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Larval husbandry (Task 19.1)

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The effect of algal turbidity on larval performance and the ontogeny of digestive tract functionality in the grey mullet (*Mugil cephalus*)

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Introduction

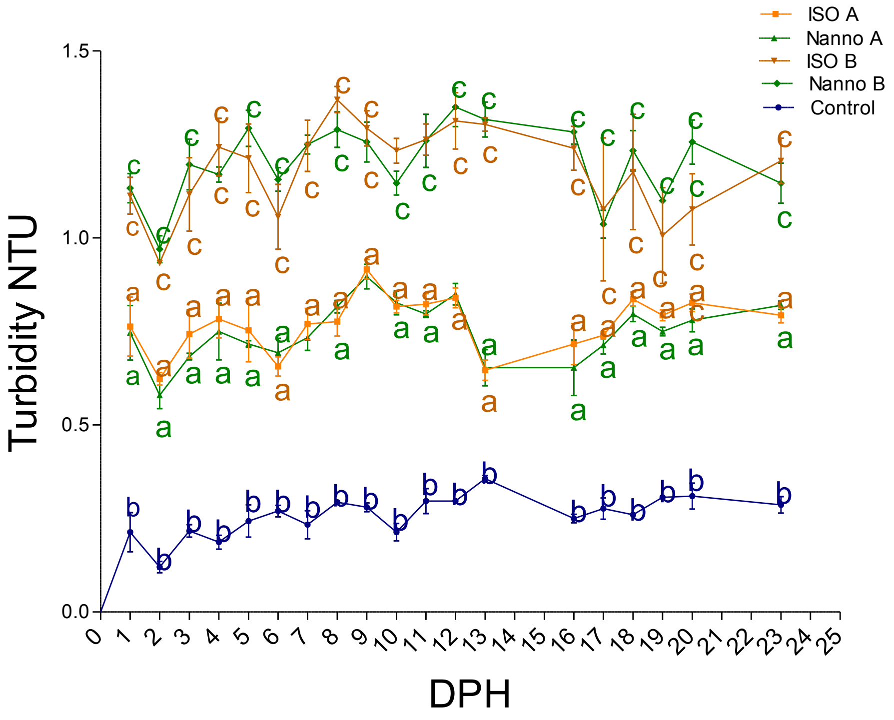
- "greening" of fish larval rearing tanks with algae → improves larval performance
- Possible reasons include

1. More rapid DT maturation.
2. Stimulation of immune system.
3. Water turbidity enhances contrast of prey against background → improved hunting success.

Aims:

- Determine if microalgal concentration provide background to facilitate prey hunting
- Compare the turbidity effect of *Nannochloropsis oculata* and *Isochrysis galbana* on larval performance

Experimental design



Treatments	Turbidity (NTU)
Control	0.26
Isochrysis A (0.0144x10^{6}cells\ml)	0.77
Nannochloropsis A (0.2x10^6 cells/ml)	0.75
Isochrysis B (0.0228x10^{6}cells\ml)	1.18
Nannochloropsis B (0.4x10^{6}cells\ml)	1.20

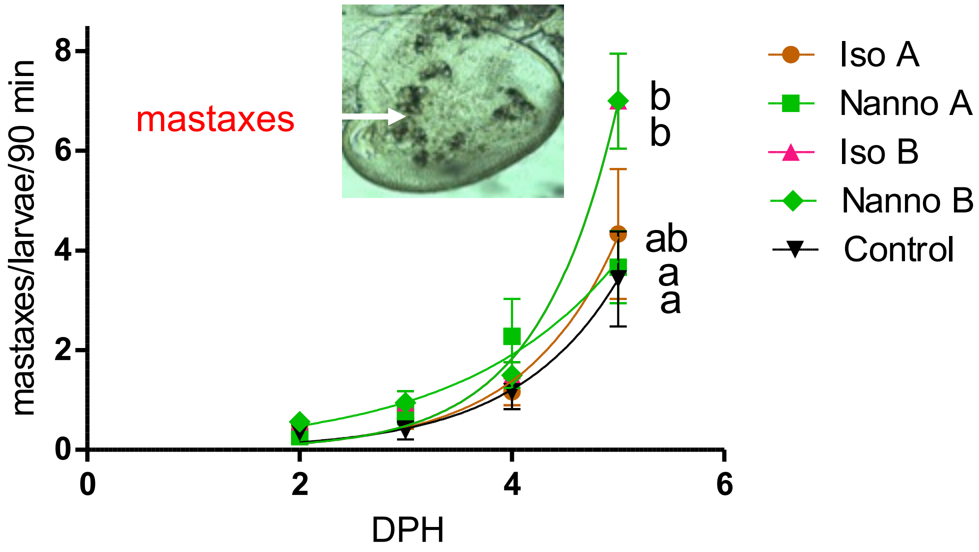
- Eggs stocked in fifteen 1.5 m^3 (100 eggs/l) in FT system using 40 ‰ , 25^{o}C.
- After hatching salinity incrementally lowered to 25 ‰.
- Two turbidity levels; A (0.76 NTU), B (1.20 NTU) and non-algal control C (0.26 NTU).
- Each turbidity level tested with two microalgal species; *Isochrysis galbana* and *Nannochloropsis oculata*
- Each of 5 treatments tested in 5 replicate tanks per treatment from first feeding (2 dph) to 23 dph.

All tanks were fed the same feeding regime

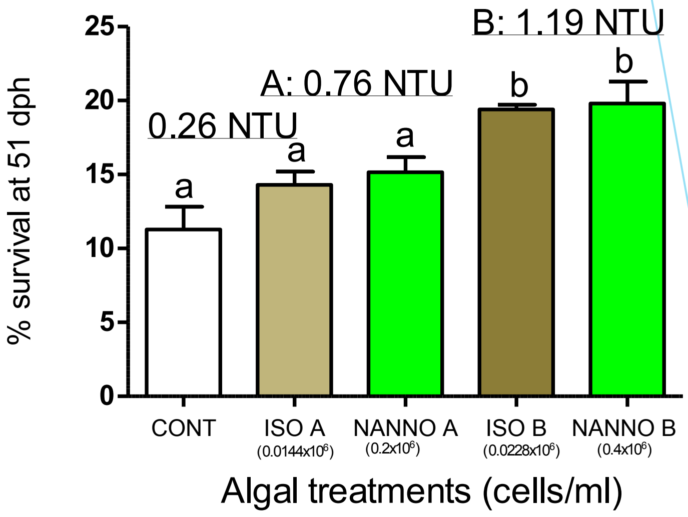
Feed	Rotifers	Unenriched Artemia	Enriched Artemia	*Caviar (Bernaqua™, Belgium)***	Ulva lactuca (IOLR,Israel)	Ranaan Dry Feed (RDF, Israel)*
				(1:1)		
Days fed (dph)	1-23	15	16-24	25-50	25-50	50-79
Protein (%)	57.3	49.1	53.1	55	34	56
Lipid (%)	27.3	12.8	28.7	15	7.4	14
Carbohydrate (%)	12.5	15	10	8	56	1
Ash	1.5	5	2	12	2.6	14.8

Results

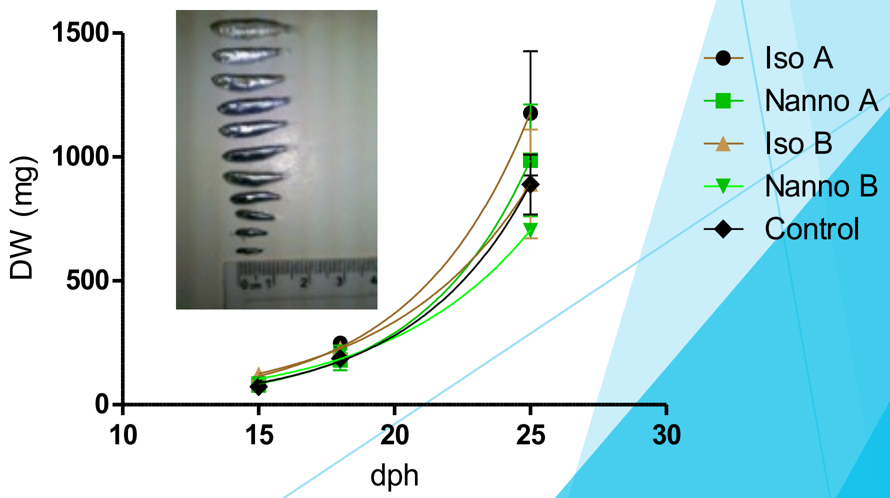
Rotifer consumption



Survival



Growth



- significant ($P < 0.05$) water turbidity effect on rotifer consumption in 5 dph grey mullet larvae independent of microalgal types tested.
- Rotifer consumption pattern from 2-23 dph was similar to that of survival in 51 dph juveniles independent of algal species.
- No significant ($P > 0.05$) treatment effect on growth performance

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Pancreatic and intestinal enzymes measured at different ages (Did turbidity treatments effect ontogeny of enzyme activity)

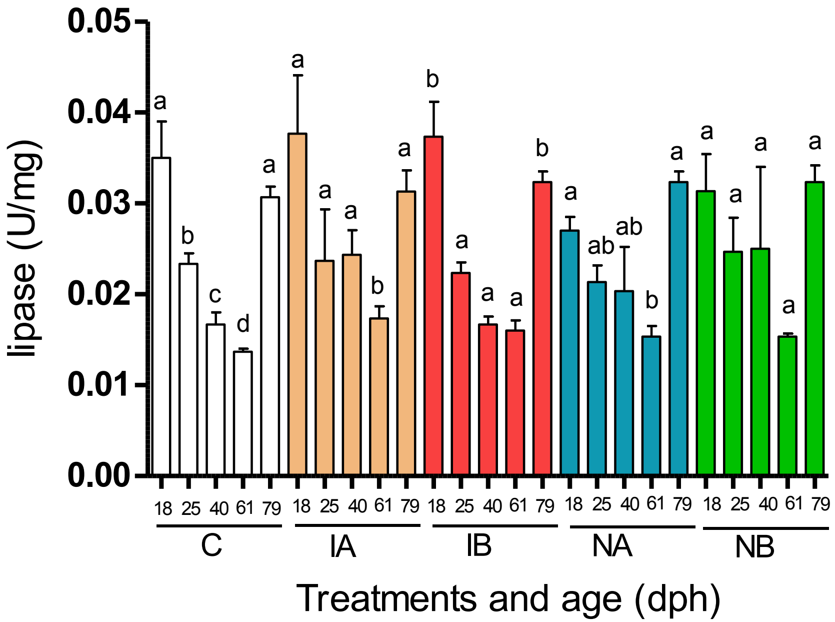
Pancreatic

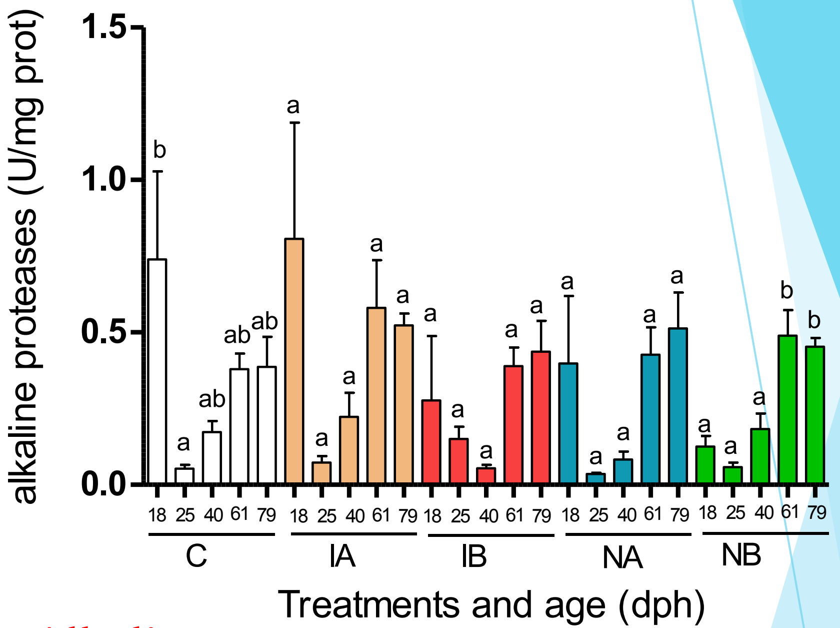
- Lipase (BSDL)
- Alkaline Protease
- Trypsin
- α-Amylase

Intestinal

- Leucine-alanine peptidase - intracellular cytosol digestion (pinocytosis)
- Alkaline phosphatase - Enterocyte brush border membrane (BBM)- marker for gut maturation.

DT enzyme ontogeny modulated by diet





Lipase

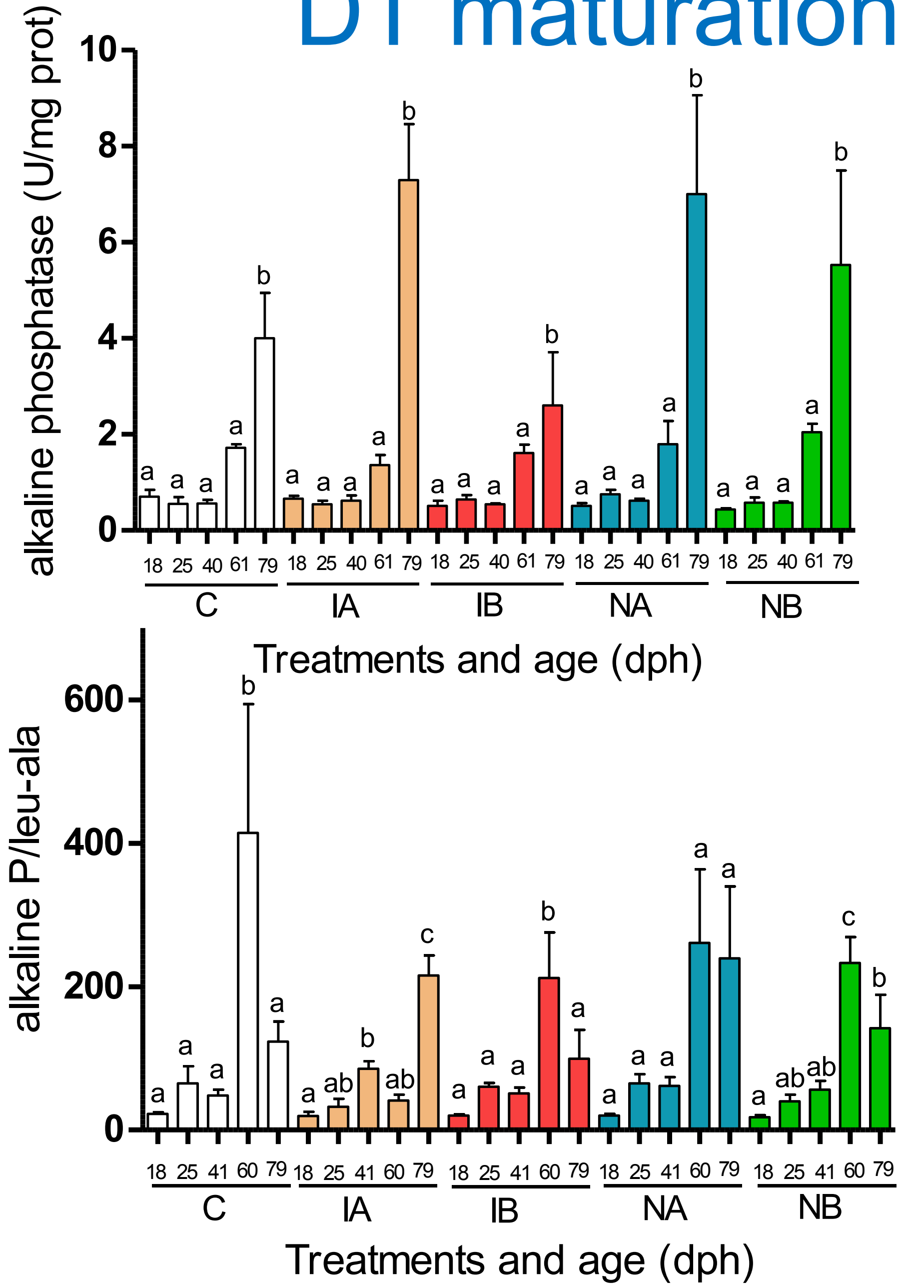
- 18 dph feeding on high lipid rotifers and *Artemia*
- 25-57 dph feeding on low lipid Caviar: *Ulva lactuca* (1:1 w/w)
- <57 dph- high lipid of RDF

Alkaline protease

- 18 dph feeding on high protein rotifers and *Artemia*
- 25-57 dph feeding on low protein Caviar: *Ulva lactuca* (1:1 w/w)
- <57 dph- high protein of RDF

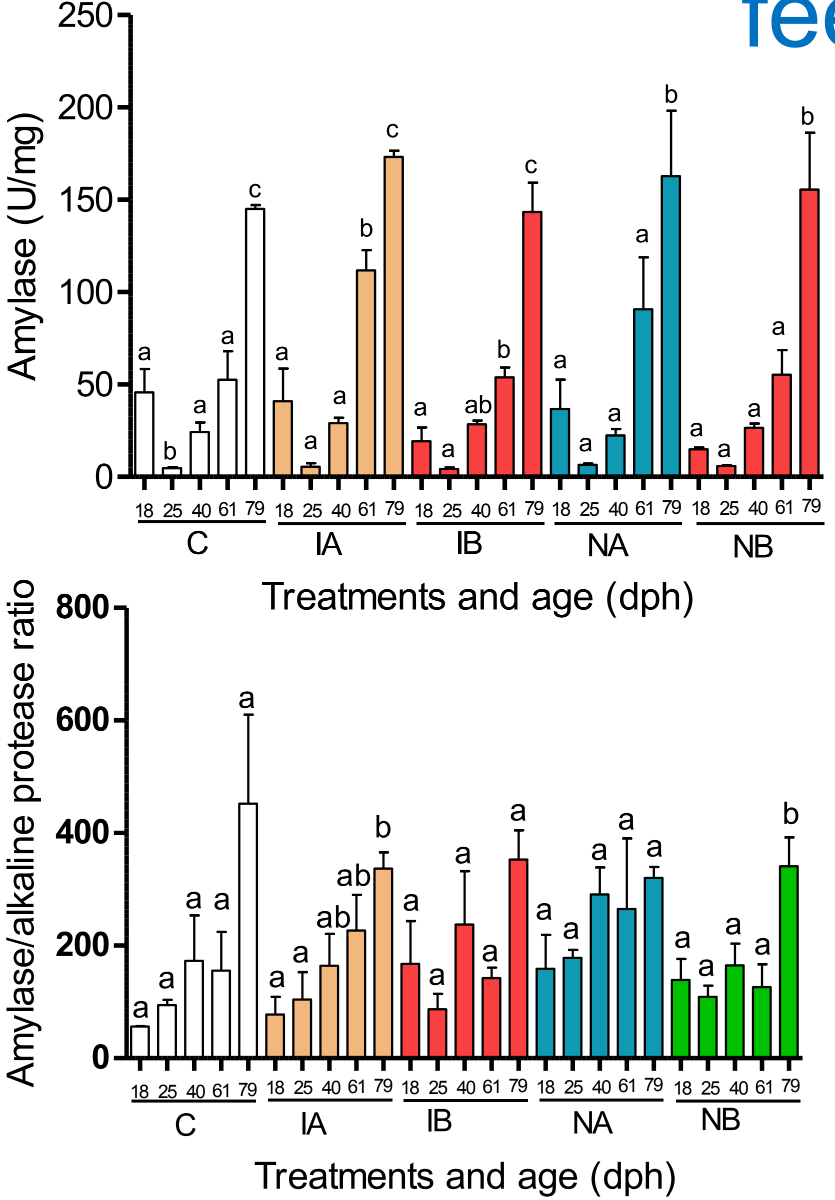
➤ **Nevertheless in general enzyme ontogeny was genetically programmed with age**

DT maturation markers



- Alkaline phosphatase (AP)-BBM marker of nutrient absorption increased over 9 fold between 61 to 79 dph
- Leucine-alanine peptidase (leu-ala)- intracellular digestion (pinocytosis) in the cytosol of enterocytes
- AP/leu-ala peptidase ratio indicator of gut maturation and peaked at 61 dph.
- Lack of acid proteases requiring the combination of both extracellular (intestinal lumen) and intracellular (enterocyte) digestion
- Possible late maturation of the BBM and/or the transition from carnivorous to omnivorous / herbivorous mode of feeding (41-60 dph).

Carnivorous-Omnivorous mode of feeding shift



Amylase

- Steadily increasing activity of α-amylase from 25-79 dph (particularly between 61 to 79 dph)
- Increasing more rapidly than alkaline proteases.
- 61-79 dph grey mullet juveniles-capacity to digest protein and starch.
- Exploit starch rich microalgae and macroalgae, as well as benthic protein rich organisms of lower salinity estuarine waters

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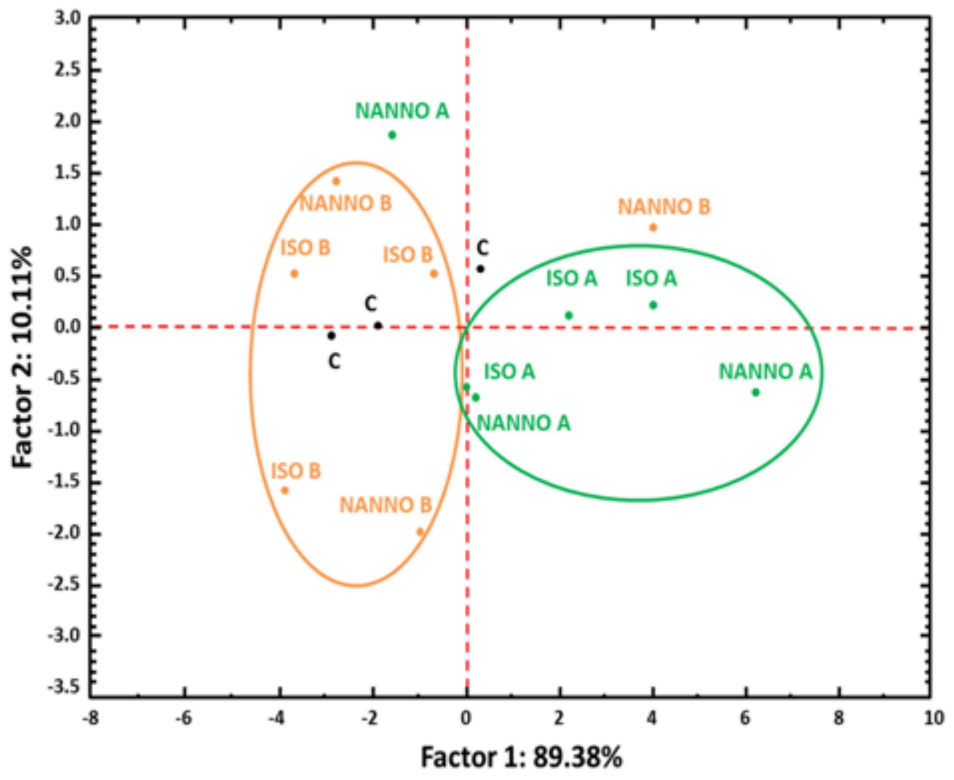
Principal component analysis (PCA) analysis

PCA analysis based on matrix of co-variations from specific activities of all pancreatic and DT enzymes



- Turbidity treatments- no lasting effect on DT enzymes from 18-79 dph fish.
- PCA - digestive profile of 79 dph juveniles, regardless of algae species, correlated to lower water turbidity.
- Possibly lower turbidity stimulated DT maturation while high turbidity increased prey ingestion and survival

Summary

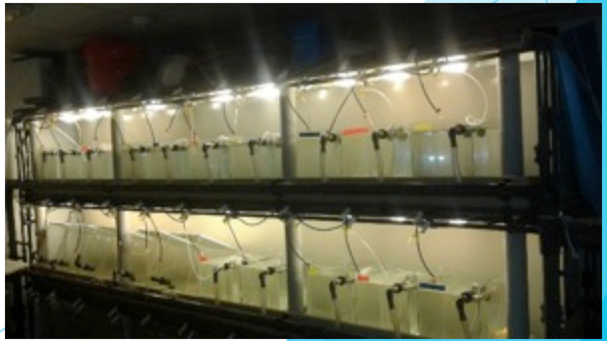
- Algal supplementation to grey mullet larval rearing tanks improves larval survival.
- “green water” improved prey ingestion, independent of microalgae species tested, at water turbidity of 1.2 NTU.
- Speculate “green water” increased larval DT enzyme activity, independent of microalgae species, at water turbidity of 0.75 NTU.
- Overall the ontogeny and activity of the P and DT enzymes genetically programmed.
- Amylase activity increased markedly between between 61-79- starch digestion (macroalgae)
- AP/leu-ala peptidase ratio peaked at 61 dph-late gut maturation.
- Alkaline protease, amylase and leu-ala peptidase activities - transition from carnivorous to omnivorous mode of feeding 40-79 dph.
- Suggests aquaculture feeds for 40-79 dph for mullet juveniles- omnivorous diets containing low cost starch sources.

Effect of weaning diet type (herbivorous, carnivorous or omnivorous) on grey mullet juveniles

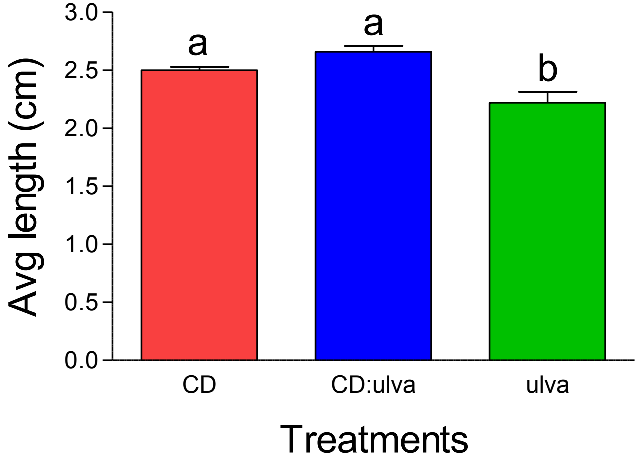
- Fifteen aquaria (17 l) were stocked with 100 larvae/l that were 23 dph.
- 15 larvae from each aquarium were sampled for wet weight determination.
- Three treatments were tested in replicates of 5 aquaria/treatment:

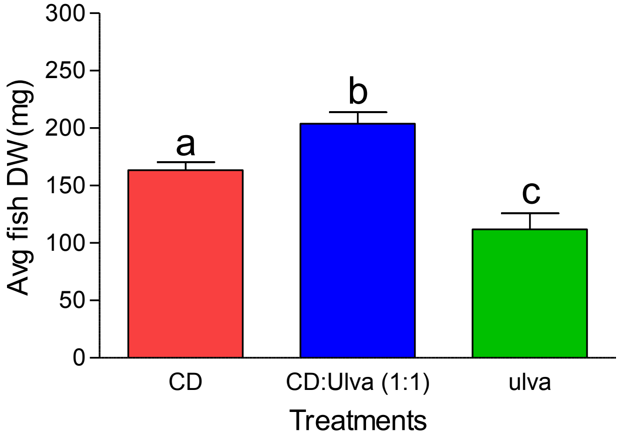
1. Ulva (IOLR)- *ca.* 34% protein
2. Caviar (Bernaqua, Belgium)-55% animal based protein (krill, squid, fish).
3. Caviar: Ulva (1:1) w/w.

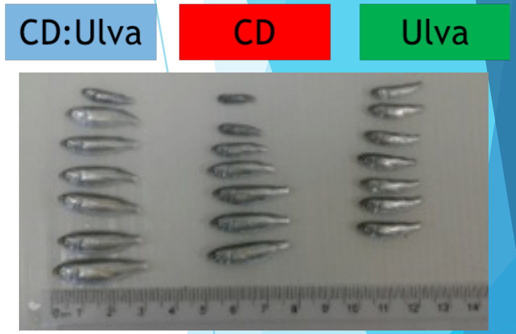
- All diets were *ad libitum*
- Larvae still to small to feed dry feed → weaned onto dry diet from 24-38 dph
- From 38-53 dph, fed only dry feed treatments.
- Samples taken for enzyme analysis at IRTA.

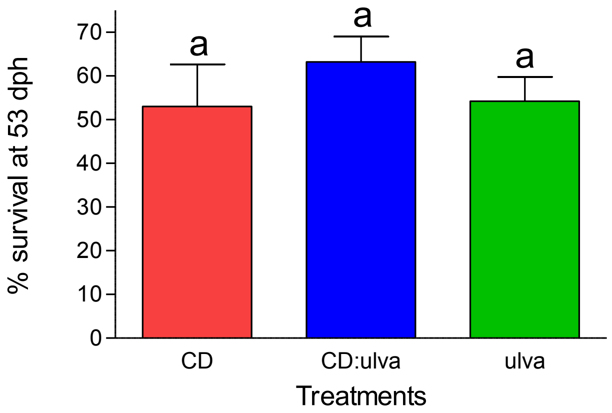


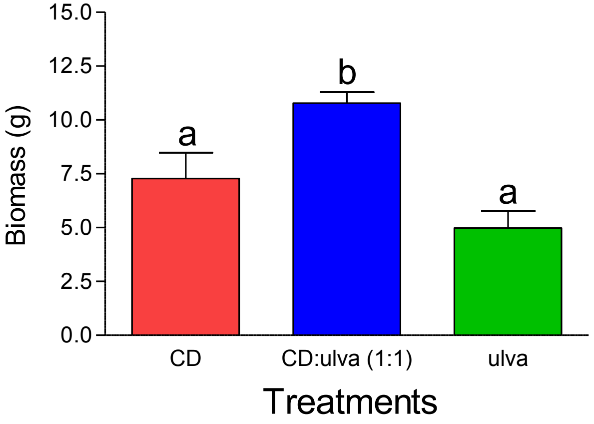
Effect of weaning diet type (herbivorous, carnivorous or omnivorous) on grey mullet juveniles





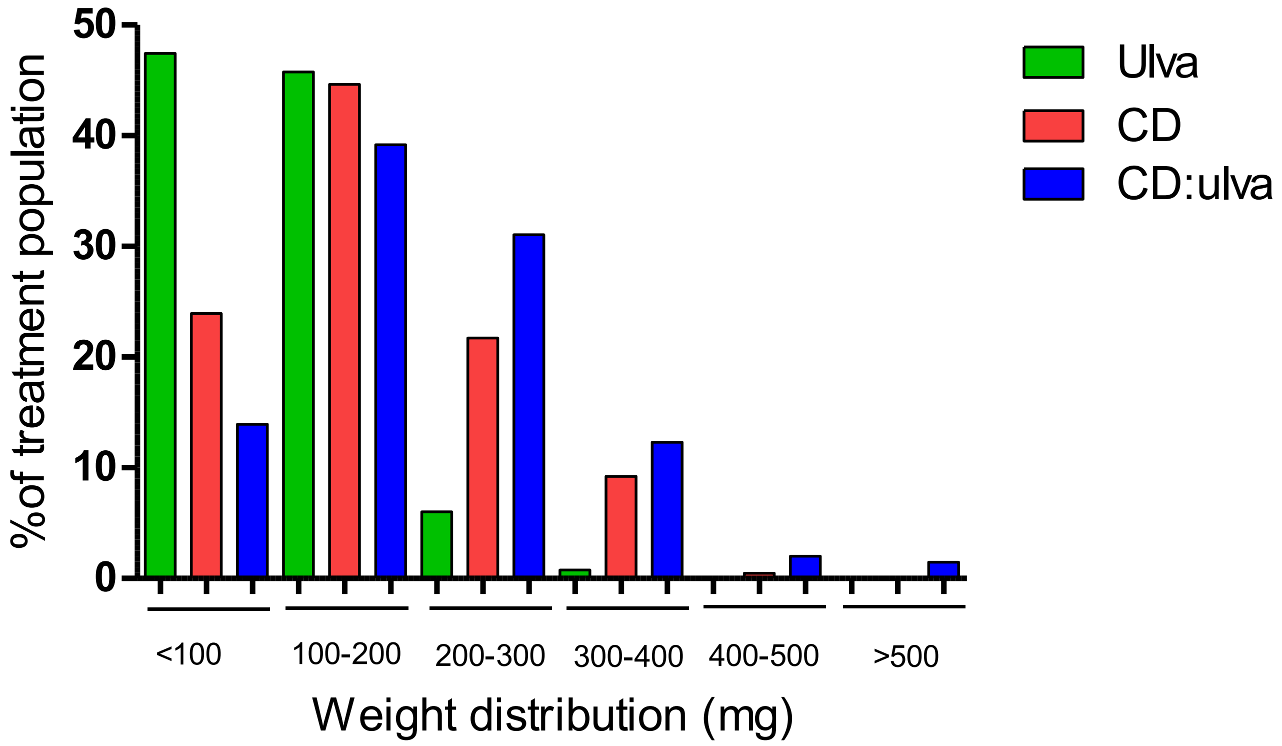


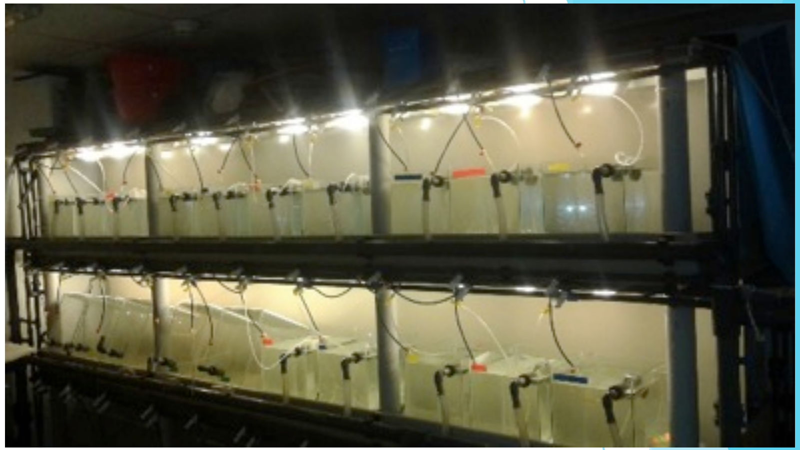




[1]CD-commercial diet-Bernaqua Caviar, where total animal protein is ca. 55% (krill, fish and squid)
[2]Ulva-produced at the IOLR, where total plant protein is ca. 35%

Diet type on treatment weight distribution





CD:Ulva | CD | Ulva

Effect of different sources of turbidity on grey mullet larval performance

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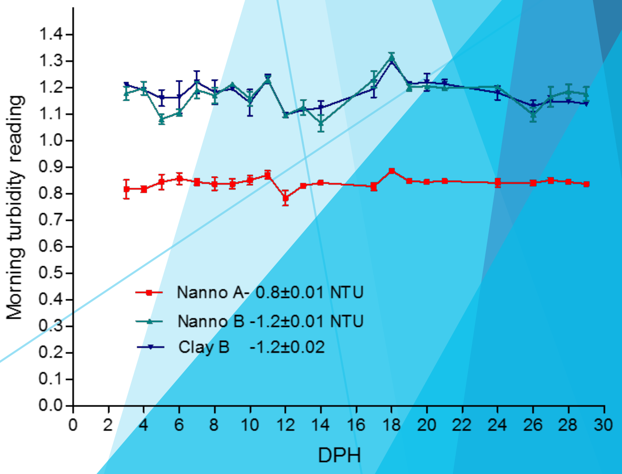
Algal turbidity vs clay turbidity

Experimental design

- Twelve 1.5 m3 tanks stocked with 100 eggs/l in a FT system where SW was 40 ‰ at 24-25 ºC.
- Three turbidity treatments tested in 4 replicate tanks/treatment until 23 dph;

1. *Nannochloropsis oculata* low (NA) turbidity (0.8±0.4 NTU; 0.25x10^{6}cells\ml)
2. *Nannochloropsis oculata* high (NB) turbidity (1.2±0.02 NTU; 0.5x10^{6}cells\ml)
3. Clay (CB) (1.2±0.02 NTU)

- Larvae were fed taurine and DHA enriched rotifers (3-13 dph) and enriched Artemia (14-21 dph). Fed the Caviar: Ulva dry feed from 22 dph

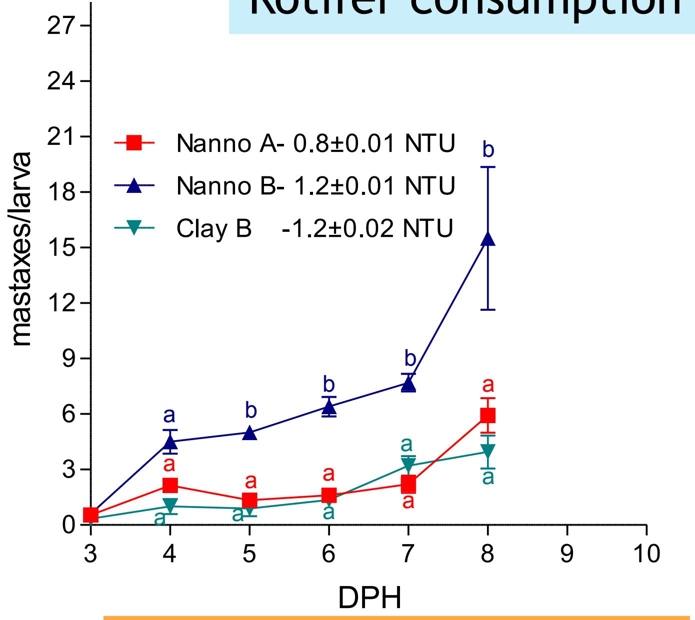


Sampling

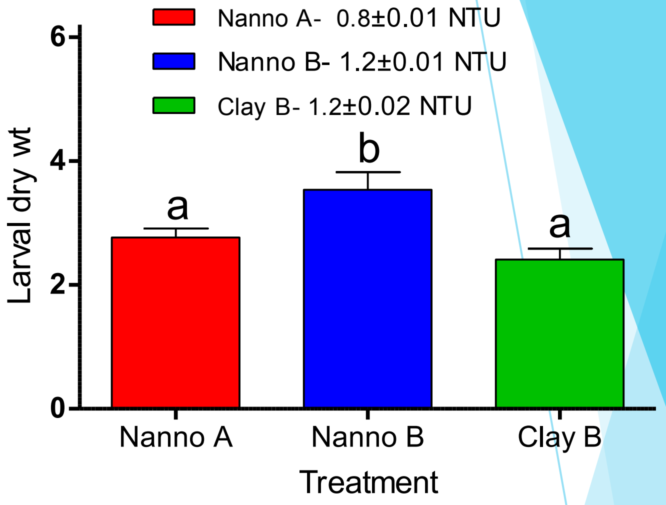
- Fatty acid profiles of egg, post hatching larvae and 28 dph fish, Growth (length and DW), rotifer ingestion, survival and samples for DT enzymes at 32 dph.

Results

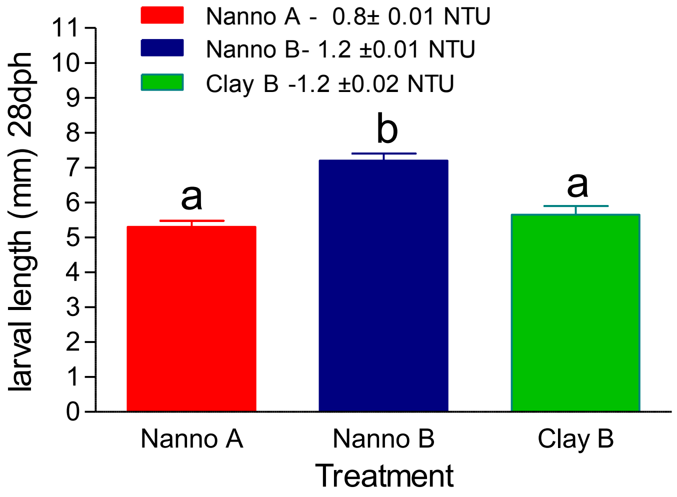
Rotifer consumption



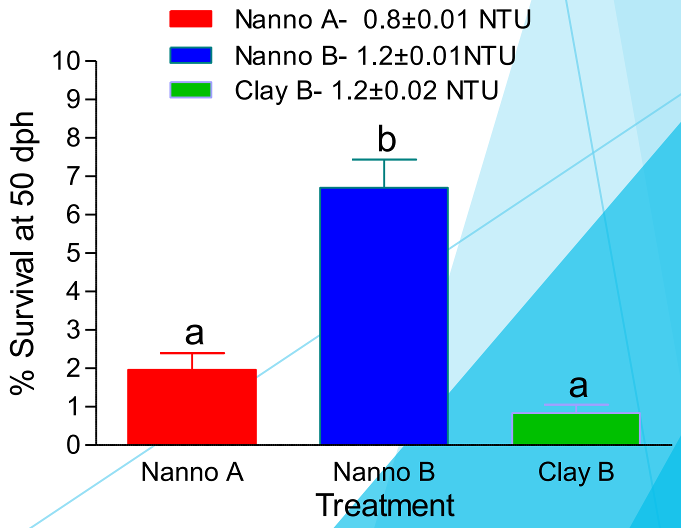
Dry Wt



Length



Survival



Summary and Conclusions

- Possible advantages of animal protein diet over plant protein diet - higher protein and taurine content (earlier studies showed a taurine requirement in this age fish).

- Skewness to larger fish described by Ulva< CD < CD:Ulva

- Amylase hydrolyze α-1, 4 glycoside bonds of starch exposing more Ulva protein for protease digestion.

- Starch digestion-source of energy-possible protein sparing.

- Sufficient production of DT amylase and protease to wean 24-38 dph juvenile grey mullet onto an omnivorous (plant and animal protein) diet for good growth and survival.

Thank you

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