DISEASE PROBLEMS IN FISH FARMING

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Introduction

Fish farming is becoming more important in the overall fishery sector. As intensive culture develops, many kinds of pathogens cause serious diseases. In this lecture, we consider the current status of the infectious diseases in fish farms and hatcheries. Overviews are given of fish health regulations.
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2. Principal infectious diseases of farmed fish
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   B. Bacterial diseases
   C. Fungal diseases
   D. Parasitic diseases

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   A. Husbandry and environment of the fish
   B. Chemotherapy
   C. Vaccination

4. Control of exotic fish pathogens
   A. Imports of aquaculture seedlings to Japan and transmission of pathogens
   B. The current legislation of fish disease control
   C. Problems of aquatic animal quarantine
Principal fish species for freshwater aquaculture

- Common Carp (*Cyprinus capio*) 11,115 tons *
- River Eel (*Anguilla japonica*) 23,211 tons *
- Ayu (*Plecoglossus altivelis*) 8,971 tons *
- Rainbow trout & other trout (*Oncorhynchus mykiss*, etc.) 6,407 tons *

(* Production in 1999)

Principal fish species for marine aquaculture

- Japanese amberjack & other amberjack (*Seriola quinqueradiata*, etc) 140,411 tons *
- Japanese sea bream (*Pagrus major*) 87,232 tons *
- Japanese flounder (*Paralichthys olivaceus*) 7,2151 tons *
- Tiger puffer & other puffer (*Takifug rubripes*, etc) 5,100 tons *
- Coho salmon (*Oncorhynchus kisutch*) 11,148 tons *

(* Production in 1999)
Estimated annual losses in income due to diseases

Table of contents - 1

1. Principal fish species for farming in Japan

2. Principal diseases of farmed fish
   A. Viral diseases
   B. Bacterial diseases
   C. Fungal diseases
   D. Parasitic diseases
### 2-A. Principal viral pathogens of fish - 1

#### RNA virus

<table>
<thead>
<tr>
<th>Virus family</th>
<th>Disease</th>
<th>Pathogen</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birnavirus</td>
<td>infectious pancreatic necrosis</td>
<td>IPNV</td>
<td>salmonids</td>
</tr>
<tr>
<td></td>
<td>yellowtail ascites</td>
<td>MBV</td>
<td>amberjack</td>
</tr>
<tr>
<td>Rhabdovirus</td>
<td>infectious hematopoietic necrosis</td>
<td>IHNV</td>
<td>salmonids</td>
</tr>
<tr>
<td></td>
<td>Hirame rhabdoviral disease</td>
<td>HIRRV</td>
<td>flounder</td>
</tr>
<tr>
<td></td>
<td>viral hemorrhagic septicemia *</td>
<td>VHS</td>
<td>salmonids</td>
</tr>
<tr>
<td></td>
<td>spring viremia of carp *</td>
<td>SVC</td>
<td>cyprinids</td>
</tr>
<tr>
<td>Nodavirus</td>
<td>viral nervous necrosis</td>
<td>VNNV</td>
<td>most marine fishes</td>
</tr>
<tr>
<td>Retrovirus (?)</td>
<td>viral whirling disease</td>
<td>VWD</td>
<td>coho salmon</td>
</tr>
<tr>
<td>Togavirus (?)</td>
<td>erythrocytic inclusion body synd.</td>
<td>EIBSV</td>
<td>coho salmon</td>
</tr>
</tbody>
</table>

* No reports in Japan

### 2-A. Principal viral pathogens of fish - 2

#### DNA virus

<table>
<thead>
<tr>
<th>Virus family</th>
<th>Disease</th>
<th>Pathogen</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iridovirus</td>
<td>lymphocystis disease</td>
<td>LCDV-2</td>
<td>most marine fishes</td>
</tr>
<tr>
<td></td>
<td>viral erythrocytic necrosis</td>
<td>VENV</td>
<td>salmonids</td>
</tr>
<tr>
<td></td>
<td>red sea bream iridoviral disease</td>
<td>RSIV</td>
<td>most marine fishes</td>
</tr>
<tr>
<td></td>
<td>epizootic hematopoietic necrosis *</td>
<td>EHNV</td>
<td>parch</td>
</tr>
<tr>
<td>Herpesvirus</td>
<td>Oncorhynchus masou virus disease = <em>Salmonid herpesvirus</em> type 2 dis.</td>
<td>OMV SaHV-2</td>
<td>salmonids</td>
</tr>
<tr>
<td></td>
<td>viral epidermal hyperplasia</td>
<td>VEHV</td>
<td>flounder</td>
</tr>
<tr>
<td></td>
<td>viral papilloma of carp</td>
<td>CyHV-1</td>
<td>carp</td>
</tr>
<tr>
<td></td>
<td>channel catfish virus disease *</td>
<td>CCHV</td>
<td>catfish</td>
</tr>
<tr>
<td>Adenovirus(?)</td>
<td>viral endothelial cell necrosis of eel</td>
<td></td>
<td>Japanese eel</td>
</tr>
<tr>
<td>Unknown</td>
<td>kuchijirosho (snout ulcer disease)</td>
<td></td>
<td>tiger puffer</td>
</tr>
</tbody>
</table>

* No reports in Japan
Infectious pancreatic necrosis (IPN) - 1

- IPN is a viral disease principally associated with salmonids.
- IPNV and IPN-like virus have been isolated from many kinds of freshwater and marine fishes, crustaceans, and molluscs.
- IPN occurs in North America, Europe, Asia (Japan, Korea, China, Taiwan, and Thailand), South Africa, New Zealand, and Chile.
- In salmonids, acute infection occur in one- to four month-old-fish.
- Fish swim rotating about the long axis or whirling violently.
- Clinical signs include dark pigmentation, exophthalmia, abdominal distension, mucoid fecal casts, and hemorrhages on the body (A).
- Marked pathological changes occur in the pancreatic tissues (B).

Infectious pancreatic necrosis (IPN) - 2

- IPNV is the first fish virus to be grown in vitro.
- Wolf and Quimby (1962) developed methods for culturing the fish cells, initiated the RTG-2 cell line (A), and noted its susceptibility to IPNV (B).
- In RTG-2 cells, CPE becomes apparent within 48 hours at 20°C.
- The identifying features of the IPNV plaques are a reticulum of elongated, shrunken dead cells, and coarsely stellate margins from which living cells radiate inward (Wolf and Quimby, 1973).
Infectious pancreatic necrosis (IPN) - 3


1) Normal, 2) IPNV, 3) IHNV, 4) Salmonid herpesvirus

Infectious pancreatic necrosis (IPN) - 4

- Negatively stained IPN virion shows a typical hexagonal profile. (A)
- The diameter is variously given as 55 to 75 nm.
- IPNV is a nonenveloped icosahedron with a single capsid containing 92 capsomeres.
- IPNV has two species of RNA, and it has an RNA-dependent polymerase that catalyzes synthesis of single-stranded RNA from the double-
- IPNV belongs to the family Birnaviridae. (B)

![Image of IPN virion](image.png)
Spring viremia of carp (SVC)

- The name of SVC was used to distinguish the viral infection from the IDC complex (Fijan et al., 1971).
- IDC had been known in pond-cultured carp in Europe since early 20th cent.
- SVC occurs in Europe and China. (not in Japan)
- Diseased fish have a distended abdomen and show exophthalmia and petechiation of skin. (fig. A)
- Petechiation is evident in the heart, liver, kidney, intestine, peritoneum, swim bladder, and skeletal muscle. (B)
- *Rhavdovirus carpio*, or SVCV, is typically bullet shaped, 60 to 90 nm wide and 90 to 180 nm long. (fig. C)
- The CPE begins as focal areas of cell rounding and sheet contraction. (fig. D)

Viral epidermal hyperplasia (VEH)

- VEH causes mortalities in hatchery-reared larvae of Japanese flounder (Iida et al., 1989; Miyazaki et al., 1989). (Fig.A)
- The affected fish showed epidermal hyperplasia on the fins (fig. B) and skin with vacuolar degeneration of the Marpighian cells, and necrosis in advanced stages of the infection (Iida et al., 1991).
- Although the causative virus (flounder herpesvirus (FHV)) has not been isolated in cultured cells, FAT using a rabbit antiserum against purified virus (fig. C) has been used for diagnosis (Nakai et al., 1991).
2-B. Principal bacterial pathogens of fish
Gram-negative bacteria - 1

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Disease</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavobacterium group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavobacterium columnare</td>
<td>columnaris disease</td>
<td>most freshwater f.</td>
</tr>
<tr>
<td>Flavobacterium psychrophilum</td>
<td>bacterial coldwater disease</td>
<td>salmonids, etc.</td>
</tr>
<tr>
<td>Flavobacterium branchiophilum</td>
<td>bacterial gill disease</td>
<td>salmonids &amp; ayu</td>
</tr>
<tr>
<td>Tenacibaculum matitimum</td>
<td>marine gliding bacterial d.</td>
<td>most marine fishes</td>
</tr>
<tr>
<td>Pseudomonadaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas anguilliseptica</td>
<td>red spot disease (Sekiten-byo)</td>
<td>eel &amp; marine fishes</td>
</tr>
<tr>
<td>Pseudomonas plecoglossicida</td>
<td>bacterial hemorrhagic ascites</td>
<td>ayu</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td>motile aeromonad septicemia</td>
<td>most freshwater f.</td>
</tr>
<tr>
<td>Aeromonas salmonicida</td>
<td>furunculosis</td>
<td>salmonids, etc.</td>
</tr>
</tbody>
</table>

2-B. Principal bacterial pathogens of fish
Gram-negative bacteria - 2

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Disease</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vibrionaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vibrio anguillarum</td>
<td>vibriosis</td>
<td>most marine fishes</td>
</tr>
<tr>
<td>Vibrio ordali</td>
<td>vibriosis</td>
<td>most marine fishes</td>
</tr>
<tr>
<td>Vibrio ichthyoenteri</td>
<td>Intestinal necrosis</td>
<td>Japanese flounder</td>
</tr>
<tr>
<td>Photobacterium damselae subsp. pisicida</td>
<td></td>
<td>various marine fishes</td>
</tr>
<tr>
<td>Enterobacteiaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Edwardsiella tarda</td>
<td>edwardsiellosis</td>
<td>freshwater &amp; marine f.</td>
</tr>
<tr>
<td>Edwardsiella ictaluri *</td>
<td>enteric septicemia of catfish</td>
<td>channel catfish, etc.</td>
</tr>
<tr>
<td>Yersinia ruckeri *</td>
<td>enteric redmouth</td>
<td>salmonids</td>
</tr>
<tr>
<td>Rickettia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piscirickettsia salmonis *</td>
<td>salmonid rickettial septicemia</td>
<td>salmonids</td>
</tr>
</tbody>
</table>

* indicates species that are not commonly encountered.
2-B. Principal bacterial pathogens of fish
Gram-positive bacteria

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Disease</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactococcus garviae</td>
<td>streptococcosis</td>
<td>various marine fishes</td>
</tr>
<tr>
<td>Streptococcus iniae</td>
<td>streptococcosis</td>
<td>various freshwater f.</td>
</tr>
<tr>
<td>Acid-fast bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycobacterium marium</td>
<td>mycobacteriosis</td>
<td>various marine fishes</td>
</tr>
<tr>
<td>Nocardia seriolae</td>
<td>nocardiosis</td>
<td>amberjack, etc.</td>
</tr>
<tr>
<td>Miscellaneous bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renibacterium salmoninarum</td>
<td>bacterial kidney disease</td>
<td>salmonids</td>
</tr>
</tbody>
</table>

Columnaris disease - 1

- Distribution of columnaris disease is probably worldwide.
- All freshwater fishes are probably susceptible to columnaris under environmental conditions favorable to the pathogen and stressful to the fish.
- Outbreaks occur when water temp. reach 15 °C and above.
- The pathogen primarily attacks the external tissues of the fish.
- Lesions are covered with a yellowish white mucoid exudate consisting of swarms of bacteria (figs. A&B).
- In the early stages of the infection, the bacteria grow on the tips of gill lamelae (fig. C), skins, and/or fins.
Columnaris disease - 2

- When pieces of infected tissues were examined microscopically in wet mount preparations, column-like masses of bacteria were observed along the margin of the tissues (fig. A). The bacteria show an active flexing movement and also display a slow gliding movement.
- Currently, the causative bacterium is renamed *Flavobacterium columnare* (Bernardet *et al.*, 1996).
- The cells are Gram-negative, slender and long bacilli, 0.3-0.5 \( \mu \text{m} \sim 3-8 \mu \text{m} \) (fig. B).
- *Cytophaga agar* (CA) (Anacker & Ordal, 1989) is the most frequently used medium for cultivation of *F. columnare*.
- On CA, it produces pale yellow colonies which are of a spreading nature with irregular margins (fig. C).

Vibriosis (*V. anguillarum* infection)

- *Vibrio anguillarum* infection occurs throughout the world.
- The disease has been reported from fishes in brackish and marine waters and from several freshwater fishes.
- Disease signs are variable, but affected fish commonly have numerous raised, hemorrhagic, inflamed areas on the skin (fig. A).
- The cells of *V. anguillarum* are Gram-negative straight or curved rods 0.3-0.5 \( \mu \text{m} \sim 1.4-2.6 \mu \text{m} \). They move by polar flagella (fig. B).
- They grow well in general purpose media with 1-2% NaCl (fig.C)
Streptococcosis (*Lactococcus garviae* infection)

- Streptococcosis (*L. garviae* infection in particular) has been associated with serious economic loss in cultured Japanese amberjack since 1974 (Kusuda, 1976; Kitao *et al*., 1979).
- Clinical signs include exophthalmia and distended abdomen, hemorrhaging in the eye (fig. A), in the opercula and at the base of the fins, and necrotic ulcer formation on the caudal peduncle (fig. B).
- The causative agent is a Gram-positive spherical or oval bacteria less than 2 μm in diameter, which occur in pairs or chain (fig. C).
- The organisms are well grow on TSA, BHIA, THBA, or blood agar. The colonies are small (0.5-1.0 mm diameter), yellowish, translucent, rounded and slightly raised (fig. D).

![A](image1.jpg) ![B](image2.jpg) ![C](image3.jpg) ![D](image4.jpg)

2-C. Principal fungal pathogens of fish

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Disease</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitidiomycetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dermocystidium koi</em></td>
<td>dermocystidiosis</td>
<td>carp</td>
</tr>
<tr>
<td>Oomycetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Saprolegnia parasitica</em></td>
<td>saprolegniasis</td>
<td>salmonids, etc.</td>
</tr>
<tr>
<td><em>Saprolegnia diclina</em></td>
<td>saprolegniasis, visceral mycosis</td>
<td>salmonids, etc.</td>
</tr>
<tr>
<td><em>Aphanomyces piscicida</em></td>
<td>mycotic granulomatosis</td>
<td>ayu</td>
</tr>
<tr>
<td><em>Aphanomyces invadans</em></td>
<td>epizootic ulcerative syndrome</td>
<td>cyprinids</td>
</tr>
<tr>
<td>Entomophthorales</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ichthyophonus hoferi</em></td>
<td>ichthyophonosis</td>
<td>trout, ayu, amberjack, etc.</td>
</tr>
<tr>
<td>Hyphomycetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ochroconis humicola</em></td>
<td>ochroconiosis</td>
<td>marine fish fry</td>
</tr>
</tbody>
</table>
Saprolegniasis

- Saprolegniasis is caused by the attachment of the zoospores (fig. C) of common water molds to the gills, skin, or fins (fig. A).
- The most prevalent species associated with infections are Saprolegnia parasitica (syn. S. diclina type 1) and S. ferax.
- Saprolegnia spp. are parasitic to fish and fish eggs of both warm-water and coldwater species.
- Fig. B: Saprolegnia sp. cultured on hemp seeds.
- Fig. C: Zoosporangium
- Fig. D: Oogonium and oosphere
- Fig. E: Gemma (chlamydospore)

2-D. Principal parasites of fish - 1

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Disease</th>
<th>Host (organ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mastigophora</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amyloodinium ocellatum</td>
<td>amyloodiniosis</td>
<td>marine fishes (gills)</td>
</tr>
<tr>
<td>Ichthyobodo necator</td>
<td>ichthyobodosis</td>
<td>freshwater f. (gills &amp; skin)</td>
</tr>
<tr>
<td>Ichthyobodo sp.</td>
<td>ichthyobodosis</td>
<td>marine fishes (gills &amp; skin)</td>
</tr>
<tr>
<td>Microspora</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glugea plecoglossi</td>
<td>glugeosis</td>
<td>ayu (viscera)</td>
</tr>
<tr>
<td>Heterosporis anguillarum</td>
<td>beko disease</td>
<td>Japanese eel (muscle)</td>
</tr>
<tr>
<td>Microposidium seriolae</td>
<td>beko disease</td>
<td>Japan. Amberjack (muscle)</td>
</tr>
<tr>
<td>Myxozoa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hoferelius carassi</td>
<td>kidney enlargement d.</td>
<td>goldfish (kidney)</td>
</tr>
<tr>
<td>Kudo amamiensis</td>
<td>kudoosis amami</td>
<td>Japan. amberjack (muscle)</td>
</tr>
<tr>
<td>Myxobolus koi</td>
<td>gill myxobolosis</td>
<td>carp (gills)</td>
</tr>
<tr>
<td>Myxobolus buri</td>
<td>myxosporean scoliosis</td>
<td>Japan. amberjack (brain)</td>
</tr>
</tbody>
</table>
### 2-D. Principal parasites of fish - 2

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Disease</th>
<th>Host (organ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciliophora</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crapitoctaryon irritans</td>
<td>white spot disease</td>
<td>marine fishes (skin)</td>
</tr>
<tr>
<td>Ichthyophthirius multifiliis</td>
<td>white spot disease</td>
<td>freshwater fishes (skin)</td>
</tr>
<tr>
<td>Scuticociliatida gen. sp.</td>
<td>scuticociliatidosis</td>
<td>flounder, etc. (skin, brain)</td>
</tr>
<tr>
<td>Monogenea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benedenia seriola</td>
<td>skin fluke disease</td>
<td>Japanese amberjack (skin)</td>
</tr>
<tr>
<td>Neobenedenia girelsea</td>
<td>skin fluke disease</td>
<td>Japan. flounder, etc. (skin)</td>
</tr>
<tr>
<td>Heteraxine heterocerca</td>
<td>gill fluke disease</td>
<td>Japan. amberjack (gills)</td>
</tr>
<tr>
<td>Heterobothrium okamotoi</td>
<td>heterobothriosis</td>
<td>tiger puffer (gills)</td>
</tr>
<tr>
<td>Trematoda</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diplodistomum (metacercaria)</td>
<td>worm cataract</td>
<td>rainbow t., etc. (eye)</td>
</tr>
<tr>
<td>Galactosomum (metacercaria)</td>
<td>trematode whirling d.</td>
<td>amberjack, etc. (brain)</td>
</tr>
<tr>
<td>Paradeontacylix grandispinus</td>
<td>blood fluke disease</td>
<td>greater amberjack (blood)</td>
</tr>
</tbody>
</table>

### 2-D. Principal parasites of fish - 3

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Disease</th>
<th>Host (organ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nematoda</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anisakis simplex</td>
<td>(harmless to fish)</td>
<td>marine fishes (viscera)</td>
</tr>
<tr>
<td>Philometra lateolabracis</td>
<td>gonad nematodosis</td>
<td>sea bream (gonad)</td>
</tr>
<tr>
<td>Anguillicola crassus</td>
<td>anguillicolosis</td>
<td>Jpn. /Eur. eel (swim bladder)</td>
</tr>
<tr>
<td>Acanthocephala</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acanthocephalus opsariichthydis</td>
<td>acanthocephalosis</td>
<td>rainbow t. etc. (intestines)</td>
</tr>
<tr>
<td>Longicollum pagrosomi</td>
<td>longicollosis</td>
<td>sea bream (intestines)</td>
</tr>
<tr>
<td>Crastacea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Argulus japonicus</td>
<td>argulosis</td>
<td>cyprinids (skin)</td>
</tr>
<tr>
<td>Lerneia cyprinacea</td>
<td>anchor worm d.</td>
<td>cyprinids (skin), eel (mouth)</td>
</tr>
<tr>
<td>Caligus spinosus</td>
<td>gill caligosis</td>
<td>greater amberjack (blood)</td>
</tr>
</tbody>
</table>
Table of contents - 2

3. Control of fish diseases
   A. Husbandry and environment of the fish
   B. Chemotherapy
   C. Vaccination

4. Control of exotic fish pathogens
   A. Imports of aquaculture seedlings to Japan and transmission of pathogens
   B. The current legislation of fish disease control
   C. Problems of aquatic animal quarantine

3. Control of fish diseases
   The relationship between a pathogen, host, and the environment

   - Serious losses only occur when a pathogen and a host are present in an environment which favors the disease.
   - When the pathogen and host are present but the environment is not favorable for the disease, no outbreak occurs.
   - Also, when the environment is favorable for disease and the host is present, no outbreak occurs unless the pathogen is also present.

   - Many diseases are difficult to treat, some are impossible. Such diseases can only be controlled by the provision of improved environmental and nutritional conditions.
   - Losses from some disease situations can be stopped or reduced by drug or chemical therapy and/or vaccination.
3-A. Husbandry and environment of the fish
Portal of entry into fish for pathogens

- Superficial abrasion of fish body, while not immediately critical, affords a portal of entry for infectious agents.
- The transport, transfer, and handling of fish should be done by the least damaging method.

Fig. The outside and inside of fish

3-A. Husbandry and environment of the fish
Adherence of bacterial cells to the fish body surface

A) Green fluorescent protein-labeled *Pseudomonas plecoglossicida* cells (A-1) and blue fluorescent microspheres (A-2) in the fin of ayu which has been immersed in a mixture of them for 15 min.

B) After bathing the microsphere suspension, the fish was dipped into a 0.05% trypan blue solution for 10 min.
B-1: Microscopic injuries in the fin are stained with trypan blue.
B-2: Fluorescent microspheres (green) are located in the micro-injuries.
3-A. Husbandry and environment of the fish
The effects of stress on fishes - 1

Social subordination paradigm:
Encounter between dominant and subordinate fish, the resulting physiological changes, and how these modulate the immune system.

Badly out-of-size fish are usually more prone to disease outbreak.

3-A. Husbandry and environment of the fish
The effects of stress on fishes - 2

- Morphological changes in the stomach of the European eel under social stress:
  - The stomach of subordinate eel shrinks, and the diameter and length of the gastric caecum decrease (fig. c & d).
  - The originally firm, deep mucous membrane folds (fig. a) become flattened in subordinate eel (fig. c).

3-A. Husbandry and environment of the fish

The effects of Stress on fishes - 3

- Defense activities of neutrophils of tilapia were investigated under the social stress.
- A large fish (about 143 g) and a small fish (about 91 g) were in an aquarium. As control a large and a small were held individually.
- Within a few hours post-pairing, the large become dominant in all pairs tested.
- The phagocytic ability (fig. A) and respiratory burst activity (fig. B) of subordinate fish were both reduced.

(Kuroki, J. & Iida, T: Fish Pathology, 34, 15-18, 1999)

B. Chemotherapy - 1

- Unlike human or cattle, which are treated on an individual basis under carefully controlled conditions, farm fish must be treated en masse and under the law of averages.
- A suitable chemotherapeutic agent must be:
  1) Effective against the pathogen;
  2) Of low toxicity, palatable, promptly and fully eliminated by the fish;
  3) Permitted by national and international regulations.
- The daily amounts of the drug must be regulated on the amount of food which is eaten by diseased fish, often a difficult assignment.
- Fish farmer must be required to know:
  1) Weight and nature of drugs to be included in the food;
  2) Amount of food to feed, in percent of fish body weight, per day;
  3) Period of treatment, in days or in weeks;
  4) Interval of time required following cessation of therapy before fish marketing.
B. Chemotherapy - 2
A list of drugs registered for fishery use in Japan (2000)

<table>
<thead>
<tr>
<th>Bactericides</th>
<th>Miloxacin</th>
<th>Antihelmintics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>Novobiocin</td>
<td>Metrifonate</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>Oleandomycin polysyrene</td>
<td>Trichlorfon</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>sulfonic acid</td>
<td>Praziquantel</td>
</tr>
<tr>
<td>Doxycycline HCl</td>
<td>Oxolinic acid</td>
<td>Anesthetics</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Oxetetracycline HCl</td>
<td>Eugenol (FA 100)</td>
</tr>
<tr>
<td>Florfenicol</td>
<td>Sodium nifurstyrenate</td>
<td>Tricainemethane-sulfonate</td>
</tr>
<tr>
<td>Flumequine</td>
<td>Spiramycin embonic acid</td>
<td>(MA 222)</td>
</tr>
<tr>
<td>Fosfomycin, Ca</td>
<td>Sulfadimethoxine, Na</td>
<td>Phenthiiazamine</td>
</tr>
<tr>
<td>Josamycin</td>
<td>Sulfamonomethoxine, Na</td>
<td>hydrobromide</td>
</tr>
<tr>
<td>Kitasamycin</td>
<td>Sulfizazole, Na</td>
<td>Disinfectants</td>
</tr>
<tr>
<td>Lincomycin HCl</td>
<td>Thiamphenicol</td>
<td>Povidone iodine</td>
</tr>
</tbody>
</table>

B. Chemotherapy - 3
Regulation on the Use of Drugs in Japan

- The regulation are in place regarding the standardized use of registered drugs.
- Fish farmers purchase drugs following the advice of the prefecture fish disease control center from veterinary drug stores.
- Prescriptions for the drugs are not necessarily required.
- Fish farmers must stick by the standardized use of the drug.
B. Chemotherapy - 4
Problems on the Use of Drugs

- The extensive uses of drugs have increased the incidence of drug resistance in fish-pathogenic and environmental bacteria.
- It gets more difficult to find effective drugs for fish diseases.
- Recently, particular attention was paid to the problem of drug residues in treated fish.
- Long interval time (2 ~4 weeks) is required following cessation of therapy before fish marketing. (Because fish is cold-blooded animal, the interval time for fish is much longer than that for cattle and poultry (1 or 2 days).)

C. Vaccination
A list of vaccine registered for fishery use in Japan (2000)

<table>
<thead>
<tr>
<th>Disease controlled</th>
<th>Pathogen</th>
<th>Type of vaccine</th>
<th>Fish applied</th>
<th>Method of application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vibriosis</td>
<td><em>Vibrio anguillarum</em> J-O-1</td>
<td>formalized</td>
<td>ayu</td>
<td>immersion</td>
</tr>
<tr>
<td>Vibriosis</td>
<td><em>Vibrio anguillarum</em> J-O-1 and J-O-3</td>
<td>formalized</td>
<td>salmonid fish</td>
<td>immersion</td>
</tr>
<tr>
<td>Streptococcosis</td>
<td><em>Lactococcus garvieae</em></td>
<td>formalized</td>
<td>Japanese amberjack</td>
<td>Oral (on food)</td>
</tr>
<tr>
<td>Iridovirus infection</td>
<td>RSIV (<em>Iridovirus</em>)</td>
<td>formalized</td>
<td>red sea bream &amp; amberjack</td>
<td>injection i.p.</td>
</tr>
<tr>
<td>Streptococcosis and vibriosis</td>
<td><em>Lactococcus garvieae</em> and <em>Vibrio anguillarum</em> J-O-3</td>
<td>formalized</td>
<td>Japanese amberjack</td>
<td>injection i.p.</td>
</tr>
</tbody>
</table>

For further information, listen to Dr. Nakanishi’s lecture, please.
Table of contents - 2

3. Control of fish diseases
   A. Husbandry and environment of the fish
   B. Chemotherapy
   C. Vaccination

4. Control of exotic fish pathogens
   A. Imports of aquaculture seedlings to Japan and transmission of pathogens
   B. The current legislation of fish disease control
   C. Problems of aquatic animal quarantine

4-A. Imports of aquaculture seedlings to Japan and transmission of pathogens

Transmission of Pathogens in Salmonid Fish - 1

<table>
<thead>
<tr>
<th>Disease - Pathogen</th>
<th>Introduction year Speculated/Confirmed</th>
<th>Suspected carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious hematopoietic necrosis (IHN)</td>
<td>1971 / 1971</td>
<td>Sockeye salmon eggs from Alaska</td>
</tr>
<tr>
<td>Bacterial kidney disease (BKD)</td>
<td>1973 / 1973</td>
<td>Coho salmon eggs from the Pacific coast</td>
</tr>
<tr>
<td>Š Renibacterium salmoninarum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial cold-water disease (BCWD)</td>
<td>1985 / 1990</td>
<td>Coho salmon eggs from the Pacific coast</td>
</tr>
<tr>
<td>Š Flavobacterium psychrophilum</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Transmission of Pathogen in Salmonids Fish - 2

BCWD of coho salmon fry caused by *Flavobacterium psychrophilum*

- The clinical signs of BCWD has been recognized since 1985 in coho salmon and ayu.
- A large number of coho salmon eggs has been imported annually from the North America for culture purpose since 1975.
- The samples of imported eggs were often positive for *F. psychrophilum* by inspection.

Transmission of Pathogen in Salmonid Fish - 2.

Detection of *Flavobacterium psychrophilum* from coho salmon eggs and fry

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Eyed egg stage</th>
<th>Fry stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Date of</td>
<td>Cultivation</td>
</tr>
<tr>
<td></td>
<td>examination</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>19/Dec/95</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>19/Dec/95</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>19/Dec/95</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>24/Dec/95</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>27/Dec/95</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>30/Dec/95</td>
<td>-</td>
</tr>
</tbody>
</table>

*1 Eggs imported from the Pacific Coast of the USA.
*2 No. of positive fish / No. of examined fish
Transmission of Pathogens in Marine Fishes - 1
Source and transportation of imported seedlings for marine fish and shellfish culture

Transmission of Pathogens in Marine Fishes - 2
Pathogens and parasites suspected to have been introduced into Japan in association with importations of marine fish seedlings from Asian countries

<table>
<thead>
<tr>
<th>Disease - Pathogen or parasite</th>
<th>Introduction year</th>
<th>Speculated/Confirmed</th>
<th>Suspected carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epitheliocystis</td>
<td>1978 / 1978</td>
<td>Speculated</td>
<td>Japanese sea-bream seedlings from Hong-Kong</td>
</tr>
<tr>
<td><em>Neobenedenia girellae</em></td>
<td>1991 / 1991</td>
<td>Speculated</td>
<td>Greater amberjack seedlings from Hainan</td>
</tr>
<tr>
<td><em>Penaeid acute viremia</em></td>
<td>1993 / 1993</td>
<td>Speculated</td>
<td><em>Penaeus japonicus</em> seedlings from Fujiang</td>
</tr>
<tr>
<td><em>Penaeid acute syn. white spot syndrome</em></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4-B. The current legislation of fish disease control -1
International Aquatic Animal Health Code - 2003
Office International des Epizooties (O.I.E)

  Section 1.1. General definitions
  Section 1.2. Notification systems
  Section 1.3. Obligations and ethics in international trade
  Section 1.4. Import risk analysis
  Section 1.5. Import/export procedure
  Section 1.6. Guidelines for contingency planning
  Section 1.7. Fallowing

Part 2. Diseases of Fish
Part 3. Diseases of Molluscs
Part 4. Diseases of Crustaceans
Part 5. Appendices

4-B. The current legislation of fish disease control - 2

The Fishery Resources Conservation Law (Japan) - 1

- The Law was amended in 1996.
- The person who intends to import seeds of aquatic animals for propagation or aquaculture purposes shall obtain license issued by the Minister of Agriculture, Forestry and Fisheries (MAFF).
- The application must be accompanied with certificates issued by the government authorities of exporting country.
### The Fishery Resources Conservation Law (Japan) - 2

**Infectious diseases of aquatic animal seeds prescribed pursuant to the Law**

<table>
<thead>
<tr>
<th>Aquatic animal seeds</th>
<th>Infectious diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carp fry</td>
<td>Spring viremia of carp (SVC)</td>
</tr>
<tr>
<td>Eyed eggs and fry of Salmonidae</td>
<td>Viral hemorrhagic septicemia (VHS)</td>
</tr>
<tr>
<td></td>
<td>Epizootic hematopoietic necrosis (EHN)</td>
</tr>
<tr>
<td></td>
<td>Piscirickettsiosis</td>
</tr>
<tr>
<td></td>
<td>Enteric redmouth disease (ERM)</td>
</tr>
<tr>
<td>Larvae, postlarvae and juvenile of Penaeus</td>
<td>Nuclear polyhedrosis vaculoviroses</td>
</tr>
<tr>
<td></td>
<td>Infectious hypodermal &amp; hematopoietic necrosis</td>
</tr>
<tr>
<td></td>
<td>Yellowhead disease</td>
</tr>
<tr>
<td></td>
<td>Penaeid acute viremia / White spot syndrome</td>
</tr>
</tbody>
</table>

### 4-B. The current legislation of fish disease control - 3

**The Law to Ensure Sustainable Aquaculture Production (Japan) - 1**

- Prevention of diffusion of specific diseases:
- The Governor can issue orders as stipulated in the following items.
  - To limit or prohibit owners of the animals which have, or suspected to have, a specific disease to transfer them.
  - To order the owners to burn or bury them.
  - To order owners of the fishing nets, cage and other equipment to which pathogens of some specific disease are attached, or suspected, to disinfect it.
The Law to Ensure Sustainable Aquaculture Production (Japan) - 2

- On-the-site inspection: The Governor can have his officials enter the aquaculture ground to inspect aquatic animals and plants.
- Fish disease prevention official (FDPO) and fish disease prevention cooperative staff (FDPCS): The governor shall assign FDPO from his staff, and can entrust FDPCS from those who have expertise on diseases of aquatic organisms.

4-C. Problems of aquatic animal quarantine - 1

- The large number of farms precludes regular inspection of each farm.
- Too many kinds of fish are cultured (approximately thirty different marine- and ten freshwater fishes).
- Most of the imported mariculture seedlings are transported by ship; the aquarium are supplied with seawater from ocean, and the effluent flows back.
- Most of the marine fish species has not yet been farmed in the exporting countries.
- Apart from aquaculture seedlings, a large number of various ornamental fishes are imported.
<table>
<thead>
<tr>
<th>Tentative plans to improve health standards for imported aquaculture seedlings</th>
</tr>
</thead>
<tbody>
<tr>
<td>▪ More precise records on the importation of seedlings from all traders and farmers.</td>
</tr>
<tr>
<td>▪ Exchange of information between the exporting countries and Japan on pathogens and parasites.</td>
</tr>
<tr>
<td>▪ Cooperative research with the exporting countries on disease of mariculture seedlings.</td>
</tr>
<tr>
<td>▪ Dissemination of epizootiological knowledge to traders and farmers.</td>
</tr>
<tr>
<td>▪ Creation of a network system providing information on epizootics in aquaculture,</td>
</tr>
<tr>
<td>▪ Development and standardization of diagnostic techniques.</td>
</tr>
</tbody>
</table>

Thank you for your attention