A Note on Length Weight Relation in Fishes

Fishes are unique among vertebrates, especially when their growth patterns are taken into account. Baring very few exceptions, fishes show an indeterminate type of growth, implying that they show continued growth throughout the life, invariably with rate of growth declining with age (Mommsen, 2001). The growth among fishes, like other organism are affected by several factors like abundance of food, ambient physical environment, internal biological cycles (e.g. reproductive cycles), etc. Growth monitoring is a key discipline in fisheries, be it capture or culture sector. Growth can be monitored either in terms of change in length or weight. Weight-length relationship enables the inter-conversion of these two measures of growth. Establishing weight-length relationships though a routine exercise in fisheries, still the relationships are available for a limited number of species, considering the enormous α-diversity among fishes (Kulbicki et al. 2005; Froese, 2006).

Historical background of length -weight relationship
The history of weight-length relationship has its conceptual origin in 'square-cube law' of Galileo Galilei, who perhaps was the first person to pronounce that volume increase as the cube of linear measurements whereas the strength only as square. Subsequently, Herbert Spencer, in his *Principles of Biology,* reaffirmed that in similarly shaped bodies the masses, and hence weights are a function of cube of linear dimension, which later became cube law. Fulton (1904) proposed what is called as Fulton's condition factor as:

$$K = 100 \frac{W}{L^3}$$

Where W = body weight in grams and L is length in cm.

He applied cube law to several fish species of North Sea and found that the law does not explicitly fit in fishes, rather most of the fishes gain more weight for length than explained by the cube law. Further, he also noticed that the variations are governed by seasons, location, and reproductive status. He thus laid the conceptual background for what is today known as 'allometric growth'.

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Subsequently, several workers like Jarvi (1920) and Weymouth (1922) have highlighted the inability of cube law to explain the weight-length relationship in fishes. Keys (1928) while working on California killifish, found cube law inefficient in explaining the weight-length relationship and established the modern relationship between weight and length in fishes as:

$W = aL^b$
Where W and L are weight and length and a and b are parameters.

He also gave the logarithmic equivalent of the above mentioned exponential function as:
$Log\ W = Log\ a + b * Log\ L$

But prior to formal publication of Keys (1928) work, Clark (1928) proposed the logarithmic form of weight-length relationship and applied the least-square regression to estimate the parameters. Clark (1928) works got a wide audience and the logarithmic function of his started being used.

Le Cren (1951) gave an exhaustive review of WLRs and condition factors and highlighted the limitation of Fulton's factor, which can be applied only when b is not significantly different from the value of 3 or the specimens were of comparable size. To address the limitation in condition factor of Fulton (1904), Le Cren (1951) proposes an index known as relative condition factor as:

$$K_{rel} = \frac{W}{aL^b}$$

Where W and L are observed weight and length and a and b are parameters of WLR.

Ricker (1958) used the term 'isometric growth' for the values of b =3, whereas Tesch (1968) introduced 'allometric growth' for values of b higher or lower than 3.

Application of weight-length relationship
- The conversion of length data in to weight and vice versa, when other measure is not available.
- To convert the growth-in-length (von Bertalanffy growth function) equation to growth-in-weight form (parameter b of LWR is required) for stock assessment.
- Calculation of biomass of the species from the available length-frequency data from commercial catches or experimental fishing.
- Conversion of length data (length) in to biomass in case of under-water surveys.
- To assess the condition of the fish in culture and capture fisheries (derivatives of WLR like condition or relative condition factor).
- Corroborate the findings of reproductive biology studies (comparison with K or K_{rel})
- Potential use of slope of regression (b) for species separation (Al-Hassan et al., 1988).

Estimation of weight-length relationship in fishes

The weight-length relationship in fishes can be estimated using least square regression method using logarithmic function, *Ln W = Ln a + b*Ln TL,* where *W* and *L* are weight and length and *a* and *b* are parameters to be estimated. There are some important things that are to be kept in mind while collecting data for estimation of weight-length relationships.

- The sample should cover different life stages of fishes. The representative samples from juveniles, sub-adults and adult phased should be covered. It is recommended to have samples from the least possible size to close of reported L_{max}. Further, it is better to have samples evenly distributed across different life-stages.
- The samples should be categorized in groups like males or females or different growth stanzas (juvenile, sub-adults, adults) based on the research question. Comparison of growth coefficient (b) across male and female are predominately practiced.
- The sufficient number of specimens must be observed to have robust and realistic estimates of the parameters. The sample size of 100, evenly distributed across different growth stanzas, should be sufficient (Froese et al., 2011).
- It is recommended to have samples collected over the entire year or all seasons of the year to capture any seasonal variations. One time sampling is mostly discouraged.

There are certain best practices in data pre-treatment and reporting (Froese et al., 2011). They are:
- Prior to fitting linear regression, the log-transformed variables must be plotted and outliers must be removed.
- The results of the analysis should include the minimum and maximum length and weight of the specimens in the sample.
- The presented results should include values for intercept (a) and slope (b) along with their 95% confidence limits, sample size (n) and coefficient of determination (r^2). The value of coefficient of determination (r^2) << 0.95 may be indicative of remaining outliers and data must be revisited to rectify the issue, if any.
- If the hypothesis of the work is to check its deviation from isometry (b = 3), it must be supported by a statistical test (student *t*-test).
- If the hypothesis of the work is to compare the WLR across different group (across sexes, growth stanzas, areas, etc.), both intercept and slopes should be tested for significant difference using appropriate statistical test (e.g. ANCOVA).

Demonstration of Correlation between length and weight of the fishes in MS-Excel
MS-Excel is most common preliminary data tabulation and analysis package familiar to most of the biologist and it can be used to basic fishery data analysis. The data for correlation and regression can be arranged in columns as shown below:

The column A and B have raw data of length (in cm) and weight (in grams) whereas column C and D have log-transformed data, here natural logarithm has been taken using function *LN().*The linear regression can be carried out using **Data Analysis** package under **DATA** tab in main menu bar. The **Data Analysis** package may not be present under **DATA** tab as a default setting. In that case, it can be added by going to *File – Options – Add-ins – Analysis Toolpak –OK*. Column A and Column B can be used as input columns for visualizing the correlation between length and weight variables of the fishes. The correlation coefficient (r) is generally high in related variables. Once we click on Data Analysis tab, a drop-down menu will appear as follows:
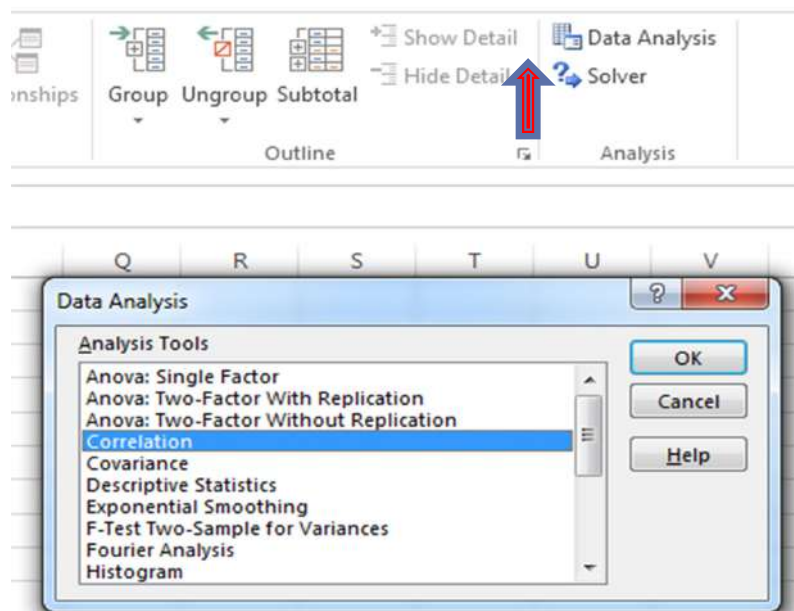
We should select **Correlation** and click **OK**. Once, we click **OK**, a new window will appear.
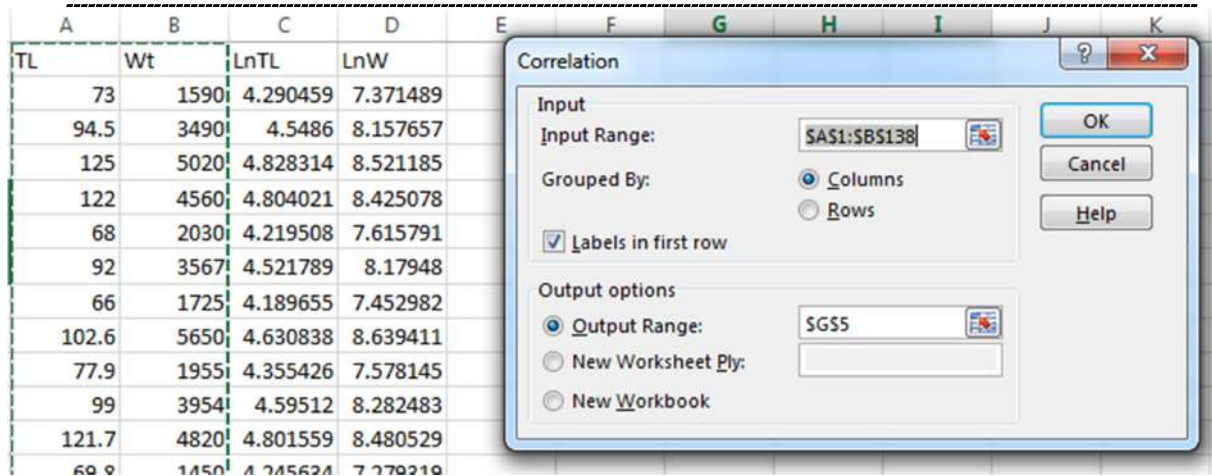
For **Input range**, we need to select entire data cells in Column A (TL) and Column B (Wt) We also have to check options **column** as variables are here arranged in columns and option **Labels in first row** as the column heading are presented in first row. We also have to select output range where the results of the analysis will appear. Here we have selected cell number G5 (results can also be taken to a new worksheet by selecting alternate options available in the window). After filling all the mandatory field, we click **OK** which leads us to the results as output.

TL	Wt	LnTL	LnW				TL	Wt
73	1590	4.290459	7.371489					
94.5	3490	4.5486	8.157657					
125	5020	4.828314	8.521185					
122	4560	4.804021	8.425078				TL	Wt
68	2030	4.219508	7.615791			TL	1	
92	3567	4.521789	8.17948			Wt	0.954334	1

The correlation coefficient (r) between total length (TL) and weight (Wt) in the present example was found to be 0.954 (cell in light orange), which is high, indicating underlying positive (as the value is +ve) relationship between the variables which will be subsequently explored using least square regression technique.

Demonstration of Linear regression for estimation of WLR in MS-Excel
Column C (x) and column (y) will be used for linear regression.
The linear regression can also be carried out using **Data Analysis** package under **DATA** tab in main menu bar. Once we click on Data Analysis tab, a drop-down menu will appear and we should select **Regression** and click **OK.**
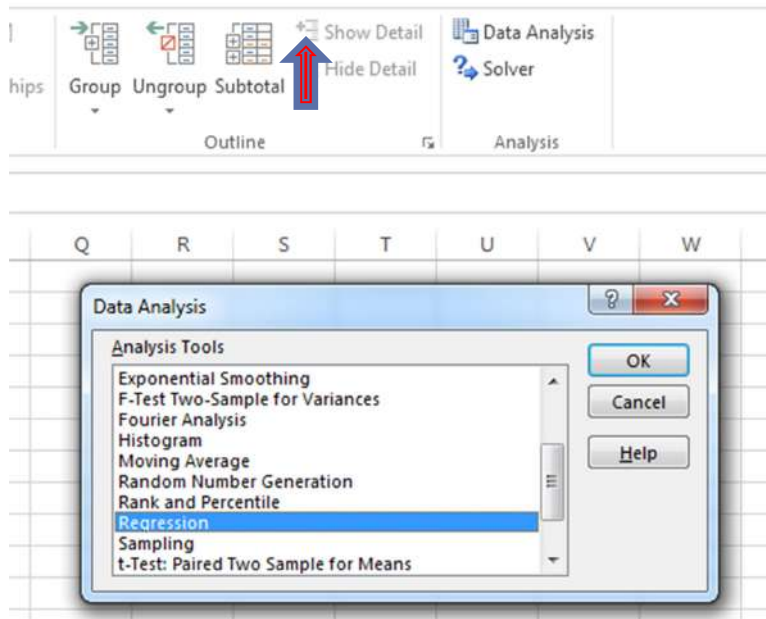
Once we click **OK**, a new window will appear asking to select data and other informations.
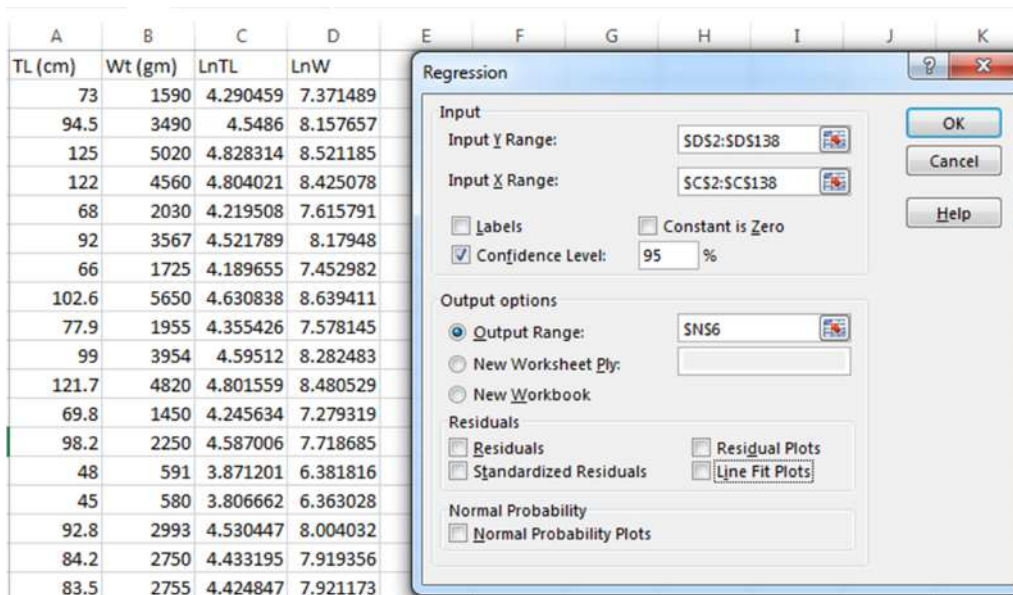
For **Input Y range**, we need to provide cells having data for LnW (here column D) and for **Input X range** we select LnTL (here column C). We have also checked Confidence Level and set as 95% (default), which will give us the 95% confidence limits for slope (b) and

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intercept (a). We also have to select output range where the results of the analysis will appear. Here we have selected cell number N6. After filling all the mandatory field, we click **OK** which leads us to the results as output.

SUMMARY OUTPUT

Regression Statistics

Multiple R	0.961337
R Square	0.924168
Adjusted R Squ	0.923607
Standard Error	0.15877
Observations	137

ANOVA

	df	SS	MS	F	Significance F
Regression	1	41.47369	41.47369	1645.258	1.74891E-77
Residual	135	3.403083	0.025208		
Total	136	44.87677			

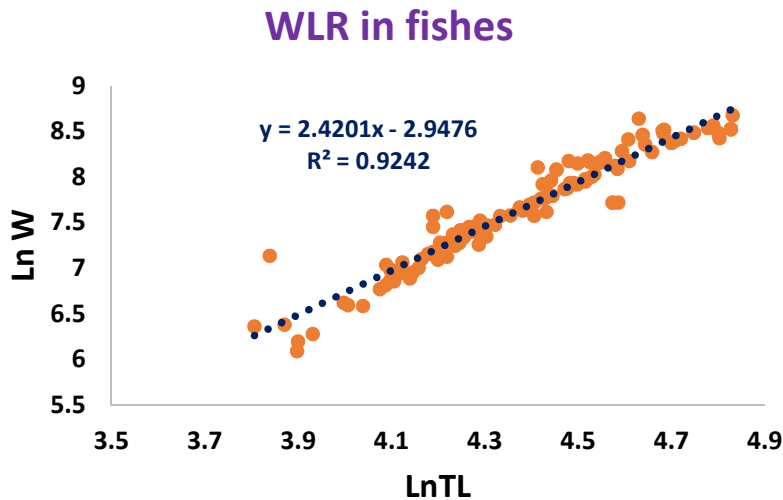|	Coefficients	Standard Err	t Stat	P-value	Lower 95%	Upper 95%
Intercept	-2.94756	0.261018	-11.2925	3.11E-21	-3.46377098	-2.431344372
X Variable 1	2.420096	0.059664	40.56178	1.75E-77	2.30209783	2.538093634

The output includes an ANOVA table and a separate table having the values of coefficients (model parameters). The cell in green represents the value of intercept (*Ln a*) while the blue cells represent the value of slope (b). The cells marked in grey are the 95% confidence limits of the corresponding coefficients and the cell in yellow gives the model fit as the coefficient of determination (r^2). The output can be written in a function form as:

$LnW = -2.947 + 2.420 * LnTL$; $r^2 = 0.924$

If we want to write the function in exponential form, first we need to convert the intercept by using an exponential function in MS-Excel (= EXP (-2.947) which gives the value of 0.052). The exponential function can be thus:

$W = 0.052 * TL^{2.42}$; $r^2 = 0.924$

The WLR relationship can also explored using graphical options in excel. The **scatterplot** options from **Charts** under **Insert** tab of main menu can be selected. The axis and other features can be customized to be presentable using **Chart tools.** Also the linear function and r^2 values cab be displayed on graph using **Add trendline option** and checking **Display equation on chart** and **Display R-squared value on chart** options appearing as pop-ups.

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WLR in fishes

The value of regression slope (b) can be interpreted as:
- Isometric growth: b = 3.0
- Positive allometric or hyper-allometeric growth: b > 3.0
- Negative allometric or hypo-allometric growth: b < 3.0

In statistical terms, the deviation from the isometry (b =3) can be tested using students t-test where the null hypothesis is H_0: b =3 and the alternate hypothesis is H_1: b ≠ 3. The test statistic need to be compared with the table value for n-2 degrees of freedom, where n is the number of observations. The t-statistics can be calculated as:

$$t = \frac{|b - 3|}{SE_b}$$

Where b is the slope of the regression (cell highlighted in blue) and SE is the standard error of the b (cell highlighted in orange). The calculated value of t in the example is 9.71 which is much higher than the table value of 1.97 (we must select the two-tailed value from the table). Hence, we have rejected the null hypothesis and accepted the alternate hypothesis of non-isometric (or allometric growth). As the value of slope is less than 3 in example, it is a case of negative allometric growth.

Note: *The demonstration excel sheet (demoLWR.xlxs) having data and analysis carried out are provided for reference and practice.*

Correlation & Linear regression for estimation of WLR in R statistical package

R statistical package is a free software environment for statistical analysis and graphical presentation of data and more and more biologist are getting familiarized with the working environment of the package. The above example of linear regression can also be done using R package and incorporated for trainees who are keen towards using the package in fisheries data analysis. The same data as above in excel can be saved as CSV file for import in R. The data can be called in to R environment and saved as name **demodata** using code:

>demodata <- read.table(file.choose(), sep = ",", header = TRUE)

The above code opens a window and prompt you to select the .csv file (data file) that you have prepared earlier. Once the data in called to the R environment, one can visualize the data just by giving code

>demodata

This will print the entire data in the R console as:

```
> demodata
        TL   Wt      LnTL      LnW
1     73.0 1590 4.290459 7.371489
2     94.5 3490 4.548600 8.157657
3    125.0 5020 4.828314 8.521185
4    122.0 4560 4.804021 8.425078
```

The correaltion coefficient between total length (TL) and wieght (Wt) can be estimated using following code which will estimate the pearson corealtion coefficient (0.954) and display in R-console. Along with the value of correaltion coeffiecnt it will also provide the 95% confidence interval for the same.

> cor.test(demodata$TL, demodata$Wt)

```
        Pearson's product-moment correlation

data:  demodata$TL and demodata$wt
t = 37.117, df = 135, p-value < 2.2e-16
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.9365133 0.9672367
sample estimates:
      cor
0.9543337
```

The linear regreesion can be carried out using following code as saved as object LWR in R environment.

>LWR <- lm(LnW ~LnTL, data = demodata)

The summarized results of the regression can be vizualized in R console using code:

>summary (LWR)

The output will be printed as:

```
Call:
lm(formula = LnW ~ LnTL, data = demodata)

Residuals:
     Min       1Q   Median       3Q      Max
-0.43475 -0.06145 -0.00609  0.05906  0.79299

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -2.94756    0.26102  -11.29   <2e-16 ***
LnTL         2.42010    0.05966   40.56   <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.1588 on 135 degrees of freedom
Multiple R-squared:  0.9242,    Adjusted R-squared:  0.9236
F-statistic:  1645 on 1 and 135 DF,  p-value: < 2.2e-16
```

The results and the values of intercept, slope, r^2, etc. will be interpreted in the same manner as the output given by MS-Excel.

***Note:** The demonstration data file (demoLWR.csv) has been provided for reference and practice.*

As pointed out by Kulbicki et al. (2005) and Froese (2006) that despite being considered routine work in fisheries science, there a large void in the information available for weight-length relationship of large number of fish species. Froese (2006) further emphasized that establishing LWR for fishes are increasingly considered work not worthy of publication in reputed scientific journals discouraging such work, which many time hampers the efforts to model aquatic ecosystem where conversion of length data to biomass is required. It is recommended to have more dedicated efforts towards establishing WLRs for fishes, especially those which are indigenous and have restricted distribution along Indian coast.

Importance of LWR

The length-weight relationship in fishes is influenced by a number of factors including season, habitat, gonad maturity, sex, diet, stomach fullness, health and differences in the length ranges, sampling amounts of the specimen caught (Tesch, 1968). It must be noted, however, that LWRs differ among fish species depending on the inherited body shape and the physiological factors such as maturity and spawning (Schneider et al., 2000). This relationship might change over seasons or even days (De Giosa et al., 2014). It is argued that "b" may change during different time periods illustrating the fullness of stomach, general condition of appetite and gonads stages (Zaher et al., 2015). In addition, the growth process can differ in the same species dwelling in diverse locations, influenced by numerous biotic and abiotic factors. LWR play a major role in describing the different stages when describing different taxonomic groups which change morphologically during their growth like flatfishes.

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