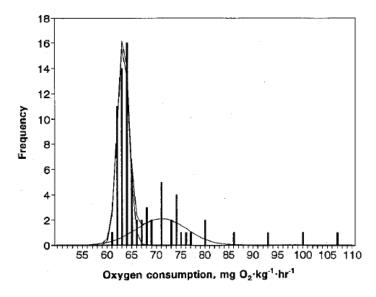


Fig. 2 An example of frequency distribution of VO<sub>2</sub> of uvak, and the fitted double normal distribution curve. The left narrow peak represent the standard VO<sub>2</sub>, whereas the right wider peak represent routine oxygen consumption

Steffensen, J. F., Bushnell, P. G. & Schurmann, H. (1994). Metabolic rate of 4 Arctic species of teleosts from Greenland. Polar Biology, 14; 49-54.



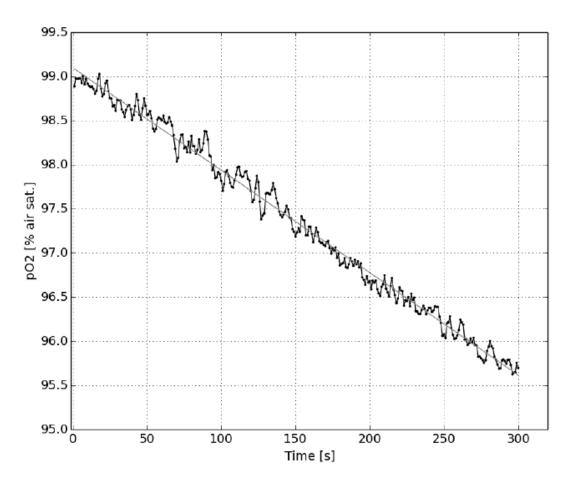


Figure 1: A plot of pO2 during one measurement period (300 seconds) of a roach (*Rutilus rutilus*) metabolic rate experiment. The dots connected with the black line are the raw data obtained from the Pyroscience Firesting logger at 1hz. The grey straight line represents the linear regression obtained from the same data (slope: -0.012; y-intercept:99.2; R<sup>2</sup>: >.99)



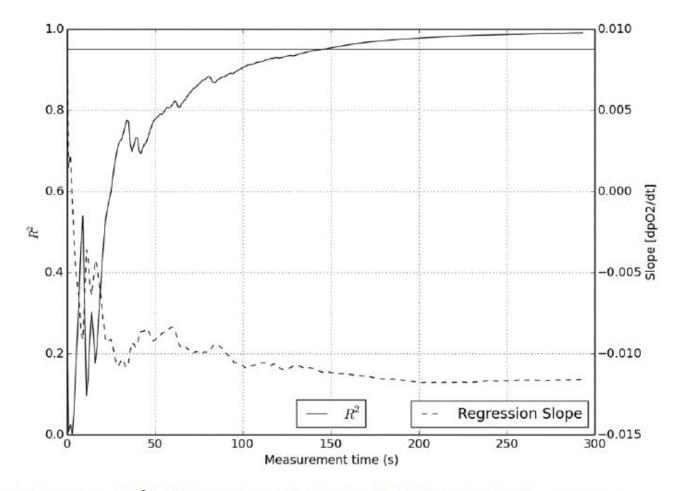


Figure 3: The change in  $R^2$  (solid line) and regression slope (dotted line) over time for the data shown shown in figure 1. Stabilization of both variants occurs after approximately 200s. The horizontal black line represents  $R^2$  = .95. This example has a Respirometer to Fish Ratio of 20.



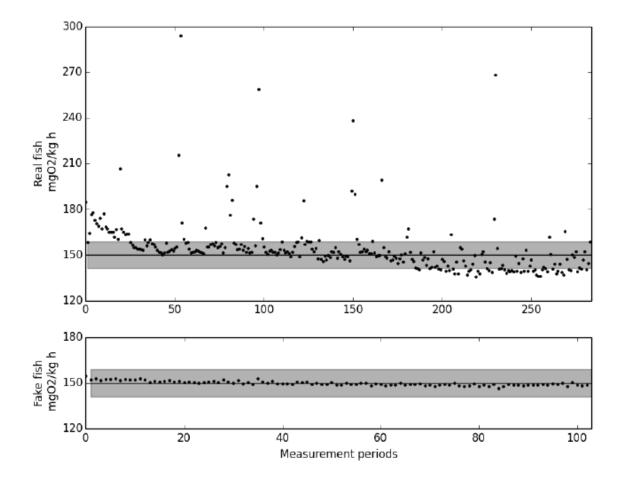


Figure 4: Oxygen consumption measurements recorded in a *Rutilus rutilus* (top panel) and an *Artifish* experiments (bottom panel). The gray box represents 3 times the standard deviations obtained from the *Artifish* experiment, thus approximating the 99th percentile. The slight variation (decline) in the *Artifish* metabolism results from change in the atmospheric pressure, air temperature and mechanical wear on the rubber tubing used in the anoxic water pump.



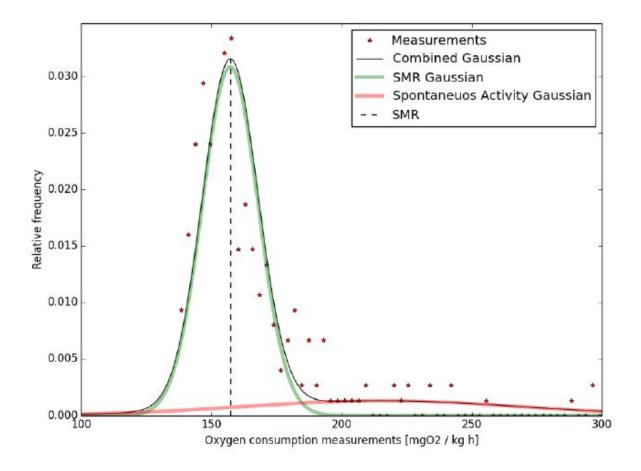


Figure 2: Determining the Standard Metabolic Rate (SMR) using a double Gaussian fit. The dots are the relative frequency distribution of the measured oxygen consumption. The solid black line is the double (combined) Gaussian curve fit consisting of the SMR-Gaussian (Green) and the Spontaneous Activity (SA) Gaussian distribution (Red). The vertical dashed line is the SMR value.



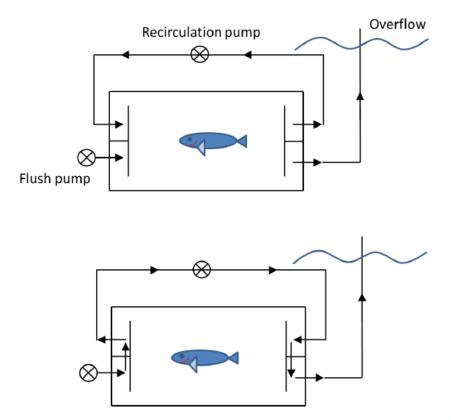


Figure 5: When a recirculating loop is needed the outflow of the flush pump and recirculating loop can be plumbed in a "non-countering" manner (top panel) where the two are in the same direction, or "countering" where the flows oppose each other (bottom panel).

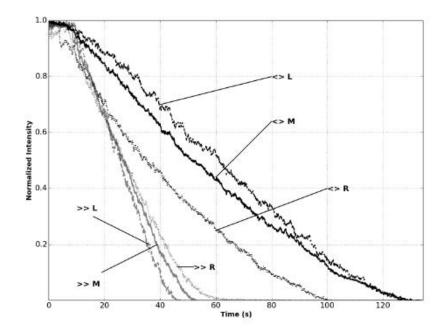


Figure 6: Fluorescent dye was used to visualize mixing and washout in a respirometer when the recirculating loop was plumbed in the same direction (non-countering) as the flush pump flow (>>) or plumbed in opposition (countering) direction to the flush pump (<>). Regardless of the flush rate a non-countering system results in much more effective flushing. L, M and R is Left, Mid and Right part of the respirometer, respectively. The flush pump is mounted to the left. Y axis is normalized fluorescent intensity, normalized to max intensity in each of the regions of interest (L,M,R).



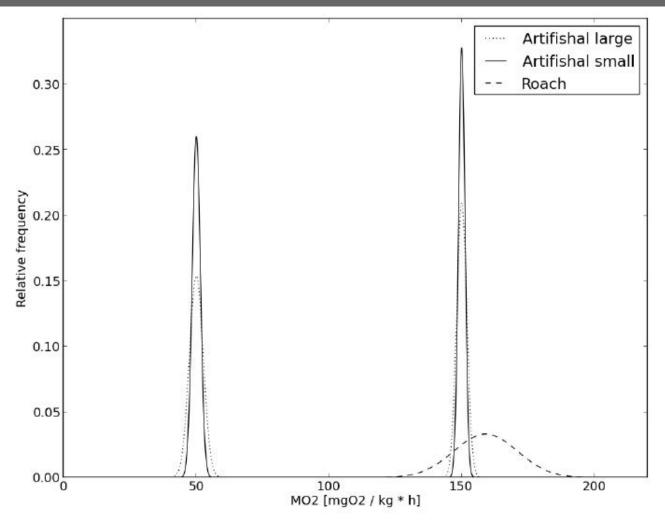


Figure 6

The Gaussian fitted histograms from *Artifish* experiments where  $MO_2$  was set to two different levels and conducted in a small (RFR=20, solid line) and large (RFR=39, dotted line) respirometer. A single *Rutilus* experiment with similar SMR in a small (RFR=26) respirometer (dashed line) is included for comparison.



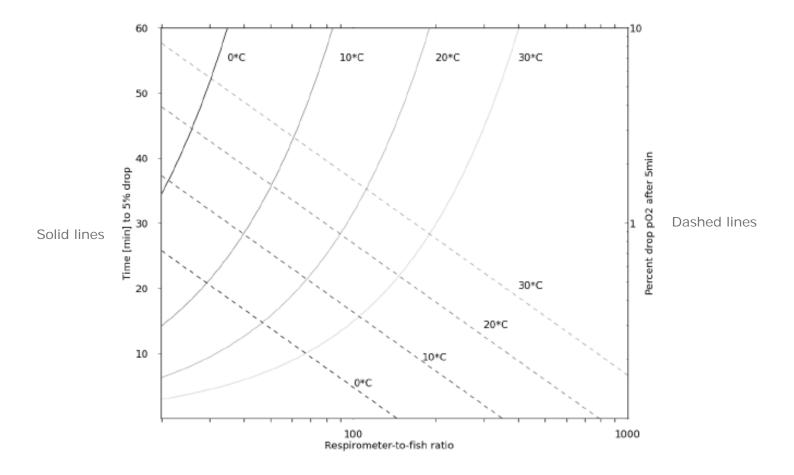


Figure 9: The amount of time needed to achieve a 5% drop in oxygen content in the respirometer for metabolic rate determinations, depends on the size of the animal relative to respirometer (respirometer to organism ratio) and its metabolic rate. This family of curves was constructed based on a metabolic rate approximation adapted from Gillooly et al (2001) in a 300g fish at 4 different temperatures. The smaller the respirometer to fish ratio and/or the higher its metabolic rate (temp) the less time the flush pump needs to be off to achieve a reasonable decline (5%) in oxygen content in the respirometer.



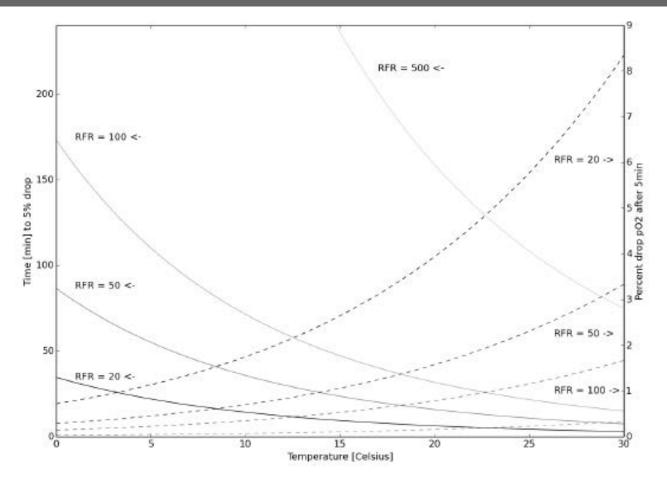


Figure 10: Because temperature has a profound effect on metabolism, the oxygen will be consumed much more quickly (time to 5% drop) in a closed respirometer at a high temperature than at a lower one. This can dramatically increase measurement times when experiments are done in large respirometers at low temperatures. Metabolic rate and Q10 assumptions as in Fig 9.



Important – respirometer volume to fish volume vs variation:

Static respirometers

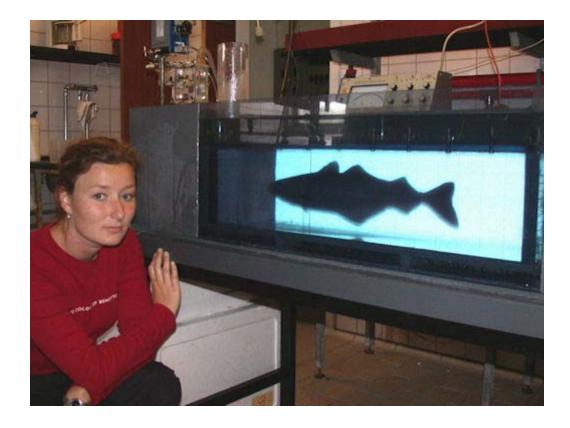
To keep standard og resting metabolic rate variation (noise) low keep static respirometer volume to fish volume as low as possible = in practice it rarely can be less than 20 but certainly less than 50

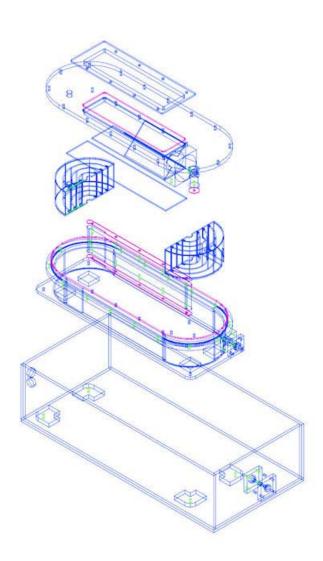
Swimming respirometers:

In practice it is difficult to design a swimming respirometer so that respirometer volume to fish volume is as low as 50, often it is 100 – 200, avoid ratios above 250.



30-legning





Steffensen Mk III Type Swim tunnel



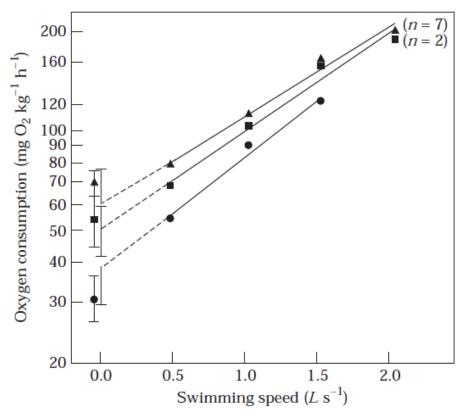


Fig. 5. Mean M<sub>O2</sub> as a function of swimming speed at 5 (●), 10 (■) and 15° C (▲). Unless otherwise indicated, n is 9, 12 and 11 at temperatures of 5, 10 and 15° C, respectively. On the left side of the figure the extrapolated SMR from the swimming experiments can be compared with the measured SMR, adjusted to the weight of the swimming fish using a weight coefficient of 0.82. The error bars around SMR predicted by the swimming experiment were obtained by extrapolating the M<sub>O2</sub> for every fish back to zero. There were no statistical differences between the measured SMR and the extrapolated SMR at any of the temperatures.

Schurmann, H. & Steffensen, J. F. (1997) Effects of temperature, hypoxia and activity on the metabolism of Atlantic cod, Gadus morhua. J. Fish Biology. 50; 1166-1180.

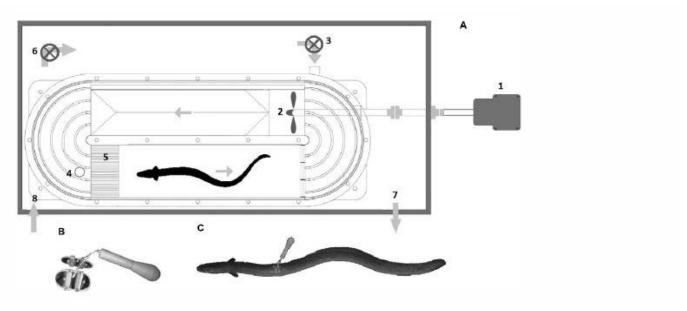




### Pop Up Satellite Tags Impair Swimming Performance and Energetics of the European Eel (*Anguilla anguilla*)

Caroline Methling<sup>1\*</sup>, Christian Tudorache<sup>2</sup>, Peter V. Skov<sup>3</sup>, John F. Steffensen<sup>1,4</sup>

1 Marine Biological Section, University of Copenhagen, Helsingør, Denmark, 2 Institute of Biology, Leiden University, Leiden, the Netherlands, 3 National Institute of Aquatic Resources, Section for Aquatic Resources, Technical University of Denmark, Hirtshals, Denmark, 4 DTU Aqua, National Institute of Aquatic Resources, Technical University of Denmark, Charlottenlund, Denmark



**Figure 1. Schematic of swim tunnel and PSAT dummy.** A. 1. Motor, 2. Propeller, 3. Flushpump (inlet), 4. Flush outlet, 5. Honeycomb, 6. Mixing pump, 7. Outlet from tank to water reservoir, 8. Inlet to tank from water reservoir. Arrows indicate water flow.B. PSAT dummy. C. PSAT dummy attached to eel. Refer to text for details. doi:10.1371/journal.pone.0020797.g001



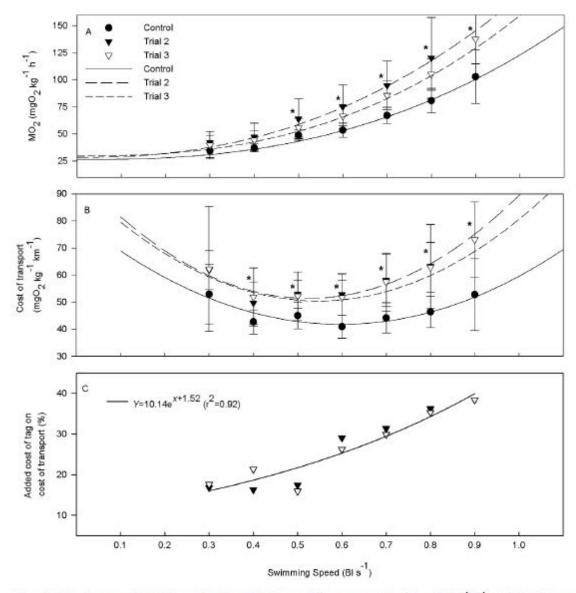


Figure 2. Swimming energetics in (A anguilla) with a PSAT dummy, A. Oxygen consumption ( $MO_2$ ,  $mgO_2$  kg $^{-1}$  k $^{-1}$ ) and B. Cost of transport (COT,  $mgO_2$  kg $^{-1}$  km $^{-1}$ ) as a function of swimming speed (U, B is  $^{-1}$ ) swimming with and without a PSAT dummy. Swim trials without (control) and with a tag (trials 2, 3) were performed on the same individual (N=9). Lines are regression lines (refer to Table 1 for regression values). An asterisk denotes significant difference between control and tagged condition (Repeated measures ANOVA, p < 0.05). Data are presented as mean  $\pm$  SD. C. Additional cost of tag on COT as a function of swimming speed (U, B is  $^{-1}$ ). Line is regression line of the average value of trials 2 and 3. doi:10.1371/journal.pone.0020797.g002



FACULTY OF SCIENCE UNIVERSITY OF COPENHAGEN

#### POSTER:



### AquaResp® - Free software for measuring MO2 in aquatic animals

Morten Bo S. Svendsen 1, Peter G. Bushnell 2 and John F. Steffensen 1

Marine Biological Laboratory, University of Copenhagen, Denmark
 Indiana University South Bend, Indiana, United States

http://www.AquaResp.com

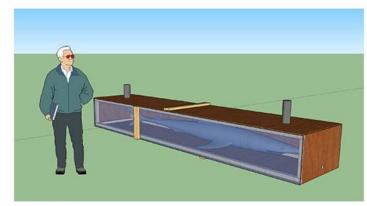
To be published in 2015 in special issue of Journal of Fish Biology.

### Design and setup of an intermittent-flow respirometry system for aquatic organisms

MBS Svendsen<sup>1</sup>, PG Bushnell<sup>2</sup>, JF Steffensen<sup>1</sup>



Metabolic rate of Greenland shark – Greenland June 2015: Respirometer volume: 1600 Liters; flush pump 5000 L/hr; recirc pump: 5000 L/hr Largest shark: 330 cm and 346 kg. Oxygen etc measured by YSI 9620 CTD.



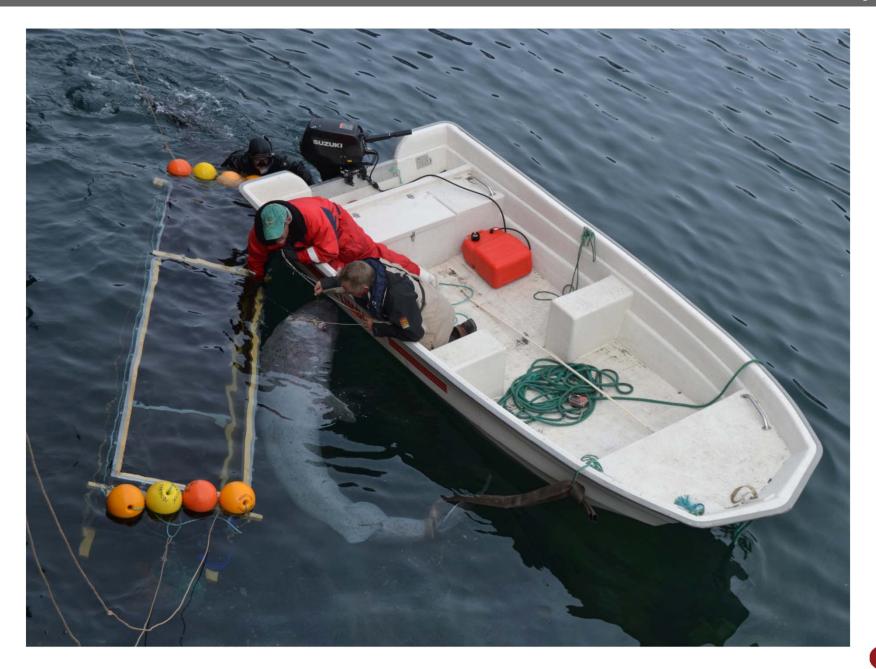
Respirometret



Respirometret under konstruktion

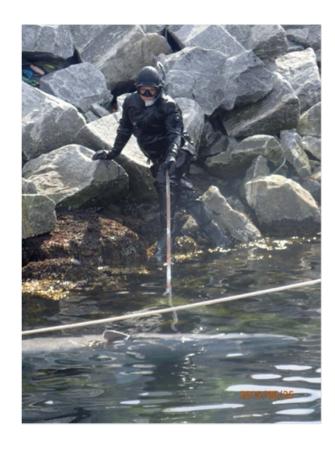


















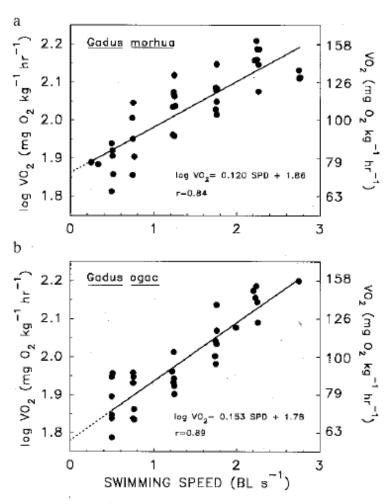


Fig. 1 Log rate of oxygen consumption of G. morhua (a) and G. ogac (b) plotted against swimming speed

Bushnell, P. G., Steffensen, J. F. & Schurmann, H. (1994). Exercise metabolism of two species of cod in Arctic waters, Atlantic cod ,Gadus morhua, and uvak ,Gadus ogak. Polar Biology. 14; 14; 43-48.



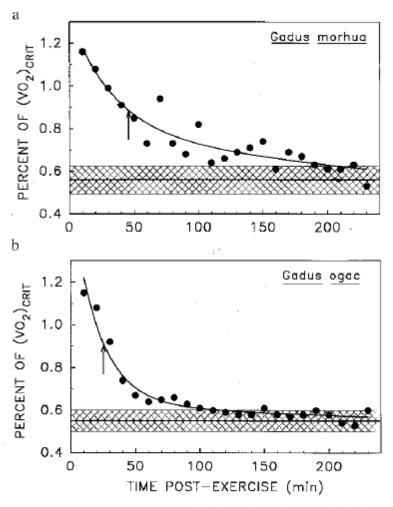
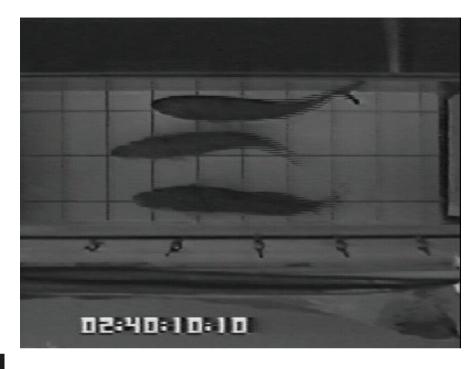


Fig. 2 Oxygen consumption as a fraction of maximum  $VO_2$  during recovery from exhaustive swimming exercise. The routine metabolic rate is given by the heavy horizontal line and the 95% confidence limits represented by the cross-hatched area. The time required to repay 50% of the oxygen debt is indicated by the arrows

Bushnell, P. G., Steffensen, J. F. & Schurmann, H. (1994). Exercise metabolism of two species of cod in Arctic waters, Atlantic cod, Gadus morhua, and uvak, Gadus ogak. Polar Biology. 14; 14; 43-48.



## Fish Physiology Swimming





#### Modes of Swimming

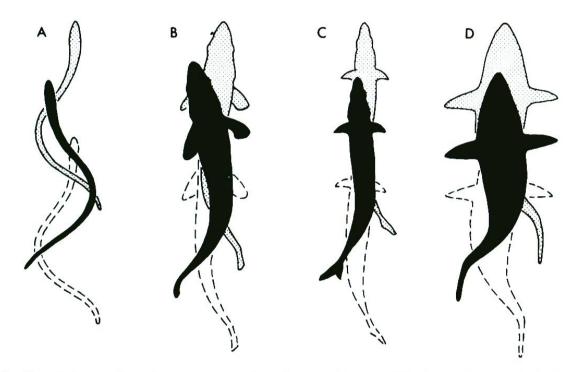


Fig. 2. Gradation of swimming modes from (A) anguilliform, through (B) subcarangiform, and (C) carangiform, to (D) thunniform. The black silhouette (dorsal view) is superimposed on successive positions one-half tail beat earlier (broken outline) and one-half tail beat later (stippled). (A) Anguilla anguilla, 7 cm long, about 1.5 beats/sec. (B) Gadus merlangus, 24 cm long, about 1.7 beats/sec. (C) Scomber scombrus, 40 cm long, about 2.4 beats/sec. (D) Euthynnus affinis, length unknown, perhaps about 40 cm, about 2.4 beats/sec. A, B, and C based on Gray (1933a, 1968); D based on Fierstine and Walters (1968).

## Sustained Swimming:

110 F. W. H. BEAMISH

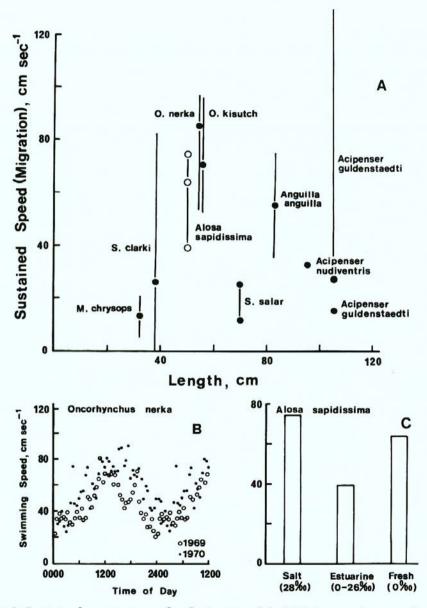


Fig. 1. Sustained cruising speeds of migrating fish. (A) Mean and range of sustained speed in relation to length (see text). (B) Diel fluctuations (redrawn from Madison et al., 1972, J. Fish. Res. Board Can.). (C) Mean speed on entry into freshwater from the estuarine and marine environment (Dodson et al., 1970, 1971, 1972).

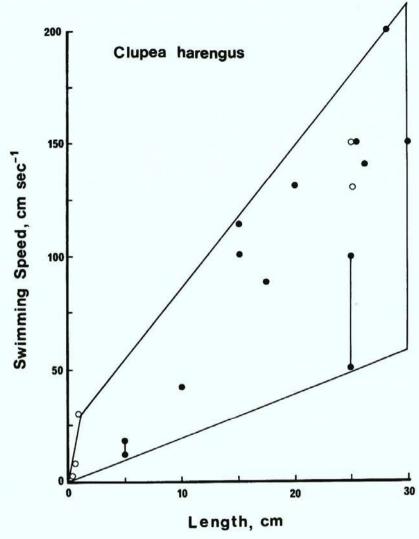


Fig. 2. Field and laboratory observations on the swimming capacity of herring, Clupea harengus, in relation to length. Sustained schooling and prolonged speeds are indicated by closed circles. Speeds reported as burst are recorded as open circles. [From Fridriksson and Aasen, 1952; Jones, 1957; Blaxter and Dickson, 1959; Bishai, 1960; Brawn, 1960; Schärfe, 1960; Boyar, 1961; Blaxter, 1962; Chestnoy (cited in Radakov, 1964); Blaxter and Parrish, 1966; High and Lusz, 1966.]

#### Annualar swimming chamber

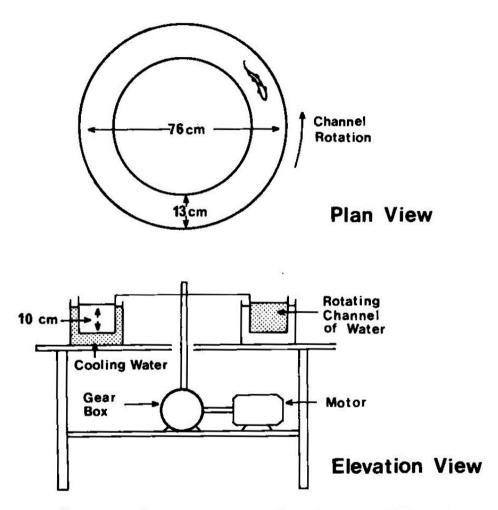
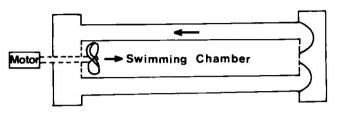
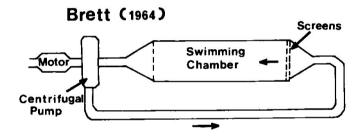


Fig. 3. Diagram of an annular swimming chamber, modified by Hammond and Hickman (1966) after that described and employed by Fry and Hart (1948). (From Hammond and Hickman, 1966, J. Fish. Res. Board Can.)

#### Swim Flumes

#### Blažka, Volf, and Cepala (1960)





#### Thomas, Burrows, and Chenoweth (1964)

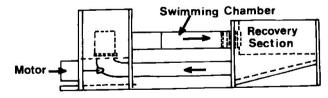


Fig. 4. Swimming flumes. The direction of flow is indicated by arrows. (Redrawn from Bishai, 1960, Thomas et al., 1964, Smith and Newcomb, 1970.)

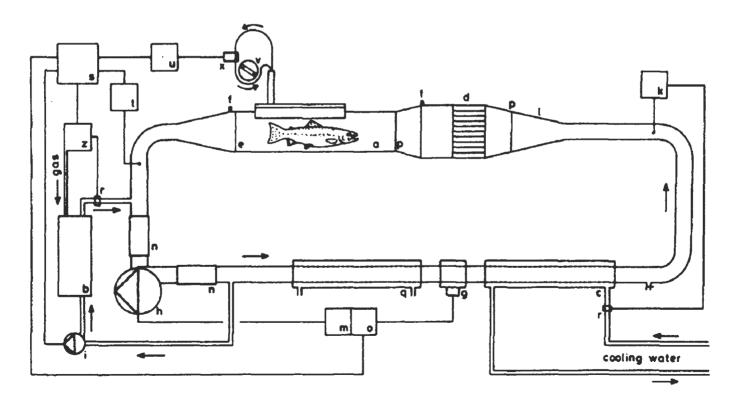
#### AN AUTOMATED SWIMMING RESPIROMETER

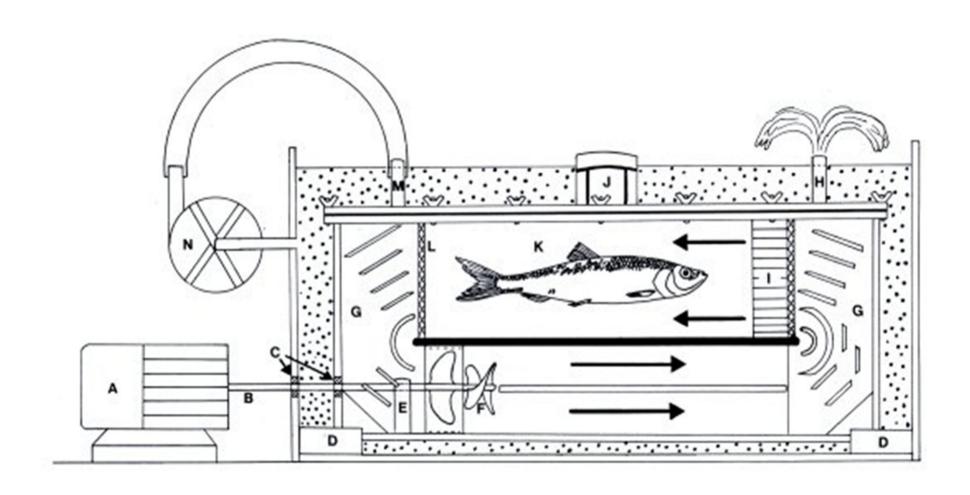
J. F. STEFFENSEN, K. JOHANSEN and P. G. BUSHNELL\*

Department of Zoophysiology, University of Aarhus, DK-8000 Aarhus C, Denmark

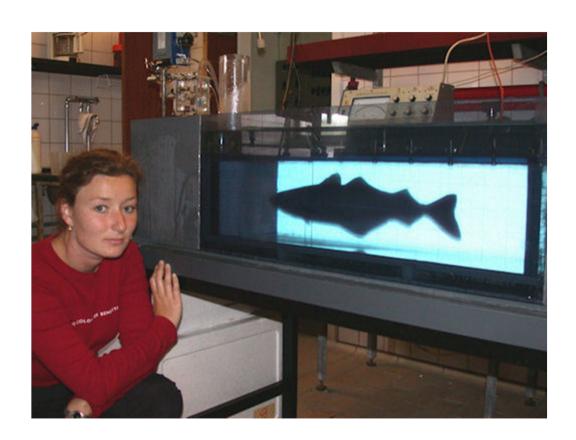
(Received 22 February 1984)

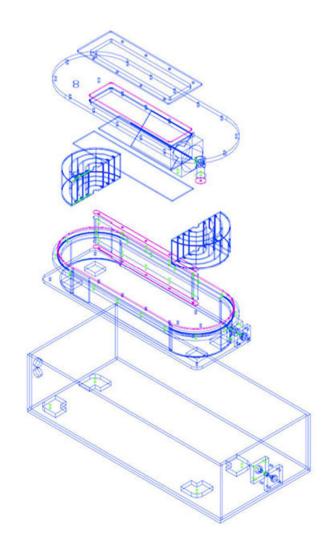
Abstract—An automated respirometer is described that can be used for computerized respirometry of trout and sharks.





"Steffensen-design Type I"





"Steffensen Design Type II"

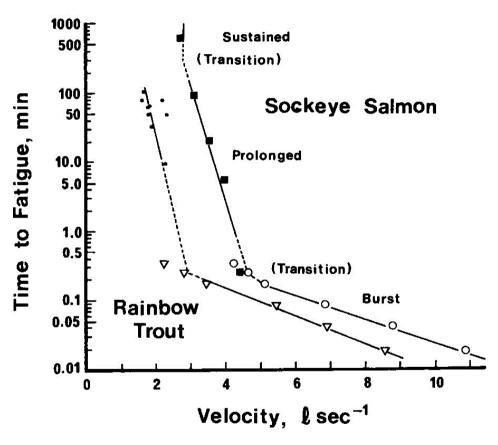


Fig. 6. Identification of sustained, prolonged, and burst speeds for rainbow trout, Salmo gairdneri, and sockey salmon Oncorhynchus nerka, on the basis of their fatigue time at different swimming velocities. Results for rainbow trout obtained from Bainbridge (1960, 1962), and for sockeye salmon from Brett (1964). (Redrawn from Brett, 1964, J. Fish. Res. Board Can.)

#### Critical swimming speed

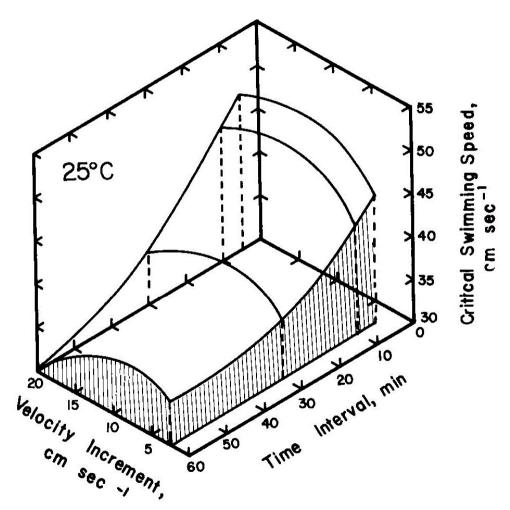


Fig. 5. Critical swimming speed of largemouth bass, *Micropterus salmoides* (10 cm), in relation to the interval between and magnitude of velocity increments. (Redrawn from Farlinger and Beamish, 1977.)

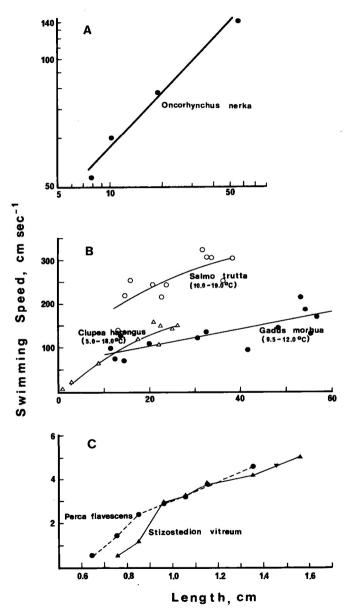


Fig. 7. Swimming speed and length. (A) Critical swimming speed of sockeye salmon (Brett, 1964). (B and C) Burst (sprint) swimming speeds (Blaxter and Dickson, 1959; Houde, 1969).

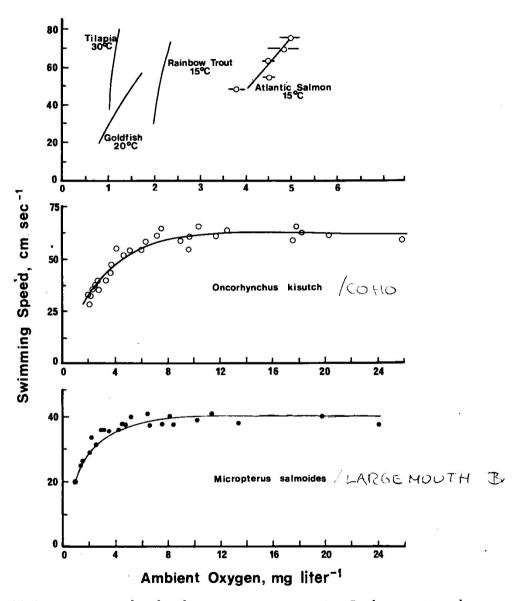


Fig. 14. Swimming speed and ambient oxygen concentration. In the upper panel, critical oxygen concentrations at which fish are unable to maintain a sustained speed (Kutty, 1968; Kutty and Saunders, 1973). In the lower two panels, the relationships between prolonged speed and oxygen (Dahlberg et al., 1968).

# Oxygen consumption versus swimming speed

#### 2. SWIMMING CAPACITY

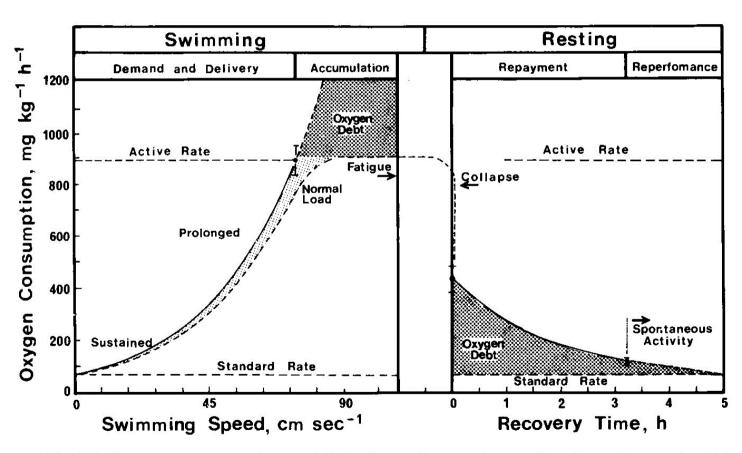


Fig. 17. Oxygen consumption and debt for sockeye salmon, *Oncohynchus nerka* (18 cm), in relation to swimming speed and recovery at 15°C. (Redrawn from Brett, 1964, *J. Fish. Res. Board Can.*)

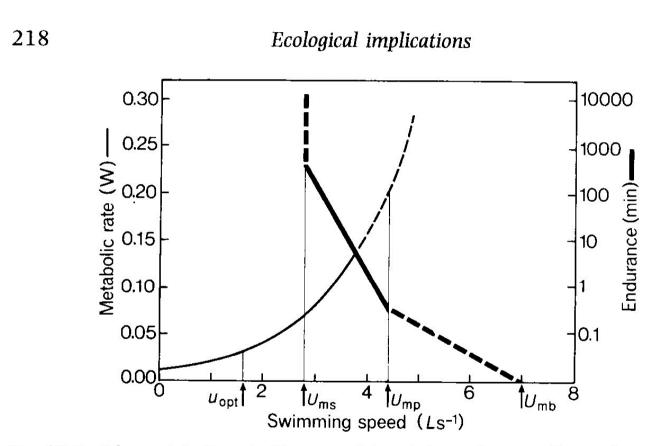


Fig. 10.3 The metabolic rate (linear scale) and the endurance (logarithmic scale) of a 0.18 m, 0.05 kg sockeye salmon as functions of swimming speed in  $Ls^{-1}$ . The water temperature was 15 °C. The optimum swimming speed ( $u_{\rm opt}$ ), the maximum sustained speed ( $U_{\rm ms}$ ), the maximum prolonged speed ( $U_{\rm mp}$ ) and an estimate of the maximum burst speed ( $U_{\rm mb}$ ) are indicated. Based on Brett (1964).

#### Metabolic scope

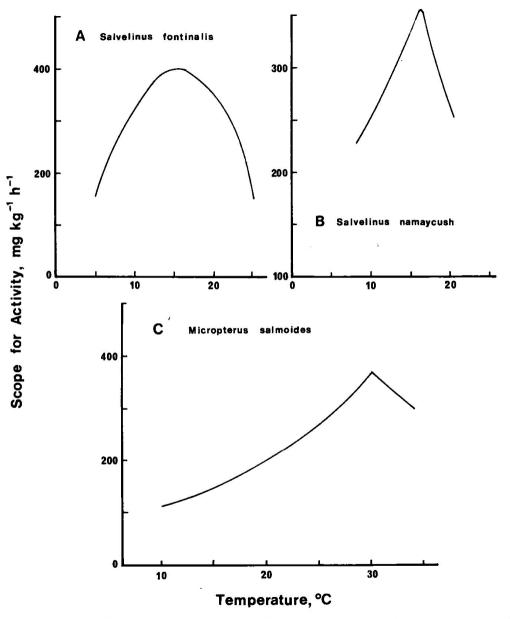


Fig. 11. Metabolic scope for activity of (A) brook trout, Salvelinus fontinalis (Graham, 1949); (B) lake trout, Salvelinus namaycush (Gibson and Fry, 1954); (C) largemouth bass, Micropterus salmoides (Beamish, 1970), in relation to temperature.

Prolonged swimming speed versus temperature

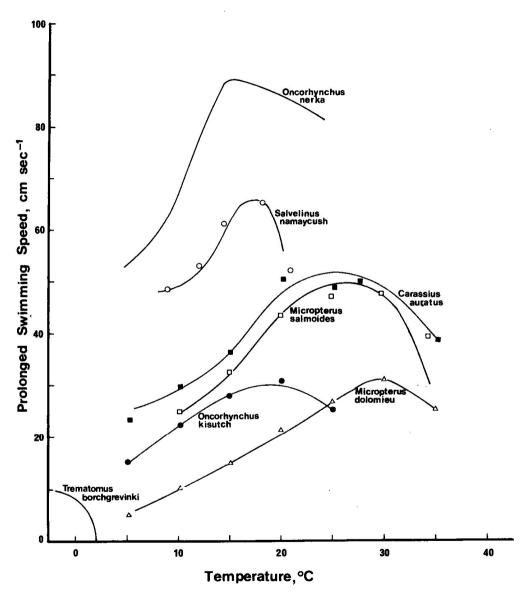


Fig. 12. Prolonged swimming speed and temperature. (From Fry and Hart, 1948; Gibson and Fry, 1954; Brett et al. 1958; Wohlschlag, 1964; Larimore and Duever, 1968; Beamish, 1970; Brett and Glass, 1973.)

### VO2 vs swim speed

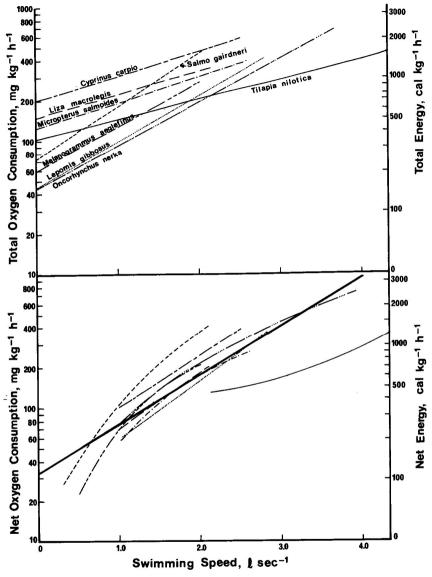


Fig. 18. Oxygen consumption, energy utilization, and swimming speed. The total oxygen consumed is presented in the upper panel. Subtraction of the basal from total metabolic rate provides a measure of the energy required for a given speed of swimming. The heavy line in the lower panel denotes the general rate of increase in net oxygen consumption and was fitted by eye. (From Basu, 1959; Beamish, 1964a, 1970; Brett, 1964; Brett and Sutherland, 1965; Farmer and Beamish, 1969; Kutty, 1969; Webb, 1971b.)

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#### The costs of swimming

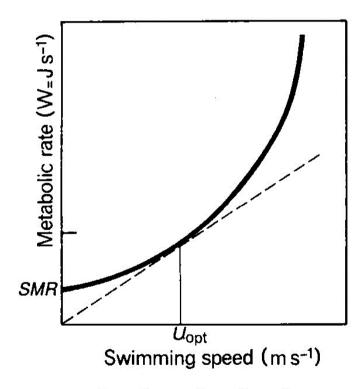


Fig. 9.1 Metabolic rate as a function of swimming speed. SMR is the standard metabolic rate at speed 0. The amount of work per unit distance covered is at a minimum at  $u_{\rm opt}$ .

#### Cost of transport

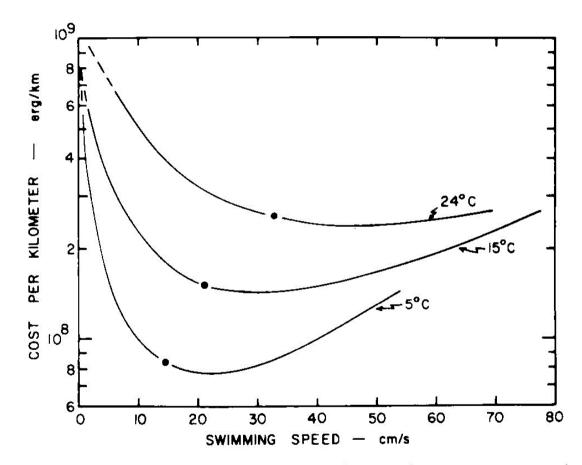


Fig. 5. Relation between energetic costs (erg/km) and swimming speed of yearling sockeye salmon at three temperatures. Optimum swimming speeds occur at the lowest point of the curve. Predicted optimum speeds (Weihs, 1973a) are solid circles. (Based on data in Brett, 1964; after Brett, 1965b; by Webb, 1975a, Bull. Fish. Res. Board Can. No. 190.)

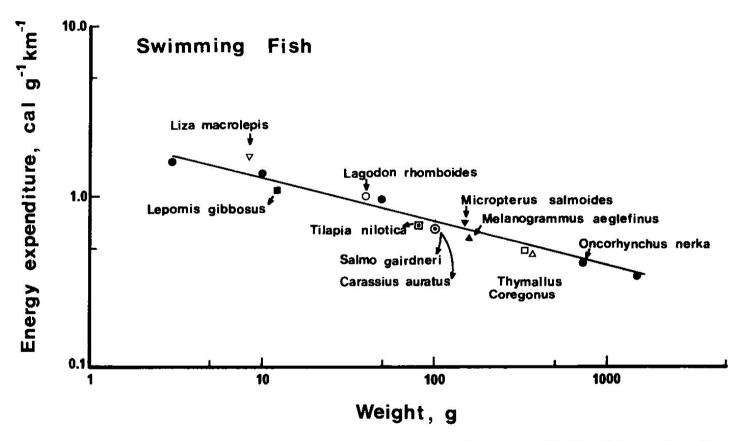


Fig. 19. Energy cost of swimming relative to body size calculated by Schmidt-Nielsen (1972) from data collected by Brett (1964), Wohlschlag et al. (1968), Matyukhin and Stolbow (1970, reported in Schmidt-Nielsen, 1972), Rao (1971), and Smit et al. (1971). In addition, measurements made by Brett and Sutherland (1965), Farmer and Beamish (1969), Kutty (1969), Tytler (1969), and Beamish (1970) have been recalculated and added to the figure.

# Swim VO2 of different salmonids

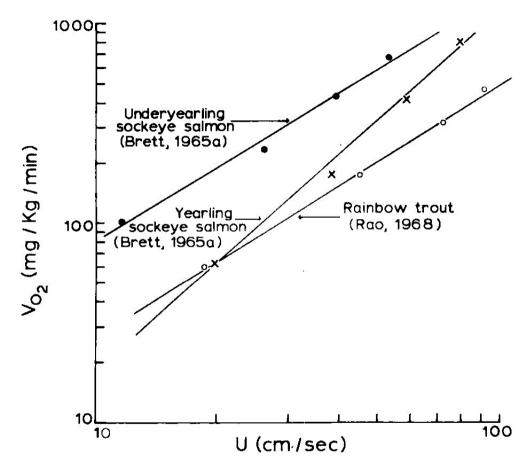


Fig. 2. Relationship between oxygen uptake (ml/kg/min) and swimming speed (U). For underyearling sockeye salmon and rainbow trout the slopes of the line are between 1.3 and 1.4, whereas for yearling sockeye salmon the slope is 1.8. (Data from Brett, 1965a; Rao, 1968.)

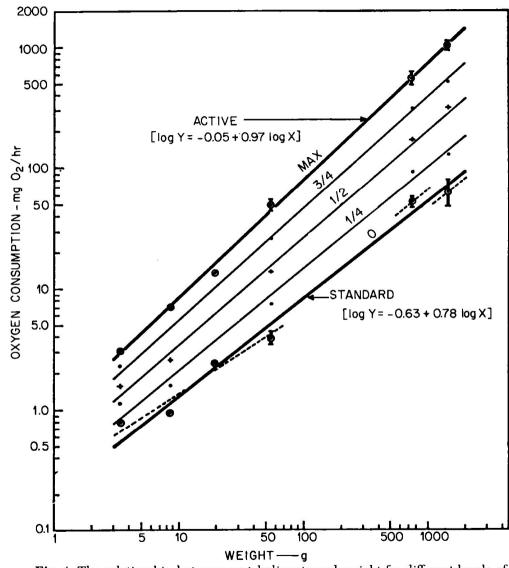


Fig. 4. The relationship between metabolic rate and weight for different levels of activity expressed as fractions of the maximum 60-min sustained speed (max) in O. nerka. Experiments performed in freshwater at 15°C. Broken lines represent possible relation of standard metabolism for immature freshwater stage and for mature fish of different sex. Whenever fish were tested singly limits of  $\pm 2$  SE are indicated. (From Brett, 1965a, J. Fish. Res. Board Can. 22, 1491.)

#### 7. THE RESPIRATORY AND CIRCULATORY SYSTEMS

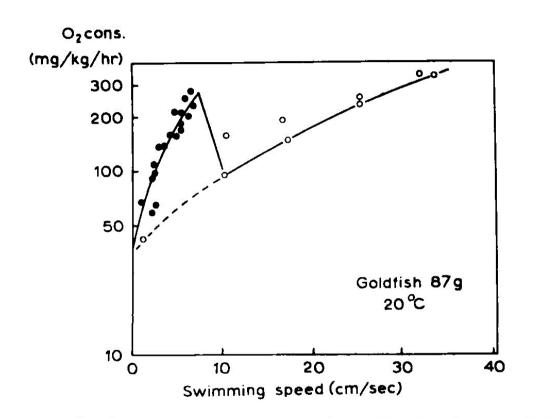
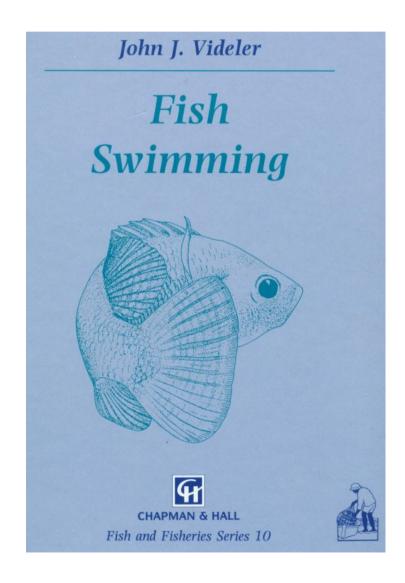


Fig. 6. Relationship between oxygen consumption and swimming speed of an 87-g goldfish (C. auratus) when spontaneously swimming in a nonrotating annular chamber (closed circles) and when forced to swim at various speeds in a rotating chamber (open circles). Throughout the experiments oxygen concentrations were at ambient. When forced to swim at 10 cm/sec the fish seemed to switch to a "thriftier use of oxygen." (From Smit, 1965, Can. J. Zool. 43, 623.)

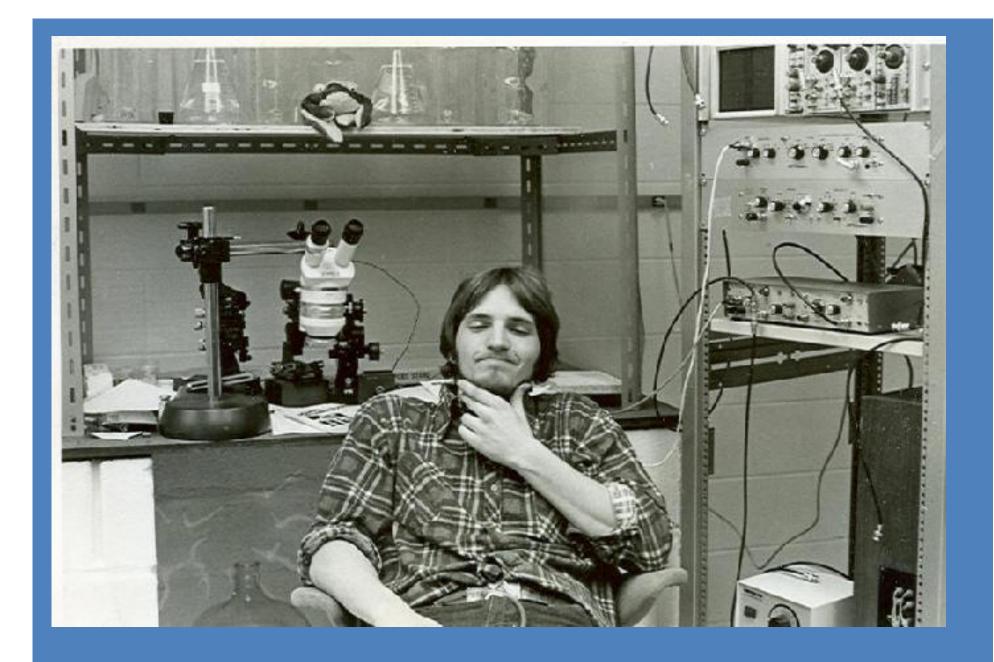
#### References

Fish Physiology, Vol. VII; Locomotion. Eds.: W. S. Hoar & D. J. Randall Academic Press, 1978



# Measuring metabolism the hard way..

Other tricks with Fick's (Principle)



### Adolf Eugene Fick (1829 - 1901)

- devised to measure cardiac output (CO)
- Fick principle-blood flow to an organ can be calculated using a marker if you know
  - Amount of marker taken up by the organ per unit time
  - Concentration of marker in arterial blood supplying the organ
  - Concentration of marker substance in venous blood leaving the organ
- In Fick's original method, the "organ" was the entire human body and the marker substance was oxygen.

$$VO_2 = (CO \times C_{AO_2}) - (CO \times C_{VO_2})$$

- CO= cardiac output
- C<sub>AO<sub>2</sub></sub> = Arterial oxygen content
   C<sub>VO<sub>2</sub></sub> = Venous oxygen content
- $CO = VO_2 / (C_{AO_2} C_{VO_2})$

 Other markers- CO2, dye, temperature pulse

$$VO_2 = (CO \times C_{A_{O_2}}) - (CO \times C_{V_{O_2}})$$

O<sub>2</sub> consumed= Amount O<sub>2</sub> in – Amount O<sub>2</sub> out

$$VO_2 = (CO \times C_{A_{O_2}}) - (CO \times C_{V_{O_2}})$$

$$VO_2 = CO \times (C_{AO_2} - C_{VO_2})$$

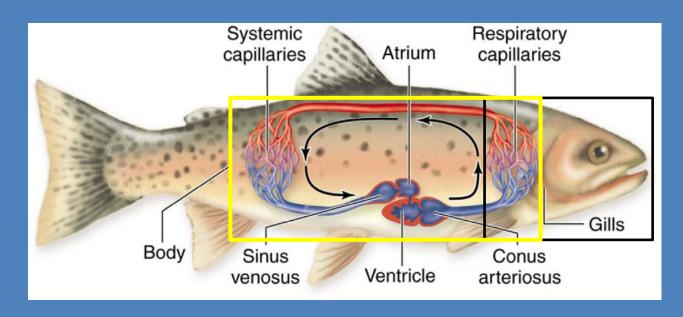
Flow through respirometer

$$VO_2$$
 = Water Flow x ( $C_{inO_2}$ -  $C_{outO_2}$ )

Stop flow respirometer

$$VO_2$$
 = Water Volume x ( $C_{startO_2}$ -  $C_{endO_2}$ )

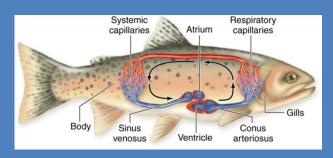
### Applying Fick in fish

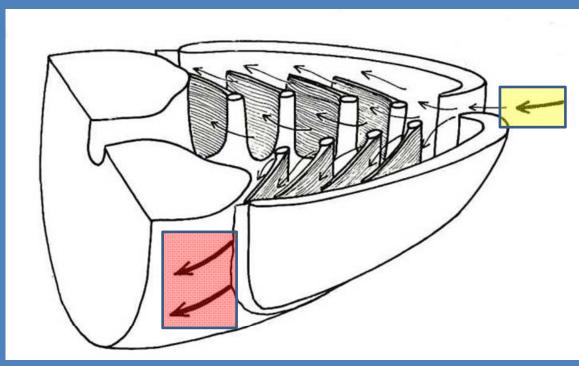


Respiratory (water) side

Cardiovascular (blood) side

### Applying Fick in fish on the water side

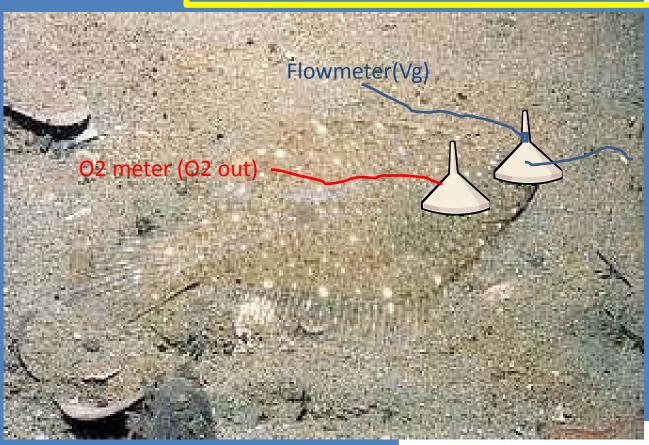




Respirometer:  $VO_2$  = Water Flow x  $(C_{inO_2} - C_{outO_2})$ 

Fish:  $VO_2$  = Ventilation Volume x ( $C_{inO_2}$ -  $C_{outO_2}$ )

# $VO_2 = Ventilation Volume(Vg) x (C<sub>inO2</sub> C<sub>outO2</sub>)$

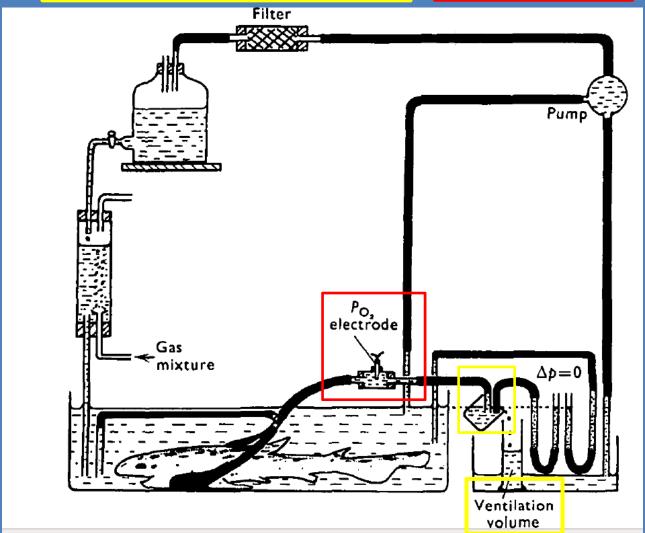


O2 meter (O2in)

Env. Biol. Fish. Vol. 7, No. 2, pp. 157-163, 1982

Gill ventilation and O2 extraction during graded hypoxia in two ecologically distinct species of flatfish, the flounder (Platichthys flesus) and the plaice (Pleuronectes platessa)



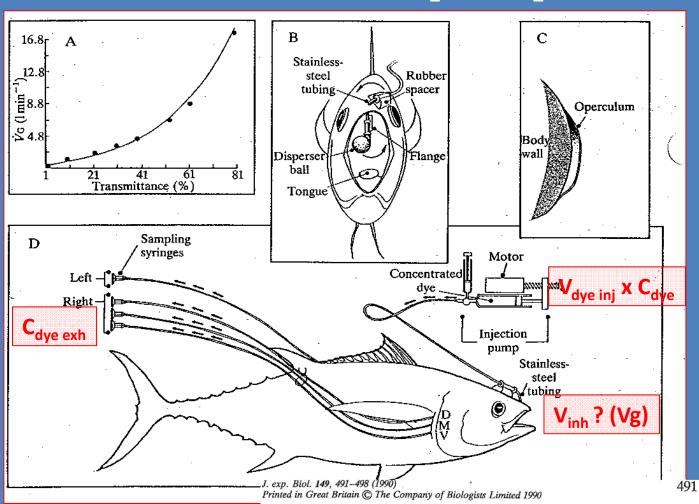


HUGHES AND UMEZAWA (1968) OXYGEN CONSUMPTION AND GILL WATER FLOW IN THE DOGFISH SCYLIORHINUS CANICULA L. J. Exp. Biol. 49: 557-564

## $VO_2$ = Ventilation Volume(Vg) x ( $C_{inO_2}$ - $C_{outO_2}$ )

Vent Vol by Dye Dilution  $V_1 \times C_1 = V_2 \times C_2$ 

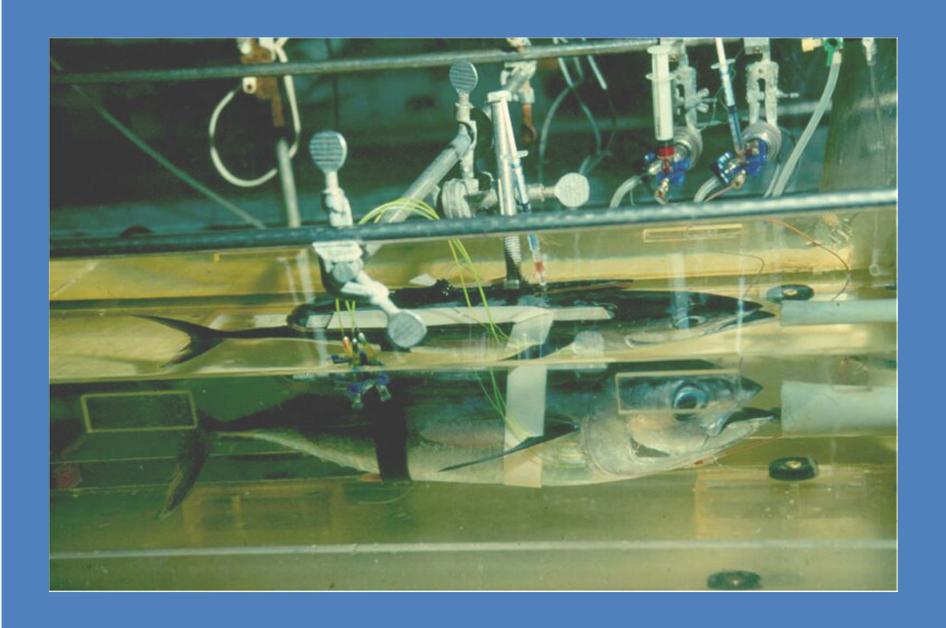
 $V_2 = V_1 \times C_1 / C_2$ 



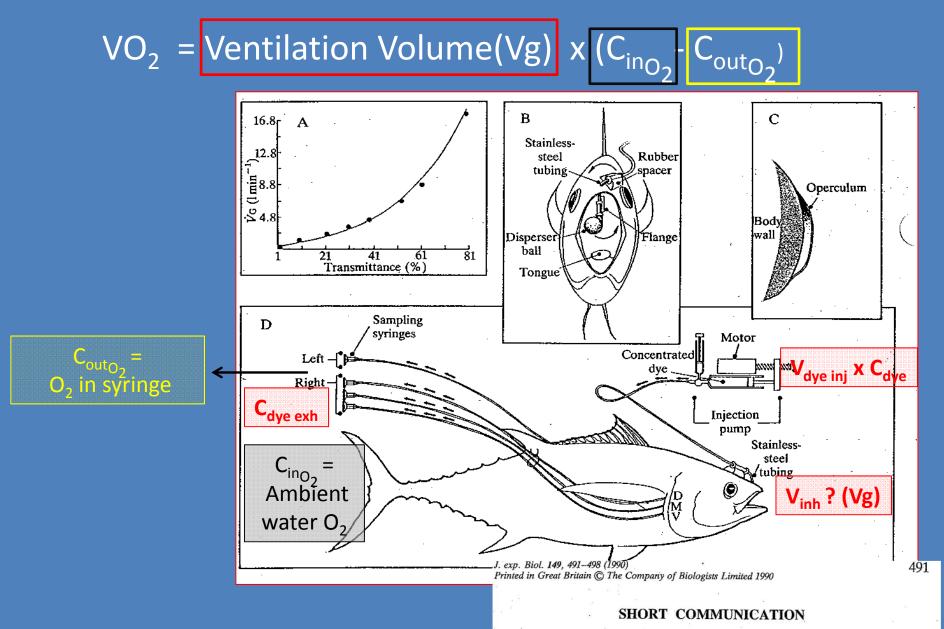
#### SHORT COMMUNICATION

MEASUREMENT OF VENTILATION VOLUME IN SWIMMING TUNAS

By DAVID R. JONES<sup>1</sup>, R. W. BRILL<sup>2</sup>, P. J. BUTLER<sup>3</sup>, P. G. BUSHNELL<sup>1</sup>
AND M. R. A. HEIEIS<sup>1</sup>





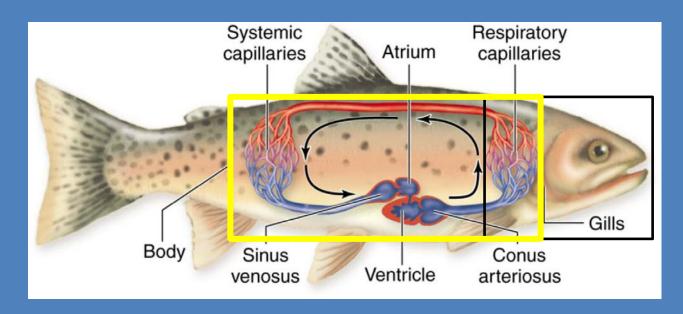


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AND M. R. A. HEIEIS<sup>1</sup>

Table 1. Ventilation volume and swimming speed of three species of tunas														
Catheter														
		pos	ition						Transmit-	Š.				
	Run		D, M		L	$P_{\rm IO_2}$	$P_{\rm E_{\rm O_2}}$	traction	tance		ition volume	$\dot{V}_{\mathrm{O}_2}$	Swimmir	
Tuna species	no.	Side	or V	(kg)	(cm)	(kPa)	(kPa)	(%)	(%)	$(1 \min^{-1})$	$(\lim^{-1} kg^{-1})$	$(ml kg^{-1} h^{-1})$	$(cm s^{-1})$	$(L s^{-1})$
Kawakawa-7	1	·L	M	2.03	46.3	18.45	6.84	62.9	78.0	12.0	5.9	1521.8	104.2	2.3
		R	M				9.96	46.0	84.0	14.9	7.3	1380.5		200000
							(8.33)	(54.4)	(81.0)	(13.4)	(6.6)	(1467.2)	(104.2)	(2.3)
	2	L	M	2.03	46.3	18.45	11.09	39.9	89.1	17.9	8.8	1436.6	102.6	2.2
×.		R	M				11.05	40.1	90.8	19.0	9.4	1535.3	- Control of the Control	10/30/121
			4				(11.07)	(40.0)	(90.0)	(18.4)	(9.1)	(1485.1)	(102.6)	(2.2)
Kawakawa-11	1	L	M	1.83	45.2	19.81	8.16	58.8	48.5	4.0	2.2	529.3	54.0	1.2
		R	D				6.07	69.4	47.0	3.8	2.1	591.7		
		R	M				7.35	62.9	46.3	3.7	2.0	523.3		
`		R	V				9.72	50.9	44.1	3.4	1.9	391.5		
							(7.83)	(60.6)	(46.5)	(3.7)	(2.0)	(506.4)	(54.0)	(1.2)
	2	L	M	1.83	45.2	20.41	9.52	53.4	48.1	4.0	2.2	473.4	73.0	1.6
		R	D				5.56	72.8	44.9	3.5	1.9	575.5		
. ^		R	M			V1	6.36	68.8	44.9	3.5	1.9	544.5		
		R	V				13.00	36.3	43.9	3.4	1.9	277.1		
						٠.	(8.61)	(57.8)	(45.4)	(3.6)	(2.0)	(466.4)	(73.0)	(1.6)
	4	L	M	1.83	45.2	18.89	9.17	51.4	45.0	3.5	1.9	408.4	84.0	1.9
		R	D				5.13	72.8	45.6	3.6	2.0	590.7		
		R	M				8.33	55.9	44.0	3.4	1.9	428.0		
		R	V				10.57	44.0	42.7	3.3	1.8	321.8		
							(8.31)	(56.0)	(44.3)	(3.5)	(1.9)	(434.3)	(84.0)	(1.9)
Kawakawa-14	1	L	M	1.38	40.5	19.23	10.52	45.3	50.9	4.4	3.2	593.1	71.0	1.8
		R	D				7.56	60.7	54.0	4.9	3.6	888.2		
		R	M				8.87	53.9	52.5	4.7	3.4	747.4		
		R	V				9.07	52.8	51.9	4.6	3.3	717.3		
							(9.01)	(53.2)	(52.3)	(4.6)	(3.4)	(733.0)	(71.0)	(1.8)

### Applying Fick in fish



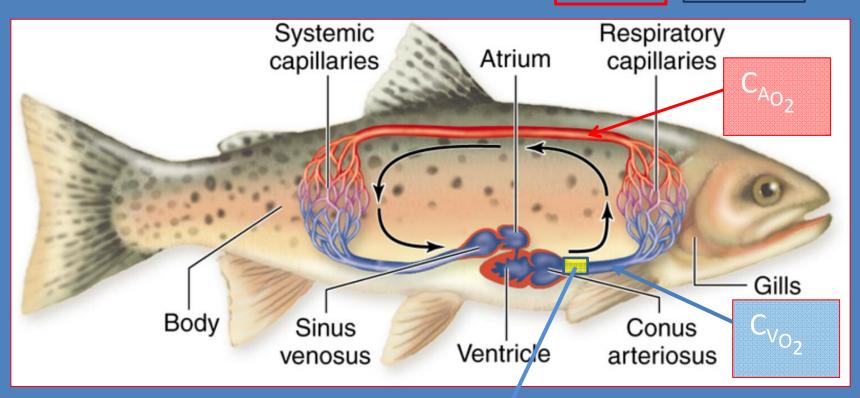
Respiratory (water) side

Cardiovascular (blood) side

RESPIROMETER:  $VO_2$  = Water Flow x  $(C_{in_{O_2}} - C_{out_{O_2}})$ 

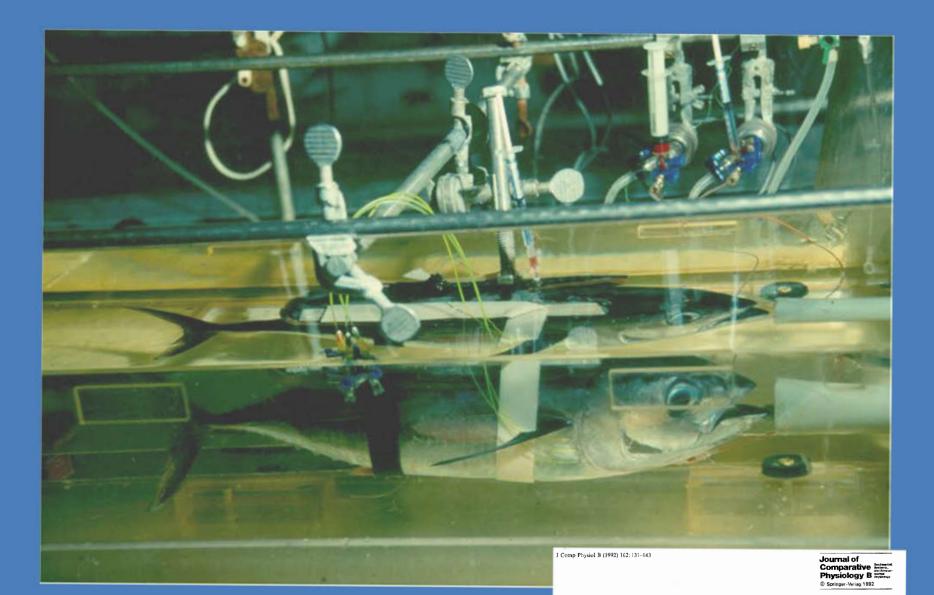
FISH:  $VO_2 = Blood flow (CO)$ 

x (C<sub>AO2</sub> - C<sub>VO2</sub>)



Doppler or EM flow probe





Oxygen transport and cardiovascular responses in skipjack tuna (Katsuwonus pelamis) and yellowfin tuna (Thunnus albacares) exposed to acute hypoxia

Peter G. Bushnell<sup>1.\*</sup> and Richard W. Brill<sup>2.\*\*</sup>

Table 1. Mean values (± SEM) of cardiorespiratory variables measured in normoxia and significant changes occurring during hypoxia in yellowfin tuna (Thunnus albacares)

Variable	Control	Hypoxia		•
	-	$(P_iO_2 \approx 130 \text{ mmHg})$	(P <sub>1</sub> O <sub>2</sub> ≈90 mmHg)	$(P_1O_2 \approx 50 \text{ mmHg})$
P <sub>i</sub> O <sub>2</sub> (mmHg)	$154.1 \pm 0.6 (10)$	$-23.6 \pm 1.8 $ (8)	$-65.5 \pm 2.2 (10)$	$-103.1 \pm 1.9 (7)$
$P_{e}O_{2}$ (mmHg)	$75.9 \pm 3.7 (10)$	NS	$-16.8 \pm 4.4 (10)$	na
$P_{\bullet}O_{2}$ (mmHg)	$74.3 \pm 6.3 (10)$	NS	$-23.6 \pm 4.3 $ (10)	$-40.5 \pm 7.8 (7)$
$P_{v}O_{2}$ (mmHg)	$32.3 \pm 3.2 (9)$	$-1.2 \pm 0.5$ (7)	$-4.5 \pm 1.0 (9)$	$-8.7 \pm 0.8$ (6)
$C_aO_2 \text{ (ml · dl - 1)}$	$13.6 \pm 1.2 (10)$	$-0.9 \pm 0.4 (8)$	$-1.7 \pm 0.7 (10)$	$-2.4 \pm 0.5$ (7)
$C_{\mathbf{v}}O_{2} \text{ (ml} \cdot \text{dl}^{-1})$	$9.0 \pm 0.8 (9)$	NS	NS	$-2.4 \pm 0.4$ (6)
$C_aO_2-C_vO_2 \text{ (ml}\cdot\text{dl}^{-1})$	$4.9 \pm 0.8 (9)$	$-1.1 \pm 0.4$ (7)	NS	NS
$P_aCO_2$ (mmHg)	$3.3 \pm 0.4 (8)$	$-0.3 \pm 0.1$ (7)	NS	$-0.8 \pm 0.3$ (7)
$P_{v}CO_{2}$ (mmHg)	$3.8 \pm 0.5 (8)$	NS	NS	$-0.6 \pm 0.2$ (6)
рНа	$7.82 \pm 0.03 (10)$	$+ 0.02 \pm 0.01$ (8)	$+ 0.05 \pm 0.01 (10)$	$+ 0.06 \pm 0.01$ (7)
рНу	$7.83 \pm 0.02 (9)$	NS	NS	NS
pHa - pHv	$-0.01\pm0.02(9)$	NS	$+ 0.04 \pm 0.01$ (8)	$+ 0.06 \pm 0.01$ (6)
O <sub>2</sub> delivery (ml·min <sup>-1</sup> ·kg <sup>-1</sup> )	$14.5 \pm 1.5 (8)$	NS	NS	$-6.3 \pm 1.7 (7)$
$VO_2$ -body (ml·min <sup>-1</sup> ·kg <sup>-1</sup> )	$4.7 \pm 0.4 (7)$	$-1.5 \pm 0.4$ (6)	NS	$-1.3 \pm 0.2$ (6)
$VO_2$ -total (ml·min <sup>-1</sup> ·kg <sup>-1</sup> )	$10.5 \pm 0.5 (10)$	$+ 1.1 \pm 0.5 (8)$	NS	na
$\dot{V}O_2$ -gill (ml·min <sup>-1</sup> ·kg <sup>-1</sup> )	$5.9 \pm 0.8 (7)$	$+ 2.5 \pm 0.7$ (6)	NS	na
$\hat{V}_{g}(1 \cdot \min^{-1} \cdot kg^{-1})$	$3.9 \pm 0.1 (10)$	$+ 1.1 \pm 0.8 $ (8)	$+ 3.0 \pm 0.1 (10)$	$+$ 5.1 $\pm$ 0.3 (8)
$ \frac{\dot{Q}}{\dot{V}_{g}}(\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}) $ $ \frac{\dot{V}_{g}}{\dot{Q}} \text{conductance} $	$115.4 \pm 17.4$ (8)	NS	NS	$-40.9 \pm 17.4$ (6)
$\dot{V}_{e}/\dot{Q}$	$43.4 \pm 8.8 (8)$	$+11.5 \pm 2.6 (7)$	$+39.2 \pm 6.7$ (8)	$+ 86.8 \pm 7.8 $ (6)
$V_s/Q$ conductance	$0.73 \pm 0.10 (8)$	$+ 0.18 \pm 0.08$ (7)	$+ 0.26 \pm 0.10$ (8)	$+ 0.58 \pm 0.08$ (6)
ΔPg (mmHg)	$62.3 \pm 4.4 (9)$	$-9.3 \pm 2.2 (7)$	$-27.6 \pm 3.4 (9)$	na
$TO_2$ (ml·min <sup>-1</sup> ·mmHg <sup>-1</sup> ·kg <sup>-1</sup> )	$0.17 \pm 0.02 (7)$	$+ 0.04 \pm 0.01$ (7)	$+ 0.22 \pm 0.06 (9)$	na
$DO_2 \text{ (ml · min^{-1} · mmHg^{-1} · kg^{-1})}$	$0.18 \pm 0.02 (9)$	$+ 0.05 \pm 0.01$ (7)	$+ 0.23 \pm 0.07 (9)$	na
U (%)	$50.8 \pm 2.3 (10)$	$-7.5 \pm 1.8 (8)$	$-18.0 \pm 4.0 (10)$	na
E <sub>w</sub> (%)	$62.6 \pm 2.7 (7)$	$-7.4 \pm 2.7 (7)$	$-15.3 \pm 5.3 $ (9)	na
E <sub>b</sub> (%)	$89.3 \pm 0.4 (7)$	NS	$-23.7 \pm 7.8 $ (9)	$-17.3 \pm 7.0$ (6)
HR (beates · min <sup>-1</sup> )	$96.7 \pm 5.8 (10)$	NS	NS	$-26.8 \pm 4.3 (7)$
SV (ml·beat <sup>-1</sup> ·kg <sup>-1</sup> )	$1.3 \pm 0.2 (8)$	NS	NS	NS
BP <sub>va</sub> (mmHg)	$89.7 \pm 8.1 (10)$	NS	NS	NS
BP <sub>ds</sub> (mmHg)	$32.6 \pm 2.4 (10)$	NS	NS	NS .
R <sub>branch</sub> (mmHg·ml <sup>-1</sup> ·min <sup>-1</sup> ·kg <sup>-1</sup> )	$0.71 \pm 0.06$ (8)	NS	NS	$+$ 0.25 $\pm$ 0.11 (6)
R <sub>system</sub> (mmHg·ml <sup>-1</sup> ·min <sup>-1</sup> ·kg <sup>-1</sup> )	$0.33 \pm 0.02 (8)$	NS	NS	$+$ 0.12 $\pm$ 0.04 (6)
R <sub>total</sub> (mmHg·ml <sup>-1</sup> ·min <sup>-1</sup> ·kg <sup>-1</sup> )	$1.05 \pm 0.06 (7)$	NS	NS	$+$ 0.37 $\pm$ 0.13 (6)
Cardiac power output (mW · kg <sup>-1</sup> )	$27.2 \pm 4.3 (8)$	NS	NS	$-11.7 \pm 4.6 (6)$
Hct (%)	$26.7 \pm 1.2 (10)$	NS	NS	NS

Number of fish is in parentheses; NS=not significantly different from normoxia values; na=data not available

Variable	Control	Hypoxia
		$(P_iO_2 \approx 130 \text{ mmHg})$

$VO_2$ -body (ml·min <sup>-1</sup> ·kg		
77	) $4.7 \pm 0.4$ (7) $-1.5 \pm$	
U() body/ml.min l.v	1	
		\-/
		/
I// tatal (mal : main = ) . ka	10 5 1 0 5 (10) 1 1 1	1 A E 101
IV ( )-a ( o) ( a)   l m )   e ) m ) m ;   c = k c		
1, 0, 10,000 (1111 1111111 111111	$10.5 \pm 0.5 (10) + 1.1 =$	** V ** ( V )

**VO2-body-** Calculated w/ Fick from <u>cardiovascular</u> side i.e.  $VO2=CO \times (C_{AO_2} - C_{VO_2})$ 

**VO2-total**= Calculated w/ Fick from respiratory side i.e. 
$$VO2 = Vg \times (C_{in_{O_2}} - C_{out_{O_2}})$$

# SO WHY THE DIFFERENCE???

Variable	Control	Hypoxia
		$(P_iO_2 \approx 130 \text{ mmHg})$

	· /	\ /	
O2-body (ml · min-	1.kg-1)	$4.7 \pm 0.4 (7)$	-15 + 04 (6)
OJ-OOGY (IIII IIIII)	ne j		1.0 4 0.1
20 tatal (ml. min -	1 . 1 11	105 1 05 (10)	1 11 105 (0)
O2-total (ml·min-	^ Kg ^)	$10.5 \pm 0.5 (10)$	T 1.1 #U.0 (0)
		£0'.00 ( 7)	1 25 107 (0)
O2-gill (ml·min-1	· Kg · l	$5.9 \pm 0.8 (7)$	+ 2.3 ±0./ (0)
- 1 0	0		

**VO2-body-** Calculated w/ Fick from <u>cardiovascular</u> side i.e.  $VO2=CO \times (C_{A_{O_2}} - C_{V_{O_2}})$ 

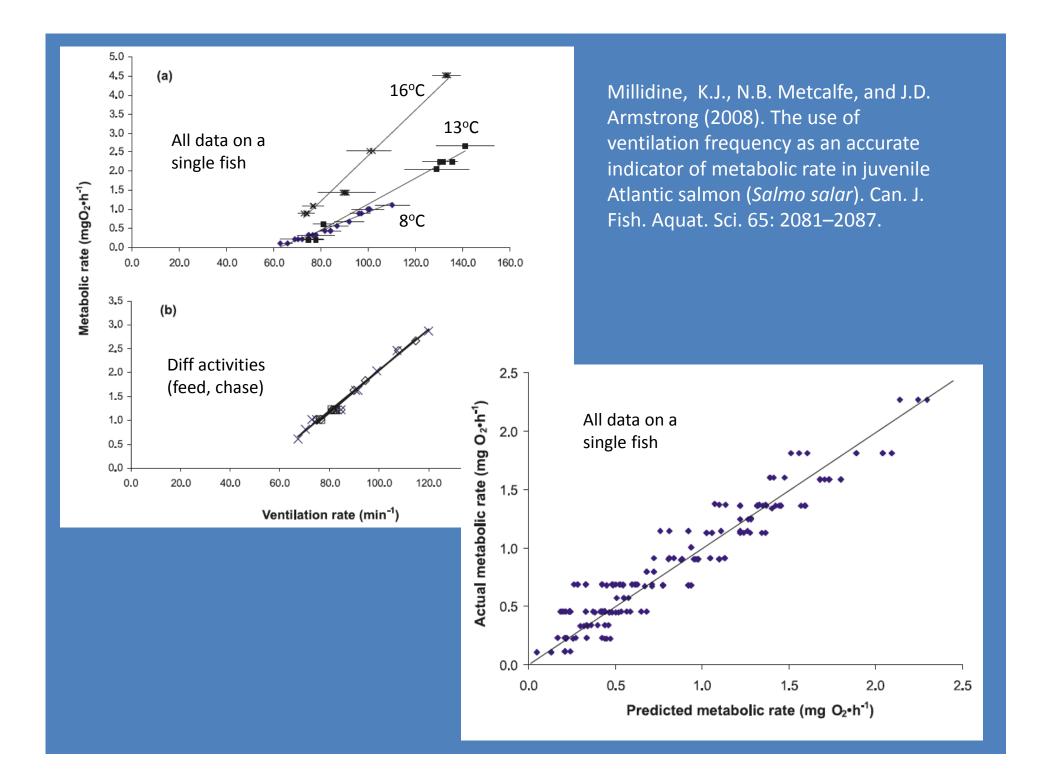
**VO2-total**= Calculated w/ Fick from respiratory side i.e. 
$$VO2 = Vg \times (C_{in_{O_2}} - C_{out_{O_2}})$$

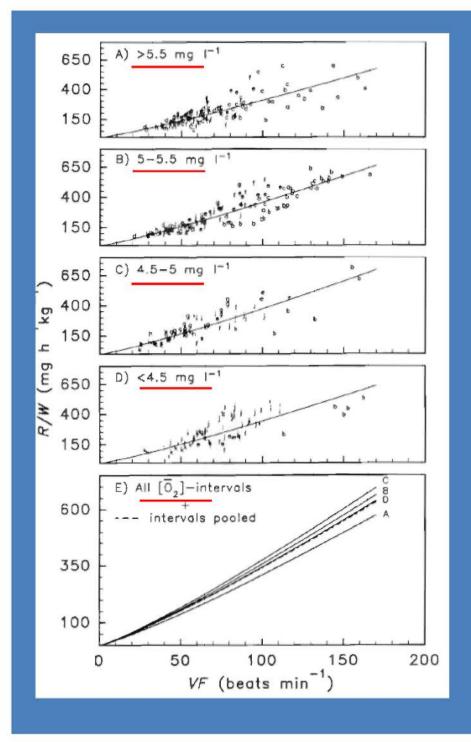
VO2-gill= VO2-total- VO2-gill

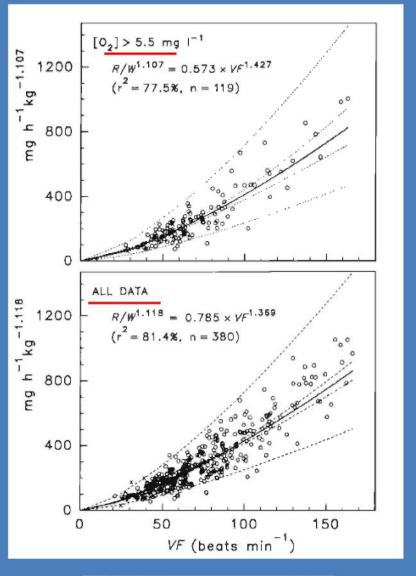
# Measuring metabolism in the field (pens, cages, tanks, etc.)

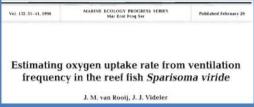
Assumes- VO2 changes primarily in response to Frequency and/or

Changes in stroke volume and extraction efficiency are closely coupled and proportional to changes in Frequency.









# Measuring metabolism in the field (pens, cages, tanks, etc.)

$$VO_2 = CO_X (C_{AO_2} - C_{VO_2})$$
Heart rate (beats/min) x stroke volume (ml/beat)

Assumes- VO2 changes primarily in response to heart rate and/or

Changes in stroke volume and extraction efficiency are closely coupled and vary in proportional to changes in heart rate.

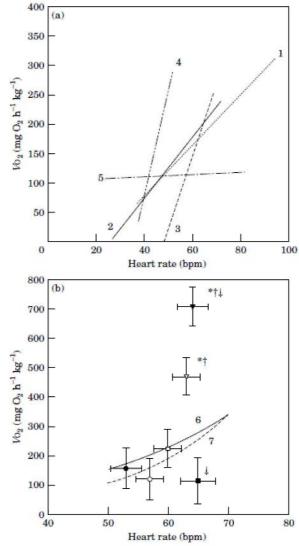
No/little skin respiration

Journal of Fish Biology (1996) 49, 226-236



#### The limitations of heart rate as a predictor of metabolic rate in fish

H. THORARENSEN\*, P. E. GALLAUGHER AND A. P. FARRELL Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6



1. (a) Heart rate and oxygen consumption of rainbow trout. 1, Regression curve from Priede & Tytler (1977). 2, Regression curve from Thorarensen, Gallaugher & Brauner (unpubl. obs.) for fish with cannula in the dorsal aorta. 3, Same group of fish as in 2, except these had a flow probe on the ventral aorta in addition to cannula in the dorsal aorta. 4, Data from Kiceniuk & Jones (1977). 5, Data from Holeton & Randall (1967) for fish during progressive hypoxia. (b) Heart rate and oxygen consumption in chinook salmon (Thorarensen & Gallaugher unpubl. obs.). Filled symbols: Fish exercise trained for 2-4 months, curve 6; Open symbols: Control fish, curve 7. ○, ♠, resting fish; ∇, ▼, maximum oxygen consumption of swimming fish; □, ■, 1 h after fish swam to Ucrit. Curves 6 and 7 are not significantly different (least squares estimate). The data for Vo<sub>2</sub> has been log transformed which gave a slightly better fit (r²=0·23) than for untransformed data (r²=0·17). Log transformation of both Vo<sub>2</sub> and f<sub>H</sub> (Lucas, 1994) gave no better fit (r²=0·23) than when only Vo<sub>2</sub> was log transformed. \*Vo<sub>2</sub> significantly higher than at rest; †, Vo<sub>2</sub> max. significantly higher in trained than in control fish; 4, fH significantly higher than at rest;

TABLE I. The relative contribution\* of heart rate, stroke volume of the heart, and extraction of O<sub>2</sub> from blood to the increase in oxygen consumption, from rest to maximum, in exercising fish

	Relative contribution to the increase in $\dot{V}_{\rm O_2}$ during exercise					
Species	Heart rate (%)	Stroke volume (%)	O <sub>2</sub> extraction (%)	Reference§		
Rainbow trout,						
Oncorhynchus mykiss	11	40	49	1		
	12	25	63	2		
	9	21	70	3, 4		
Chinook salmon,						
Oncorhynchus tshawytscha	6	34	60*	3, 4		
	10	23	67	3, 4		
Dogfish,						
Scyliorhinus stellaris L. Leopard shark,	13	111	<b>- 24</b>	5		
Triakis semifasciata Girard	10	43	47	6		
Mean	10	42	48			

<sup>\*%</sup> contribution of heart rate, stroke volume or  $O_2$  extraction was calculated as the log of the factoral increase in the variable over the sum of the logs of the factorial increase for all variables, e.g. for heart rate % contribution =  $100 \cdot \log(f_{\rm Hmax}/f_{\rm Hrest})/(\log(f_{\rm Hmax}/f_{\rm Hrest}) + \log(SV_{\rm Hmax}/SV_{\rm Hrest}) + \log(Eo_{\rm 2max}/Eo_{\rm 2rest}))$ .

<sup>†</sup>Control fish [as in Fig. 1(b)].

<sup>‡</sup>Trained fish [as in Fig. 1(b)].

<sup>§</sup>References: 1, Kiceniuk & Jones (1977); 2, Thorarensen, Gallaugher & Brauner, unpubl. obs.; 3, Thorarensen (1994); 4, Gallaugher (1994); 5, Piiper et al. (1977); 6, Lai et al. (1990).

TABLE II. Percent contribution\* of heart rate to increases in O<sub>2</sub> consumption measured telemetrically and the perturbations which increased O<sub>2</sub> consumption

Species	Percent contribution of heart rate	Perturbation	Reference†
Rainbow trout,			
Oncorhynchus mykiss	50	Spontaneous activity	1
Pike,			
Esox lucius	122	Temperature (2–17° C), spontaneous activity, feeding	2
Leopard shark,			
Tirakis semifascia	32	Forced activity (swim tunnel)	3
Lemon shark,			
Negaprion brevirostris Poey	20	Forced activity (swim tunnel)	4
Atlantic salmon,	15 17	Constant and activity	-
Salmo salar Atlantic cod,	45–47	Spontaneous activity	5
Gadus morhua	32	Forced activity (swim tunnel)	6

<sup>\*</sup>Calculated as in Scharold and Gruber (1991).

<sup>†</sup>References: 1, Priede & Tytler (1977); 2, Armstrong (1986); 3, Scharold et al. (1989); 4. Sharold & Gruber (1991); 5, Lucas (1994); 6, Claireaux et al. (1995a).

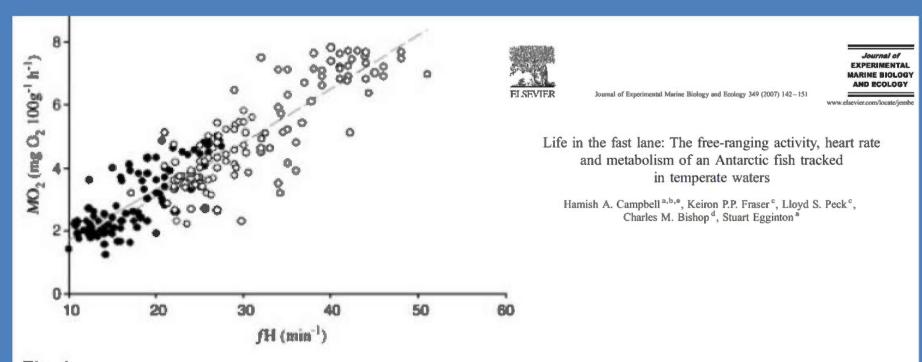


Fig. 1. The relationship between fH and  $MO_2$  in N. coriiceps (closed circles, n = 94, N = 6) and N. angustata (open circles, n = 128, N = 6). The instantaneous electrocardiogram (ECG) and oxygen consumption were recorded simultaneously in a closed circuit respirometer for three 10 min periods every 24 h after each fish was fed a 5% bd. wt. ration. Measurements were taken until  $MO_2$  did not change between successive days. Recording time was 168 h for N. coriiceps and 96 h for N. angustata. Linear regression lines are fitted to the data for each fish (N. angustata = 0.16 X + 0.15,  $r^2$  = 0.61; N. coriiceps = 0.17X - 0.35,  $r^2$  = 0.74). There was no significant difference between the slopes of fitted regression lines (ANCOVA, F = 2.66, P = 1.049).

# **CONCLUSIONS**????



# FitFish 2016 Gases, aeration and degassing

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Hirtshals



### Contents

Fundamentals of dissolved gases

Solubility

Total gas pressure and supersaturation

**Aeration** 

Degassing

Exercise



# Fundamentals of dissolved gases

Composition of atmospheric air

Gas	% volume	% mass	MW
Nitrogen	78.084	75.6	28.0
Oxygen	20.946	23.2	32.0
Carbon dioxide	0.033	0.05	44.0
Argon	0.934	1.3	39.9

The gases that make up atmospheric air will always strive to reach equilibrium with water.

At 20° C and 760 mmHg the mass of 1m³ of air is 1.2754 kilograms



In atmospheric air, the partial pressure of a gas is given by its volume percentage and barometric pressure.

Thus, at a barometric pressure of 772 mmHg, the partial pressure of each gas type can be calculated as

$$pO_2 = \frac{20.946}{100} \times 772 \text{ mmHg} = 161.70 \text{ mmHg}$$

$$pN_2 = \frac{78.084}{100} \times 772 \text{ mmHg} = 602.81 \text{ mmHg}$$

$$pCO_2 \qquad \frac{0.03}{100} \times 772 \text{ mmHg} = 0.23 \text{ mmHg}$$



### Water vapour pressure

A fraction of the air is occupied by moisture in the air – this is termed water vapour pressure.

Water vapour pressure is dependent on temperature and can be calculated using the formula derived by Colt (1984)

$$P_{\text{WV}} = A_0 \times e^{0.0645 \times T}$$

where  $A_0$  is a constant of 4.7603



#### Example

At a temperature of 7° C water vapour pressure is

$$P_{WV} = 4.7603 \times e^{0.0645 \times 7}$$
  
= 7.48 mmHg

And at 28° C

$$P_{WV}$$
 = 4.7603 ×  $e^{0.0645 \times 28}$   
= 28.97 mmHg



So in reality at 28° C at a barometric pressure of 772 mmHg the partial pressure of a gas is given by its volume percentage and barometric pressure minus water vapour pressure.

$$pO_2$$
  $\frac{20.946}{100}$  × (772 mmHg – 28.97 mmHg) = 155.64 mmHg

$$pN_2$$
  $\frac{78.084}{100}$  × (772 mmHg – 28.97 mmHg) = 580.19 mmHg

$$pCO_2$$
  $\frac{0.03}{100}$  × (772 mmHg – 28.97 mmHg) = 0.22 mmHg



#### Elevation

Increase in altitude above sea level causes a decrease in barometric pressure. Barometric pressure can be corrected for elevation by the following equation

$$BP_{ALT} = BP_{SL} \times 10^a / 760$$

Where a = 2.880814 - 
$$\frac{h}{19748.2}$$
 (h is height in meters)

So at sea level a barometer reads a BP of 772 mmHg. At an elevation of 432 meters corrected BP is 734 mmHg.

Water vapour pressure is calculated after correction for elevation.



# Solubility

Gases, like any other chemical compound, are different in how much can be dissolved in water, so the partial pressure of each gas (although proportional to atmospheric pressure) does not translate directly into how much of that gas is in solution.

Simply stated, the amount of a gas species present in solution depends on

- Solubility
- Barometric pressure
- Water vapour pressure
- Concentration of other chemicals in solution



With changes in barometric pressure and temperature the saturation (s) solubility of a gas (i) changes accordingly, and can be calculated as

$$C_{s,i} = 1000 \text{ K}_{i} \beta_{i} X_{i} \frac{P_{BP} - P_{WV}}{760}$$

#### Where

 $K_i$  is the solubility constant  $X_i$  is the mole fraction  $\beta_i$  is the Bunsen coefficient

Table 10.3 Constants Used in Gas Solubility Equations and Mole Fraction for Standard Air

Gas Species	Mole Fraction	Ki	Aı	$A_2$	$A_3$	$J_{i}$
Oxygen	0.20946	1.42903	58.3877	85.8079	23.8439	0.5318
Nitrogen	0.78084	1.25043	59.6274	85.7661	24.3696	0.6078
Carbon Dioxide	0.00032	1.97681	58.0931	90.5069	22.2940	0.3845



#### Bunsen coefficients

The Bunsen coefficient is temperature and salinity dependent.

$$O_2$$
 and  $N_2$   $\beta_i = \exp[-A_1 + A_2 (100/T) + A_3 \ln(T/100)]$ 

$$CO_2$$
  $\beta_i = K_0 (22.263)$ 

Where 
$$K_0 = \exp[-A_1 + A_2 (100/T) + A_3 \ln (T/100)]$$

T in Kelvin =  $^{\circ}$  C + 273.15

Bunsen's coefficient for oxygen as a function of temperature and salinity

**Table 10.3** Constants Used in Gas Solubility Equations and Mole Fraction for Standard Air

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Carbon Dioxide	0.00032	1.97681	58.0931	90.5069	22.2940	0.3845

	Salinity, parts per thousand (ppt)					
Temperature (°C)	0	10	20	30	40	
0	0.049	0.046	0.043	0.040	0.037	
2	0.047	0.043	0.041	0.038	0.036	
4	0.044	0.041	0.039	0.036	0.034	
6	0.042	0.039	0.037	0.034	0.032	
8	0.040	0.037	0.035	0.033	0.031	
10	0.038	0.036	0.034	0.032	0.030	
12	0.036	0.034	0.032	0.030	0.028	
14	0.035	0.033	0.031	0.029	0.027	
16	0.034	0.032	0.030	0.028	0.026	
18	0.032	0.030	0.029	0.02%	0.025	
20	0.031	0.029	0.028	0.026	0.025	
22	0.030	0.028	0.027	0.025	0.024	
24	0.029	0.027	0.026	0.024	0.023	
26	0.028	0.026	0.025	0.024	0.022	
28	0.027	0.026	0.024	0.023	0.022	
30	0.026	0.025	0.024	0.022	0.021	



## Total gas pressure

The sum of pressures of gases in solution is termed the total gas pressure.

$$TGP = pO_2 + pN_2 + pCO_2 + pAr + pWV$$

Based on the barometric pressure (BP) we can assess whether water is less than saturated or oversaturated with gases

Saturation =  $TGP / BP \times 100\%$ 



#### However -

# TGP provides no information on the partial pressures of individual gas species.

Thus a saturation of 123% caused by oxygen (eg pO2 = 326 mmHg) is not a problem, whereas if it were caused by other gases it would present a problem.

 $O_2$  supersaturation is considered safe up to approximately 300%  $N_2$  supersaturation has adverse effects above 104 – 120% depending on species and life stage (more deleterious to fry)  $CO_2$  supersaturation has undesirable effects at low P but not toxic until fairly high concentrations



While it may be desirable to have high  $pO_2$  levels it must be kept in mind that

#### Every mole of O<sub>2</sub> consumed produces 1 mole of CO<sub>2</sub>

As well as nitrogen waste products.

Cumulative oxygen consumption may not always be the limiting parameter with build up of other gases and further addition of oxygen will not help!!

It is advisable to measure pCO<sub>2</sub> and TGP



# When is gas tension a problem?

Since RAS typically recycle >95% of the water only 5% of the water will be replaced while the rest undergoes mechanical treatment and is recycled to the fish.



Oxygen becomes depleted



Carbon dioxide accumulates



Aeration and possibly degassing (stripping) becomes necessary



# Example

#### High fish stocking densities

Example: fish are stocked at a density of 50 kg m<sup>3</sup> at 15° C. Assuming a BP of 760 mmHg oxygen solubility is 10 mg L<sup>-1</sup>. In 1 m<sup>3</sup> there will be

1000 liters – (50 kg × 1.06 l kg<sup>-1</sup>) = 947 liters, containing 947 × 10.07 mg l<sup>-1</sup> = 9536 mg 
$$O_2$$

Fish that are digesting a meal might consume >150 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>

50 kg of fish are consuming 7500 mg O<sub>2</sub> per hour

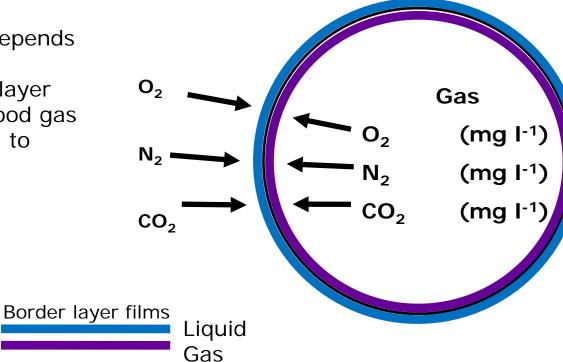
or more than 75% of the oxygen content!



#### Gas transfer

Regardless of interface (water surface or bubbles) the exchange of gas species between gas and water follows the two film theory where two border layer films (one gas film and one water film) inhibit the transport

Thickness of the films depends on turbulence: much turbulence reduces the layer thickness. To achieve good gas transport it is important to have a thin film





Mechanisms of gas transport through the two film barrier

#### > From gas to gas film

Combined diffusion and convection. Rapid process due to high gas mixing in air.

- - > From gas interface to liquid

Convection. Velocity depends on water movement



Gas transfer per unit time across a surface is given by

$$\frac{\mathrm{d}c}{\mathrm{d}t} = K_{L} \times A \times (C^* - C_0)$$

Where

dc / dt is change in concentration over time (mg  $I^{-1}$   $h^{-1}$ )  $K_L$  is the coefficient for gas transfer (cm  $h^{-1}$ ) A is the contact of surface area in relation to volume  $C^*$  is the saturation concentration for the gas in liquid  $C_0$  is the starting concentration of the gas in liquid



$$\frac{\mathrm{d}c}{\mathrm{d}t} = K_{L} \times A \times (C^{*} - C)$$

The two variables that can more easily be changed are

C\* (although a decrease in C would also increase the flux this is obviously not desirable). This can be achieved by gas with a high content of oxygen (for example pure oxygen)

A Increasing the diffusion area

 $\mathbf{K}_{\mathsf{L}}$  Changing the configuration of the system, i.e. water or air flow rates (G:L ratio)

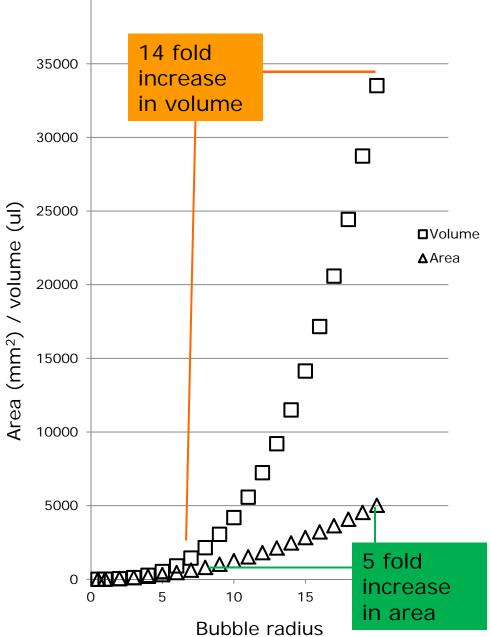


Bubble volume (V)

$$V = r^3 \times 4/3 \times \pi$$

Bubble area (A)

$$A = 4 \times \pi \times r^2$$



40000



#### Properties of bubbles

If injecting 2 m<sup>3</sup> per minute

Radius	Volume	Area	Area to volume	Number of	Total area	Change
(mm)	(ml)	(mm²)	(mm²/ml)	bubbles	(m²)	r=0,5
0,5	0,0005	3,1	6000	4,77E+08	6000	50,00
1	0,0042	12,6	3000	5,97E+07	3000	25,00
2	0,0335	50,3	1500	1,77E+07	2000	16,67
3	0,1131	113,1	1000	7,46E+06	1500	12,50
4	0,2681	201,1	750	3,82E+06	1200	10,00
5	0,5236	314,2	600	2,21E+06	1000	8,33
6	0,9048	452,4	500	1,39E+06	857	7,14
7	1,4368	615,8	429	9,33E+05	750	6,25
8	2,1447	804,2	375	6,55E+05	667	5,56
9	3,0536	1017,9	333	4,77E+05	600	5,00
10	4,1888	1256,6	300	3,59E+05	545	4,55
11	5,5753	1520,5	273	2,76E+05	500	4,17
12	7,2382	1809,6	250	2,17E+05	462	3,85
13	9,2028	2123,7	231	1,74E+05	429	3,57
14	11,4940	2463,0	214	1,41E+05	400	3,33
15	14,1372	2827,4	200	1,17E+05	375	3,13
16	17,1573	3217,0	188	9,72E+04	353	2,94
17	20,5795	3631,7	176	8,19E+04	333	2,78
18	24,4290	4071,5	167	6,96E+04	316	2,63
19	28,7309	4536,5	158	5,97E+04	300	2,50

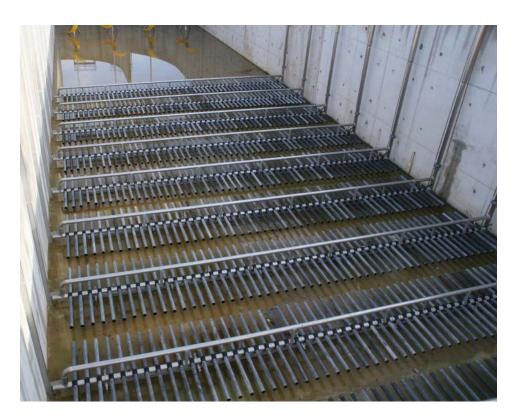
$$\frac{\mathrm{d}c}{\mathrm{d}t} = K_{L} \times A \times (C^* - C)$$

Note that bubble volume and area are calculated at atmospheric pressure



### Air diffuser types

The airlift pumps installed in model 3 aquaculture facilities are typically perforated PVC tubes with an outer cladding of fabric which determines the hole size for air diffusion.





In principal, a variety of different diffuser types may be employed, but all face the risk of clogging with time, due to biofouling. In many instances they also serve a purpose of water movement and must allow for the passage of water from below, and bubble size must allow for rapid rising



FitFish 2016 Dissolved gases





#### I. Changing the pressure of the supplying gas

At 15° C compressed air is added at a depth of 1 meter and a pressure of 2 bar

$$\frac{\mathrm{d}c}{\mathrm{d}t} = K_{L} \times A \times (C^{*} - C)$$

What is the saturation solubility of oxygen?

$$C_{s,i} = 1000 \text{ K}_{i} \beta_{i} X_{i} \frac{P - P_{WV}}{760}$$
  
 $1000 \times 1.42903 \times 0.0345 \times 0.20946 \times \frac{1520 - 12.53}{760} = 20.483 \text{ mg I}^{-1}$ 

If dry air is being added water vapour pressure is 0

What about nitrogen and carbon dioxide??



What if we use pure oxygen?

At 15° C oxygen is added at a depth of 1 meter and a pressure of 1 bar (760mmHg)

What is the saturation solubility of oxygen?

$$C_{s,i} = 1000 \text{ K}_i \beta_i X_i$$

$$1000 \times 1.42903 \times 0.0345 \times 1 \times \frac{760}{760} = 49.302 \text{ mg I}^{-1}$$

$$pO_2 = 477\%$$



#### Advantages and disadvantages

Air should only be added at pressure slightly higher than ambient to prevent supersaturation of other gases.

Using pure greatly increase the partial pressure of oxygen, but has little capacity to degas  $CO_2$  and  $N_2$  due to a very low G:L ratio.



## $K_{L}$

One way to change the  $K_L$  is to change the gas: liquid ratio. All systems will have an optimal ratio of gas to liquid to perform at its best, depending on its intended use – aeration or degassing.

Aeration G:L < 3:1

Degassing G:L > 5:1

Why?



#### Sources of oxygen

Oxygen can be obtained from commercial suppliers in pressurised tanks or as liquid oxygen.

Liquid oxygen is produced by cooling compressed air until it liquefies and then distilling it to fractionate gases. Oxygen is supplied to fixed tanks in liquid form at a temperature of -183° C.





#### Aeration devices

Aeration devices can be classified as either

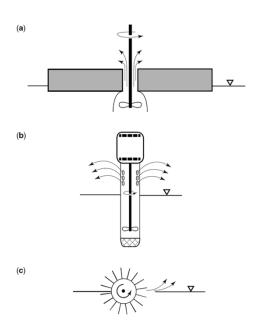
- Surface aerator
- Subsurface aerator
- Gravity aerator

Depending on whether they spray water into the air, add air to the water underneath the surface or use the gravity head to transfer gas into the water



#### Surface aerators

Typical surface aerators: (a) floating aerator; (b) surface aerator with draft tube; (c) brush, rotor, or paddlewheel aerator.

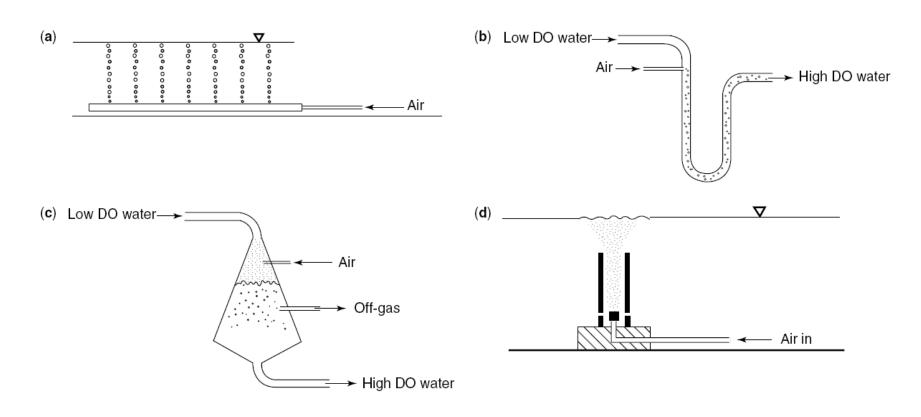








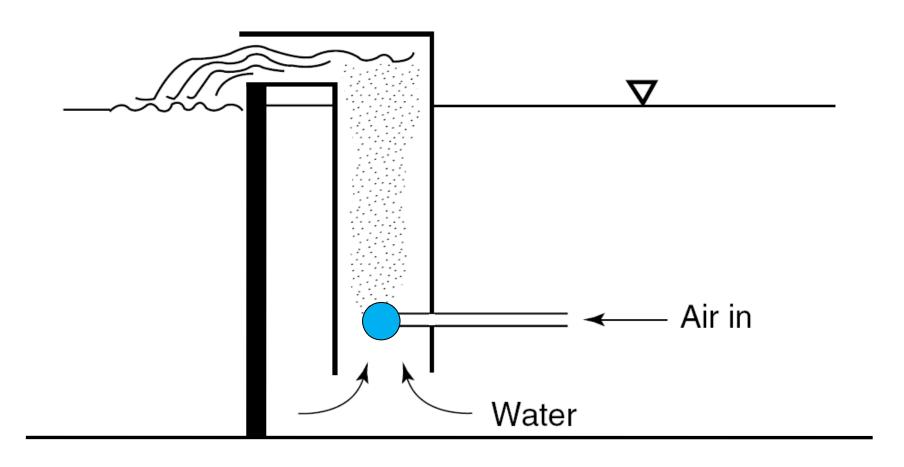
#### Subsurface (submerged) aerators



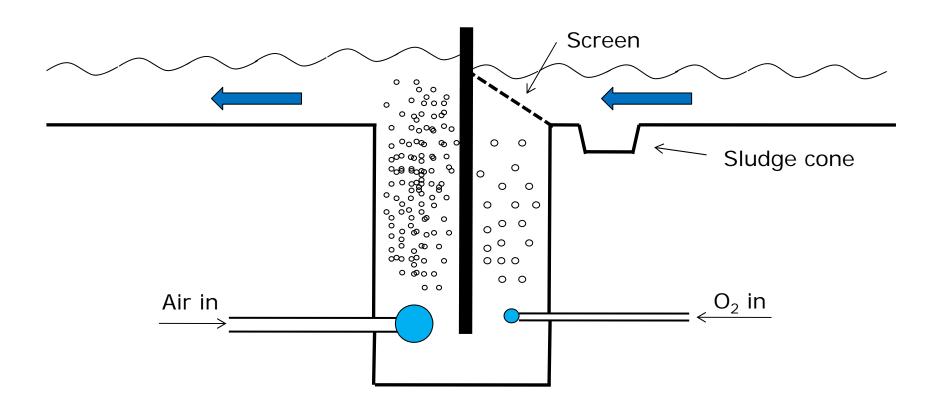
(a) diffused, (b) U-tube, (c) aerator cone, (d) static tube



## Air lift pumps





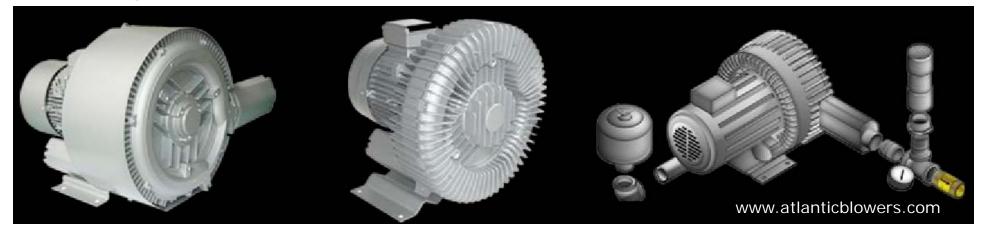




#### Air sources

Air for aeration devices can be supplied by blowers, air pumps or regenerative blowers

❖Regenerative blowers

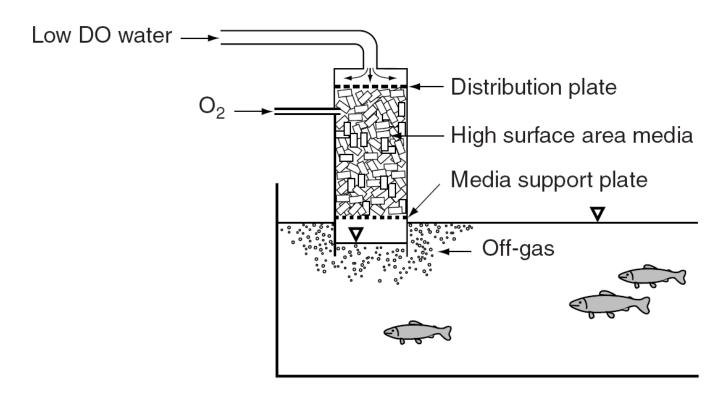


Blowers have the advantage of providing large volumes of air at low pressure, they are energy efficient, reliable and low purchase cost



### Low head oxygenator (packed column)

Exists in many different versions operating on the same principles with or without media





## Degassing (CO<sub>2</sub> or N<sub>2</sub> stripping)

Constructed in a similar manner as aerators, but operate at a much higher air flow because air quickly becomes saturated with  ${\rm CO_2}$  leaving the water.

Inexpensive to provide large volumes of air because there is no large hydraulic loading (back pressure)

$$\ln\left(\frac{C^* - C_{\rm in}}{C^* - C_{\rm out}}\right) = (K_L a)t \tag{1}$$

where

 $C^* = ext{equilibrium saturation concentration at local}$   $ext{temperature and pressure (mg/L),}$   $C_{ ext{in}} = ext{influent concentration (mg/L),}$   $C_{ ext{out}} = ext{effluent concentration (mg/L),}$   $K_L = ext{overall liquid-phase mass-transfer coefficient (m/hr),}$   $a = ext{interfacial surface area (m^2/m^3),}$   $t = ext{aeration time (hr).}$ 



## Degassing CO<sub>2</sub>

What is important to consider?

- 1. Carbonate chemistry
- 2. Dehydroxylation speed (HCO<sub>3</sub>- to CO<sub>2</sub>)



#### Efficiency

For design and operational purposes it is desirable to know the efficiency of a unit. Factors with a large impact on efficiency are

- Media type in packed columns (A)
- ♦ Number of chambers / volume of media (A + K<sub>L</sub>)
- ❖ Flow rate of air (K₁)
- ♦ Hydraulic loading (K<sub>L</sub>)

Changes in these variables can greatly influence performance



#### Testing the efficiency of aerators

While the efficiency of the different aerators may be calculated / modelled with some accuracy, it is desirable to test their efficiency in practice.

Commonly an application can be tested under clean water conditions. The test is performed under unsteady state. This means water is deoxygenated chemically and the aeration device is started



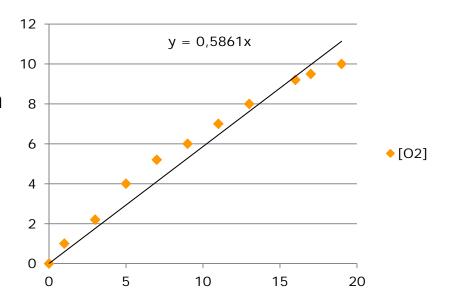


using

$$\frac{\mathrm{d}c}{\mathrm{d}t} = K_{L} \times A \times (C^{*} - C)$$

The change in oxygen concentration over time is used to calculate  $K_LA$  (since it is rarely possible to determine each on its own).

The relevant part of the curve can be used to calculate the maximum rate of transfer of oxygen into the water





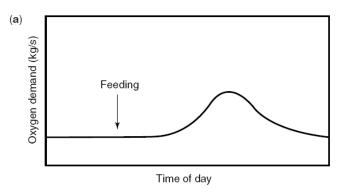
The maximum rate of oxygen transfer is termed the standardised oxygen transfer rate (SOTR) measured in kg h<sup>-1</sup>.

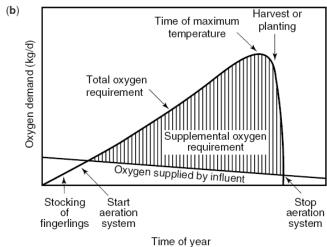
Using the SOTR and the measured energy consumption of the device ( $P_{IN}$  in kW or hp), the standardised aeration efficiency (SAE kg  $O_2$  kW<sup>-1</sup> h<sup>-1</sup>) can be calculated as

 $SAE = SOTR / P_{IN}$ 



However – you should take into consideration the diurnal and seasonal variation

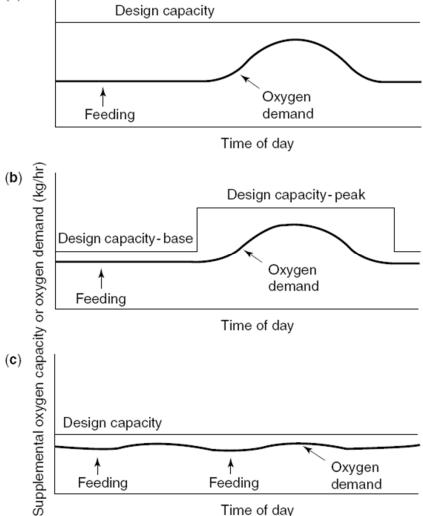




**Figure 6.** Variation of oxygen demand in a flow-through system with (**a**) time of day (**b**) season of year.

Supplemental oxygen supply can be configured to daily cycles or maintained constantly.

(a)



Time of day



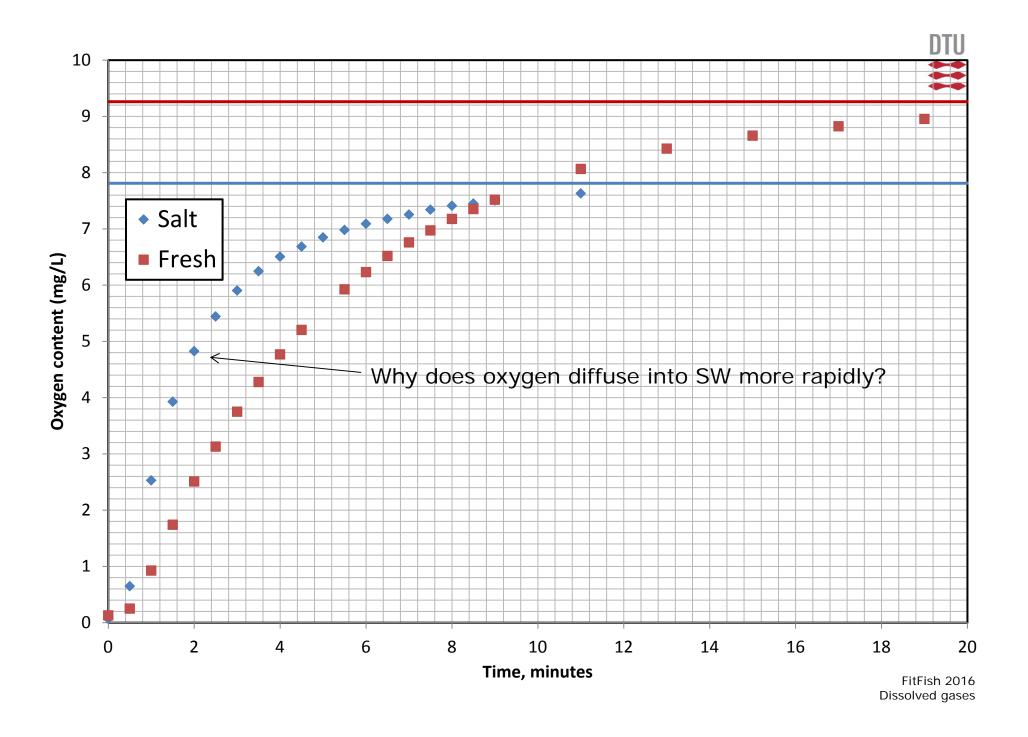
#### Freshwater or saltwater

What are the major differences between freshwater and saltwater in terms of aeration and degassing efforts?

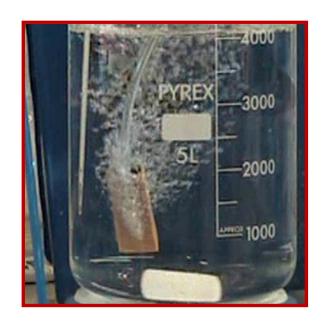
$$\frac{\mathrm{d}c}{\mathrm{d}t} = K_{L} \times A \times (C^{*} - C)$$

Bunsen's coefficient for oxygen as a function of temperature and salinity

	Salinity, parts per thousand (ppt)					
Temperature (°C)	0	10	20	30	40	
0	0.049	0.046	0.043	0.040	0.037	
2	0.947	0.043	0.041	0.038	0.036	
4	0.044	0.041	0.039	0.036	0.034	
6	0.042	0.039	0.037	0.034	0.032	
8	0.040	0.037	0.035	0.033	0.031	
10	0.038	0.036	0.034	0.032	0.030	
12	0.036	0.034	0.032	0.030	0.028	
14	0.035	0.033	0.031	0.029	0.027	
16	0.034	0.032	0.030	0.028	0.026	
18	0.032	0.030	0.029	0.02%	0.025	
20	0.031	0.029	0.028	0.026	0.025	
22	0.030	0.028	0.027	0.025	0.024	
24	0.029	0.027	0.026	0.024	0.023	
26	0.028	0.026	0.025	0.024	0.022	
28	0.027	0.026	0.024	0.023	0.022	
30	0.026	0.025	0.024	0.022	0.021	





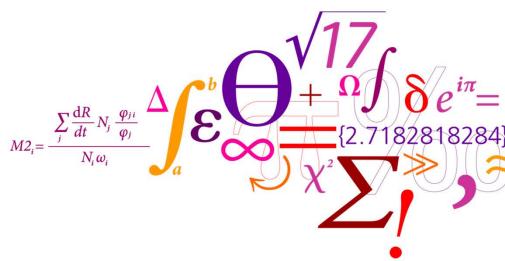






## FitFish 2016 Energy and metabolism

Peter Vilhelm Skov
Technical University of Denmark
DTU Aqua
Section for Aquaculture
Hirtshals

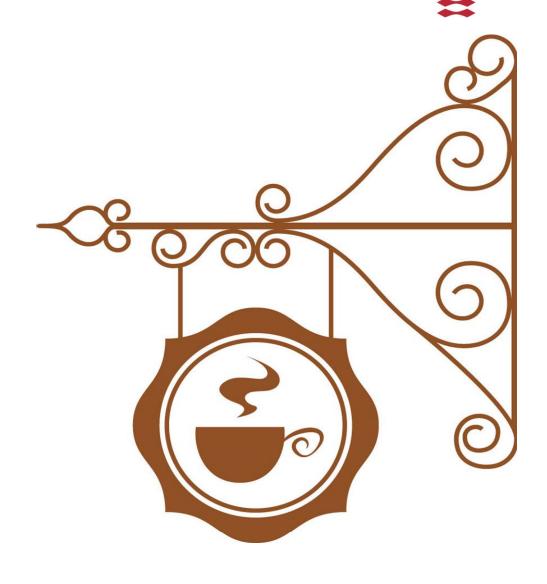


DTU Aqua

National Institute of Aquatic Resources

# Energy

1 kcal = 4.18 kJ





## Energy requirements

#### **Mechanical work:**

Muscle contraction and cellular movement

#### **Transport:**

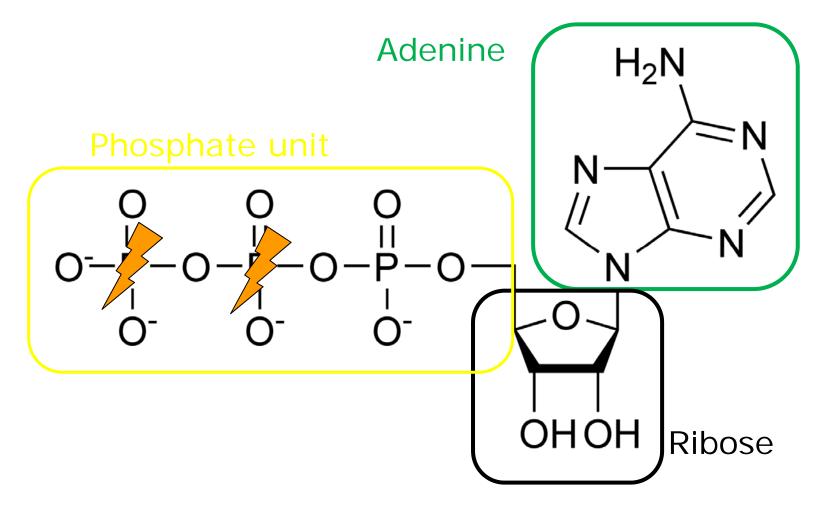
Transport of molecules and ions

#### Synthesis:

Synthesis of complex molecules from simple precursors



## ATP is the energy currency



ATP is the energy currency

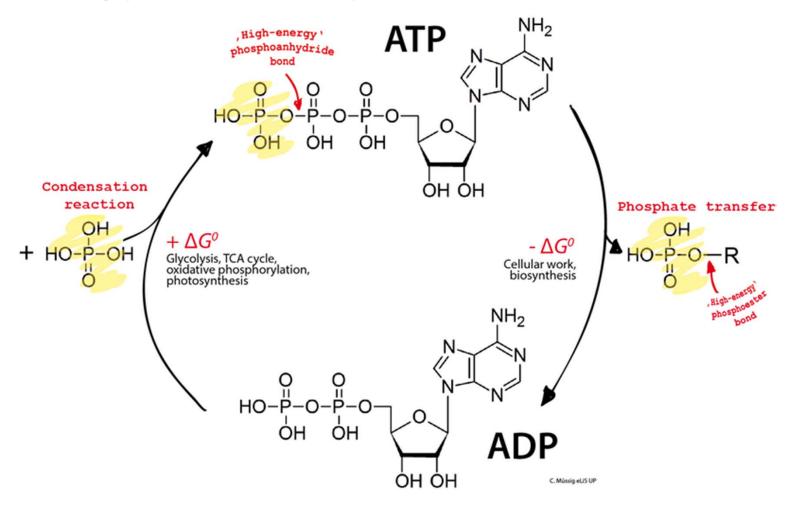
 $H_2N$ 

ATP + 
$$H_2O \rightleftharpoons ADP + P_i + H^+$$

 $\Delta G^{\circ}$  of ATP to ADP = ~30.5 kJ/mol

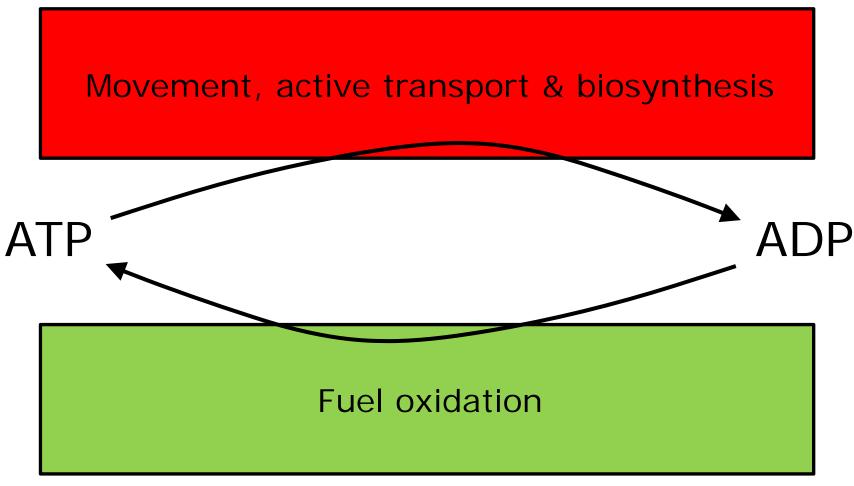


## Energy currency





## Energy exchange

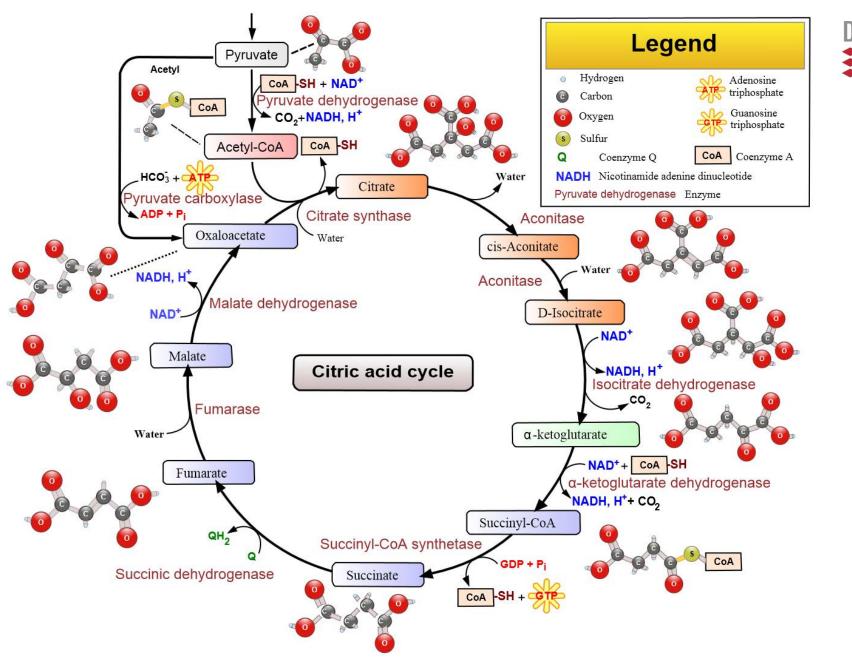




#### Fuel oxidation

The chemical oxidation of nutrients for energy is divided into 3 stages:

- Breakdown of larger molecules into smaller units; proteins are hydrolysed to their 20 constituent amino acids, polysaccharides are hydrolysed into simple sugars, and fats are hydrolysed into glycerols and fatty acids
- II. These units are degraded into simple units for metabolic processing mostly the acetyl unit of acetyl CoA
- III. Acetyl enters the citric acid cycle by acetyl CoA where they are oxidized to CO<sub>2</sub>.





The net reaction of the citric acid cycle is

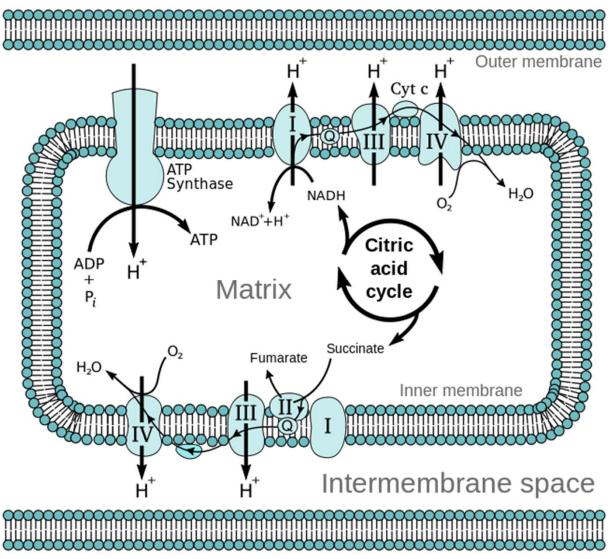
Acetyl CoA + 3 NAD
$$^+$$
 + FAD + ADP + Pi + 2 H $_2$ O  
2 CO $_2$  + 3 NADH + FADH $_2$  + ATP + 2 H $^+$  + CoA

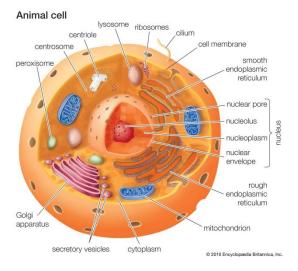


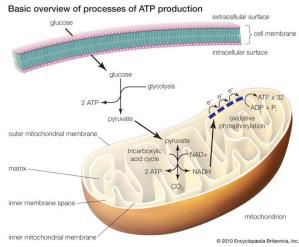
NADH and FADH<sub>2</sub> are energy rich molecules. Each contains a pair of electrons that are donated to molecular oxygen, whereby a large amount of free energy is liberated for the generation of ATP

### Oxidative phosphorylation

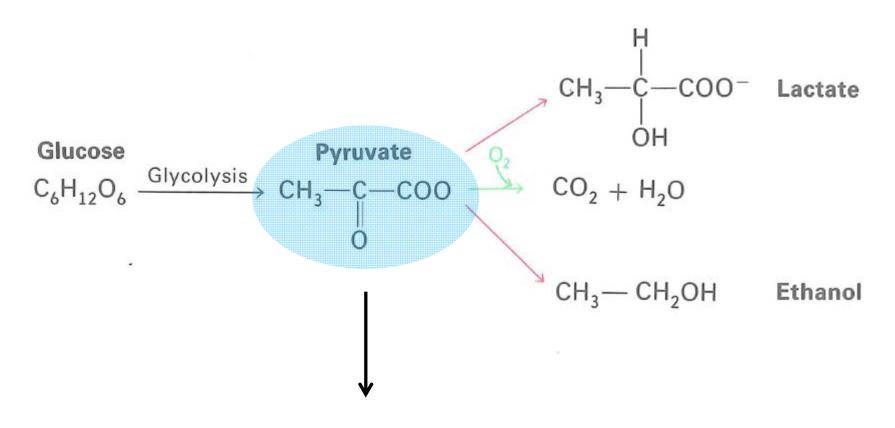










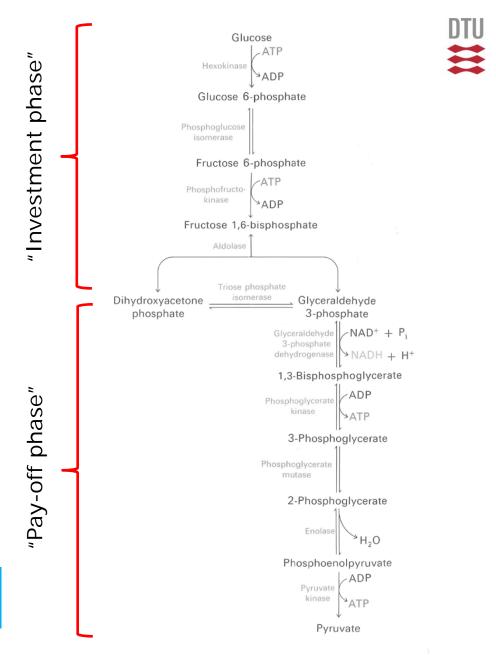


Glucose + 2 
$$P_i$$
 + 2  $ADP$  + 2  $NAD^+$   $\longrightarrow$  2 pyruvate + 2  $ATP$  + 2  $NADH$  + 2  $H^+$  + 2  $H_2O$ 



Gain 4 ATP

Net yield 2 ATP



# A series of enzymatic reactions drive glycolysis in the cytoplasma

Step	Reaction	Enzyme
1	Glucose + ATP → glucose 6-phosphate + ADP + H <sup>+</sup>	Hexokinase
2	Glucose 6-phosphate ==== fructose 6-phosphate	Phosphoglucose isomerase
3	Fructose 6-phosphate + ATP $\longrightarrow$ fructose 1,6-bisphosphate + ADP + H <sup>+</sup>	Phosphofructokinase
4	Fructose 1,6-bisphosphate ====================================	Aldolase
5	Dihydroxyacetone phosphate ⇒ glyceraldehyde 3-phosphate	Triose phosphate isomerase
6	Glyceraldehyde 3-phosphate $+ P_i + NAD^+ \rightleftharpoons$ 1,3-bisphosphoglycerate $+ NADH + H^+$	Glyceraldehyde 3-phosphate dehydrogenase
7	1,3-Bisphosphoglycerate + ADP ==== 3-phosphoglycerate + ATP	Phosphoglycerate kinase
8	3-Phosphoglycerate === 2-phosphoglycerate	Phosphoglyceratmutase
9	2-Phosphoglycerate ⇒ phosphoenolpyruvate +H <sub>2</sub> O	Enolase
10	Phosphoenolpyruvate + ADP + H <sup>+</sup> → pyruvate +ATP	Pyruvate kinase

http://programs.northlandcollege.edu/biology/Biology1111/animations/glycolysis.html



#### ATP yield from the complete oxidation of glucose

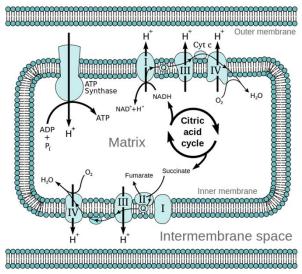
	TP yield r glucose
Glycolysis: Glucose into pyruvate (in the cytosol)	
Phosphorylation of glucose	-1
Phosphorylation of fructose 6-phosphate	-1
Dephosphorylation of 2 molecules of 1,3-BPG	+2
Dephosphorylation of 2 molecules of phosphoenolpyruvate	+2
2 NADH are formed in the oxidation of 2 molecules of glyceraldehyde 3-phosphate	
Conversion of pyruvate into acetyl CoA (inside mitochondria)  2 NADH are formed	
Citric acid cycle (inside mitochondria)	
<ul><li>2 molecules of guanosine triphosphate are formed from</li><li>2 molecules of succinyl CoA</li></ul>	+2
6 NADH are formed in the oxidation of 2 molecules each of isocitrate, $\alpha$ -ketoglutarate, and malate	
2 FADH <sub>2</sub> are formed in the oxidation of 2 molecules of succinate	
Oxidative phosphorylation (inside mitochondria)	
2 NADH formed in glycolysis; each yields 1.5 ATP (assuming transport of NADH by the glycerol phosphate shuttle)	+3
2 NADH formed in the oxidative decarboxylation of pyruvate; each yields 2.5 ATP	+5
2 FADH <sub>2</sub> formed in the citric acid cycle; each yields 1.5 ATP	+3
6 NADH formed in the citric acid cycle; each yields 2.5 ATP	+15
NET YIELD PER GLUCOSE	+30



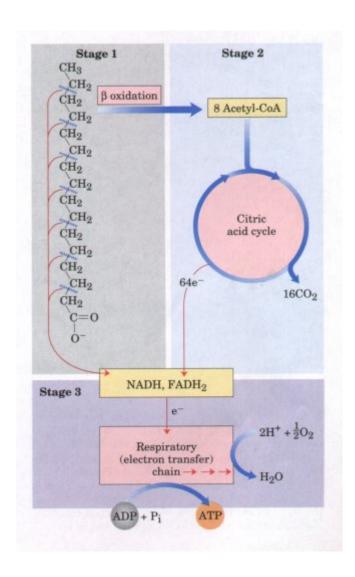
#### Oxidation of lipid

Fatty acids are transported across the outer mitochondrial membrane by carnitine acyl transferases (for e.g. carnitine-palmitoyl transferase I (CPT-I)), and then couriered across the inner mitochondrial membrane by carnitine.

Once inside the mitochondrial matrix, the fatty acyl-carnitine (such as palmitoylcarnitine) reacts with coenzyme A to release the fatty acid and **produce acetyl-CoA**.









#### How much ATP from substrates?

Complete oxidation of palmitate

Palmitoyl CoA + 7 FAD + 7 NAD<sup>+</sup> + 7 H<sub>2</sub>O + 7 CoA  

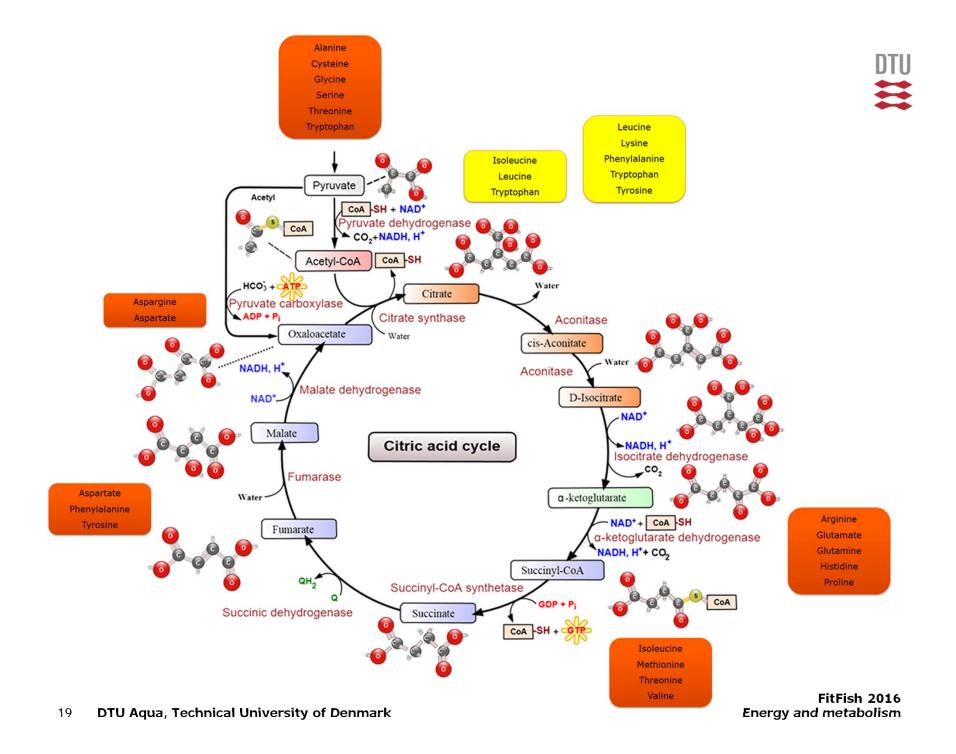
$$\longrightarrow$$
 8 acetyl CoA + 7 FADH<sub>2</sub> + 7 NADH + 7 H<sup>+</sup>

Each CoA yields 10 ATP = 80 ATP Each FADH<sub>2</sub> yields 1.5 ATP = 10.5 ATP Each NADH yields 2.5 ATP = 17.5 ATP

= 108 ATP

However, the activation of palmitate costs 2 ATP, so the net yield for the complete oxidation of palmitate is

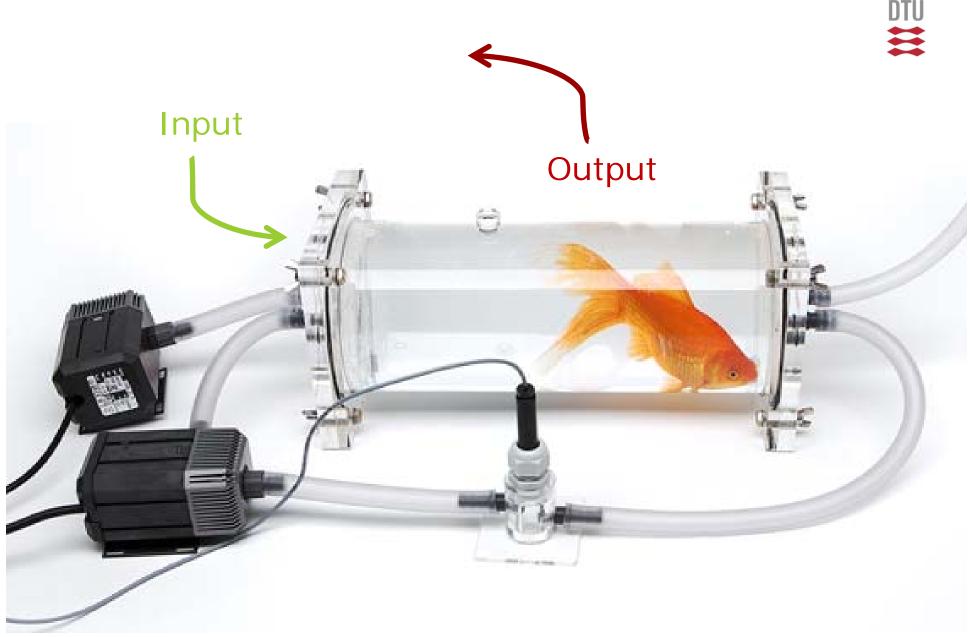
**106 ATP** 

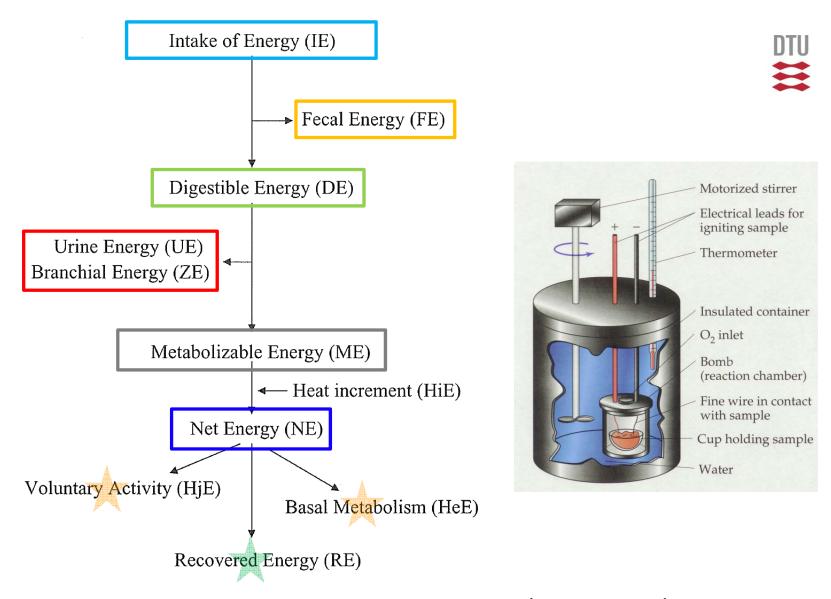




# Metabolism





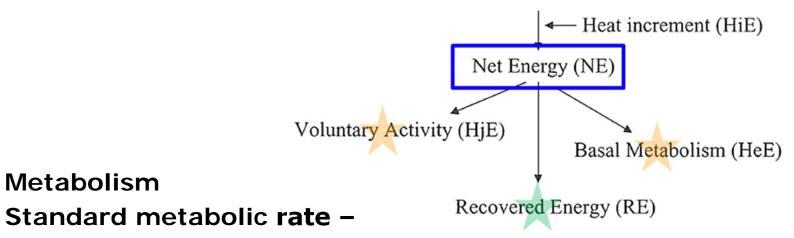


Energy partitioning scheme and nomenclature (NRC, 1981)



## Energy content in nutrients

Nutrient	Energy content (kJ/g)	Oxycal. coefficient (kJ/g O <sub>2</sub> )
Protein	23.7	13.36
Fat	39.6	13.72
Carbohydrate	17.2	14.76





Oxygen consumption or energy expenditure in a non-feeding fish

#### Routine metabolic rate -

Oxygen consumption in a fish performing routine activity

#### Active metabolic rate –

Oxygen consumption in a swimming fish

#### Maximum metabolic rate –

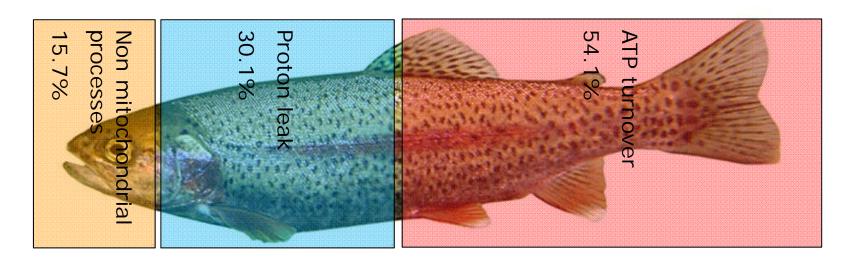
The maximum oxygen uptake in a fish

#### Specific dynamic action –

Oxygen consumption during processing a meal



### What does SMR in fish pay for?



Modified from Brand et al., 1990



# Contributors to metabolic rate

	Size range	P =	$P = kW^s$		$QO_2 = cW^d$	
Organ or part	examined (g)	k	S	Size range examined (g)	С	d
Head	0.021-1234	0.355	0.963			
Brain	0.0033 - 0.027*	0.113 <sup>§</sup>	1.026			
Brain	0.027*-55.6*	0.0251	0.610	0.074-970	31.58	-0.052
Brain	55.6*-1234	0.0489	0.444			
Gill filaments	0.48-5.02*	0.00486	1.217	0.26-911	47.54	-0.071
Gill filaments	5.02*-1234	0.00613	1.073 }	0.26-911	47.54	-0.071
Trunk	0.068 - 1.84*	0.492	1.148			
Trunk	1.84*-1234	0.533	1.017			
White muscle				0.097-985	0.80	-0.035
Scales <sup>†</sup> of trunk	1.2-1080	0.0704	0.895	0.66-970	9.54	-0.248
Skin of trunk	20-1080	0.0477	0.947	0.66-970	3.56	-0.009
Stomach	0.64-1234	0.0202	0.801	0.48-930	22.45	-0.064
Pyloric ceca	1.1-1234	0.00626	0.72	1.1–970	27.54	-0.005
Intestine	1.1-1234	0.0124	0.898	0.48-930	20.70	-0.051
Hepatopancreas	0.0033–95	0.0170 <sup>§</sup>	1.008 <sup>§</sup>	0.12-985	13.39	-0.130
Spleen	0.082-2.54*	0.000778	1.195	31–985	47.63	-0.202
Spleen	2.54*-1234	0.00100	0.925 }	31–985	47.03	-0.202
Head kidney	1.1-1234	0.00165	0.966	4.8-985	32.68	-0.088
Body kidney	0.18-1234	0.00173	0.996	4.8-985	70.69	-0.109
Atrium	0.64-1234	0.000246	0.917	348 <sup>¶</sup>	14.22 <sup>¶</sup>	ND
Ventricle	0.64 - 1234	0.00120	0.863	325 <sup>¶</sup>	13.34 <sup>¶</sup>	ND
Arterial bulb	1.1-1234	0.000294	0.905	310 <sup>¶</sup>	8.30 <sup>9</sup>	ND
Gonads	156-1234	0.000080#	1.642#	338 <sup>¶</sup>	7.47	ND
Fins	0.068 - 1234	0.0243	0.939			
Pectoral fins	1.1-1234	0.00401	0.947	0.45 - 488	20.54	-0.153
Ventral fins	1.1-1234	0.00269	1.009			
Dorsal fin	1.1 - 1234	0.00625	0.903	0.45 - 450	13.96	-0.177
Anal fin	1.1 - 1234	0.00258	0.906			
Caudal fin	1.1 - 1234	0.00553	1.032	0.45-450	20.04	-0.215

**Table 1** Allometric relationships used for the calculation of summated tissue respiration of the porgy between mass of an organ or part (P; in g) and body mass (W; in g) and between tissue respiration ( $QO_2$ ; in  $\mu$ mol/g per h) and body mass (W; in g)

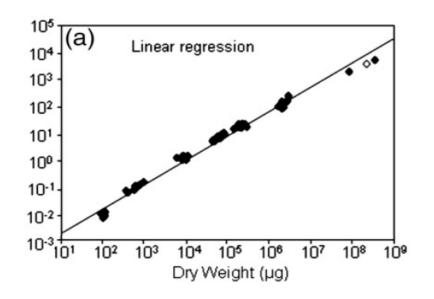


### Does body size matter?

Usually metabolic rate is expressed in mass specific terms.

For example a 500 gram trout might have a metabolic rate of 87 mg O<sub>2</sub>/kg/h

Does this also apply for a 1500 gram trout?



$$MR = CM_b^{0.67-0.75}$$



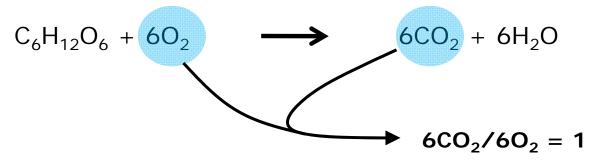
# Measuring on a larger scale



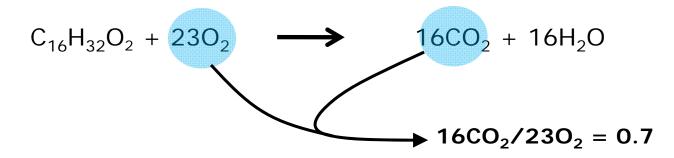


### What is fuelling metabolism?

#### Carbohydrate (glucose)



#### Fat (palmitic acid)





#### Amino acid oxidation

Nitrogen quotient

Similarly, a nitrogen quotient can be calculated

$$NQ = MN / MO_2$$

Where MN is the sum of all nitrogen excretion via UN + ZN

Theoretically, if all metabolism is fuelled by protein, then

$$NQ = 0.27$$

To calculate the fraction of metabolism fuelled by protein you therefore divide by 0.27 (eg. If NQ is 0.16, then =0.16/0.27 = 59% is fuelled by protein)

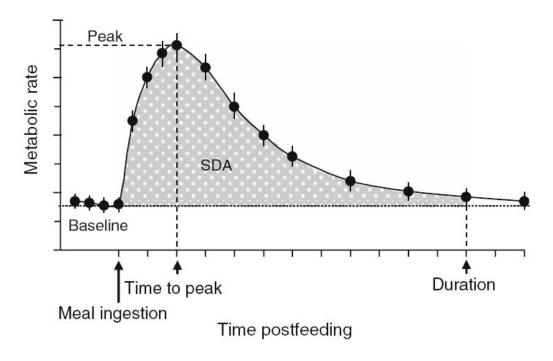


### Energy expenditure from feeding

- Physical processing of food, digestion, and the absorption of nutrients
- Biosynthesis, turnover, and deposition of tissue macromolecules
- Deamination of amino acids and synthesis of excretory products



### Measuring the cost of feeding

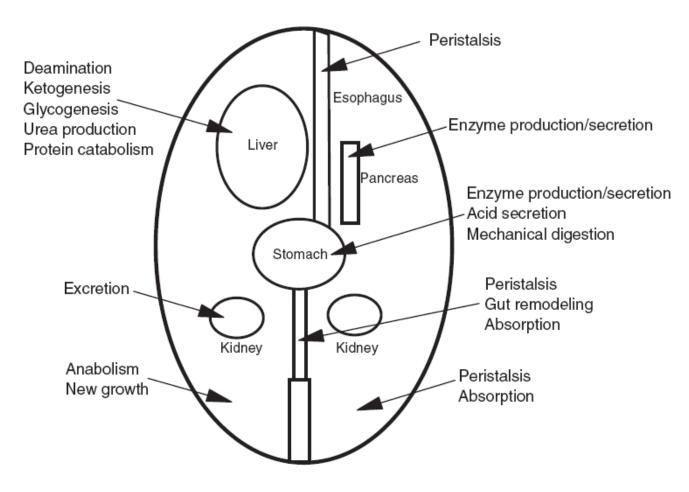


**Fig. 1** Hypothetical postprandial metabolic profile of metabolic rate plotted against time postfeeding. Noted are variables commonly quantified in characterizing and comparing the SDA response

Variable	Definition
Baseline	Metabolic rate of postabsorptive individuals, quantified as standard metabolic rate (SMR, ectotherms), basal metabolic rate (BMR, endotherms), resting metabolic rate (RMR) or routine metabolic rate (fishes swimming)
Meal size	Wet mass and/or as a percentage of body mass
Meal energy	Meal energy (calories or kilojoules) determined by bomb calorimetry
Peak	Postprandial peak in metabolism
Time to peak	Duration from time of feeding to peak metabolic rate
Scope	Postprandial peak divided by baseline
Duration	Time from feeding when metabolic rate is no longer significantly greater than baseline
SDA	Accumulated energy expended above baseline for duration of SDA response
SDA coefficient	SDA divided by meal energy

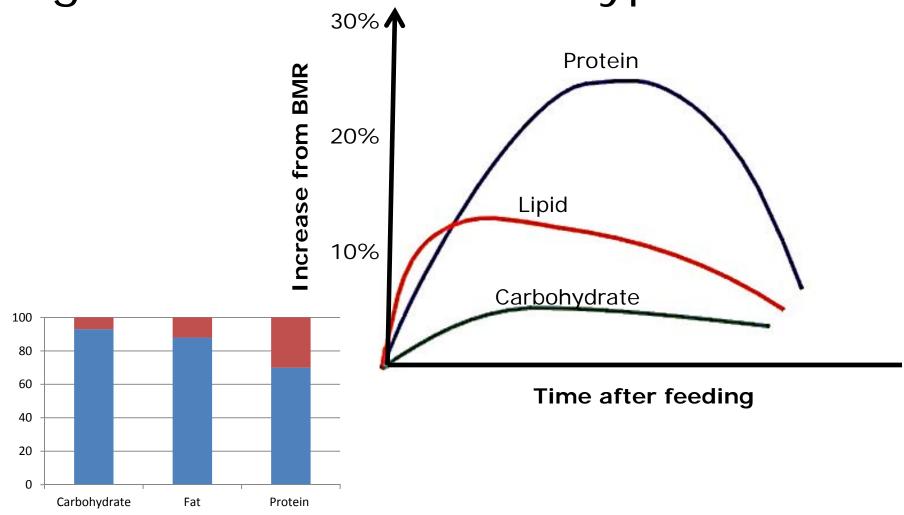


#### SDA contributors





Significance of nutrient type



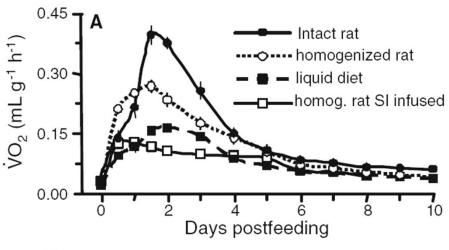


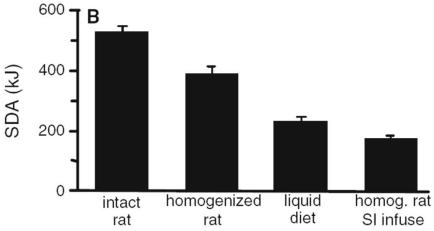
# Significance of physical property

Postprandial metabolic profile of *Python molurus*, following the ingestion of an intact rat, the gastric infusion of homogenized rat and liquid diet, and small intestinal infusion of homogenized rat. All meals equal in mass to 25% of snake body mass.

SDA generated from each of these meal treatments.

Magnitude and duration of elevated postprandial metabolism decline with a decrease in the structural integrity of the diet and the bypassing of gastric digestion.







#### Meal size

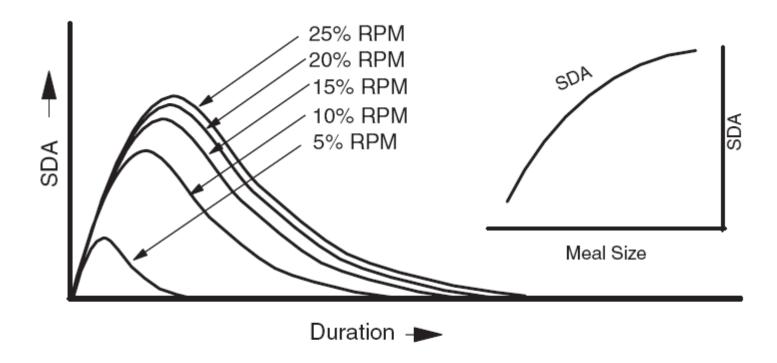
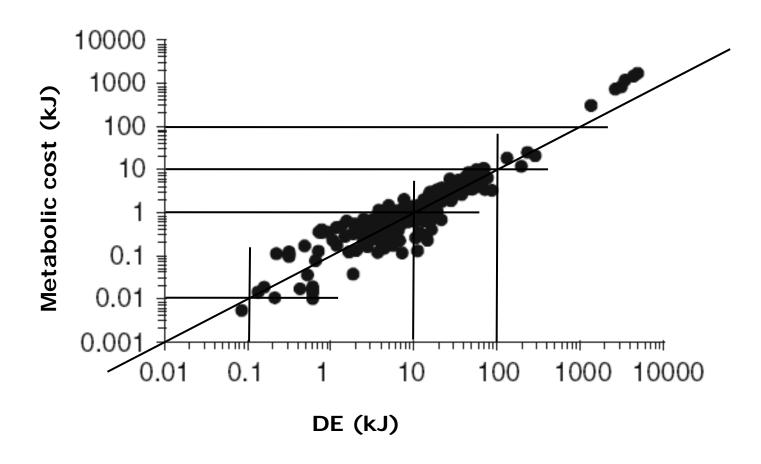


Fig. 7. Schematic illustrating the nonlinear relationship between relative meal size or relative prey mass (RPM) and SDA response typical in ectotherms.



### Meal energy content





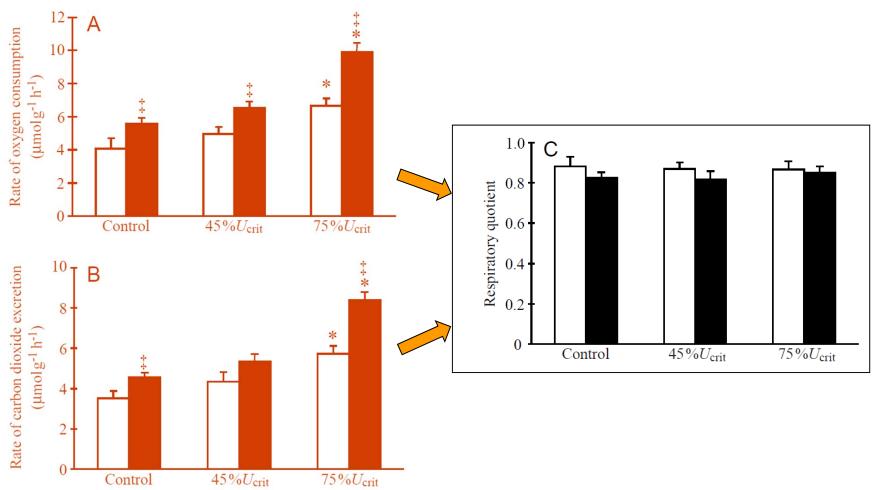
#### Effects of temperature

Many physiological and biochemical processes are modified when fish are acclimated to different temperatures. At the cellular level temperature acclimation affects

- Membrane fluidity
- Enzyme activities
- Substrate for energy production
- Mitochondrial density
- Cell recruitment (muscle)
- Contractile properties

Exercise: Discuss how a 10°C increase in temperature will affect overall energetics and feed utilization in fish

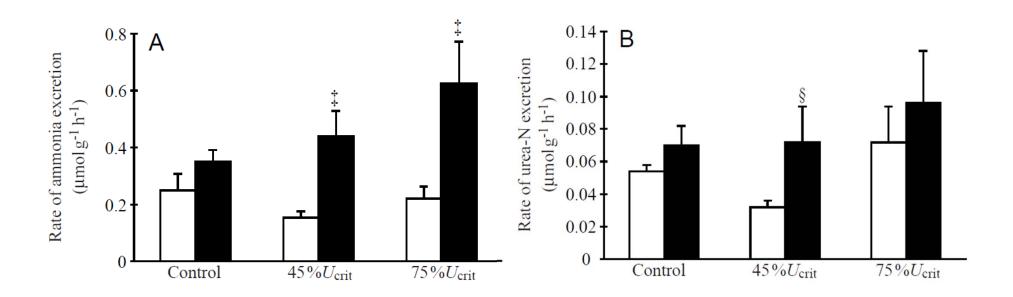




A RESPIROMETRIC ANALYSIS OF FUEL USE DURING AEROBIC SWIMMING AT DIFFERENT TEMPERATURES IN RAINBOW TROUT (ONCORHYNCHUS MYKISS)

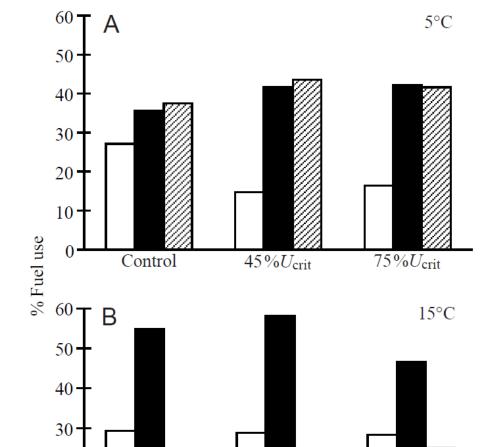
JAMES D. KIEFFER<sup>1,\*</sup>, DEREK ALSOP<sup>2</sup> AND CHRIS M. WOOD<sup>2</sup>





#### A RESPIROMETRIC ANALYSIS OF FUEL USE DURING AEROBIC SWIMMING AT DIFFERENT TEMPERATURES IN RAINBOW TROUT (ONCORHYNCHUS MYKISS)

JAMES D. KIEFFER  $^{1,\ast},$  DEREK ALSOP  $^2$  and CHRIS M. WOOD  $^2$ 



 $45\% U_{\rm crit}$ 

75%Ucrit



A RESPIROMETRIC ANALYSIS OF FUEL USE DURING AEROBIC SWIMMING AT DIFFERENT TEMPERATURES IN RAINBOW TROUT (ONCORHYNCHUS MYKISS)

Control

20-

10+

JAMES D. KIEFFER  $^{1,\ast},$  DEREK ALSOP  $^2$  and CHRIS M. WOOD  $^2$