



## ALLOMETRIC SCALING IN GROWTH AND REPRODUCTION OF A FRESHWATER LOACH *NEMACHEILUS MOOREH* (SYKES, 1839)

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**Abstract:** Self-similar systems are best described as power functions, known as allometric scaling relationships in biological context. Understanding the relationships among changes in various somatic and gonadal (reproductive) tissues with increasing body size can provide valuable ecological information and may shed light on evolutionary trends. In this paper we have discussed how different somatic and gonadal tissues of a freshwater loach *Nemacheilus mooreh* (Sykes, 1839) scale with each other. We observed that tissues, which directly influence the reproductive success, show non-isometric relationships, i.e. relations not scaled as per the Euclidian geometry, with high values of exponents than expected; while tissues, which directly do not influence the reproductive behavior scale as per the Euclidian geometry. This finding suggests that the scaling exponents could be subject to selection towards higher reproductive efficiency. As different species have different r and K selection strategies we believe that the allometric scaling may not be universal as has been advocated before.

**Key words:** Allometric Scaling, Length-weight relationship, Reproductive biology, Freshwater loach, *Nemacheilus mooreh*.

### Introduction

If a system is self-similar, i.e. there exists some feature that is constant on all scales, then it can be represented by a mathematical expression called a power or scaling equation (Brown *et al.* 2002; Harte *et al.* 1999; West *et al.* 1997). Scaling equations simply describe how a system's feature changes in proportion to the scale of the system. In biology, scaling equations describe a variety of allometric relationships. The general equation of allometric scaling is given by,

$$Y = aX^b \quad (1)$$

Where, 'Y' is a dependent variable, 'a' is normalization constant, 'X' is the

independent variable, and 'b' is the scaling exponent. Taking the logarithms of both sides of this equation gives the expression for a straight line:

$$\log Y = \log a + b \log X \quad (2)$$

Thus, the statistics of linear regression can be used to fit scaling functions to data.

The exponent b is of particular interest as it can depict two important outcomes. Firstly, whether X and Y are related as expected by Euclidian geometry, i.e. are they isometric, for instance whether mass scales as cube of length, area as square of length, etc. Secondly, while comparing two variables belonging to same scalar quantity, for example, length of head and length of

body, mass of brain and mass of body, etc., whether one variables grows more rapidly than the other ( $b > 1$ ), less rapidly than the other ( $b < 1$ ) or grows in proportion ( $b = 1$ ).

Studies in allometric scaling have attracted both ecologist and evolutionary biologists for a variety of reasons. Ecologists have used allometric relationships to characterize growth patterns in organisms. For instance, especially in fish, the allometric relationship between length and weight is used for determining the conditioning factor, a measure of well being of the live stock in the given environment (Kozul *et al.* 2001; Peck *et al.* 2005). Evolutionary significance of allometric scaling has focused on identifying universal scaling laws, which can explain fundamental structural, metabolic and physiological rules that span over 21 orders of magnitude in size of biological diversity (West and Brown, 2005).

Current research on allometric scaling laws is influenced by three schools of thoughts that have emerged from observations on scaling between basic metabolic rate (BMR) and mass (M) of an organism. One school of thought suggests that the BMR scales with M as a power  $2/3$  as expected by Euclidian geometry of 3D objects (White and Seymour, 2003), while the other school of thought suggests that BMR scales with M with the power  $3/4$  as expected from fractal like network of metabolic exchange in the body (West *et al.* 1997; Banavar *et al.* 1999; Brown *et al.* 2002; West and Brown, 2005). Both these schools rely on single cause explanations of scaling exponent. The third school of thought suggests that there are multiple causes for the scaling exponent and that the exponent is not a fixed value but rather a follows a distribution selected evolutionarily based on the metabolic activities of the organism (Darveau *et al.* 2002; Kozlowski and Konarzewski, 2005). Interestingly, all three claims are supported experimentally under different sets of conditions. The scaling laws have been used further to infer about body temperature, biological clocks,

ontogenetic growth, home ranges of animals and species diversity patterns (West *et al.* 2001; Haskell *et al.* 2002, Enquist *et al.* 2002). Both single cause and multiple cause explanations of allometric scaling have been debated at length (Kozlowski and Konarzewski, 2005; Darveau *et al.* 2002; Banavar *et al.* 2003; Brown *et al.* 2005; West *et al.* 2003) and these debates are far from being settled. Especially the model based on fractal geometry (West *et al.* 1997), which explains why allometric scaling does not follow Euclidian geometry, is severely criticized.

In this paper we take this debate further by studying different allometric relationships in a freshwater loach, *Nemacheilus mooreh* (Sykes, 1839). We have addressed two major concepts, firstly, how do various tissues, which increase in conjunction with the growth of the fish, scale with each other, and secondly, how do tissues associated with reproductive organs, which shows seasonal growth as per the reproductive cycle, scale with the body parameters. We show that the scaling exponents of characters directly related to the reproductive success show non-isometric relationships, probably due to their selection for maximum reproductive output, while scaling exponents of characters that are not directly related to reproductive success scale isometrically. We have also discussed probable reasons that can lead to the selection of non-isometric relations in the allometric scaling.

## Materials and Methods

Freshwater loach, *Nemacheilus mooreh*, was collected throughout the year from Mutha River ( $18^{\circ}31' N$  and  $73^{\circ}51' E$ ), Pune, India. The total length of the fish (L) from snout tip to the tip of caudal fin, and the length of the alimentary canal (AL) from opening of the mouth to the vent opening was determined using vernier calipers. The wet weight (W) of the fish was measured on an electric balance. The fish were dissected and gonads (testis or ovary) were removed and their weight was measured.

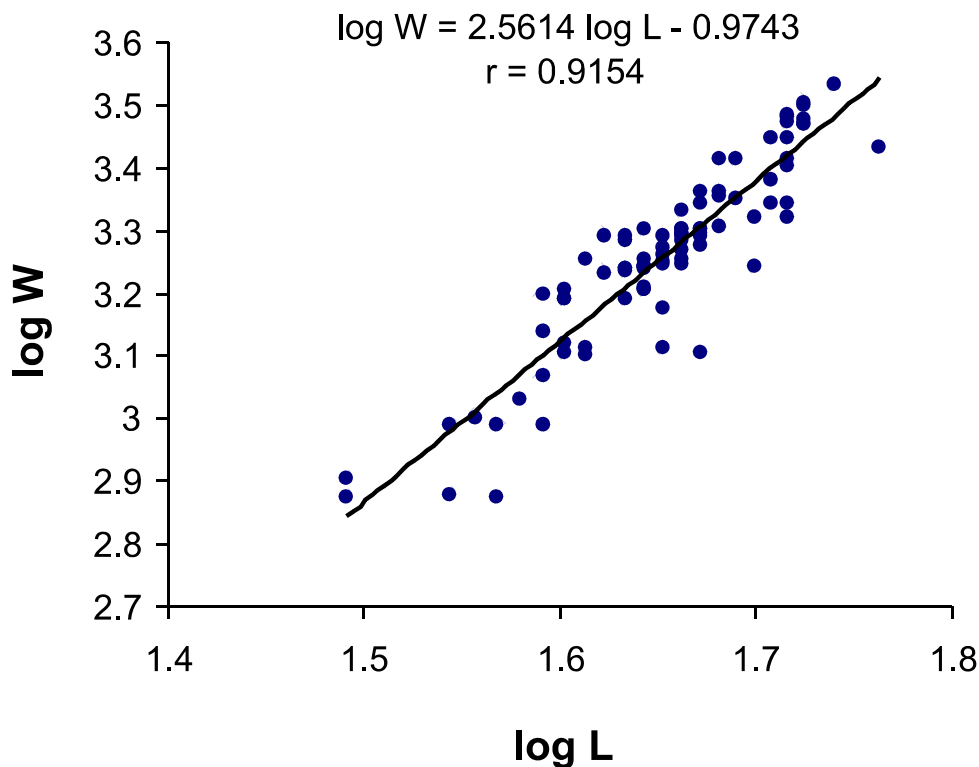
A subset of 26 gravid ripe females was considered for determining the relationship between fecundity (F), weight of the ovary ( $W_o$ ), W and L. Ovaries were immersed in Gilson's fluid (80% nitric acid, 15 ml; Glacial acetic acid, 9 ml; 60% alcohol, 100 ml; mercuric chloride, 20 g and distilled water, 880 ml) and fecundity (number of eggs) was calculated by separating each and every egg.

Relationships between L, AL, W, F and  $W_o$  were determined by plotting the data on log-log scale. A least square line, given by Eq. (2) was fitted to the scatter of the data. The significance of the relationship was determined from coefficient of regression (r) and uncertainty in the prediction of the exponent was determined by calculating standard error of the exponent (SEE). The

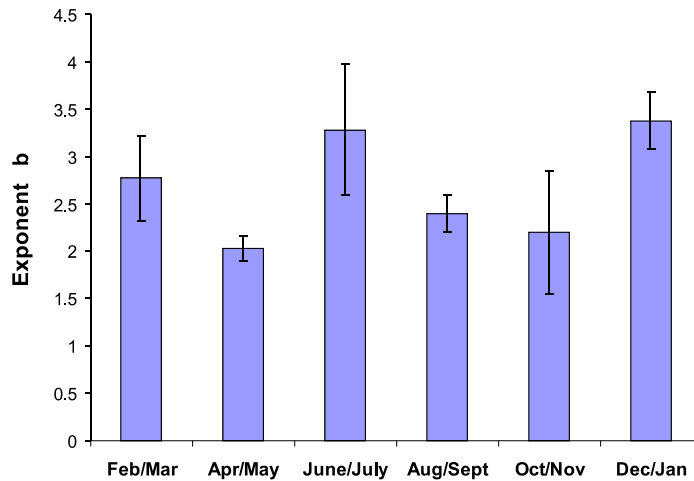
parameters derived from best-fit line were used to express the relation between biological variables in the form of Eq. (1).

## Results and Discussions

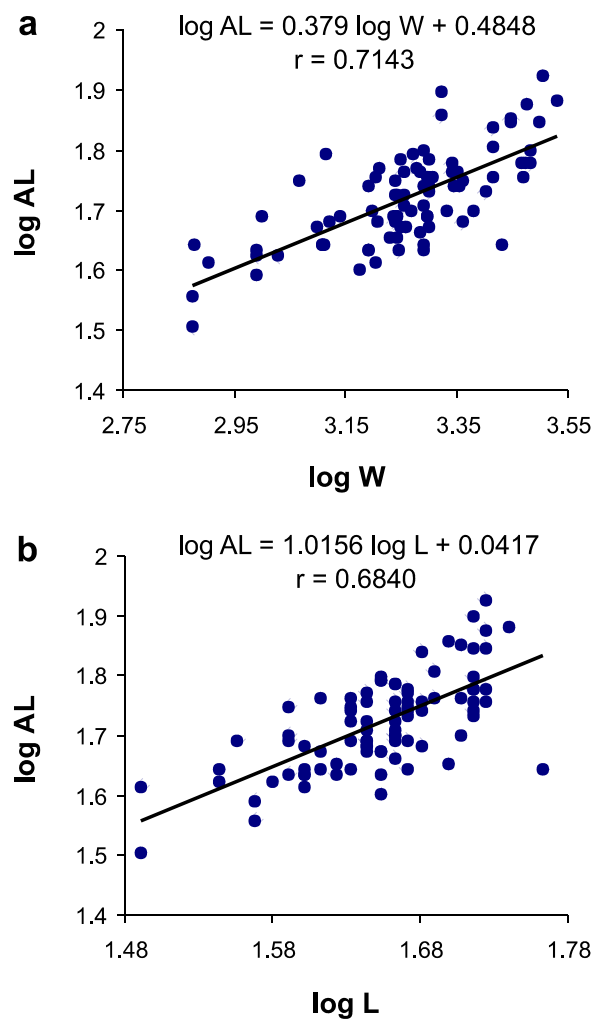
For a constant density, the mass of the body (considered here as weight of fish) is expected to scale as cube of length as per the Euclidian geometry. Thus, the exponent b in a length-weight relationship should be close to 3. However, the length-weight relationship of *N. mooreh* for the pooled data of one year showed a best fit curve defined by the equation,  $W = 0.1061 L^{2.5614}$  (Figure 1.,  $r = 0.9154$ ,  $p < 0.001$ ). This exponent 2.5614 (SEE 0.1214) is far lesser than the expected cubic value.



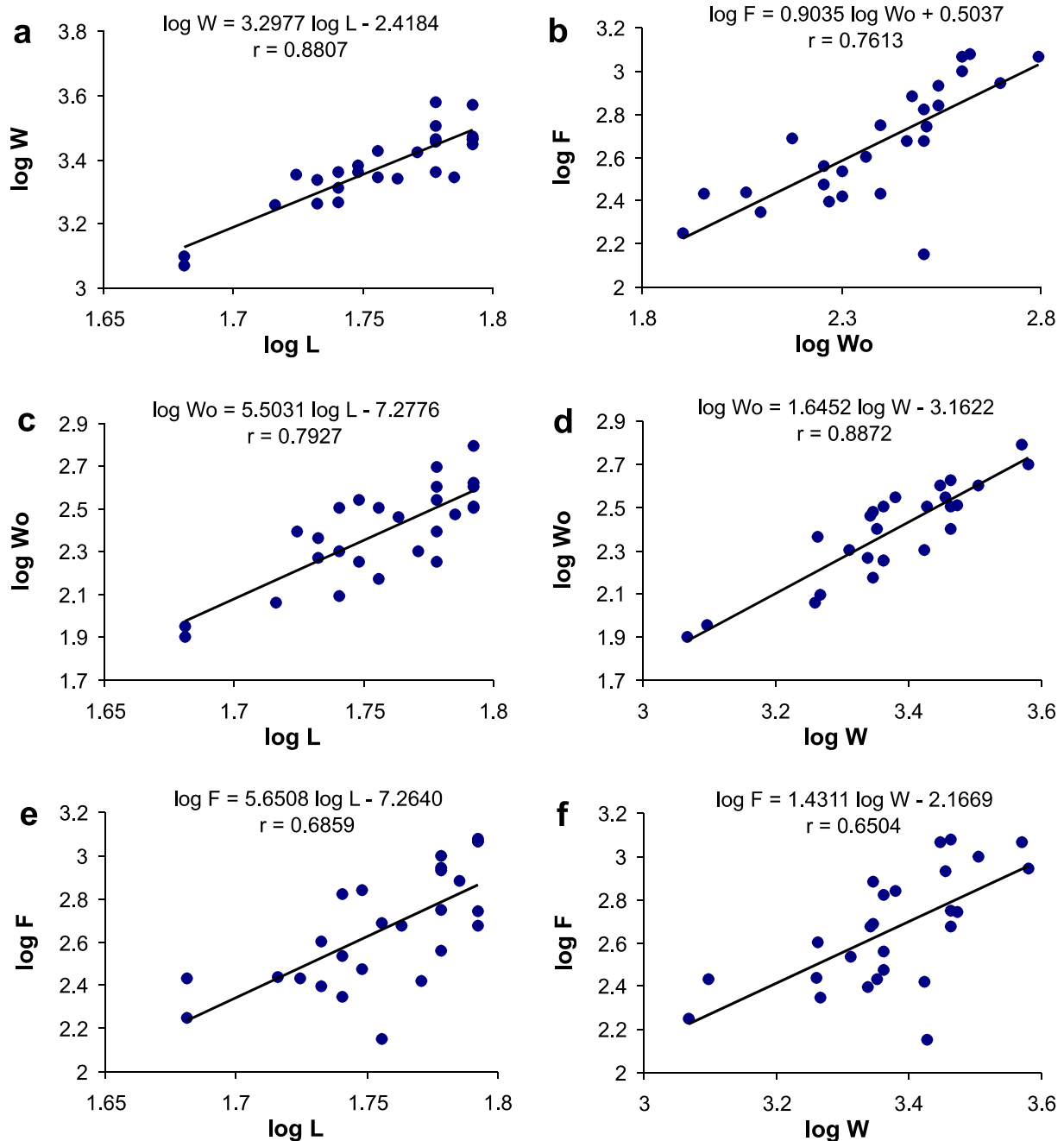
**Figure 1:** The length – weight relationship in *Nemacheilus mooreh* from the pooled data of one year.



**Figure 2:** Month wise change in the exponent  $b$  of the Length-Weight relationship,  $W = aL^b$ . All relationships for respective exponents are significant at  $p < 0.05$ . Bars indicate standard error in the estimation of  $b$ .



**Figure 3:** Relation of alimentary canal length (AL) with (a) total length (L) and (b) weight (W) of the fish for the pooled data of one year. Both relationships are significant at  $p < 0.001$ .



**Figure 4:** Relations between total length (L), body weight (W), weight of ovary (Wo) and fecundity (F) of 26 fully ripe females. All relationships are significant at  $p < 0.001$ .

In the length-weight relationship of fish the exponent  $b$  show a normal distribution on both sides of the cubic value with little deviation (ND, unpublished). Turkmen *et al.* (2001) has argued that the exponent  $b$  in fish differ according to the species, sex, age, season and fish feeding. Peck *et al.* (2005) has shown the effect of ontogenic changes on the allometric scaling of mass

and length relationship in a fish *Sprattus sprattus*. While, Moutopoulos and Stergiou (2002) also attributed the variation in exponent  $b$  from its expected cubic value to differences in the number of specimen examined, area or season effect and differences in the observed length ranges of the specimen caught. We, however, suspect that the major factor, which affects the

exponent  $b$  in *N. mooreh*, could be the degree of sexual maturity of the fish, and that the found value of 2.5614 (Figure 1) is an additive effect of high variation in gonad weight during various stages of sexual maturity.

To substantiate this argument we plotted the exponent  $b$  for the data of two months (Figure 2). The months were selected taking into consideration the reproductive behaviour of *N. mooreh*. Kharat (2001) has shown that *N. mooreh* reproduces twice in a year once during August-September and during February-March. Figure 2 clearly depicts that there is a discrete pattern in change in the exponent  $b$  and is correlated with the reproductive phase of the fish. The exponent is highest in pre-spawning months (June/July and Dec/Jan) and decreases substantially during the spawning period (Feb/Mar and Aug/Sept). The least exponent is seen during the post-spawning months (Apr/May and Oct/Nov), which probably are the months during which the fish starts preparing for the next reproductive cycle.

Based on these findings we put forth a hypothesis that the low value of exponent  $b$  during post spawning season could be an evolutionary adaptation. *N. mooreh* has a behavioral tendency to migrate upstream for reproduction (SSK and ND, personal observations). If the weight scaled as cube of length in post spawning seasons then it will scale more than cube in pre-spawning and spawning period, due to the weight of the gonads. This may lead to hampered moment of the fish, as the streamline structure of the fish will be distorted. Thus, the adaptation of the fish to a smaller exponent during the post-spawning months not only renders it rapid moments in post-spawning months but also with the advent of reproductive cycle (i.e. as the pre-spawning and spawning period) the exponent approached cube or slightly more than the cube making it possible that the fish maintains streamline body for the upstream migration during spawning.

In Euclidian geometry, as the scale of the body increases the relationship depicting change in lengths of two tissues should show an exponent of one and the relationship depicting change in length versus weight should show an exponent of 1/3 or 0.3333. Both the relationships are consistent with our finding  $AL = 1.1008 L^{1.0156}$  (Figure 3a,  $r = 0.6840$ ,  $p < 0.001$ , SEE 0.1168) and  $AL = 3.0535 W^{0.379}$  (Figure 3b,  $r = 0.7143$ ,  $p < 0.001$ , SEE 0.0401). These isometric relationships suggest that among tissues that are not under the pressure of selection for maximum reproductive efficiency, the relationships can follow Euclidian geometry.

To study the scaling laws during reproductive phase of the fish, 26 gravid fully ripe females were considered. The length-weight relationship of these females had an exponent of more than 3 as per our expectation (Figure 2). The length-weight relationship was given by the equation  $W = 3.82 \times 10^{-6} L^{3.2977}$  (Figure 4a,  $r = 0.8807$ ,  $p < 0.001$ , SEE 0.3620).

If we assume that the eggs are tightly packed in the ovary and have a constant volume  $V_{egg}$  then the total volume of the ovary  $V_o$  will be equal to,  $V_o = V_{egg} \times F$ , where,  $F$  is the fecundity. If  $V_{egg}$  is constant, then  $F \propto V_o$  and for constant density  $F \propto V_o \propto M_o$ , where,  $M_o$  is the mass of the ovary. We therefore expect that the fecundity should scale as unity when compared with the mass of the ovary. Our observed relation is  $F = 3.1893 W_o^{0.9035}$  (Figure 4b,  $r = 0.7613$ ,  $p < 0.001$ , SEE 0.1571) which is not statistically different from unity.

Most interestingly, the literature survey on the relation of  $F$  and  $W_o$  of other fish showed marked deviation from the unity (Table 1). In all these cases the fecundity has not been measured by counting each and every egg, as we did, instead, weighing the ovary and a sub-sample and then multiplying the number of eggs in a sub-sample accordingly have made the gross estimate of fecundity. Demartini (1987) has shown that in case of *Purulubrux nebulifer* the difference in fecundity estimate from any given sub-sample is not statistically insignificant. However, it is not mentioned

**Table 1: Scaling exponents for the relationship between fecundity and two somatic tissue related parameters (total length and weight of the fish) and a gonadal tissue related parameter (weight of the ovary).**

Fish species	Scaling exponent			Reference
	Total length	Weight of fish	Weight of Ovary	
<i>Cyprinus carpio</i>	2.5800	0.8600	0.8800	Crivelli (1981)
<i>Euryglossa orientalis</i>	4.3890	1.1740	1.1000	Khan and Hoda (1998)
<i>Ilisha africana</i>	3.2910	1.0490	-	Marcus and Kusemiju (1984)
<i>Lota lota lota</i>	2.8129	1.0144	0.8482	Bryliska <i>et al</i> (2002)
<i>Mystus cavasious</i>	2.4330	1.1503	-	Sharma <i>et al.</i> (1996)
<i>Perca fluviatilis</i>	2.8280	0.8499	0.7494	Treasurer <i>et al.</i> (1981)
<i>Tilapia nilotica</i>	2.0170	0.8300	-	Dadzie and Wagila (1980)
<i>Tilapia zilli</i>	2.3600	1.0061	-	Babiker and Ibrahim (1979)
<i>Tinca tinca</i>	2.9230	0.7920	-	Alas and Solak (2004)
<i>Nemacheilus mooreh</i>	5.6508	1.4311	0.9035	This study

how much error is incorporated when the fecundity is measured by this method. We argue that the deviation of exponent from unity in this relationship probably could be attributed to the error incorporated during this sub-sampling method. It is less likely that the lower than unity exponent in this relationship is an outcome of selection. If we expect that the ovary grow in proportion to the body growth, then isometry suggests that  $W_o$  should scale as cube of L and as unity with W. However, the found relationships are  $W_o = 5.28 \times 10^{-8} L^{5.5031}$  (Figure 4c,  $r = 0.7927$ ,  $p < 0.001$ , SEE 1.2240) and  $W_o = 59.33 W^{1.6452}$  (Figure 4d,  $r = 0.8872$ ,  $p < 0.001$ , SEE 0.3412). These relations further reflect the relationship between F, L and W by the equations  $F = 5.45 \times 10^{-7} L^{5.6508}$  (Figure 4e,  $r = 0.6859$ ,  $p < 0.001$ , SEE 0.8640) and  $F = 133.78 W^{1.4311}$  (Figure 4f,  $r = 0.6504$ ,  $p < 0.001$ , SEE 0.1746).

The non-isometric growth of ovary as compared to somatic tissues can have evolutionary significance. Our relationship predicts that the weight of ovary scales 1.6 times the weight of the body. That is, with every unit increment in body weight, the increase in ovary weight is drastic. Interestingly, Kharat (2001) has noted that

during the spawning period the stomach of a gravid female is normally empty and there is high content of oil in liver tissue, which probably serves as a reserved energy supply. This arrangement suggests that the fish devotes its entire abdominal space for the growing ovary. We suspect that this adaptation could be an outcome of maximization of fitness in terms of reproductive output, because with unit increment in the body weight the weight of ovary that is carried by the female increases by a factor 1.6. Nonetheless, such adaptations will not give universal scaling exponent because each fish will differ in its reproductive cycle and r and K selection. Thus, it can be seen from Table 1 that the scaling exponent for relationship between F and L or F and W is variable in different fish species.

Recent debate on the allometric scaling revolves around the concept that the scaling exponents are universal (West and Brown, 2005). Their inferences are based mainly on the relationship between metabolic rate and body mass and the factorial like geometry of the organisms (West *et al.* 1997). Darveau *et al.* (2002) however argued that allometric scaling exponents can show deviation from

universal exponents depending upon the state of the organism leading to an additive effect of allometric cascades. Their point was severely criticized by West *et al.* (2003) and Banavar *et al.* (2003). Along with other reports of deviation from allometric relationships (Peck *et al.* 2005) our findings supports the view of Darveau *et al.* (2002) that the allometric scaling can show deviations from universal exponents. Furthermore, Kozłowski and Konarzewski (2005) have criticized the single cause explanation given by West *et al.* (1997) a pluralistic approach to scaling, founded-on the life history theory, can explain the scaling relationships. Our findings supports Kozłowski and Konarzewski's (2005) claim by suggesting that the scaling exponent are subject to change from isometry depending on the reproductive cycle,  $r$  and  $K$  selection and the selection pressure on characters from the point of view of maximizing reproductive outcome.

Allometric scaling will be subject to selection especially if it is directly relevant for the reproductive efficiency of the organism. In our study we observed isometric relationship, which could be fairly constant, between parameters, which are not directly relevant in the reproduction of the fish. In the case relationship between AL versus L and AL versus W we observed isometric relationship. Interestingly we observed a non-isometric exponent in the relationship between L and W. In *Nemacheilus mooreh*, the loach that migrates up-streams for the reproduction, maintaining the streamline structure of the loach is essential and thus the non-isometric exponent could be an adaptation as described before.

In case of other gonadal tissues that are associated with the reproductive behavior of the fish we observed non-isometric exponents, which are also not universal in other fish species. The relationship between  $W_0$  and L that gives extraordinary high deviation from the cubic value clearly indicates that the gonadal tissues are subject for selection towards high reproductive

efficiency. Further more an interesting relationship that showed isometric exponent between  $W_0$  and F suggests that all the reproduction related parameters could be under the same selection pressure.

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