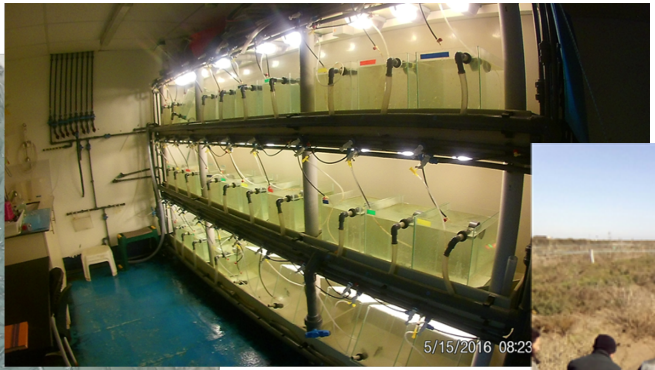
The grey mullet (*Mugil cephalus*): **Objectives and Progress**

Bill Koven, Hanna Rosenfeld, Oriya Nixon, Iris Meiri-Ashkenazi, Enric Gisbert,

Alicia Estevez, Yannis Kotzamanis, Rocio Robles, Aldo Corriero, Amos Tandler



Background and rational

- Grey mullet euryhaline, eurythermal, found all over the world.
- Present IOLR formula-Omnivorous based diet with no fish meal-Sustainable, reduced environmental impact.
- Offers an economical source of protein as well as the high end added value (200 €/kg) product bottarga (karasumi in Japan).
- Changing demographics in Europe to populations that traditionally eat mullet represents an expanding European market

Israel Oceanographic & Limnological Research

Reproduction

Mullets do not spawn spontaneously in captivity

The problem

❖Females often do not progress past early stages of vitellogenesis and do not undergo final maturation & spawning

❖Spermiating males are rarely observed. In most cases the produced milt is highly viscous and fails to fertilize the eggs

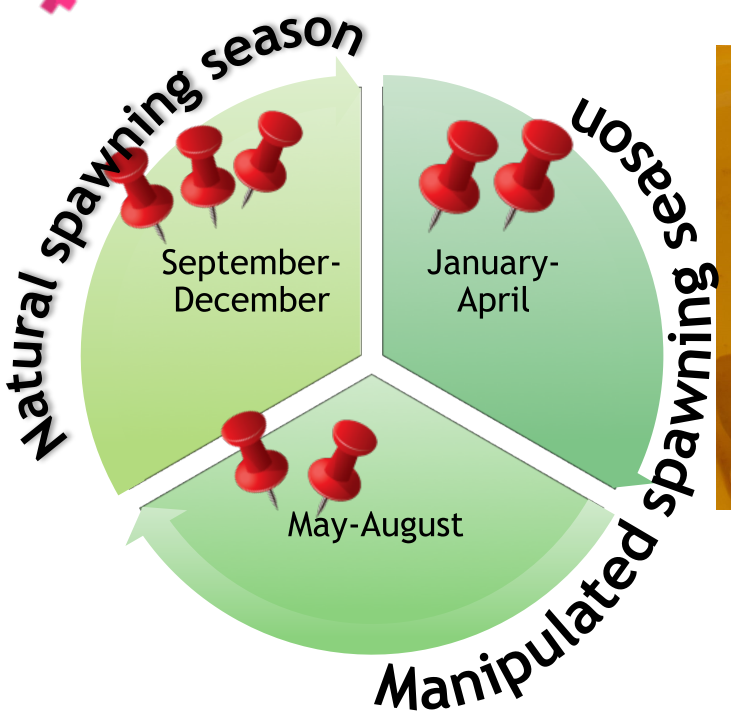
The objectives:

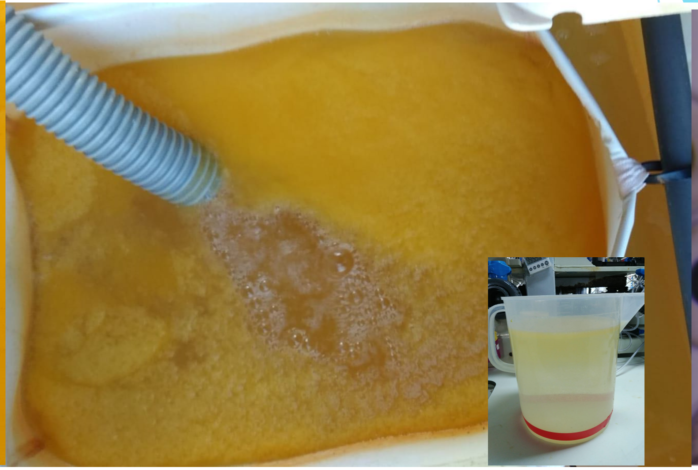
1. To increase the abundance of spermiating males exhibiting high quality milt
2. To synchronize gonadal development in- and between sexes
3. To induce spawning

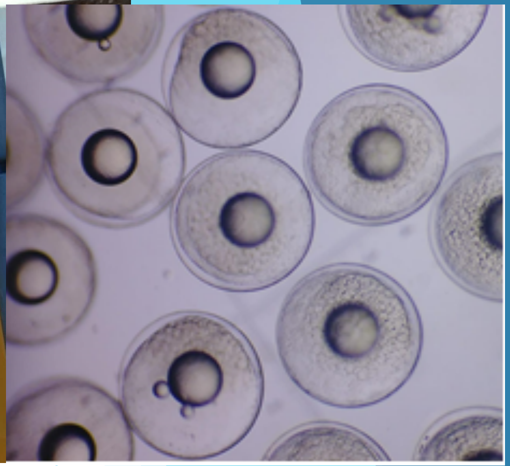
Hormonal treatment

- **Implementation of hormone- therapy at the onset of gametogenesis successfully enhanced gonadal development.**
- **Timed administration of recombinant FSH and antagonist for dopamine D2 receptors at critical stages.**
- **Towards the completion of the reproductive cycle, a second hormone-based treatment, consisting of GnRh and dopamine antagonist, gave rise to successful spawning.**

Summary of the spawning data





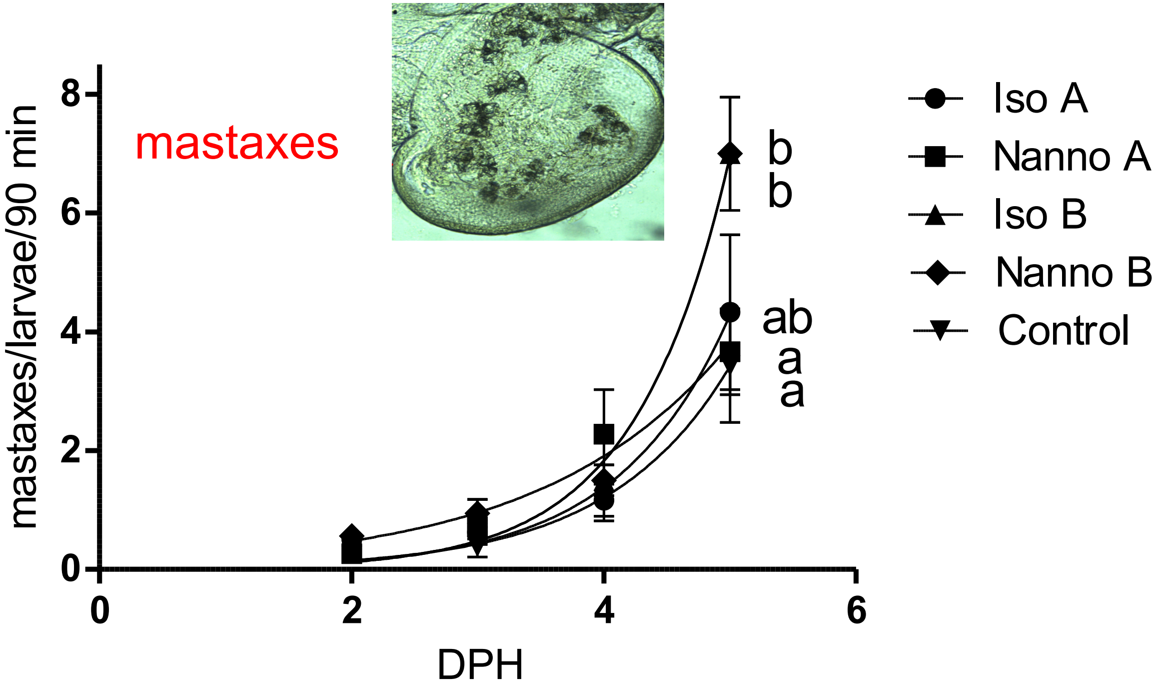


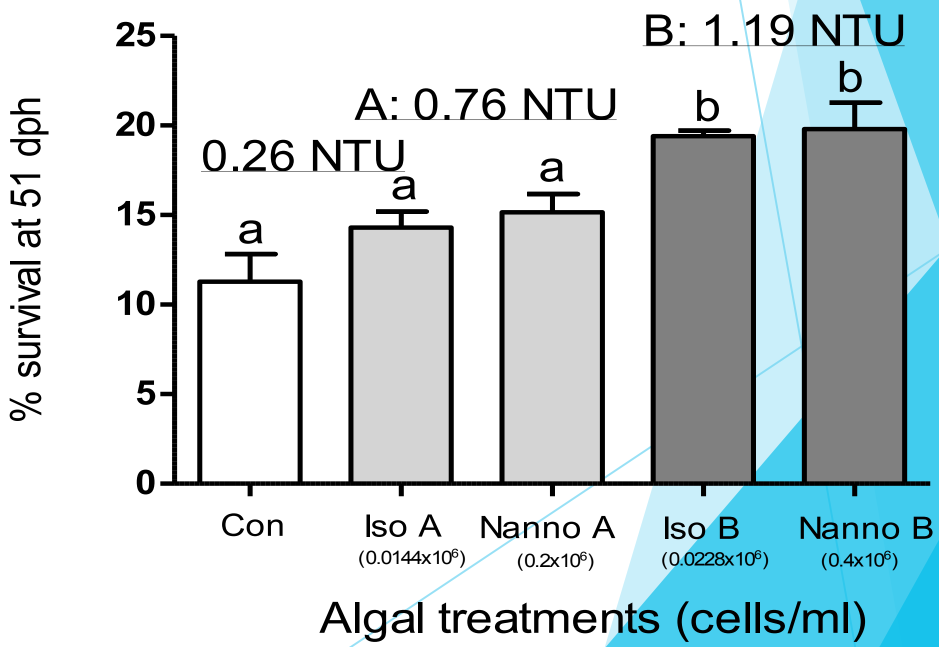
- A relatively extended natural spawning season (~3 months).
- Shifted spawning season can be easily achieved via photo-thermal manipulation.
- Improved spawning success (60%) among hormonally induced females.
- Improved synchronization among breeding units increased fertilization rate (>70%).
- Relatively high fecundity (Av. 1.76 ±0.52 million eggs/kg)
- Hatching rate: 78.84 ± 11.93 %
- Over two hundred thousand fingerlings were produced in 2017

Larval Rearing

Effect of turbidity treatments on first feeding mullet larval prey ingestion and juvenile survival

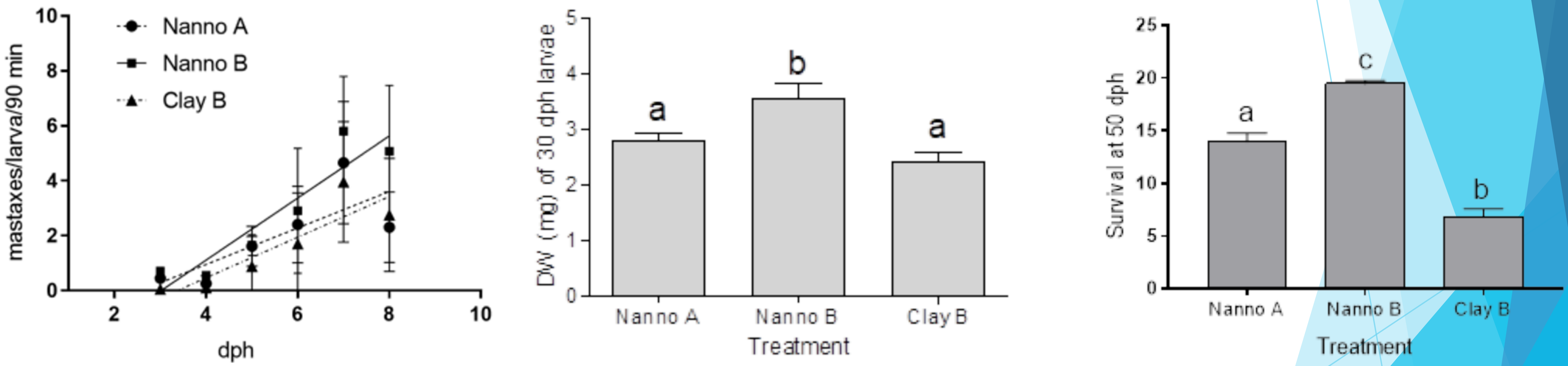
Treatments	Designation	Turbidity (NTU)
Control (no microalgae)	Control	0.26 ± 0.01[a]
Isochrysis galbana A (0.0144 x 10^6 cell/ml)	Iso A	0.77 ± 0.01[b]
Nannochloropsis oculata A (0.2 x 10^6 cells/ml)	Nanno A	0.75 ± 0.01[b]
Isochrysis galbana A (0.0288 x 10^6 cell/ml)	Iso B	1.18 ± 0.02[c]
Nannochloropsis oculata A (0.4 x 10^6 cells/ml)	Nanno B	1.20 ± 0.02[c]





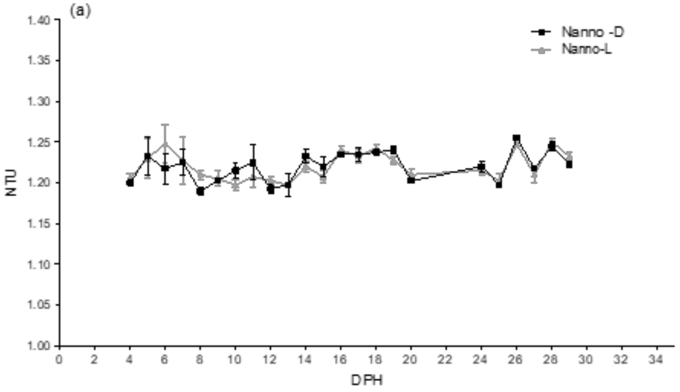
Larval Rearing

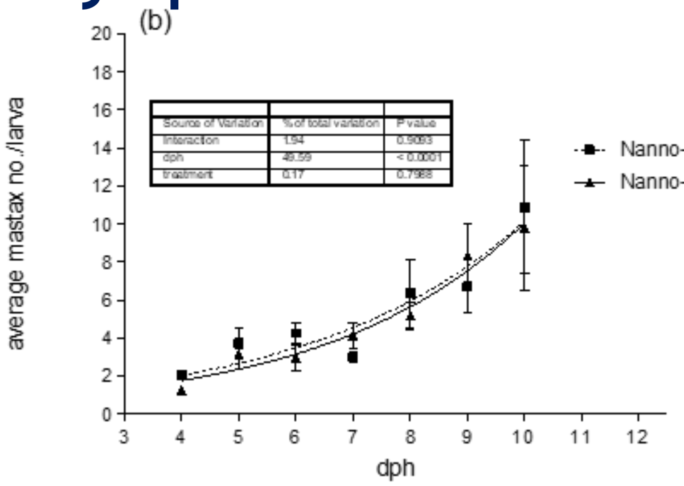
Algae produced vs clay produced turbidity



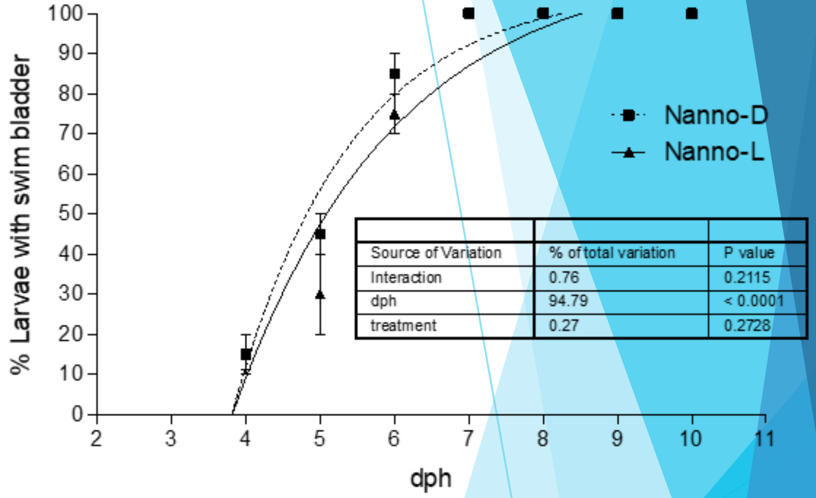
- Algal biochemical content more dominant than turbidity affecting larval performance in grey mullet

Comparing the selected microalgae type and protocol with lyophilized substitute

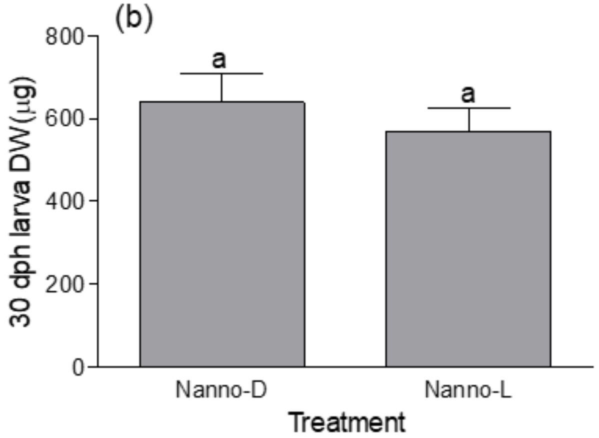


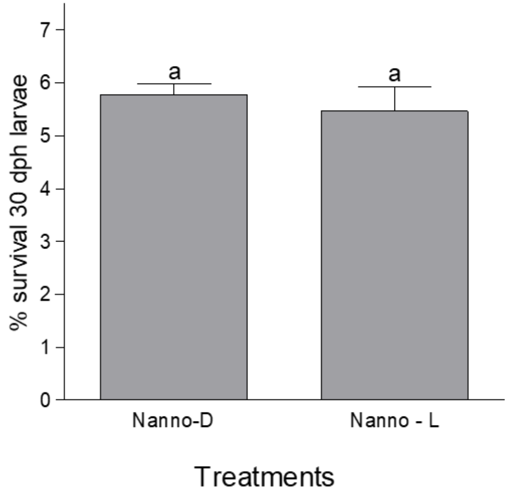


Source of Variation	% of total variation	P value
Interaction	1.94	0.9093
dph	49.59	< 0.0001
treatment	0.17	0.7988



Source of Variation	% of total variation	P value
Interaction	0.76	0.2115
dph	94.79	< 0.0001
treatment	0.27	0.2728





- Can use more economical and convenient dry algae instead of live algae to green tanks

Israel Oceanographic & Limnological Research

DIVERSIFY

IRTA RESEARCH & TECHNOLOGY FOOD & AGRICULTURE

Juvenile rearing

Pancreatic enzymes-amylase

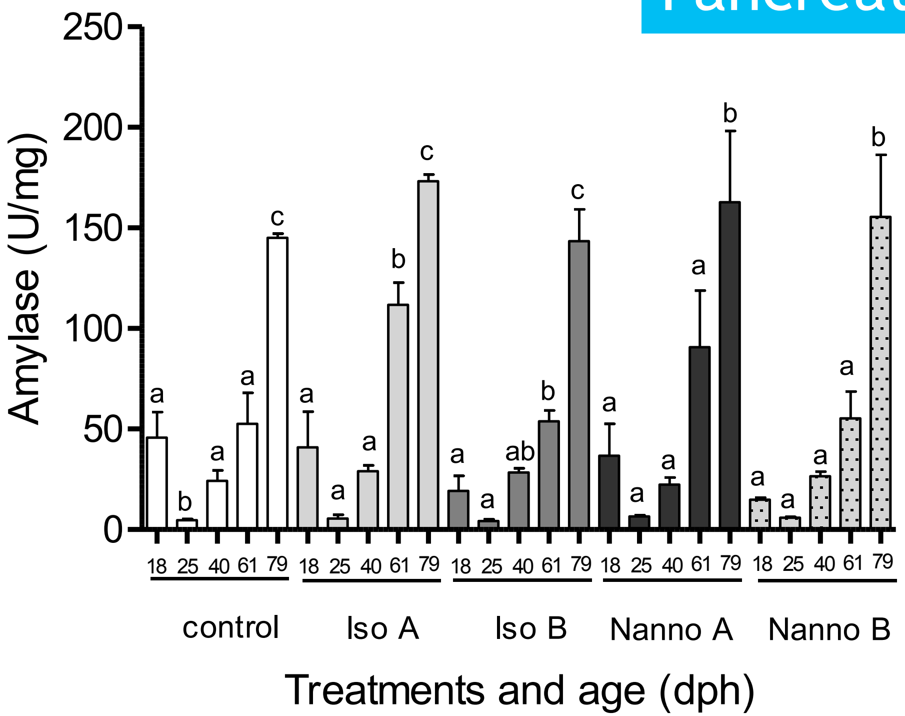


Table 19.1.5 Composition (%) of food used to feed the grey mullet at different stages of development.

Feed	Enriched Rotifers*	Unenriched *Artemia***	Enriched *Artemia****	Caviar (Bernaqua, Belgium) †	*Ulva lactaca* (IOLR, Israel)****	Ranaan Dry feed (RDF, Israel)†
Days fed (dph)	1-23	15	16-24	25-50	25-50	50-79
Protein	48.2	56.2	53.1	55	34	56
Lipid	14.0	17.0	28.7	15	7.4	14
Carbohydrate	18.5	3.6	3.5	8	56	1
Ash	9.3	7.6	2	12	2.6	14.8

*Demir and Diken 2011, **Garcia-Ortega et al. 1998, ***Koca et al 2015, ****analysis at IOLR, †according to manufacturer

- No effect of microalgae treatments
- Mullet amylase production increased 30x from 25-79 dph
- Suggests fish transiting from carnivorous to herbivorous or omnivorous mode of feeding

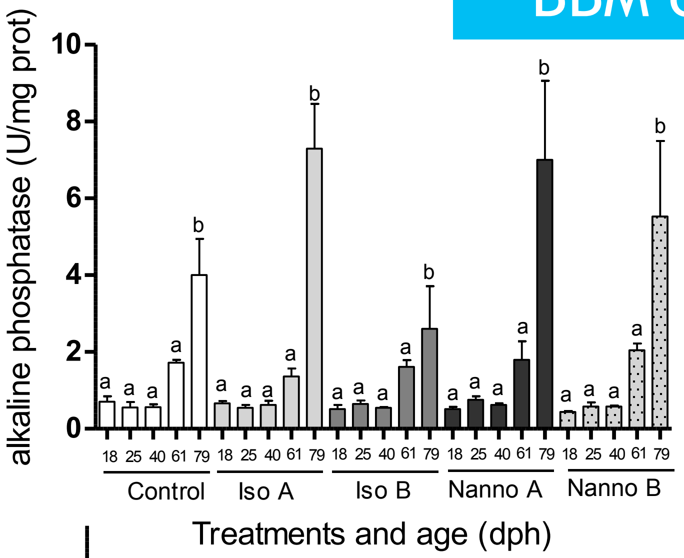
Israel Oceanographic & Limnological Research

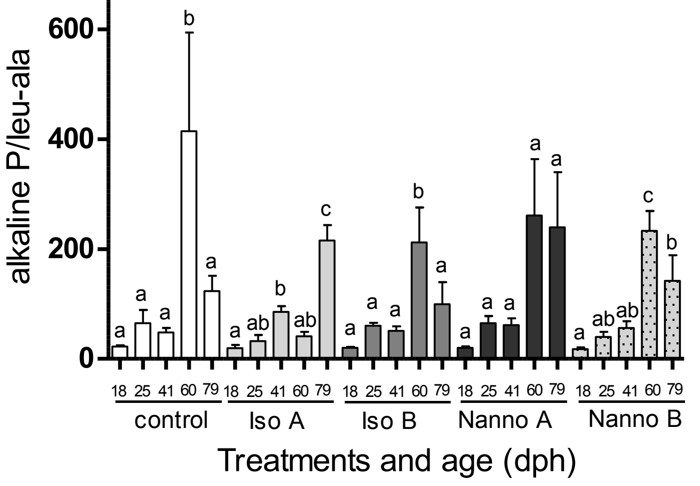
DIVERSIFY

IRTA
RESEARCH & TECHNOLOGY
FOOD & AGRICULTURE

Juvenile rearing

BBM enzyme alkaline phosphatase

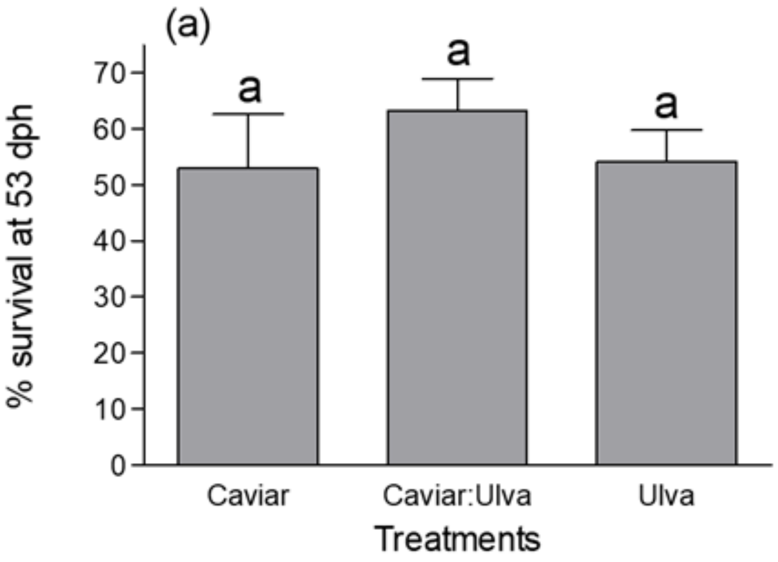


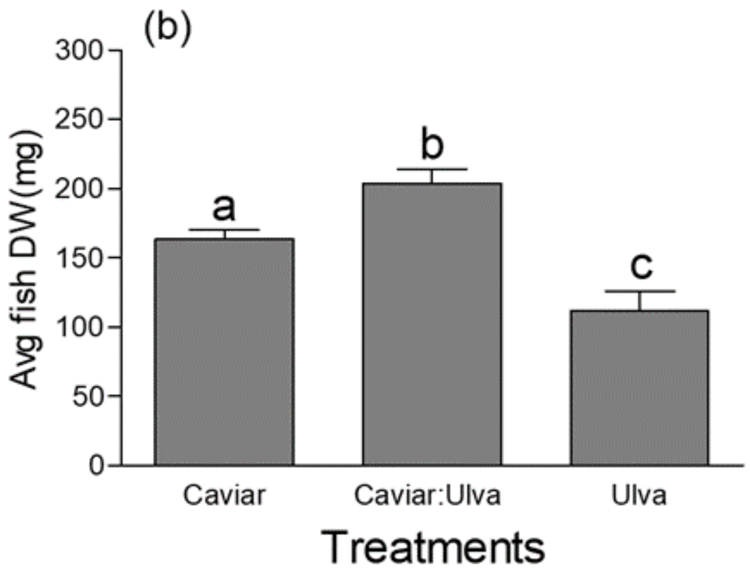


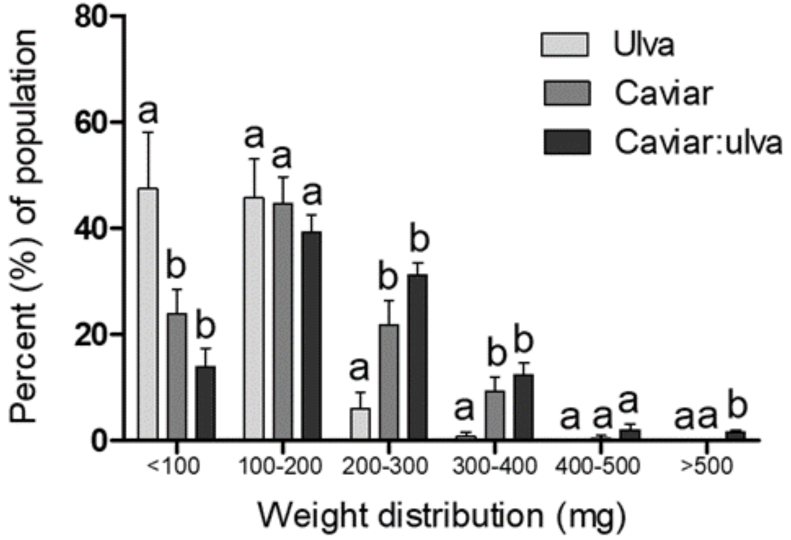
- Mullet BBM Alkaline phosphatase (AP) is a marker for nutrient absorption and BBM development. Peaked at 79 dph.
- Leucine alanine aminopeptidase (LAP) is a marker for intracellular digestion, which is dominant in early development.
- Mullet AP/LAP is (indicator of gut maturation) peaked at 61 dph
- BBM and pancreatic enzyme production largely genetically programmed

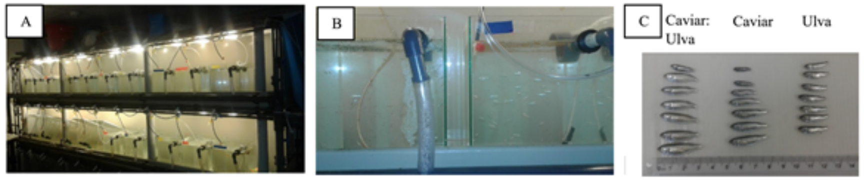
Weaning

Determine if weaning feed type should be carnivorous, omnivorous or herbivorous









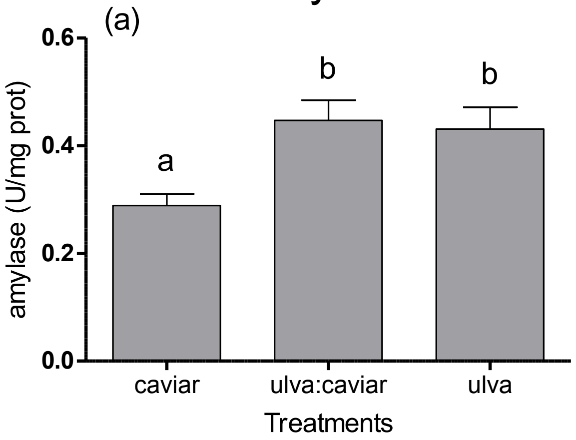
- Omnivorous diet resulted in significantly better survival, growth and production of more larger fish.

Pancreatic and brush border enzymes stimulated by weaning diet in 51 dph fish

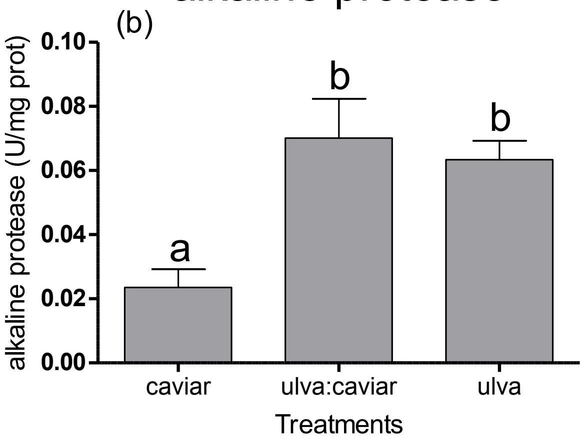
pancreatic

Brush border

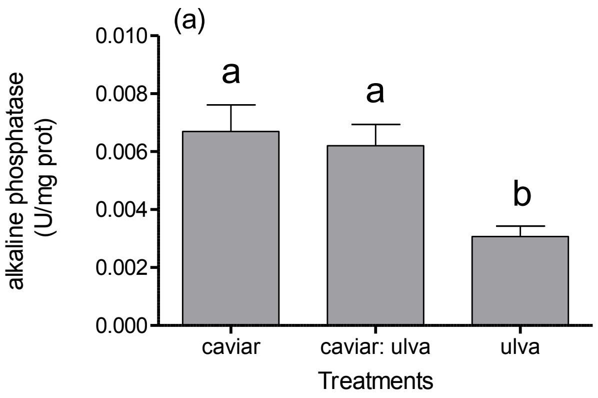
amylase



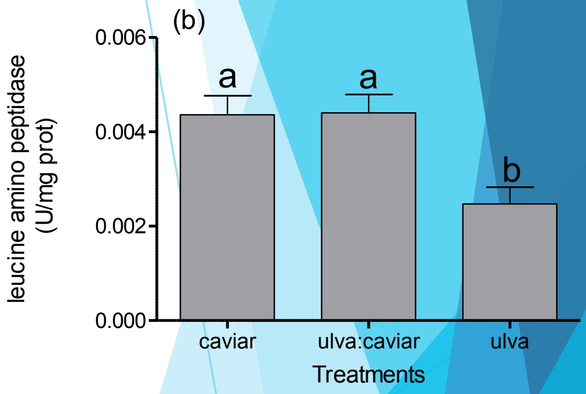
alkaline protease



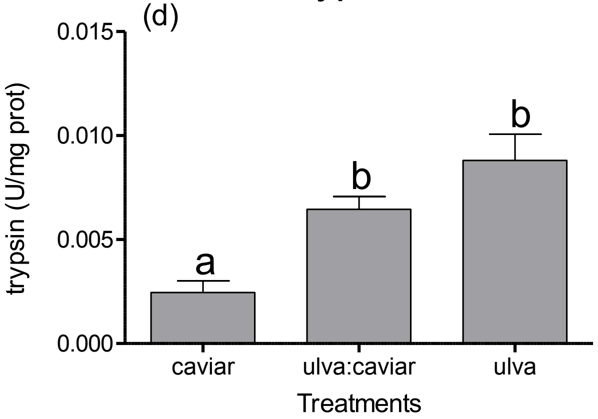
alkaline phosphatase



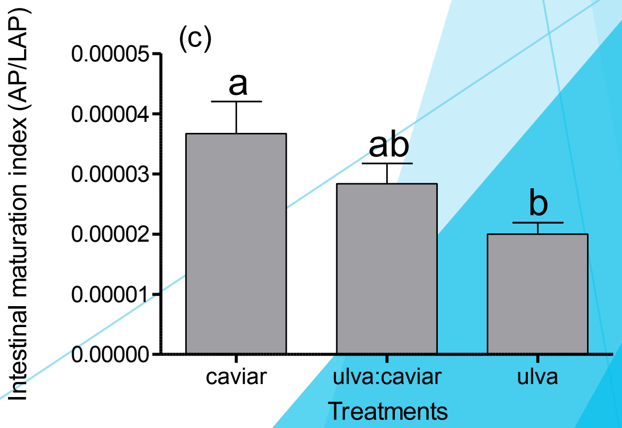
leu-ala aminopeptidase



trypsin



Intestinal maturation index

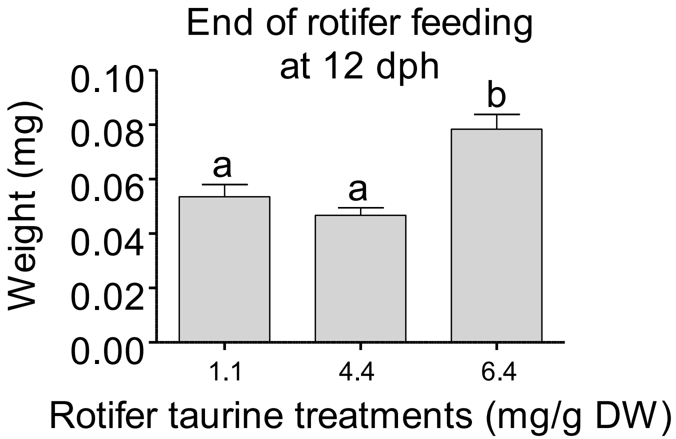


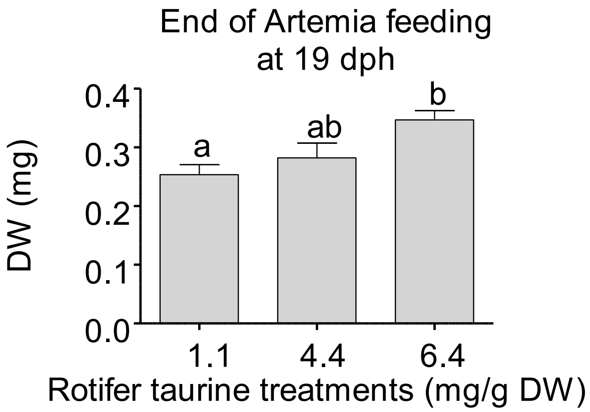
Israel Oceanographic & Limnological Research

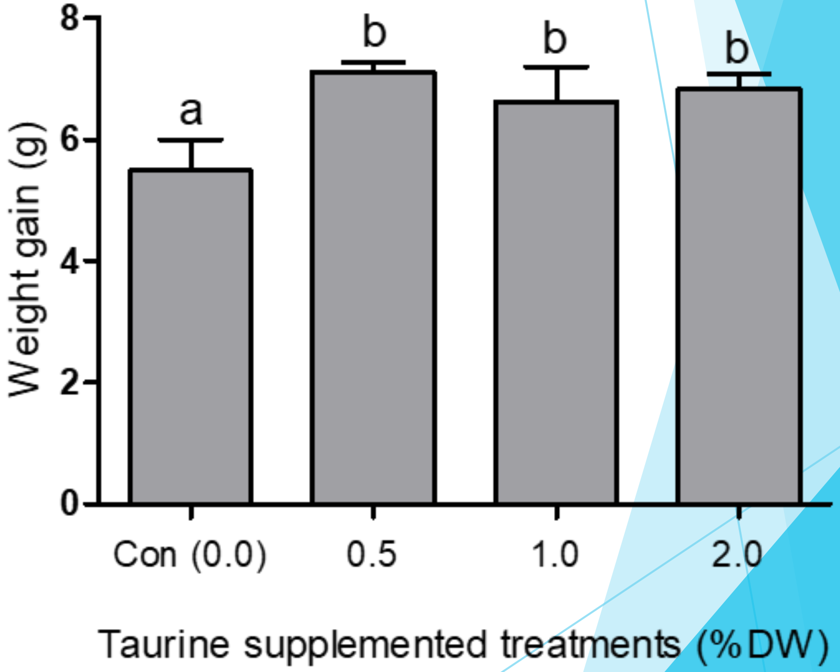
Nutrition

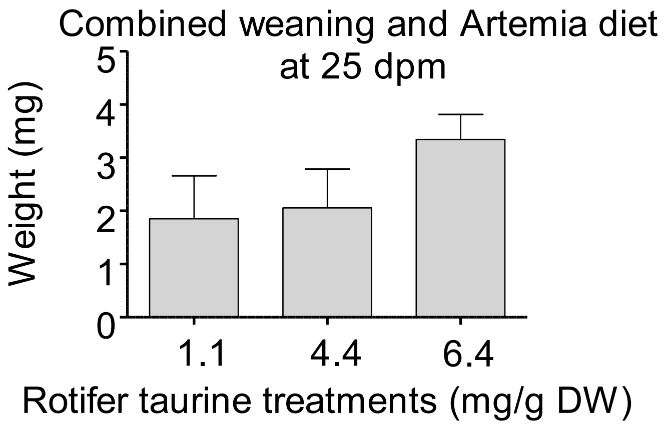
DIVERSIFY

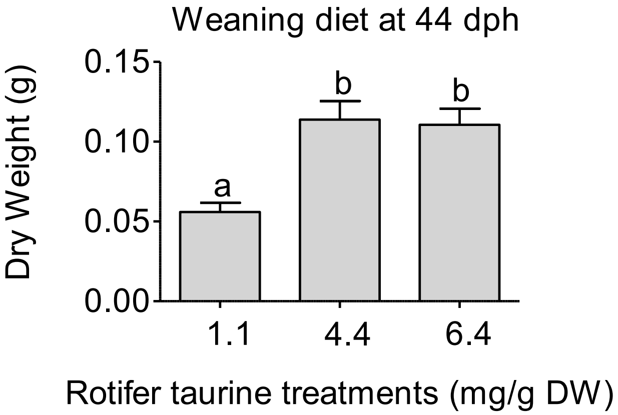
The effect of dietary taurine on growth in different age larvae and juveniles











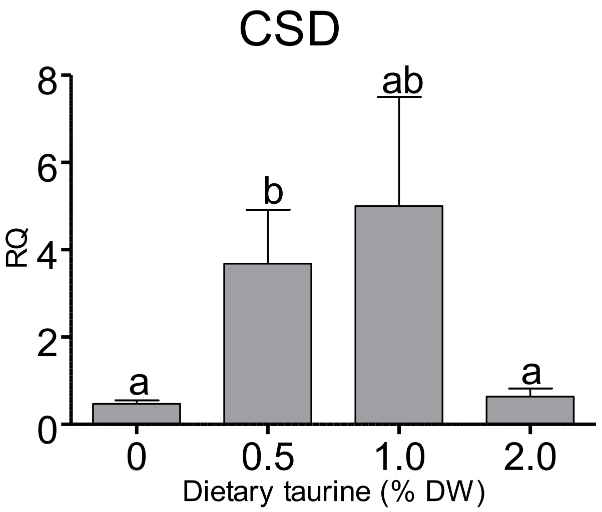
- Further supports that mullets are omnivores- taurine is an animal product.

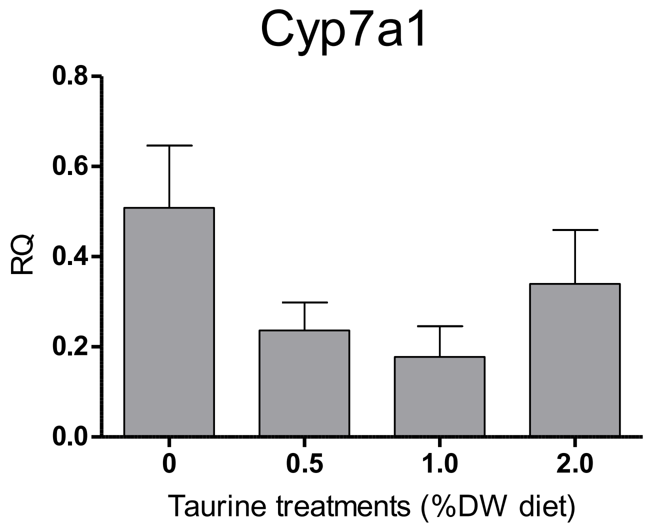
Israel Oceanographic & Limnological Research

DIVERSIFY

Nutrition

Expression of rate limiting enzyme for taurine synthesis; cysteine sulfinate decarboxylase (CSD) and rate limiting enzyme for bile salt; Cyp7a1

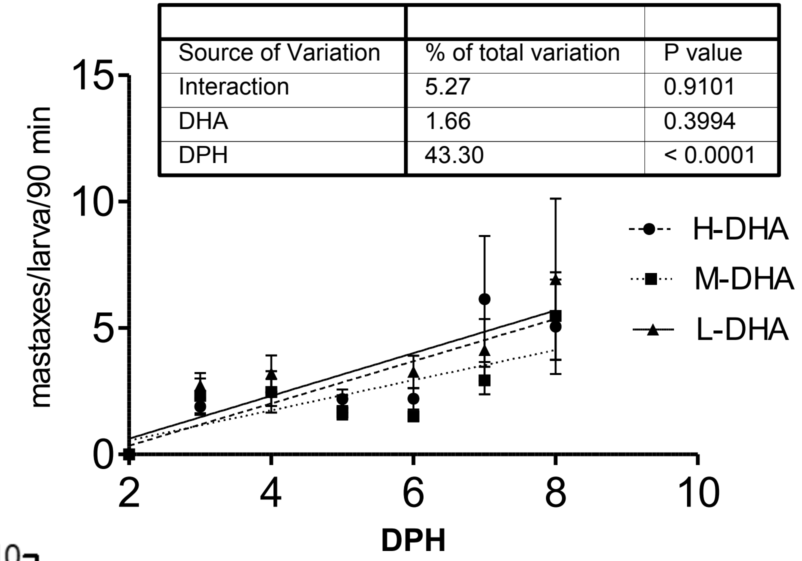




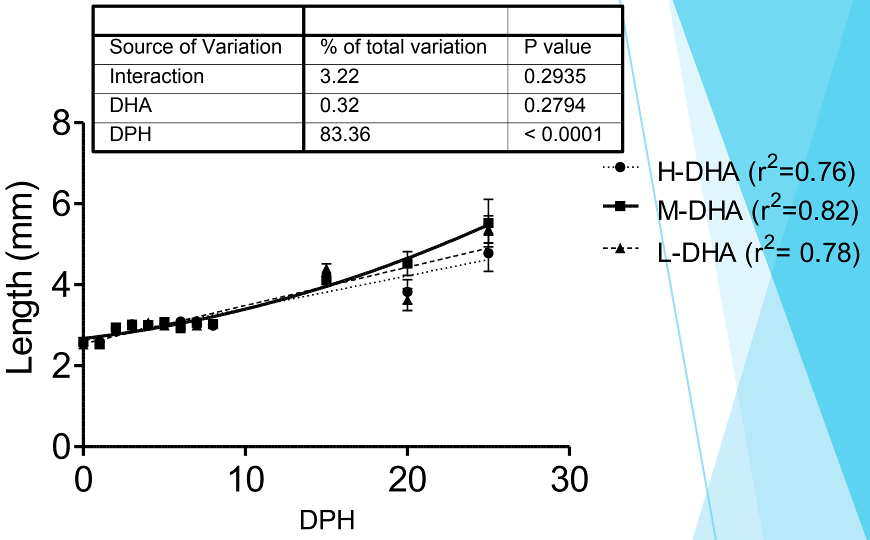
- Taurine dose dependent, significant ($P<0.05$) response of CSD to 1% taurine (8x) and then decrease at 2%.
- no clear, significant ($P>0.05$) response by dietary taurine on the expression of fish liver CYP7A1
- Taurine may function as osmolyte to maintain cell volume-increased taurine in blood reduce osmotic pressure across membrane in liver
- Synthetic capability sufficient for osmolyte and Cyp7a1 and bile salt production.

Nutrition

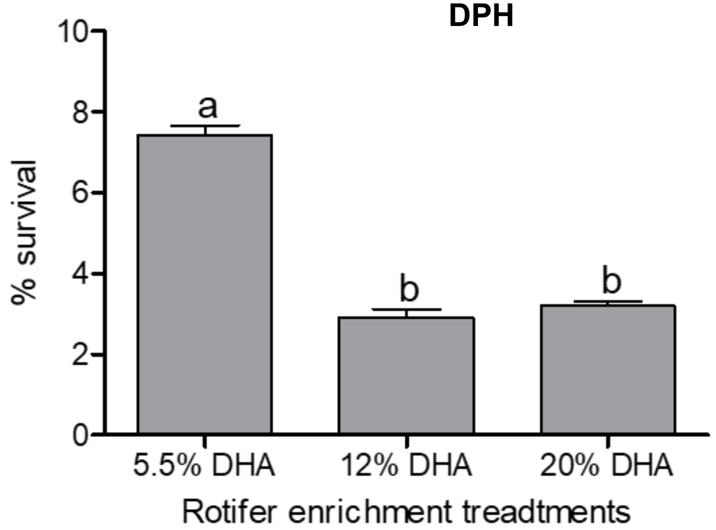
Effect of DHA on larval performance



Source of Variation	% of total variation	P value
Interaction	5.27	0.9101
DHA	1.66	0.3994
DPH	43.30	< 0.0001



Source of Variation	% of total variation	P value
Interaction	3.22	0.2935
DHA	0.32	0.2794
DPH	83.36	< 0.0001



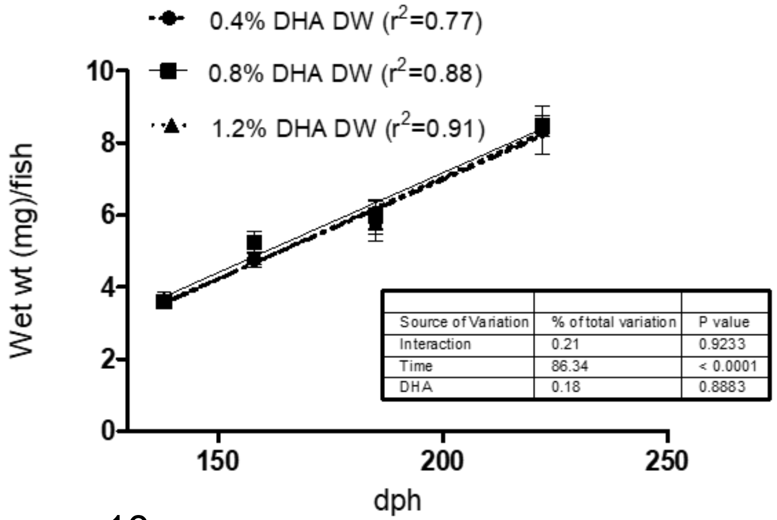
- DHA requirement preparations (12 and 20% of TFA) does not exceed commercial enrichment preparations (e.g. 5.5% in “Red Pepper”)
- Too high DHA may reduce survival

Israel Oceanographic & Limnological Research

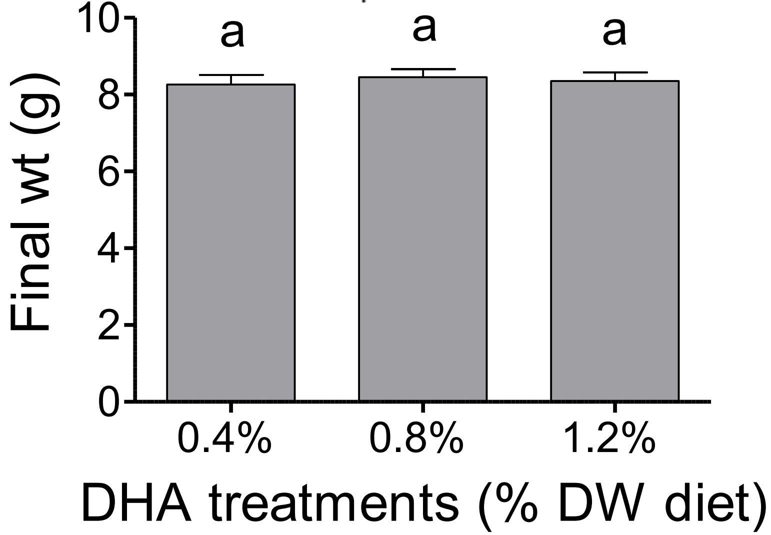
DIVERSIFY

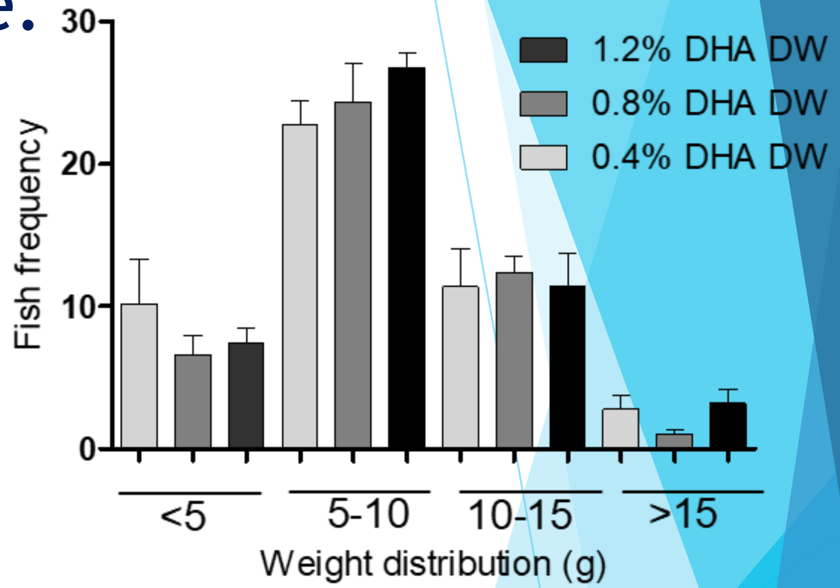
Grow-out of juveniles

Effect of DHA in non-fish meal grow-out diets on fish performance.



Source of Variation	% of total variation	P value
Interaction	0.21	0.9233
Time	86.34	< 0.0001
DHA	0.18	0.8883





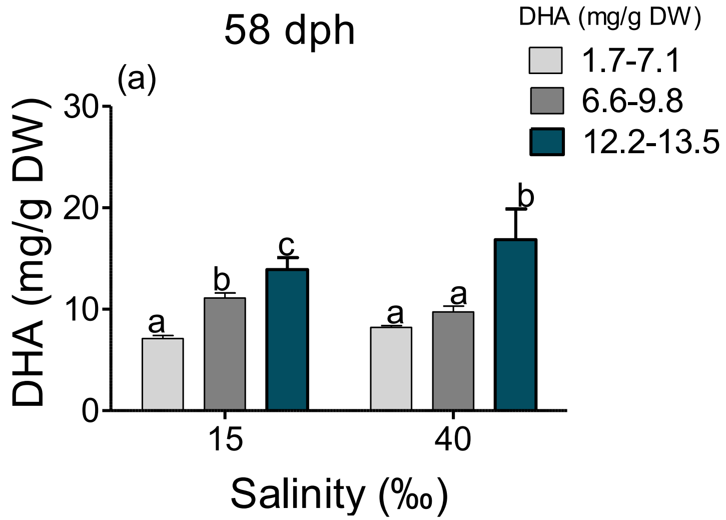
- There was no DHA dose dependent effect on growth rate, final weight and weight distribution.
- DHA requirement is ≤ 0.4% DW diet.

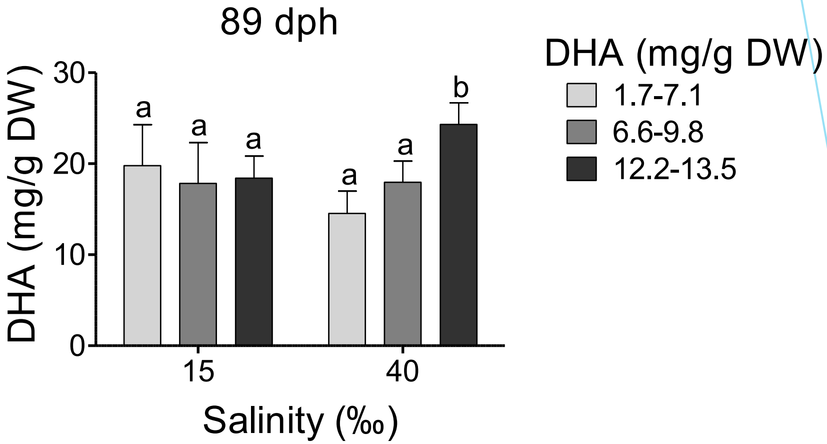
Israel Oceanographic & Limnological Research

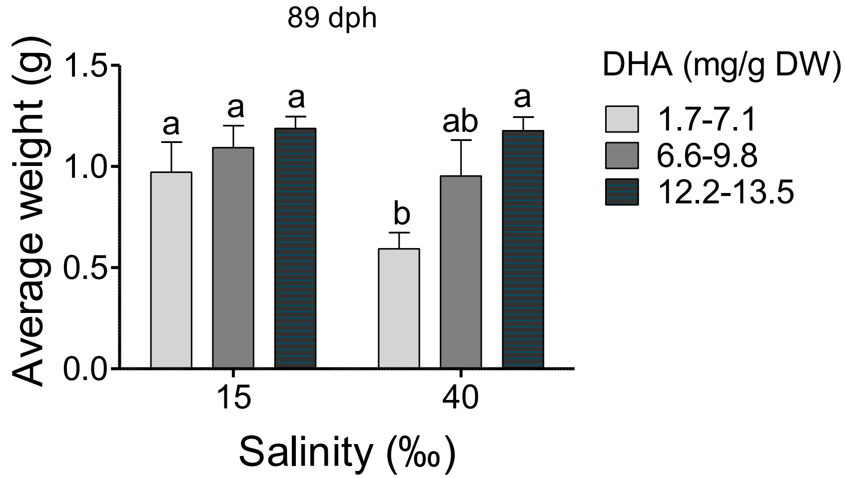
Grow-out of juveniles

DIVERSIFY

Salinity as the driving force for DHA requirement in grey mullet







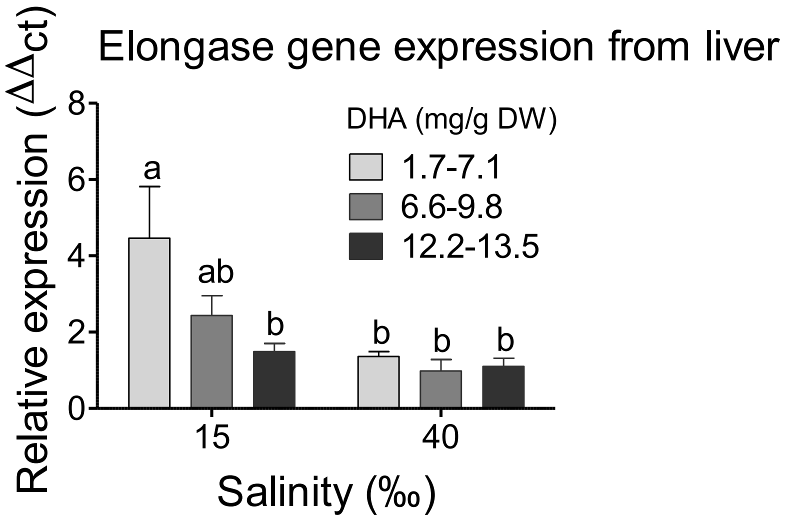
- At 58 dph-dietary DHA dose dependent effect on tissue DHA in both 40 and 15 ‰.
- At 89 dph-dietary DHA dose dependent effect on tissue DHA only in 40 ‰ group. In 15 ‰ group tissue DHA independent of diet.
- At89 dph-dietary DHA dose dependent effect on growth only in 40 ‰ group.
- Suggests that mullet have DHA synthetic ability at low salinity.

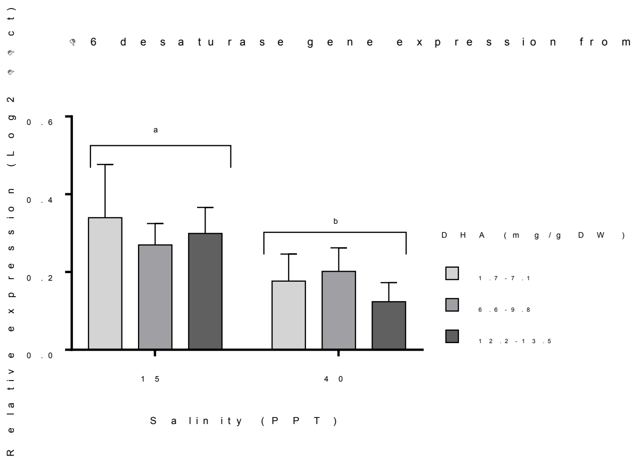
Israel Oceanographic & Limnological Research

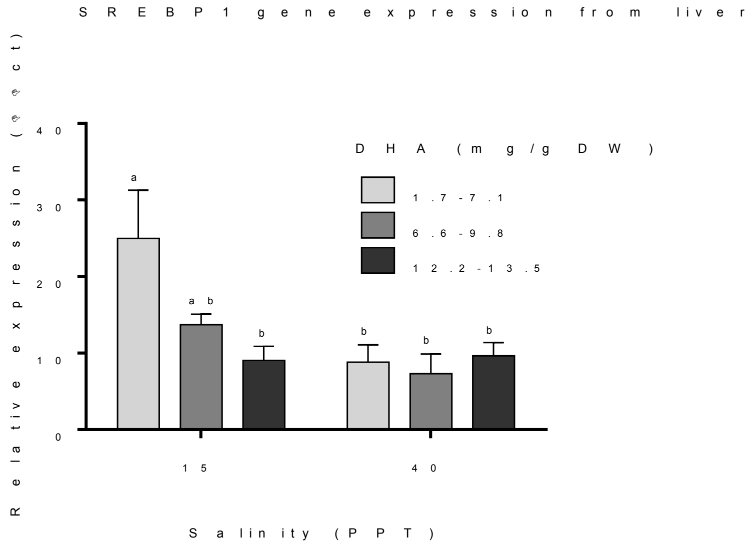
DIVERSIFY

Nutrition

Gene expression of key enzymes in DHA synthesis



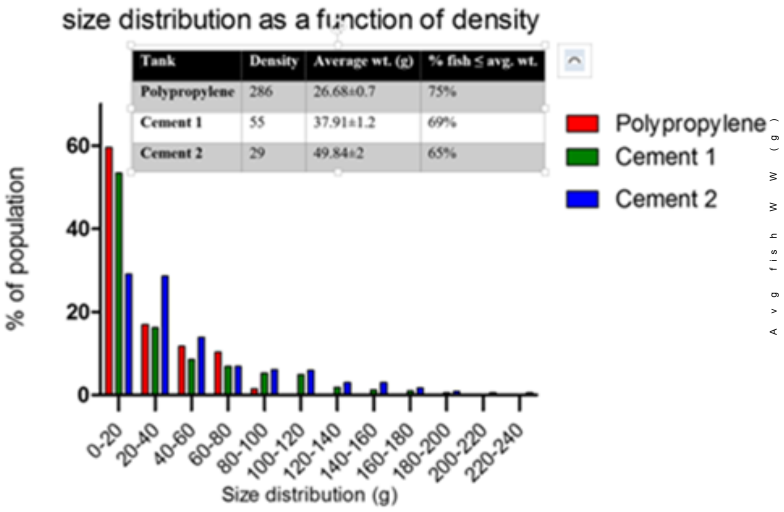




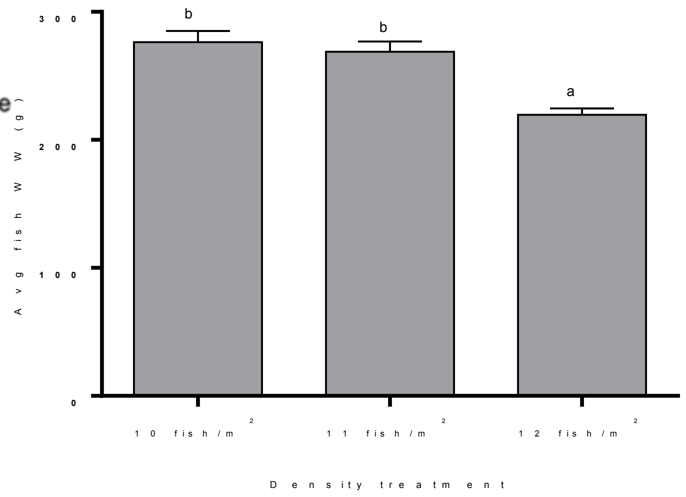
- Inverse dietary DHA dose dependent effect on elongase gene expression in low salinity of 15 ‰.
- Δ6 desaturase gene expression independent of dietary DHA but activated by low 15 ‰ salinity.
- Inverse dietary DHA dose dependent effect on transcription factor SREBP1
- In nature mullet moving to estuaries-less rich in LCPUFA and more abundant in smaller chain PUFA precursors
- Dietary DHA can be decreased in juvenile mullet at low salinity

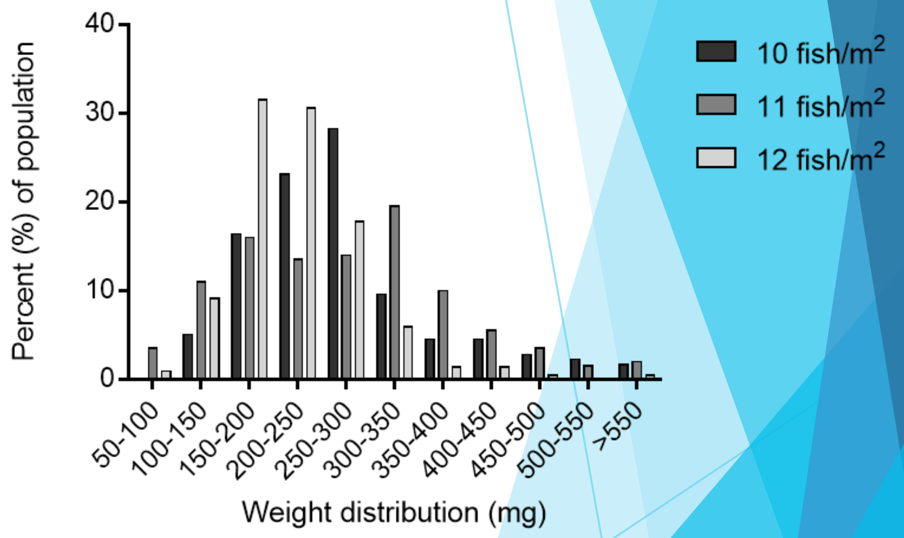
Grow out

Effect of stocking density on weight distribution in cement and polypropylene tanks



Tank	Density	Average wt. (g)	% fish ≤ avg. wt.
Polypropylene	286	26.68±0.7	75%
Cement 1	55	37.91±1.2	69%
Cement 2	29	49.84±2	65%

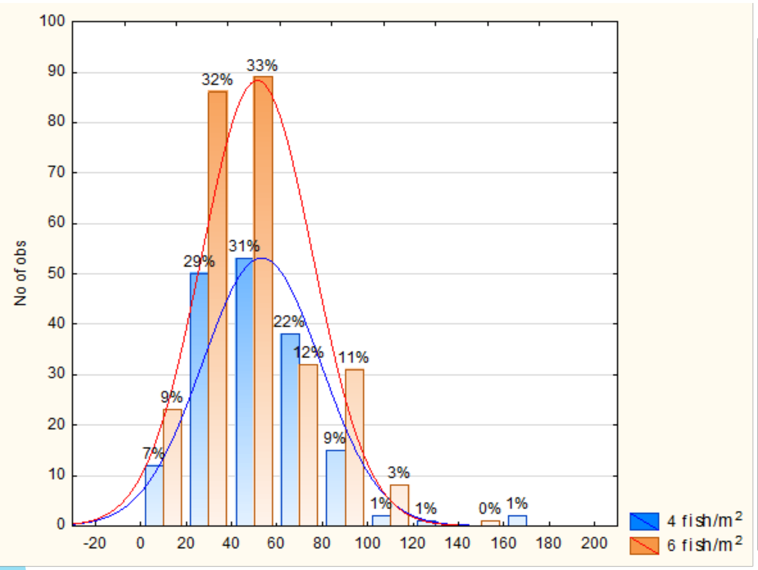


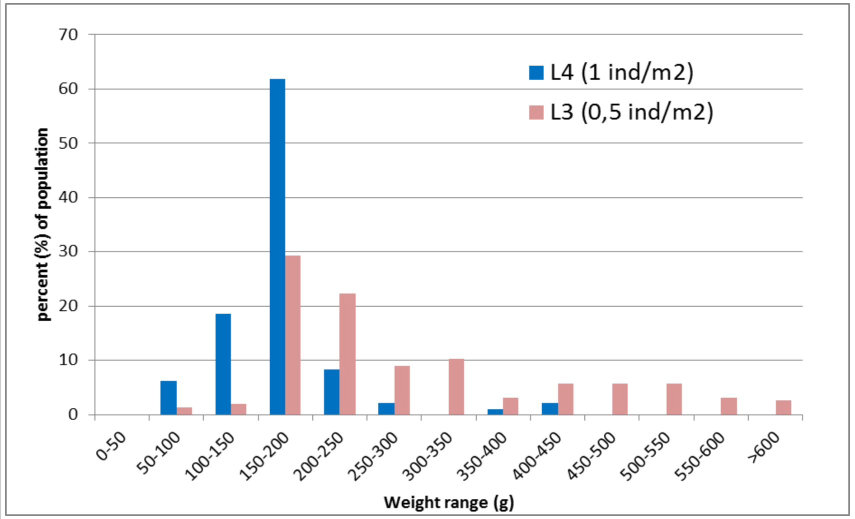


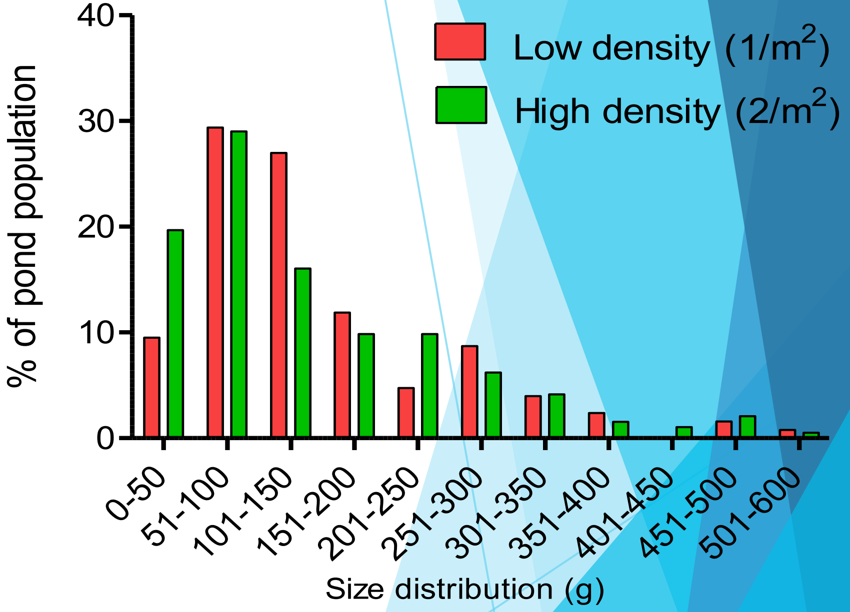
- As stocking density increases, the size distribution skewed to smaller fish
- Reduced feed utilization (FCR↑). Feed is the highest cost in grow out.
- Obstacle to efficient monoculture

Grow out

Effect of stocking density using IOLR formula extruded diet under commercial grow-out conditions







HCMR -Greece

- 6 cement 14^3 ponds
- 15 months
- No significant differences in survival, growth and size distribution.
- Poor growth-30 g/fish gain

CTAqua-Spain

- 1100 and 800 m^2 ponds
- 18 months
- Avg. wt; 294.2 (0.5/m^2), 174.5 (1/m^2)
- Density effect

IOLR-Israel

- 2 plastic line earthen ponds (144 m^2)
- Avg. wt.; 154 g (1/m^2), 145 g(2/m^2)
- 17 months
- Density effect

Practical **take-home messages** for grey mullet culture

- Improved spawning performance, quality and extended season through timed hormonal therapy.
- Greening of larval tanks with *Nannochloropsis oculata* (0.5×10^6 cells/ml) most effective. Microalgae content and not turbidity is dominant factor affecting larval performance.
- Can replace live microalgae with dry microalgae but not clay. More economical.
- Omnivorous weaning diet most effective, in terms of growth, survival and production of larger fish. Not a carnivorous or herbivorous diet.
- Gut maturation ca. 60 dph. Grow-out diet based on fish's capacity to digest high carbohydrate and low protein diets. Lower feed costs.
- Taurine requirement in larval (0.6%) and juvenile (0.5% DW diet) stages, despite some capacity to synthesize .

Practical **take-home messages** for grey mullet culture

- Commercial enrichment preparations containing 5.5% DHA (of TFA) are sufficient during larval rearing while 0.4% DHA DW diet sufficient for juvenile grow-out.
- Salinity is the driving force for DHA (LCPUFA) synthesis and requirement during grow-out of juvenile mullet. Suggests lower levels of dietary DHA needed. More economical.
- As pond stocking density increases, the size distribution skewed to smaller fish whether in cement or earthen ponds or polypropylene tanks. Obstacle to efficient monoculture.
- **The entire juvenile production at P4.IOLR for 2017 was *ca.* 200,000 fish.**

Publications

Israel Oceanographic & Limnological Research

DIVERSIFY

Aquaculture 462 (2016) 92–100

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

ELSEVIER

Weaning wild flathead grey mullet (*Mugil cephalus*) fry with diets with different levels of fish meal substitution

CrossMark

Enric Gisbert [a,*], Mansour Torfi Mozanzadeh [b], Yannis Kotzamanis [c], Alicia Estévez [a]

[a] IRTA, Centre de Sant Carles de la Ràpita (IRTA-SCR), Unitat de Cultius Experimentals, Crta. del Poble Nou Km 5.5, 43540 Sant Carles de la Rápita, Spain
[b] Fisheries Department, Faculty of Marine Natural Resources, Khoramshahr Marine Science and Technology University, Iran
[c] Institute of Marine Biology, Biotechnology and Aquaculture, Athens, Greece

ELSEVIER

Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology

Volume 228, February 2019, Pages 71-80

This article is a part of the Special Issue on Aquaculture

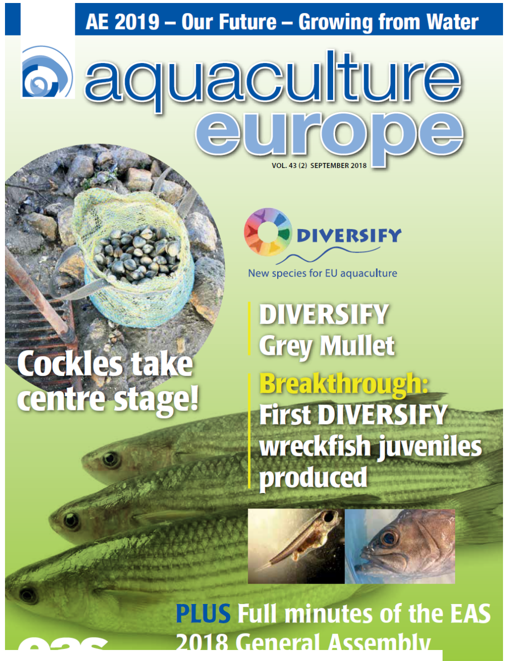
The effect of algal turbidity on larval performance and the ontogeny of digestive enzymes in the grey mullet (*Mugil cephalus*) ☆

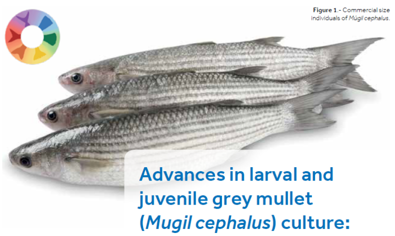
William Koven [a], Enric Gisbert [b], Oriya Nixon [a], Mikhail M. Solovyev [c, d], Aviad Gaon [a], Guy Allon [a], Iris Meiri-Ashkenazi [a], Amos Tandler [a], Hanna Rosenfeld [a]

Show more

https://doi.org/10.1016/j.cbpa.2018.11.005

Get rights and content





Advances in larval and juvenile grey mullet (*Mugil cephalus*) culture: The DIVERSIFY project

W. KOVEN[1], O. NIXON[1], I. MEIR-ASHKANAZI[1], R. ROTEM[1], A. TANDLER[1], D. ISRAELI[1], H. BRACHA[1], A. CORREIRO[2], E. GISBERT[3], H. ROSENFELD[1], M. BANOVIC[4], L. GUERRERO[5], G. TACKEN[6], R. ROBLES[7]

The grey mullet *(Mugil cephalus)* (Fig. 1) is one of 6 species selected for the European program DIVERSIFY (FP7, GA 603121), a five-year project to advance our knowledge and its practical application in the culture of new and emerging finfish species, with the potential of satisfying an expanding sustainably the European market for a variety of fresh sea food. There is increasing interest in the culture of the omnivorous grey mullet as a high quality source of protein and as a species that requires little or no dietary fishmeal (FM). Moreover, the salted and dried roe (bottarga) from gravid females is considered a highly prized delicacy in the southern Mediterranean and an added value product from the culture of this species.

The future growth of the grey mullet aquaculture is limited by a number of bottlenecks, which have been addressed in DIVERSIFY. The control of the reproductive cycle and improving egg quality via broodstock management and nutrition was necessary not only for the production of robust larvae, but also for obtaining high value bottarga. A very important issue has been the development of a larval rearing protocol necessary to reduce early mortalities and size dispersion, as well as increas-

NEWS

Market-size grey mullet (Credit: DIVERSIFY)

EU experiment "designs" effective weaning diet for grey mullet juveniles

Grey mullet (*Mugil cephalus*) fingerlings fed an omnivorous weaning diet, which has moderate carbohydrate and protein levels, exhibited superior growth and biomass gain compared to carnivorous and herbivorous weaning feeds.

This was a main finding of a grey mullet study within the framework of a EU funded project through DIVERSIFY. The project explores the biological and socio-economic potential of new/emerging candidate fish species for expansion of the European aquaculture industry.

Carnivorous feeds have low carbohydrate and high protein levels while herbivorous feeds have high carbohydrate and low protein levels.

The study was carried out by Dr. William Koven and his team at the Israel Oceanographic and Limnological Research Institute, the National Center for Mariculture (IOLR/NCM) in Eilat, Israel with the help of Dr. Enric Gisbert and his group at the Institute for Food and Agricultural Research & Technology (IRTA) in Spain.

"The mullet at the fingerling or juvenile stage is increasingly producing significant levels of amylase with age for carbohydrate digestion as well as proteases to break down animal and plant protein suggesting an omnivorous mode of feeding.

"Amylase is a pancreatic enzyme that digests starch to maltose, which is ultimately broken down by brush border enzymes to glucose units that are absorbed into the enterocytes of the intestine.

"Pancreatic proteases digest proteins to oligopeptides, which are further hydrolyzed through brush border enzymes to di- and tri-peptides and free amino acids to be absorbed, through different mechanisms, into the enterocytes as well," Dr. William Koven, associate professor at the IOLR/NCM in Israel, told Hatchery International.

The ability to breakdown considerable levels of starch to glucose might also provide energy that would be protein sparing and allow more protein deposition in the tissues and further promote growth.

Koven explained, "This makes sense as grey mullet undergo a trophic shift at this stage of development from carnivorous larvae to omnivorous fingerlings which move towards less saline estuaries and are exposed to a diet of invertebrates and carbohydrate rich micro and macroalgae.

"These results will contribute to improved juvenile performance during weaning in fish farms, leading to higher fish production and faster growth," he said of the study's impact on the program.

– Ruby Gonzalez

In preparation

1. Koven, W., Gisbert, E., Estevez, A., Nixon, O., Meiri-Ashkenazi, I., Tandler, A., Rosenfeld, H. **Comparing the efficacy of adding live or lyophilized *Nannochloropsis oculata* on grey mullet (*Mugil cephalus*) larval performance.**
2. Koven, W., Gisbert, E., Meiri-Askenazi, I., Nixon, O., Tandler, A., Rosenfeld, H. **Determine the efficacy of weaning diet type during the carnivorous-omnivorous transition of juvenile grey mullet (*Mugil cephalus*).**
3. Koven, W., Meiri-Ashkenazi, I., Nixon, O., Tandler, A., Rosenfeld, H. **The importance of dietary taurine at different developmental stages during the larval rearing of the grey mullet (Mugil cephalus).**
4. Koven, W., Meiri-Ashenazi, I., Nixon, O., Tandler, A., Rosenfeld, H. **The effect of dietary taurine on growth, survival and fatty acid distribution of selected tissues in juvenile grey mullet (*Mugil cephalus*).**
5. Koven, W., Meiri-Ashenazi, I., Nixon, O., Tandler, A., Rosenfeld, H. Grey mullet (*Mugil cephalus*) have a moderate Docosahexaenoic acid requirement during larval and juvenile rearing.
6. Israeli, D., Bitan, A., Nixon, O., Tandler, A., Koven, W. **Salinity as the driving force of DHA synthesis and requirement in juvenile grey mullet.**
7. Rosenfeld, H., Corriero, A., Rodriguez, C.et al. **Advances in broodstock nutrition of grey mullet enhances larval performance.**
8. Corriero, A., Rodreguez, C., Rosenfeld, H. et al. **Effect of captivity on the timing of the onset of first sexual maturity in grey mullet (*Mugil cephalus*).**
9. Rosenfeld et al. **Overcoming reproductive dysfunction in captive grey mullet (*Mugil cephalus*) broodstock.**

Israel Oceanographic & Limnological Research

Further challenges and studies for grey mullet

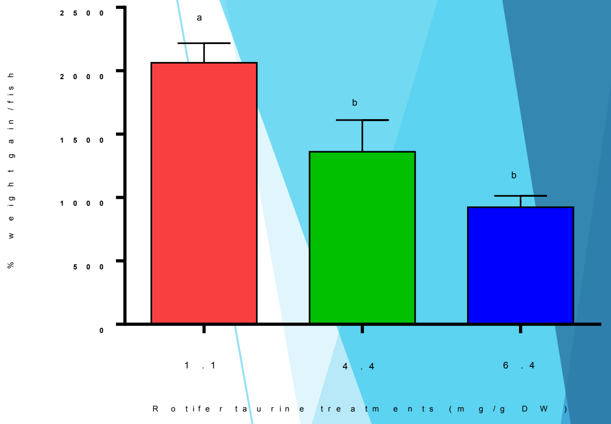
1. Reducing mortality during the first 10 days of larval rearing.

a. Improve broodstock diet.

b. Investigate specific algal nutrients that stimulates gut maturation.

d. Ciliate pre-rotifer feeding to improve survival past PNR.

(A)



2. Poor growth and size variability during grow-out

a. Major obstacle for the future of grey mullet monoculture.

b. Exacerbated with increased stocking density of ponds.

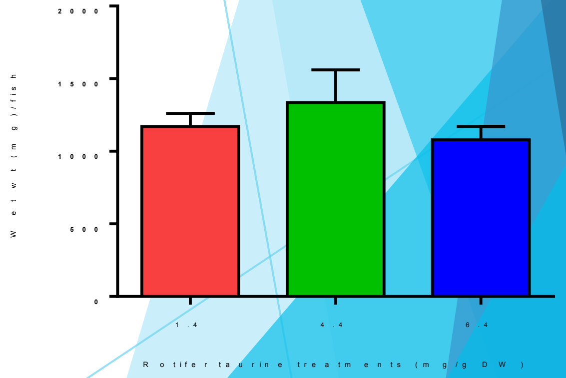
c. Small fish separated from large fish grow significantly faster than larger fish; Growth compensation (**see Figure a,b**).

d. Possible stress interaction between individuals. Modulate the stress response through the diet.

e. May lead to female skewed populations, which grow faster.

f. Improve grow-out diet-fish also feeding on natural productivity as well as feed.

(B)



Israel Oceanographic & Limnological Research

hcmr
ΕΛΚΕΘΕ

IRIDA

IRTA
RESEARCH & TECHNOLOGY
FOOD & AGRICULTURE

ctaqua
CENTRO TECNOLÓGICO DE LA ACUICULTURA

DIVERSIFY

This project has received funding from the European Union's Seventh Framework Programme for Research, technological development and demonstration (KBBE-2013-07 single stage, GA 603121, DIVERSIFY