



*Effects of phospholipids and HUFA levels on ontogenetic development and performance of pikeperch (*Sander lucioperca*) larvae*



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The outcome of the experiments should provide information leading to the WP 10.2 deliverable:

“Providing a protocol for optimal enrichment to optimize performance and reduce stress sensitivity of pike perch larvae”

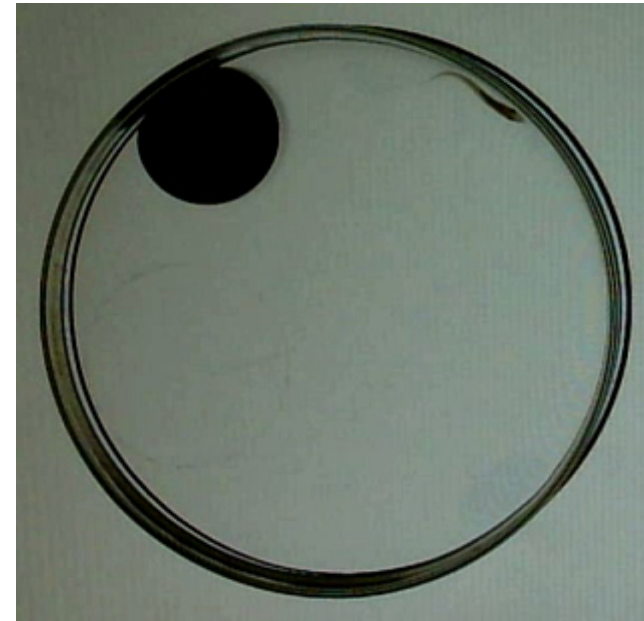
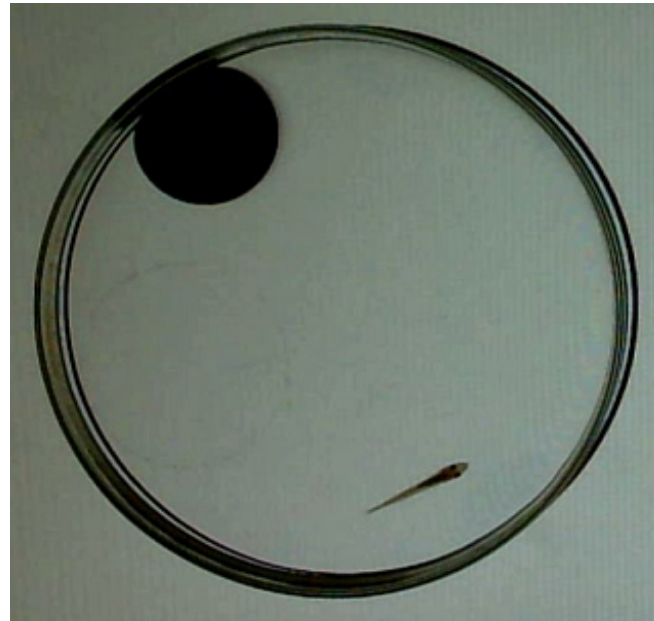
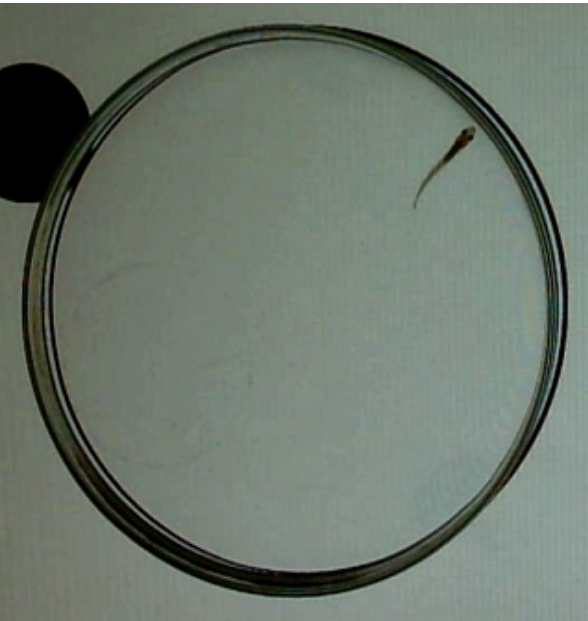
From previous pike perch larval live feed emulsion studies we know that:

- ✓ n-3 HUFAs - especially DHA has a positive effect on stress sensitivity
- ✓ n-3 HUFAs increase neural development and brain size
- ✓ Dietary phospholipids improve growth of larvae

- Levels of phospholipids in formulated diets ?
- Possible physiol. effects by additional n-3 HUFA supplementation ?

Previous studies

Behaviour of larvae in absence and presence of a simulated predator



Dietary composition of the 6 experimental diets.



Diet Ingredients (%)	PL1	PL2	PL3	PL1H1	PL2H2	PL3H3
MicroNorse Fish Meal^a	45	45	45	45	45	45
CPSP 90^b	7	7	7	7	7	7
Squid meal^c	13	13	13	13	13	13
Fish gelatin^d	1	1	1	1	1	1
Wheat Gluten^e	4.4	4.4	4.4	4.4	4.4	4.4
Wheat meal^f	6.1	5.9	5.6	6.1	5.9	5.6
Algatrium DHA70^g	0.0	0.0	0.0	0.6	2.0	3.4
Olive oil^h	18.9	12.1	3.4	18.4	10.1	0.0
Vitamin & Mineral Premix PV01ⁱ	1.0	1.0	1.0	1.0	1.0	1.0
Soy lecithin powder^j	3.0	10.0	19.0	3.0	10.0	19.0

Analytical composition of exp. diets

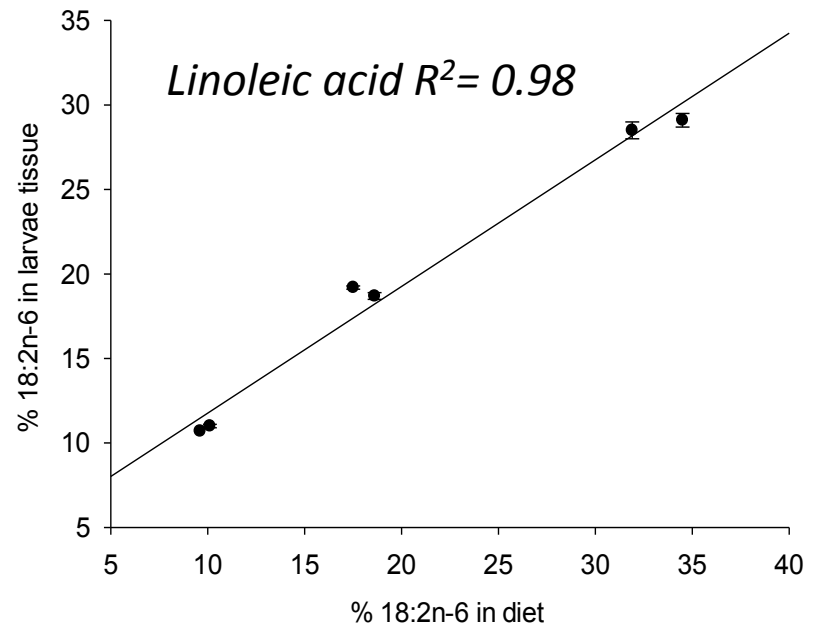
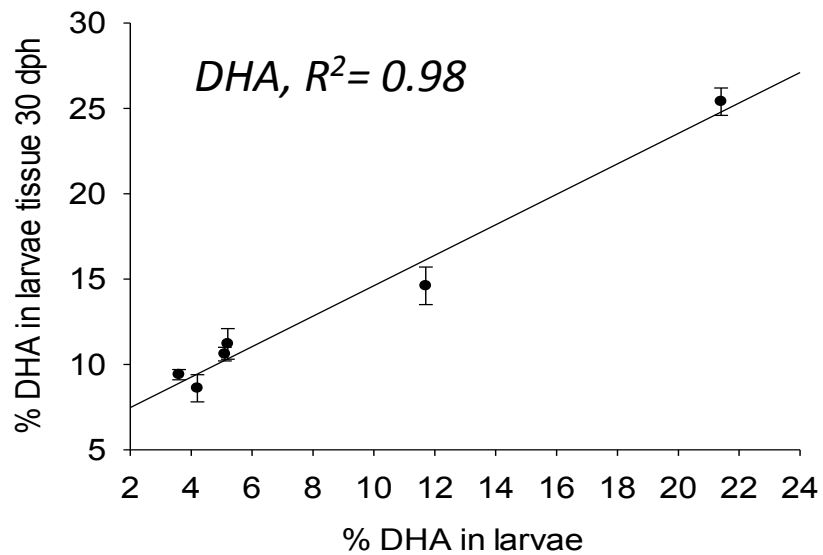
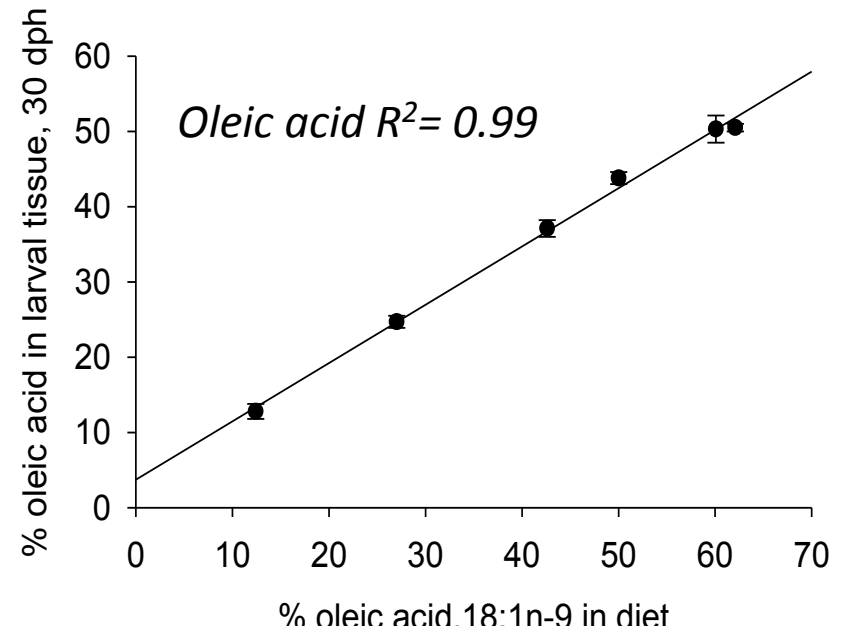
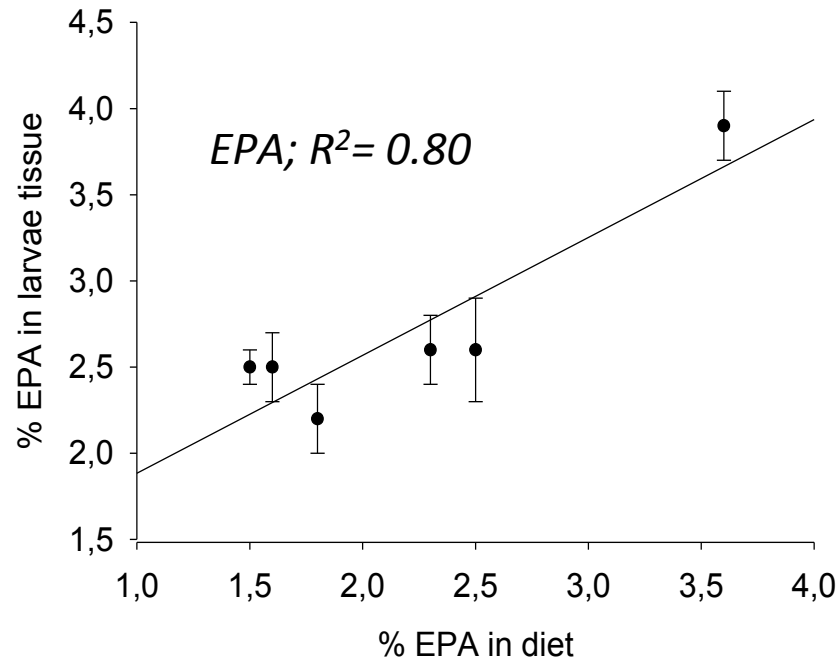


Analysed content (% DM)	PL1	PL2	PL3	PL1H1	PL1H2	PL1H3
% dry matter (DM)						
Crude protein	52,5	51,7	52,0	51,8	52,4	52,1
Crude lipid	27,0	27,0	27,0	27,0	26,9	27,0
NFE + fibre (substracted)	4,0	3,9	3,7	4,0	3,9	3,7
Ash	8,1	8,1	8,1	8,1	8,1	8,1
Gross Energy	24,0	23,3	22,5	24,0	23,3	22,5
EPA (% ww, as fed)	0,41	0,41	0,41	0,47	0,61	0,75
DHA (% ww, as fed)	0,66	0,66	0,66	1,04	2,06	3,04
PC	1,40	2,61	4,31	1,42	2,68	4,29
PE	0,43	1,22	2,20	0,40	1,14	1,87
PI	0,44	1,28	2,44	0,43	1,28	2,48
TPL						
(% total phospholipid levels)	3.73	8.19	14.38	3.70	8.32	14.51

Analysed TFA content (mg g^{-1} d.w.) and FA composition (% of TFA) of the 6 experimental feed types



	PL1	PL2	PL3	PL1H1	PL2H2	PL3H3
TFA	119.2±5.7	118.0±26.0	134.1±8.2	187.5±30.6	116.3±19.6	146.7±17.9
FA						
18:1 (n-9)	62.1±0.2	50.0±0.1	27.0±0.0	60.1±0.4	42.6±0.1	12.4±0.0
Total MUFAs	65.5±0.2	53.5±0.1	30.7±0.0	64.2±0.4	46.0±0.0	15.9±0.0
18:2 (n-6)	10.1±0.0	18.6±0.1	34.5±0.0	9.6±0.2	17.5±0.1	31.9±0.1
Total (n-6) PUFA	10.4±0.0	18.9±0.1	34.9±0.0	9.9±0.2	17.9±0.0	32.4±0.1
20:5 (n-3) EPA	1.5±0.0	1.8±0.0	2.3±0.0	1.6±0.1	2.5±0.1	3.6±0.0
22:6 (n-3) DHA	3.6±0.1	4.2±0.0	5.1±0.1	5.2±0.2	11.7±0.0	21.4±0.2
Total (n-3) PUFA	6.2±0.1	7.7±0.0	10.5±0.0	7.9±0.3	15.9±0.0	27.9±0.1
DHA/EPA	2.4±0.1	2.3±0.0	2.2±0.1	3.3±0.0	4.8±0.2	5.9±0.1
(n-3)/(n-6)	0.6±0.0	0.4±0.0	0.3±0.0	0.8±0.0	0.9±0.0	0.9±0.0



Larval lipid class composition

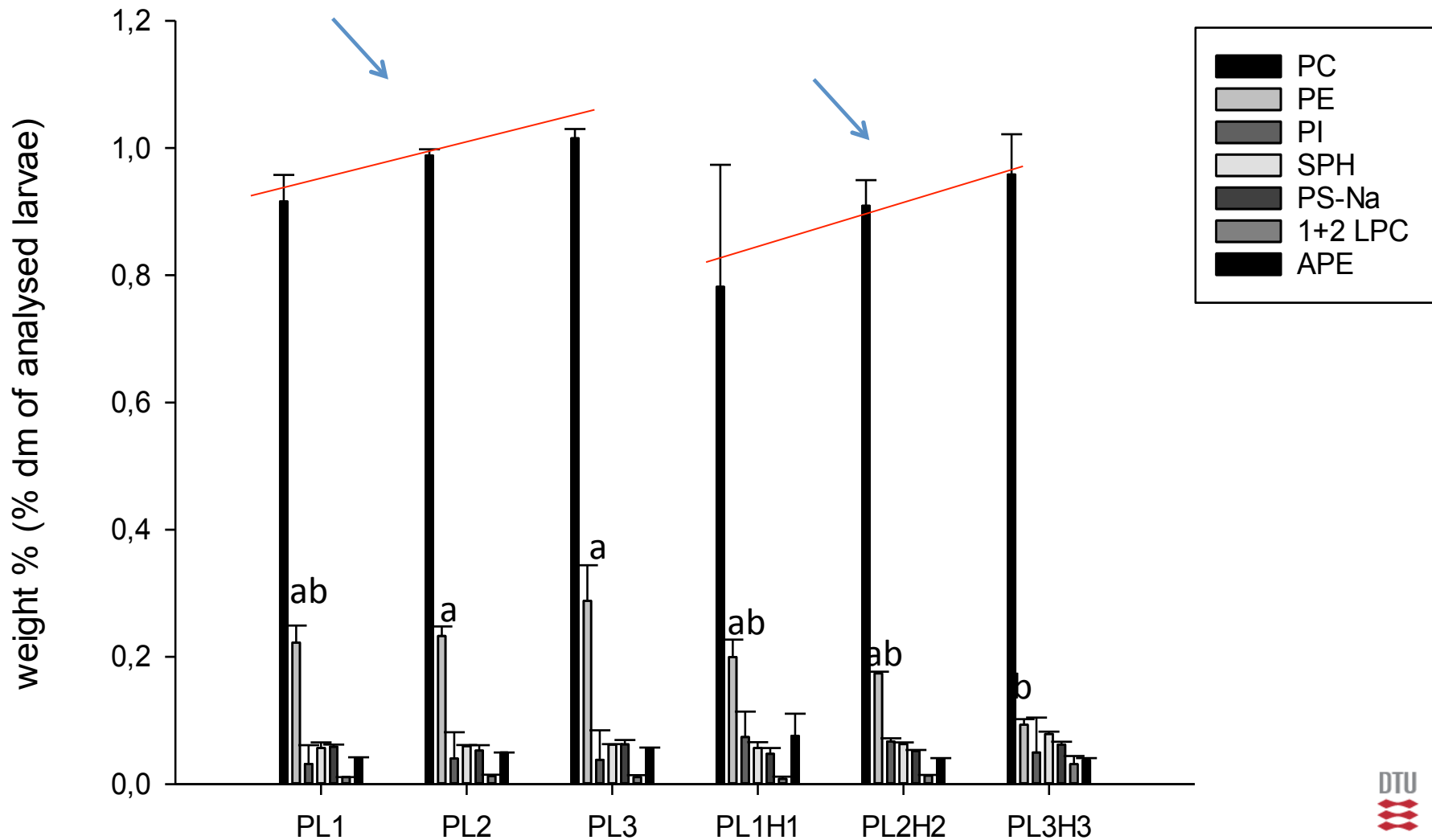


Fig. 2. Phospholipid class tissue composition in larvae at 30 days post hatching (dph) fed on the six experimental diets.

Growth performance

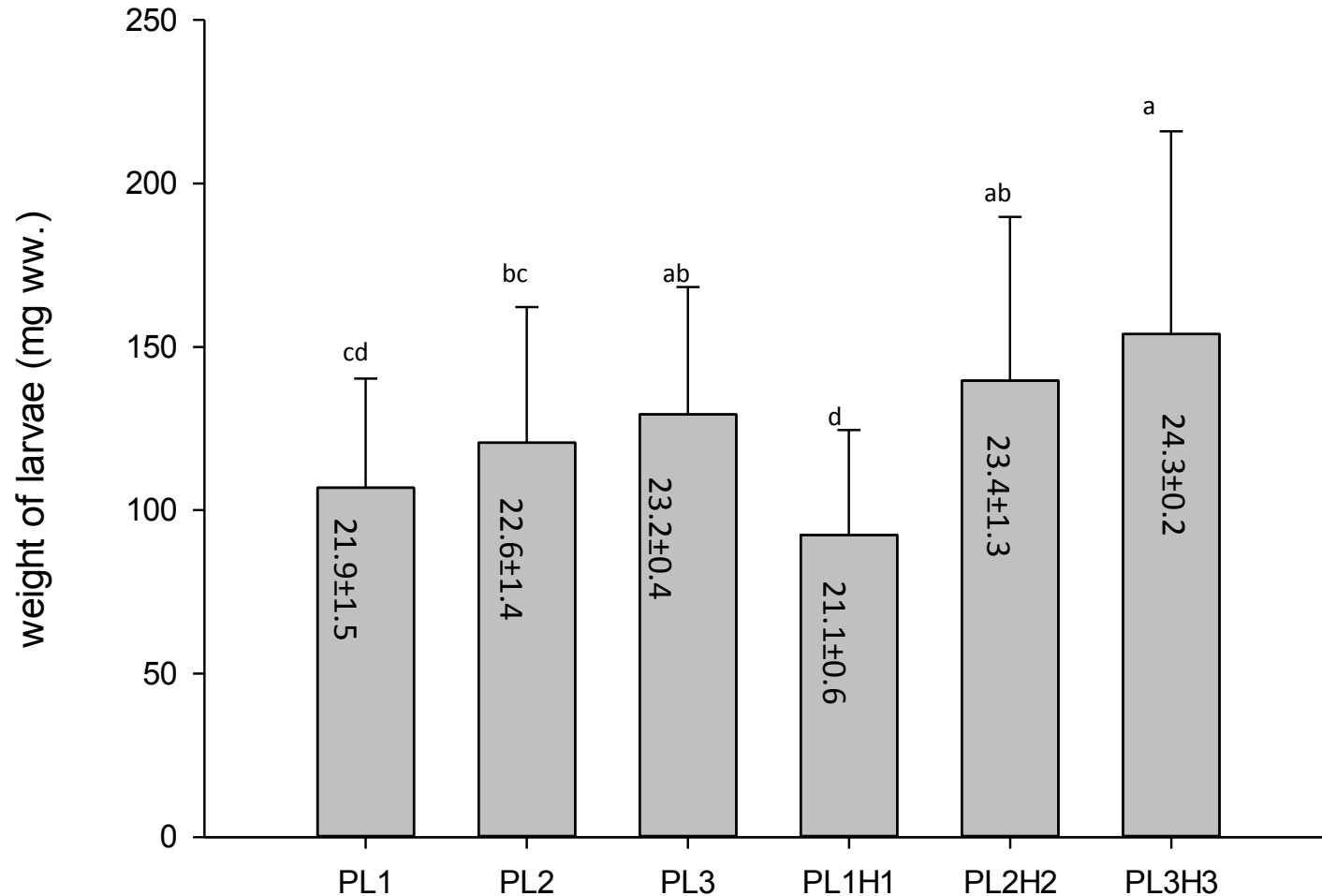


Fig. 1. Weight of pikeperch larvae at 30 days post hatching (dph) fed on the six experimental diets. SGR \pm sd illustrated on each graph.

Larval digestive enzymatic activity

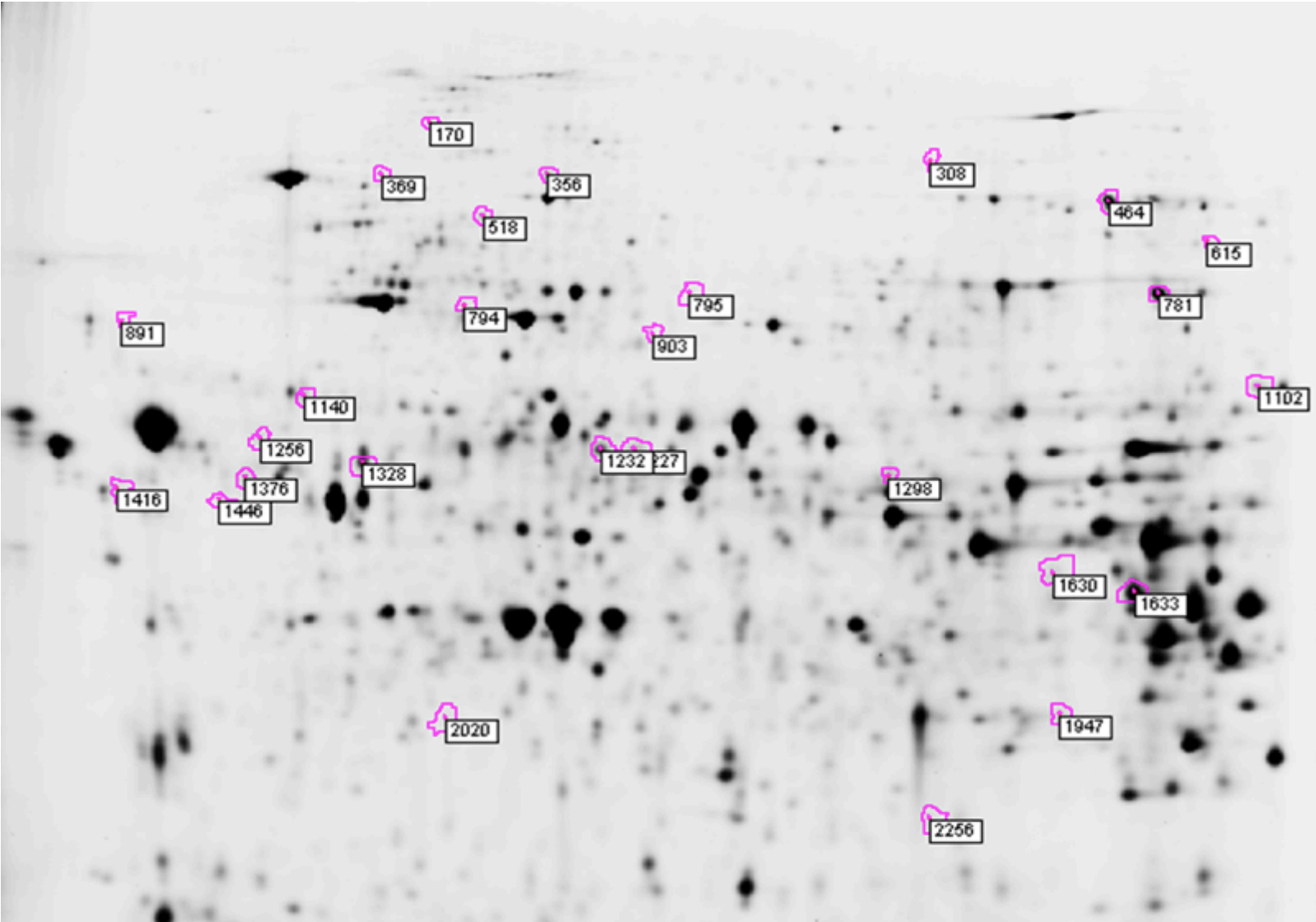


Specific activity	PL1	PL2	PL3	PL1H1	PL2H2	PL3H3
Pepsin (mU mg protein⁻¹)	48.0±7.9	49.8±9.0	56.3±7.4	51.8 ±7.9	55.2 ±2.9	63.0±3.2
Trypsin (mU mg protein⁻¹)	10.1±3.1 ^{abc}	6.4±1.7 ^a	8.5±2.3 ^{ab}	16.4±3.9 ^c	14.2±1.7 ^{bc}	8.8±1.0 ^{ab}
Aminopeptidase N (mU mg protein⁻¹)	5.9±0.8 ^{abc}	3.3±0.6 ^a	6.9±0.3 ^{bc}	5.3±0.7 ^{ab}	6.2±0.5 ^{bc}	8.0±1.9 ^c
Alkaline phosphatase AP (mU mg protein⁻¹)	24.7±2.8 ^{ab}	20.8±4.8 ^{ab}	21.1±3.2 ^{ab}	18.7±1.0 ^a	20.5±3.7 ^{ab}	28.8±3.9 ^b
Leu-ala (U mg protein⁻¹)	376.2±39.0	350.9±39.9	395.2±36.2	371.0±52.3	364.4±14.0	361.1±58.9
Amylase (U mg protein⁻¹)	5.2±0.6	4.4±0.7	5.35±0.52	4.70±0.5	4.5±0.9	4.3±1.0

Ref. FUNDP, Najlae Kertaoui

Table 1 Larval enzymatic activity of 30 dph pikeperch larvae fed different PL / n-3
Values in a row followed by a different superscript are significantly different P<0.05

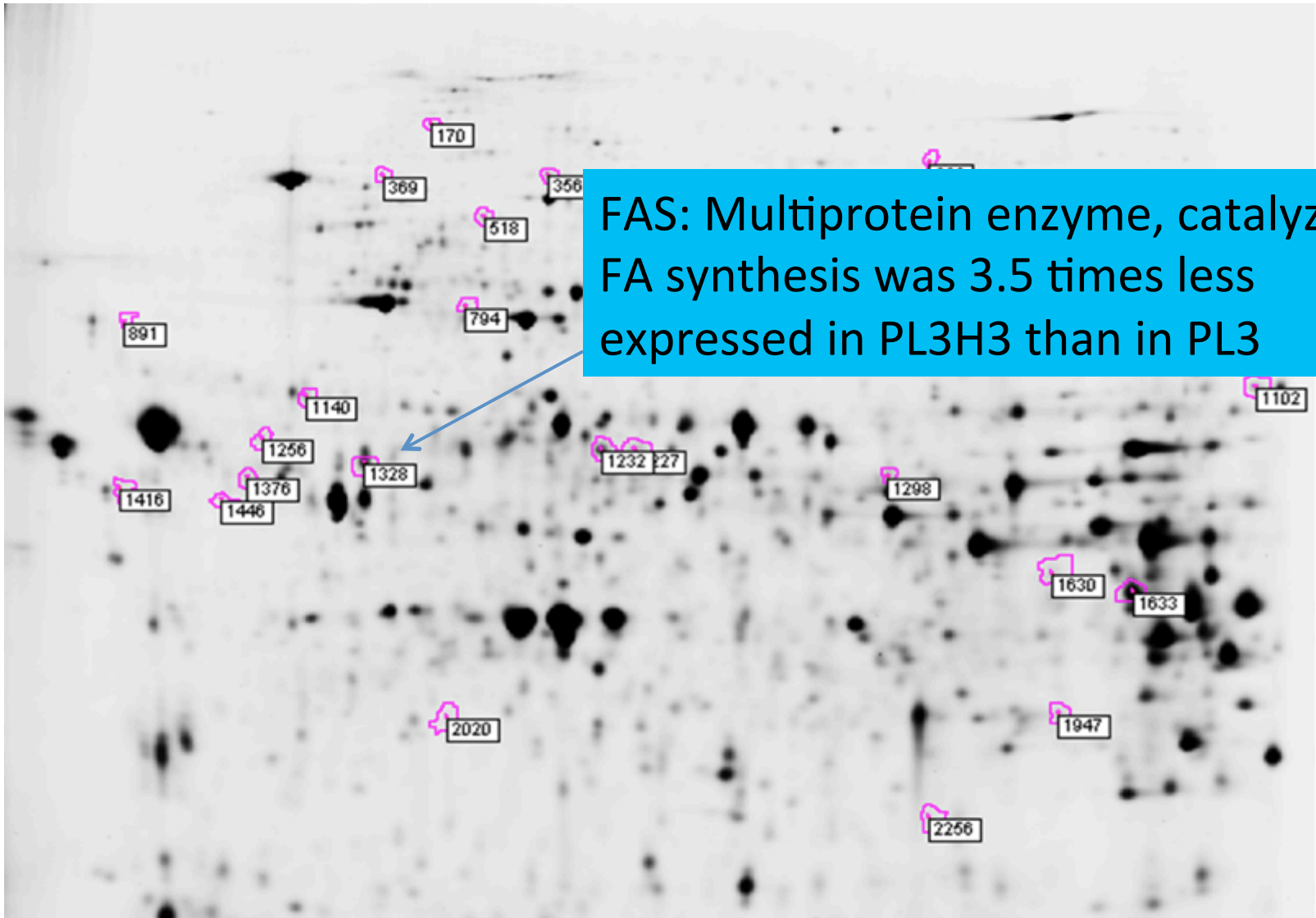
Liver Proteomics: Protein expression



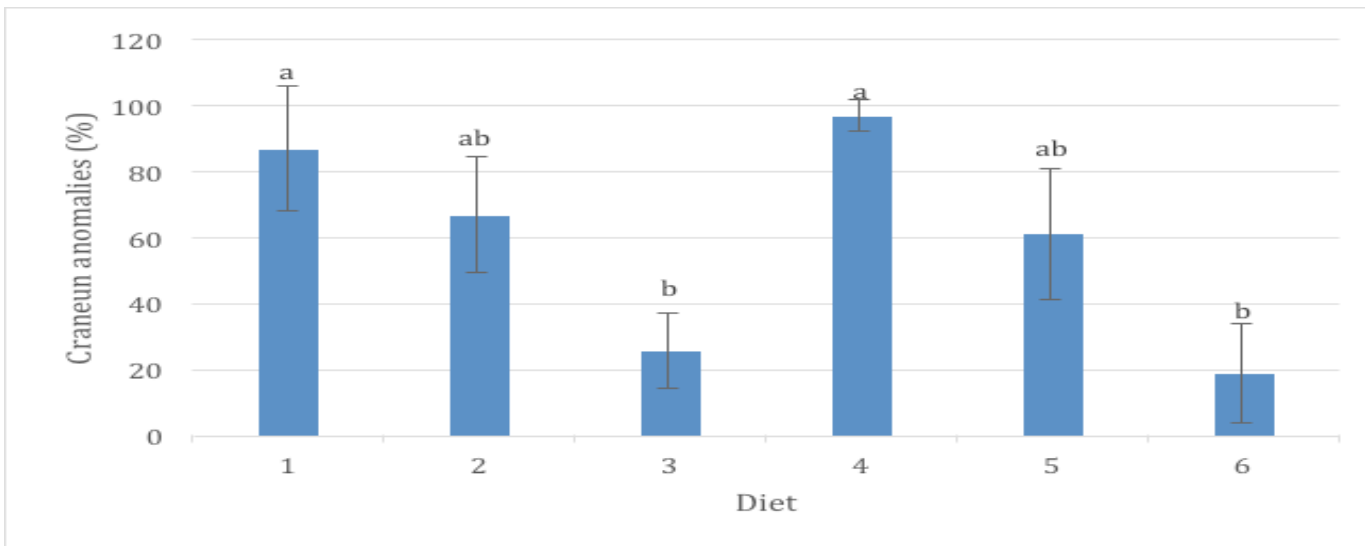
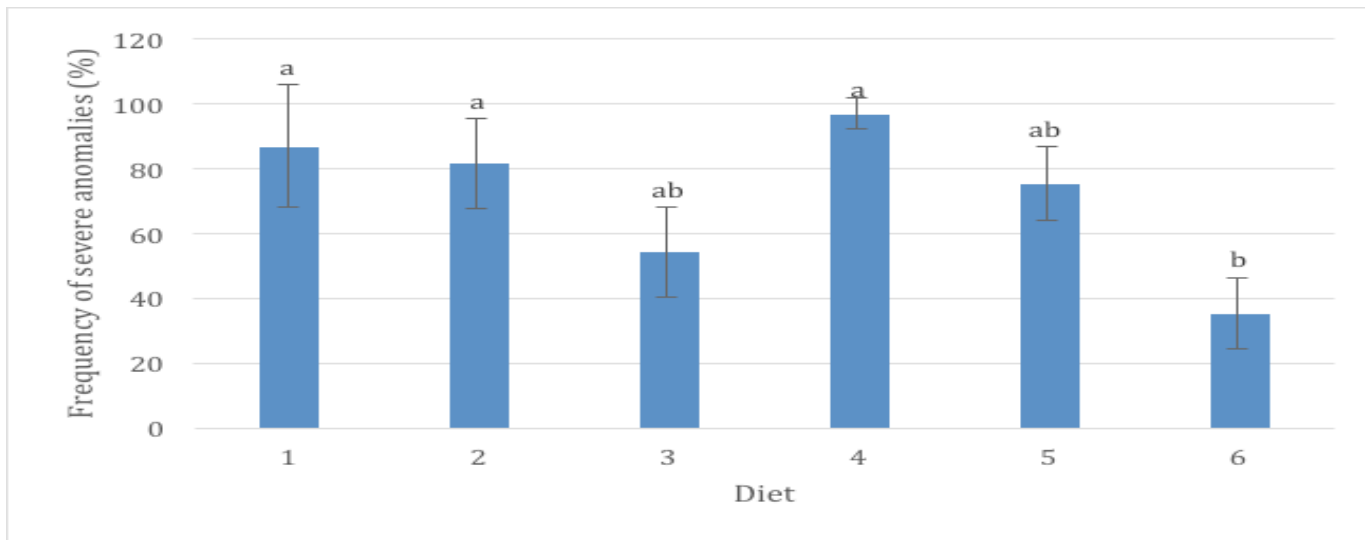
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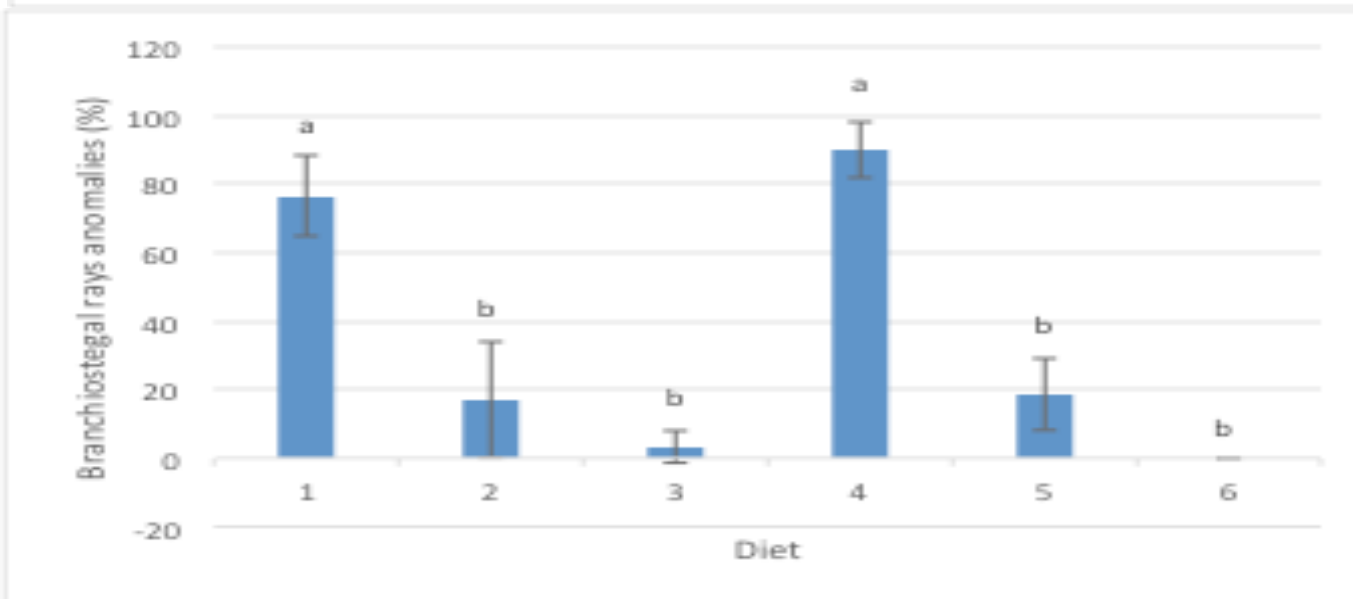
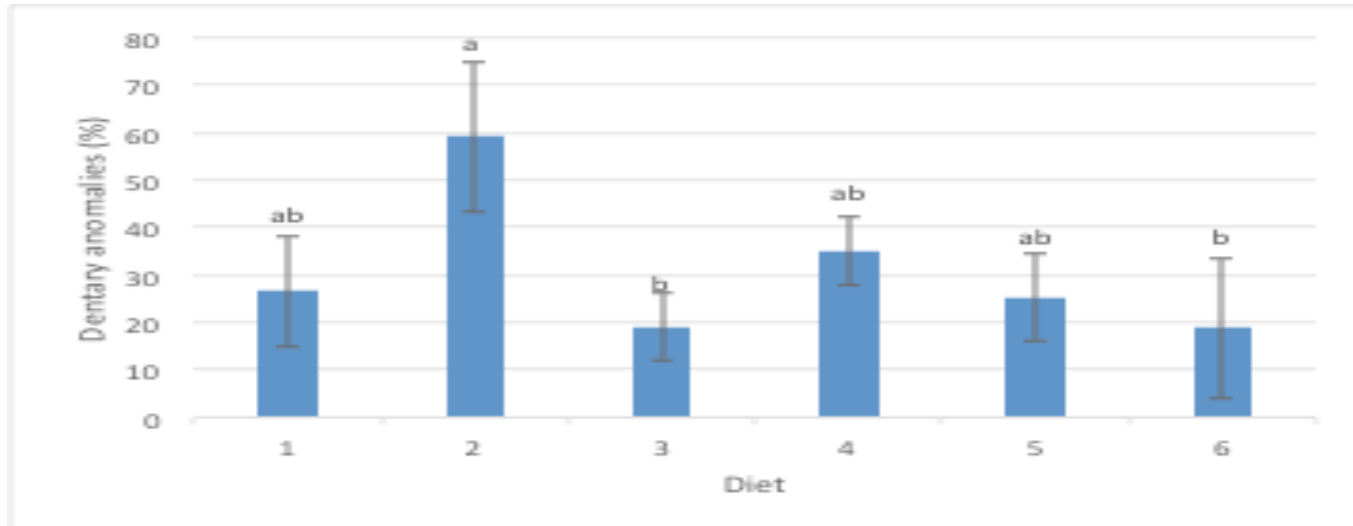
Liver Proteomics: Protein expression



Larval anomalies



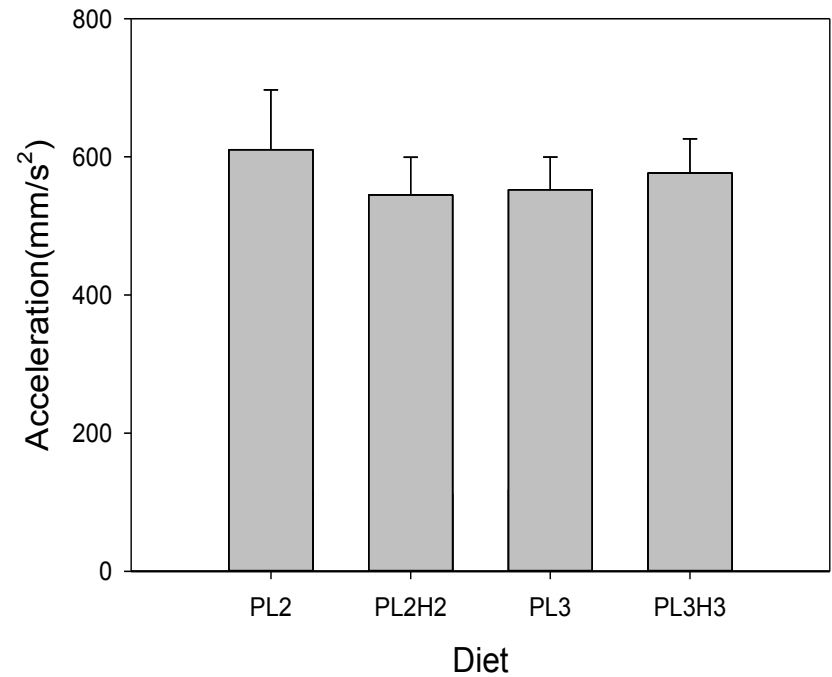
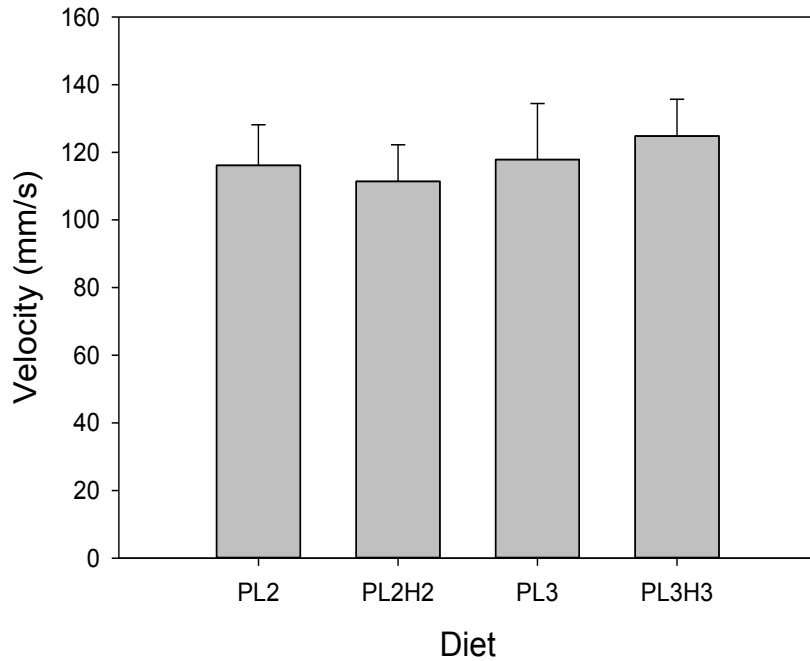
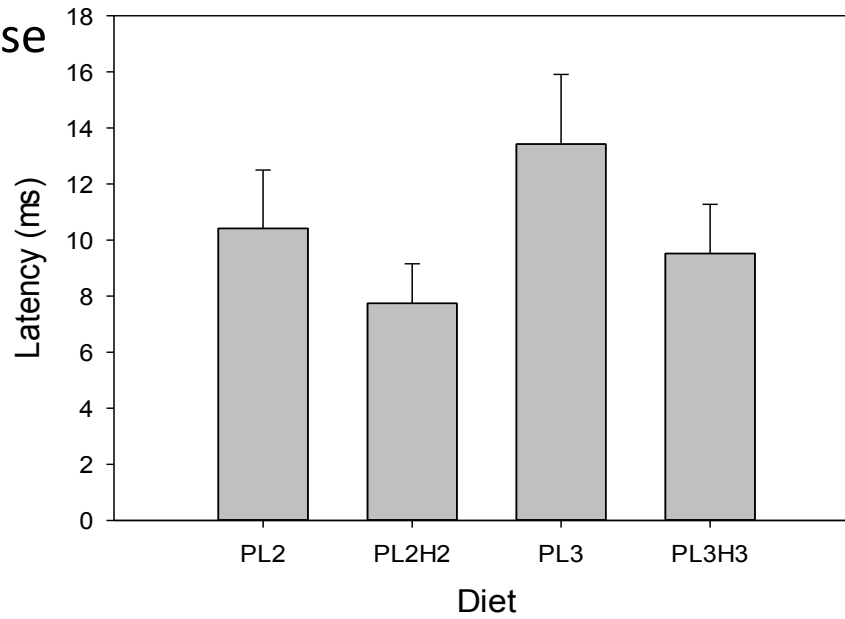
Larval anomalies



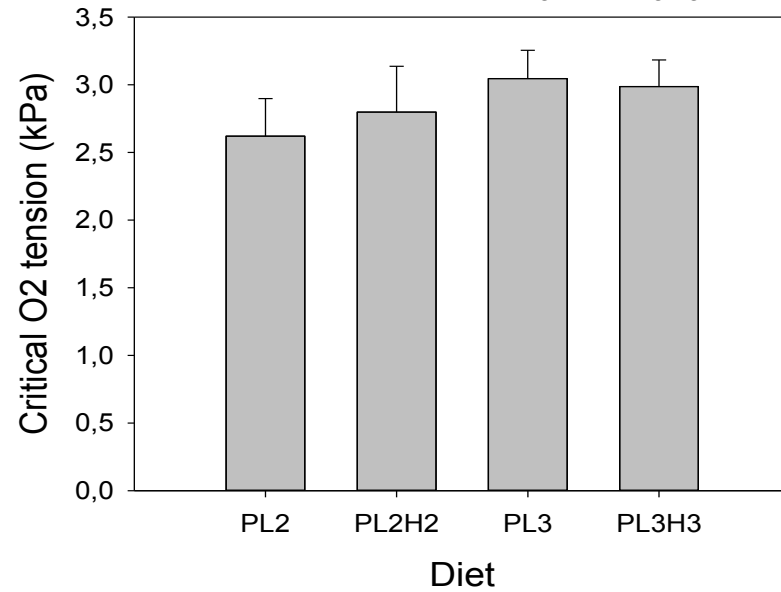
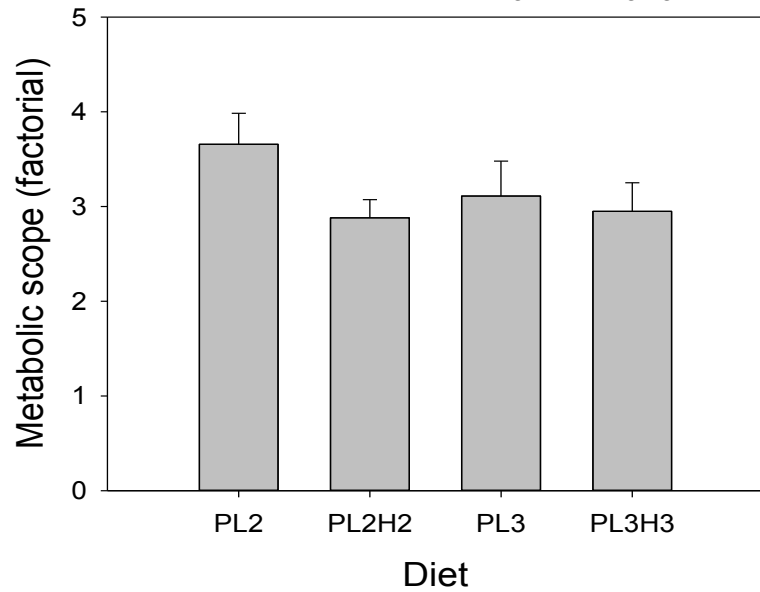
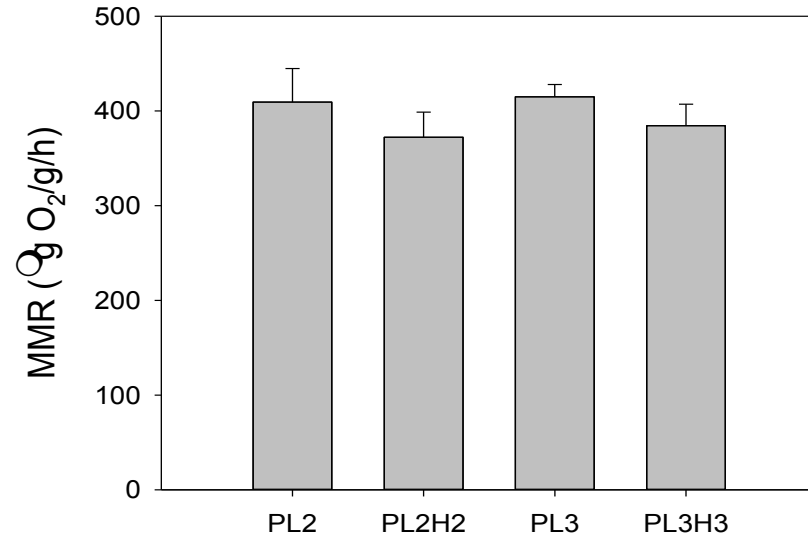
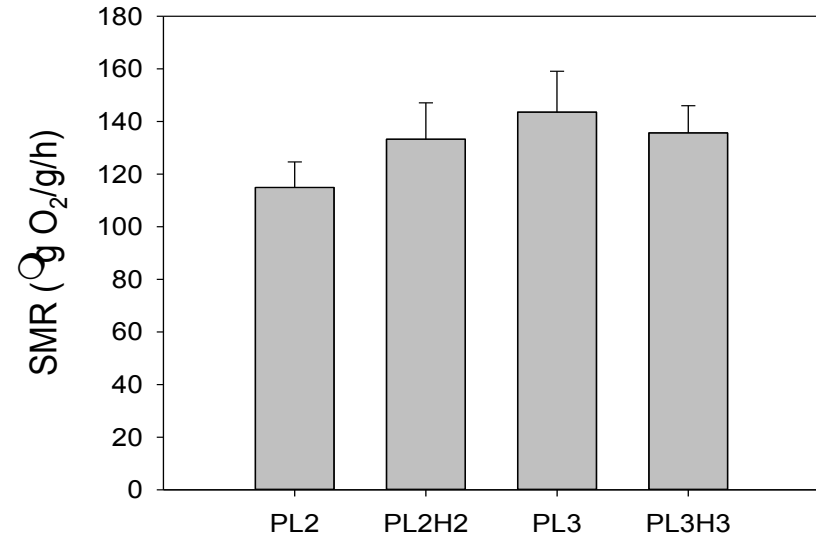
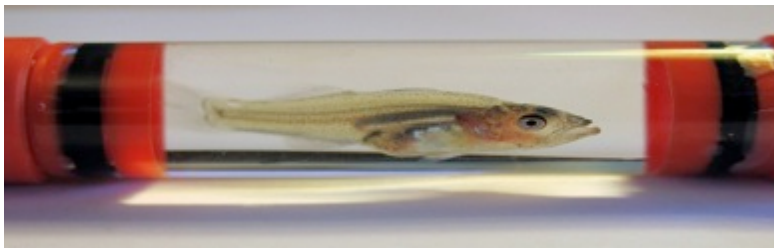
Fast escape response



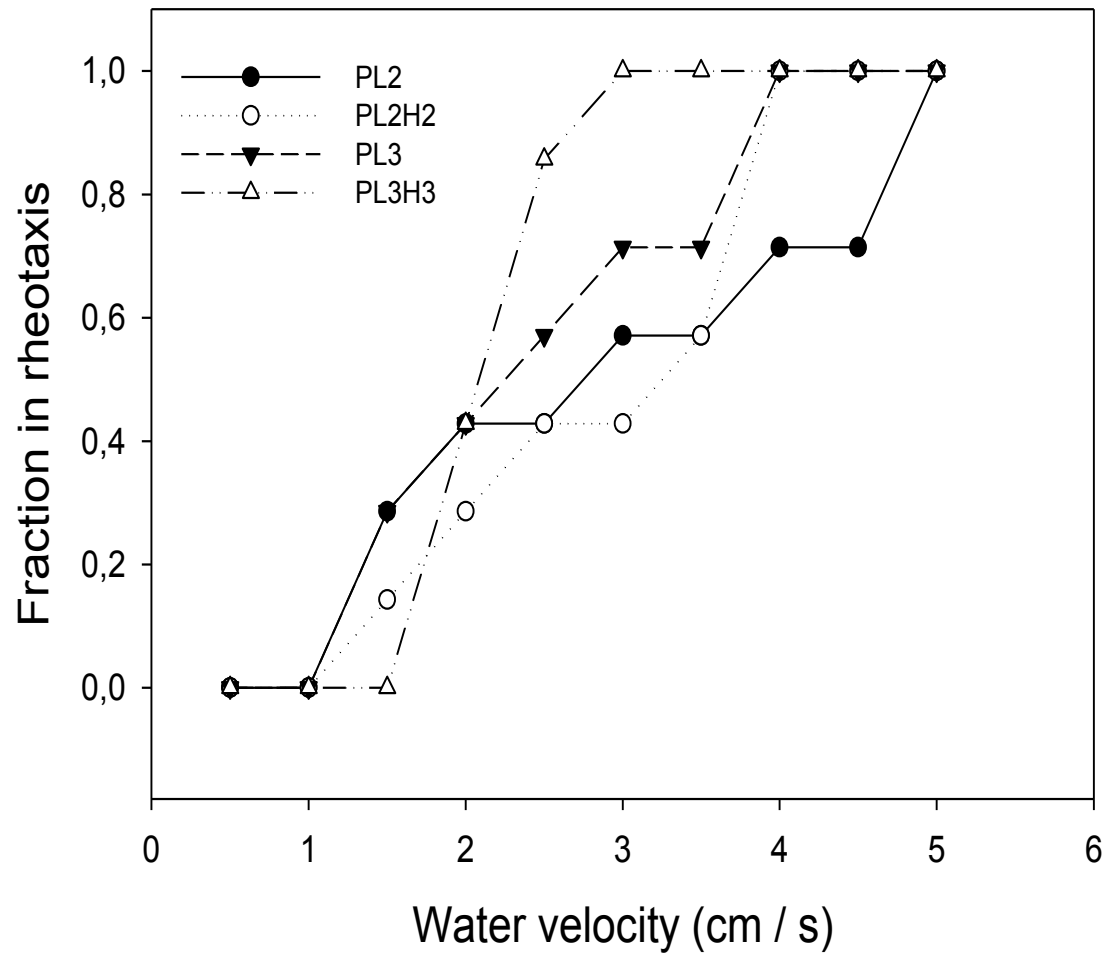
Fast escape response

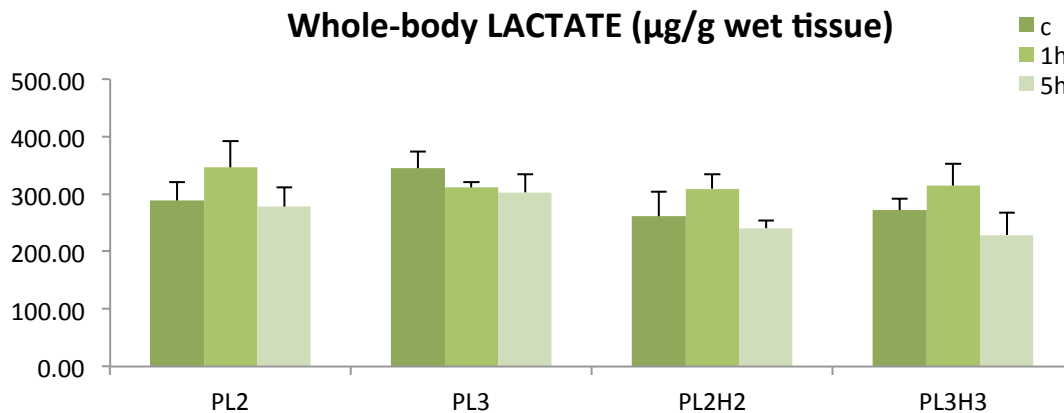
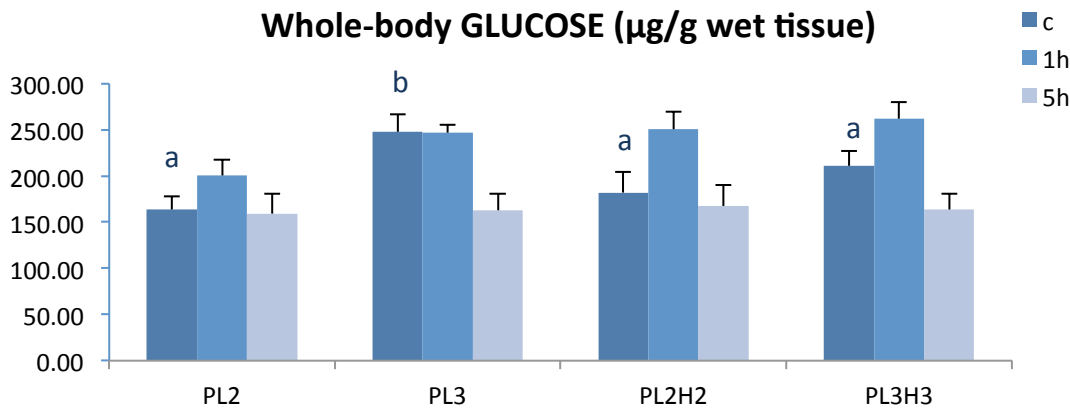
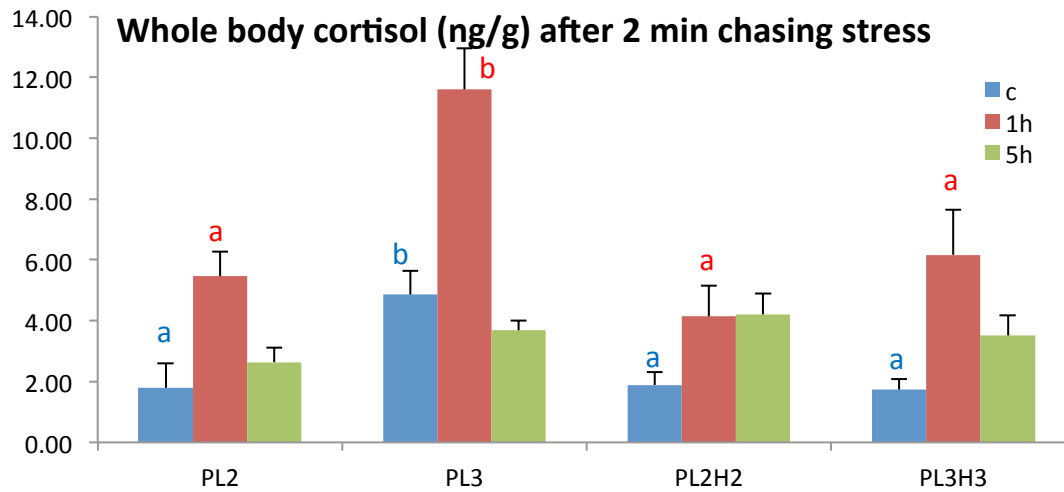


Respiration



Rheotaxis





Conclusions:

- ✓ 14% phospholipids (soy lecithin) significantly improves growth performance and reduces deformities compared with 3 - and 9 % phospholipids in diets high in marine protein and with vegetable oils
- ✓ n-3 HUFAs may have an additional positive effect
- ✓ Effect on digestive enzymatic activity
- ✓ Protein expression of important several important enzyme proteins are affected by phospholipid level and n-3 HUFA level. Anomalies decrease by an Increase in PL and n-3 Hufa level.
- ✓ No effect on stress markers, escape response or metabolic respiration for larvae fed diets with ≥ 8 % phospholipids with or without n-3 HUFA supplementation
- ✓ There may be some positive influence of n-3 HUFAs on sensory systems and sensitivity towards rheotaxis.