



# **Communication Influence of Salinity on the Survival Rate of Fertilized Chum Salmon (***Oncorhynchus keta***)** Eggs

Jong Won Park <sup>1</sup>, Beom Sik Kim <sup>1</sup>, Ju Kyoung Kim <sup>1,2</sup>, Hae Kun Jung <sup>3</sup>, Hyun Je Park <sup>1</sup>, and Chung Il Lee <sup>1,\*</sup>

- <sup>1</sup> Department of Marine Ecology and Environment, Gangneung-Wonju National University, Gangneung 25457, Republic of Korea; po7639@gwnu.ac.kr (J.W.P.); 20131078@gwnu.ac.kr (B.S.K.); logonkjk@fira.or.kr (J.K.K.); phj13579@gwnu.ac.kr (H.J.P.)
- <sup>2</sup> Aquatic Living Resources Center of South Sea, Korea Fisheries Resources Agency, Wando 59110, Republic of Korea
- <sup>3</sup> East Sea Fisheries Research Institute, National Institute of Fisheries Science, Gangneung 25435, Republic of Korea; hkjung85@korea.kr
- \* Correspondence: leeci@gwnu.ac.kr; Tel.: +82-33-640-2855

**Abstract:** Chum salmon (*Oncorhynchus keta*) exhibit a remarkable ability to adapt to changes in salinity during their life cycle. However, the fertilized egg stages are sensitive to salinity, affecting ontogeny and hatching. This study investigated the effect of salinity (0, 1, 3, and 5 PSU) on the survival of two developmental stages (<1 day after fertilization and <1 day after the eyed-egg stage) of fertilized eggs. Based on the experimental results, we assessed the spawning ground environment using the in situ salinity data of the Namdae River from 1997 to 2002, where the largest number of salmon in Korea migrate to spawn. Survival of the <1-day-old fertilized eggs decreased sharply at 3 PSU or more, and all eggs died at 5 PSU. Hatching of the eyed-egg stage occurred under all environmental conditions. After 2010, the salinity of the layer of water in contact with the sediment in the lower reaches of the river increased (>6.9 PSU) with the frequency of high waves. Overall, the function of the lower river in spawning and hatching is weakening. This study enhances our understanding of the effects of climate change, including increased wave activity, on salmon spawning grounds.

**Keywords:** chum salmon; fertilized eggs; salinity tolerance; hatching rate; spawning ground; Namdae River

# 1. Introduction

Chum salmon (*Oncorhynchus keta*) is one of the six species (chum, coho, chinook, sockeye, pink, and cherry salmon) of salmonid fish that inhabit the North Pacific. All species of salmon are anadromous, i.e., they hatch in a river, migrate to the sea for growth, and then return to the river to spawn [1–5]. The early life stage of a salmon (hereafter referred to as "salmon") begins in a wild or artificial hatchery in freshwater. After growth, it moves to a brackish water environment and, when adapted to seawater, migrates seaward [5,6]. Therefore, salmon are euryhaline [7]; they inhabit freshwater and seawater environments. However, the fertilized egg period is highly sensitive to environmental changes such as salinity, which can affect egg development and hatching [8]. Fertilized eggs of Atlantic salmon (*Salmo salar*) are adversely affected by osmotic pressure when exposed to saline water (0.5–5 PSU) during the prehatching development stage [9].

During the spawning migration, salmon move to the upper reaches of rivers to find the optimal spawning environment. For example, in the Skagit River (WA, USA), salmon travel more than 170 km upstream to spawn. In the Yukon River (AK, USA) and the Amur River (border of China and the Russian Federation), salmon move more than 2500 km upstream before they reach their spawning grounds [6]. Most salmon-spawning migrations in Korea occur in rivers on the eastern coast, which are short with steep slopes from the headwater to the river mouth [10,11]. In particular, the Namdae River is where most salmon in Korea



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). migrate to spawn, and their major spawning period is between October and November [12]. The watershed size of the Namdae River is 478.8 km<sup>2</sup>, and it experiences significant flow rate variations during the rainy season [13]. The downstream of the Namdae River exhibits a slower flow rate compared to the middle and upstream, with more than 80% of the sediments in the downstream consisting of sand and silt [14]. The topographical characteristics limit the range of salmon distribution in these rivers to a narrow space (approximately 10 km in the case of the Namdae River) upstream from the estuary [13], and artificial structures such as weirs can also interrupt salmon movement to the upstream areas [11]. Moreover, reduced winter precipitation results in low stream flow and water levels, restricting the area available for salmon migration. This leads to frequent exposure of fertilized eggs to the atmosphere after spawning [15–17]. Because of these environmental factors, in the case of rivers located on the eastern coast of Korea, including the Namdae River (Yangyang), salmon spawning grounds are formed downstream [11,15], and seawater movements, such as high waves and tides, can cause environmental changes (e.g., salinity) in the lower reaches.

Owing to recent global climate changes, regional sea levels have risen, which has exposed more regions worldwide to the risk of coastal overtopping [18]. The recent significant increase in wave height along the eastern coast of Korea has caused wave overtopping [19,20]. The influx of seawater in the spawning ground can affect the survival (hatching) rate of salmon eggs. For example, the wave overtopping that occurred immediately after the 2011 Tohoku earthquake and tsunami affected the lower reaches of rivers near the Pacific Ocean in Japan. The salinity of artificial spawning grounds in Iwate and Miyagi Prefectures rose to 14.5 PSU, affecting salmon incubation and discharge rates [8]. In addition, long-term sea level rise as a result of global warming can be a major cause of environmental change in the lower reaches of rivers and, as a result, can cause changes in the Namdae River's resources that salmon use as spawning grounds.

This study hypothesized that changes in salinity in the lower reaches of the river, owing to the influence of environmental changes, such as high waves, could affect the survival rate of salmon eggs. The purpose of this study was to understand how changes in river water salinity affect the development of fertilized chum salmon eggs. To achieve this, we observed the survival rate of fertilized eggs before and after the eyed-egg stage exposed to various salinities.

## 2. Materials and Methods

## 2.1. Salinity in the Namdae River

Salinity data were provided by the Water Environment Information System (WEIS; http://water.nier.go.kr/, accessed on 1 October 2023), Kwon et al. [14], and the Gangneung Wonju National University Fisheries Oceanography Lab (hereafter referred to as GWNU lab; Figure 1). The salinity concentration from WEIS was measured weekly at the surface from 1997 to 2022 (excluding January 2001 and 2016), and for this study, the monthly averages were calculated. The data from Kwon et al. covered April to November 2008, excluding June and September [14]. The data from the GWNU lab were collected at a depth of 1 m from June to December 2017 (excluding October and November) and October 2019 to April 2020, and at the surface in October and November 2017, and from October 2019 to April 2020. GWNU lab and Kwon et al. [14] data were measured once a month.

#### 2.2. Experiment Design

Fertilized and eyed eggs were provided by the Korea Fisheries Resources Agency (FIRA) in November and December 2019, respectively. These eggs were artificially fertilized in freshwater (0 PSU). FIRA provided the equipment to conduct the outdoor experiments using an Atkins incubator equipped with a water-recirculating system.

The experimental conditions involved controlling salinity using freshwater from the Namdae River, where salmon migrate for spawning, to create four environments (0, 1, 3, and 5 PSU; salinities were created by adding salt to the river water; Red Sea Aquatics Ltd.,

Coral Pro Salt, Essex, UK). Supplemental water was periodically added to maintain the salinity concentration. During the experiment, the water temperature was not artificially controlled and was affected by changes in the atmospheric environment in the same manner as in the natural spawning grounds (Figure 2).



**Figure 1.** Stations for salinity measurement along the lower reaches of the Namdae River (**top**) and a representation of the topographical characteristics and water level difference at the mouth of the Namdae River (**bottom**). Colored circles represent the stations for salinity measurement [14].

Two experiments were performed based on the egg development process. This involved assessing, in response to various salinities, the rate of survival to the eyed-egg stage of <1-day-old fertilized eggs (Exp 1) and the hatching rate of early eyed-eggs (Exp 2). Exp 1 was concluded when all eggs were at either the eyed-egg stage or dead, and Exp 2 was concluded when all eyed-eggs had hatched or were dead. For each experiment, 100 eggs were placed, and dead eggs (which are discolored) were removed daily. All experiments were performed in triplicate, and during the experiments, the Atkins incubators were covered with a mesh to prevent threats from predators such as rodents. During Exp 1 and Exp 2, the water temperature ranged from 3.99 to 10.98 °C (7.43 ± 1.87 °C) and 2.12 to 6.85 °C (4.86 ± 1.19 °C), respectively (Figure 2). The survival rate to the eyed-egg stage and the hatching rate are expressed as percentages.

# 2.3. Statistical Analysis

We used IBM SPSS Statistics for Windows (v. 28.0; IBM Corp., Armonk, NY, USA) for statistical analyses and conducted one-way ANOVAs to examine the relationship between salinity concentration and the survival rate to eyed-egg stage and hatching rate. When statistical significance was confirmed, we performed a post-hoc analysis using Tukey's HSD.



**Figure 2.** Water and air temperatures recorded during the experimental period (from 15 November 2019 to 4 February 2020). During Exp 1, the water temperature ranged from 3.99 to 10.98 °C ( $7.43 \pm 1.87$  °C). In Exp 2, it ranged from 2.12 to 6.85 °C ( $4.86 \pm 1.19$  °C). Water temperature data were provided by the Gangneung Wonju National University Fisheries Oceanography Lab, and air temperature data were provided by the Korea Meteorological Administration.

## 3. Results

## 3.1. Changes in the Salinity Concentration of the Namdae River

During the experiment, the surface water salinity at station A, located approximately 3.6 km from the Namdae River estuary, ranged from 0.06 to 1.97 PSU ( $\bar{x} = 0.26$  PSU). The average salinity was 0.09, 0.25, and 0.30 PSU from 1997 to 2006, 2007 to 2016, and 2017 to 2022, respectively. At stations B, C, D, and E, salinity ranged from 1.36 to 20.08 PSU ( $\bar{x} = 13.81$  PSU), 0.21 to 3.01 PSU ( $\bar{x} = 0.87$  PSU), 0.11 to 11.17 PSU ( $\bar{x} = 2.71$  PSU), and 0.21 to 10.14 PSU ( $\bar{x} = 2.14$  PSU), respectively (Figures 1 and 3).



**Figure 3.** Salinity recorded at five stations in the Namdae River from 1997 to 2022. The left axis is for station A (provided by the Water Environment Information System; WEIS), and the right axis is for stations B–E. Data from stations B and C were provided by Kwon et al. [14]. Data from stations D and E were provided by the Gangneung Wonju National University Fisheries Oceanography Lab (GWNU lab).

## 3.2. Effect of Salinity on the Rate of Survival to the Eyed-Egg Stage and Hatching Rate

The average ( $\pm$ SD) rate of survival to the eyed-egg stage of the fertilized eggs in Exp 1 was 91.3  $\pm$  0.47%, 92.3  $\pm$  2.49%, and 56.0  $\pm$  2.16% at 0, 1, and 3 PSU, respectively. However, at 5 PSU, all eggs died 10 days post-fertilization and did not develop into the eyed stage (Figures 4 and 5).



**Figure 4.** Mean ( $\pm$ SD, n = 3) mortality rates of fertilized chum salmon eggs over 39 days under various salinity conditions.



**Figure 5.** Mean ( $\pm$ SD, n = 3) rate of survival of fertilized chum salmon eggs to the eyed-egg stage under various salinity conditions (*p* < 0.05). Alphabetic letters indicate significant differences between the treatment groups.

The average hatching rates of the eyed-egg stage in Exp 2 were  $82.7 \pm 1.15\%$ ,  $80.7 \pm 3.79\%$ ,  $68.3 \pm 10.69\%$ , and  $72.0 \pm 12.49\%$  at 0, 1, 3, and 5 PSU, respectively (Figures 6 and 7). In Experiment 2, egg development progressed to the hatching stage at all salinities.



**Figure 6.** Mean ( $\pm$ SD, n = 3) mortality rate of chum salmon eyed-eggs over 43 days under various salinity conditions.



**Figure 7.** Mean ( $\pm$ SD, n = 3) hatching rate of chum salmon eyed-eggs under various salinity conditions (*p* > 0.05).

# 4. Discussion

The Namdae River, where the largest number of salmon migrate for spawning in Korea, passes through a small coastal city. In this river, saline water is distributed in the lower layers of the lower reaches because of the influence of domestic sewage and wave overtopping caused by high waves [13,14,19,20]. Salmon adapt to freshwater and seawater environments at each growth stage throughout their life cycle. Generally, during the spawning and hatching seasons, they spend their early life in the middle and upper reaches of the river, where they are unaffected by seawater.

In this study, the eyed-egg stage was more salinity-tolerant than the early fertilized egg stage (Figures 4–7). This may result from the exposure to saline water before the chorionization of the egg envelope during development to the eyed-egg stage. Exposure to saline water in the early stage of fertilization can affect the hardening process by weakening the egg envelope and causing premature hatching [21,22]. Ban et al. [8] exposed chum salmon eggs to various salinities (0, 0.5, 1, 2.1, 4.1, 8.3 PSU) before and after fertilization at a water temperature of 10 °C. For eggs exposed before fertilization, the hatching rate was less than 2% when the salinity was 4.1 PSU or higher. However, the hatching rate of eggs exposed after fertilization was more than 90% in all salinities [8]. Similarly, in this study, 1-day-old fertilized eggs were directly influenced by salinity. However, the low hatching rate of eyed eggs associated with salinities above 3 PSU in our study contrasts

with the results of Ban et al. [8]. This may be associated with the effect of water temperature on hatching. The optimal hatching temperature of fertilized salmon eggs is between 8 and 10 °C [8,23,24]. Ban et al. found that eggs exposed to freshwater (0 PSU) between 7 and 13 °C had a very high hatching rate (approximately 90%) [8]. The hatching rate was relatively lower, approximately 78% and 86% at 4 °C and 16 °C, respectively.

Most early life stages of aquatic animals are highly vulnerable [25] and are known to be sensitive to changes in the physical environment of their spawning grounds [26]. Most salmon that migrate to the Namdae River in Korea cannot move to the upper reaches of the river and spawn in the middle or lower reaches [16]. In particular, many salmon are prevented from accessing the middle or upper reaches of the river through the presence of weirs and catch nets installed for artificial seedling production [12]. Similarly, in the Lamprey and Oyster Rivers in New Hampshire (USA), anadromous river herring (alewives (*Alosa pseudoharengus*) and blueback herring (*Alosa aestivalis*)) typically spawn in the upper reaches. However, dam construction has led to the destruction of spawning grounds, eventually resulting in a decrease in the resource volume [27].

Owing to the geographical characteristics of the lower Namdae River, when seawater flows in, it tends to remain in the lower layer of the river for a long time because it is denser than the freshwater ([20]; Figures 1 and 3). The high salinity in the lower reaches of the Namdae River has appeared continuously since 2012. It is considered to be related to the recent trend of increasing significant waves along the eastern coast of Korea during winter [19] (Figure A1). In addition, sea levels along the eastern coast of Korea are expected to rise by approximately 0.58 cm/year from 2005 to 2040 [28], and the frequency of high waves is simultaneously increasing [19,20]. These phenomena are significant causes of frequent wave overtopping and the consequent changes in the salinity of rivers.

This study found that exposure of fertilized salmon eggs to saline water in the preeyed stage negatively affected survival and hatching rates. The gradual increase in salinity in the lower reaches of the Namdae River is expected to affect not only the survival rate of salmon in early life but also change salmon stocks in the long run [12].

This study investigated the effects of salinity on eggs immediately after fertilization and at the eyed stage. However, it is necessary to continuously monitor changes as the hatched larvae grow in this environment. For example, exposure to saline water during egg development can also impact survival and growth after hatching [8]. Additionally, Korea continues to experience severe drought in winter, and these environmental changes result in a decrease in the flow rate and water levels, increasing the likelihood of spawning areas forming in the lower reaches of rivers.

## 5. Conclusions

In this study, we analyzed the effect of salinity on the survival rate of fertilized eggs and found that the stage-before-eyed eggs were vulnerable to salinity. Due to anthropogenic environmental factors, the spawning grounds of the Namdae River have gradually moved to the lower reaches of the river, where high-salinity water is distributed. This phenomenon is expected to undermine spawning and hatching capabilities and impact the sustainability of salmon stocks. The results of this study could contribute to the establishment of an environmental management plan for the sustainability of salmon resources and changes in salmon resource abundance influenced by precipitation patterns, water temperature, and the increasing frequency of high waves due to climate change.

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## Appendix A



**Figure A1.** Seasonal change in significant wave height measured at the ocean buoy near the Namdae River mouth (orange-colored star in Figure 1). Ocean buoy data was provided by the Korea Meteorological Administration (https://data.kma.go.kr/cmmn/main.do, accessed on 1 October 2023).

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