Aquaculture Biology Laboratory
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Nagasaki University

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Research projects and plan-do-action of our laboratory

Seedling production (Live feeds & larval rearing) \(\rightarrow\) Aquaculture

Research Projects

Produce good quality of new target species from empirical to scientific approach

I. Development of live feed planktons

1) Breeding and stock culture of live feed planktons
   - Culture collection of rotifers \(\ldots\ldots\ldots1\)
   - Gene analysis of rotifers \(\ldots\ldots\ldots\ldots2\)
   - Resting eggs of rotifers \(\ldots\ldots\ldots\ldots3\)
   - Health diagnosis of planktons \(\ldots\ldots\ldots4\)
   - Biologically active substances \(\ldots\ldots\ldots5,6\)

2) New species for live feed \(\ldots\ldots\ldots7\)

II. Larval rearing

3) Diagnosis of fish health \(\ldots\ldots\ldots8\)
4) Optimal feeding regime \(\ldots\ldots\ldots\ldots9\)
5) Optimal rearing tank \(\ldots\ldots\ldots\ldots10\)

III. Publications and Patents \(\ldots\ldots\ldots11,12\)
1. Breeding project of rotifers

Goal: Development of new rotifer strains efficient for larval rearing

Outcomes:
- Cross breeding between rotifers of different strains
- Biological characteristics in cross-breeding strains

Culture collection of rotifers; they were collected from all around the world

Various strains of rotifers and males (down left)

Strain selection

Cross Breeding

Fertilized eggs (resting eggs)

Hatch

Lorica size (um)

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<td>Lorica size</td>
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2. Construction of expressed sequence tag (EST) and development of transformation method on rotifer

Goal:
1) To construct rotifer EST in order to create cDNA database.
2) To isolate and identify specific genes on rotifer life cycle (male, amictic female and mictic female) by differential display PCR.
3) To create new rotifer strain by chemical manipulation based on the above genetic data.

Outcomes:
1) We established an axenic culture method of rotifers by initially using antibiotics. This culture is useful to prevent other organismal DNA in constructing EST (pat. 2003-382155).
2) We isolated mRNA from axenic rotifer culture and constructed cDNA library.
3) We established a transformation technique for rotifer resting eggs using micro manipulator (under patent application and preparation).
3. Rotifer resting eggs: preserved product of live feed for marine fish larvae

Goal: Development of techniques to mass-produce rotifer resting eggs

Outcomes
- Rotifer resting egg production by regulating life cycle (Pat-465050, Pat 2003-323257)
- Artificial sea water for maximizing rotifer resting egg production (Pat 2003-072203)
- Resting egg production at the order of $10^{10}$ and preservation by canning

Rotifer resting eggs

Three female types (A,B,D) and male (C) of marine rotifers

Rotifer resting eggs mass-produced in a 50 m³ tank

Resting egg can containing 10 million resting eggs
4. Diagnosis of culture status of zooplankton used as live food for rearing fish larvae

Goal: To develop techniques to assess the physiological status of mass cultured marine rotifers used for feeding fish larvae.

Outcomes:

Environmental changes affect physiological status of rotifers, resulting in the change of life span and fecundity. We confirmed that such changes in demographic parameters correlate with the change in ingestion rate and swimming speed of rotifers.

We further tested the effects of environmental changes on rotifer enzyme activity, and found that it correlates well with rotifer demographic parameters. The use of fluorescent substrate simplifies the process for enzyme activity measurement.

Health check of rotifers

“More fluorescence from viable rotifers”

“Less fluorescence from weak rotifers…”
5. Effect of hormone treatments on life history of marine rotifers

Goal: There are few information about endocrine system of marine rotifers. We examined the effects of the addition of vertebrate and invertebrate hormones on rotifer demographic parameters.

Outcomes
- Among 16 hormones tested (including neurotransmitters), the addition of γ-aminobutyric acid (GABA) and porcine growth hormone (GH) promote rotifer population growth. Serotonin (5-HT) and juvenile hormone treatments induced resting egg formation.
- Effective GABA effects were seen when rotifer cultures are not viable under stressed condition.
- GABA and 5-HT exist in rotifers (HPLC analysis), probably indicating that these function as neurotransmitters in rotifers. GH like substance was found in rotifers, which immuno-react with the antibody of porcine growth hormone.

Histological section of marine rotifers; rotifers are metazoeans composed of about 800 cells.
6. Effects of known and suspected endocrine disrupting chemicals (EDCs) on marine zooplankton

Goal:
To investigate how EDCs affect zooplankton.

Outcomes:
1) Some EDCs (e.g. 17β-estradiol) increases fecundity of cladoceran.
2) Estrogen and estrogenic compounds affect development of copepods.
3) Some pesticides affect the hatchability of resting eggs of rotifers.
7. Development of culture methods for marine copepods

Goal: Development of novel live feed for fish larva

Outcomes:
- Establishment of culture method for marine copepods
- Long-term culture of copepods from one batch
- Selection of micro algae for marine copepods

Marine copepod, *Acartia tsuensis* (adult)

Nauplius of *Acartia tsuensis*

Small scale (5 L) culture system

20 L-scale copepod culture system with continuous feeding system

Mass culture system for copepods (400 L)
8. Development of health diagnosis for marine fish larvae

Mangrove killifish
the only known self-fertilizing vertebrate

Larva of devil stinger
(day 7, 5 mm BL)

Rearing experiments in Nagasaki Prefectural Fisheries Experimental Station

Diagnosis of larval quality using enzyme assay and behavioral analysis

Individual based enzyme assay

We use fluorescent substrate

Enzyme activity is calculated by micro-plate reader

Behavioral analysis

Video recording of fish behavior
9. Development of optimal feeding regime for marine fish larvae

Goal:
To establish the optimal feeding regime during the live feed period of marine fish larvae.

Outcomes:
□ We revealed the size-selectivity on live feeds (rotifer and *Artemia*) for the larvae of the seven-band grouper (*Epinephelus septemfasciatus*) and devil stinger (*Inimicus japonicus*).
□ We tested a new feeding regime for seven-band grouper based on its feeding selectivity, and found that survival, growth and quality (DHA composition) of larvae are better than the former feeding regime.