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Replication of Four Aquatic Reoviruses in Experimentally Infected Golden Shiners (*Notemigonus crysoleucas*)

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ABSTRACT: Golden shiners (*Notemigonus crysoleucas*) experimentally infected with four reoviruses supported replication of golden shiner virus as well as chum salmon virus, reovirus $13p_2$ and catfish reovirus at temperatures of 23 and 28 C. All four reoviruses replicated in golden shiners in this study. Natural infections of the golden shiner virus are known only in golden shiners.

Key words: Golden shiner virus, chum salmon virus, reovirus $13p_2$, catfish reovirus, golden shiner, *Notemigonus crysoleucas*, experimental infections.

Golden shiner virus (GSV) was first isolated in 1977 from golden shiners taken from a hatchery in Arkansas; the previously undescribed virus was placed into the reovirus group (Plumb et al., 1979). Chum salmon virus (CSV) which causes a low martality in chum salmon (*Oncho-ryncus keta*) has only been isolated from salmonids in Japan (Winton et al., 1981). Reovirus $13p_2$, originally isolated from oyster homogenates, is experimentally pathogenic to bluegill (*Lepomis macrochirus*) fingerlings causing a 40 to 50% mortality rate (Meyers, 1980). Catfish reovirus has only been isolated from cultured channel catfish (*Ictalurus punctatus*) undergoing chronic low mortality rates in California (Amend et al., 1982). Brady and Plumb (1988) reported that GSV, CSV, and $13p_2$ were significantly related serologically based on cross neutralization studies, and that cross reactions were significant between CRV and GSV, but not with other viruses. The purpose of this study was to determine if CSV, $13p_2$ and CRV could replicate in the golden shiner at 23 and 28 C.

Adult golden shiners averaging 6 cm in length were acclimated for 48 hr in a 250 L fiberglass trough containing flow through dechlorinated water supplemented with compressed air. Five replicate groups of 25 golden shiners each were used in four separate trials at 23 and 28 C. The optimum temperature for replication of CSV and $13p_2$ is 23 C; the optimum temperature for GSV replication is 28 C, while CRV replicates within a temperature range of 23 to 28 C. Each group of 25 fish was anesthetized with MS-222 (Argent Chemical Laboratories, Redmond, Washington 98052, USA) before being injected intraperitnoneally with 0.1 ml of HBSS (Hanks' balanced salt solution; Grand Island Biological Co., Grand Island, New York 14072, USA) containing 100 tissue culture infective doses ($TCID_{50}$) of the test virus. Group I was injected with 0.1 ml HBSS only and served as the control; Group II was injected with GSV; Group III was injected with CSV; Group IV was injected with $13p_2$; and Group V was injected with CRV. Each group of fish was placed in static aquaria containing 40 L of aerated, dechlorinated municipal water with activated charcoal filtration. Fish were fed to satiation with a commercial diet of crumbled fish chow (Doane, Birmingham, Alabama 35202, USA) on alternate days. Fish were observed daily for abnormal behavior and clinical signs of infection.

At 10, 20 and 30 days post-injection, five fish from each group were sampled. Only two fish were assayed for virus replication. The remaining fish from each sample were processed for histological examination (Brady, 1985). Kidneys, liver and spleen were removed from two fish from each group and pooled. Approximately 1 g of the pooled organs was placed in 9 ml of HBSS. The tissues were homogenized using a Brinkman® Polytron

(Brinkman Instruments, Westbury, New York 11590, USA), filtered through a 0.45 μm membrane filter, serially diluted in 10-fold steps and placed in the appropriate cell line for virus replication. All cell lines for this study were stock cultures obtained from the fish disease laboratory at Auburn University. Golden shiner virus replicates only in fathead minnow (FHM) cells (Gravell and Malsberger, 1965; Plumb et al., 1979). These cells were grown at 30 C in a 50:50 mixture of Leibovitz's L-15 with 10% fetal calf serum (Grand Island Biological Co.) and Medium 199 (M-199); (Grand Island Biological Co.) with 10% fetal calf serum. Chum salmon virus and catfish reovirus replicate in salmonid and brown bullhead cells (Winton et al., 1981; Amend et al., 1984). In this study chum salmon virus was grown in chinook salmon embryo cells (CHSE-214) (Nims et al., 1970) at 20 C in minimal essential medium (Grand Island Biological Co.) with 5% fetal calf serum. Catfish reovirus was grown in channel catfish ovary (CCO) cells (Bowser and Plumb, 1980) at 30 C in minimal essential medium with 10% fetal calf serum. Reovirus $13p_2$ replicates in bluegill fry cells (BF-2) (Wolf and Quimby, 1969). BF-2 cells were grown at 30 C in Eagle's minimal essential medium with 10% fetal calf serum. All media were supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin, and 50 μg/ml gentamycin. The tissue culture infectious dose-50% end point ($TCID_{50}$) was calculated by the Reed and Muench (1938) equation. All virus titrations were performed in 96-well microtiter plates (Flow Laboratories, McLean, Virginia 22102, USA).

Golden shiners supported replication of GSV, CSV, $13p_2$ and CRV when held at 23 and 28 C (Table 1). Golden shiner virus replicated in golden shiners as expected. GSV was not detected in the control group. There was no significant difference in virus replication at different temperatures in any of the trials. Chum salmon virus,

TABLE 1. Virus recovery from pooled golden shiner kidneys, liver and spleen, expressed as log 10 $TCID_{50}$/g of tissue.[a]

Sampling	Virus[b]							
	GSV		CSV		$13p_2$		CRV	
	23 C	28 C	23 C	28 C	23 C	28 C	23 C	28 C
Day 10	3.8×10^{3c}	3.2×10^{3c}	7.7×10^{2c}	1.8×10^{2c}	3.1×10^{2c}	2.3×10^{3c}	1.2×10^{2c}	2.8×10^{2c}
Day 20	2.3×10^{3}	4.8×10^{3}	4.8×10^{3}	3.1×10^{3}	2.7×10^{3}	3.0×10^{3}	8.8×10^{3c}	1.4×10^{4c}
Day 30	5.8×10^{4c}	1.2×10^{5d}	1.2×10^{4}	5.1×10^{3}	3.6×10^{3}	5.2×10^{3}	7.0×10^{2c}	1.7×10^{3c}

[a] All values represent the average of two pooled organ samples with four trials.
[b] GSV, golden shiner virus; CSV, chum salmon virus; $13p_2$, reovirus $13p_2$; CRV, catfish reovirus.
[c] Average of three trials.
[d] Average of two trials.

$13p_2$ and CRV also were recovered in golden shiners.

The 10-day average titer of three trials of GSV at 23 C was 3.8×10^3 $TCID_{50}/g$ and 3.2×10^3 $TCID_{50}/g$ at 28 C. One 10-day sample was omitted to allow for adequate numbers of fish at later sample intervals. The 20-day average titer of four trials at 23 and 28 C was 2.3×10^3 $TCID_{50}/g$ and 4.8×10^3 $TCID_{50}/g$, respectively. The average 30-day titer of three trials at 23 C was 5.8×10^3 $TCID_{50}/g$. The average of two trials at 28 C was 1.2×10^5 $TCID_{50}/g$. All fish were dead by day-30 of trial 1 at 28 C and day-30 trial 2 at 23 and 28 C. Fish were not assayed for virus because of advanced decomposition of internal organs (Table 1).

The 10-day average titer of three trials of CSV at 23 C and 28 C was 7.7×10^2 $TCID_{50}/g$ and 1.8×10^2 $TCID_{50}/g$, respectively. The 20-day average titers for four trials at 23 and 28 C were 4.8×10^3 $TCID_{50}/g$ and 3.1×10^3 $TCID_{50}/g$, respectively; the 30-day average titers for four trials were 1.2×10^4 $TCID_{50}/g$ at 23 C and 5.1×10^3 $TCID_{50}/g$ at 28 C. One 10-day sample was omitted to allow for adequate numbers of fish at later sample intervals (Table 1).

The day-10 average of three trials of $13p_2$ at 23 and 28 C was 3.1×10^2 $TCID_{50}/g$ and 2.3×10^3 $TCID_{50}/g$, respectively. The day-20 average titer of four trials at 23 C was 2.7×10^3 $TCID_{50}/g$ and 3.0×10^3 $TCID_{50}/g$ at 28 C. The 30-day average of four trials at 23 and 28 C was 3.6×10^3 $TCID_{50}/g$ and 5.2×10^3 $TCID_{50}/g$, respectively. Only one day-10 sample was omitted (Table 1).

There were only three trials for CRV due to an aeration system failure. The day-10 average titers of three trials at 23 and 28 C were 1.2×10^3 $TCID_{50}/g$ and 2.8×10^2 $TCID_{50}/g$, respectively. The average 20-day titers were 8.8×10^3 $TCID_{50}/g$ at 23 C and 1.4×10^4 $TCID_{50}/g$ at 28 C. The average 30-day titers were 7.0×10^2 $TCID_{50}/g$ at 23 C and 1.7×10^3 $TCID_{50}/g$ at 28 C (Table 1).

Following injection, all four reoviruses showed some replication in golden shiners. However, it is not known if CSV, $13p_2$ and CRV could infect golden shiners by normal means of exposure. In the golden shiner, GSV causes a relatively low mortality rate except under crowded conditions when temperatures are high (Schwedler and Plumb, 1982).

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