Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente, Ciclo XXXVII

Use of flavorings to improve aquafeed palatability in aquaculture: a multidisciplinary approach to better understand teleost physiological responses

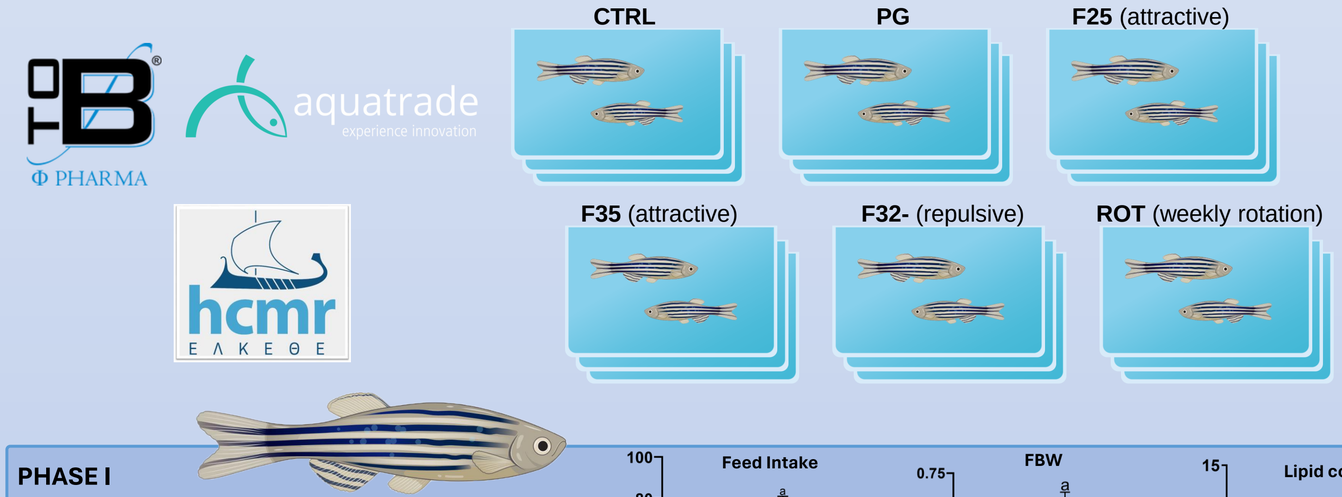
PhD student: Federico Conti

DiSVA, Laboratorio Biologia dello Sviluppo e della Riproduzione *Tutor: Prof. Ike Olivotto*

The inclusion of novel ingredients in aquafeeds often impairs palatability, affecting fish feed intake and growth, with implications for farm economics and the environment. In this regard, feed attractants (FAs) are generally included in fish diets to improve feed acceptability. However, while marine-derived FAs pose unsustainability issues, alternative attractive substances have led to controversial results. In this regard, synthetic flavors are emerging as a novel and sustainable alternative to improve feeding strategies for a sustainable production.

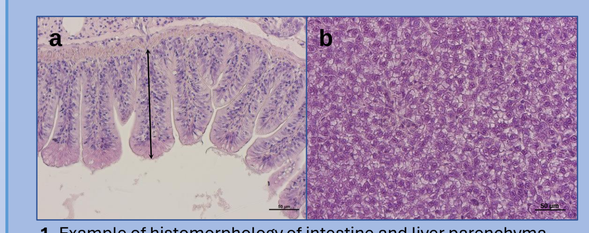
The aim of this PhD project is to identify and test different synthetic flavors, in the zebrafish (*Danio rerio*) whole life cycle, to assess their potential role as feed attractants by evaluating the fish physiological responses. Subsequently, knowledge obtained in the zebrafish model, an omnivorous species, can be transposed, using a multi multidisciplinary approach, to the most commercially relevant species for the mediterranean aquaculture, as European seabass (*Dicentrarchus labrax*) and Gilthead Seabream (*Sparus aurata*).

Materials and Methods



PHASE I

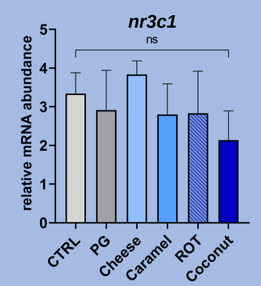
Zebrafish (*Danio rerio*)



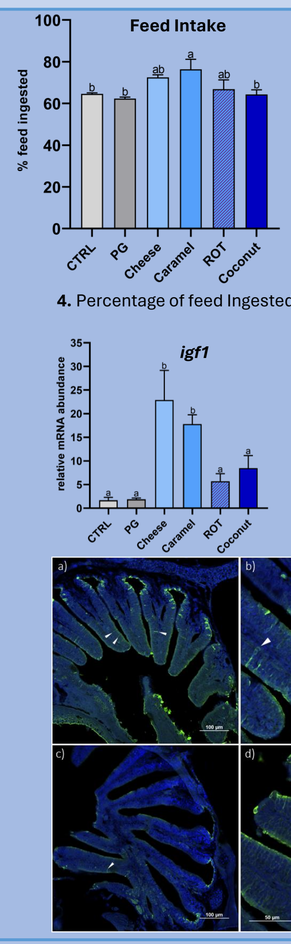
1. Example of histomorphology of intestine and liver parenchyma of adult zebrafish.

	CTRL	PG	F25	F35	ROT	F32-
Mucosal folds height	197.9 ± 8.6	213.3 ± 41.4	222.8 ± 54.3	180.2 ± 11.1	177.4 ± 16.1	183.5 ± 20.6
Inflammatory influx	+	+	+	+	+	+
Mucosal folds fusion	+	+	+	+	+	+

2. Histological indexes measured in the intestine of adult zebrafish fed the experimental diets.

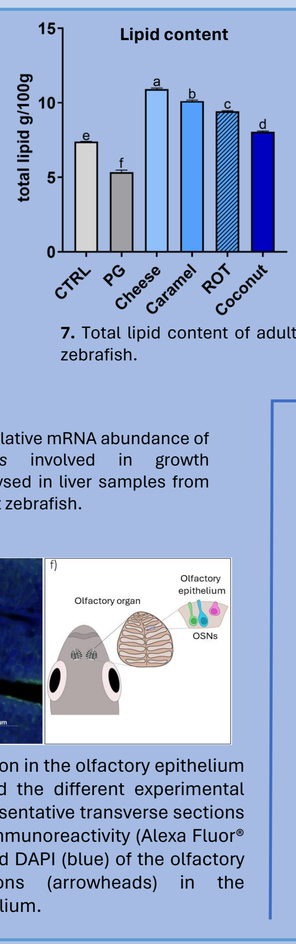


3. Relative mRNA abundance of *nr3c1* involved in stress response analysed in liver samples from adult zebrafish.

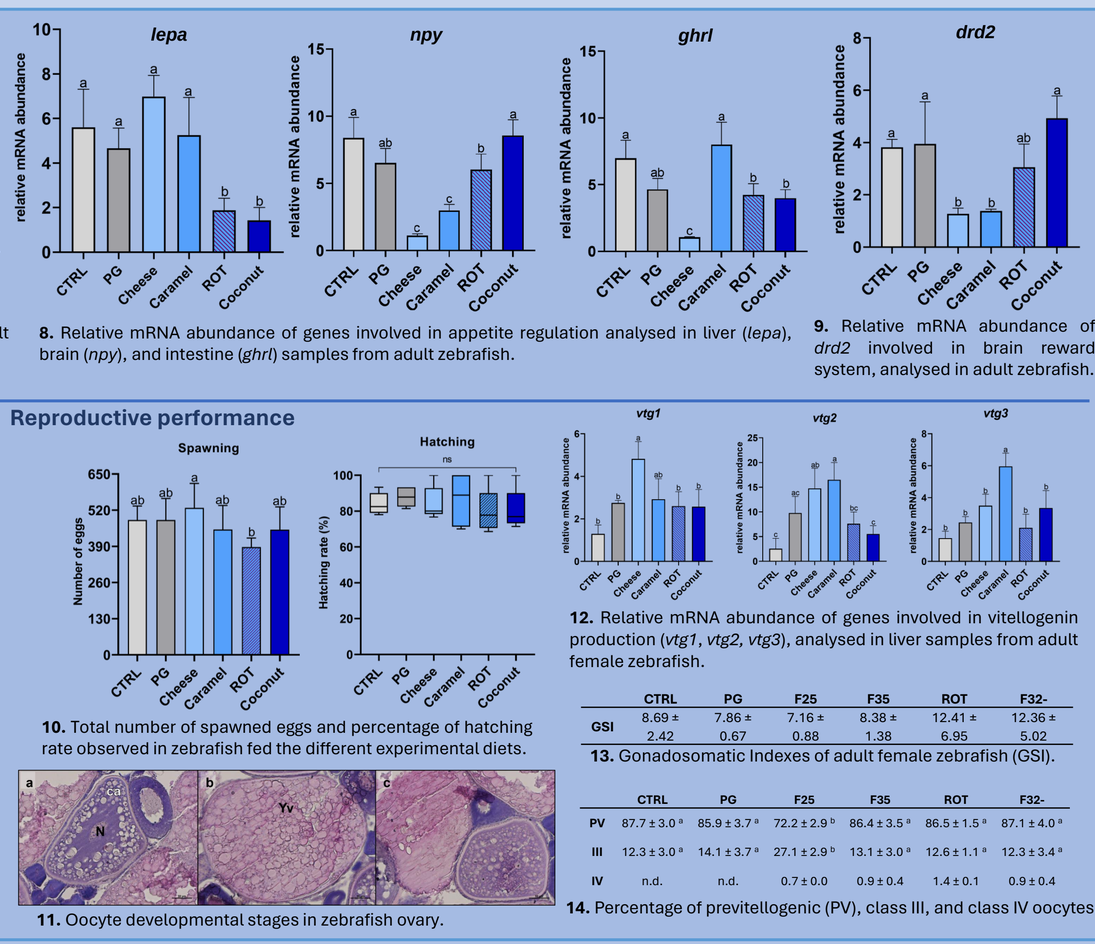


4. Percentage of feed Ingested.

5. Final body weight.



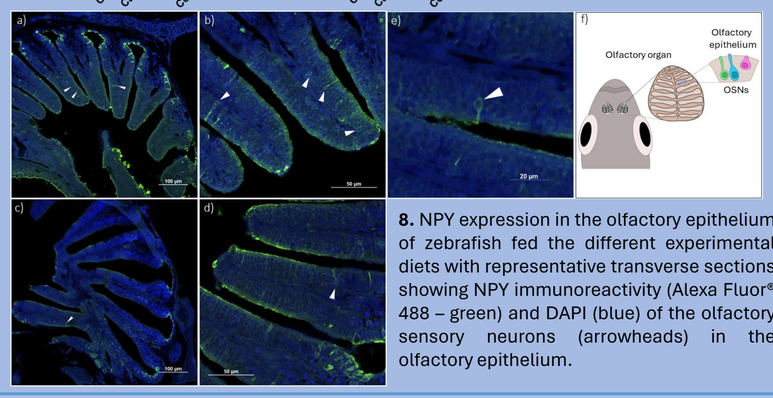
7. Total lipid content of adult zebrafish.



8. Relative mRNA abundance of genes involved in appetite regulation analysed in liver (*lepa*), brain (*npy*), and intestine (*ghrl*) samples from adult zebrafish.

9. Relative mRNA abundance of *drd2* involved in brain reward system, analysed in adult zebrafish.

6. Relative mRNA abundance of *genes* involved in growth analysed in liver samples from adult zebrafish.



8. NPY expression in the olfactory epithelium of zebrafish fed the different experimental diets with representative transverse sections showing NPY immunoreactivity (Alexa Fluor® 488 – green) and DAPI (blue) of the olfactory sensory neurons (arrowheads) in the olfactory epithelium.

Reproductive performance

10. Total number of spawned eggs and percentage of hatching rate observed in zebrafish fed the different experimental diets.

11. Oocyte developmental stages in zebrafish ovary.

12. Relative mRNA abundance of genes involved in vitellogenin production (*vtg1*, *vtg2*, *vtg3*), analysed in liver samples from adult female zebrafish.

	CTRL	PG	F25	F35	ROT	F32-
GSI	8.69 ± 2.42	7.86 ± 0.67	7.16 ± 0.88	8.38 ± 1.38	12.41 ± 6.95	12.36 ± 5.02

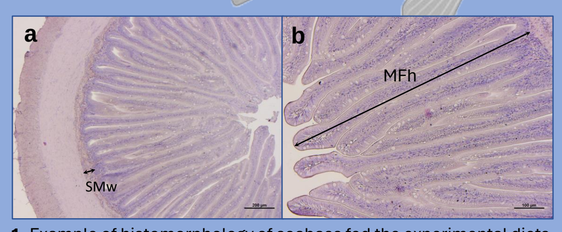
13. Gonadosomatic Indexes of adult female zebrafish (GSI).

	CTRL	PG	F25	F35	ROT	F32-
PV	87.7 ± 3.0 [a]	85.9 ± 3.7 [a]	72.2 ± 2.9 [b]	86.4 ± 3.5 [a]	86.5 ± 1.5 [a]	87.1 ± 4.0 [a]
III	12.3 ± 3.0 [a]	14.1 ± 3.7 [a]	27.1 ± 2.9 [b]	13.1 ± 3.0 [a]	12.6 ± 1.1 [a]	12.3 ± 3.4 [a]
IV	n.d.	n.d.	0.7 ± 0.0	0.9 ± 0.4	1.4 ± 0.1	0.9 ± 0.4

14. Percentage of previtellogenic (PV), class III, and class IV oocytes.

PHASE II

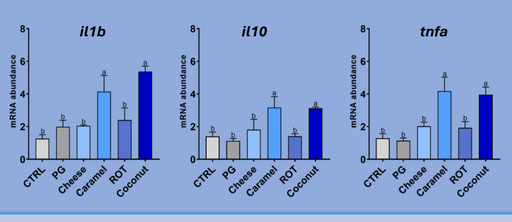
European seabass (*Dicentrarchus labrax*)



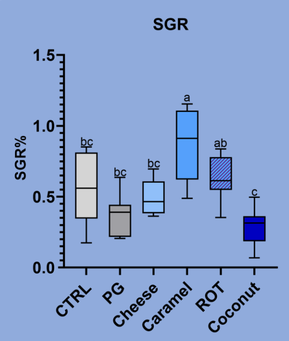
1. Example of histomorphology of seabass fed the experimental diets.

	CTRL	PG	F25	F35	ROT	F32-
Mucosal fold height	1023.0 ± 73.9	969.4 ± 52.0	977.7 ± 71.9	942.2 ± 75.2	1097.0 ± 40.2	918.0 ± 30.1
Submucosa width	38.98 ± 0.045	40.92 ± 2.5	45.03 ± 2.64	40.31 ± 2.05	42.34 ± 1.54	45.86 ± 2.04
Mucosal fold fusion events	+	+	+	+	+	+
Basal inflammatory influx	-	-	-	-	-	-
Supranuclear vacuoles	-	-	-	-	-	-

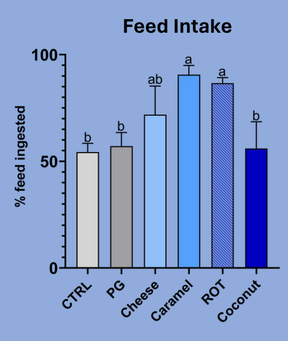
2. Histological indexes measured in the intestine of seabass fed the experimental diets.



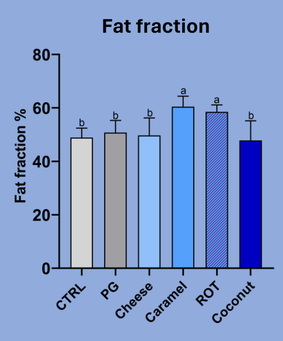
3. Relative mRNA abundance of genes involved in immune response (*il1b*, *il10*, and *tnfa*) analysed in intestine samples from seabass.



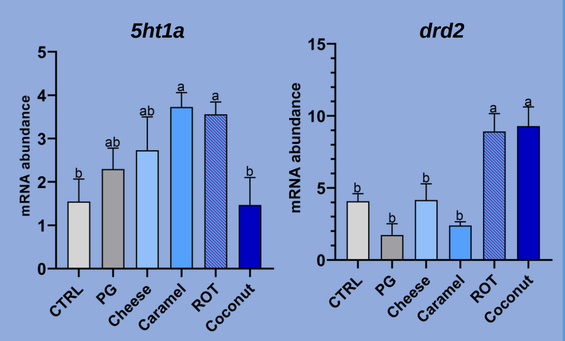
4. Specific Growth Rate of seabass fed the experimental diets.



5. Percentage of feed Ingested by seabass.



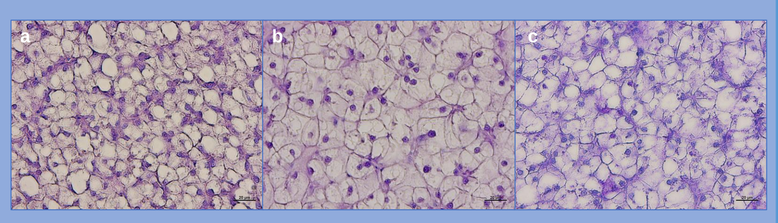
7. Hepatic lipid accumulation of seabass.



9. Relative mRNA abundance of genes involved in serotonergic (*5ht1a*) and dopaminergic (*drd2*) activity analysed in brain samples from seabass.

	CTRL	PG	F25	F35	ROT	F32-	*p-Value*
WG	120.9 ± 25.8 [b]	108.1 ± 17.6 [b]	122.2 ± 15.2 [b]	194.1 ± 26.1 [a]	167.9 ± 32.7 [ab]	110.4 ± 13.9 [b]	0.0026
IBW (g/fish)	67.8 ± 6.3	71.5 ± 8.9	72.6 ± 8.1	75.3 ± 7.8	71.6 ± 7.8	75.9 ± 8.3	0.8291
FBW (g/fish)	196.0 ± 35.4 [b]	180.1 ± 17.6 [b]	194.1 ± 15.1 [b]	256.1 ± 16.1 [a]	219.9 ± 19.7 [ab]	176.2 ± 10.8 [b]	0.0043
FCR	0.96 ± 0.08	1.17 ± 0.09	1.17 ± 0.09	0.96 ± 0.10	1.04 ± 0.09	1.20 ± 0.15	0.0359
RGR (%)	181.6 ± 22.7 [b]	160.1 ± 24.5 [b]	169.7 ± 21.0 [b]	251.9 ± 20.2 [a]	210.3 ± 27.2 [ab]	176.6 ± 19.3 [b]	0.0034
SGR (%)	1.7 ± 0.1 [bc]	1.5 ± 0.2 [bc]	1.6 ± 0.1 [bc]	2.2 ± 0.2 [a]	1.8 ± 0.2 [ab]	1.3 ± 0.1 [c]	0.0003

6. Zootechnical performance of European seabass fed the experimental diets.



8. Example of histomorphology of liver from European seabass fed the experimental diets.

PHASE III – analyses still ongoing

On the basis of the results obtained during the PhD project, the technology used for the synthetic flavors inclusion in the feed was implemented via the cooperation of two companies (To Be Pharma S.r.l. and Aquatrade) that were involved in the Aquaexcel European project, in collaboration with the **Hellenic Centre for Marine Research (HCMR)**, in Crete, Greece. The experiment was carried out on the model species Gilthead seabream (*Sparus aurata*). This allowed the evaluation of a new technology which trough the use of natural compounds was able to include the flavors in the wall of microcapsules. These microcapsules are able to stick to any aquafeed and to release the flavor once in contact with water.

Conclusions. The enhanced feed palatability stimulate a higher ingestion thereby promoting fish growth and welfare, with positive implications for production yields. These results demonstrated the promising effectiveness of synthetic flavors in advancing aquaculture feeding practices, paving the way for more sustainable and cost-effective progress in the aquaculture sector.

Discussion

Histology. All the flavors had no adverse effects on the overall health of the fish, showing no morphological alteration in tissue's architecture nor inflammations.

Feed intake. An higher feed intake was observed highlighting the attraction of flavored diets. The growth rate analysis further supported the results.

Lipid storage. A higher lipid content in zebrafish, and higher hepatic lipid accumulation in seabass fed attractive diets were observed, highlighting a higher energy reserves availability.

Appetite. The expression of appetite-related signals (*npy, ghrl,* and *lepa*) in zebrafish fed the attractive diets, as well as of serotonin receptor (*5ht1a*) in seabass, evidenced a higher satiety, supporting the feed intake results.

Brain reward system. The dopaminergic activity observed in both the model species may be associated to the reinforcement effects of a long-term exposure to a positive stimulus, related to the reward mechanisms thereby promoting the desire to eat.

Reproduction. Higher feed ingestion promoted reproduction, although optimal reproductive performance was evidence in all groups.