Effects of Water Temperature on the Susceptibility of Rainbow Trout to *Streptococcus agalactiae*

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ABSTRACT

**Background:** The outbreak of diseases is a limiting factor in fish culture. Among different bacterial agents, *Streptococcus agalactiae* is a major problem, causing heavy loss in cultured and wild fish species from fresh and marine water. Outbreaks with considerable mortalities in different fish species have been associated with multiple environmental factors, including warm water temperatures, increased ammonia levels and low dissolved oxygen levels. The effects of various water quality factors on streptococcal infections have been previously shown. To date, there is no available data on the effects of water temperature on the infection of rainbow trout with *S. agalactiae*. Therefore, present study evaluated the effect of two water temperatures (12 and 18°C) on clinical signs, relative percent survival (RPS), cumulative mortality and LD50 in rainbow trout against *S. agalactiae*.

**Materials, Methods & Results:** Three hundred and sixty rainbow trout (56 ± 4.1 g) were acclimatized for 2 weeks and fed the commercial fish diet at 2% body weight twice daily. The experiment was conducted in two different water temperatures (12 ± 1°C and 18 ± 1°C). In each temperature, fish were injected with 0.2 mL of five different concentrations (10^8, 10^7, 10^6, 10^5 and 10^4 CFU mL^-1) of *S. agalactiae* (RTCC2051). In control group, the same amount of physiological saline was injected instead of the bacterial suspension. Mortality rates and clinical signs were recorded for 14 days. Throughout the experiment, different signs such as eye abnormalities, erratic swimming and body lesions were observed in fish which were exposed to high bacterial doses at 18°C while fish kept at 12°C were completely asymptomatic. No mortality was observed within 2 or 3 days in fish at 12°C while at 18°C, the mortality started growing rapidly even from first day of bacterial injections. Results also revealed that the trend of mortalities in 18°C was quite different from 12°C. The median lethal dose (LD50) on the 14th day was also calculated at 12 and 18°C as 7.22 × 10^7 CFU mL^-1 and 1.711 × 10^7 CFU mL^-1, respectively.

**Discussion:** In this study, the relationship between temperature and infection with *Streptococcus agalactiae* was pronounced. When the water temperature was 18°C, mortality due to *S. agalactiae* was higher in rainbow trout than 12°C. It was previously showed that the optimum temperature for growth provide the best performance of fish immune system. Non-specific and specific immune parameters in teleost decrease significantly when the water temperature is lower or higher than normal physiological range of the fish. According to current study, the higher mortality in fish kept at 18°C might be affected by the reduction of rainbow trout immunogenic activity. On the other hand, the effects of water temperature on the progress of infection in fish species are also important. For example, manipulation of temperature and/or salinity can be used to reduce the growth rate of *Flavobacterium psychrophilum*. The change of virulent *S. agalactiae* related to water temperature in fish species needs more consideration. In conclusion, there was a clear relationship between temperature and mortality in rainbow trout against *Streptococcus agalactiae* and the total cumulative mortality at 18°C was higher compared to 12°C for every single concentration used.

**Keywords:** water temperature, *Streptococcus agalactiae*, LD50, rainbow trout, relative percent survival.
INTRODUCTION

Fish in production facilities are exposed to stressful conditions, diseases and deteriorating environmental conditions, that can have serious economic losses [2]. Among different bacterial agents, *Streptococcus agalactiae* is the cause of streptococcosis in numerous fish species, including tilapia, *Oreochromis niloticus* (L.), and mullet, *Liza klunzingeri* [6], golden shiners, *Notemigonus crysoleucas* [13], menhaden, *Brevoortia patronus* [10], and bullminnows, *Fundulus grandis* [11]. The pathogen can produce septicemia and meningoencephalitis in diseased fish, which show various clinical signs such as skin hemorrhage, exophthalmia, ascites and erratic swimming concerned as a typical sign for streptococcosis [9].

There have been a number of studies examining the effects of various water quality factors such as low oxygen levels and increased nitrite, over crowding on streptococcal infections in fish species [4,15]. For aquatic animals, water temperature also play an important role on the prevalence of diseases in cultured fish. It was shown that there is a strong association between water temperature and increased mortality, especially between 25 and 28ºC in barramundi (*Lates calcarifer*) [3]. Rodkhum *et al.* [14] recently showed that Nile tilapia reared in high water temperature is highly susceptible to *S. agalactiae*. To date, there is no available data on the effects of water temperature on the infection of rainbow trout with *S. agalactiae*. Therefore, this study was carried out to investigate the effects of two water temperatures (12 and 18˚C) on clinical signs, relative percent survival (RPS), cumulative mortality and LD50 in rainbow trout against *Streptococcus agalactiae*.

MATERIALS AND METHODS

**Conditions of the experiment**

Healthy rainbow trout with average body weight 56 ± 4.1 g were obtained from Mahisara fish farm (Karaj, Iran). Study was started from September 2010 and specimens were exposed to a natural photoperiod (12L : 12D). They were acclimated in 300 L tanks filled with chlorine-free tap water and provided with continuous aeration using an electric air pumping compressor for 2 weeks. During the experiment, the following conditions were maintained: dissolved oxygen concentration 6.0 ± 0.6 mg L⁻¹ and pH 7.0 ± 0.5. 50% of the water was exchanged twice a week to remove waste feed and fecal materials. Throughout the acclimation period, the fish were fed the commercial fish diet at 2% body weight twice daily and continued two weeks after injections.

**Bacterial preparations**

*Streptococcus agalactiae* (RTCC2051) was cultured on brain heart infusion agar (BHI) in 37°C for 24 h. Cells were harvested, suspended in physiological saline, to obtain a final concentration of 10⁸ CFU mL⁻¹. From an initial bacterial concentration of 10⁸ CFU mL⁻¹, 10-fold serial dilutions were prepared (10⁸, 10⁷, 10⁶, 10⁵ and 10⁴ CFU mL⁻¹).

**Experimental infection trial**

Three hundred and sixty fish were divided into 2 groups with subgroup of 5 different concentrations (T5:10⁸, T4:10⁷, T3:10⁶, T2:10⁵ and T1:10⁴ CFU mL⁻¹) along with the control group in triplicate with 10 fish in each tank. The experiment conducted in two different water temperatures (12 ± 1 and 18 ± 1ºC). In each experiment, fish were injected with 0.2 mL of each dilution intraperitoneally. In control group, the same amount of physiological saline was injected instead of the bacterial suspension. Mortalities were recorded daily for 14 days. Dead fish were removed from the tanks daily and their livers and kidneys were subjected to bacterial identification testing to confirm the presence of *Streptococcus agalactiae* and the clinical signs were also reported. The LD50 was calculated and expressed as colony forming units per milliliter (CFU mL⁻¹) [12]. RPS was calculated by the following formula of Ellis [5]:

\[
RPS = 1 - \left( \frac{\text{Percent mortality in treated group}}{\text{Percent mortality in control group}} \right) \times 100
\]

**Statistical analysis**

All statistical analyses were conducted by SPSS. Determination of the LD50 was conducted using Probit analysis, and mortality schedules were compared using the survival function with pairwise comparison.

RESULTS

In the current study, rainbow trout challenged with *Streptococcus agalactiae* at 18ºC exhibited various signs and lesions such as exophthalmia, haemorrhaging at the base of fins, visceral haemorrhage and ascitic fluid accumulation in the peritoneal cavity present in all fish dying from exposure at 18ºC while there was no clinical sign observed in 12ºC (Figure 1).

This study showed that, cumulative mortality at 12°C was considerably lower than 18°C in all treatment groups while in each temperature, highest cumulative mortality was occurred in T5 (10⁹) group. In fish kept at 12°C, no mortality was shown within 2 or 3 days after injection. On the other hand, in fish held at 18°C, mortality started growing rapidly from the first day post injection (Figure 2-3). RPS in each water temperature also showed that in each treatment dose, lower survival was related to fish reared at 12°C in comparison with rainbow trout kept at 18°C. The LD50 on the 14th day was calculated at 12 and 18°C as 7.22 × 10⁷ CFU mL⁻¹ and 1.711 × 10⁷ CFU mL⁻¹, respectively (Table 1).
DISCUSSION

In this study, fish kept at 18°C showed various signs such as exophthalmia (which may be bilateral), corneal opacity, melanosis, lethargy, loss of orientation, swimming erratically, dorsal rigidity, vertebral deformity, tachypnoea, anorexia, emaciation, or sudden death with few accompanying signs. Besides, the most consistent findings were intracranial edema, ocular alterations resulting from a severe haemorrhagic panophthalmitis, Hemorrhaging at the base of fins, exophthalmy (“pop-eye”), ascitic fluid in the abdominal cavity and haemorrhaging in/on the internal organs. These signs with bacterial identification testing indicated the experimental infection with *Streptococcus agalactiae*.

Noteworthy, fish kept at 12°C did not show any clinical signs of Streptococcosis. Moreover, no mortality was observed within 2 or 3 days in fish at 12°C while at 18°C, the mortality started growing rapidly even from first day of bacterial injections. In this study, the LD₅₀ and RPS results also showed that fish which were kept at 12°C were much more resistant to this bacterial infection in comparison with 18°C. The change of virulent *S. agalactiae* related to water temperature in fish species needs more consideration.

Overall, it can be concluded that there was a clear relationship between temperature and mortality in rainbow trout against *Streptococcus agalactiae* and the total cumulative mortality at 18°C was higher compared to 12°C for every single concentration used.

## Table 1.

<table>
<thead>
<tr>
<th>Bacterial Dose (CFU mL⁻¹)</th>
<th>Water temperature</th>
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<tbody>
<tr>
<td></td>
<td>12°C</td>
</tr>
<tr>
<td>10⁴</td>
<td>86.6</td>
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<tr>
<td>10⁵</td>
<td>70</td>
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<tr>
<td>10⁶</td>
<td>70</td>
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<tr>
<td>10⁷</td>
<td>53.3</td>
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<tr>
<td>10⁸</td>
<td>43.3</td>
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<tr>
<td>LD₅₀ (CFU mL⁻¹)</td>
<td>7.22 × 10⁷</td>
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</table>

SOURCE AND MANUFACTURER

1Razi Vaccine and Serum Institute, Karaj, Iran.
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REFERENCES


