Advances in Agricultural, Animal and Fisheries Sciences (*Eds: Devi Dayal and Shamsudheen M*), 2022, ZNAN Publishers, Society for Technology, Environment, Science& People, Kozhikode, India, Pages: 20-29.
ISBN : 978-81-956227-2-6

Nutrigenomics tools to address the emerging issues in marine fish larviculture

Chapter
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ABSTRACT

It is of note that the capture fisheries are under stress for many targeted species. To reduce the pressure on the wild, research on mariculture had been practicing for decades. Even though technologies for captive breeding are standardized, the growth and survival of the larvae of candidate marine fish species are still not up to the mark. It is necessary to focus and encourage new approaches to standardize the hatchery protocol for enhanced production and sustainable management towards future demands. The major concern facing the marine fish hatchery is the delivery of nutritionally enriched feeds to the larvae. Inadequate nutrition is one of the major reasons for early mortality, weaning mortality, cannibalism, inadequate growth, lowered immunity etc. In India, nutrigenomics research is an up-and-coming field, especially the transcriptomics approach that can be applied to understand the gene expression profile of the larvae and juveniles with respect to nutritional interventions. By means of RNA sequencing (RNA-seq) technology, a clear understanding of the growth, development, metabolism, immune function, stress, adaptation and differential gene expression is possible. In addition, the specific effects of selected nutrients on targeted genes can be studied to alter the diet composition accordingly for improving the growth, condition and survival of candidate species of marine fish larvae. This novel approach has the potential to unravel valuable information required to address the issues in marine fish larval nutrition and for the development of species and life-stage specific micro-feeds for sustainable larviculture.

Keywords: Larviculture, Mariculture, mRNA, Nutrigenomics, Transcriptomics

Introduction

Globally aquaculture is one of the fast-growing and demanding sectors through which a nation's food crises are secured nutritionally. Due to the tremendously increasing demand for seafood and declining trend in capture fisheries, an innovative and sustainable hatchery technology is required to operate the industries (FAO, 2018). One of the major problems in marine aquaculture operations is sudden mortality, especially during the early larval and weaning stages (Dessen, 2020). This could be due to more than one factor, but the most important concern is the nutritional imbalance which leads to disease outbreaks (bacterial, fungal, virus), cannibalism, etc (Shefat and Karim, 2018). Even though marine aquaculture is a growing industry, the problems in the hatcheries at their early stages are tricky and it is a challenging task to overcome. Advancement in research towards nutrient delivery through suitable feed may increase marine fish production (Roques *et al.*, 2020). Researchers have been trying to solve the issues in various approaches based on nutrition as a solution (Liang *et al.*, 2001; Hixon, 2014). To achieve this, basic knowledge about the growth and development process with the advancement in research like nutrigenomics is needed to enhance sustainable production (Hakim *et a.l*, 2018).

Nutrigenomics especially in marine organisms is the advanced and emerging technology used in aquaculture research to overcome the problems faced by aqua-farming sector. It involves the study of the effects of diets and their constituent ingredients on gene expression through metabolism. There are many ‘omics’ tools that can be applied in marine fish research which includes transcriptomics, proteomics, and metabolomics. The nutrients for an organism have been identified as important elements which modifies the gene expression, normal metabolism and affect the health (Corthésy-Theulaz *et al.*, 2005; Heras *et al.*, 2020). Considerable reduction in early weaning mortality of marine larvae via making dietary recommendations based on its genome could be considered as a breakthrough in the larval rearing sector. In India, nutrigenomics research is an up-and-coming field, especially the transcriptomics approach that can be applied to understand the gene expression profile of the larvae and juveniles due to nutritional interventions.

Application of Nutrigenomics in aquaculture

The scientific approach plays an important role in the growing aquaculture industry. Nutrigenomics tools which are an emerging field, especially in marine fish species involve transcriptomics, proteomics and metabolomics, each with specific applications (Mutch *et al.*, 2005). The technique is applied in marine research to sort out the issues involved in the hatchery phase with respect to the deficiency in nutrition. The applications of nutrigenomics can be used to study the marine fish' response to the nutrients, and diet development according to the nutrient acceptance, understanding cell response to the provided nutrients, deliver sustainable hatchery protocol, pointing out the metabolism according to the environmental factor, identifying the various nutrient requirement of an organ and tissue of an organism, metabolic changes of candidate species (Hakim *et al.*, 2018). Most of the tropical marine fish larvae depend on the live feed to grow starting from day one to more than a month. Even though researchers are working towards micro-diets for the early-stage larvae, most marine fish hatcheries still rely on live feed (rotifers, *Artemia* sp. and copepods). This is mostly due to the knowledge gap in the nutritional requirement of the marine fish larvae species (Hamre *et al.*, 2013). Application of nutrigenomics tools in aquaculture has the potential to crack these issues.

Transcriptomics

Transcriptomics is the study of whole RNA transcripts at a specific point of time. It describes a clear understanding of the growth, development, metabolism, immune function, stress, adaptation and differential gene expression with the help of whole transcriptome shotgun sequencing or RNA sequencing (RNA-seq) technology (Leduc *et al.*, 2018; Chandhini and Kumar, 2019). The previous studies of transcriptome analysis were done with economically important aquaculture species which including, *Lates calcarifer*, *Macrobrachium rosenbergii*, *Epinephelus coioides*, *Ctenopharyngodon idella*, *Oreochromis niloticus*, *Fenneropenaeus chinensis*, *Eriocheir sinensis*, and *Crassostrea virginica* (Chandhini and Kumar, 2019). Effect of specific nutrients in fish larval metabolism and survival could be revealed with the help of transcriptomic analysis (Panserat and Kaushik, 2010). There are studies which established the early developmental transcriptome profiles of marine larvae and identified candidate nutritional markers in the development of digestive system and organogenesis (Hilerio-Ruiz *et al.*, 2021; Martínez-Burguete *et al.*, 2021).

Proteomics

Fish plays a pivotal role in providing animal protein in the human diet. Proteomics is the study of proteomes, which is a set of proteins produced in an organism. This tool is used to investigate the expression of the proteins, amount of protein produced, degraded and stabilized, modification of ways, and its interactions with one another (Nissa *et al.*, 2021).

The proteins responsible for metabolic pathways are important tools in the aquaculture industry that is used to study nutrition, health, environmental quality (pollutants, toxins and temperature fluctuations) and safety (Tomenek, 2011; Nissa *et al.*, 2021). The modern technology applied is the high throughput proteomics and metaproteomics which are necessary for aquaculture research to achieve a high standard of quality food production and environmental sustainability (Rodrigues *et al.*, 2017). Proteomics helps researchers to find out the scientific reasons behind the altered cellular and/or tissue level phenomena as the effect of nutritional factors. The technique reveals communications involved in complex intracellular signalling pathways by which the cell maintains its fates as proliferation, migration, recognition and differentiation (Tian, 2014). Thus, specific biological queries could be addressed with the application of various types of proteomic approaches and technologies in larval nutrition.

Metabolomics

Among the progressive technology, metabolomics is important which allows us to understand the effects of feed on fish metabolism. Metabolomics is the study of the set of life-sustaining non-targeted chemical reactions of metabolites in cells, biofluids and tissues of an organism and how this changes the physiological disturbance (Wishart, 2019). The metabolome is the final most product of gene transcription and its changes are relative to transcriptomic and the proteomic changes (Horgan and Kenny, 2011). It is a useful methodology which helps to understand the complex molecular interactions in biological systems (Hall *et al.*, 2002). Application of metabolomic studies based on muscle lipid profiling can even identify the origin of the fish stock (Melis *et al.*, 2014). Gil-Solsona *et al.* (2019) identified nutritional deficiency markers in gilthead sea bream. Application of this technology will aid in the development of species-specific metabolic network maps with differential expression of metabolites in fish fed with special or targeted nutrients.

A combination of transcriptomics, proteomics and metabolomics approaches will enhance the quality and survival of marine larviculture by improved feed formulation, disease management etc. The path ahead appears full of hope as the development of proteomic and transcriptomic database of different species become available leading to emergence of new hatchery management strategies, and also helps researchers to characterise and design larval fish feeds based on molecular level expression of the nutrients in order to get desired nutritional quality, growth and health of fish.

Nutrigenomics studies initiated at ICAR- Central Marine Fisheries Research Institute

To address the issues in larval nutrition, a proper understanding of the nutritional requirements (stage and species-wise) *viz.*, protein, carbohydrates, fatty acids, peptides, vitamins, and minerals is necessary. By keeping this in focus, the Central Marine Fisheries Research Institute initiated a project entitled 'Dr. E. G. Silas Centre of Excellence and Innovations in Marine Fish Microbiome and Nutrigenomics' (Dr. EGS-CoEI), supported by the Department of Biotechnology, Government of India (http://210.212.232.215/egscei/index.php). Under this project, the economically important marine species such as Cobia (*Rachycentron canadum*), Silver pompano (*Trachinotus blochii*) and Orange spotted Grouper (*Epinephelus coioides*) were selected. The Dr. EGS-CoEI is the first of its kind in India, dedicated to address the poor larval growth and survival rates of commercially important mariculture finfish species of the country.

Standardization of sampling, processing and storage of Cobia (*R. canadum*) larvae

As an initial step towards RNA-seq, marine fish larvae (*R. canadum*) of different life stages were collected, preserved and analysed to evaluate the RNA quantity and quality. The larval samples were freshly collected from the Marine Fish Hatchery at Mandapam Regional Centre of the Central Marine Fisheries Research Institute, Tamil Nadu and (Fig. 1 a, b).

(a) (b)

Fig. .1 (a) Larval sampling at the Marine fish hatchery at ICAR- CMFRI, Mandapam; (b) Collection of cobia larvae

The larval samples were processed in five different methods for transcriptomics analysis on different trials (Fig. 3) as follows: Trial 1– the fresh/ live larval sample was collected and directly stored at - 20 °C, Trial 2– the fresh larval sample was collected in a vial, 5 volumes of Trizol added and stored in - 20 °C, Trial 3– the fresh larval sample was collected in a vial, 5 volumes of RNAlater added and stored at - 20 °C, Trial 4– the fresh larval sample was collected in a vial, 5 volumes of RNAlater added, kept at 4 °C for 12 hours and stored at - 20 °C, Trial 5– the fresh larval sample was collected in cryovials, 5 times of RNAlater added, kept at 4 °C for 12 hours and stored in Liquid Nitrogen (LN2).

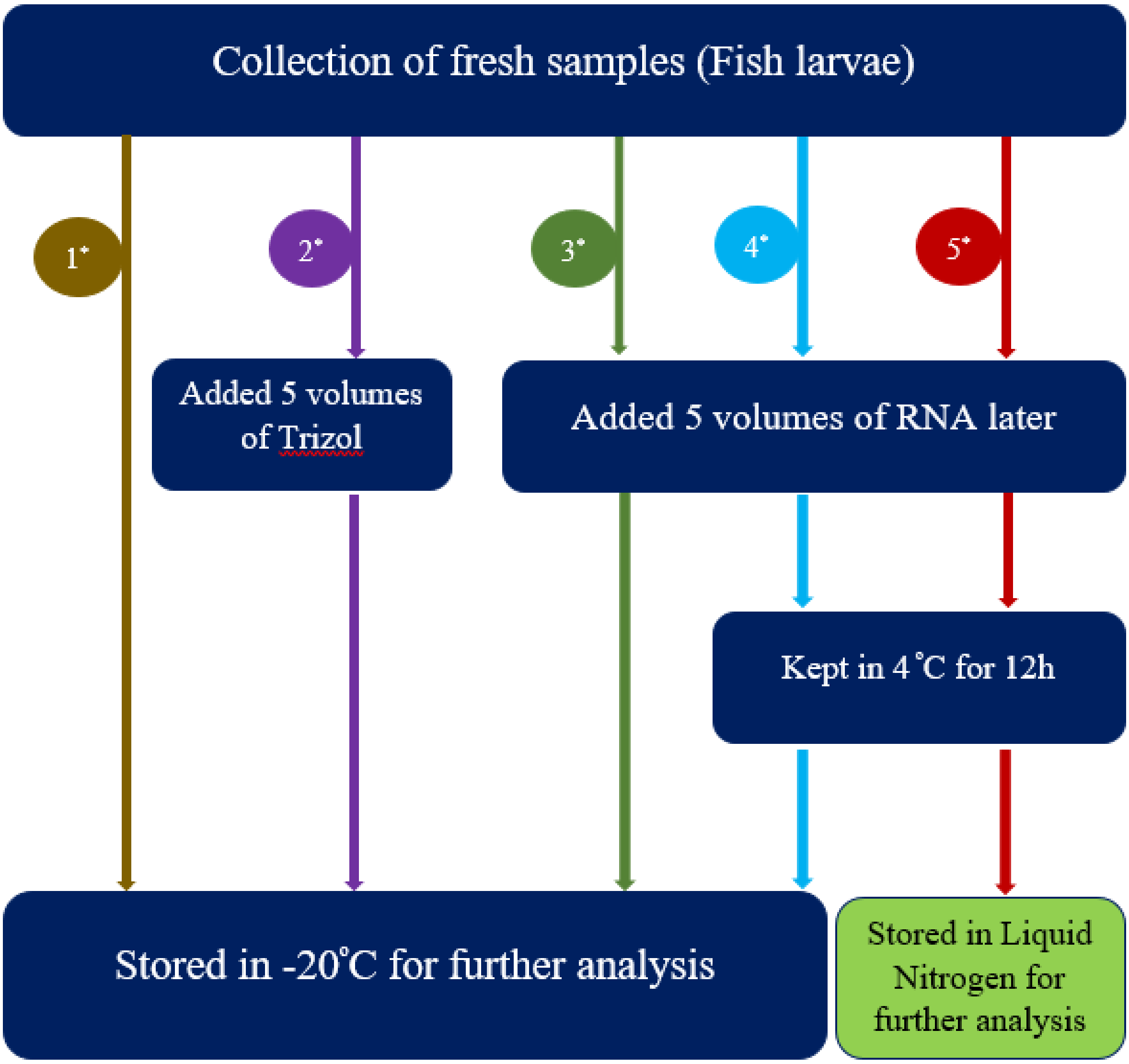

Fig. 3. Pictorial representation of sample collection and analysis methods used for the study

Different methods employed

Results

Among the different methods used, the Trial 5, in which the samples were collected and treated with RNA later and incubated at 4 ºC for 12h and finally stored in liquid nitrogen, maintained superior RNA quality and found suitable for further processing for transcriptomic analysis as compared to other methods.

Conclusion and future prospects

Nutrigenomics is the most appropriate tool to understand the effects of nutrients on the growth and metabolism in marine fish larvae and to decipher the major reasons resulting in early mortality *viz.*, weaning mortality, cannibalism, inadequate growth, lowered immunity etc. By using the RNA-seq technology, a clear understanding of the growth, development, metabolism, immune function, stress, adaptation and differential gene expression is possible. In addition, the cues leading to early mortality in the different weaning periods can be predicted and also envisage the contribution of the genotype responsible for mortality. The specific effects of selected nutrients on targeted genes can be studied to alter the diet composition accordingly for improving the growth, condition and survival of candidate species of marine fish larvae. This novel approach has the potential to unravel valuable information required to address the issues in marine fish larval nutrition and for the development of species and life-stage specific micro-feeds for sustainable larviculture.

Acknowledgement: The financial support for this project was provided by the Department of Biotechnology, Govt. of India (Project Number: BT/AAQ/3/SP29267/2018).

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