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INTRODUCTION

The life styles and needs of the different species have a major influence on its physiological adaptations. Active pelagic fish tend to have higher metabolic rates than sluggish benthic species, with a relatively more inactive life style (Webb, 1993). However, few studies have directly compared the metabolic capacity and the metabolic response to exercise in species with different lifestyles.

The ability of fish to exercise, recover and swim again without hindrance has important ecological ramifications. Even though fish apparently spend most of their time swimming at slow speeds, a fish's ability to successfully forage, escape predation, to maintain position in a current and to migrate upstream, depends on the capacity to sustain and recover from high levels of exercise (Milligan, 1996; Farrell *et al.,* 1998). Therefore, depending on the kind of exercise being performed (routine swimming, foraging or migrating upstream), a different metabolic response, or metabolic rate, is expected to meet the challenge imposed to the animal.

Resting metabolic rate (RMR) is the measure of the minimum oxygen consumption of fish at rest, and is that required for basic maintenance functions that sustain life (Cech, 1990; Webb, 1993). Activity is a factor that greatly affects an animal's energy expenditure, and consequently its metabolism. Measurements of metabolic rate during different types of exercise help to understand the energy costs of such activities (Randall *et al.*, 1997). As activity level increases, O_2 consumption rises to meet increased demand for ATP (adenosine triphosphate) produced in the muscles. Maximum aerobic metabolic rate is defined as the active metabolic rate (AMR) and sets an upper limit on this kind of sustainable behaviour (Bennet, 1991; Webb, 1993). The energy available for aerobic swimming activities is the difference between the resting and active metabolic rate, traditionally called metabolic scope (MS) or scope for activity (Fry, 1957; Webb, 1993).

The aim of this study is to determine the range of metabolic capacity, or metabolic scope, of 5 North Atlantic teleost species with different life styles (*Tautogolabrus adspersus*, cunner; *Macrozoarces americanus*, ocean pout; *Gadus morhua*, Atlantic cod; *Osmerus mordax mordax*, Atlantic rainbow smelt; and *Mallotus villosus*, capelin) by measuring oxygen consumption (MO_2) while at rest, swimming at low velocities and during intense exercise. The hypotheses tested are: 1) The RMR, AMR and MS depend on the species life style; 2) The metabolic rate within each species depends on the type of activity being performed.

MATERIAL AND METHODS

Experimental animals

Five species of North Atlantic teleost fish were used in this study, and information on their origin, diet, mass and length are presented in Table I. Before and during the experiments, the fish were maintained indoors in fiberglass tanks supplied with 8±1ºC seawater, exposed to a natural photoperiod. Feeding was suspended 48 hours before experimentation, and only fish in good condition were used in experiments. These experiments were performed from May to August 2003.

Table I. Physical characteristics, capture/rearing information, nº of animals (N) and diet of the five species used in this study. Values for mass and length are presented as means ± standard error.

Species	Common name	N	Mass (g)	Length (cm)	Origin	Feeding
Tautogolabrus adspersus	Cunner	10	111.7 ± 11.4	19.3 ± 0.9	Wild, collected in Logy Bay and at Middle Cove	Chopped herring*; 2x a week
Macrozoarces americanus	Ocean pout	10	50.5 ± 2.8	22.4 ± 0.3	Wild parents, eggs collected in the wild 2002, reared at OSC	Commercial pellets + chopped herring*, 1x a week
Gadus morhua	Atlantic cod	10	73.1 ± 5.8	20.6 ± 0.6	Reared at ARDF\OSC; 2002 spawning	Commercial pellets
Osmerus mordax mordax	Atlantic rainbow smelt	7	34.0 ± 4.4	18.0 ± 0.7	Wild, collected in fresh water by Ian Bradbury, June 2003	Chopped herring*; 2x a week
Mallotus villosus	Capelin	10	22.9 ± 1.1	15.6 ± 0.3	Wild, collected in Middle Cove, NFLD, July 2003	Chopped herring*; 2x a week

* - Atlantic herring (*Clupea harengus*).

Experimental protocol

The metabolic capacity of fish is normally examined using a critical swimming speed (U_{crit}) test (Brett, 1964). This test is performed by increasing the swimming speed of the fish by 0.25 to 0.5 bl s^{-1} (body lengths per second) every 15–30 minutes until the fish is exhausted. However, the ocean pout is a relatively inactive benthic species, and the cunner does not swim continually in its natural habitat. Thus, a protocol was developed which would allow the metabolism of all five species to be directly compared at rest, while swimming slowly, during intense (maximal) exercise, and during

recovery from intense exercise. Metabolic rates (at 8ºC) were measured in a Blazka-type respirometer.

1) Resting Metabolic Rate - To make measurements of RMR, the fish were placed in the respirometer and left for 18 hours (overnight) to recover from handling, and to habituate to the conditions within the respirometer. A water current of 3-5 cm s^{-1} was maintained to help the fish orient, and to reduce stress. During the habituation period, and all measurements of oxygen consumption, the swimming section of the respirometer was completely covered with a black plastic to minimize visual disturbance.

2) Swimming Metabolic Rate - Following the measurement of RMR, each fish was forced to swim at velocities of 10 cm s^{-1} (SwMR 10) and 15 cm s^{-1} (SwMR 15) for 15 minutes. The current velocity was gradually increased from the velocity used in the resting period to 10 cm s^{-1}, and from 10 cm s^{-1} to 15 cm s^{-1}, over a period of 1-2 minutes. This gradual increase in current velocity allowed the fish to remain calm during each increase in velocity. During SwMRs periods, electrical stimulation (< 5 volts) was only used if the fish rested on the rear partition of the swimming section for more than 10 seconds.

3) Active Metabolic Rate - Active metabolic rate (AMR) is the maximum aerobic metabolic rate (MO_2 max), and is associated with swimming at the greatest sustainable velocity (Cech, 1990). To obtain the MO_2 max of all species, the fish were subjected to a stress period - 15 minutes of burst exercise. Burst exercise was accomplished by increasing the current velocity to a point where the fish began “burst swimming”. This velocity was then maintained until apparent exhaustion occurred (ie. until the fish ceased swimming and rested against the rear partition of the swimming section of the respirometer). At this point, the current velocity was rapidly decreased to encourage swimming. As soon as the fish began to swim again, the current velocity was rapidly increased to a speed where the fish began burst swimming. If the fish took more than approximately 10 seconds to begin swimming again, electrical stimulation (5-10 volts) was applied. This cycle of burst swimming and brief rests was maintained for the entire period, and the animals were clearly exhausted by this protocol.

After the swim trials, the fish were removed from the respirometer and their mass and length (total length) were recorded before they were returned to the holding tank.

Respirometer

Metabolic rates (at 8±1 ºC) were measured in a Blazka-type respirometer, and water speed was controlled by an electrical motor with calibrated controller. Water temperature and oxygen concentration in the respirometer were continuously monitored during the experiment by drawing water from the respirometer through an external circuit. Oxygen concentration was measured using a galvanic oxygen electrode with thermal sensor (model CellOx 325, WTW, Weilheim, Germany) that was housed in a flow chamber. This oxygen electrode was connected to an oxygen meter (model Oxi 342, WTW) with automatic temperature compensation so that water oxygen readings could be taken in mg l^{-1}.

Measurements and Calculations

Oxygen consumption (MO_2) was measured in every period by stopping the flow of fresh water into the tunnel for 10 minutes (20 min during the resting period), and recording the drop in water oxygen content in the 6.8 liter respirometer. The first 2 minutes of readings were ignored due to pressure variations inside the tunnel. Between periods the tunnel was open for 5 minutes.

Oxygen consumption was calculated as:

$$MO_2 = [(Ci – Cf) \times V \times m] / T \text{ , where:}$$

MO_2 = Oxygen consumption (mg O_2 kg^{-1} h^{-1})

Ci = Water O_2 content at the start of MO_2 measurement (mg O_2 l^{-1})

Cf = Water O_2 content at the end of MO_2 measurement (mg O_2 l^{-1})

V = volume of respirometer and external circuit (6.81l)

m = fish mass (kg)

T = duration of the measurement period (h)

MO_2 values were converted to mass-independent values using mass exponents calculated from the data, by regressing Ln MO_2 *vs* Ln body mass. Metabolic scope (MS) was calculated as AMR-RMR.

RESULTS

Table II – Metabolic rates of 5 North Atlantic teleost species while at rest – Resting metabolic rate (RMR) and during exhausting exercise – Active metabolic rate (AMR). Metabolic Scope is calculated as AMR-RMR.

Species	Cunner	Ocean pout	Atlantic cod	Rainbow smelt	Capelin
Resting metabolic rate ($mg.kg^{-1}.h^{-1}$)	16.7	19.6	24.4	23.9	24.9
	21.3	18.8	28.5	28.1	24.6
	19.6	24.7	33.9	13.5	28.2
	10.1	18.2	30.2	19.3	54.5
	24.8	27.7	24.6	15.7	21.6
	15.9	22.2	30.2	24.3	22.4
	28.9	19.4	34.8	19.7	32.9
	28.3	19.8	28.4		24.9
	20.3				
Active metabolic rate ($mg.kg^{-1}.h^{-1}$)	125.3	165.1	156.2	302.8	433.4
	93.5	149.5	142.0	304.5	438.3
	78.2	142.8	183.1	237.2	370.6
	148.6	143.5	157.1	316.7	373.5
	134.7	168.2	160.6	254.8	293.1
	113.2	148.2	164.3	235.5	325.7
	123.8	159.2	161.2	219.8	308.1
	150.9	140.3	164.8		370.6
	127.8				
Metabolic Scope ($mg.kg^{-1}.h^{-1}$)	93.8	116.0	100.3	228.7	350.2
	50.9	105.2	78.9	236.3	359.8
	38.7	86.5	111.1	195.7	284.3
	124.7	97.4	83.0	260.3	208.6
	90.7	99.9	104.0	214.8	229.9
	81.1	95.1	96.3	172.4	256.1
	73.9	110.8	90.7	160.2	203.2
	100.1	87.9	107.5		
	85.1				

Table III – Metabolic rates of 5 North Atlantic teleost species while swimming at low velocities – Swimming metabolic rate, at 10 cm s^{-1} (SwMR 10) and at 15 cm s^{-1} (SwMR 15).

Species	Cunner	Ocean pout	Atlantic cod	Rainbow smelt	Capelin
Swimming metabolic rate ($mg.kg^{-1}.h^{-1}$) SwMR 10	46.0	150.3	74.6	104.4	99.2
	45.1	114.6	76.3	217.5	76.2
	46.6	145.1	76.3	95.9	73.0
	79.4	122.1	126.2	111.5	90.8
	92.5	114.0	75.6	93.2	78.2
	56.6	124.8	146.5	93.5	66.9
	52.6	132.7	77.2	97.7	67.9
	109.5	133.5	101.6		162.9
	35.2				
Swimming metabolic rate ($mg.kg^{-1}.h^{-1}$) SwMR 15	68.2	144.4	88.6	109.7	120.1
	58.0	119.6	86.9	233.8	108.0
	59.9	145.1	87.7	106.0	101.1
	130.7	128.2	123.4	116.0	95.9
	84.3	151.1	87.4	111.9	117.2
	88.2	140.4	153.2	96.8	98.1
	88.7	141.5	83.9	122.1	67.9
	112.0	147.2	101.6		202.2
	64.8				

Hypothesis 1

Problem: Does the resting metabolic rate (RMR), active metabolic rate (AMR) and metabolic scope (MS) depend on the species life style?

I am going to follow an *a priori* approach to analyze my data, based in the knowledge that in teleost fish, species with a more active life style tend to have higher metabolic rates than more sluggish species. Thus, based on their life style, I expect that the metabolic rates of the species used in this study will behave as follow: Cunner < Ocean pout < Atlantic cod < Rainbow smelt < Capelin.

My statistic is a ratio of 2 variances and my explanatory variable is categorical, with 2 groups. Therefore, for each of the following types of metabolic rates: RMR, AMR and MS; I'll run 4 t-test (one way ANOVA) to analyze each one of my 4 H_A/Ho pairs in each type of MR.

H_A: Var (β_{Sp}) > 0, the means differ between the 2 species ($\mu_{Sp\ n} \neq \mu_{Sp\ n+1}$)

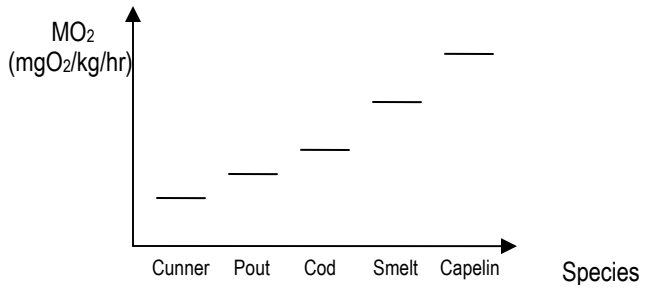
Ho: Var (β_{Sp}) = 0 ($\mu_{Sp\ n} = \mu_{Sp\ n+1}$), for the 5 species (Cunner – Sp 1; Ocean pout – Sp 2; Atlantic cod – Sp 3; Rainbow smelt – Sp 4; Capelin – Sp 5)

I'll use the GLM to perform my analysis (t-test, one-way ANOVA). I'll use the F-statistic, F-distribution and $\alpha = 0.05$ for hypothesis testing.

Analysis 1 – Resting metabolic rate (RMR)

Verbal model: RMR depends on the species.

Graphical model:



Response variable: MO2 – Metabolic rate (mg O_2 kg^{-1} hr^{-1}) on a ratio type scale.

Explanatory variable: Sp - Species (Sp n or Sp n+1) on a nominal type scale.

Cunner – Sp 1; Ocean pout – Sp 2; Atlantic cod – Sp 3; Rainbow smelt – Sp 4; Capelin – Sp 5

Formal model: $MO_2 = \beta_0 + \beta_{Sp} * Sp + \epsilon$

Analysis 1.1 – RMR Cunner < RMR Ocean pout

H_A: Var (β_{Sp}) > 0 The means differ between the 2 species

Ho: Var (β_{Sp}) = 0

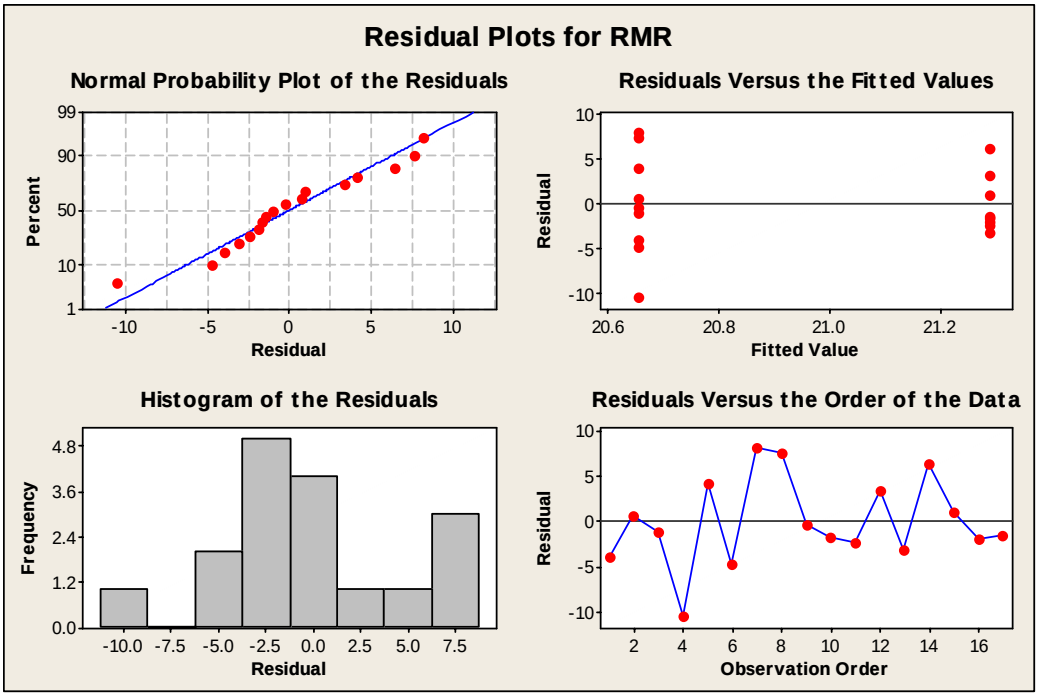


Figure 1 – Residual plots for the analysis of resting metabolic rate (mg O_2 kg^{-1} hr^{-1}) of 2 species (cunner and ocean pout).

Evaluate the model (evaluation of the residuals) (see fig. 1)

Homogeneity of residuals – There are no strong cones in the residuals versus fitted values plot, so residuals are homogeneous.

Independency – The residuals versus order of the data plot shows no evident relation so the residuals are taken to be independent.

Normality – The histogram (values do not cluster around a mean value) and the normal probability plot (s-shaped) of the residuals show some problems and therefore the residuals deviate from normality.

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	1.70	1.70	1.70	0.07	0.797
Error	15	372.58	372.58	24.84		
Total	16	374.28				

Although the assumptions about the residuals were not met, n = 17 and the p-value obtained is quite far from α, and it is very unlikely that a p-value re-computed by randomization will change our decision. So, I'll trust the decision and accept the Ho: Var (β_{Sp}) = 0, rejecting H_A.

There is no difference between the RMR of cunner and ocean pout. ($F_{1,15}$ = 0.07 p = 0.797)

Analysis 1.2 – RMR Ocean pout < RMR Atlantic cod

H_A: Var (β_{Sp}) > 0 The means differ between the 2 species

Ho: Var (β_{Sp}) = 0

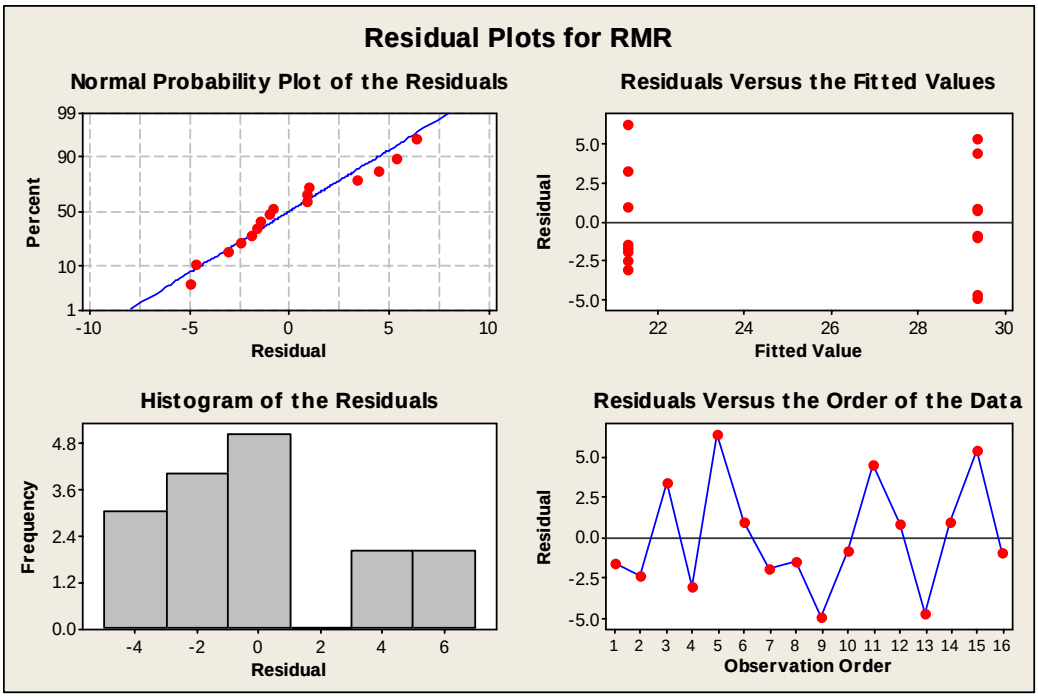


Figure 2 – Residual plots for the analysis of resting metabolic rate (mg O_2 kg^{-1} hr^{-1}) of 2 species (ocean pout and Atlantic cod).

Evaluate the model (evaluation of the residuals) (see fig. 2)

Homogeneity of residuals – There are no strong cones in the residuals versus fitted values plot, so the residuals are taken to be homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals cluster around a central value in a close to symmetrical shape and the normal probability plot of the residuals shows an almost straight line, so the residuals are taken to be normal.

ANOVA Table

Source	DF	Seq SS	Adj SS		Adj MS	F	P
Species	1	260.42	260.42		260.42	20.56	0.000
Error	14	177.33	177.33	12.67			
Total	15	437.75					

The p-value < α (0.05) so, I accept the H_A: Var (β_{Sp}) > 0, rejecting Ho.

The RMR of Atlantic cod is significantly higher than ocean pout's RMR.

($F_{1,14}$ = 20.56 p < 0.001)

Analysis 1.3 – RMR Atlantic cod < RMR Rainbow smelt

H_A: Var (β_{Sp}) > 0 The means differ between the 2 species

Ho: Var (β_{Sp}) = 0

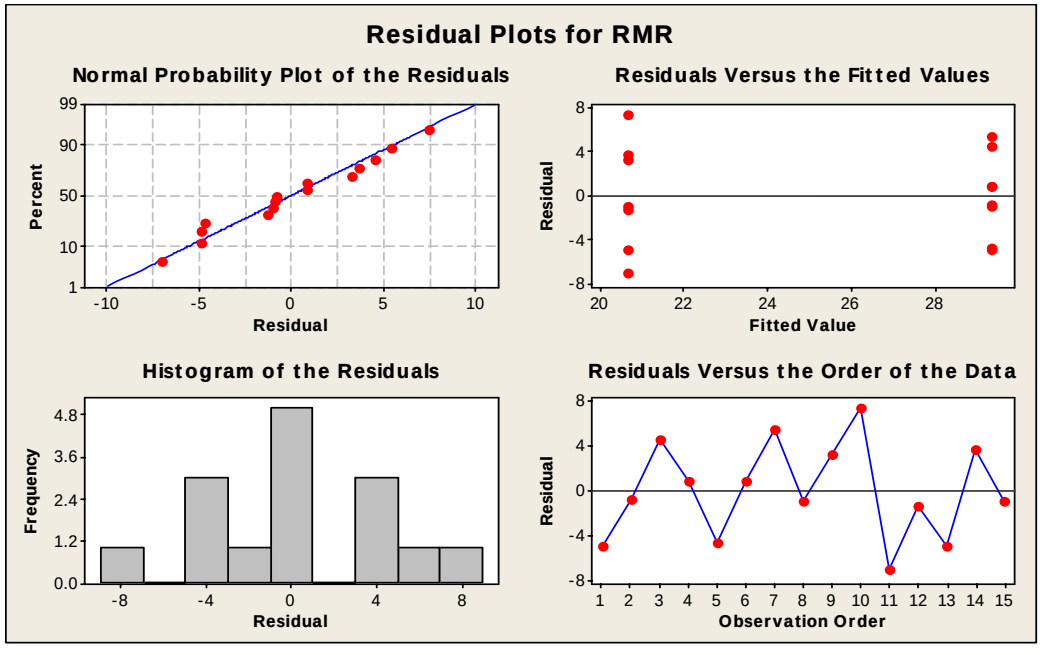


Figure 3 – Residual plots for the analysis of resting metabolic rate (mg O_2 kg^{-1} hr^{-1}) of 2 species (Atlantic cod and rainbow smelt).

Evaluate the model (evaluation of the residuals) (see fig. 3)

Homogeneity of residuals – There are no strong cones in the residuals versus fitted values plot, so the residuals are taken to be homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals cluster around a central value in close to symmetrical shape and the normal probability plot of the residuals shows an almost straight line, so the residuals are taken to be normal.

ANOVA Table

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Species	1	282.68	282.68	282.68	14.27	0.002
Error	13	257.58	257.58	19.81		
Total	14	540.26				

The p-value < α (0.05) so, I accept the H_A: Var (β_{Sp}) > 0, rejecting Ho.

The RMR of rainbow smelt is significantly higher than Atlantic cod's RMR.

($F_{1,13}$ = 14.27 p = 0.002)

Analysis 1.4 – RMR Rainbow smelt < RMR Capelin

H_A: Var (β_{Sp}) > 0 The means differ between the 2 species

Ho: Var (β_{Sp}) = 0

Evaluate the model (evaluation of the residuals) (see fig. 4)

Homogeneity of residuals – Although there is a strong cone in the residuals versus fitted values plot, so the residuals are not homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals cluster around a central value, but normal probability plot of the residuals does not show a straight line, so the residuals deviate from normality.

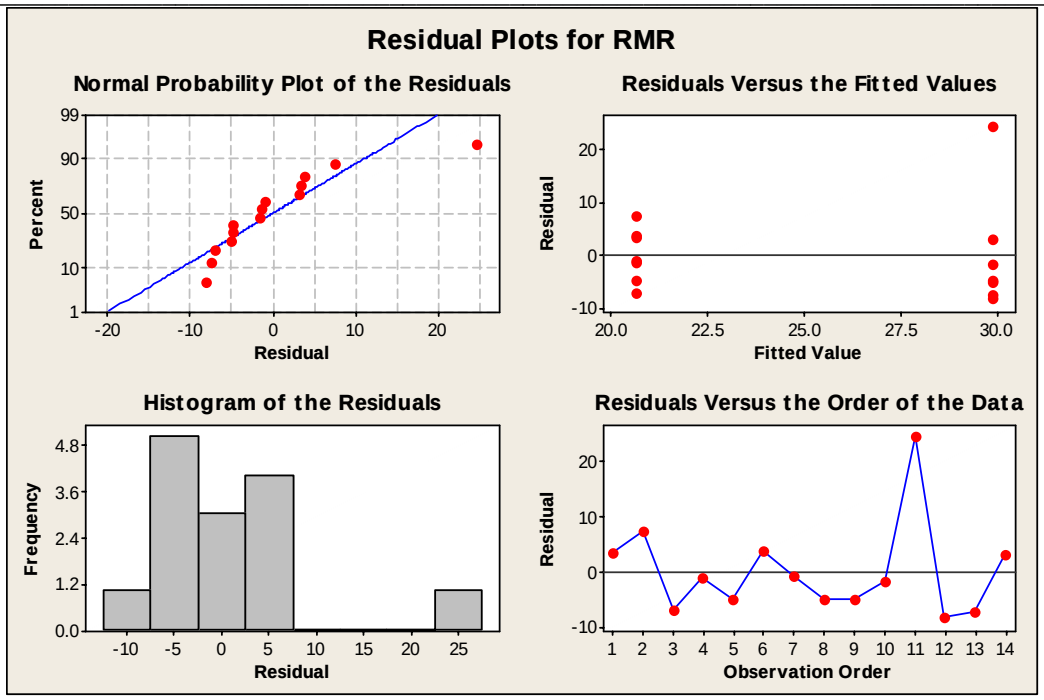


Figure 4 – Residual plots for the analysis of resting metabolic rate (mg O_2 kg^{-1} hr^{-1}) of 2 species (rainbow smelt and capelin).

ANOVA Table

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Species	1	298.36	298.36	298.36	3.76	0.076
Error	12	951.12	951.12	79.26		
Total	13	1249.48				

The assumptions were not met (normality and homogeneity), the n = 15 and the p-value (0.076) is close to α (0.05) so, I'll re-compute a p-value free of assumptions by randomization.

Re-compute the p-value

After 2000 randomizations, 88 F values were higher than the F_{obs} = 3.76, so the new p-value is equal to 0.044 (see also fig. 5).

The p-value < α (0.05) so, I accept the H_A: Var (β_{Sp}) > 0, rejecting Ho.

The RMR of capelin is significantly higher than rainbow smelt's RMR.

($F_{1,12}$ = 3.76 p = 0.044 by randomization)

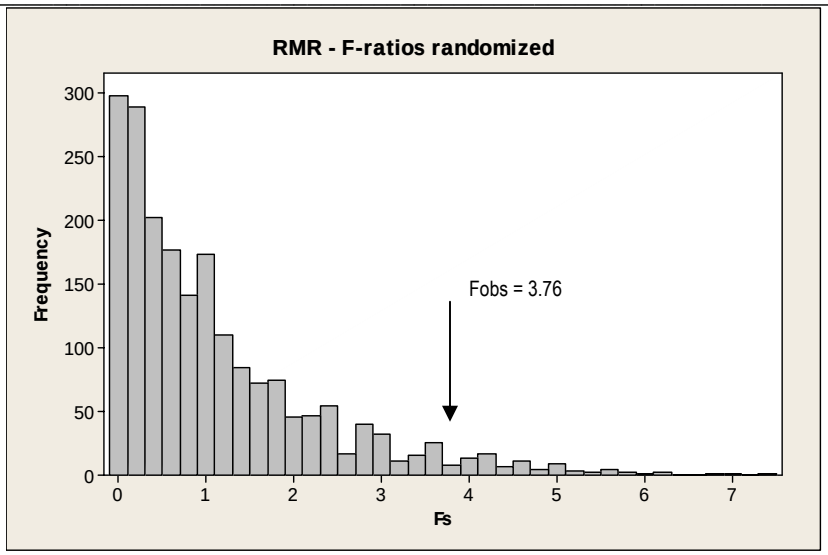


Figure 5 – Histogram of randomized F-ratios for the analysis of resting metabolic rate (mg O_2 kg^{-1} hr^{-1}) of 2 species (rainbow smelt and capelin).

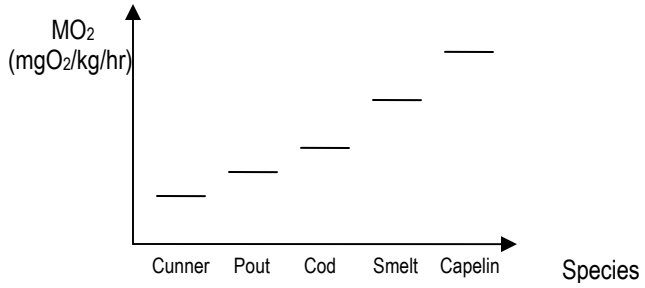
Relatively to resting metabolic rate we found that:

Cunner = Ocean pout < Atlantic cod < Rainbow smelt < Capelin

Analysis 2 – Active metabolic rate (AMR)

Verbal model: AMR depends on the species.

Graphical model:



Response variable: MO_2 – Metabolic rate (mg O_2 kg^{-1} hr^{-1}) on a ratio type scale.

Explanatory variable: Sp - Species (Sp n or Sp n+1) on a nominal type scale.

Cunner – Sp 1; Ocean pout – Sp 2; Atlantic cod – Sp 3; Rainbow smelt – Sp 4; Capelin – Sp 5

Formal model: $MO_2 = \beta_0 + \beta_{Sp} * Sp + \epsilon$

Analysis 2.1 – AMR Cunner < AMR Ocean pout

H_A: Var (β_{Sp}) > 0 The means differ between the 2 species

Ho: Var (β_{Sp}) = 0

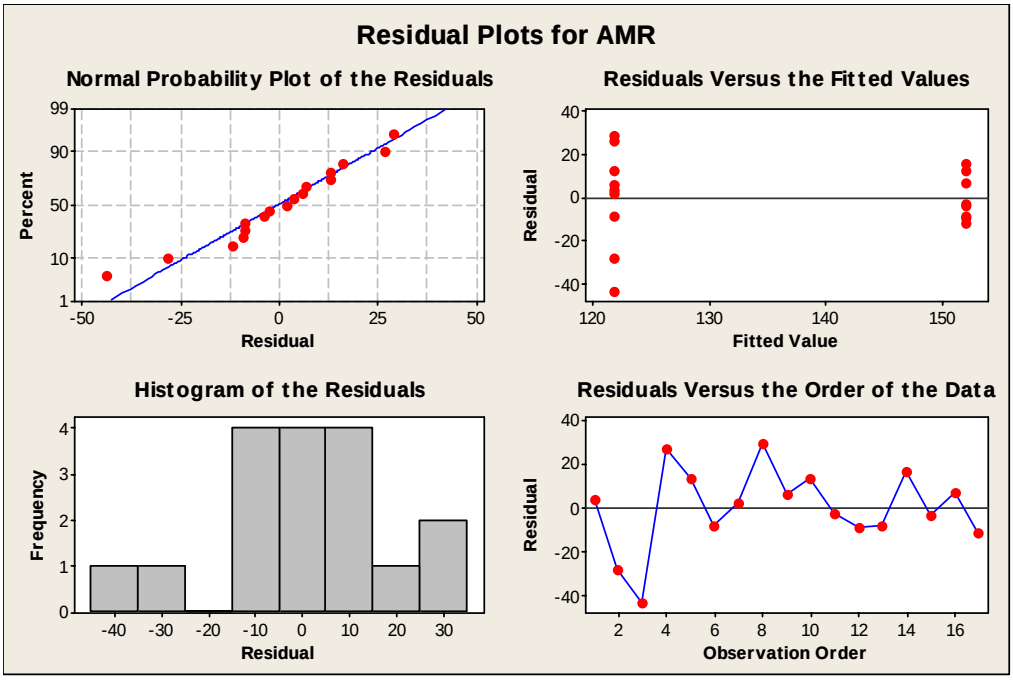


Figure 6 – Residual plots for the analysis of active metabolic rate (mg O_2 kg^{-1} hr^{-1}) of 2 species (cunner and ocean pout).

Evaluate the model (evaluation of the residuals) (see fig. 6)

Homogeneity of residuals – There is a strong cone, opening to the left, in the residuals versus fitted values plot, so the residuals are not homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals cluster around a central value in a close to symmetrical shape and the normal probability plot of the residuals shows an almost straight line, so the residuals are taken to be normal.

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	3887.3	3887.3	3887.3	10.88	0.005
Error	15	5361.1	5361.1	357.4		
Total	16	9248.5				

Although the assumption of homogeneity of the residuals was not met, n = 17 and the p-value obtained is quite far from α, so I'll trust the decision and accept the H_A: Var (β_{Sp}) > 0, rejecting Ho.

The AMR of ocean pout is significantly higher than cunner's AMR. ($F_{1,15}$ = 10.88 p = 0.005)

Analysis 2.2 – AMR Ocean pout < AMR Atlantic cod

H_A: Var (β_{Sp}) > 0 The means differ between the 2 species

Ho: Var (β_{Sp}) = 0

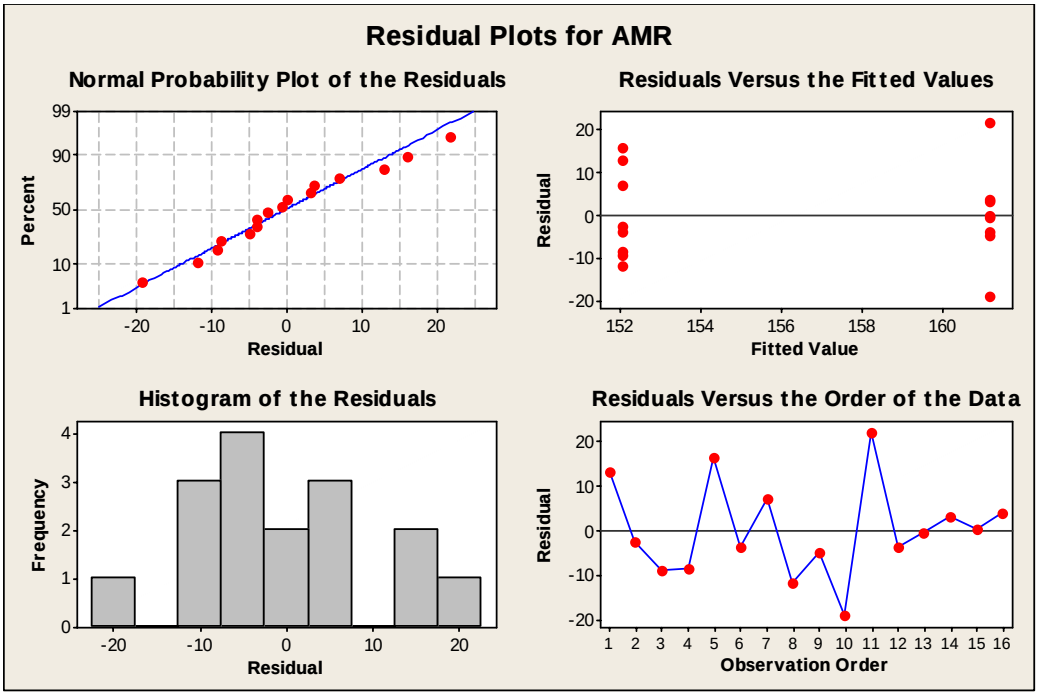


Figure 7 – Residual plots for the analysis of active metabolic rate (mg O_2 kg^{-1} hr^{-1}) of 2 species (ocean pout and Atlantic cod).

Evaluate the model (evaluation of the residuals) (see fig. 7)

Homogeneity – There are no strong cones in the residuals versus fitted values plot, so the residuals are taken to be homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals cluster around a central value in close to symmetrical shape and the normal probability plot of the residuals shows an almost straight line, so the residuals are taken to be normal.

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	329.5	329.5	329.5	2.69	0.123
Error	14	1713.7	1713.7	122.4		
Total	15	2043.2				

The p-value > α (0.05) so, I accept the Ho: Var (β_{Sp}) = 0, rejecting H_A.

There is no difference between the AMR of ocean pout and Atlantic cod.

($F_{1,14}$ = 2.69 p = 0.123)

Analysis 2.3 – AMR Atlantic cod < AMR Rainbow smelt

H_A: Var (β_{Sp}) > 0 The means differ between the 2 species

Ho: Var (β_{Sp}) = 0

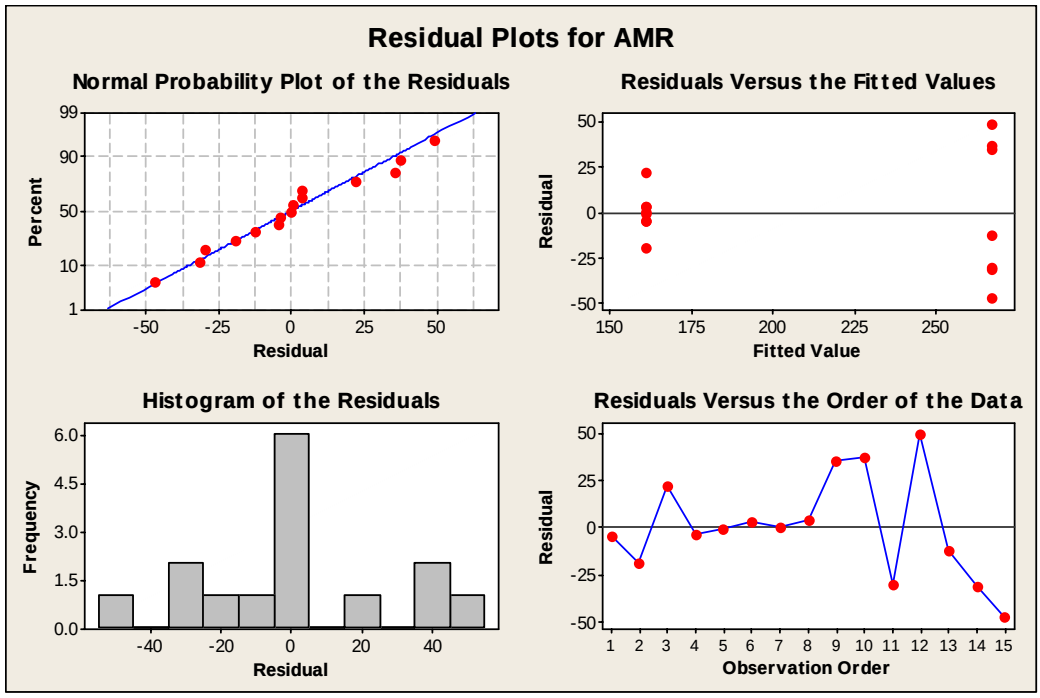


Figure 8 – Residual plots for the analysis of active metabolic rate (mg O_2 kg^{-1} hr^{-1}) of 2 species (Atlantic cod and rainbow smelt).

Evaluate the model (evaluation of the residuals) (see fig. 8)

Homogeneity – There is a strong cone, opening to the right, in the residuals versus fitted values plot, so the residuals are not homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so the residuals are taken to be independent.

Normality – In the histogram the residuals cluster around a central value in a close to symmetrical shape and the normal probability plot of the residuals shows an almost straight line, so the residuals are taken to be normal.

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	42090	42090	42090	52.93	0.000
Error	13	10337	10337	795		
Total	14	52427				

Although the assumption of homogeneity of the residuals was not met, n = 14 and the p-value obtained is quite far from α, so I'll trust the decision and accept the H_A: Var (β_{Sp}) > 0, rejecting Ho.

The AMR of rainbow smelt is significantly higher than Atlantic cod's AMR.

($F_{1,13} = 52.93$ $p < 0.001$)

Analysis 2.4 – AMR Rainbow smelt < AMR Capelin

H_A: Var (β_{Sp}) > 0 The means differ between the 2 species

Ho: Var (β_{Sp}) = 0

Evaluate the model (evaluation of the residuals) (see fig. 9)

Homogeneity – There are no strong cones in the residuals versus fitted values plot, so the residuals are taken to be homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals cluster around a central value in close to symmetrical shape and the normal probability plot of the residuals shows an almost straight line, so the residuals are taken to be normal.

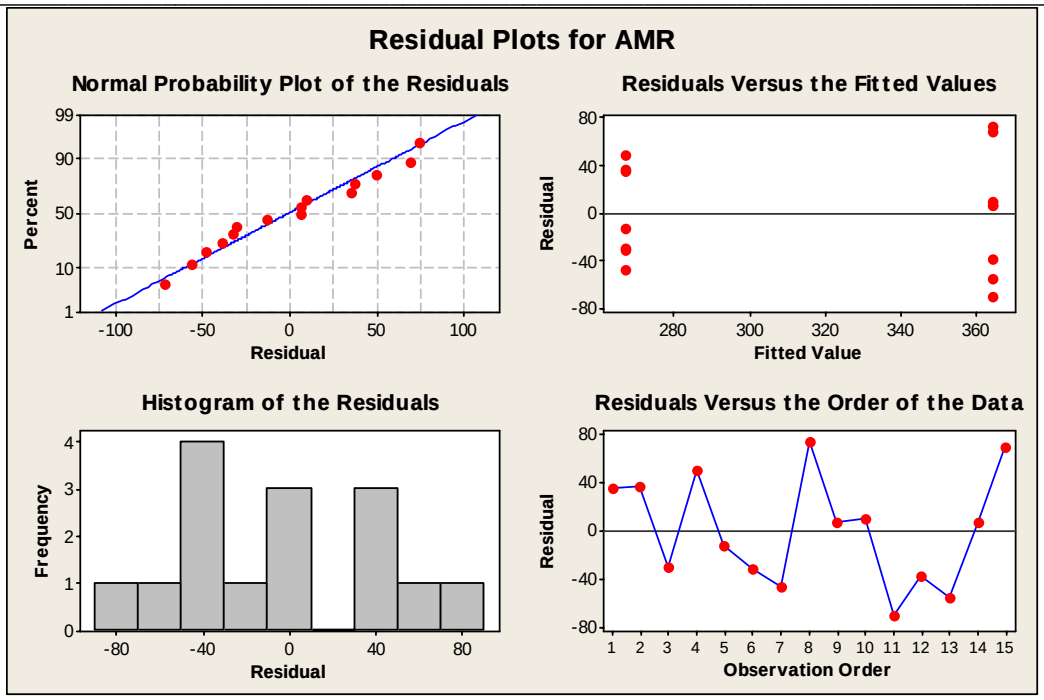


Figure 9 – Residual plots for the analysis of active metabolic rate (mg O_2 kg^{-1} hr^{-1}) of 2 species (rainbow smelt and capelin).

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	34997	34997	34997	15.38	0.002
Error	13	29572	29572	2275		
Total	14	64568				

The p-value < α (0.05) so, I accept the H_A: Var (β_{Sp}) > 0, rejecting Ho.

The AMR of capelin is significantly higher than rainbow smelt's AMR.

($F_{1,13}$ = 15.38 p = 0.002)

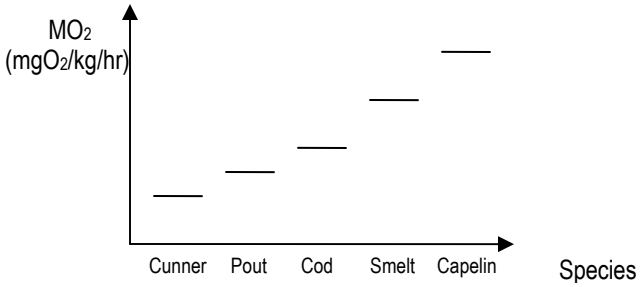
Relatively to active metabolic rate we found that:

Cunner < Ocean pout = Atlantic cod < Rainbow smelt < Capelin

Analysis 3 – Metabolic scope (MS)

Verbal model: MS depends on the species.

Graphical model:



Response variable: MO_2 – Metabolic rate (mg O_2 kg^{-1} hr^{-1}) on a ratio type scale.

Explanatory variable: Sp - Species (Sp n or Sp n+1) on a nominal type scale.

Cunner – Sp 1; Ocean pout – Sp 2; Atlantic cod – Sp 3; Rainbow smelt – Sp 4; Capelin – Sp 5

Formal model: $MO_2 = \beta_0 + \beta_{Sp} * Sp + \epsilon$

Analysis 3.1 – MS Cunner < MS Ocean pout

H_A: Var (β_{Sp}) > 0 The means differ between the 2 species

Ho: Var (β_{Sp}) = 0

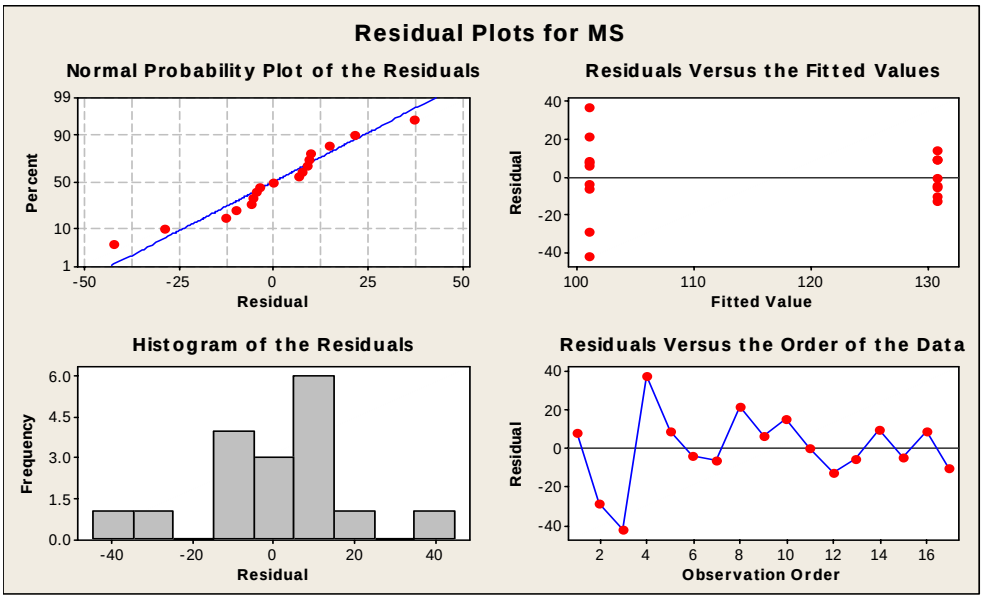


Figure 10 – Residual plots for the analysis of metabolic scope (mg O_2 kg^{-1} hr^{-1}) of 2 species (cunner and ocean pout).

Evaluate the model (evaluation of the residuals) (see fig. 9)

Homogeneity – There is a strong cone, opening to the left, in the residuals versus fitted values plot, so the residuals are not homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals cluster around a central value in a close to symmetrical shape and the normal probability plot of the residuals shows an almost straight line, so the residuals are taken to be normal.

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	3726.2	3726.2	3726.2	10.26	0.006
Error	15	5446.9	5446.9	363.1		
Total	16	9173.2				

Although the assumption of homogeneity of the residuals was not met, n = 17 and the p-value obtained is quite far from α, so I'll trust the decision and accept the H_A: Var (β_{Sp}) > 0, rejecting Ho.

The MS of ocean pout is significantly higher than cunner's MS. ($F_{1,15} = 10.26$ p = 0.006)

Analysis 3.2 – MS Ocean pout < MS Atlantic cod

H_A: Var (β_{Sp}) > 0 The means differ between the 2 species

Ho: Var (β_{Sp}) = 0

Evaluate the model (evaluation of the residuals) (see fig. 11)

Homogeneity – There are no strong cones in the residuals versus fitted values plot, so the residuals are taken to be homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals cluster around a central value in close to symmetrical shape and the normal probability plot of the residuals shows an almost straight line, so the residuals are taken to be normal.

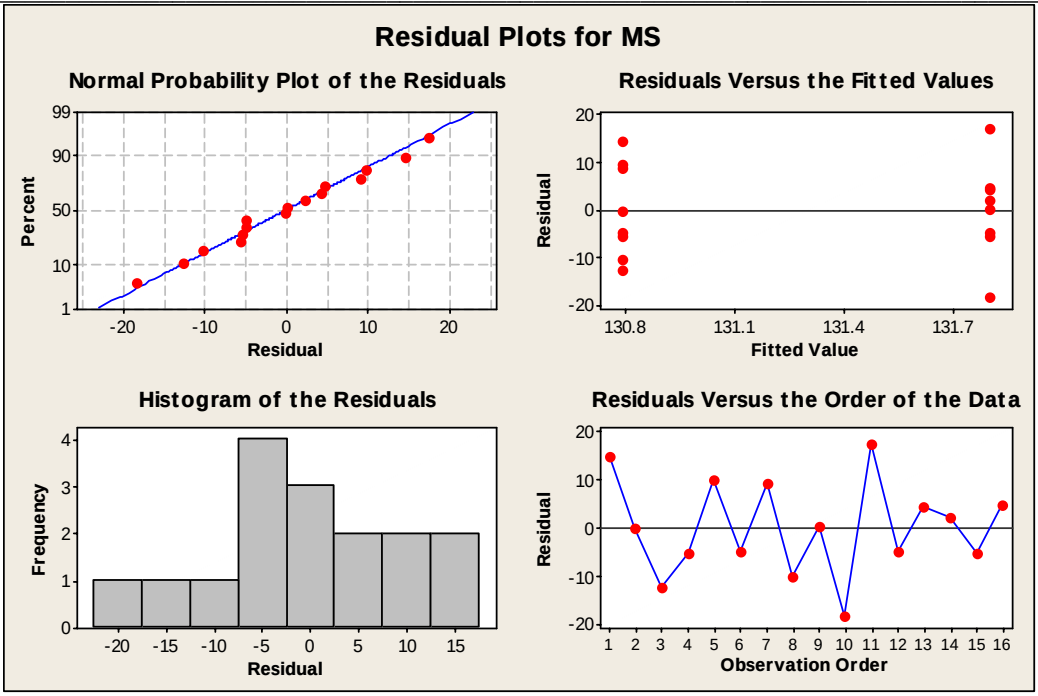


Figure 11 – Residual plots for the analysis of metabolic scope (mg O_2 kg^{-1} hr^{-1}) of 2 species (ocean pout and Atlantic cod).

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	4.1	4.1	4.1	0.04	0.846
Error	14	1449.5	1449.5	103.5		
Total	15	1453.6				

The p-value > α (0.05) so, I accept the Ho: Var (β_{Sp}) = 0, rejecting H_A.

There is no difference between the MS of ocean pout and Atlantic cod.

($F_{1,14}$ = 0.04 p = 0.846)

Analysis 3.3 – MS Atlantic cod < MS Rainbow smelt

H_A: Var (β_{Sp}) > 0 The means differ between the 2 species

Ho: Var (β_{Sp}) = 0

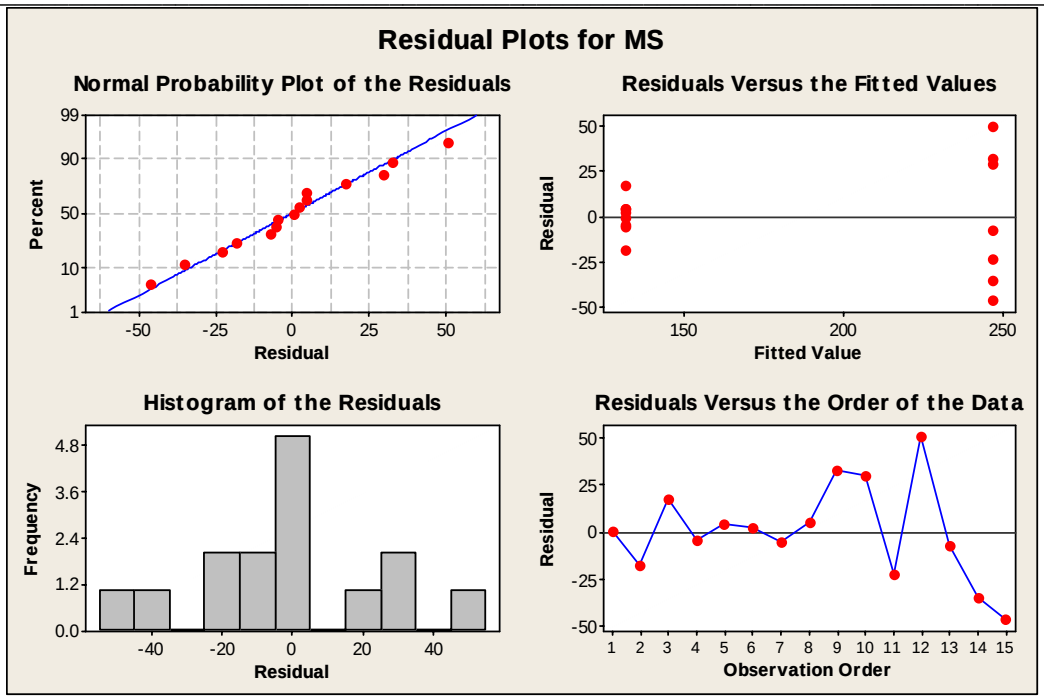


Figure 12 – Residual plots for the analysis of metabolic scope (mg O_2 kg^{-1} hr^{-1}) of 2 species (Atlantic cod and rainbow smelt).

Evaluate the model (evaluation of the residuals) (see fig. 12)

Homogeneity – There is a strong cone, opening to the right, in the residuals versus fitted values plot, so the residuals are not homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals cluster around a central value in a close to symmetrical shape and the normal probability plot of the residuals shows an almost straight line, so the residuals are taken to be normal.

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	49272	49272	49272	69.17	0.000
Error	13	9260	9260	712		
Total	14	58532				

Although the assumption of homogeneity of the residuals was not met, n = 15 and the p-value obtained is quite far from α, so I'll trust the decision and accept the H_A: Var (β_{Sp}) > 0, rejecting Ho.

The MS of rainbow smelt is significantly higher than Atlantic cod's MS.

($F_{1,14}$ = 69.17 p < 0.001)

Analysis 3.4 – MS Rainbow smelt < MS Capelin

H_A: Var (β_{Sp}) > 0 The means differ between the 2 species

Ho: Var (β_{Sp}) = 0

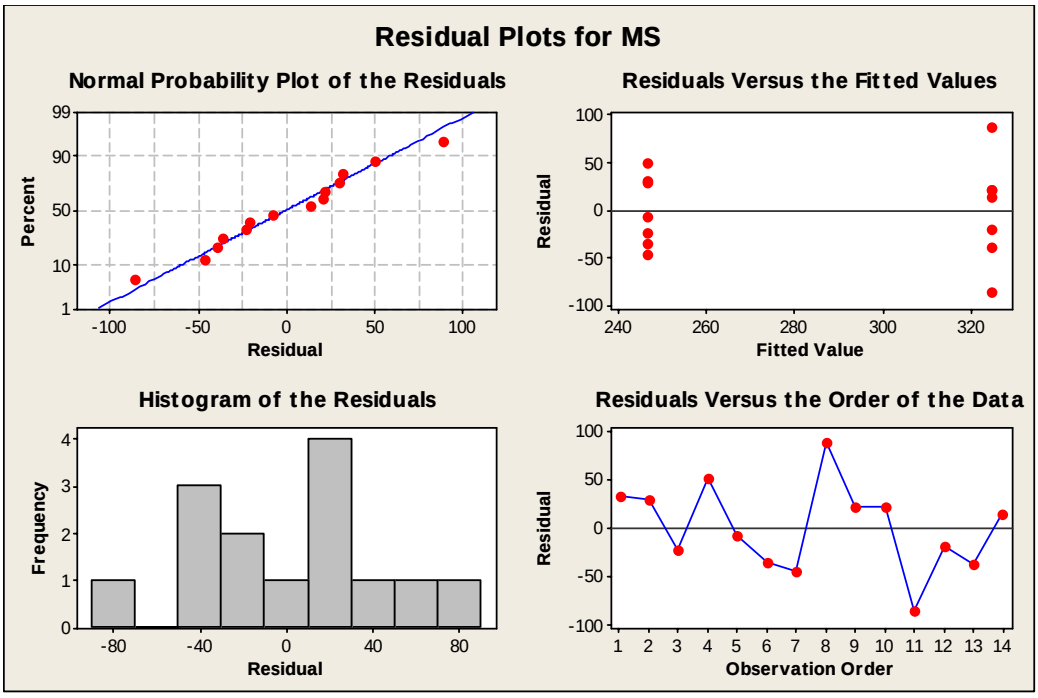


Figure 13 – Residual plots for the analysis of metabolic scope (mg O_2 kg^{-1} hr^{-1}) of 2 species (rainbow smelt and capelin).

Evaluate the model (evaluation of the residuals) (see fig. 13)

Homogeneity – There are no strong cones in the residuals versus fitted values plot, so the residuals are taken to be homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals do not cluster around a central value and normal probability plot of the residuals is s-shaped, so the residuals deviate from normality.

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	21129	21129	21129	9.46	0.010
Error	12	26809	26809	2234		
Total	13	47938				

The assumptions of normality was not met, the n = 14 and the p-value (0.01) is close to α (0.05) so, I'll re-compute a p-value free of assumptions by randomization.

Re-compute the p-value

After 2000 randomizations, 21 F values were higher than the F_{obs} = 9.46, so the new p-value is equal to 0.011 (see also fig. 14).

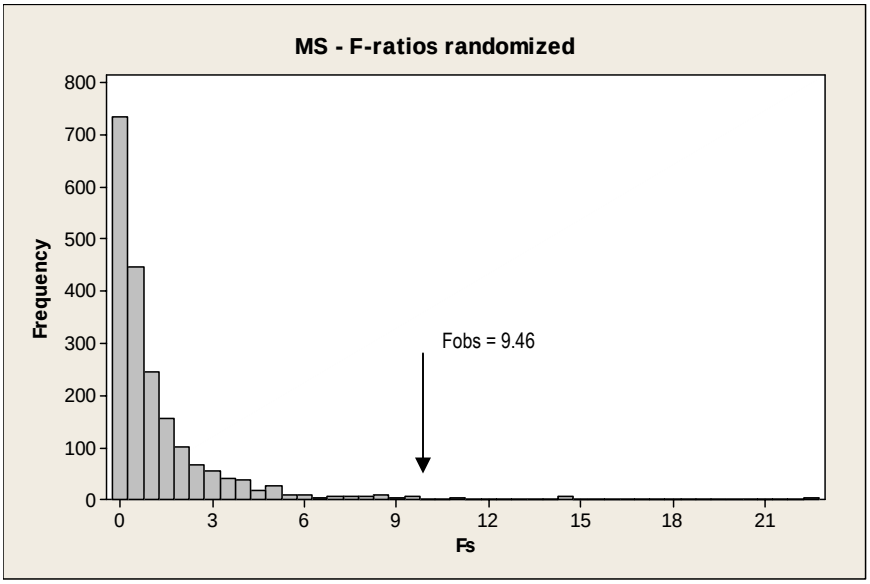


Figure 14 – Histogram of randomized F-ratios for the analysis of metabolic scope (mg O_2 kg^{-1} hr^{-1}) of 2 species (rainbow smelt and capelin).

The p-value < α (0.05) so, I accept the H_A: Var (β_{Sp}) > 0, rejecting Ho.

The MS of capelin is significantly higher than rainbow smelt's MS.

($F_{1,12}$ = 9.46 p = 0.011 by randomization)

Relatively to metabolic scope we found that:

Cunner < Ocean pout = Atlantic cod < Rainbow smelt < Capelin

Analysis of parameters in model

Table IV – Overall means (βo) of metabolic rates (resting, active and metabolic scope) for each analysis, with standard error (SE).

	Analysis	Overall mean - βo	SE
Resting metabolic rate ($mg\ O_2\ kg^{-1}\ hr^{-1}$)	1.1 - Cunner < O. pout	21.0	1.2
	1.2 - O. pout < A. cod	25.3	1.4
	1.3 - A. cod < R. smelt	25.3	1.6
	1.4 - R. smelt < Capelin	25.3	2.6
Active metabolic rate ($mg\ O_2\ kg^{-1}\ hr^{-1}$)	2.1 - Cunner < O. pout	136.0	5.8
	2.2 - O. pout < A. cod	156.6	2.9
	2.3 - A. cod < R. smelt	210.7	15.8
	2.4 - R. smelt < Capelin	310.8	16.7
Metabolic scope ($mg\ O_2\ kg^{-1}\ hr^{-1}$)	3.1 - Cunner < O. pout	115.1	5.8
	3.2 - O. pout < A. cod	131.3	2.5
	3.3 - A. cod < R. smelt	185.4	16.7
	3.4 - R. smelt < Capelin	285.5	16.2

Table V – Mean metabolic rates (resting, active and metabolic scope) of each species with standard error (SE) and 95% confidence intervals.

	Species	Mean	SE	Confidence limits	
				Lower	Upper
Resting metabolic rate ($mg\ O_2\ kg^{-1}\ hr^{-1}$)	Cunner	20.7	2.0	16.4	24.9
	Ocean pout	21.3	1.2	18.8	23.8
	Atlantic cod	29.4	1.3	26.5	32.2
	Rainbow smelt	20.7	1.9	16.4	24.9
	Capelim	29.9	4.3	20.4	39.4
Active metabolic rate ($mg\ O_2\ kg^{-1}\ hr^{-1}$)	Cunner	121.8	8.0	104.9	138.7
	Ocean pout	152.1	3.8	144.0	160.2
	Atlantic cod	161.2	4.0	152.5	169.8
	Rainbow smelt	267.3	15.0	234.7	300.0
	Capelim	354.3	18.7	313.5	395.0
Metabolic scope ($mg\ O_2\ kg^{-1}\ hr^{-1}$)	Cunner	101.1	8.1	83.9	118.3
	Ocean pout	130.8	3.6	123.1	138.4
	Atlantic cod	131.8	3.6	124.0	139.6
	Rainbow smelt	246.7	14.2	215.6	277.7
	Capelim	324.4	20.9	278.9	369.8

Confidence limits:

$$P\left[\beta_{Sp} - t_{\alpha/2\,(n-1)} * SE \leq \mu \leq \beta_{Sp} - t_{\alpha/2\,(n-1)} * SE\right] = 1-\alpha$$

Hypothesis 2

Problem: Does the metabolic rate (MO_2) depend on the level activity being performed and on species life style?

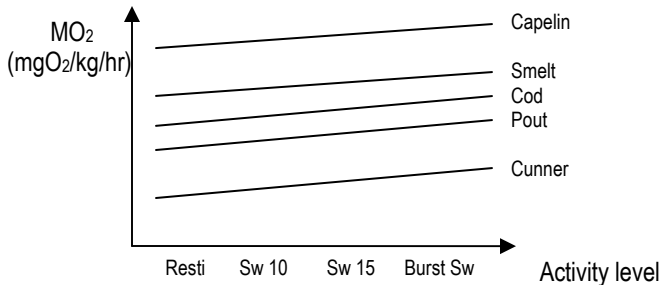
I am going to follow an *a priori* approach to analyze my data, based in the knowledge that activity tends to increase the metabolic rate and that in teleost fish, species with a more active life style tend to have higher metabolic rates than more sluggish species. Thus, based on the levels of activity used I expect that the MO_2 will behave as follow: Resting < Swimming at 10 cm/s < Swimming at 15 cm/s < Burst swimming.

My statistic will be variances and I have 2 explanatory explanatory variable, both categorical (species and activity level), therefore I'll use a 2-way ANOVA to analyze my data. I'll use the GLM to perform my analysis (2-way ANOVA). I'll use the F-statistic, F-distribution and $\alpha = 0.05$ for hypothesis testing.

Analysis 4 – MO_2 depends on activity level and species

Verbal model: MO_2 depends on activity level being performed and species.

Graphical model:



Response variable: MO2 – Metabolic rate (mg O_2 kg^{-1} hr^{-1}) on a ratio type scale.

Explanatory variables:

1) ActL – Activity level (Resting – Rest, Swimming at 10 cm/s – Sw 10, Swimming at 15 cm/s – Sw 15 or Burst swimming – Burst Sw) on a nominal type scale;

2) Sp - Species (Cunner, Ocean pout, Atlantic cod, Rainbow smelt or Capelin) on a nominal type scale.

Formal model: $MO_2 = \beta_0 + \beta_{ActL} * ActL + \beta_{Sp} * Sp + \beta_{ActL*Sp} * ActL*Sp + \epsilon$

H_A: Var ($\beta_{ActL*Sp} * ActL*Sp$) > 0 Is there variance due to the interaction term?

Ho: Var ($\beta_{ActL*Sp} * ActL*Sp$) = 0

H_A: Var (β_{ActL}) > 0 Is there variance due to the activity level?

Ho: Var (β_{ActL}) = 0

H_A: Var (β_{Sp}) > 0 Is there variance due to the species?

Ho: Var (β_{Sp}) = 0

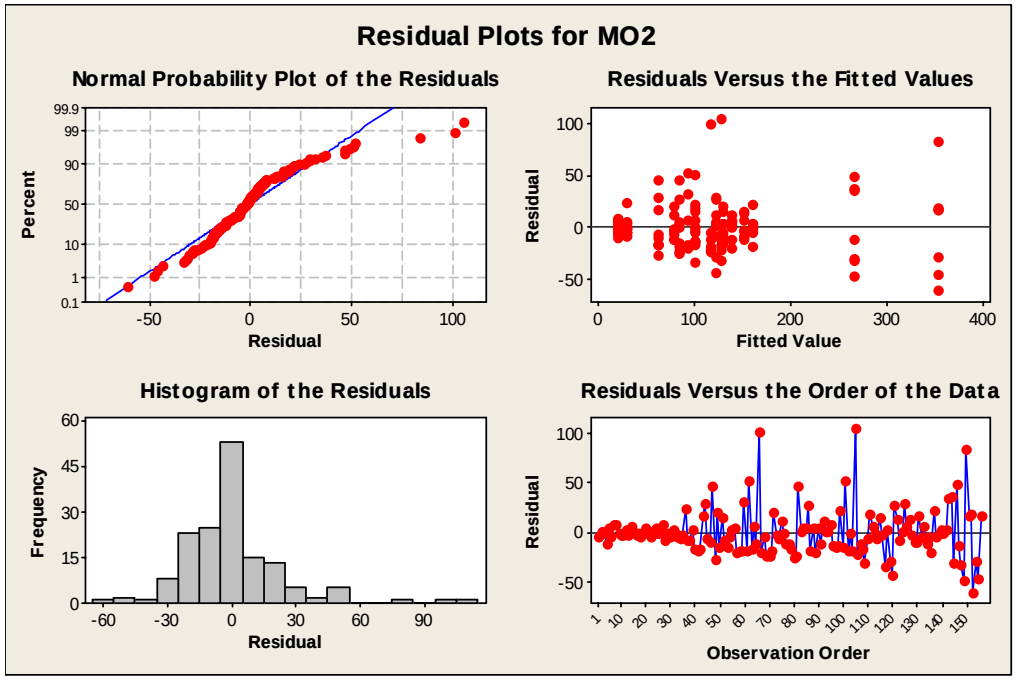


Figure 15 – Residual plots for the analysis of metabolic rate (mg O_2 kg^{-1} hr^{-1}) of 5 North Atlantic teleost species during 4 different levels of activity (Rest, Sw 10, Sw 15 and Burst Sw).

Evaluate the model (evaluation of the residuals) (see fig. 15)

Homogeneity of residuals – There is a strong cone, opening to the right in the residuals versus fitted values plot, so the residuals are somewhat heterogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals cluster around a central value in a close to symmetrical shape and the normal probability plot of the residuals shows an almost straight line, so the residuals are taken to be normal.

ANOVA Table

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Species	4	98609	98609	24652	40.98	0.000
Activity level	3	638909	687403	229134	380.88	0.000
Species*Activity level	12	225902	225902	18825	31.29	0.000
Error	136	81817	81817	602		
Total	155	1045237				

Although the assumption of homogeneity of the residuals was not met, N is quite large (156) and the p-value obtained is far from α, so it is unlikely that the decision will change with p-value re-computed by randomization. Therefore I will trust the decision.

Interaction term

The p-value < α (0.05) so, I accept the H_A: Var ($\beta_{ActL*Sp}$ * ActL*Sp) > 0, rejecting Ho.

The interaction term is significant; we cannot proceed and interpret main effects. We cannot interpret one main effect independent of another. ($F_{12,136} = 31.29$ p < 0.001)

I'll have to analyze the effect of activity level on the MO_2 separately for each species. I'll follow the same type of analysis used in the analysis of hypothesis 1. For each species I'll have 3 H_A/Ho pairs, and I'll analyze each one with a t-test (one-way ANOVA).

H_A: Var (β_{ActL}) > 0, the means differ between the 2 activity levels ($\mu_{ActL\ n} \neq \mu_{ActL\ n+1}$)

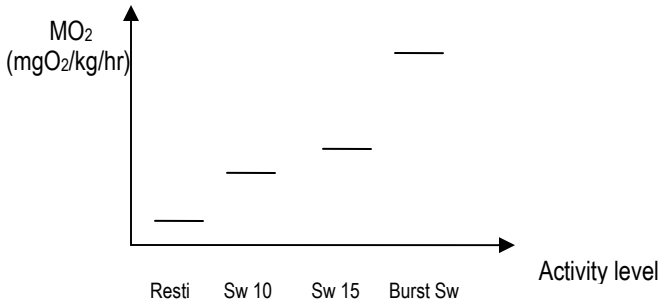
Ho: Var (β_{ActL}) = 0 ($\mu_{ActL\ n} = \mu_{ActL\ n+1}$), for the 4 activity levels tested (Resting – ActL 1; Sw 10 – ActL 2; Sw 15 – ActL 3; Burst Sw – ActL 4).

I'll use the GLM to perform my analysis and the F-statistic, F-distribution and α = 0.05 for hypothesis testing.

Analysis 5 – MO_2 of species X depends on activity level

Verbal model: MO_2 depends on activity level being performed.

Graphical model:



Response variable: MO2 – Metabolic rate (mg O_2 kg^{-1} hr^{-1}) on a ratio type scale.

Explanatory variable: ActL – Activity level (ActL n or ActL n+1) on a nominal type scale.

Resting – ActL 1; Sw 10 – ActL 2; Sw 15 – ActL 3; Burst Sw – ActL 4

Formal model: $MO_2 = \beta_0 + \beta_{ActL} * ActL + \epsilon$

Analysis 5.1 – MO_2 of Cunner

H_A: Var (β_{ActL}) > 0 the means differ between the 2 activity levels ($\mu_{ActL\ n} \neq \mu_{ActL\ n+1}$)

Ho: Var (β_{ActL}) = 0 ($\mu_{ActL\ n} = \mu_{ActL\ n+1}$)

Analysis 5.1.1 – Rest < Sw 10

Evaluate the model (evaluation of the residuals) (see fig. 16)

Homogeneity of residuals – There is a strong cone, opening to the left, in the residuals versus fitted values plot, so the residuals are not homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals cluster around a central value in a close to symmetrical shape and the normal probability plot of the residuals shows an almost straight line, so the residuals are taken to be normal.

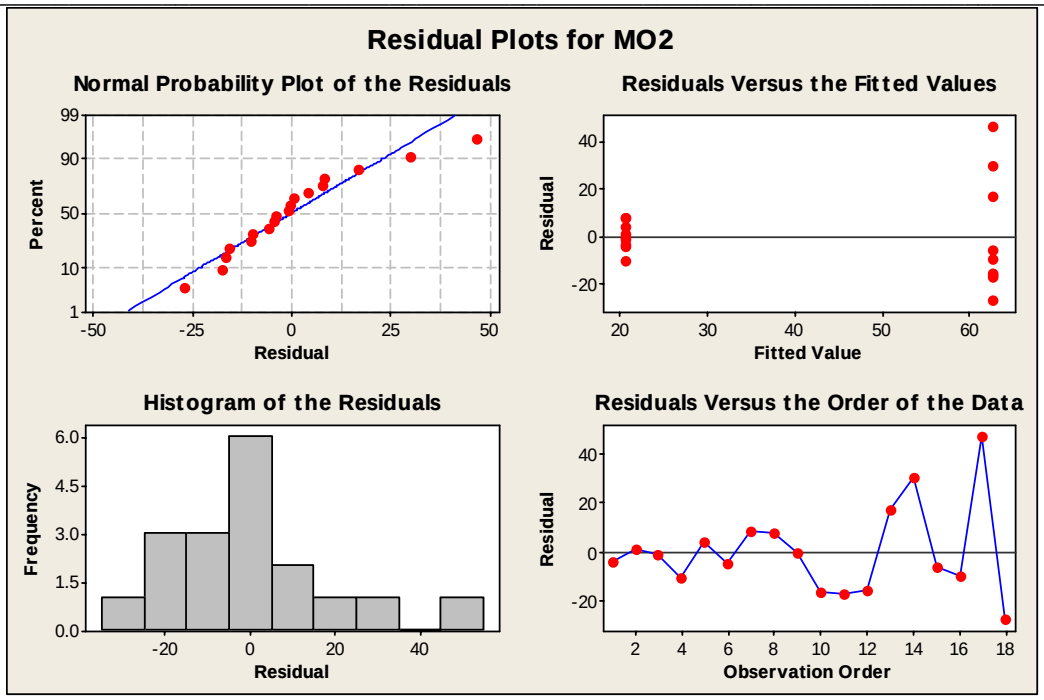


Figure 16 – Residual plots for the analysis of metabolic rate (mg O_2 kg^{-1} hr^{-1}) of cunner during 2 different levels of activity (Rest < Sw 10).

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	7923.7	7923.7	7923.7	23.47	0.000
Error	16	5402.3	5402.3	337.6		
Total	17	13326.1				

Although the assumption of homogeneity of the residuals was not met, n = 18 and the p-value obtained is quite far from α, so I'll trust the decision and accept the H_A: Var (β_{ActL}) > 0, rejecting Ho.

The MO_2 of cunner when it is Sw 10 is significantly higher than when at rest.

($F_{1,16}$ = 23.47 p < 0.001)

Analysis 5.1.2 – Sw 10 < Sw 15

Evaluate the model (evaluation of the residuals) (see fig. 17)

Homogeneity – There are no strong cones in the residuals versus fitted values plot, so the residuals are taken to be homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals do not cluster around a central value and normal probability plot of the residuals is s-shaped, so the residuals deviate from normality.

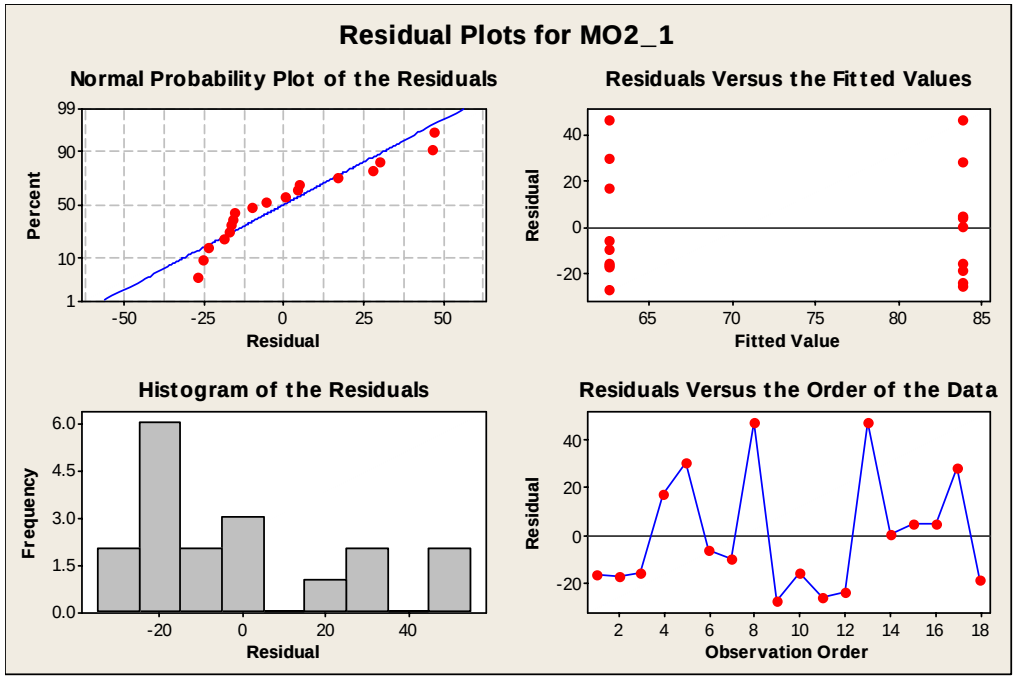


Figure 17 – Residual plots for the analysis of metabolic rate (mg O_2 kg^{-1} hr^{-1}) of cunner during 2 different levels of activity (Sw 10 < Sw 15).

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	2035.4	2035.4	2035.4	3.26	0.090
Error	16	9979.6	9979.6	623.7		
Total	17	12015.0				

The assumption of normality was not met, the n = 18 and the p-value (0.09) is close to α (0.05) so, I'll re-compute a p-value free of assumptions by randomization.

Re-compute the p-value

After 2000 randomizations, 181 F values were higher than the F_{obs} = 3.26, so the new p-value is equal to 0.091 (see also fig. 18).

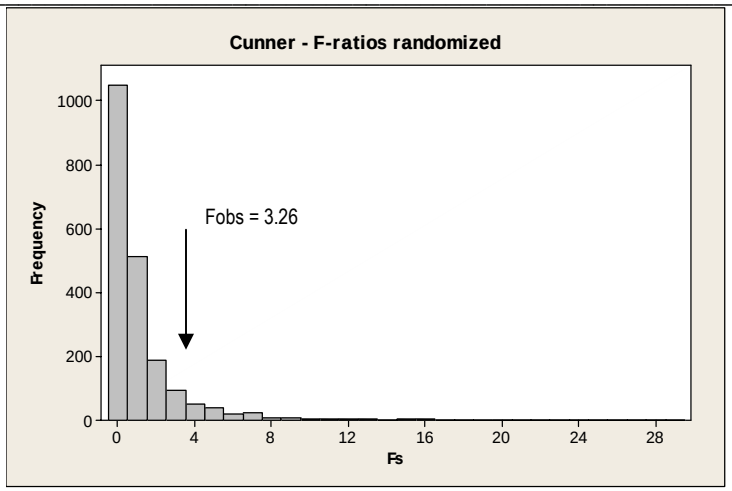


Figure 18 - Histogram of randomized F-ratios for the analysis of metabolic rate (mg O_2 kg^{-1} hr^{-1}) of cunner during 2 different levels of activity (Sw 10 < Sw15).

The p-value > α (0.05) so, I accept the Ho: Var (β_{ActL}) = 0, rejecting H_A.

There is no difference in the MO_2 of cunner while swimming at 10 or 15 cm/s.

($F_{1,16}$ = 3.26 p = 0.091 by randomization)

Analysis 5.1.3 – Sw 15 < Burst Sw

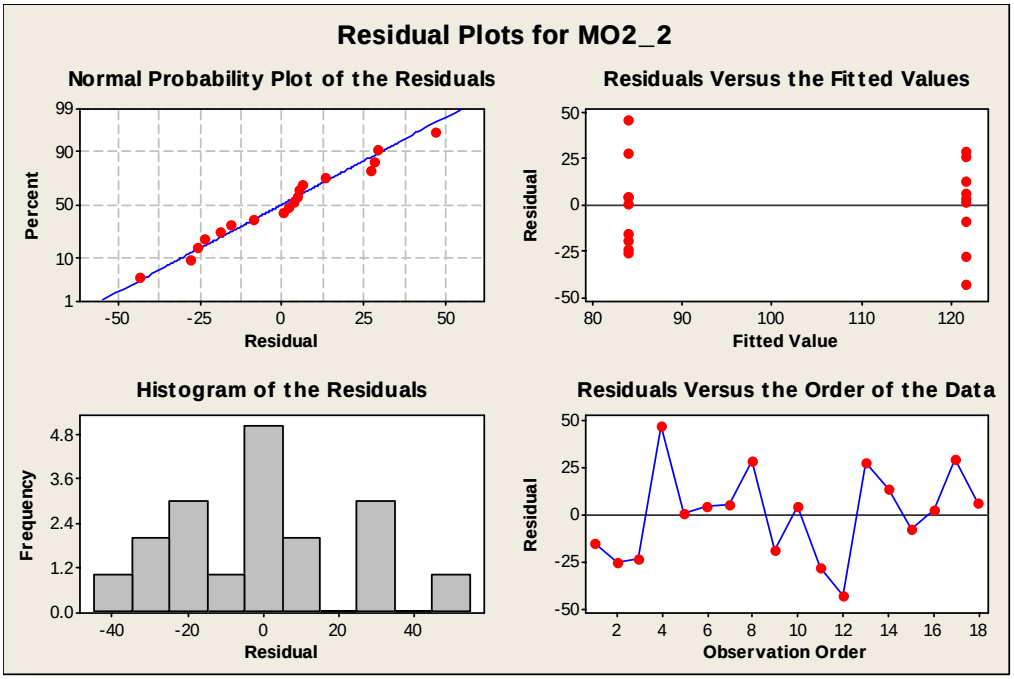


Figure 19 – Residual plots for the analysis of metabolic rate (mg O_2 kg^{-1} hr^{-1}) of cunner during 2 different levels of activity (Sw 15 < Burst Sw).

Evaluate the model (evaluation of the residuals) (see fig. 19)

Homogeneity – There are no strong cones in the residuals versus fitted values plot, so the residuals are taken to be homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals cluster around a central value in close to symmetrical shape and the normal probability plot of the residuals shows an almost straight line, so the residuals are taken to be normal.

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	6464.6	6464.6	6464.6	10.97	0.004
Error	16	9432.2	9432.2	589.5		
Total	17	15896.8				

The p-value < α (0.05) so, I accept the H_A: Var (β_{ActL}) > 0, rejecting Ho.

The MO_2 of cunner when it is burst Sw is significantly higher than when swimming at 15 cm/s.

($F_{1,16}$ = 10.97 p = 0.004)

Relatively to MO_2 in cunner we found that:

Resting < Swimming at 10 cm/s = Swimming at 15 cm/s < Burst swimming

Analysis 5.2 – MO_2 of Ocean pout

H_A: Var (β_{ActL}) > 0 the means differ between the 2 activity levels ($\mu_{ActL\ n} \neq \mu_{ActL\ n+1}$)

Ho: Var (β_{ActL}) = 0 ($\mu_{ActL\ n} = \mu_{ActL\ n+1}$)

Analysis 5.2.1 – Rest < Sw 10

Evaluate the model (evaluation of the residuals) (see fig. 20)

Homogeneity of residuals – There is a strong cone, opening to the right, in the residuals versus fitted values plot, so the residuals are not homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals cluster around a central value in a close to symmetrical shape and the normal probability plot of the residuals shows an almost straight line, so the residuals are taken to be normal.

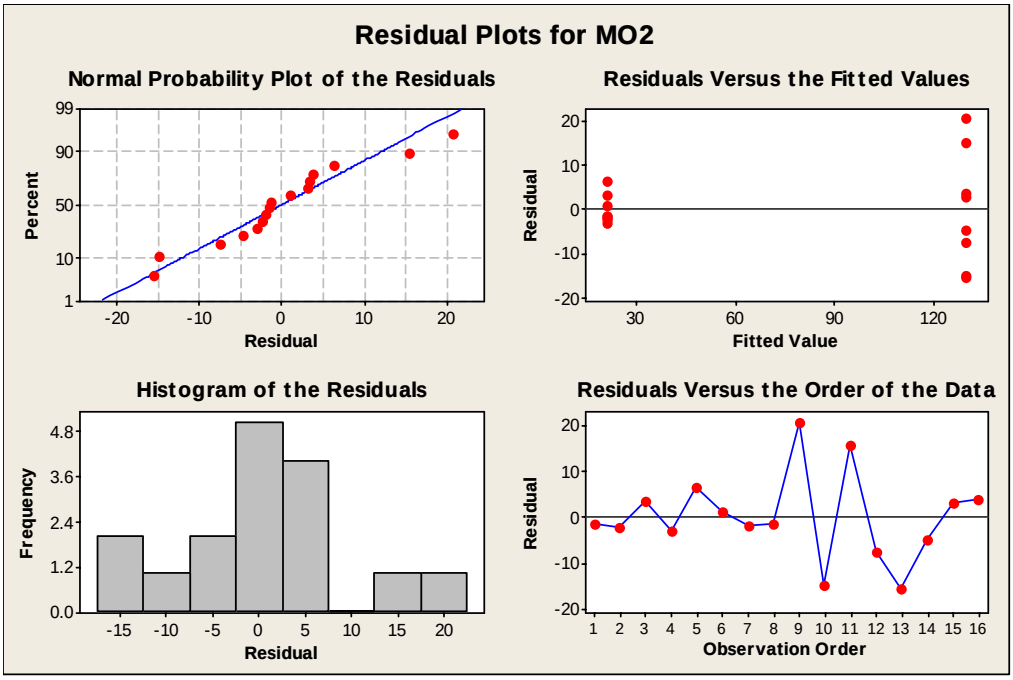


Figure 20 – Residual plots for the analysis of metabolic rate (mg O_2 kg^{-1} hr^{-1}) of ocean pout during 2 different levels of activity (Rest < Sw 10).

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	46951	46951	46951	498.91	0.000
Error	14	1318	1318	94		
Total	15	48269				

Although the assumption of homogeneity of the residuals was not met, n = 16 and the p-value obtained is quite far from α, so I'll trust the decision and accept the H_A: Var (β_{ActL}) > 0, rejecting Ho.

The MO_2 of ocean pout when it is Sw 10 is significantly higher than when at rest.

($F_{1,14}$ = 498.91 p < 0.001)

Analysis 5.2.2 – Sw 10 < Sw 15

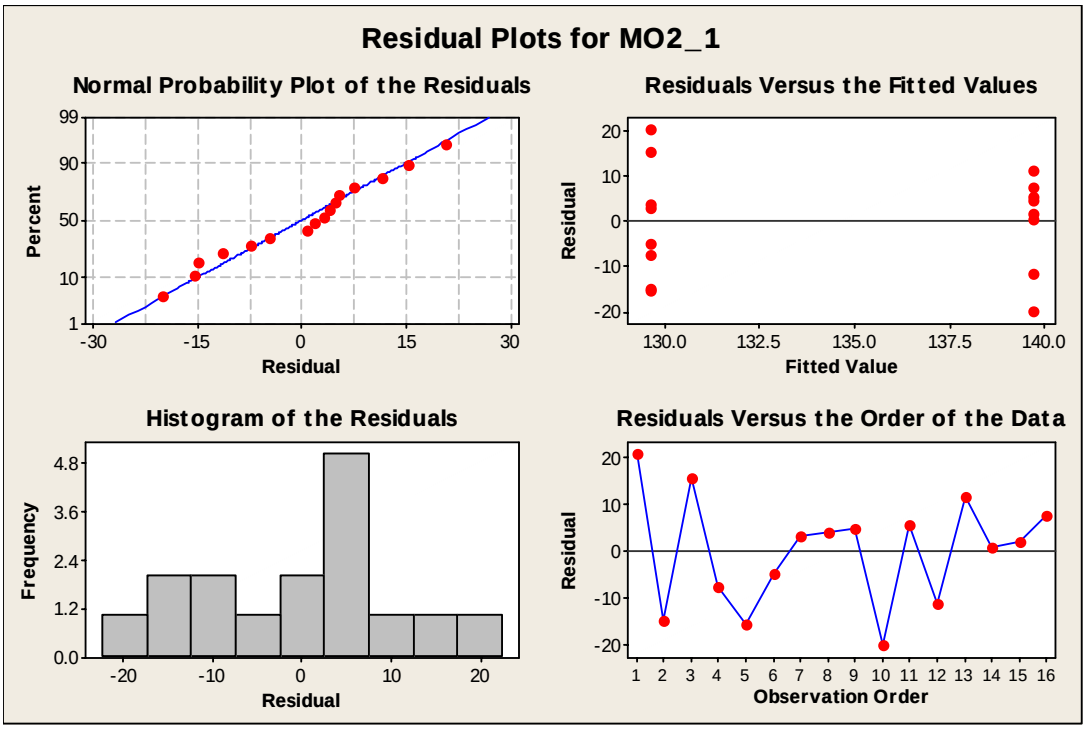


Figure 21 – Residual plots for the analysis of metabolic rate (mg O_2 kg^{-1} hr^{-1}) of ocean pout during 2 different levels of activity (Sw 10 < Sw 15).

Evaluate the model (evaluation of the residuals) (see fig. 21)

Homogeneity – There are no strong cones in the residuals versus fitted values plot, so the residuals are taken to be homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals do not cluster around a central value and normal probability plot of the residuals is s-shaped, so the residuals deviate from normality.

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	403.8	403.8	403.8	2.80	0.116
Error	14	2017.9	2017.9	144.1		
Total	15	2421.7				

Although the assumption of normality of the residuals was not met, n = 16 and the p-value obtained is quite far from α, so I'll trust the decision and accept the Ho: Var (β_{ActL}) = 0, rejecting H_A.

There is no difference in the MO_2 of ocean pout while swimming at 10 or 15 cm/s.

($F_{1,14}$ = 2.8 p = 0.116)

Analysis 5.2.3 – Sw 15 < Burst Sw

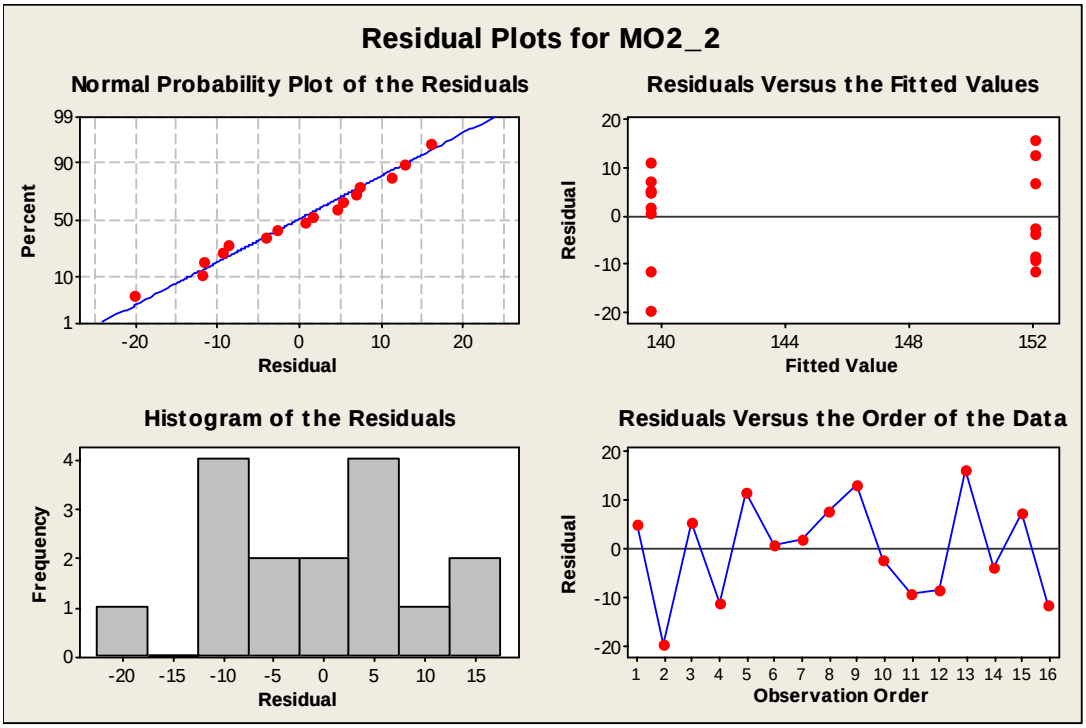


Figure 22 – Residual plots for the analysis of metabolic rate (mg O_2 kg^{-1} hr^{-1}) of ocean pout during 2 different levels of activity (Sw 15 < Burst Sw).

Evaluate the model (evaluation of the residuals) (see fig. 22)

Homogeneity – There are no strong cones in the residuals versus fitted values plot, so the residuals are taken to be homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals do not cluster around a central value and normal probability plot is not a straight line, so the residuals deviate from normality.

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	615.5	615.5	615.5	5.46	0.035
Error	14	1579.1	1579.1	112.8		
Total	15	2194.7				

The assumption of normality was not met, the n = 16 and the p-value (0.035) is close to α (0.05) so, I'll re-compute a p-value free of assumptions by randomization.

Re-compute the p-value

After 2000 randomizations, 79 F values were higher than the F_{obs} = 5.46, so the new p-value is equal to 0.039 (see also fig 23).

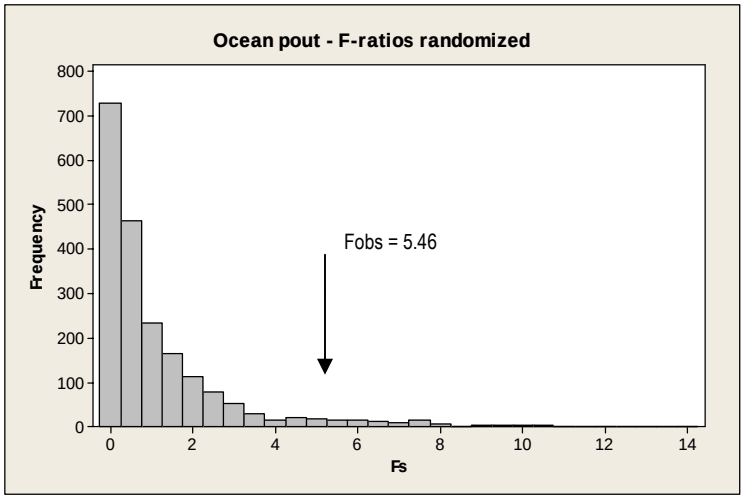


Figure 23 - Histogram of randomized F-ratios for the analysis of metabolic rate (mg O_2 kg^{-1} hr^{-1}) of ocean pout during 2 different levels of activity (Sw 15 < Burst Sw).

The p-value < α (0.05) so, I accept the H_A: Var (β_{ActL}) > 0, rejecting Ho.

The MO_2 of ocean pout when it is Burst Sw is significantly higher than when Sw 15.

($F_{1,14}$ = 5.46 p = 0.039 by randomization)

Relatively to MO_2 in ocean pout we found that:

Resting < Swimming at 10 cm/s = Swimming at 15 cm/s < Burst swimming

Analysis 5.3 – MO_2 of Atlantic cod

H_A: Var (β_{ActL}) > 0 the means differ between the 2 activity levels ($\mu_{ActL\ n} \neq \mu_{ActL\ n+1}$)

Ho: Var (β_{ActL}) = 0 ($\mu_{ActL\ n} = \mu_{ActL\ n+1}$)

Analysis 5.3.1 – Rest < Sw 10

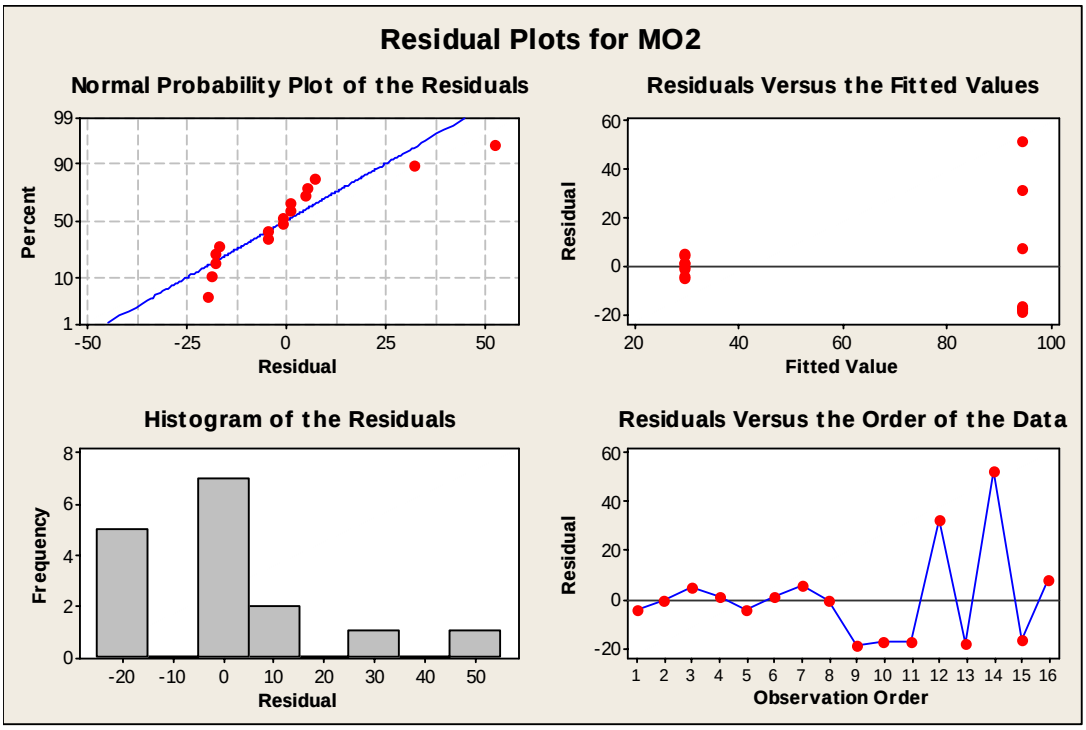


Figure 24 – Residual plots for the analysis of metabolic rate (mg O_2 kg^{-1} hr^{-1}) of Atlantic cod during 2 different levels of activity (Rest < Sw 10).

Evaluate the model (evaluation of the residuals) (see fig. 24)

Homogeneity of residuals – There is a strong cone, opening to the right, in the residuals versus fitted values plot, so the residuals are not homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals do not cluster around a central value and normal probability plot is not a straight line, so the residuals deviate from normality.

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	16864	16864	16864	42.33	0.000
Error	14	5577	5577	398		
Total	15	22441				

Although the assumptions of homogeneity and normality of the residuals were not met, n = 16 and the p-value obtained is quite far from α, so I'll trust the decision and accept the H_A: Var (β_{ActL}) > 0, rejecting Ho.

The MO_2 of Atlantic cod when it is Sw 10 is significantly higher than when at rest.

($F_{1,14} = 42.33$ $p < 0.001$)

Analysis 5.3.2 – Sw 10 < Sw 15

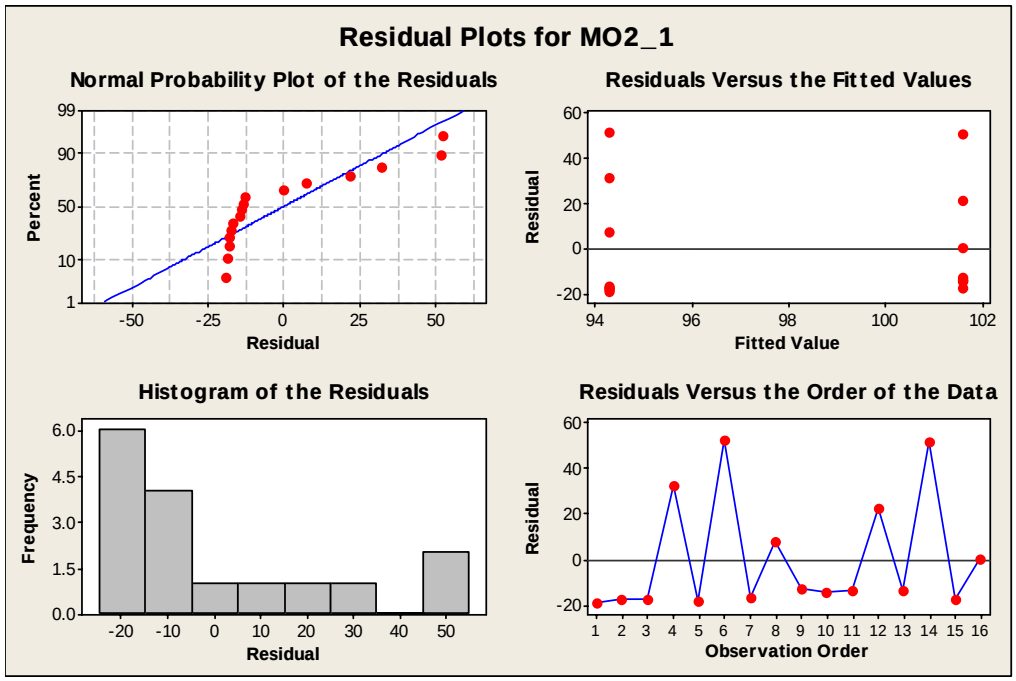


Figure 25 – Residual plots for the analysis of metabolic rate (mg O_2 kg^{-1} hr^{-1}) of Atlantic cod during 2 different levels of activity (Sw 10 < Sw 15).

Evaluate the model (evaluation of the residuals) (see fig. 25)

Homogeneity – There are no strong cones in the residuals versus fitted values plot, so the residuals are taken to be homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals do not cluster around a central value and normal probability plot of the residuals is s-shaped, so the residuals deviate from normality.

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	213.2	213.2	213.2	0.31	0.588
Error	14	9705.2	9705.2	693.2		
Total	15	9918.4				

Although the assumption of normality of the residuals was not met, n = 16 and the p-value obtained is quite far from α, so I'll trust the decision and accept the Ho: Var (β_{ActL}) = 0, rejecting H_A.

There is no difference in the MO_2 of Atlantic cod while swimming at 10 or 15 cm/s.

($F_{1,14}$ = 0.31 p = 0.588)

Analysis 5.3.3 – Sw 15 < Burst Sw

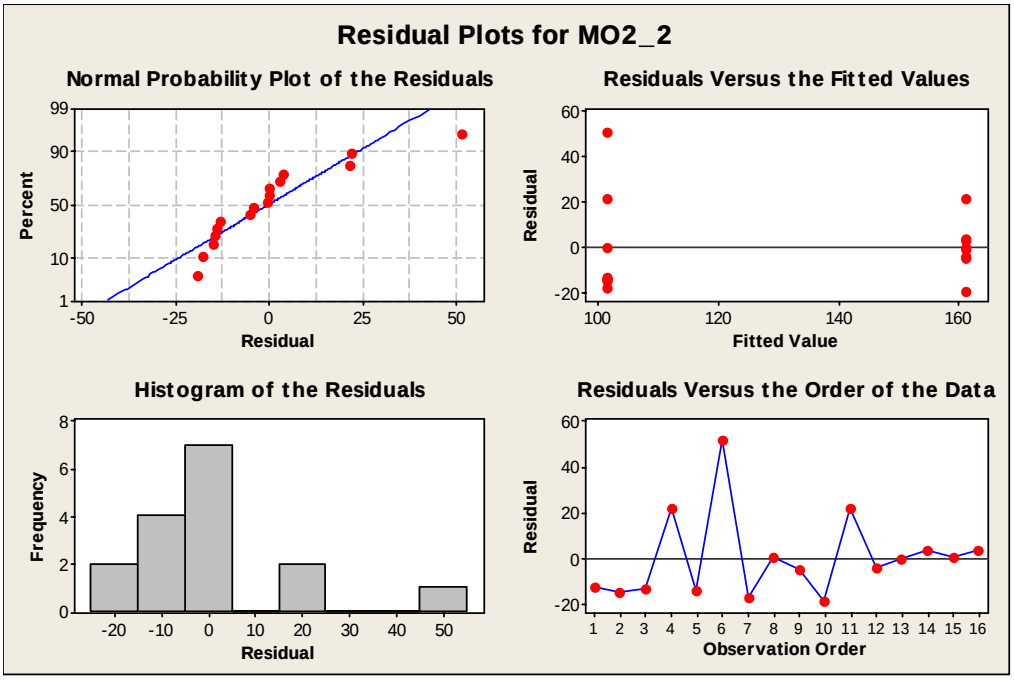


Figure 26 – Residual plots for the analysis of metabolic rate (mg O_2 kg^{-1} hr^{-1}) of Atlantic cod during 2 different levels of activity (Sw 15 < Burst Sw).

Evaluate the model (evaluation of the residuals) (see fig. 26)

Homogeneity – There are no strong cones in the residuals versus fitted values plot, so the residuals are taken to be homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals cluster around a central value but the normal probability plot of the residuals there is several deviations from the straight line, so the residuals deviate from normality.

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	14194	14194	14194	38.66	0.000
Error	14	5140	5140	367		
Total	15	19334				

Although the assumption of normality of the residuals was not met, n = 16 and the p-value obtained is quite far from α, so I'll trust the decision and accept the H_A: Var (β_{ActL}) > 0, rejecting Ho.

The MO_2 of Atlantic cod when it is burst Sw is significantly higher than when Sw 15.

($F_{1,14} = 38.66$ $p < 0.001$)

Relatively to MO_2 in Atlantic cod we found that:

Resting < Swimming at 10 cm/s = Swimming at 15 cm/s < Burst swimming

Analysis 5.4 – MO_2 of rainbow smelt

H_A: Var (β_{ActL}) > 0 the means differ between the 2 activity levels ($\mu_{ActL\ n} \neq \mu_{ActL\ n+1}$)

Ho: Var (β_{ActL}) = 0 ($\mu_{ActL\ n} = \mu_{ActL\ n+1}$)

Analysis 5.4.1 – Rest < Sw 10

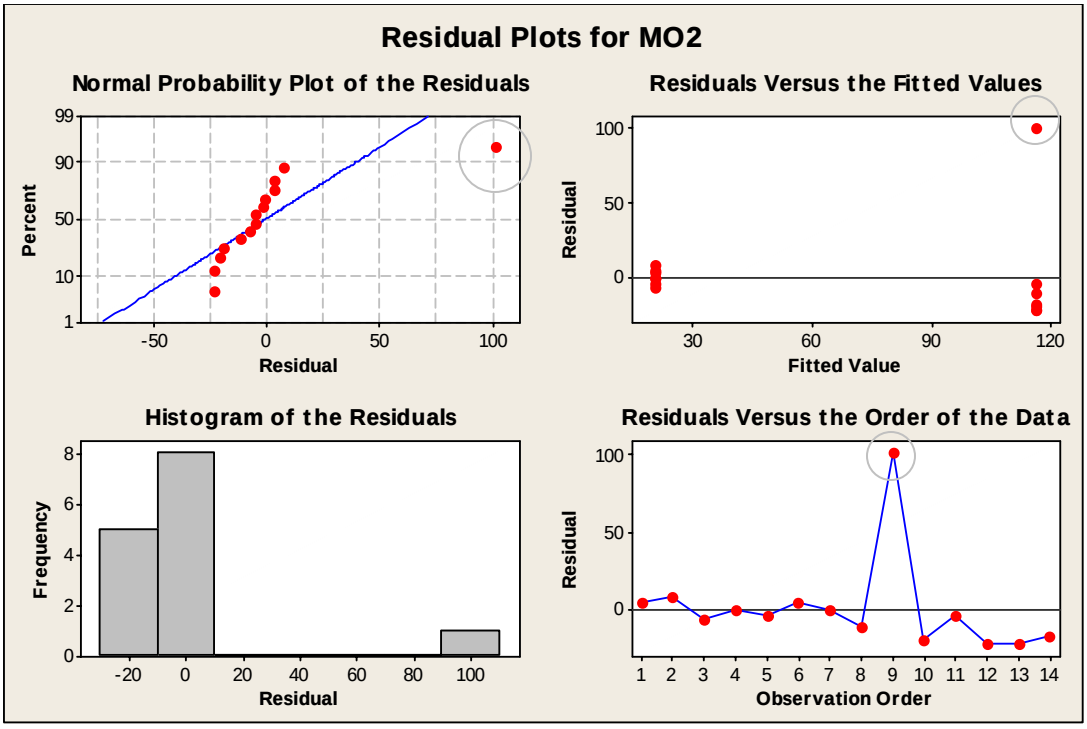


Figure 27 – Residual plots for the analysis of metabolic rate (mg O_2 kg^{-1} hr^{-1}) of rainbow smelt during 2 different levels of activity (Rest < Sw 10).

Evaluate the model (evaluation of the residuals) (see fig. 27)

Homogeneity of residuals – There is a strong cone (opening to the right) in the residuals versus fitted values plot, due mostly to a single outlier. Therefore, the residuals are not homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals do not cluster around a central value and normal probability plot is not a straight line, due to one outlier, so the residuals deviate from normality.

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	31989	31989	31989	31.02	0.000
Error	12	12376	12376	1031		
Total	13	44365				

Although the assumptions of homogeneity and normality of the residuals were not met, n = 14 and the p-value obtained is quite far from α, so I'll trust the decision and accept the H_A: Var (β_{ActL}) > 0, rejecting Ho.

The MO_2 of rainbow smelt when it is Sw 10 is significantly higher than when at rest.

($F_{1,12} = 31.02$ $p < 0.001$)

Analysis 5.4.2 – Sw 10 < Sw 15

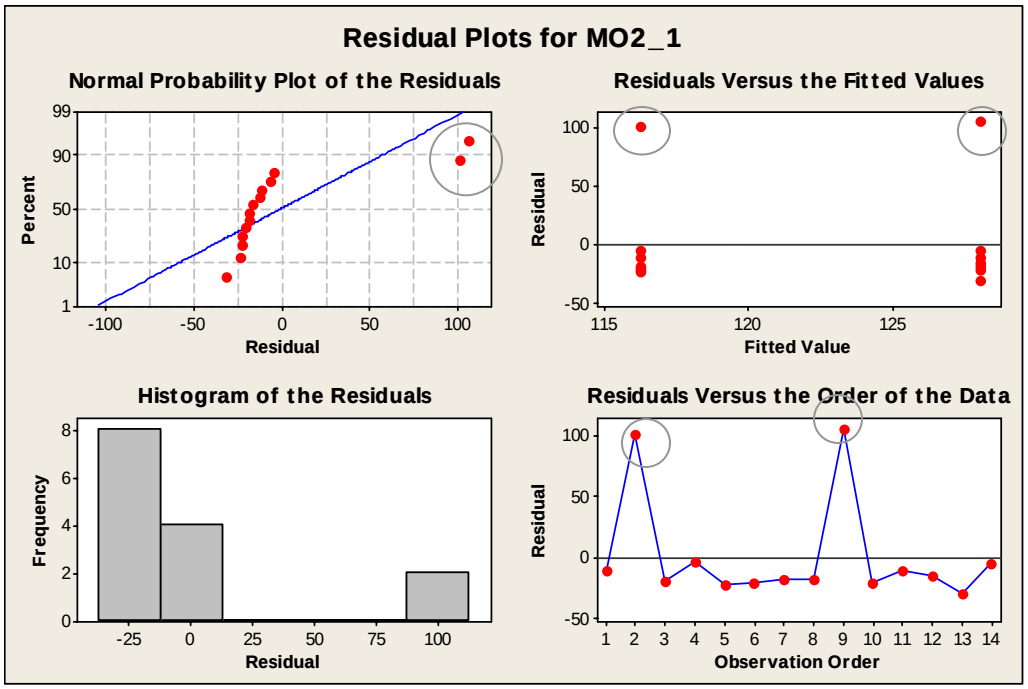


Figure 28 – Residual plots for the analysis of metabolic rate (mg O_2 kg^{-1} hr^{-1}) of rainbow smelt during 2 different levels of activity (Sw 10 < Sw 15).

Evaluate the model (evaluation of the residuals) (see fig. 28)

Homogeneity – There are no strong cones in the residuals versus fitted values plot, so the residuals are taken to be homogeneous. There are 2 outliers, one in each group.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals cluster around a central value but the normal probability plot of the residuals is s-shaped due to the presence of the 2 outliers mentioned before, so the residuals deviate from normality.

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	485	485	485	0.23	0.643
Error	12	25653	25653	2138		
Total	13	26137				

Although the assumption of normality of the residuals was not met, n = 14 and the p-value obtained is quite far from α, so I'll trust the decision and accept the Ho: Var (β_{ActL}) = 0, rejecting H_A.

There is no difference in the MO_2 of rainbow smelt while swimming at 10 or 15 cm/s.

($F_{1,12} = 0.23$ $p = 0.643$)

Analysis 5.4.3 – Sw 15 < Burst Sw

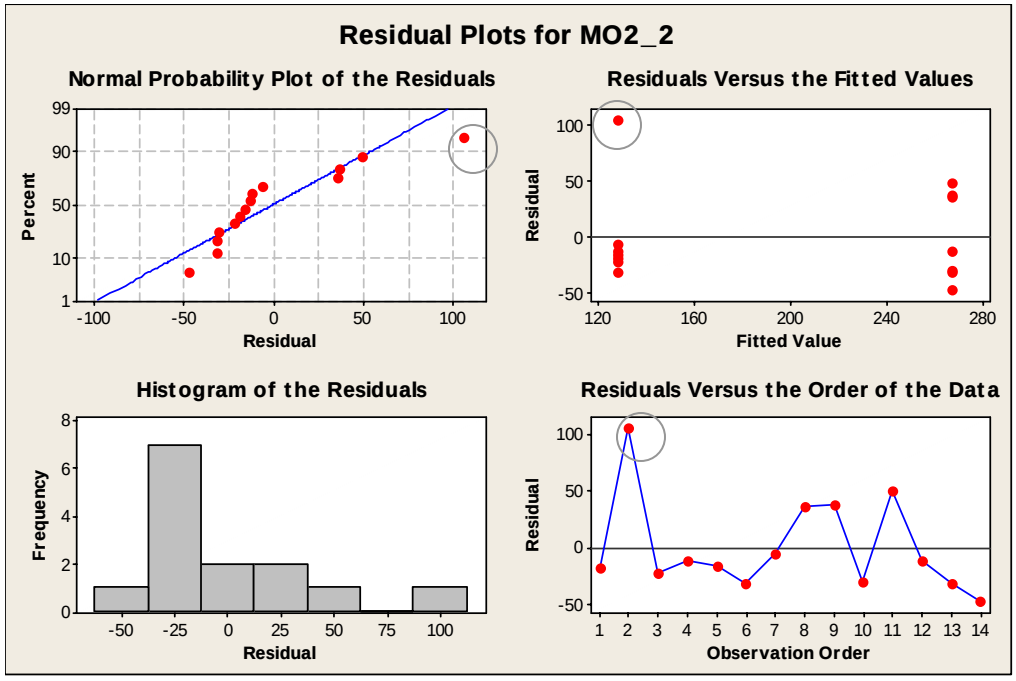


Figure 29 – Residual plots for the analysis of metabolic rate (mg O_2 kg^{-1} hr^{-1}) of rainbow smelt during 2 different levels of activity (Sw 15 < Burst Sw).

Evaluate the model (evaluation of the residuals) (see fig. 29)

Homogeneity – There are no strong cones in the residuals versus fitted values plot due to the presence of one outlier, so the residuals have some problems with homogeneity.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals do not cluster around a central value and the normal probability plot of the residuals is s-shaped, so the residuals deviate from normality.

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	67930	67930	67930	35.66	0.000
Error	12	22859	22859	1905		
Total	13	90789				

Although the assumptions of homogeneity and normality of the residuals were not met, n = 14 and the p-value obtained is quite far from α, so I'll trust the decision and accept the H_A: Var (β_{ActL}) > 0, rejecting Ho.

The MO_2 of rainbow smelt when it is burst Sw is significantly higher than when Sw 15.

($F_{1,12} = 35.66$ $p < 0.001$)

Relatively to MO_2 in rainbow smelt we found that:

Resting < Swimming at 10 cm/s = Swimming at 15 cm/s < Burst swimming

Analysis 5.5 – MO_2 of capelin

H_A: Var (β_{ActL}) > 0 the means differ between the 2 activity levels ($\mu_{ActL\ n} \neq \mu_{ActL\ n+1}$)

Ho: Var (β_{ActL}) = 0 ($\mu_{ActL\ n} = \mu_{ActL\ n+1}$)

Analysis 5.5.1 – Rest < Sw 10

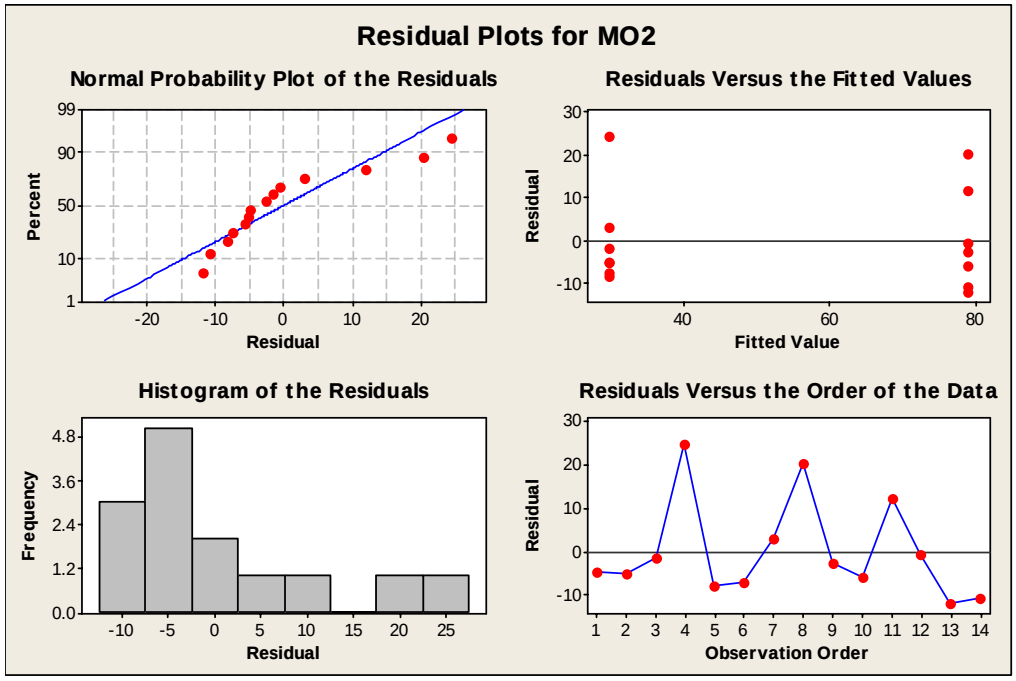


Figure 30 – Residual plots for the analysis of metabolic rate (mg O_2 kg^{-1} hr^{-1}) of capelin during 2 different levels of activity (Rest < Sw 10).

Evaluate the model (evaluation of the residuals) (see fig. 30)

Homogeneity – There are no strong cones in the residuals versus fitted values plot, so the residuals are taken to be homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals do not cluster around a central value and the normal probability plot of the residuals is s-shaped, so the residuals deviate from normality.

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	8403.5	8403.5	8403.5	60.89	0.000
Error	12	1656.1	1656.1	138.0		
Total	13	10059.6				

Although the assumption of normality of the residuals was not met, n = 14 and the p-value obtained is quite far from α, so I'll trust the decision and accept the H_A: Var (β_{ActL}) > 0, rejecting Ho.

The MO_2 of capelin when it is Sw 10 is significantly higher than when at rest.

($F_{1,12} = 60.89$ $p < 0.001$)

Analysis 5.5.2 – Sw 10 < Sw 15

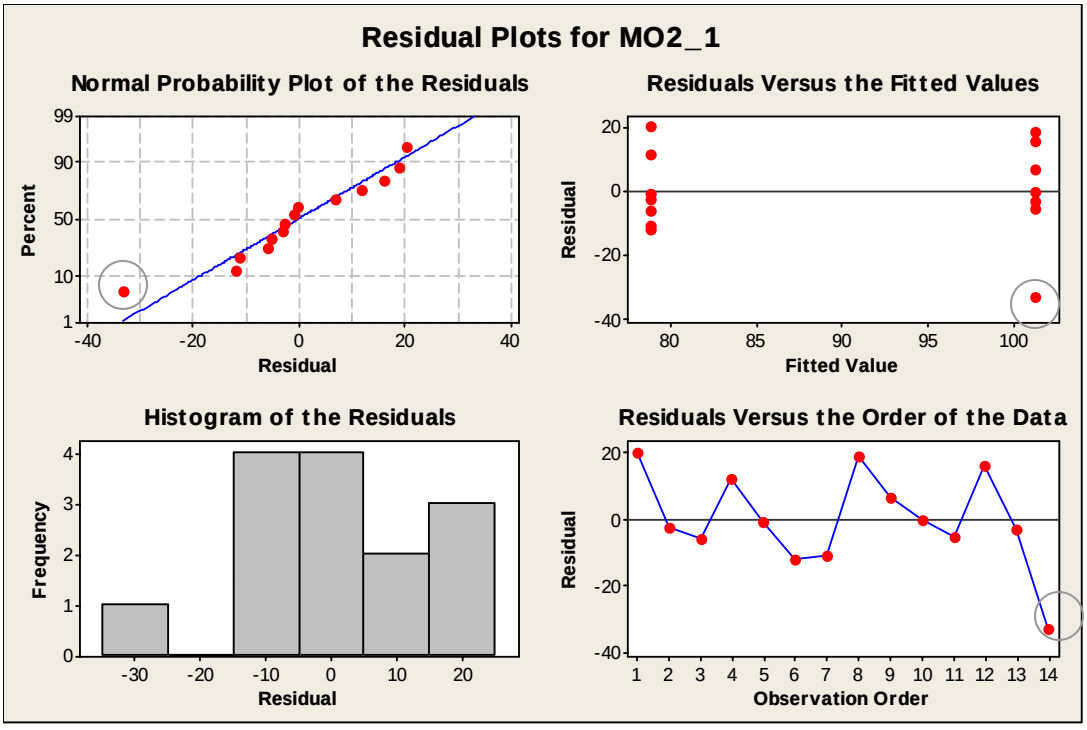


Figure 31 – Residual plots for the analysis of metabolic rate (mg O_2 kg^{-1} hr^{-1}) of capelin during 2 different levels of activity (Sw 10 < Sw 15).

Evaluate the model (evaluation of the residuals) (see fig. 31)

Homogeneity – There are no strong cones in the residuals versus fitted values plot due to the presence of one outlier, so the residuals have some problems with homogeneity.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals do not cluster around a central value and the normal probability plot of the residuals is s-shaped due to the presence of an outlier, so the residuals deviate from normality.

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	1740.3	1740.3	1740.3	7.82	0.016
Error	12	2670.3	2670.3	222.5		
Total	13	4410.6				

The assumptions of homogeneity and normality were not met, the n = 14 and the p-value (0.016) is close to α (0.05) so, I'll re-compute a p-value free of assumptions by randomization.

Re-compute the p-value

After 2000 randomizations, 42 F values were higher than the F_{obs} = 7.82, so the new p-value is equal to 0.021 (see also fig. 32).

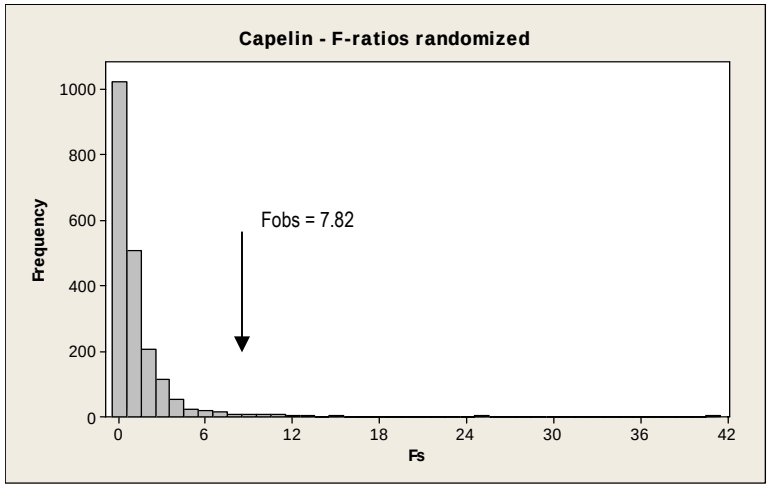


Figure 32 - Histogram of randomized F-ratios for the analysis of metabolic rate (mg O_2 kg^{-1} hr^{-1}) of capelin during 2 different levels of activity (Sw 10 < Sw15).

The p-value < α (0.05) so, I accept the H_A: Var (β_{ActL}) > 0, rejecting Ho.

The MO_2 of capelin when it is Sw 15 is significantly higher than when Sw 10.

($F_{1,12}$ = 7.82 p = 0.021 by randomization)

Analysis 5.5.3 – Sw 15 < Burst Sw

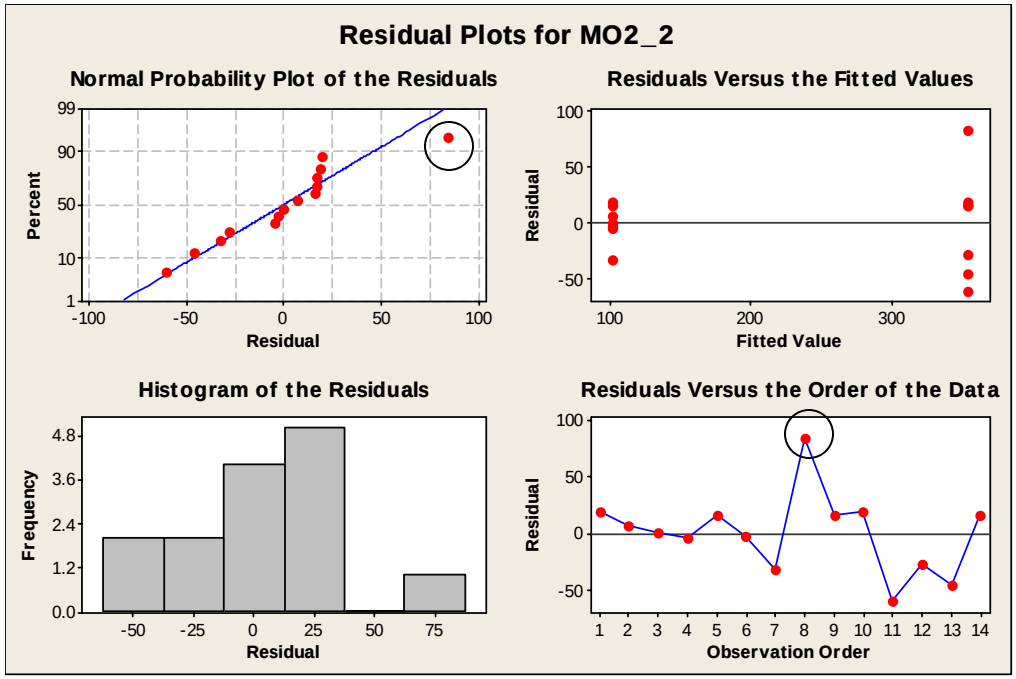


Figure 33 – Residual plots for the analysis of metabolic rate (mg O_2 kg^{-1} hr^{-1}) of capelin during 2 different levels of activity (Sw 15 < Burst Sw).

Evaluate the model (evaluation of the residuals) (see fig. 33)

Homogeneity of residuals – There is a strong cone, opening to the right, in the residuals versus fitted values plot, so the residuals are not homogeneous.

Independency – The residuals versus order of the data plot shows no obvious trend, so residuals are taken to be independent.

Normality – In the histogram the residuals cluster around a central value in a close to symmetrical shape but the normal probability plot of the residuals is s-shaped due to the presence of an outlier, so the residuals deviate from normality.

ANOVA Table

Source	DF	Seq SS	AdjSS	Adj MS	F	P
Species	1	224176	224176	224176	163.26	0.000
Error	12	16478	16478	1373		
Total	13	240653				

Although the assumption of homogeneity and normality of the residuals were not met, n = 14 and the p-value obtained is quite far from α, so I'll trust the decision and accept the H_A: Var (β_{ActL}) > 0, rejecting Ho.

The MO_2 of capelin when it is Burst Sw is significantly higher than at when Sw 15.

($F_{1,12} = 163.26$ $p < 0.001$)

Relatively to MO_2 in capelin we found that:

Resting < Swimming at 10 cm/s < Swimming at 15 cm/s < Burst swimming

Table VI – Comparison of p-values obtained by F-distribution and randomization for the analysis that showed some problems with assumption and p-values close to α (0.05).

Figure	p (F-distribution)	p (randomization)	Violation	Decision
4	0.076	0.044	Normality + Homogeneity	Changed
13	0.010	0.011	Normality	Same
17	0.090	0.091	Normality	Same
22	0.035	0.039	Normality	Same
31	0.016	0.021	Normality + Homogeneity	Same

Looking to the table VI, it is seems that for having a significant change in the p-value obtained with the F-distribution we have to have at least 2 violations in the assumption and a p-value different from α by approximately a factor of 2 (e.g. figure 2). Thus, unless we have large violation of the assumptions and p-value close to α by a factor of 2, we can trust the p-values and confidence limits obtained in the analysis.

Analysis of parameters in model

Table VI – Overall means (βo) of metabolic rates (mg O_2 kg^{-1} hr^{-1}) for each analysis for each of the 5 species studied, with standard error (SE).

	Analysis	βo	SE
Cunner	5.1.1 - Rest < Sw 10	41.6	6.6
	5.1.2 - Sw 10 < Sw 15	73.3	6.3
	5.1.3 - Sw 15 < Burst Sw	102.8	7.2
Ocean pout	5.2.1 - Rest < Sw 10	75.46	14.18
	5.2.2 - Sw 10 < Sw 15	134.65	3.18
	5.2.3 - Sw 15 < Burst Sw	145.88	3.02
Atlantic cod	5.3.1 - Rest < Sw 10	61.8	9.7
	5.3.2 - Sw 10 < Sw 15	97.9	6.4
	5.3.3 - Sw 15 < Burst Sw	131.4	9.0
Rainbow smelt	5.4.1 - Rest < Sw 10	68.5	15.6
	5.4.2 - Sw 10 < Sw 15	122.1	12.0
	5.4.3 - Sw 15 < Burst Sw	197.7	22.3
Capelin	5.5.1 - Rest < Sw 10	54.4	7.4
	5.5.2 - Sw 10 < Sw 15	90.0	4.9
	5.5.3 - Sw 15 < Burst Sw	227.7	36.4

Table VII – Mean metabolic rates (mg O_2 kg^{-1} hr^{-1}) of each species at several activity levels with standard error (SE) and 95% confidence intervals.

	Species	Mean	SE	Confidence limits	
				Lower	Upper
Resting	Cunner	20.7	2.0	16.4	24.9
	Ocean pout	21.3	1.2	18.8	23.8
	Atlantic cod	29.4	1.3	26.5	32.2
	Rainbow smelt	20.7	1.9	16.4	24.9
	Capelim	29.9	4.3	20.4	39.4
Sw 10	Cunner	62.6	8.4	44.8	80.5
	Ocean pout	129.6	4.7	119.5	139.7
	Atlantic cod	94.3	9.9	73.1	115.5
	Rainbow smelt	116.3	17.1	79.1	153.4
	Capelim	78.9	4.5	69.0	88.8
Sw 15	Cunner	83.9	8.2	66.4	101.3
	Ocean pout	139.7	3.7	131.7	147.7
	Atlantic cod	101.6	8.7	83.0	120.2
	Rainbow smelt	128.0	17.9	89.1	167.0
	Capelim	101.2	6.6	86.9	115.5
Burst Sw	Cunner	121.8	8.0	104.9	138.7
	Ocean pout	152.1	3.8	144.0	160.2
	Atlantic cod	161.2	4.0	152.5	169.8
	Rainbow smelt	267.3	15.0	234.7	300.0
	Capelim	354.3	18.7	313.5	395.0

Confidence limits:

$$P\left[\beta_{Sp} - t_{\alpha/2\,(n-1)} * SE \leq \mu \leq \beta_{Sp} - t_{\alpha/2\,(n-1)} * SE\right] = 1-\alpha$$

DISCUSSION

Both cunner and ocean pout, the species in this study with the most inactive and sluggish life styles, had similar resting metabolic rates (RMR), while rainbow smelt and Atlantic cod, more active species, showed higher RMR. Usually a relatively inactive life style is reflected by a low RMR that allows a saving in maintenance costs and is an advantage in energy saving (Duthie, 1982). Thus, in conclusion the RMR does depend on the species life style, since we had increasing values from the more sluggish to the more active species: cunner+pout < cod < smelt < capelin.

The results obtained in this study show a 3 fold difference in the metabolic scope of the 5 species of North Atlantic teleost fish, with the cunner being the species with the lowest and the capelin the species with the highest metabolic scope, respectively. The cunner showed an active metabolic rate (122±8.0 mg O_2 kg^{-1} h^{-1}) similar to flatfish species reported by Duthie (1981) and Priede & Holliday (1980) and relatively low when compared to other members of the Labridae family, with generally active fish. However, this species is known to reach a state of seasonal torpor during the winter months (Green & Farwell, 1971). Therefore, the metabolic rates of cunner are probably related to metabolic efficiency, or a consequence of being able to go into torpor (severely depressed metabolism). Both AMR and SA of ocean pout were higher than the ones reported for other benthic, sluggish species and similar to those measured for the cod, a relatively active schooling marine species. The relatively high AMR of the benthic ocean pout is possibly related to digestive costs/demands (i.e. consuming difficult to digest prey).Schurmann & Steffensen (1997) suggest that the low metabolic scope of Atlantic cod of this species when compared to other fish groups with similar life styles, like the salmonids, may be related with their relatively low amount of aerobic red muscle. Therefore, neither AMR, or MS depended on the life style of the species in this study,

All species increased their metabolic rate (MR) when subject to a 10 cm/s water current and all species increased their MR when subjected to burst swimming. Although all species, except for capelin, maintained the same MR while swimming at 10 and 15 cm/s, showing that higher velocities are necessary to increase SwMR in these species, MR does increase with increasing level of activity, in a similar way between the species studied.

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