Occurrence of *Ichthyophthirius multifiliis* (White spot) infection in snow trout, *Schizothorax richardsonii* (Gray) and its treatment trial in control condition

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**ABSTRACT**
High altitude raceways reared *Schizothorax richardsonii* stocks were monitored periodically for white spot infection at experimental coldwater fish farm, Chhirapani, Champawat, Uttarakhand. Total 1268 numbers of fishes with mean length (cm) ranging from 15.53 ± 0.20 to 16.86 ± 0.08, and mean weight (g) 24.70 ± 0.05 to 26.73 ± 0.06 were examined from January - December 2009. Presence of pinhead sized white spots (0.4 to 0.8 mm) was observed on dorsal body surface and caudal fins of sampled fishes during month of April. The causative agent was identified to be a ciliate parasite, *Ichthyophthirius multifiliis*. A temperature dependant infection pattern was noted with maximum prevalence (%) of 84.80 ± 1.83 in July followed by a decline in December (21.99 ± 2.13). Prevalence varied significantly (p< 0.05) between the months as water temperatures dropped with onset of winter. Trophont counts following dip treatment for five consecutive days under laboratory conditions with 2% common salt, 250 ppm formalin and a combination of the two, of samples of the snow trout, *Schizothorax richardsonii*, infected with *Ichthyophthirius multifiliis*, indicated that treatment with combination of salt and formalin was significantly (p< 0.05) effective than either salt or formalin alone in controlling the infection.

**Key words:** Common salt, Dip treatment, Formalin, *Ichthyophthirius multifiliis*, *Schizothorax richardsonii*.

**INTRODUCTION**
White spot (Ich disease) is an ectoparasitic disease caused by ciliate protozoan, *Ichthyophthirius multifiliis*. This ectoparasitic disease is reported to be occurring in any freshwater fish, indicating extremely low host specificity (Jessop, 1995; Traxler et al., 1998; and Buchmann et al., 2001). Infection of white spot disease may cause considerable mortalities in both cultured and wild fish population (Buchmann et al., 2001). It is reported that the mortality rate due to this disease might reach up to 100% leading to severe economic loss in fish farms. The infection is mainly initiated by theront (one of the developmental stage of *I. multifiliis*) with penetration in fish epithelium (Xu and Klesius, 2004) and is characterized by presence of white spot all over the body surface of fish.

Observing strict preventive measures may not completely stop *I. multifiliis* infecting fish stock in rearing system. In past, many experiments were conducted using different chemicals to control the infection. Malachite green, malachite green in combination with salt or formalin, formalin, chloramine T, KMnO₄ and CuSO₄ were applied as control measures for this infection (Johnson 1961; Ljunberg 1963; Prost and Studnicka 1972; Cross and Hursey 1973; Straus 1993 and Straus and Giffin 2002). The European Union has only permitted use of formalin and Chloramine-T against *I. multifiliis*. Malachite green, being mutagenic in nature, is prohibited in treatment of fish diseases by European Council. Toltrazuril, Sodium percarbonate and garlic extract had been tried in Spain and Denmark. But they were found not effective in controlling the infection (Tojo et al., 1994 and Buchmann et al., 2003).

In India, white spot infections were observed in tropical aquarium fishes as well as in carp culture practices. In two districts of West Bengal, Ich infection was recorded in carp fingerlings (Banerjee and Bandyopadhyay, 2010). High intensity of *Ichthyophthirius* infection in goldfish, *Carassius auratus* during summer was also reported in three district of West Bengal, India (Chanda et al., 2011). *Labeo rohita* (size > 10 cm in length) collected from river Song in Doon valley, Uttarakhand were recorded harbouring *I. multifiliis* in their gills (Upadhyay et al., 2012). But its occurrence in high altitude coldwater aquaculture practices was not studied extensively. The present manuscript describes incident of white spot infection in Snowtrout, *Schizothorax richardsonii* and its treatment trial in control condition at experimental coldwater fish farm, Champawat, Uttarakhand.

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MATERIALS AND METHODS

Sample collection: The stocks of Snowtrout, *S. richardsonii* were examined thrice a month over a period of 1 year (January - December 2009) for *I. multifiliis* infection. During each sampling, more than 40% of stock was sampled randomly. The incident of white spot disease was noticed during April, 2009.

Data Collection: A total sample size (cumulative sampling data over the sampling period after detection of infection in April 2009) of one thousand two hundred sixty-eight (1268) *S. richardsonii* was collected for examination of white spot disease. Data on average length and weight of *S. richardsonii*, number of sample collected every month and mean water temperature (°C) are presented in Table -1. White spots from infected samples were scraped and examined at 400X magnification (LEICA DM 2500). The prevalence was calculated as per the method stated by Bhuiyan et al. (2008). The water temperature (°C) of culture system was also recorded during each sampling with help of a mercury thermometer.

Treatment trial: One hundred thirty five (135) infected *S. richardsonii* (16.16 ± 0.06 cm length and 25.53 ± 0.033 g weight) were collected from rearing pond and stocked in 9 FRP tanks @15 no. of fish per tank (0.5 ton capacity each) with flow-through arrangement. The infected fish were subjected to 3 treatments processes; common salt-2% (T1), formalin -250 ppm (T2), and combination of common salt - 2% and formalin 250 ppm (T3) in three experimental replicates. Three tanks, C1, C2 and C3, stocked @15 numbers of infected fish samples each, were kept separately as controls for the treatment T1, T2, and T3 respectively for the study. A consecutive 5 days dip treatment trial, lasting for 20 seconds each, was carried out to study the effect of NaCl, Formalin and their combination. Water, supplied to the FRP tanks, was carbon-filtered and the average water temperature within the tanks was maintained at 20 °C throughout the trial period. Fish were examined externally after 5th day of treatment. Ten fish from each tank were sacrificed to take skin scraping from 60% of area of the dorsal body surface of freshly killed fish and *Ichthyophthirius multifiliis* trophonts were counted (LEICA DM 2500).

Data analysis: The relationship between prevalence and water temperature was evaluated with Spearman correlation (SPSS 19.0). The effect of months and water temperatures on prevalence as well as treatment effect (P<0.05) between the chemicals were assessed.

RESULTS AND DISCUSSIONS

Fish sampling: Presences of white spots (0.4 to 0.8 mm small cyst) were observed on the body surface including dorsal and caudal fins of fishes. Trophonts of *I. multifiliis* were detected in microscopic observations of skin scraping. The macronucleus (C- shaped) surrounded by a thin rounded, transparent jelly mass changing its shape frequently using tiny hairs called cilia was recorded (Fig 1). Marked hyperplasia of infected fins was also observed. This marked hyperplasia of infected fins was due the presence of trophonts in tissues.

![Fig 1: Matured trophonts of *Ichthyophthirius multifiliis* showing presence of C- shaped macronucleus](image)

<table>
<thead>
<tr>
<th>Month</th>
<th>No. of Host Examined</th>
<th>No. of Host Infected</th>
<th>Length (g)(Mean±SE)</th>
<th>Weight (g)(Mean±SE)</th>
<th>WT (°C)(Mean±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>140</td>
<td>53</td>
<td>15.53±0.202</td>
<td>24.70±0.057</td>
<td>12.4±0.202</td>
</tr>
<tr>
<td>May</td>
<td>149</td>
<td>81</td>
<td>16.00±0.057</td>
<td>24.76±0.088</td>
<td>17.4±0.593</td>
</tr>
<tr>
<td>June</td>
<td>154</td>
<td>110</td>
<td>16.16±0.088</td>
<td>25.16±0.120</td>
<td>20.1±0.375</td>
</tr>
<tr>
<td>July</td>
<td>145</td>
<td>123</td>
<td>16.16±0.066</td>
<td>25.53±0.033</td>
<td>21.4±0.120</td>
</tr>
<tr>
<td>August</td>
<td>139</td>
<td>90</td>
<td>16.10±0.057</td>
<td>25.70±0.057</td>
<td>19.9±0.425</td>
</tr>
<tr>
<td>September</td>
<td>138</td>
<td>79</td>
<td>16.63±0.145</td>
<td>26.06±0.218</td>
<td>18.3±0.145</td>
</tr>
<tr>
<td>October</td>
<td>136</td>
<td>66</td>
<td>16.46±0.145</td>
<td>26.43±0.173</td>
<td>16.2±0.173</td>
</tr>
<tr>
<td>November</td>
<td>135</td>
<td>47</td>
<td>16.80±0.208</td>
<td>26.70±0.100</td>
<td>11.9±0.472</td>
</tr>
<tr>
<td>December</td>
<td>132</td>
<td>29</td>
<td>16.86±0.088</td>
<td>26.73±0.066</td>
<td>8.1±0.260</td>
</tr>
</tbody>
</table>

WT: water temperature
I. multifiliis infection leading to pronounced hyperplasia was also reported by Buchmann and Bresciani (1997). No mortality was evident due to the presence of this ciliate parasite.

**Prevalence and effect of water temperature:** Observations on prevalence and water temperature revealed, a temperature dependant mode of infection pattern, indicating severe infections in July followed by June in the year 2009 (Fig 2). Decline in I. multifiliis infections was recorded from August onwards. This pattern of infection depicts favorable water temperature for completion of life cycle of the parasite. It is reported that the encysted tomont within 24 hour of its becoming theront, the infective stage of this parasite, seeks a host for epithelial infection. Theront grows in the epithelium and becomes matured trophonts in 5-6 days at water temperature 20-25°C. In present study, the average water temperature recorded during June and July were 20.1°C and 21.4°C respectively. This indicated a favorable condition for progression of life cycle of the parasite. Similar infection pattern in the host rainbow trout, Oncorhynchus mykiss, was also recorded by Buchmann and Bresciani in 1997. Prevalence (%) varied significantly (p<0.05) among the months as water temperature started decreasing owing to winter (Table 2). Effect of water temperature was positively correlated (r = 0.91) with prevalence (%) in the study.

**Treatment:** The numbers of trophonts present in skin scraping were counted to evaluate the efficacy of chemicals used in the study (Table 3). Similarly Tojo-Rodriguez and Santamarina, M.T. (1998) evaluated the efficacy of metronidazole and seemidazole by trophonts counts in skin scraping. In life cycle of I. multifiliis, only tomonts and theronts are free swimming in nature and susceptible for chemical treatment. Hence, one time application of any chemical may kill the organism, before they get encysted or burrowed into skin of the host. Durborow et al. (1998) reported administration of multiple treatments (3 to 7), as all stages in life cycle of I. multifiliis were not affected by treatments. Thus, a dip treatment trial was executed successively for 5 days to control the infection. It was also reported by Durborow et al. (1998) that formalin could be used as a bath treatment for up to an hour at 125 to 250 ppm. But in the present dip treatment trial, formalin was applied @250 ppm for 20 seconds. As higher dosage of formalin removed oxygen rapidly from water (Swann and Fitzgerald, 1991) the duration for treatment trial was kept for 20 second only to reduce stress on the treated fish. Use of common salt@0.2–0.3%, as a preventive measure, in tank and recirculation system for a long period, was found effective against I. multifiliis infection (Durborow et al., 1998). Common salt was applied @3% for 30 seconds to control I. multifiliis infection in small volume of water (Flyod and Reed, 2009). Application of 2% common salt for more than 7 days in dip treatment trial was also found effective in treating the said infection (Sharma et al., 2012).

In present study, combination of formalin @250 ppm and common salt @2% was found significantly (p<0.05) effective than either salt or formalin alone in controlling the infection. Kinnunen et al. (2005) also experienced effectiveness of combination of chemical Desirox (containing 20% H2O2) and formalin for treating infection of I. multifiliis.

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