4. RESULTS

4.1. Survival rate

Figure 4.1 indicates the survival rate of the different groups of trout during the biological trial. Considerable variability in survival is evident between groups and the main cause of the difference is related to a high initial mortality. For example the triploid control group has a high mortality in the first 14 days of the trial (approximately 30%) and subsequently mortality is low. This trend is also observed for the other groups in which most of the mortality occurred within the first 14 days although it is not as high as the triploid control the exception is the triploid group receiving the P poor diet which had a mortality of ~20%.

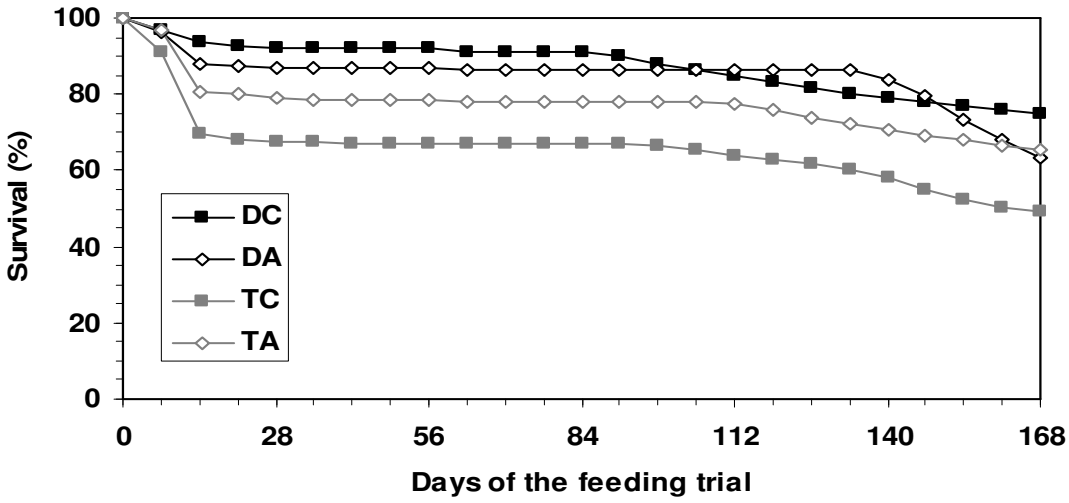


Figure 4.1 – Survival rate (%) in each experimental group during the feeding trial experiment. Data kindly provided by Drª. Stephanie Fontagné, INRA, France.

4.2. Biochemical analysis

4.2.1. Whole alevin Ca and P content

The biochemical parameters analyzed were total calcium concentration and total inorganic phosphorous concentration of whole alevin or specific bones using an

Nomenclature adopted: DC – Diploid control diet; DA – Diploid diet A; TC – Triploid control diet; TA – Triploid diet A; Body regions: VCR – Vizcero cranial; TCR – Trunco cranial; TCA- Trunco caudal; CA – Caudal region.

optimised colorimetric method. The calcium and phosphorus concentration for the whole body of trout alevin, 51, 64, 79 and 107 dpf and the results are presented in figure 4.2 (A and B). Trout with 219 dpf, 3 vertebras taken from the middle of each region of the vertebral column was determined the calcium and phosphorus concentration. In all experimental groups during early ontogeny, 51 dpf – 107 dpf a gradual accumulation of calcium was noticed with age (fig 4.2 A), this was not evident for phosphorus which from 64 dpf onwards was very similar P content in all experimental groups.

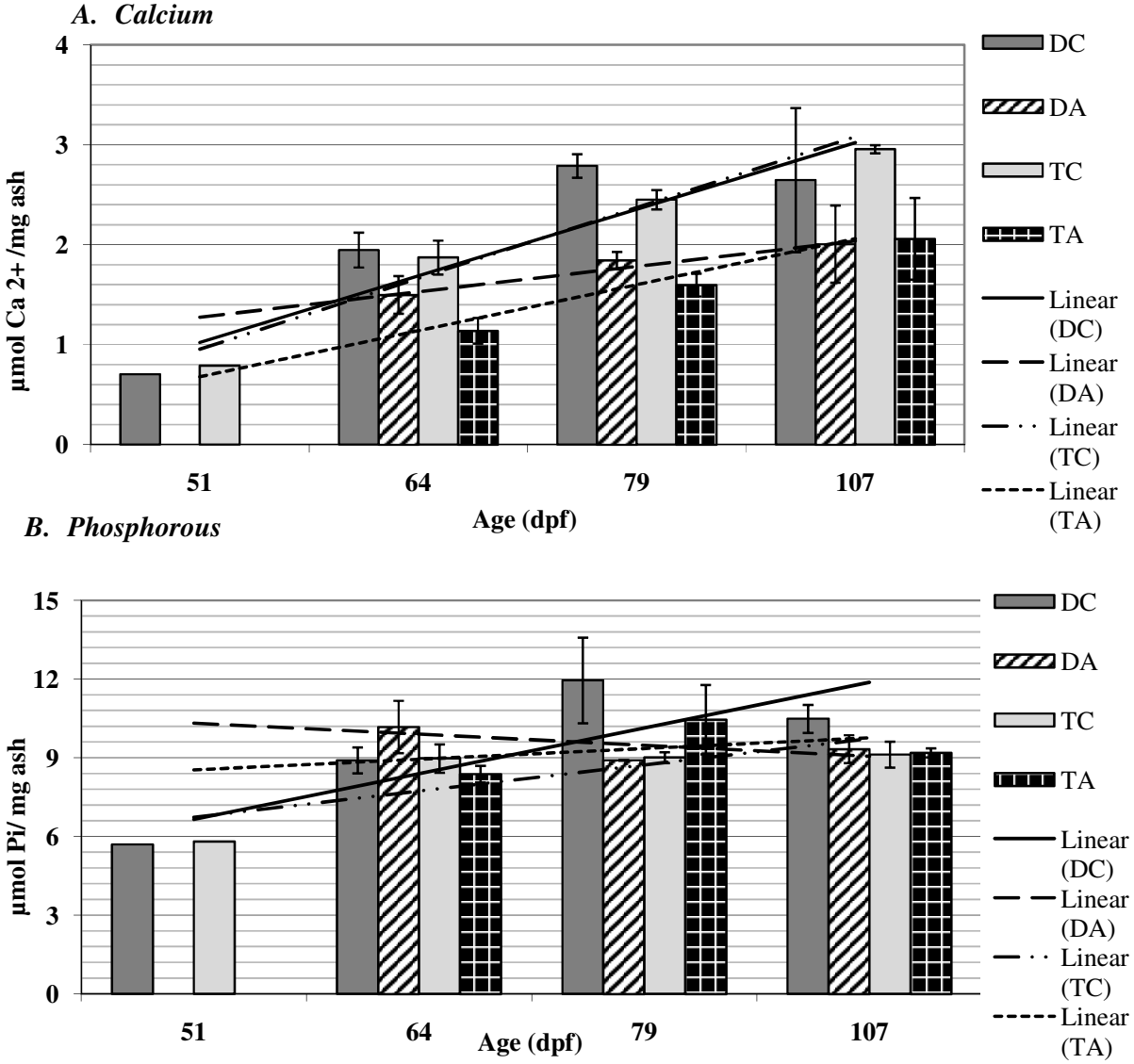


Figure 4.2 – Whole body calcium (A) and phosphorus (B) in rainbow trout alevin at 51, 64, 79 and 107 dpf**.** The mean calcium concentration per group is given as µmolCa/mg Ash and the mean phosphorus concentration is given as µmolCa/mg ash. The standard error is indicated for each group and a linear plot gives the trend for each group.

The accumulation of calcium with age occurs at a similar rate (slope) in DC and TC trout alevin. In contrast, the accumulation of calcium in TA and DA is significantly slower and the final concentration of Ca measured is lower than trout fed with control diets. Comparison of phosphorus and calcium reveals that the P content per larvae is much higher in the begeening of the experiment (~ 6 µmol P /mg ash) compared to less than 1 µmol Ca^{2+} /mg ash. The phosphorus content of the body increases to a maximum of 9-11 µmol P /mg ash while the calcium content gradually increases to reach a maximum of ~3 µmol Ca^{2+} /mg ash. The relatively high concentration of P relative to Ca is a reflection of the presence of significant levels of phosphorus in muscle and a number of other tissues while the Ca probably reflects its accumulation in developing scales and bones.

As animals grow they tend to gradually accumulate more calcium and phosphorous in their skeleton and this is evident even with the feeding restriction (figure 4.2 A), suggesting that increased efficiency of P uptake in P restricted diets. The phosphorous content is not significantly different between samples, although the Ca content varies significantly at 64 and 79 dpf between alevin of groups TC and TA groups and DC and TA ($p < 0.05$, table 4.1).

Table 4.1 – Results of the statistical analysis applied (student t- test) for Ca^{2+} samples. The heading of the table indicates the groups which were compared. = means no significant differences were detected; groups significantly different and their p (test significance) value are indicated.

Age (dpf)	DC vs DA	TC vs TA	DA vs TA	DC vs TC	DC vs TA	DA vs TC
64	=	$p < 0.05$	=	=	$p<0.05$	=
79	=	$p < 0.05$	=	=	$p<0.05$	=
107	=	=	=	=	=	=

The results obtained indicate that TC have significantly more Ca than TA and DC also has significantly more Ca than TA. In contrast, at 107 dpf, when the skeleton is fully formed no statistically significant differences are found. The different in calcium in early probably reflects the delay in skeletal formation when dietary minerals are restricted. The higher variability in the (TC) group may reflect difference in the timing of completion of skeletal ontogeny.

4.1.2. Body region calcium and phosphorous quantification results

In this section the results for the determination of the Ca and P content in ash prepared from vertebra that are situated in the middle point of each specific vertebral region (figure 4.3) are presented.

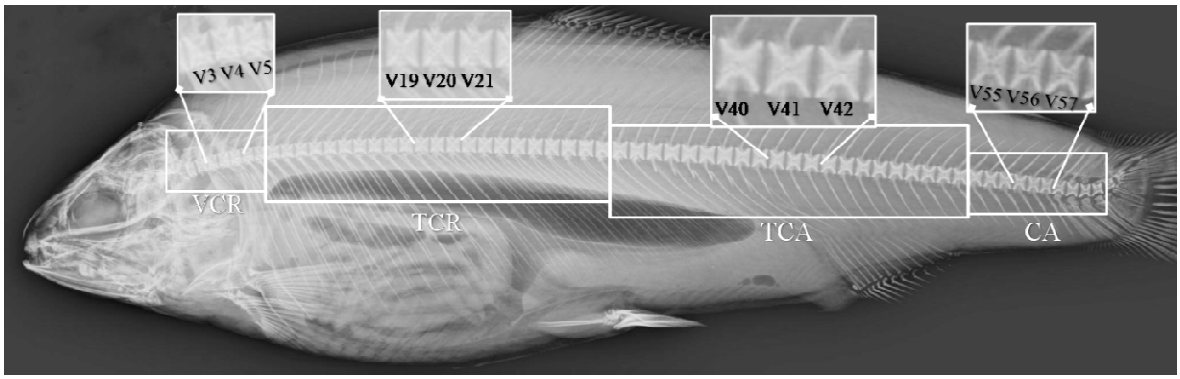


Figure 4.3 –Radiography of a rainbow trout diploid control of 219 dpf. Each body region considered is boxed and the verterba removed for subsequent analysis of Ca and P are presened in higher amplification. VCR – Vizcero cranial region corresponds to vertebra 1 – 8 and verterba taken for analysis were 3, 4 and 5; TCR - Trunco Cranial region, corresponds to vertebra 9 - 30 and the vertebra taken for analysis correspond to 19, 20 and 21; TCA – Trunco Caudal, corresponds to vertebra 31 - 51 and vertebra taken for analysis correspond to 40, 41 and 42; CA – caudal region includes vertebra 52 - 61 and the vertebra taken for analysis correspond to 55, 56 and 57.

Figure 4.4 presents the results for Ca and P content in the vertebra from the different body regions. The calcium content is variable between vertebra of the different regions and diet also causes a significant difference in some regions. The most variable regions in relation to Ca content are VCR and TCA and significant differences exist between groups DC and DA and also between DA and TC (table 4.1). Revealing that in diploid trout fed with a P deficient diet for 3 month, and fed with a complete diet subsequently "overcompensated" and incorporated significantly higher levels of Ca into vertebra compared to trout fed a control diet or triploid trout fed P deficient or a control diet.

The P content of trout fed with P deficient diet for 3 months and then fed with a diets with a normal P content for 3 months also was significantly modified in the vertebra of the vizcero- cranial and trunco-caudal region. The P content in vertebra of the VCR and CR was significantly higher in diploid trout fed the P deficient diet compared to DC which is similar to what was observed for Ca. In the triploid fish fed a P deficient diet

the level of P in the vertebra of the VCR also appeared to be significantly increased in relation to the control diet compared to the DC and TC trout.

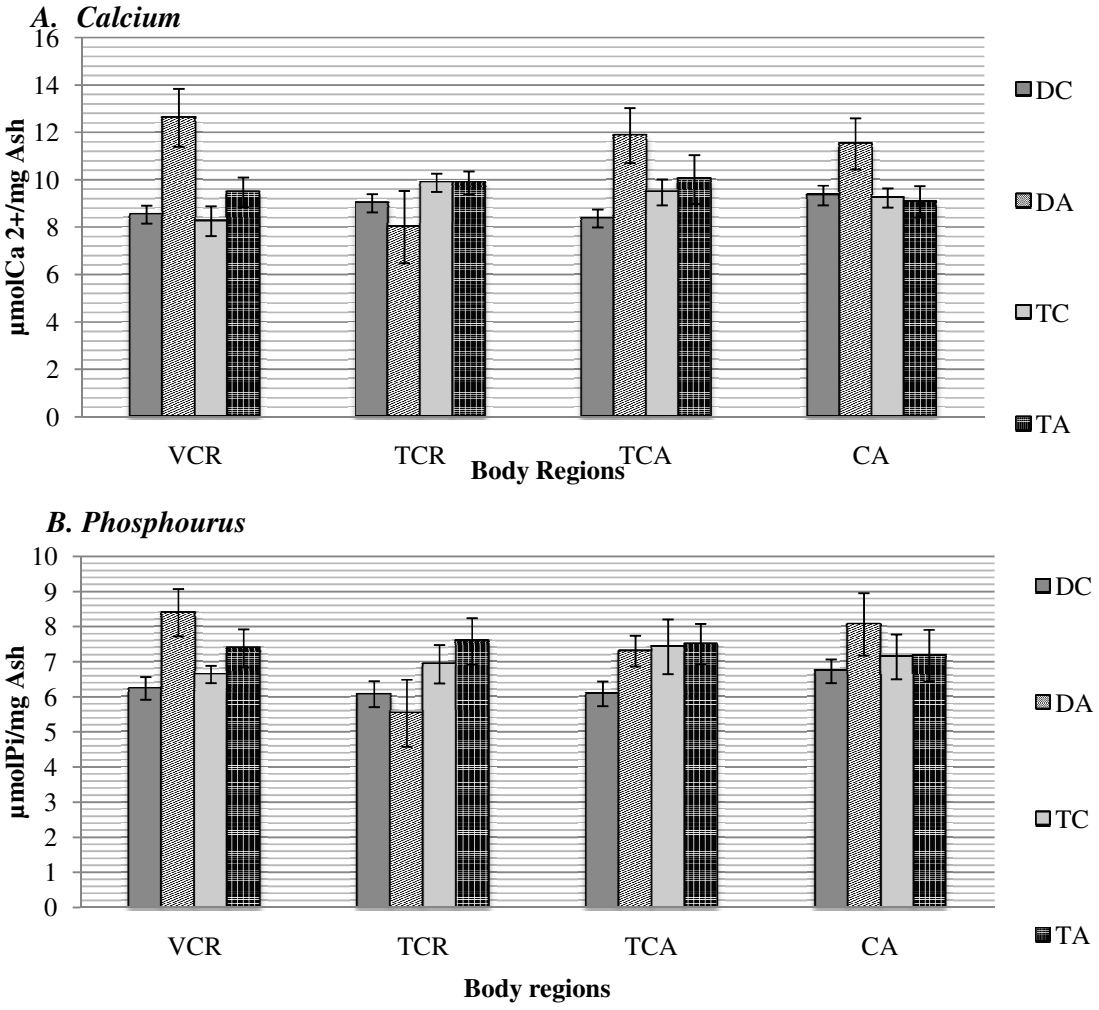


Figure 4.4 – A. Total mean Ca concentration (µmolCa^{2+}/mg Ash) and the SEM from 3 vertebra from the mid-point of each region identified in rainbow trout (n = 10) of 219 dpf (168 days and 6 month of feeding). B. Total mean Pi concentration (µmolPi/mg Ash) and the SEM from 3 vertebra from the mid-point of each region identified in rainbow trout (n = 10) of 219 dpf (168 days and 6 month of feeding).Body regions: VCR - vizcero cranial; TCR – Trunco cranial; TCA – Trunco caudal; CA – Caudal.

It appears that the vertebra of both diploid and triploid trout fed diets deficient in P are able to recover both Ca and P content when fed an adequate diet for 3 months after 3 months on a P poor diet and that an "overcompensation" occurs as levels of the minerals are significantly higher in DA compared to both DC and TC (table 4.2). The effect of

modified P availability is different according to the region of the vertebral column studied and the VCR and TCA region are most susceptible to change although the basis for the difference in response in vertebra along the vertebral column remains to be established.

Table 4.2 – Results of the t-student performed to establish if Ca or P in vertebra of the different regions of the vertebral column differed signifcantly between groups fed with diets with a different P content. (=) no significant differences detected; significant differences (test significance) value are indicated.

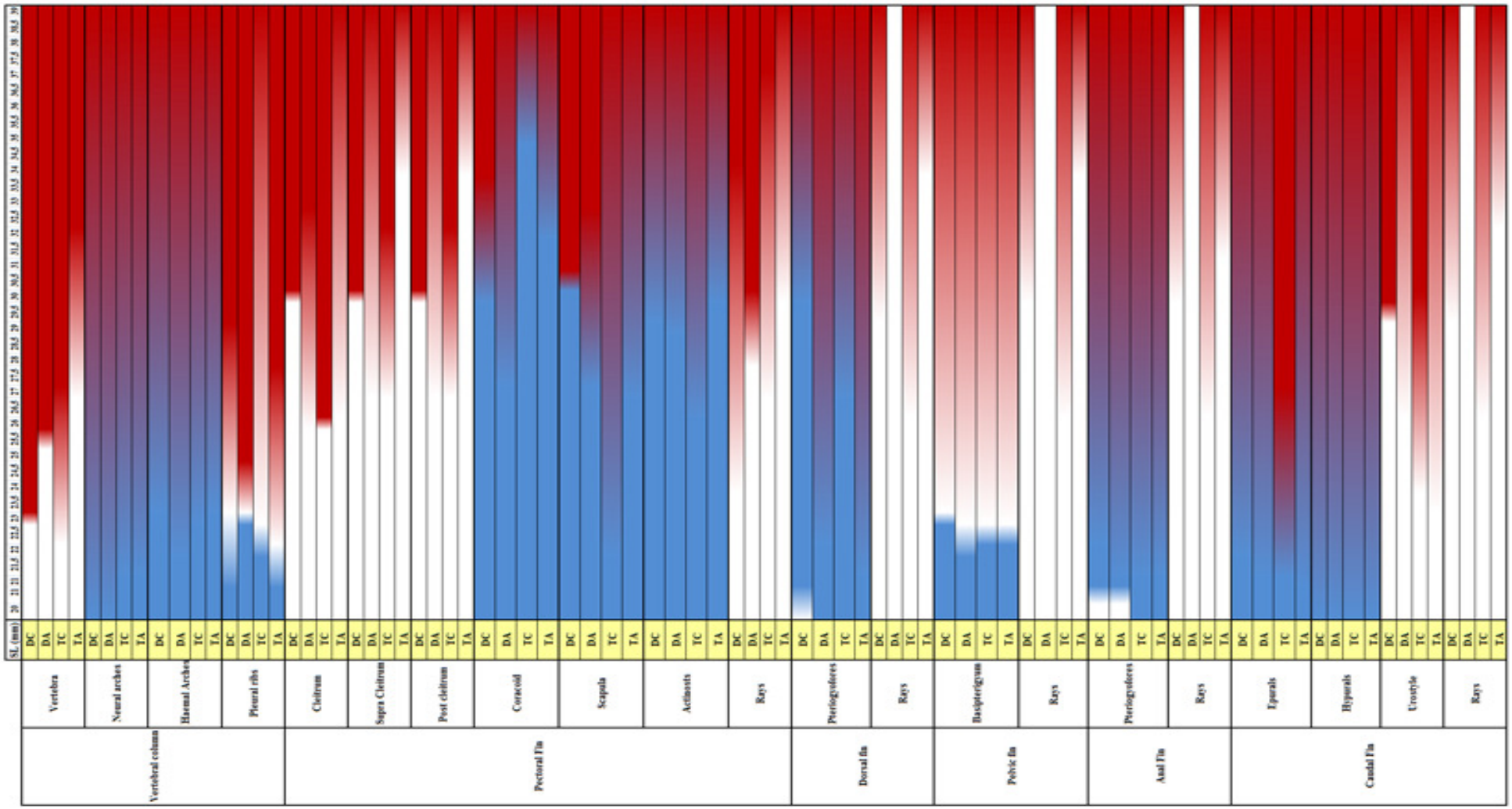
t-student results summary	VCR	TCR	TCA	CA
A. Calcium	DA>DC p<0.05 DA>TC p<0.05	=	DA>DC p<0.05 DA>TC p<0.05	=
B. Phosphorous	DA>DC p<0.05 DA>TC p<0.05	=	DA>DC p<0.05 TA>DC p<0.05	=

4.2 Morphological and Biometric Analysis

4.2.1. Ontogenic Evolution

Whole-mount differential cartilage and bone staining allowed the establishment of the ontogeny of the main skeletal structures considered (see 3.Methodology, table 3.4) in the trout alevin from the experiment. The skeletal ontogeny was characterised in 10 specimens from each experimental group (DC, DA, TC and TA) from 51 dpf until 79 dpf using as the reference between groups standard length (SL). Figure 4.5 presents a schematic representation of the results obtained, with cartilage shown in blue, bone indicated by red and transition structures shown by faded blue or red. The analysis was not exhaustive as samples were not taken daily but the results of the analysis give an overview of the effect of modified P availability and triploidization on skeletal ontogeny.

Figure 4.5 - Diagram representing the ontogenic development of rainbow trout skeleton in relation to SL. Blue colour represents cartilage; red colour represents the ossified bone; intermediate colour represents transition phase. Body structures that vary from blue to red are endocondral bone. Structures that vary from white to red represent dermal bone.


SL (mm)
DC
DA
TC
TA
Vertebra
Vertebral column
Neural arches
Haemal Arches
Pleural ribs
Cleithrum
Supra Cleithrum
Post cleithrum
Coracoid
Scapula
Actinosts
Rays
Pectoral Fin
Pterygiophores
Rays
Dorsal fin
Basipterigium
Rays
Pelvic fin
Pterygiophores
Rays
Anal Fin
Epurals
Hypurals
Urostyle
Rays
Caudal Fin

Overall the skeleton of diploid trout fed a normal diet start to ossify before any of the other groups and the triploid trout regardless of dietary manipulation present a delayed ossification in relation to the diploid trout. In all experimental groups a general pattern emerges in relation to the time phase of development of the different structures considered. The vertebra (in all vertebral column) and pelvic fin develop earlier in relation to other structures presumable because of their importance for locomotion and their feeding, predator avoidance and thus survival. In contrast (the accessory structures of the pectoral fin, the cleithrum etc of the pectoral fins) is much delayed and as it is a dermal structure emerges immediately as bone. The anal fin is the last fin to ossify. A complex pattern emerges in relation to the effect of ploidy level and diet on the skeleton formation which is considered in more detail in the subsequent section in which cumulative counts (total number of ossified or transition structures, whether endocondral or dermal) are used (figure 4.6).

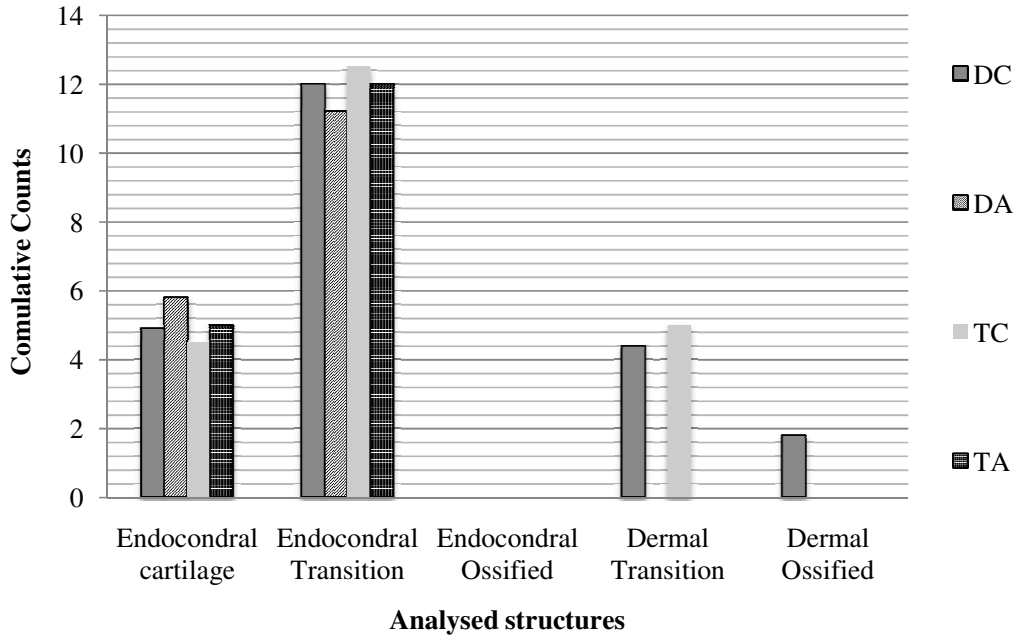


Figure 4.6 – Cumulative counts ± SEM, sum of all analyzed structures in each experimental group at 64 dpf [Relation with figure 4.5 - Endocondral cartilage (blue colour), Endocondral Transition (transition between blue and pink), Endocondral Ossified (Pink or red) and Dermal transition (light red) and Dermal ossified(red)].

Nonetheless, overall the skeletal ontogeny is conserved between the diploid and triploid fish, although diploid trout (DC and DA) are more similar and triploid trout (TC and TA) are more similar. The feeding of a diet deficient in P to triploid trout appears to cause a significant delay in skeletal formation and ossification which is not so evident in diploid trout fed the same diet.

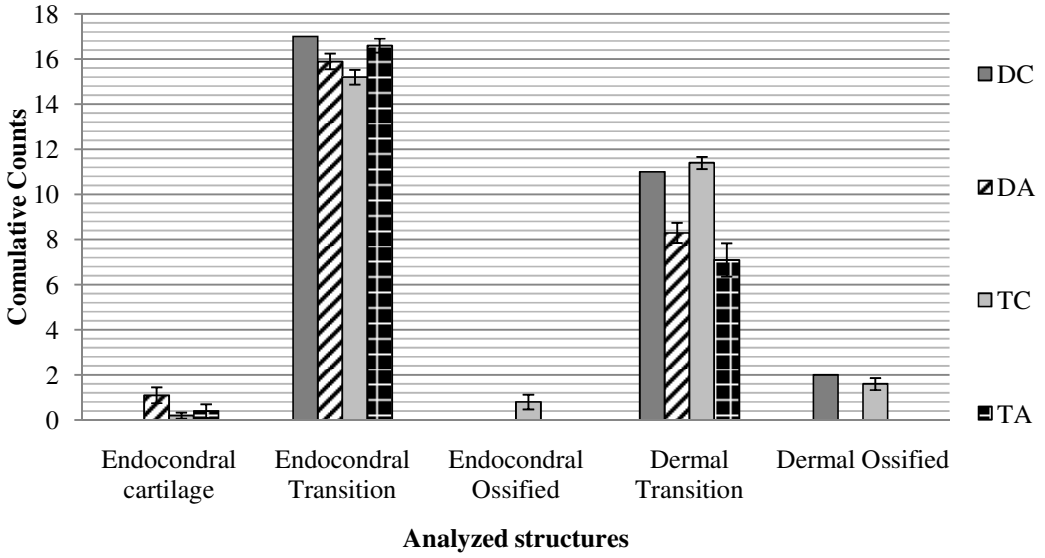


Figure 4.7 – Cumulative counts± SEM, sum of all analyzed structures present in each treatment evaluated, in animals with 79 dpf. The bars represent a standard error in each group. [Connection with figure 4.5 - Endocondral cartilage (blue colour), Endocondral Transition (transition between blue and pink), Endocondral Ossified (Pink or red) and Dermal transition (light red) and Dermal ossified (red)].

Interestingly the dermal structures seem to be most significantly (table 4.3) affected by the P deficient diet and group DC and TC have a significantly greater number of dermal transition structures and calcified structures in relation to the DA and TA groups. The effect of the experimental treatments on endochondral ossification is less evident but an interesting observation is the significantly lower number of endochondral transitional structures in the TC group which is the only group with ossified endochondral structures, at 79 dpf (figure 4.7).

The results of statistical analysis of the results presented in figures 4.6 and 4.7 are summarised in table 4.3. It is clear that there is a significant difference in dermal transitional structures caused by the P deficient diet fed to diploid and triploid trout. A similar significant delay is observed in the appearance of ossified structures.

Table 4.3 –Results presented for endochondral and dermal ossification. **A** Student t-test was performed to compare the experimental groups in relation to status of endochondral and dermal ossification. (=) no significant differences detected; p - the level of significance p<0.05.

Age dpf	Hypothesis tested	Endocondral Cartilage	Endocondral Transition	Endocondral Ossified	Dermal Transition	Dermal Ossified
64	**DC vs DA**	=	=	=	p<0.05	p<0.05
	TC vs TA	=	=	=	p<0.05	=
	DC vs TC	=	=	=	=	p<0.05
	DA vs TA	=	=	=	=	=
	DC vs TA	=	=	=	p<0.05	p<0.05
	DA vs TC	=	=	=	=	=
79	**DC vs DA**	p<0.05	p<0.05	=	p<0.05	p<0.05
	TC vs TA	p<0.05	p<0.05	p<0.05	=	p<0.05
	DC vs TC	=	p<0.05	p<0.05	=	=
	DA vs TA	=	=	=	=	=
	DC vs TA	=	=	=	p<0.05	p<0.05
	DA vs TC	=	=	p<0.05	=	p<0.05

Analyzing the results in figure 4.6 it is clear that DC is the only group with ossified dermal structures and dermal structures in transition are only present in DC and TC. None of the trout fed with the P deficient diet (A) have ossified dermal structures and none of the trout analyzed at 64 dpf had ossified endochondral structures.

The data presented in figure 4.7 for trout at 79 dpf reveals that in DC group all endochondral structures are in transition and neither cartilage only or totally ossified structures are detected. DC and TC are the only groups in which ossified dermal structures are present at 79 dpf.

4.2.2 X-rays analysis

Radiographies of trout from 135 dpf to 219 dpf were used to establish meristic counts and to analyse the skeleton. Table 3.5 in Methodology indicates the number of radiographed trouts from each experimental group. A number of different parameters were measured by analyzing radiographies and measuring specific parameters which are presented in the following figures.

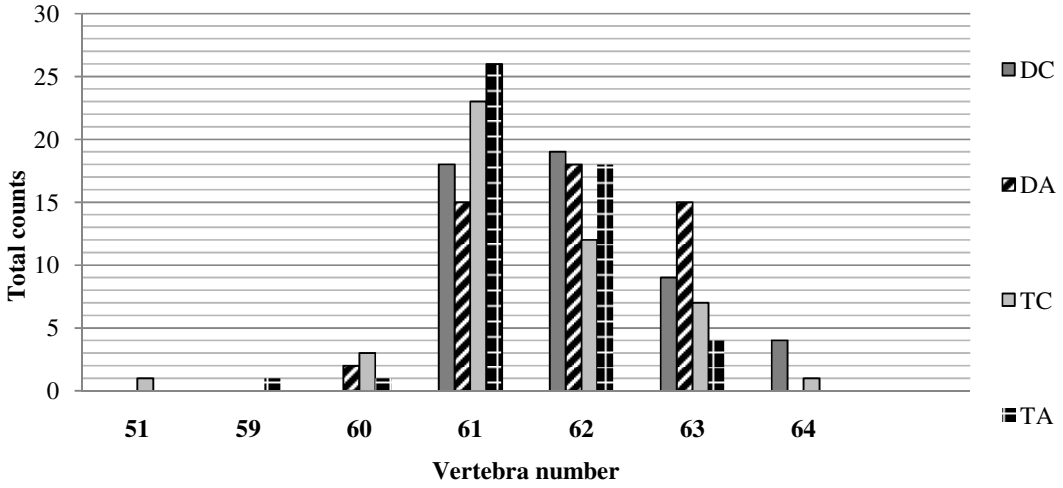


Figure 4.8 - Total number of vertebra determined in individuals with completely ossified vertebral column (considered samples between 135 and 219 dpf) in DC, DA, TC and TA samples.

In general the total number of vertebra were similar in each of the experimental groups. A major difference observed was that the triploid trout generally had 1 less vertebra (n=61) in the vertebral column than the diploids (n=62) and this was even more evident in the triploids fed with P deficient diet (figure 4.8). In the diploid trout fed with the P deficient diet the effect was contrary to that seen in the triploid trout as the number of vertebra increased and 15 specimens had 63 vertebra compared to 9 specimens in DC, 7 animals in TC and 4 in TA.

Other parameter studied was the dimension of the vertebra in the 4 main body regions (Vizcero cranial, Trunco cranial, Trunco caudal and Caudal region) considered in trout (figure 4.9). Taking into consideration the growth of the trout during the experiment

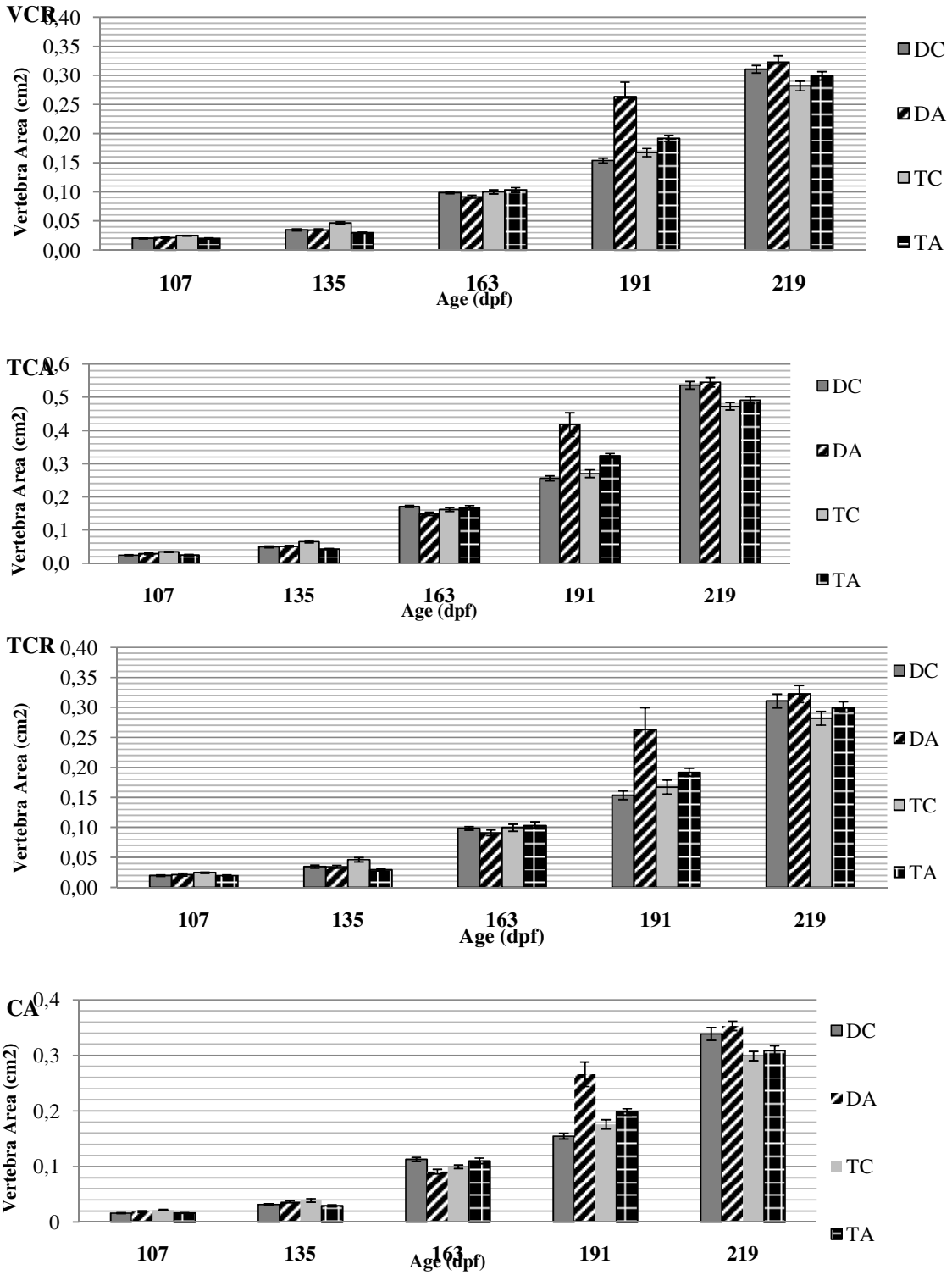


Figure 4.9 – Mean area ± SEM (cm^2) of vertebra from each vertebral region: VCR, TCR, TCA and CA, for specimens from treatments DC, DA, TC and TA and in each age studied 107, 135, 163, 191 and 219 dpf.

each of the different age groups analyzed was considered independently and as expected a gradual increase in the area of vertebra occurred as the trout grew. The modification in P availability had a small but noticeable effect on vertebral area in the VCR and this was particularly notable for DA at 191 dpf. In fact, the area of the vertebra in all body areas in DA was significantly greater than those of the other experimental groups and may represent a compensatory response to the increase in phosphate availability after feeding with a diet with adequate P content at 135 dpf. The larger vertebral area was still evident in DA trout compared to all other groups (DC, TC, TA) at 219 dpf, although was not as notable. In general, the vertebra of triploid trouts irrespective of the diet, was significantly smaller than diploid trouts, at 219, dpf and this was particularly evident in vertebra in the TCA and CA regions.

In table 4.4 statistically significant differences in the area of the vertebra between groups is presented. A very interesting observation arising from the data is the very well conserved area of the vertebra at a given age and this is evident from the extremely small error bars encountered within each group. The mechanisms which regulate the growth of the vertebra and the final size achieved are largely unstudied and understanding this mechanism represents an important challenge.

It remains to be established if some of the differences observed between specimens of the various experimental groups are reflective of allometric differences as all the trout analysed are still developing and may present different growth patterns. Such differences in growth might be expected to be also found associated with the internal structures and further work will be required to establish if this is a factor and then to integrate this factor into the general analysis.

Table 4.4 - Results of t-student and each hypothesis tested for vertebra area for different experimental groups and restricted to a specific age group. (=) no significant differences detected; the level of significance differences between groups p are indicated, p<0.01; p<0.05 and P<0.001.

Body regions	Hypothesis tested	107	135	163	191	219
VCR	DAvsDC	=	=	p < 0.05	p <0.05	=
	TAvsTC	p < 0.05	p < 0.05	=	p < 0.05	=
	DCvsTC	p < 0.05	p < 0.05	=	=	p < 0.05
	DAvsTA	=	p < 0.05	=	=	=
	DCvsTA	=	p < 0.05	=	p < 0.05	=
	DAvsTC	=	p < 0.05	=	=	p < 0.05
TCR	DAvsDC	p < 0.05	=	=	p < 0.05	=
	TAvsTC	p<0.05	p<0.05	=	p < 0.05	p < 0.05
	DCvsTC	p<0.05	p <0.05	=	=	p < 0.05
	DAvsTA	=	p <0.05	p < 0.05	=	p < 0.05
	DCvsTA	p < 0.05	=	=	p < 0.05	=
	DAvsTC	=	p<0.05	=	P <0.05	p < 0.05
TCA	DAvsDC	=	=	p < 0.05	=	=
	TAvsTC	p <0.05	p<0.001	=	p <0.05	=
	DCvsTC	p<0.05	p <0.001	=	=	p < 0.05
	DAvsTA	=	p < 0.005	=	=	p < 0.05
	DCvsTA	=	p < 0.05	=	p < 0.05	p < 0.05
	DAvsTC	=	p < 0.005	=	=	p < 0.05
CA	DAvsDC	=	=	p < 0.05	p < 0.05	=
	TAvsTC	p <0.05	p < 0.05	=	p < 0.05	=
	DCvsTC	p<0.05	p < 0.05	p < 0.05	p < 0.05	
	DAvsTA	=	p <0.05	p < 0.05	=	p < 0.05
	DCvsTA	=	=	=	p < 0.05	p < 0.05
	DAvsTC	=	=	=	p < 0.05	p < 0.05

Nonetheless, despite the difference in vertebral area the general growth curves were very similar for each group, although there was a trend in which the triploid trout were bigger than the diploid trout in early stages (107 - 191 dpf) but on the end of the experiment the diploid trout were always bigger (figure 4.10). The supply of P in the diet did not appear to affect the growth performance of diploid or triploid trouts.

On the whole the reduction of length in the triploid trout was also associated with a change in the relative length of the body and head and the results are presented as a ratio head length : column length (figure 4.11).

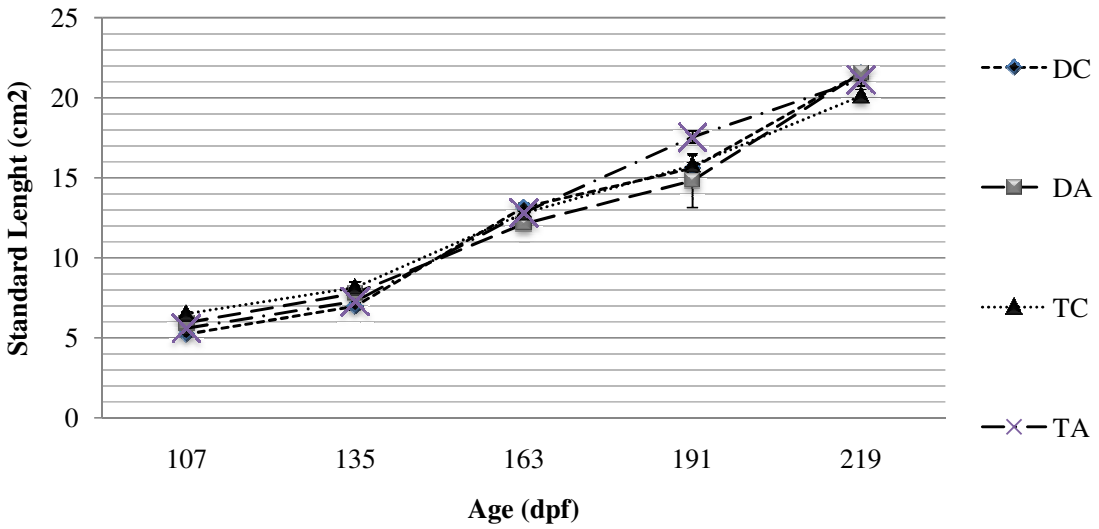


Figure 4.10 – Standard length measurements mean (cm^2) from each group of trout measured in each studied group, in every sampled age. The bars represent a standard error in each group.

Significant differences existed between the experimental groups in the ratio of head length: column length and this relationship varied with age, suggesting different patterns of allometric growth in the experimental groups (table 4.5). At 219 dpf, a significant difference is observed in head length: column length between triploid and diploid control trout and also between DC and TA, confirming the general observation that triploids are smaller than diploids and have modified body dimensions.

In summary, significant differences in length were observed between experimental groups at each age studied and this was particularly evident at several different ages (table 4.5). Comparison of the ratio of head to body length revealed the greatest differences between experimental groups were observed at 191 and 219 dpf. By 219 dpf, significant differences were mainly observed between diploid and triploid trout suggesting that ploidy manipulation may be a key factor in changing body form.

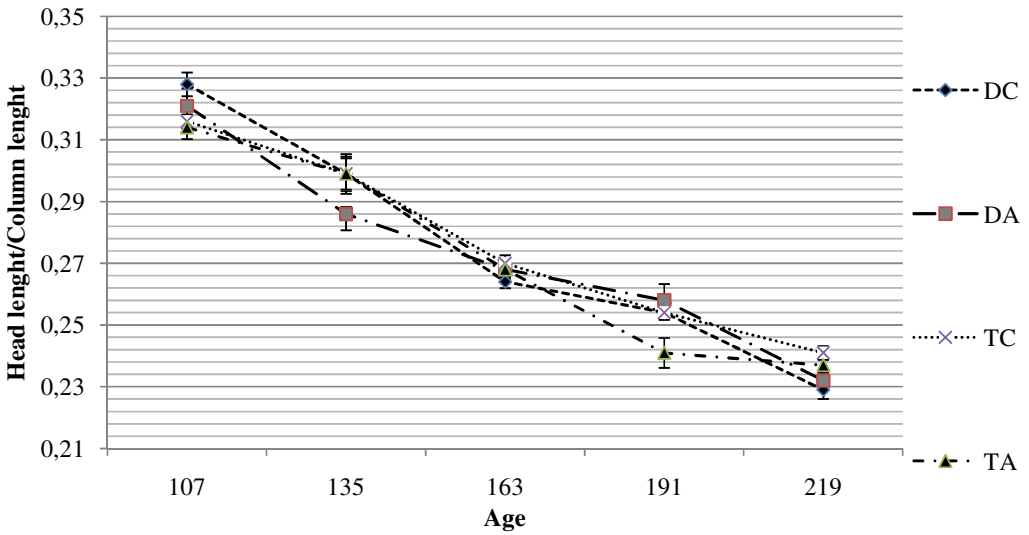


Figure 4.11 –Ratio of head length: body length presented as mean ± SEM (cm^2) for each of the experimental groups analysed at each age.

Table 4.5 - T-student test results and each hypothesis tested for the measurement taken for the standard length and ration head length: vertebral column, for the different treatments and ages considered. = there are no significant differences; if there are differences between groups p indicates the level of significance $p < 0.05$.

AGE	hypotheses tested	107	135	163	191	219
SL	DAvsDC	=	DA>DC p < 0.05	DC>DA p < 0.05	=	=
	TAvsTC	TC>TA p < 0.05	TC>TA p < 0.05	=	TA>TC P < 0.05	=
	DCvsTC	TC>DC p < 0.05	TC>DC p < 0.05	=	=	DC>TC P < 0.05
	DAvsTA	=	=	=	=	=
	DCvsTA	=	=	=	TA>DC p < 0.05	=
	DAvsTC	=	=	=	=	=
Ratio Head Lenght: Column lenght	DAvsDC	=	=	=	=	=
	TAvsTC	=	=	=	TC>TA p < 0.05	=
	DCvsTC	DC>TC p < 0.05	=	=	=	TC>DC P<0.05
	DAvsTA	=	=	=	DA>TA p < 0.05	=
	DCvsTA	DC>TA p <0.05	=	=	DC>TA p < 0.05	TA>DC p < 0.05
	DAvsTC	=	=	=	=	TC>DA p < 0.05

4.3 Histology - muscle myotome total counts

The total number of myotomes where determinate in each experimental group at 64 and 79 dpf and the results are presented in table 4.6.

Table 4.6 – Mean and standard deviation and standard error of the mean (SEM) of the total myotome number per in transverse sections of trout from each treatment group. (n= individuals and X sections/ individual).

Treated Groups	64 dpf			79 dpf		
	Mean	**Std Dev**	**SEM**	**Mean**	**Std Dev.**	**SEM**
DC	42	2,40	0,49	47	1,73	0,35
DA	41	1,95	0,38	45	1,56	0,31
TC	40	2,26	0,44	47	2,31	0,47
TA	38	1,67	0,33	43	1,76	0,34

Hypothesis tested

Age	**DAvsDC**	**TAvsTC**	**DCvsTC**	**DAvsTA**	**DCvsTA**	**DAvsTC**
64 dpf	DC>DA p <0.05	TC>TA p<0.05	DC>TC p<0.05	DA>TA p<0.05	DC>TA p<0.05	=
79 dpf	DC>DA p<0.05	TC>TA p<0.05	=	DA>TA p<0.05	DC>TA p<0.05	TC>DA p<0.05

Table 4.6 – Mean and standard deviation and standard error of the mean (SEM) of the total myotome number in transverse sections of trout from each treatment group. The asterisk indicates the groups where some of the tissue sections where damaged and impossible to count (3 individuals and 30 sections/ individual).

Treated Groups	64 dpf			79 dpf		
	Mean	**Std Dev**	**SEM**	**Mean**	**Std Dev.**	**SEM**
DC	42	2,40	0,49	47	1,73	0,35
DA	41	1,95	0,38	45	1,56	0,31
TC	40	2,26	0,44	47	2,31	0,47
TA	38	1,67	0,33	43	1,76	0,34

Hypothesis tested

Age	**DAvsDC**	**TAvsTC**	**DCvsTC**	**DAvsTA**	**DCvsTA**	**DAvsTC**
64 dpf	DC>DA p <0.05	TC>TA p<0.05	DC>TC p<0.05	DA>TA p<0.05	DC>TA p<0.05	=
79 dpf	DC>DA p<0.05	TC>TA p<0.05	=	DA>TA p<0.05	DC>TA p<0.05	TC>DA p<0.05

There are significant differences in the myotomes number at both ages analyzed. Results presented in table 4.6 shows that at 64 dph diploid trout have, in average, an increased total number of myotomes. In each group there are also differences regarded the differences in diets. These are statistically significant ($p < 0.05$) differences.

In contrast at 79 dph animals fed with control diet have the same number of myotomes. Significant differences inside each group (DC vs DA and TC vs TA), are highly significant.

The group that presented less myotomes were the triploids fed with diet A, which have a total of 38 myotomes at 64 dpf and 43, at 79 dpf.

4.4 Molecular Analysis- Gene expression

4.4.1 β_actin

To establish the best gene for normalisation of the quantity of cDNA introduced into PCR reactions a comparison between the genes 18S and β-actin was carried out. It was observed that 18S was variable between groups and that β-actin did not vary significantly between experimental groups. For this reason β-actin was used for normalization of the other genes which were amplified.

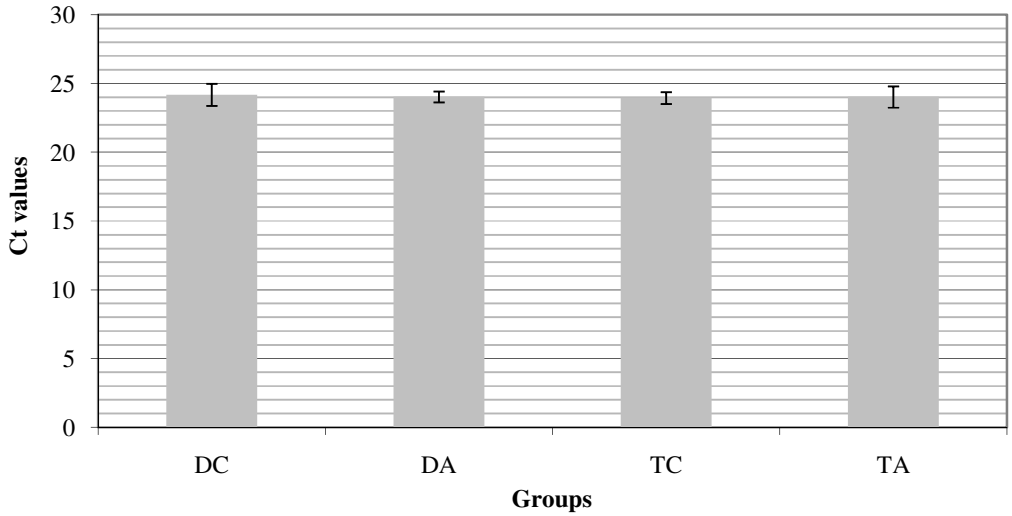


Figure 4.12 – Representation of the Ct value for the sample expression of β-actin gene. Mean for the total Ct values obtained for each treatment group (n= 6). The bars represent a standard error in each group.

In general, the Ct value results of each group were found to be approximately 24 (Figure 4.12) and relatively little variation was observed between any of the experimental groups making β-actin an excellent choice for normalisation. The SEM is slightly higher for the groups DC and TA as they both have one individual with a Ct value substantially different from the other individuals (table 4.7).

Table 4.7 – CT values results, mean value Standard error and SEM, for a threshold of 20, for the β-actin gene analyzed in every group.

Group	Ct values						Mean	Std Dev	SEM
DC	24.1	27.9	23.7	23.7	23.7	22.1	24.18	1.96	0.79
DA	23.9	24.1	23.1	24.9	23	25.4	24.04	0.96	0.39
TC	24.5	24.9	24.5	22.1	24.5	23.3	23.96	1.06	0.43
TA	26.9	22.9	23.3	24.4	22.7	-	24.03	1.73	0.77

4.4.2 Osteocalcin

The level of osteocalcin expression in the caudal region of 64 dpf animals is low (figure 4.13). This is one of the reasons for the use of q RT-PCR, due to the sensitivity of the method. Although due to variation (SEM) in the total average of the log Square means of each samples, there are no statistical differences detected between groups.

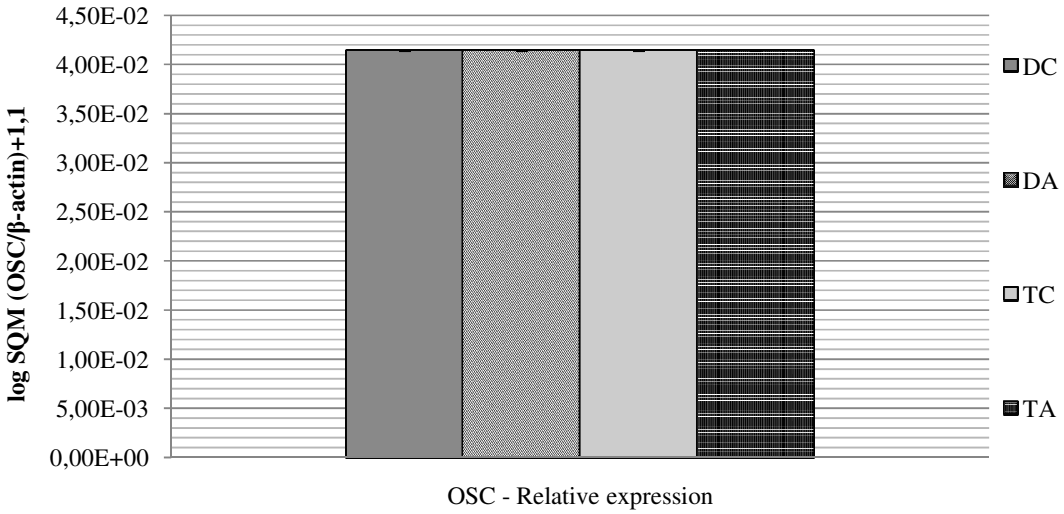


Figure 4.13 – Logarithm of the ratio of the square means of the expression of osteocalcin versus relative expression of β-actin. Each column represent the average of the logarithm of the relative expression from the OCN gene**.** The bars represent the SEM in each group.

4.4.3 Osteonectin

Osteonectin qRT_PCR results revealed that this is a transcript with a variable expression in the sample of RNA from the caudal region of the fish which is composed of muscle, skin, bones and rays. No significant differences are found in transcript abundance due to its variable expression in different individuals (see the relatively high

SEM) which are presented as log Square mean of each treatment. A general trend that is evident (figure 4.14) is the lower expression of osteonectin transcripts in DC and TC relative to DA and TA. This suggests that diets poor in phosphorous may the expression of the osteonectin gene.

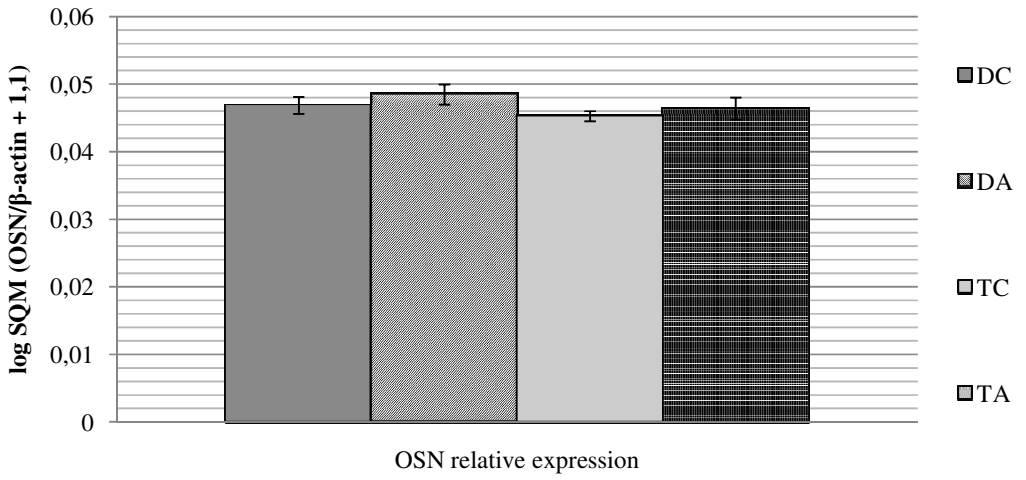


Figure 4.14 – Logarithm of the ratio of the square means of the expression of Osteonectin versus relative expression of β-actin. Each column represents the average of the logarithim of the relative expression from the OSN gene**.** The bars represent the SEM in each group.

4.4.4 Osteopontin

The expression of osteopontin transcripts as determined using qRT-PCR reveals that this transcript is fairly abundant. Moreover, the expression shows relatively little variation in individuals from DA and in TC and TA is slightly more variable. No significant difference in transcript abundance was identified between any of the groups as a consequence of the low SEM only the diploid control have a small increase in the SEM bar (figure 24).

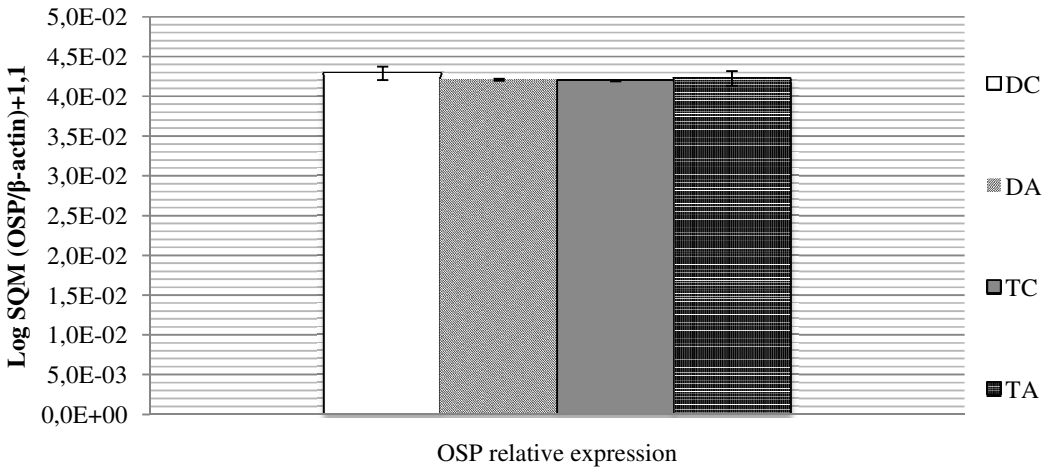


Figure 4.15 – Logarithm of the ratio of the square means of the expression of Osteopontin versus relative expression of β-actin. Each column represents the average of the logarithim of the relative expression from the OPN gene**.** The bars represent the SEM in each group.

The transcript abundance of osteopontin is slightly higher in DC which is opposite to the lower transcript abundance of osteocalcin results in the same group.