AQUACULTURE

DEVELOPMENT AND USE OF PRIMARY FISH CELL CULTURES IN FISH DISEASE DIAGNOSIS

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"THE PRIMARY CELL CULTURES OF COMMERCIALLY IMPORTANT FISH SPECIES COULD FUNCTION AS VERSATILE AND COST-EFFECTIVE MODEL SYSTEMS IN STUDIES RELATING TO FISH VIROLOGY, TOXICOLOGY, IMMUNOLOGY, PHYSIOLOGY, BIOTECHNOLOGY, AND DISEASE CONTROL."

David Wise

Cell culture refers to cultured cells with a uniform genetic makeup. Fish cell cultures have proven to be a successful biological alternative to the use of whole animals in research and are most commonly used to test for viruses in bioassays. The cell lines commonly used in research are established cell lines, which are immortal and can be maintained indefinitely by passage to new media. While established cell lines are easy to maintain, they have lost many properties of the parental cell tissue. Primary cell cultures more closely represent host tissue and represent a more appropriate model of tissues *in vivo*. Development of primary fish cell cultures will greatly aid in the detection of unknown viruses, parasites and toxins causing fish death.

Several of the fish viruses are host specific and some are even tissue specific, which makes the establishment of new cell lines from different organs and tissues of a host species essential for proper monitoring of viral/ myxosporean/ microsporidian diseases. There are more than 30,000 different species of fish and yet the fish cell culture field remains largely unexplored. There is a scarcity of the host specific cell lines in the aquaculture research field, and most of the time researchers rely on general fish cell lines, which might not be conducive for the replication and growth of the pathogens. The primary fish cell culture could be initiated from a broad range of tissues and fish species allowing to study species-specific responses. In addition to testing the virus susceptibility, several bacterial/fungal toxins could also be tested on different cell cultures.

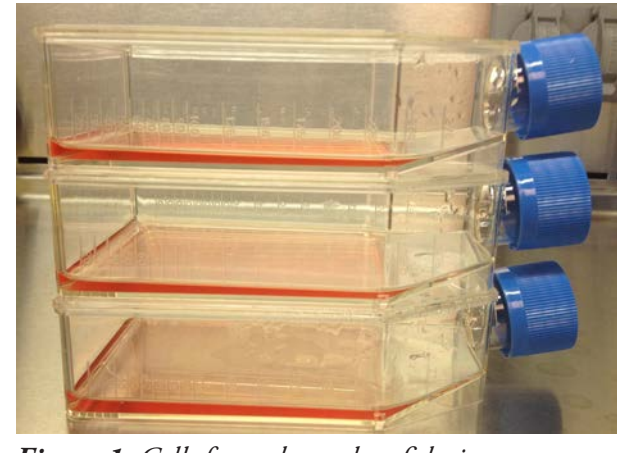

Figure 1. *Cells from channel catfish tissues are grown in tissue culture media and maintained in culture flasks at 25^0 C.*

Scientists were able to use cultured cells to determine the cause of death after conducting a feed study. A pond study at the National Warmwater Aquaculture Center research facilities evaluated two commercially-produced experimental diets. Anemia-related losses occurred in both treatments but was isolated to this specific pond trial. Anemia was also induced by feeding laboratory fish the

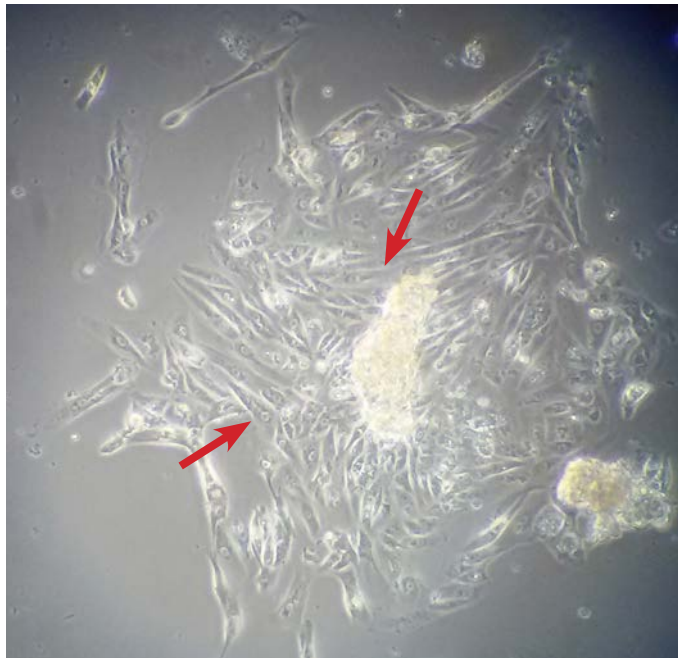

Figure 2A. *Channel catfish kidney cells four days post incubation visualized though an inverted phase contrast microscope at 200x magnification.*

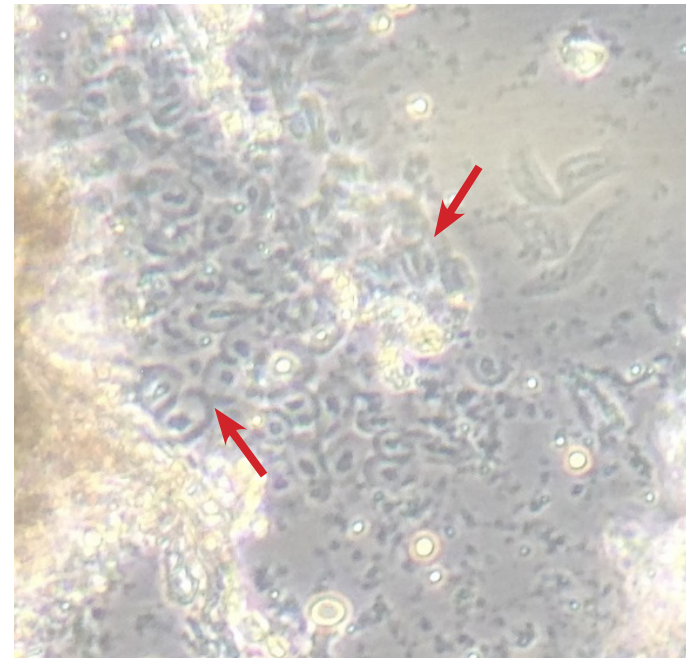

Figure 2B: *Channel catfish fin cells five days post incubation. The red arrows indicate individual cells growing in the single cell suspension.*

experimental diets, identifying a feed-related anemia. Feed samples were negative for pesticides, heavy metals, mycotoxins and other potential chemical contaminants. The water soluble feed extract was inoculated onto channel catfish ovary (CCO) cells. Cell death was observed after 48 hours indicating the presence of possible toxins in the feed. Similarly, fish serum from affected fish inoculated onto CCO cells also caused cell death. While the bioassay does not identify the causative agent, it is suggestive of the presence of a bio-toxin not detected by feed analysis.

The goal of this research was to test two commercially-produced experimental diets and then use cell culture to determine the problems. Healthy channel catfish weighing ~20 grams, was used for the cell culture. Tissues were harvested and washed with antibiotic media, homogenized, and incubated with 500 Units/mL collagenase for two hours with frequent mixing. The samples were centrifuged with serum free media and the supernatant was seeded onto two 75 cm^2 flasks with fetal bovine serum (FBS), antibiotics, and fungizone, and incubated at 25° C overnight. The following day, half of the media was replaced with complete L-15 medium (Figure 1). Adherence to culture flasks occurred after three days. In the initial growth stages, the catfish kidney, and fin cell cultures were composed of elongated fibroblast-like cells and epithelial-like cells respectively (Figure 2).

In conclusion, primary cell cultures were developed from channel catfish kidney and fin tissues. The cells were found to be free of bacterial and fungal contamination. These primary cell cultures can be used as a diagnostic tool for isolation, propagation and detection of fish viruses including channel catfish virus. The cells will also facilitate pathogenesis for significant pathogens and antiviral drug identification studies.