（学位第３号様式）

学 位 論 文 要 旨

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題　目	**Study on the ontogenetic development of the digestive system of marine fish larvae for diet optimization** **(餌の最適化に向けた海産魚類仔魚の個体発生における消化機構の研究)**

Fish larvae hatch with a rudimentary digestive system, but demand a highly efficient enzymatic digestive machinery that is able to process the impressive amount of ingested nutrients required for supporting their high growth and development. This study aimed to investigate the early ontogeny of marine fish larvae through an integrative investigation to understand the general patterns of activity through the effect of feeding status, morphological changes, and gene expression on final enzymatic capacity. Three marine fish species were evaluated including red seabream (*Pagrus major*), Japanese flounder (*Paralichthys olivaceus*) and Kawakawa tuna (*Euthynnus affinis*). The results showed that most of digestive enzymes (trypsin, chymotrypsin, lipase and amylase) were genetically programmed and activated at hatching. When the larvae begin exogenous feeding, the digestive tract gradually differentiates into specific-functional organs and digestive capacity improves with larval age. Energy metabolism (*cox, atp),* growth (*igf1)* and peptide regulatory factors (*cck, ny*) related genes were transcribed and translated at hatching to assist upon the onset of exogenous feeding. Detection of gastric glands and pepsin activity indicated the functionality of the stomach. The detectable point of gastric glands and pepsin varied in different fish species such as at 15 days after hatching (DAH) in *P. major*, 25 DAH in *P. olivaceus* and 10 DAH in *E. affinis*. However, the activation of functions of the gastric gland does not necessarily imply full functioning of the stomach and therefore weaning from live feed into formulated feed should be considered coinciding with the peak of pepsin activity. Dramatic drops of proteolytic and lipolytic enzyme activity could result in mass larvae mortality, these were observed at 15 to 20 DAH and 25–30 DAH in *P. major*, 20-30 DAH in *P. olivaceus* and 6 - 10 DAH in *E. affinis*. Delay in enzymatic precursor gene transcription and expression of different isoforms should be considered during the investigation.

Most fish larvae still rely on live feed as primary feed during their early ontogenetic stages. In this study, the feeding regime of rotifer *Brachionus plicatilis* species complex L-type enriched with *Chlorella vulgaris* containing DHA followed by *Artemia* nauplii showed efficient nutritional effects on the larvae. Larvae fed micro-diet responded positively to the digestion but failed to induce the secretion of digestive enzyme and therefore was found to be not effective in feed hydrolysis consequently leaving larvae malnourished. However, a co-feeding protocol (live fed and micro-diet) from 15 DAH accompanied with a 5 day co-feeding transitionary period could improve the growth and survival rate of *P. major* larvae. Investigation into novel uses of Shochu distillery by-product (SDBP) highlighted that it can be considered as a potential supplementary enrichment for live feed in fish larviculture, especially at 5 % and 10 % rotifer enrichment dosage. At 5 % and 10 % doses of SDBP showed significant improvement in nutritional qualities of enriched rotifers, especially protein, carbohydrate and HUFA. Larvae fed with rotifers enriched with salmon oil supplemented with SDBP also displayed enhanced enzymatic profiles and energy metabolism at early metamorphosis; which was reflected in improved larviculture performance of *P. olivaceus* larvae.