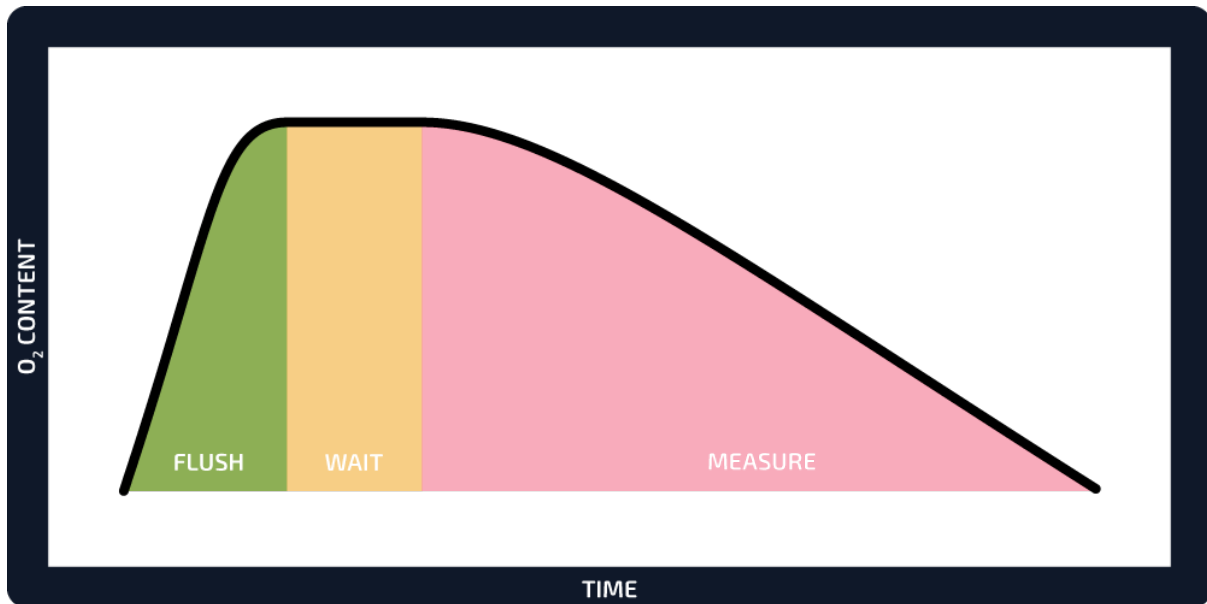


## AutoResp™ 3 – Algorithms summary

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**$MO_2$ -calculations (Metabolic Rate)**

$MO_2$  values are calculated by selecting the time series of  $O_2$  values during a *Measurement Period* and fitting a straight line through these data. The *slope* equals the rate of change in the dissolved oxygen content,  $\frac{dO_2}{dt}$ . This value is multiplied with the volume of water inside the closed chamber, the recirculation pump, and the attached tubing ( $V_{O_2}$ ) to give the total rate of change of oxygen which equals the metabolic rate. The goodness-of-fit ( $R^2$ -value) is also provided and can be used to filter away noisy data.

$$MO_2 = \frac{dO_2}{dt} \cdot V_{O_2}$$

Glossary:

**$MO_2$**  is the *Metabolic Rate* which is the amount of oxygen consumed in a given period of time.  $MO_2$  may be expressed in units such as  $mg\ O_2/h$ ,  $mg\ O_2/kg/h$  or similar.

**$V_{O_2}$**  is the volume of water inside the closed chamber, the recirculation pump, and the attached tubing, i.e. the total volume of water from which oxygen is consumed.

### **$MO_2$ -transforms (Allometric scaling & $Q_{10}$ )**

$MO_2$ -measurements can be automatically normalized to a normalized weight (allometric scaling) or to a normalized temperature ( $Temp_{norm}$ ).

It is assumed that organismal metabolic rates obey the relationship.

$$\widehat{MO_2} = MO_2 \cdot Q_{10}^{\frac{\hat{T} - T}{10}} \cdot \left(\frac{\widehat{M}}{M}\right)^c$$

Glossary:

$c$  is the species dependent *Allometric Scaling Constant*, typically around 0.7.

**$Q_{10}$**  is the *Temperature Coefficient*, which is the factor with which the metabolic rate scales for every 10<sup>th</sup> degree, typically around 2.0.

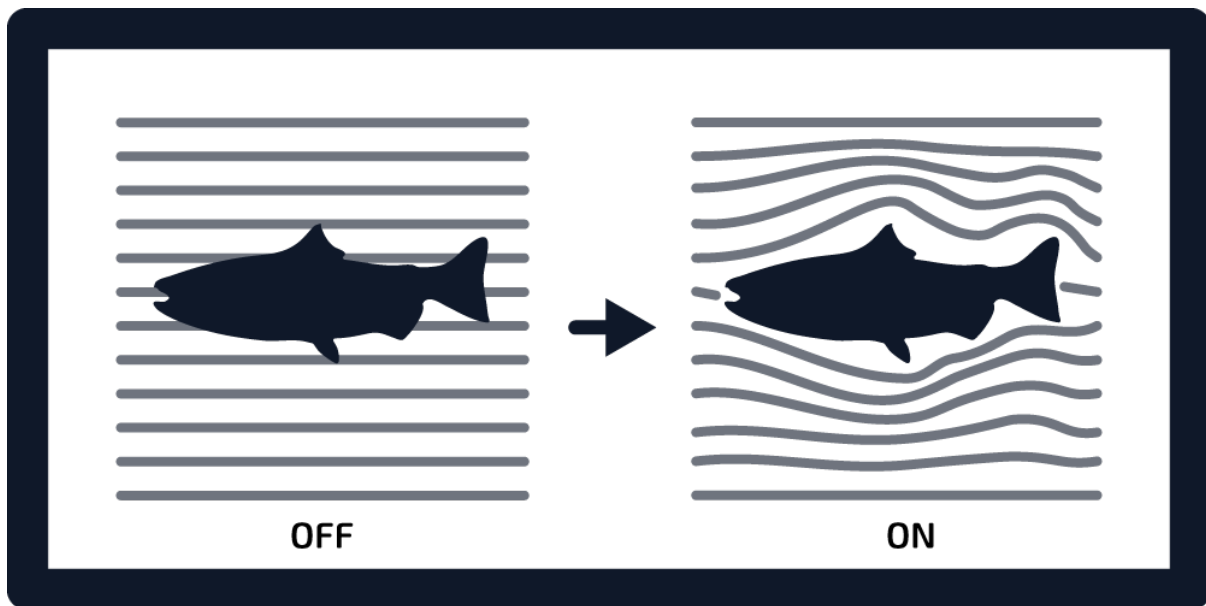
**$M$**  is the mass of the measured organism.

**$\widehat{M}$**  is the *Normalized Mass*, the organism mass at which one wants to examine the metabolic rate,

**$\widehat{MO_2}$**  is the *Standard Metabolic Rate* i.e., the *transformed* or *normalized* metabolic rate at a *Normalized Temperature* and for a *Normalized Mass*.

**$T$**  is the temperature (in Kelvin) at which the measurement was made.

**$\hat{T}$**  is the *Normalized Temperature* ( $Temp_{norm}$ ), the temperature (in Kelvin) at which one want to examine the metabolic rate of the organism.

**Solid Blocking-corrections**

Liquid passing around an organism in a swim chamber changes velocity, and AutoResp™ 3 can automatically compensate for this. (Bell, W.H. & Terhune, L.D.B. (1970)) estimates a correction term which is used to calculate a *Solid Blocking*-compensated flow speed. According to Bell & Terhune, a correction term can be calculated as:

$$\epsilon_s = \lambda \cdot \tau \cdot F^{\frac{3}{2}} = 0.8 \cdot 0.5 \cdot \frac{l_{fish}}{2 \cdot r_{fish}} \cdot \left( \frac{A_{fish}}{A_{swim\ tunnel}} \right)^{\frac{3}{2}}$$

The corrected flow speed is then calculated to be  $V_F = V_T \cdot (1 + \epsilon_s)$ , where  $V_T$  is the measured flow speed in the empty sections.

Glossary:

$r_{fish}$  is the radius of the fish, often estimated as half the mean of its width and height.

$A_{fish}$  is the cross-sectional area of the fish which is generally modelled as  $A_{fish} = \pi \cdot r_{fish}^2$ .

$A_{swim\ tunnel}$  is the cross-sectional area of the swim tunnel.

$F$  is a blocking term equal to the proportion of the swim tunnel cross-section ( $A_{swim\ tunnel}$ ) blocked by the animal.

$\lambda = 0.8$  is a shape term describing a fusiform fish.

$\tau$  is half the length of the fish divided by its thickness ( $0.5 \cdot \frac{l_{fish}}{2 \cdot r_{fish}}$ ).

$V_F$  is the corrected flow speed equivalent to the flow speed of water experienced by the fish.

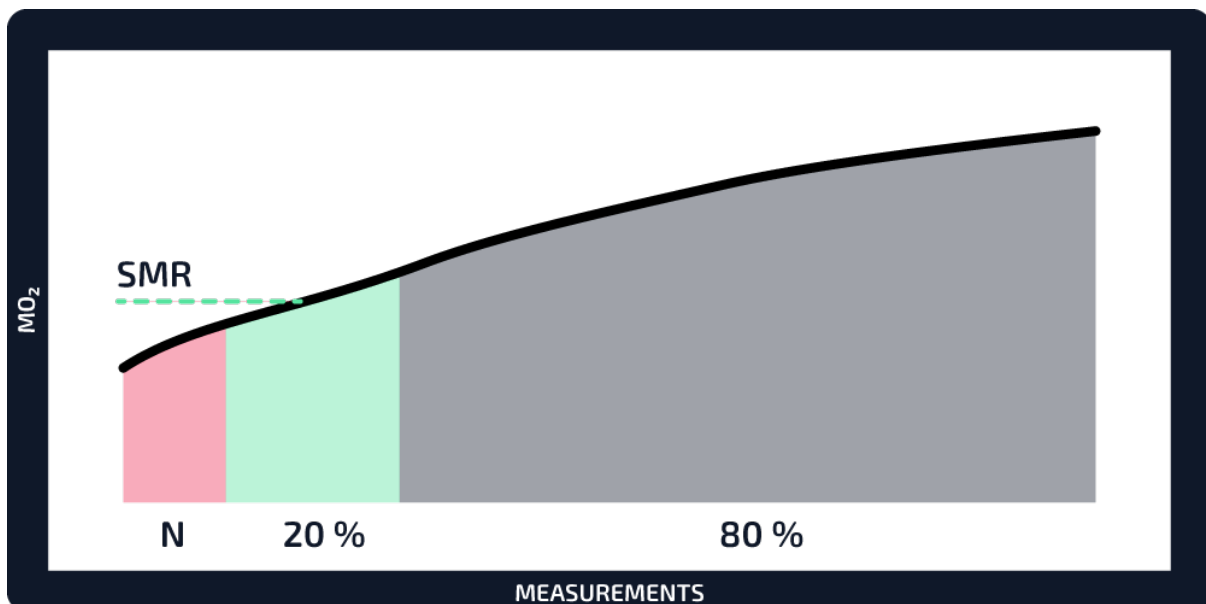
$V_T$  measured flow speed of the water in the empty sections.

$\epsilon_s$  is an error term describing the extra fraction of the measured flow speed experienced by the fish.

### SMR-calculations (Standard Metabolic Rate)

AutoResp™ 3 offers several ways of calculating the *Standard Metabolic Rate (SMR)*. For all of them, it is possible to select a minimum and a maximum value for oxygen content which will filter away  $MO_2$ -values outside these ranges:

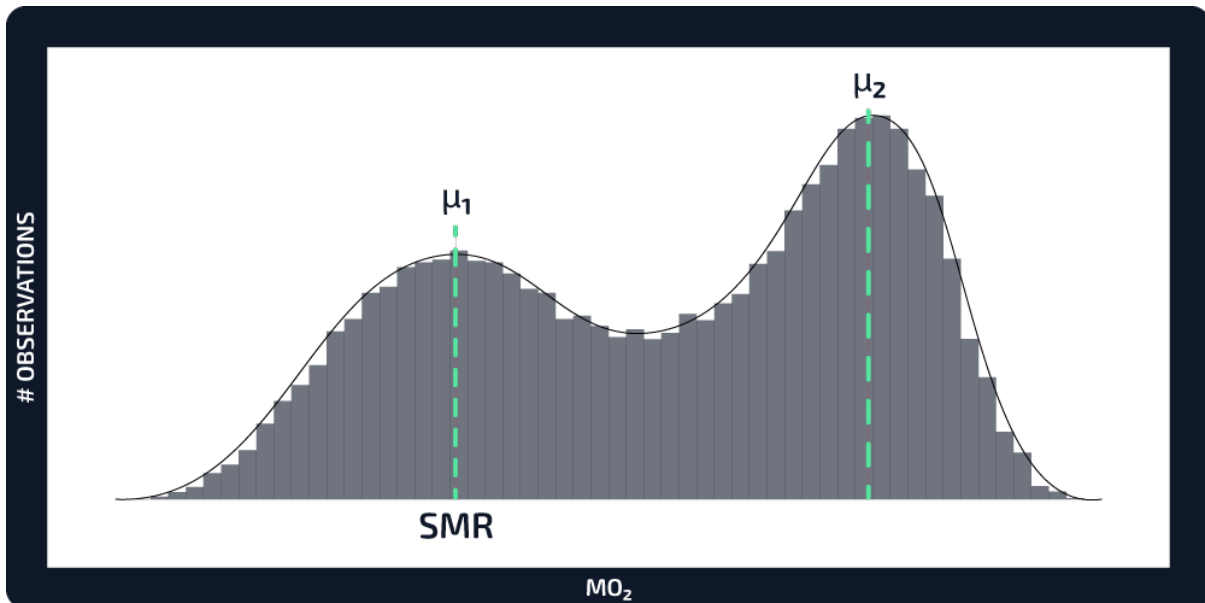
- *Average of lowest N measurements:*  
Estimates the *SMR*-value as the average of the lowest  $N$   $MO_2$ -measurements or the average of all measurements if  $N$  is greater than the number of measurements.
- *Average of lowest P% minus N lowest measurements:*  
For the purposes of this calculation, the lowest  $N$   $MO_2$ -measurements are discarded, and the mean of the lowest  $P\%$  measurements are returned. If, for example,  $N = 5$  &  $P = 20$ , the lowest 5 measurements are discarded, and the *SMR* value is estimated as the mean of the remaining 20% lowest measurements.



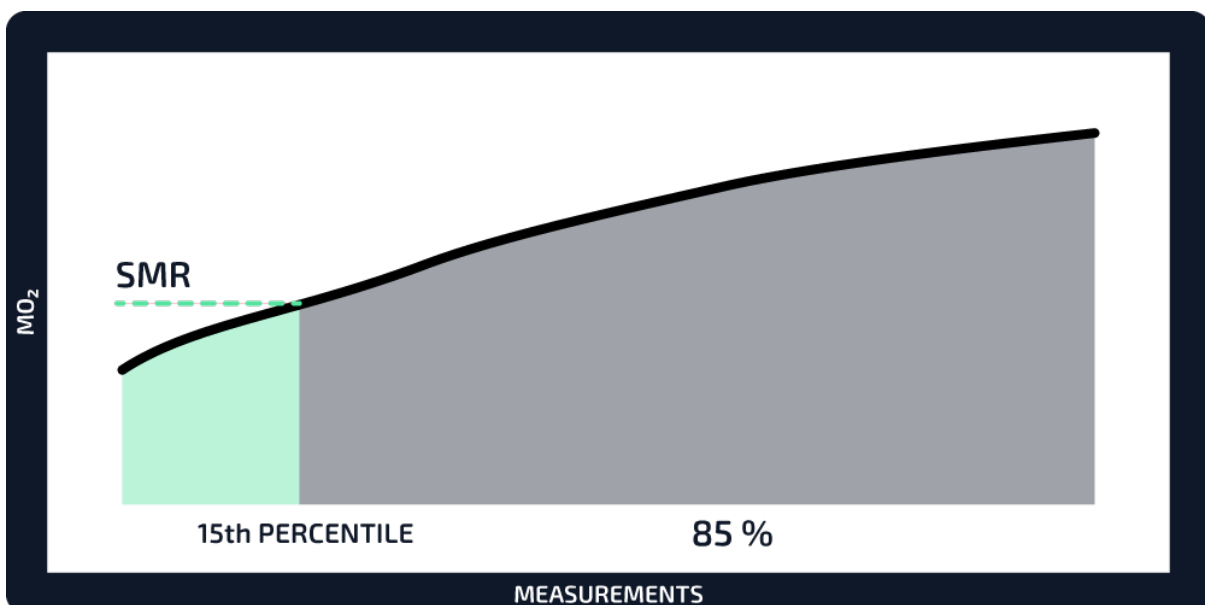
- *Mean of lowest bimodal normal distribution:*  
A bimodal Gaussian (Normal) distribution is fitted to the  $MO_2$ -data. The function has the form:

$$f(x) = a_1 \frac{1}{\sigma_1 \cdot \sqrt{2\pi}} e^{-\frac{1}{2} \left( \frac{x-\mu_1}{\sigma_1} \right)^2} + a_2 \frac{1}{\sigma_2 \cdot \sqrt{2\pi}} e^{-\frac{1}{2} \left( \frac{x-\mu_2}{\sigma_2} \right)^2},$$

and the lowest of  $\mu_1, \mu_2$  is used to estimate the *SMR*-value. (Korsmeyer et. al., 2002)  
Expectation-Maximization (EM) is used to fit the parameters of the function above.



- *N<sup>th</sup> percentile of measurements:*  
Either the 5<sup>th</sup> 10<sup>th</sup> 15<sup>th</sup> 20<sup>th</sup> or 25<sup>th</sup> percentile of the (sorted)  $MO_2$ -measurements can be selected as the *SMR*-value. For example, the 15<sup>th</sup> percentile of 40  $MO_2$ -values would return the 6<sup>th</sup>-lowest value. ( $15\% \cdot 40 = 6$ ). This method was inspired by (Drown et. al., 2020).



Glossary:

**SMR** is the *Standard Metabolic Rate*, the  $MO_2$ -value of the animal at rest and undisturbed.

$\mu_1$  and  $\mu_2$  are the means of the two Gaussian distributions.

### MMR-calculations (Maximum Metabolic Rate)

These calculations are much like *SMR*, except that the *highest* values are generally used instead of the *lowest*. Please refer to *SMR* for these calculations.

### $P_{crit}$ -calculations (Critical Oxygen Tension)

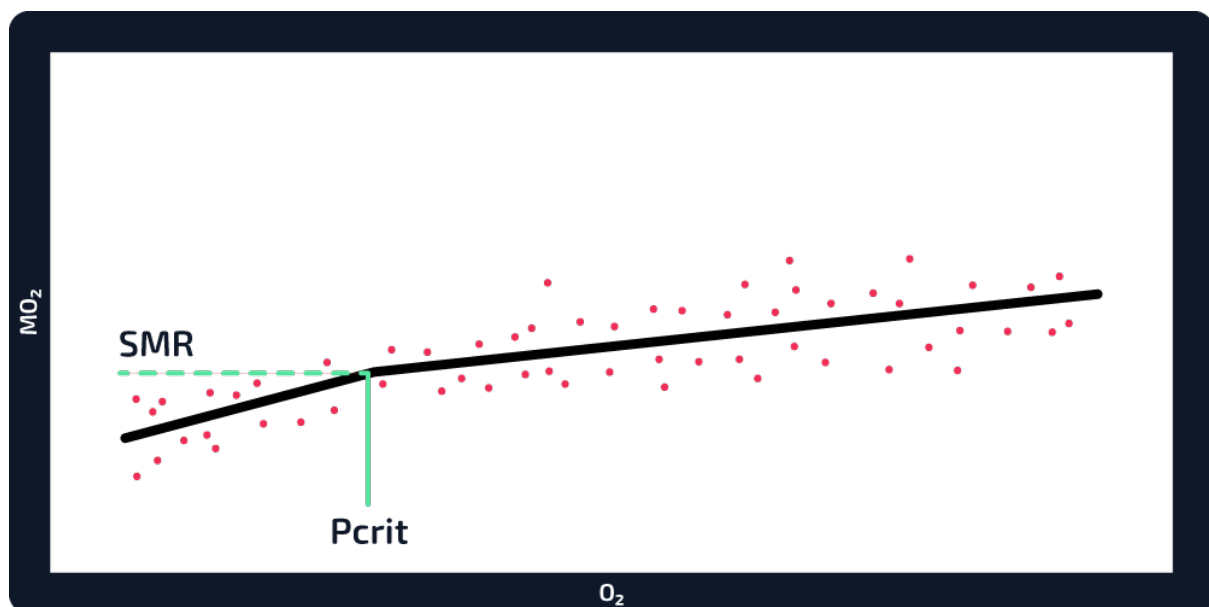
AutoResp™ 3 offers several ways of calculating a  $P_{crit}$ -value. The  $P_{crit}$ -value is generally defined as the partial pressure of oxygen ( $P_{O_2}$ ) below which the animal can no longer maintain a stable metabolic rate ( $MO_2$ ). For all methods of calculating  $P_{crit}$  it is possible to select a minimum and a maximum  $O_2$ -value.  $MO_2$ -values with  $O_2$ -values outside this range will not be included in the calculations of  $P_{crit}$ . For all the methods,  $x$  represents the partial pressure of oxygen, or, if applicable, the corresponding oxygen value in some other units.

- *Broken-Stick*:  
Performs a non-linear parameter fit to a function of the form:

$$MO_2(x) = \begin{cases} a_1 \cdot x + b_1 & \text{for } x \leq P_{crit} \\ a_2 \cdot x + b_2 & \text{for } x \geq P_{crit}' \end{cases}$$

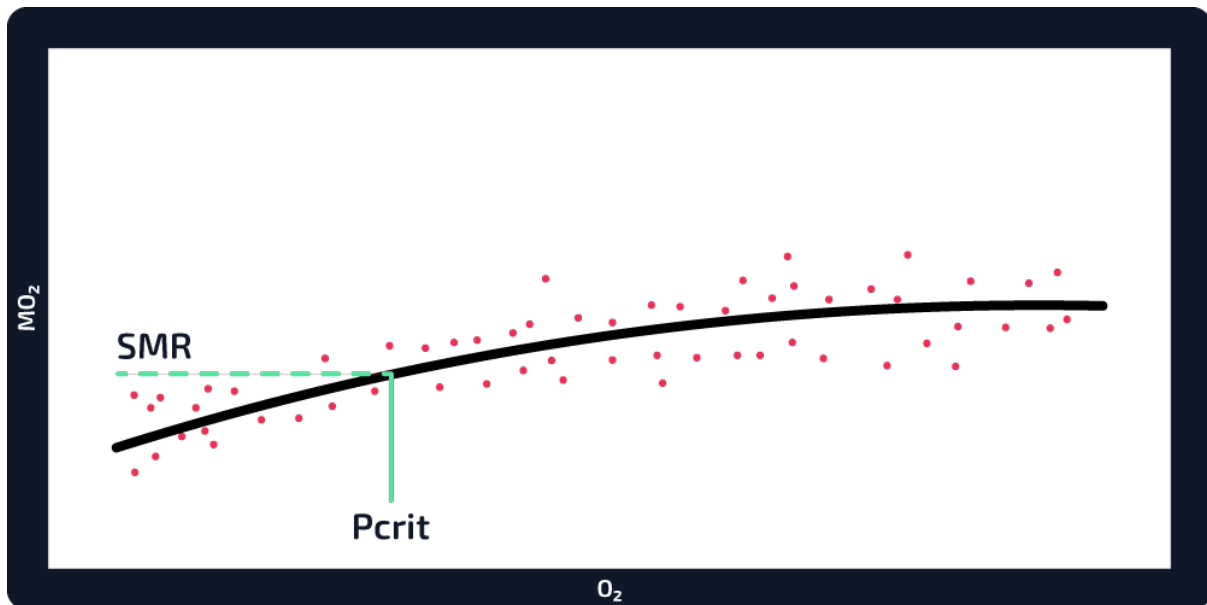
where  $a_1 \cdot P_{crit} + b_1 = a_2 \cdot P_{crit} + b_2$  which ensures continuity at the critical value. The implicit value of  $P_{crit}$  is extracted and returned. There are a couple of options which restricts the possible parameters of this function.

- If an *SMR*-value is provided, either by manually entering it or by using the value automatically calculated for each experiment, the function is restricted such that  $MO_2(P_{crit}) = SMR$ , i.e.,  $a_1 \cdot P_{crit} + b_1 = a_2 \cdot P_{crit} + b_2 = SMR$ .
- If the *Force through 0* checkbox is checked,  $b_1 = 0$ , and the function passes through 0 at  $P_{O_2} = 0$ .
- If the *Slope 2 is horizontal* checkbox is checked,  $a_2 = 0$ .





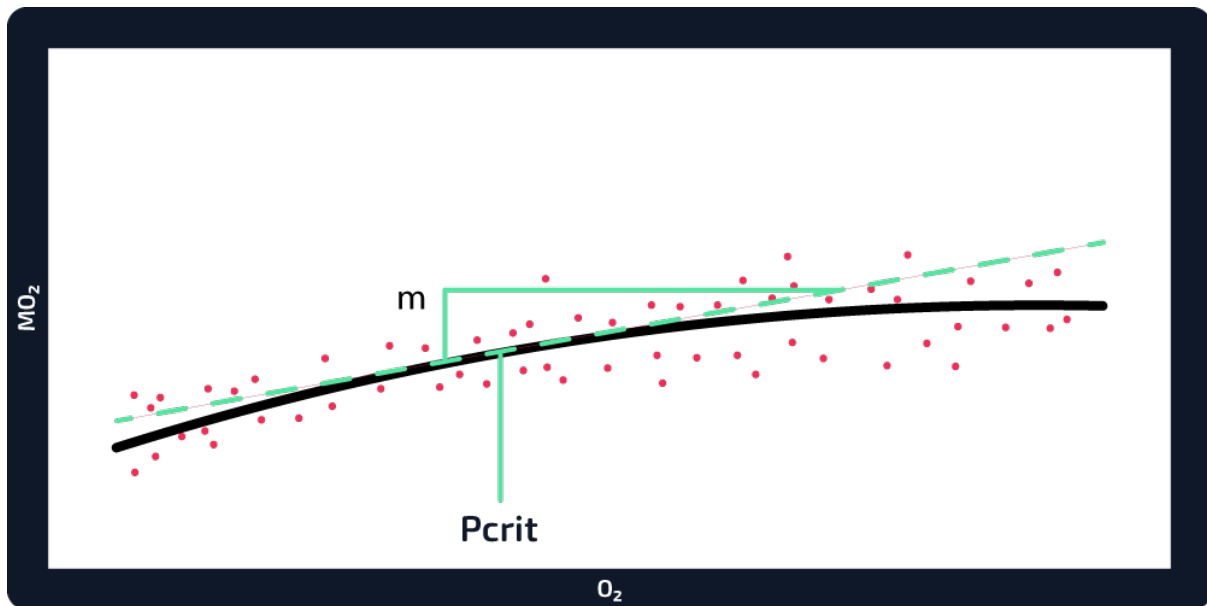
- *Functions Asymptote, MM and W:*  
These methods fit different asymptote functions to the  $(O_2, MO_2)$ -data. The  $P_{crit}$ -value is then estimated by one of two methods:
  1. The intersection with the *SMR*-value is found, and the corresponding oxygen value is determined as the  $P_{crit}$ . This method comes from by (Reemeyer & Rees, 2019). In their study, they use the *MM*-function and the *W*-function described below.



2. The  $MO_2$  data are normalized with  $MO_2^{max} = 1$ , and the oxygen value for which the slope of the function is equal to a predetermined slope,  $m$ , is determined to be  $P_{crit}$ . That is,

$$\frac{dMO_2}{dO_2}(P_{crit}) = m.$$

This method comes from (Marshall *et al.*, 2013) which also uses the *MM* and *W*-functions described below in addition to some others. The critical slope value,  $m$ , used by Marshall *et al* is  $m = 0.065$ .



The different available models are described below. Some of these differ slightly across different articles, but the ones below are the ones used by AutoResp™ 3.

- *Function Asymptote:*  
This model has the form:

$$MO_2(x) = a \cdot (1 - e^{-e^K \cdot (x-l)}),$$

where  $l$  is the intercept at the  $x$ -axis and  $a$  is an asymptotic value approached at high oxygen values. This form is derived from (Zhang *et al.*, 2022), which mentions it as a hypoxic model although not as a candidate for estimating  $P_{crit}$ .

- *Function MM (Michaelis-Menten):*  
This model has the form:

$$MO_2(x) = \frac{a \cdot x}{b+x}$$

This model naturally passes through 0 at  $x = 0$  and approaches  $MO_2 = a$  at higher  $O_2$ -values.

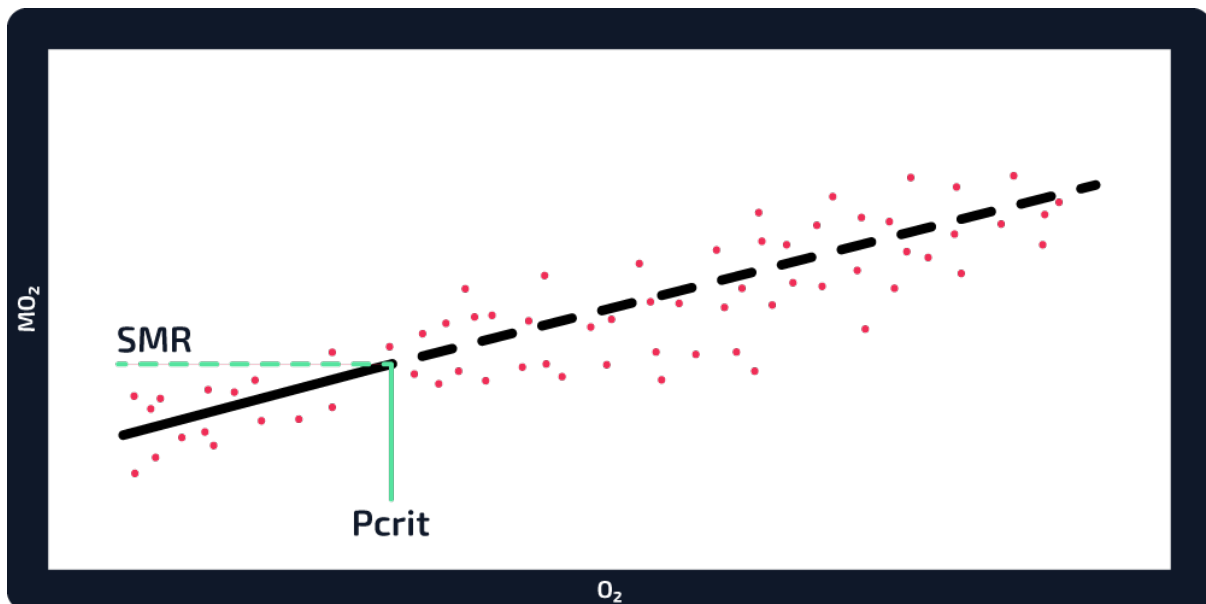
- *Function W (Weibull):*  
This model has the form<sup>1</sup>

$$MO_2(x) = a \cdot \left(1 - e^{-\frac{x}{b}}\right) + d$$

where  $a + d$  is the value asymptotically approached at high values of  $O_2$ , and  $d$  is the  $MO_2$  value at  $O_2 = 0$ .

- *Low value linear fit:*  
This method selects  $(O_2, MO_2)$  values for which  $MO_2 \leq SMR$  and performs a linear fit to these data with a model of the form  $MO_2(x) = a \cdot x + b$ . The value of  $x$  for which  $a \cdot x + b = SMR$  is then estimated to be the  $P_{crit}$  value. That is:

$$P_{crit} = \frac{SMR - b}{a}.$$



Glossary:

$P_{crit}$  is the critical partial pressure of oxygen below which the animal can no longer maintain a stable metabolic rate (it *crashes*). Besides partial pressure, it can be expressed in different equivalent units such as % air saturation.

$MO_2^{max}$  is the maximum  $MO_2$ -measurement in the dataset.

<sup>1</sup> The model as referenced originally has the form  $MO_2(x) = a \cdot \left(1 - e^{-\left(\frac{x}{b}\right)^c}\right) + d$ , but (Reemeyer & Rees, 2019) finds that fitting the exponent  $c$  stops the fits from converging and that it makes no appreciable difference when this parameter is set to 1.

**$U_{crit}$ -calculations (Critical Swim Speed)**

The *Critical Swim Speed* ( $U_{crit}$ ) is found by letting the fish swim at an increasing series of swim speeds until it fatigues. It is calculated according to Brett (1964):

$$U_{crit} = U_f + U_i \cdot \frac{T_f}{T_i}$$

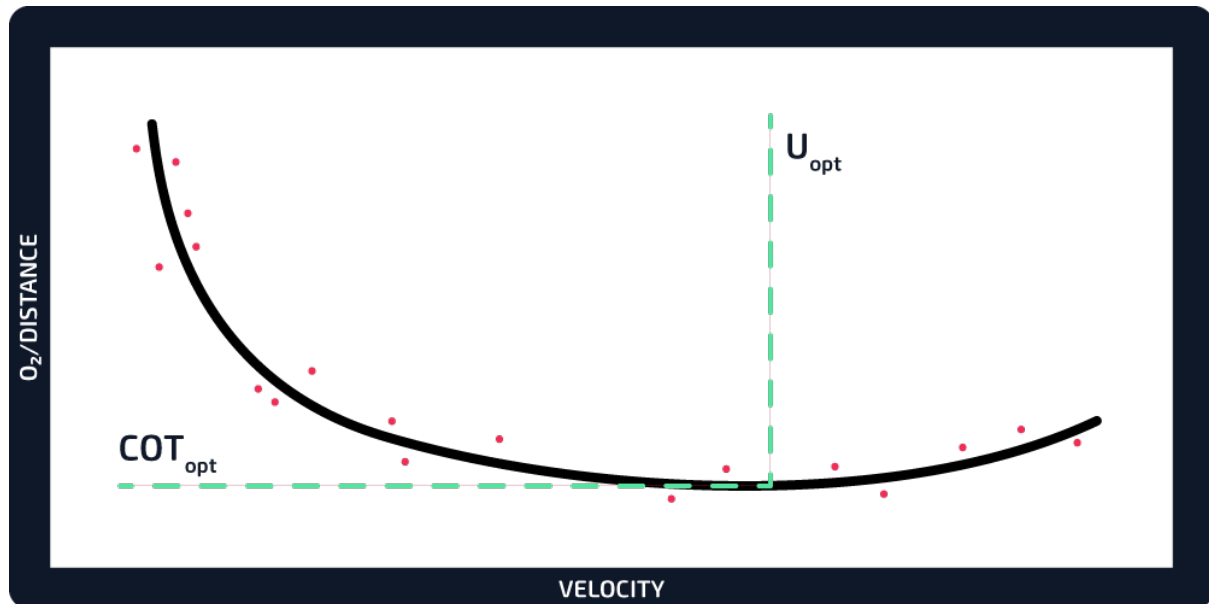
Glossary:

$T_f$  is the time spent at the swim speed at which the fish fatigued.

$T_i$  is the *Time Increment*, the time assigned to each swim speed.

$U_f$  is the highest sustained swim speed of the fish, equal to the highest swim speed completed without the fish fatiguing.

$U_i$  is the *Incremental Swim Speed*, the increase between each successive swim speed in the series.

**COT- and  $U_{opt}$ -calculations (Cost Of Transport and Optimal Swim Speed)**

Moving at a certain speed  $U$  with a certain metabolic rate  $MO_2$  means that the *Oxygen Cost* of moving a certain distance  $d$  is equal to  $MO_2 \cdot \frac{d}{U}$ . Expressing the metabolic rate as a function of the movement speed allows us to calculate the (oxygen) cost of moving a certain constant distance  $d$  at different speeds:

$$COT(U) = MO_2(U) \cdot \frac{d}{U}.$$

It is assumed that the metabolic rate can be described by the exponential relation<sup>2</sup>

$$MO_2(U) = SMR \cdot e^{b \cdot U}$$

or by the hydrodynamics-based power-curve<sup>3</sup>

$$MO_2 = SMR + b \cdot U^c.$$

The first of these curves can be seen in the  $MO_2$  vs  $U$  tab, and in the  $\text{Log } MO_2$  vs  $U$  tab where it is linear, since

$$\log(MO_2(U)) = \log(SMR) + b \cdot U.$$

<sup>2</sup> (Brett, 1964; Webb, 1975; Beamish, 1978)

<sup>3</sup> (Korsmeyer et. al., 2002; Wu, 1977; Videler, 1993)

The parameter  $b$  and an estimated  $SMR$ -value is found by performing a linear regression to this relation.

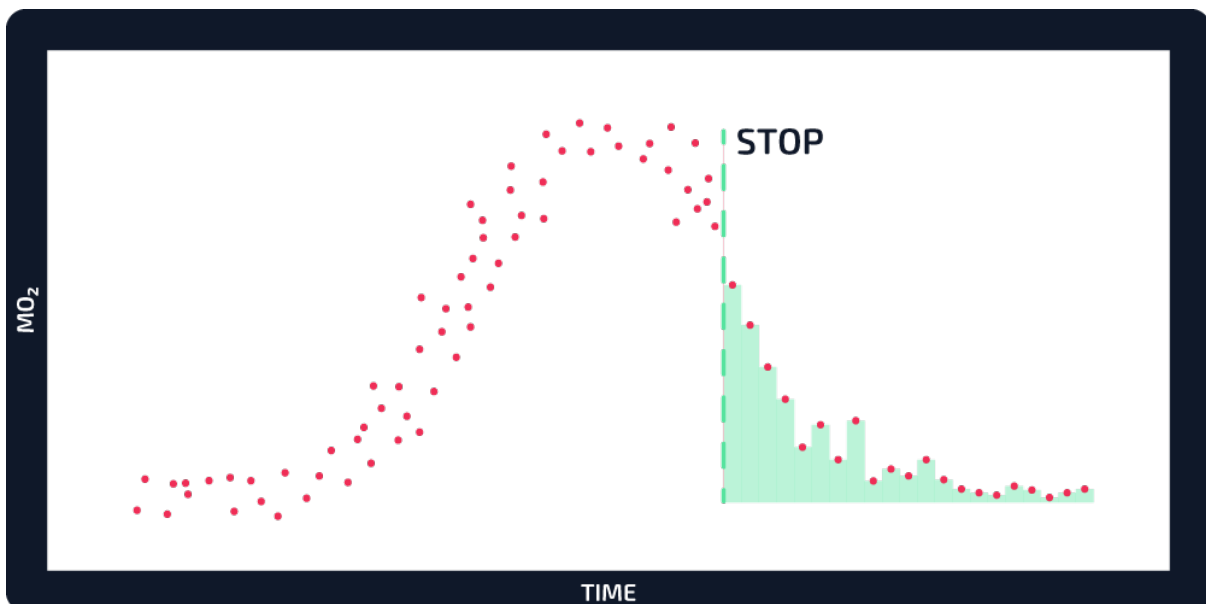
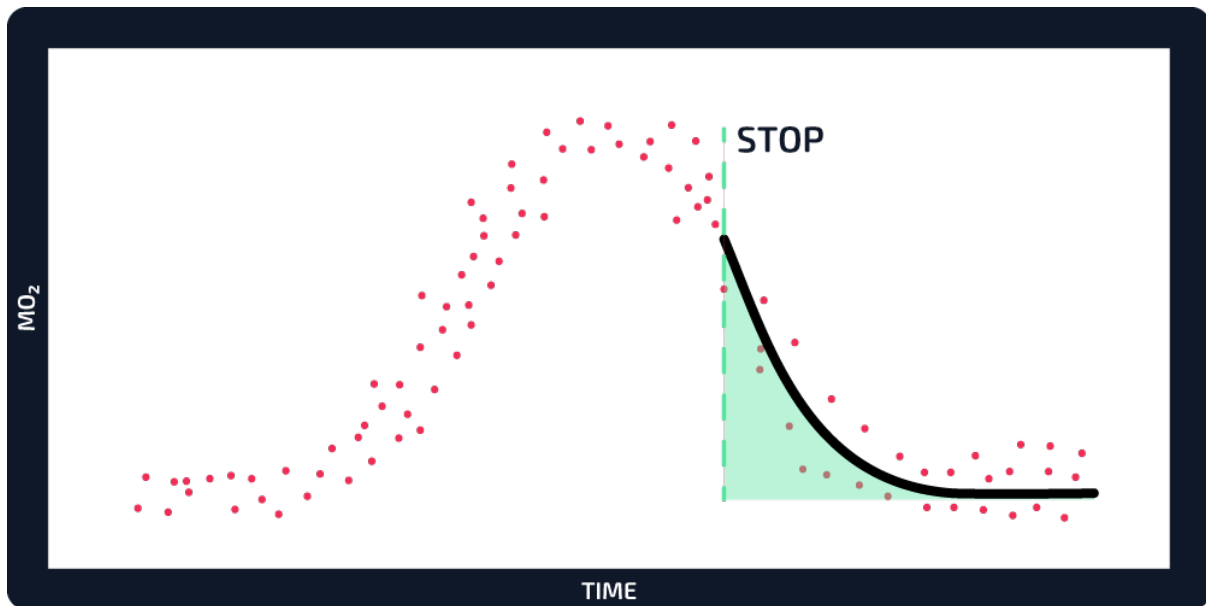
An *Optimal Swim Speed* ( $U_{opt}$ ) can be found, since  $COT(U) = SMR \cdot e^{b \cdot U} \cdot \frac{d}{U}$  has a minimum at  $U = b^{-1}$ , which yields  $U_{opt} = b^{-1}$ . This is the swim speed at which the organism moves furthest while using a certain amount of oxygen or equivalently the speed at which the organism uses the least amount of oxygen to move a certain distance.

Glossary:

***COT*** is the *Cost Of Transport*, which is the oxygen cost of moving a certain distance at a given speed.

***U<sub>opt</sub>*** is the *Optimal swim speed*, which is the speed at which the organism moves furthest while using a certain amount of oxygen or equivalently the speed at which the organism uses the least amount of oxygen to move a certain distance.

## ***EPOC***-calculations (Excessive Post-exercise Oxygen Consumption)



The *EPOC*-value measures the oxygen 'debt' after exercise and is the total amount of excess (above *SMR*) oxygen absorbed after exercise, i.e.

$$EPOC = \int_{t_1}^{t_2} MO_2(t) - SMR dt.$$

The AutoResp™ 3 graphs contain a slider which determines the time of 'exercise-stop' ( $t_1$ ), and this value is also estimated by the software.

AutoResp™ 3 provides two methods of estimating this value: By *Numeric Integration* or by fitting an exponentially decaying function. In both cases,  $t_2$  is assumed to be  $\infty$  or at least to correspond to the end of the experiment.

The numeric integration simply sums all values of  $MO_2 - SMR$  after the  $t_1$  and multiplies them with their respective durations.

The *Exponential Decay* option fits a model of the form:

$$MO_2(t) = SMR + m \cdot e^{-k \cdot t}$$

to the data, where  $m$  may be thought of as the *Absolute Aerobic Scope (AAS)* at the end of exercise, and where  $t$  is time passed since the end of exercise. Internally, it performs a log-transform ( $\log(MO_2(t) - SMR) = \log(m) - kt$ ), which enables a linear regression to be performed. To do this, *all  $MO_2$ -values below  $SMR$  are discarded*. The *EPOC* is then calculated as

$$EPOC = \int_{t_1}^{t_2} MO_2(t) - SMR dt = \int_0^{\infty} m \cdot e^{-k \cdot t} dt = \frac{m}{k}.$$

Glossary:

**EPOC** is the *Excessive Post-exercise Oxygen Consumption*, which is the oxygen ‘debt’ after exercise and is the total amount of excess (above *SMR*) oxygen absorbed after exercise.

### A note on confidence intervals

The various 95%-confidence intervals are *numerically estimated* assuming that the fitted parameters are uncorrelated, that the selected model correctly describes the data with a best fit and without considering uncertainties in the underlying data. These confidence bands should not be seen as the true ranges of the underlying parameters but as the confidence ranges of the fits of the selected models, given the provided data.