# GAPS IN OCTOPUS FIRST LIFE STAGES NUTRITION

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## Background

**Despite of the recent advances in common octopus (*Octopus vulgaris*) rearing, nutrition remains as the main bottleneck along the first life stages of this species. In the present communication, we discuss different approaches to deal with this problem focusing on the one hand in a deeper knowledge of the physiology and nutrition of the first life stages and on the other hand the selection and/or optimization of diet based on the data obtained before**

# Characterization of nutritional requirements and biomarkers through:

## Metabolic pathways and nutritional requirements

### Key techniques

- ✓ Transcriptomics
- ✓ Proteomics
- ✓ Enzyme activities
- ✓ Pathway modelling
- ✓ Metabolomics

### Previous data

- ✓ Different studies have showed the essentiality of long chain polyunsaturated fatty acids such as ArA, EPA and DHA. The presence of ω3 desaturase has been described (Navarro *et al*., 2014. In Cephalopod culture 77-95, Springer. Garrido *et al*., 2019 *BBA-Mol. Cell. Biol. Lipids* 1864, 1134–1144)
- ✓ The pathways and biomarkers related with improved diet have been characterized and the results showed that most of the proteins and genes over-expressed are related to: cell cycle and replication, production of structural components, and development of the nervous system (García-Fernández *et al*., 2019. *Sci. Rep*. 9, 10312; Varó *et al*., 2022. *Front. Mar. Sci*. 8:817701)
- ✓ The nutritional regulation of genes and proteins confirms that proteins and carbohydrates as the preferred fuels for cephalopods. An increase in fatty acid oxidation would be indicative of an unbalanced lipid profile of preys (Morales *et al*., 2017. *Front. Physiol.* 8:427; Varó *et al*., 2022. *Front. Mar. Sci.* 8:817701)

### Future challenges

- ✓ To study the essentiality of other nutrients such as amino acids, vitamins and trace elements.
- ✓ Further study of the requirements during the settlement phase
- ✓ To determine how is the digestive, metabolic and nutritional status of the octopus at tissue level

## Digestive physiology

- ✓ In vitro and in vivo digestibility.
- ✓ Histological and histochemical studies.
- ✓ Genetic expression of digestive enzymes.
- ✓ Nutrient radiolabelling.
- ✓ Diet fluorescence markers.
- ✓ Digestive enzyme assays.

- ✓ Trajectory of food from mouth to stomach - crop - stomach - caecum – digestive gland (Nande *et al*., 2017. *Front. Physiol.* 8, 573)
- ✓ Alkaline proteases mainly in the salivary glands; and cathepsin-type enzymes in gastric juice (Hamdan *et al*., 2014. *Aquac. Res.* 45(12), 2048-2056)
- ✓ Enzymatic activity influenced by the animal size, temperature or feeding (Linares *et al*., 2015. Aquat. Biol. 24(2), 127-140)
- ✓ Characterization of the digestive system and digestive enzyme activity throughout development and under the effect of different diets (Fernández-Gago *et al*., 2017. *Front. Physiol.* 8: 462 ; Martín *et al*., 2015. *Aquaculture Europe 2015*)

- ✓ Timing of digestion, absorption and assimilation of nutrients
- ✓ Gut transit times for different preys.
- ✓ Relationship between temperature and gut transit.
- ✓ Markers for absorption of different proteins and lipids.
- ✓ Protocols for determining nutrient digestibility.
- ✓ Measurement of pH along the digestive tube.
- ✓ Regulation of the secretion of digestive enzymes.
- ✓ Enzymatic profile of digestive organs throughout digestion.
- ✓ Factors affecting the activity of digestive enzymes.

## Microbiome

- ✓ DNA metabarcoding of gut contents
- ✓ Bioinformatic analysis

- ✓ A meta-analysis among different studies showed that there is no common microbiota for the different groups of cephalopods, but there could be a bias depending on the species (Sanz JC. Thesis Master ULPGC, Spain 2021)
- ✓ Paralarvae reared in captivity with Artemia showed a depletion of bacterial diversity compared to wild paralarvae (Roura *et al*., 2017. *Front. Physiol*. 8:362)

- ✓ To understand how the natural microbiota of octopus may be affected under culture conditions and how these variations in turn affect the development of paralarvae and juveniles.
- ✓ Isolate, identify and select different microbial strains from biological samples of octopus with probiotic potential.
- ✓ To analyse the potential relationship between microbiota and immune system in cephalopods (Aitken, 2019. Animal Sentience 26( 11))

# Development of diets:

## Inert diets

- ✓ Design and formulation
- ✓ Biochemical composition
- ✓ Buoyancy
- ✓ Acceptability
- ✓ Ingestion measures

- ✓ Paralarvae attack and ingest inert diets. Effective ingestion can be tested adding 10 µm fluorospheres to the inert diet. (de Moraes Lenz, *et al*., 2019. *Aquac. Res.* 50(10), 3070-3073)
- ✓ A gelatine based diet for *O. vulgaris* was patented by Estefanell *et al*., 2018 (ES2599603)
- ✓ Alginate-crab diets increases average weight at day 14. (Delgadillo, A. 2018. Master Thesis. Univ. of La Laguna)

- ✓ Formulate inert diets with commercial ingredients
- ✓ Designing inert diets to assess nutritional requirements
- ✓ Effects of heated ingredients in growth rate
- ✓ Effects of exogenous enzymes and emulsifiers

## Artemia enrichments

- ✓ Design and formulation
- ✓ Biochemical composition
- ✓ Buoyancy
- ✓ Acceptability
- ✓ Ingestion measures

- ✓ A meta-analysis showed that enrichment based in marine phospholipids improved the growth in *O. vulgaris* paralarvae (Garrido *et al*., 2018. *Rev. Aquac.* 10(1), 3-14)
- ✓ A radiolabelling study showed that Artemia may not be the most appropriate vehicle to provide DHA to paralarvae.(Reis *et al*., 2019. *Aquaculture* 500, 264-271)
- ✓ An alternative way to transport bioactive molecules bond to exoesqueleton through quitine binding domains ha s been developed, (Talens-Perales *et al*., 2017. *Aquaculture* 474, 95-100)

- ✓ Design of new *Artemia* enrichment protocols, with phospholipids, n-3 HUFA, copper and antioxidants, for use in co-feeding with other live preys

## Alternative preys

- ✓ Prey selection
- ✓ Protocols for prey rearing
- ✓ Ingestion measures

- ✓ Decapod zoeae such as spider crab (*Maja* sp.) have showed the best results in paralarvae viability (Iglesias and Fuentes. 2014. In Cephalopod culture, pp. 77-95, Springer; Garrido *et al*., 2018. *Rev. Aquac.* 10(1), 3-14)
- ✓ It is necessary to search for a live prey easy to obtain and maintain in the laboratory that meets the nutritional requirements of the octopus paralarvae and adapts to its predatory behaviour (Reis *et al*., 2021. *Aquac. Res.* 52(1), 105-116.
- ✓ Paralarvae are specialist predators at least during the first weeks of their life cycle and showed seasonal and spatial variability in the diet (Roura *et al*., 2012. *Mar. Biol.* 159(6), 1335-1345; Olmos-Pérez *et al*., 2017. *Front. Physiol.* 8:321.)

- ✓ Search for alternative prey with an adequate nutritional profile, easy to capture and keep in captivity or commercially available
- ✓ Look for preys suitable for both the paralarval and settlement phases.

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