THE ESTABLISHMENT OF TOTAL DNA QUANTITY VARIABILITY INTERAVALS, FOR FIVE TYPES OF TISSUE, FROM *Carassius auratus gibelio* BLOCH 1783, INDIVIDUALS

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Abstract: In present experiments, we establish the total DNA quantity and the variability intervals for this parameter (by extraction with phenol : chloroform : izoamylalcohol and spectrophotometrical dosing) for five types of tissue (gills, liver, muscle, spleen and kidneys), sampled from *Carassius auratus gibelio* Bloch. 1783 individuals.

Key words: *DNA, Carassius*

MATHERIAL AND METHOD

The first objective of our research was to establish the individual groups' homogeneity, followed by total DNA quantity establishment for individuals of *Carassius* genera, and the determination of total DNA quantity variation intervals for different tissues, sampled from different individuals.

Measurements have been made on the used individuals following on series of phenotypic parameters (Lt=total length; Ls=standard length; g=individual weight; C=circumference; Hd=maximum height; Ha=minimum height; Lc=head's length) and phenotypic indexes (profile index=Lt/Hd; circumference index (Kiselev)=Ls/C. (Voican et. al., 1975, Gorgan et. al., 2000).

To obtain information regarding the population's uniformity degree as well as on statistic signification level, biometrical measurements were made for 50 individuals and the statistical parameters values were calculated (Snedecor, 1968): $\bar{x}$ = average; S= standard deviation; Es = standard error; C.V. % = variation coeficient.

Phenol : chloroform : isoamil alcohol extraction and purification technique was used to extract total DNA from fresh, frozen or ethanol conserved tissues (Ausubel et. al., 1995). All determinations were made on 20 individuals of *Carassius auratus gibelio* Bloch. species.

1.5ml Eppendorf tubes were labeled, and in each of them 500µl lisys buffer were used.

Between 20-200mg fresh, frozen or conserved (in 95% ethylic alcohol) tissue were laid on a sterile plate for cutting.

The small fragments of tissue were introduced in the Eppendorf tubes, containing 500µl lissys buffer. In each tube 10µl K proteinase were added. All samples were shacked on a vortex and kept over night at 37 degrees.

In the next day, in each tube, 600µl phenol:chloroform:izoamilic alcohol (25:24:1) were added. The tubes were agitated up to 60 seconds, than centrifuged for 4 minutes at 8000rpm. After centrifuging, the tubes present a yellow shaped inferior layer, which contains the organic solvent, a superior layer with DNA and an intermediary phase, which contains unlissed tissue fragments.

The superior layers (700µl) were separated in new labeled tubes and at the end, 550µl chloroform were added. The tubes were agitated up to 60 seconds, and then centrifuged for three minutes at 8000rpm.

The superior phases were again separated in new tubes, without taking something from intermediary separation phase.

1ml cold 95% ethanol (kept at -20 C degrees) was added in the new tubes after separation. The tubes were agitated and left for 30-60 minutes at -20 C degrees. At the end of this time on the bottom of the tubes a white precipitate appeared (total DNA) (Figure 1). After the DNA precipitation, the tubes were centrifuged for 5 minutes at 10000rpm for sedimentation. The superior layer was eliminated and the pellets were dried by vacuum centrifugation, 10 minutes, with the temperature "in low". If in this interval the pellets are not dry, the tubes are centrifuged 5minutes more.

After the pellets dried, in each tube 200µl TE solution (pH=8.0) were added and manually or pipeting resuspended. For a better resuspension, the tubes can be left up to 30 minutes at room temperature, 37 C degrees or longer at 4 C degrees.

Using the average's $(\bar{x})$ and standard deviation's *(S)* values, counted based on the samples from one population, the appropriate values can be estimated for the entire population (or species). Estimating the population's average involves the average's standard error calculation $(S_{\bar{x}})$, which depends on the samples' variability and size (Varvara et. al., 2001).

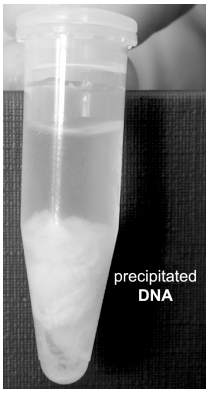

Figure 1. Precipitated DNA

A critical value is searched for certain *α* confidence degrees and a certain number of *t(α, n-1)* liberty degrees, then, the probability's estimation will be given by *1-α*, which means *α=0,05*. The *t* value represents the critical value of *t* distribution for a certain confidence level *α* and *n-1* liberty degrees. The signification threshold represents the arbitrary chosen probability, for the calculated confidence interval to include population's average. For the majority of studies, the 95% probability, meaning *α=0,05*, is considered to be satisfying. The average's standard error is used to calculate the population's average confidence interval, which includes, with some probability, the population's average.

$\mu = \bar{x} \pm t(\alpha, n-1) \cdot S_{\bar{x}}$, where μ represent the population's average and $\bar{x}$ samples' mean values.

Interval's limit: inferior limit *(LI)* and superior limit *(LS)*, are given by the next formulas:

$$LI = \bar{x} - S_{\bar{x}} \cdot t(\alpha, n-1)$$

$$LS = \bar{x} + S_{\bar{x}} \cdot t(\alpha, n-1)$$

In this way, the LI-LS interval includes the population's average, with a *1-α* probability.

RESULTS AND DISCUSSIONS

The main directions we focused on were to determine the individuals' homogeneity, to dose the DNA quantity and to establish the variability intervals.

It was considered as necessary to determine the individuals' homogeneity, primarily to exclude a variable given by morphological differences and growth conditions, which could determine errors in establishing total DNA variability intervals.

Analyzing the observed data for *Carassius* genera's species (Table 1), we found out that the standard error values were very small, which signified a grouping of all data very close to the average value per sample and the investigated parameter.

Table 1 Calculated phenotypic parameters, for the analyzed individuals

Analyzed parameter	Lt (cm)	Ls (cm)	Lc (cm)	Ha (cm)	Hd (cm)	G (g)	C (cm)
Average	19.02	15.83	4.12	2.04	5.96	267.5	15.20
Standard deviation	1.17	0.99	0.34	0.34	0.46	29.4	0.97
Variation coefficient	2.34	1.99	0.67	0.67	0.91	58.9	1.93
Standard error	0.16	0.14	0.04	0.04	0.06	4.1	0.13
Superior limit of confidence interval	19.38	16.18	4.47	2.39	6.32	267.9	15.56
Inferior limit of confidence interval	18.66	15.46	3.75	1.68	5.60	267.2	14.84

Lt=total length, **Ls**=standard length, **Lc**=head's length, **Ha**=high on the anal fin, **Hd**=high on the dorsal fin, **G**=weight, **C**=circumference

Based on average's standard error and *t*'s value given by $\alpha=0,05$ (which means a 95% probability) and *n-1* liberty degrees (where *n* represents the number of values from each sample), the variability intervals' limits were calculated for all investigated phenotypic parameters and indexes. From the intervals' graphic representation (Figure 2), one can observe the reduced limits of all parameters which signify a uniformity of all analyzed individuals. Also, by analyzing the profile index, (Table 2) one can notice it's variability between very low limits for all 20 analyzed individuals (2.30 – 3.02cm). All this values confirm once again the uniformity of analyzed individuals, morphologically speaking.

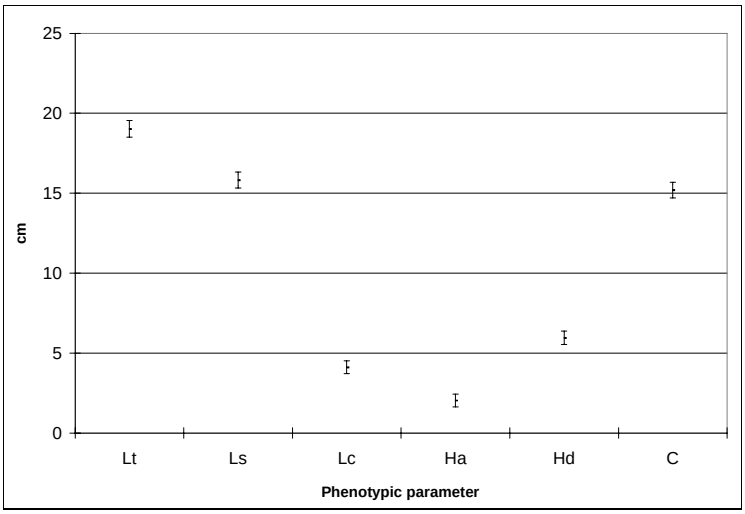

Figure 2 *Carassius* auratus gibelio's variability intervals for the analyzed phenotypic parameters (**Lt**=total length, **Ls**=standard length, **Lc**=head's length, **Ha**=high on the anal fin, **Hd**=high on the dorsal fin, **G**=weight, **C**=circumference)

Table 2 Calculated phenotypic indexes

Investigated parameters	Profile index	Circumference index
Average	2.66	1.04
Standard deviation	0.17	0.07
Variation coefficient	0.34	0.13
Standard error	0.02	0.01
Superior limit of confidence interval	3.02	1.40
Inferior limit of confidence interval	2.30	0.68

The first objective for total DNA concentration determination was to build the etalon curve; in this purpose, we used pure DNA, as starting point for concentrations between 10µg – 50µg DNA/ml. The absorptions were read on a $\lambda=270nm$ length wave. For counting the DNA concentration values, an etalon graphic curve was made (Figure 3).

Based on the graphic curve, the regression line was indicated and the regression equation was calculated, which gave us the DNA quantity for each tissue from different individuals. Afterwards, all these values were reported to the quantity of used tissue (DNA µg corresponding to 100mg of tissue) (Gorgan et. al., 2005).

In this way, we analyzed 20 individuals of *Carassius auratus gibelio* Bloch. from Movileni population by sampling five types of tissue from each of them (gills, spleen, liver, muscle and kidneys). We used five different types of tissue, to be able to realize average values and to establish variability intervals as well as their dynamics with the smallest error possible (Gorgan et. al., 2004).

Regarding all individuals from *Carassius auratus gibelio* species, one can notice that individual values have the same variation tendency, and that they are grouped around the average value, which shows a high central tendency. The highest total DNA quantity (262,411ug DNA/100mg tissue) was recorded for Ca11 individual for hepatic tissue and the smallest value (44,505µg DNA / 100mg tissue) for Ca04 individual, for muscular tissue. The highest medium value 227,012µg ADN/100mg tissue, was observed for kidney tissue which also has a higher variation amplitude comparing to muscular tissue which has the smallest medium value (58,293µg DNA/100mg tissue).

Considering the average values for all five types of tissue for all analyzed individuals and standard deviation values, the total DNA quantity standard error and variability interval limits were calculated (Table 3).

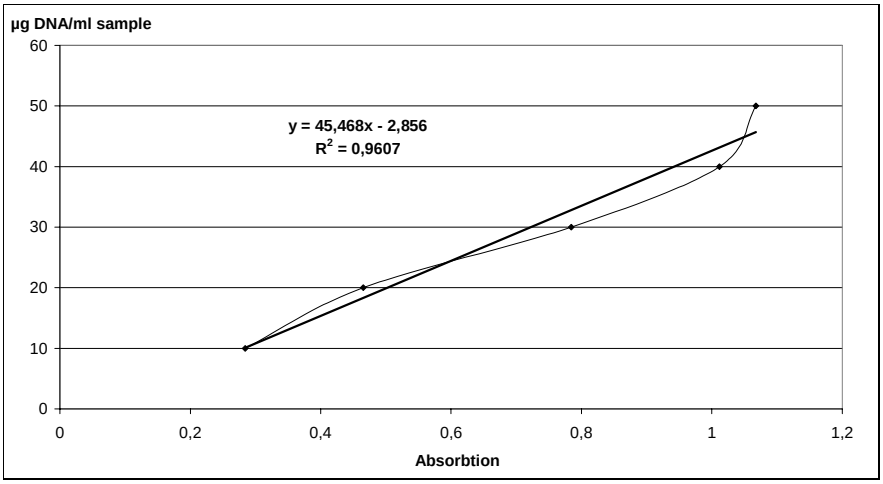

Figure 3 Etalon curve for DNA quantity dosing, on *λ=270nm* wave length (Gorgan et. al., 2005)

Table 3 Total DNA quantity variability limits intervals
for five tissues from *Carassius auratus gibelio* Bloch. (µg DNA/100mg tissue)

Tissue	Superior limit	Inferior limit	Average	Standard error
Gills	105.543	90.377	97.960	7.583
Spleen	201.789	188.351	195.070	6.718
Liver	226.367	193.672	210.019	16.347
Muscle	62.892	53.693	58.293	4.599
Kidney	238.680	215.344	227.012	11.667

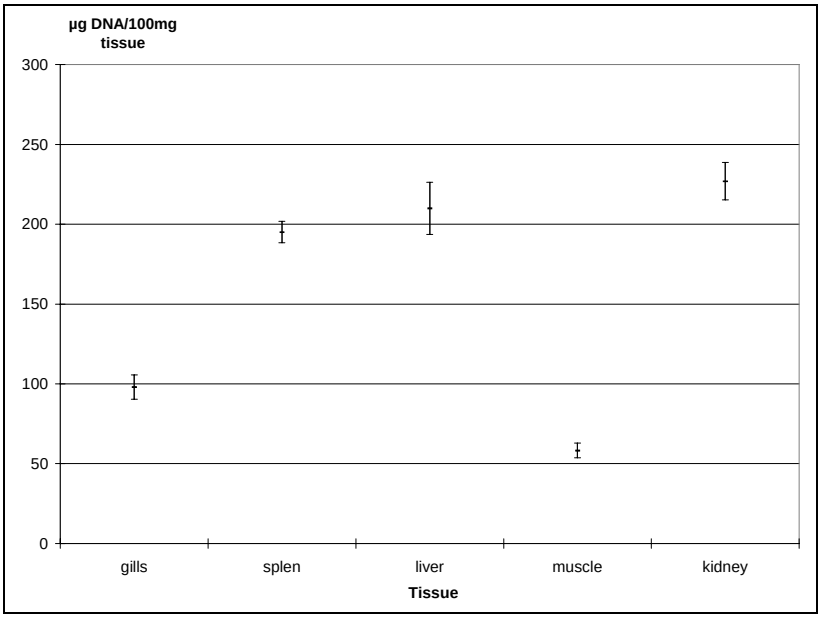

Figure 4 Total DNA quantities variability intervals for *Carassius auratus gibelio* Bloch.

Subsequently, using the average, superior and inferior limits values, the total DNA quantity variability intervals were graphic represented for all individuals (Figure 4).

From Figure 4, we observe that the variability intervals have in general grouped values. In this case, the highest variability is recorded for hepatic tissue which has its limits in the 226,367 – 193,672µg DNA/100mg tissue interval and the tissue with the smallest variability interval as well as the smallest total DNA concentration fluctuation, is the muscular tissue (62,892 – 53,693µg DNA/100mg tissue).

CONCLUSIONS

From the intervals' graphic representation, we observe that the variability limits for total DNA quantities are generally grouped around the average for all types of tissue, which shows once again their uniformity for different tissues.

For *Carassius auratus gibelio* Bloch. individuals we noticed that individual values have the same variation tendency and that they are grouped around the average value, showing a high central tendency.

Total DNA quantity average values, vary between 227,012µg DNA/100mg tissue for renal tissue and 58,293µg DNA/100mg tissue for muscular tissue

The highest variability for all analyzed individuals, was recorded for hepatic tissue, which limits are included in the 226,367 – 193,672µg DNA/100mg tissue interval, and the tissue with the smallest variability interval and implicit the smallest total DNA concentration fluctuations, is the muscular tissue (62,892 – 53,693µg DNA/100mg tissue).

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