# Fish and Shellfish Diseases in Culture Systems

## VIII. Isolation of Pathogen(s)

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In disease investigation, isolation of pathogen(s) is an essential aspect of work in order to systematically diagnose microbial cases, to provide rapid remedial measures and to adapt suitable prophylaxis. The signs and symptoms of a disease aid to provisionally diagnose a disease, but microscopical examination of the pathological sample facilitates to take proper steps for culture and isolation of the probable aetiological agent(s), and for concrete case diagnosis.

If bacteria is suspected based on the results of the microscopical examination, the aseptically removed pathological sample(s) may be directly inoculated (or after suitable dilution in filtered and sterile habitat water) on to the plated medium. There is no single medium in use to culture and isolate all the pathogens present in a sample. However, a suitable all purpose culture medium is fish infusion nutrient agar (Fina) for isolating bacterial pathogens from finfishes.

FISH INFUSION NUTRIENT AGAR

| | | |
|---|---|---|
| Peptone | : | 1.0 gm |
| Agar agar | : | 1.5 gm |
| Beef extract | : | 0.01 gm |
| Fish infusion* | : | 100 ml |

(pH adjusted to 7.2 and sterilized at 121°C for 15 minutes)

*Preparation of fish infusion

454 gm of fresh fish meat is minced and mixed into one litre of water**. The mixture is kept overnight in a refrigerator at 4 °C. Then, the mixture is boiled using a water bath for about 30 minutes. The precipitated and coagulable protein in the mixture can be removed by filtering through a lint and filter paper.

**In the case of water, aged and filtered sea water is preferred for isolation of bacterial pathogens from marine fish and distilled water with 0.5% sodium chloride for fresh water fish.

Shrimp infusion nutrient agar, crab infusion nutrient agar and mussel infusion nutrient agar are prepared in similar manner replacing the fish muscle by shrimp, crab or mussel meat in the fish infusion nutrient agar, for isolating bacterial pathogens from the respective species. Fresh media may always be used as the cost of these media is cheap and the best results are obtained.

The inoculated culture media may be incubated aerobically at room temperature, 28+2 °C for 24—72 hours.

The predominating bacteria represented by most numerous colonies similar in colonial characteristics are to be selected as representative agents, studying their colonial characteristics such as size, shape, margin, elevation, consistency, opacity and colour. A colony may be transferred into a suitable fish infusion nutrient broth (Fina excluding agar) aseptically. The isolate has to be finally checked for its purity by streaking over the fish infusion nutrient agar. The purified isolate is then subjected for its identification.

To ascertain that a particular isolate is the causative agent of a disease the Koch's postulates are to be satisfied.

Part VII of this series, sampling techniques for disease diagnosis, is being published in *Mar. Fish. Infor Serv. T and E Serv.*, a CMFRI publication.

# Why do some Marine Fishes Luminesce with the aid of Bioluminescent Bacteria?

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"The shrewd guess, the fertile hypothesis, the courageous leap to a tentative conclusion - these are the most valuable coin of the thinker at work.....".

*JEROME S. BRUNER*

The preponderance of luminous fishes in marine environment is obvious. Though fishes belonging to about 25 families generate their own light, only members of 10 families of teleost fishes possess specialised organs to harbour luminescent bacterial symbionts to produce light. Information available on physiological capabilities and responses to luminescence among fishes suggests that they probably exceed those of any other group of terrestrial or aquatic organisms. There is a great diversity of luminescent organs in fishes; it occurs not only in different species but also varies even within a single species of fish.

## TELEOST FISHES-LIGHT ORGAN BACTERIAL SYMBIONTS:

Fishes which are associated with bioluminescent bacteria are found mostly in Indo-Pacific waters.

### Family : CHLOROPHTHALMIDAE

A perianal groove harbouring luminous bacteria acts as a light organ in *Chlorophthalmus albatrossi* and *C. nigromarginatus*. The luminous bacteria are easily culturable in the laboratory but yet to be identified.

### Family : MACROURIDAE

Species of the genera *Cetonurus, Coelorhynchus, Hymenocephalus, Lepidorhynchus, Malacocephalus, Nezumia, Odontomacrurus, Sphagemacrurus, Trachonurus* and *Ventrifossa* produce bacterial associated light from the light organ situated ventrally in front of the anus. The luminescent bacteria which are maintained in the glandural part of the organ belong to the psychrotrophic species *Photobacterium phosphorium*.

### Family : MORIDAE

Morids belonging to the genera *Physiculus, Brosmiculus, Gadella, Tripterophycis, Antimora* and *Lotella* possess luminescent organs similar to those of macrurids. The luminescent bacteria present are *P. Phosphoreum.*

Family : MERLUCCIIDAE

Under this family the species *Steindachneria argentea* is found to luminesce. The donut shaped light organ surrounding the rectum is situated just below the anus. The luminous bacteria *P. phosphroeum* are harboured within the lumen.

Family : TRACHICHTHYIDAE

Members of the genus *Paratrachichthys* consist of a bacterial housed light organ around rectum below the anus. The bacteria *P. Phosphoreum* are in association with these fishes.

Family : ANOMALOPIDAE

These small dark fishes are grouped under 3 genera and 4 species viz., *Anomalops kataptron, Kryptophnaron alfredi, K. harveyi* and *Photoblepharon palpebratus*. All these species have a prominent sub ocular light organ under each eye like a torch. The histological studies confirmed that the light organs are packed with luminescent bacteria; however, repeated attempts failed to culture the bacteria in laboratory.

Family : MONOCENTRIDAE

Four species *Cleidopus gloria-maris, Monocentris, japonicus, M. neozelanicus* and *M. reedi* are lumiscent. In *Monocentris* a pair of light organs is situated as small ventral protruberances on the lower jaw. The light organ in *Cleidopus* is large and laterally placed. The bacterial cultures of the light organs of monocentrids are the pure cultures of *Photobacterium fischeri*.

Family : APOGONIDAE

Species under the genus *Siphamia* are luminiscent. The light organs is bulbous and situated below pyloric

stomach and liver. Accessory diffusers are also present. The culturable symbiotic bacteria of the light organ seem to be *Photobacterium leiognathi.*

Family : ACROPOMATIDAE

In two species of *Acropoma* viz., *A. japonicum* and *A. hanedai* an internal glandular light organ is present. The gland is U-shaped in *A. joponicum* around the anus with the blind part of U extending well anterior to the anus. In *A. hanedai,* the light organ is almost similar except that it is much longer and the loop faces the opposit direction. Though the associated luminescent bacteria packed in the light organs are culturable, no published account is available on their characterestics.

Family : LEIOGNATHIDAE

All the species of the family Leiognathidae grouped under the genera *Leiognathus, Secutor* and *Gazza* possess a well developed internal luminescent system. This glandular organ houses the bacteria *P. leiognathi* which are easily grown on artificial media.

## FUNCTIONS OF BACTERIAL SOURCE OF LIGHT IN FISHES :

Several theories have been proposed on the significance of bioluminescence in various living systems. A number of authors have hypothesised the light production to one or more of the three major functions such as attracting the prey, aiding in escaping or diverting the predators and in intraspecific communication. In fishes associated with symbiotic luminescent bacteria, the functional value is attributed to their behaviour. These luminescent fishes are capable of controlling the light emitted by the symbiotic bacteria present in their light organs.

The bacterial source of light in deep water fishes seems to be useful to attract the prey organisms. To avoid predation, the light is used in various ways including frightening and diverting the predators. The above functions involve luminescence during night. Interes tingly, in fishes like lieognathids the ventral emission of light during day time can serve to counter illuminate the downwelling ambient light to obscure their silhouette. In anomalopids, monocentrids and in few species of leiognathids the luminescence also appears to help in intraspecific communication, to lure or attract potent mate and to indicate sex of the possessor apart from illuminating their surroundings.

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## Pattern of Marine Products

| Items | | 1972 | 1973 | 1974 | 1975 |
|---|---|---|---|---|---|
| Frozen Shrimp | Q: | 30,550 | 358,95 | 34,361 | 46,831 |
| | V: | 5,08,843 | 6,58,122 | 6,37,326 | 9,43,386 |
| Frozen Froglegs | Q: | 1,823 | 2,698 | 1,454 | 1,317 |
| | V: | 21,709 | 44,979 | 28,652 | 27,983 |
| Frozen Lobster Tails | Q: | 369 | 380 | 456 | 402 |
| | V: | 12,794 | 10,663 | 12,573 | 15,760 |
| Fresh & Frozen Fish | Q: | 21 | 146 | 66 | 134 |
| | V: | 123 | 731 | 767 | 1,884 |
| Frozen Cuttle Fish & Fillets | Q: | — | 14 | 141 | 1,017 |
| | V: | — | 190 | 1,979 | 29,071 |
| Frozen Squids | Q: | — | — | -- | 46 |
| | V: | — | — | — | 305 |
| Canned Shrimp | Q: | 1,058 | 2,199 | 1,516 | 261 |
| | V: | 21,026 | 52,369 | 47,842 | 5,999 |
| Dried Shrimp | Q: | 139 | 284 | 116 | 99 |
| | V: | 1,380 | 3,230 | 1,426 | 1,132 |
| Dried Fish | Q: | 3,478 | 3,388 | 1,748 | 2,295 |
| | V: | 7,971 | 10,955 | 6,658 | 9,061 |
| Shark Fins & Fish Maws | Q: | 294 | 252 | 259 | 307 |
| | V: | 6,027 | 6,569 | 8,464 | 9,822 |
| Other Items | Q: | 539 | 3,530 | 6,512 | 703 |
| | V: | 1,444 | 7,955 | 17,440 | 4,660 |
| TOTAL | Q: | 38,271 | 48,785 | 46,629 | 53,412 |
| | V: | 5,81,317 | 7,95,763 | 7,63,127 | 10,49,063 |

## Exports from India (1972-1981)

Q: Quantity in Tonnes.
V: Value Rs. in '000

| 1976 | 1977 | 1978 | 1979 | 1980 | 1981 |
|---|---|---|---|---|---|
| 47,952 | 47,239 | 51,223 | 53,511 | 47,762 | 54,538 |
| 16,06,499 | 15,62,206 | 17,90,644 | 22,31,273 | 18,33,661 | 24,85,210 |
| 3,170 | 2,834 | 3,570 | 3,764 | 3,095 | 4,368 |
| 77,970 | 65,967 | 84,251 | 87,150 | 73,200 | 1,19,570 |
| 513 | 596 | 691 | 752 | 501 | 636 |
| 31,802 | 38,804 | 45,668 | 53,465 | 27,889 | 47,003 |
| 1,583 | 3,765 | 9,931 | 24,126 | 11,195 | 8,565 |
| 16,383 | 38,566 | 63,396 | 1,15,581 | 1,11,939 | 94,526 |
| 648 | 1,089 | 979 | 1,339 | 1,603 | 1,488 |
| 13,334 | 17,315 | 16,591 | 35,310 | 30.326 | 32,525 |
| 497 | 607 | 2,428 | 2.107 | 2,179 | 1,314 |
| 6,162 | 6,501 | 32,677 | 28,033 | 25,084 | 15,690 |
| 102 | 128 | 204 | 139 | 365 | 100 |
| 3,935 | 5,221 | 9,149 | 6,428 | 15,794 | 4,900 |
| 36 | 235 | 4 | 19 | 124 | 56 |
| 385 | 1,711 | 75 | 222 | 1,349 | 809 |
| 4,668 | 4,221 | 6,311 | 3,728 | 4,340 | 1,523 |
| 17,341 | 22,730 | 32,135 | 18,934 | 20,802 | 14,408 |
| 268 | 287 | 423 | 372 | 332 | 406 |
| 15,294 | 22,469 | 34,676 | 29,342 | 32.526 | 38,811 |
| 2,714 | 3,963 | 2,182 | 2,327 | 3,046 | 2,381 |
| 9,515 | 15,884 | 12,312 | 14,554 | 16,186 | 13,676 |
| 62,151 | 64,964 | 77,946 | 92,184 | 74,542 | 75,375 |
| 17,98,620 | 17,97,374 | 21,21,574 | 26,20,292 | 21,88,756 | 28,67,128 |