Recent trends in *Enteromyxum leei* surveillance through seawater filtration in Jeju island, Korea

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Abstract

To prevent disease and establish an early warning system, we monitored the presence of *Enteromyxum leei,* the causative agent of emaciation symptom of the olive flounder, in seawater from six fish farms in Jeju Island on a monthly basis. The seawater from the fish farms was sampled, filtered through a 5 μm filter, and then gDNA was extracted. To quantify *E. leei*, qPCR was performed using specific primers based on the 28S ribosomal gene. Among the six fish farms, we analyzed the four with the most severe *E. leei* infections. The results showed some regional variation, but the gene quantity began to increase in May, peaking between July and October. The concentration in the rearing water was higher than in the inflow water, likely due to the release of parasites into the rearing water after multiplying within infected flounders. The data from 2017 to 2020 indicated that the parasites infiltrated through inflow water infected the flounders, with infection rates increasing from September and peaking in November, followed by a gradual decrease, showing a seasonal pattern. However, the 2023 data showed that the infection persisted from spring through early summer, weakening the seasonality, and once a group was infected, it maintained a consistently high infection status. This suggests that the parasite infection and resulting damage to farmed flounders could occur throughout the year. Infection rates by flounder size were highest in the 21-40 cm range, while fish larger than 40 cm and smaller than 20 cm had lower infection rates. The lower infection rates in fish under 20 cm are likely due to the shorter exposure period to the parasites.

Introduction

Jeju island contributes to over half of the total production of Olive Flounder (*Paralichthys olivaceus*) in Korean aquaculture industry. However, the rapid growth of olive flounder aquaculture in Jeju has also brought challenges in the form of frequent disease outbreaks, with mortality rates peaked at 47.5% of production in 2019. Recently, *E. leei*, a myxozoan parasite causing enteromyxosis, has emerged as a major concern due to its role in causing severe mortality, especially among adult flounders, resulting in significant economic losses. To prevent the disease and establish an early alert system, we conducted monthly monitoring of the occurrence of *E. leei* in seawater at six fish farms in Jeju island.

Materials and Methods

Sample seawater collected area

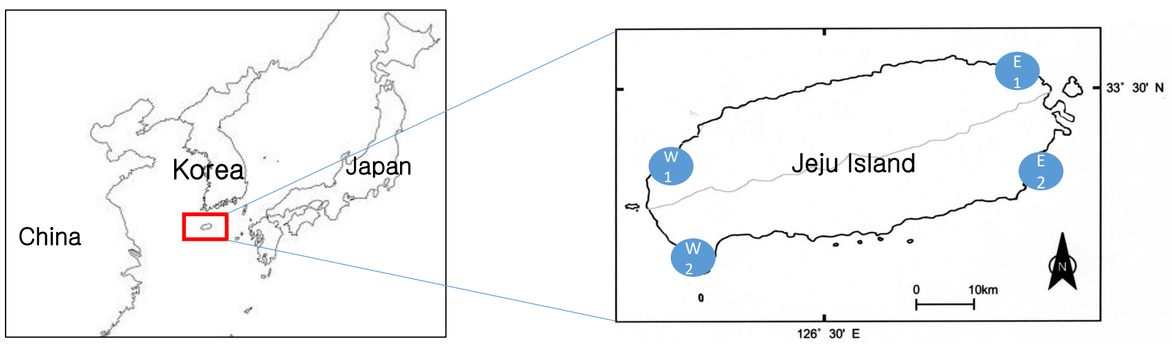
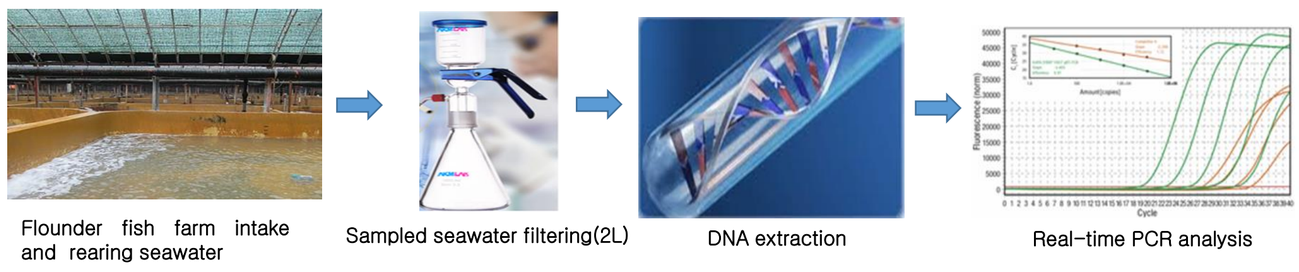


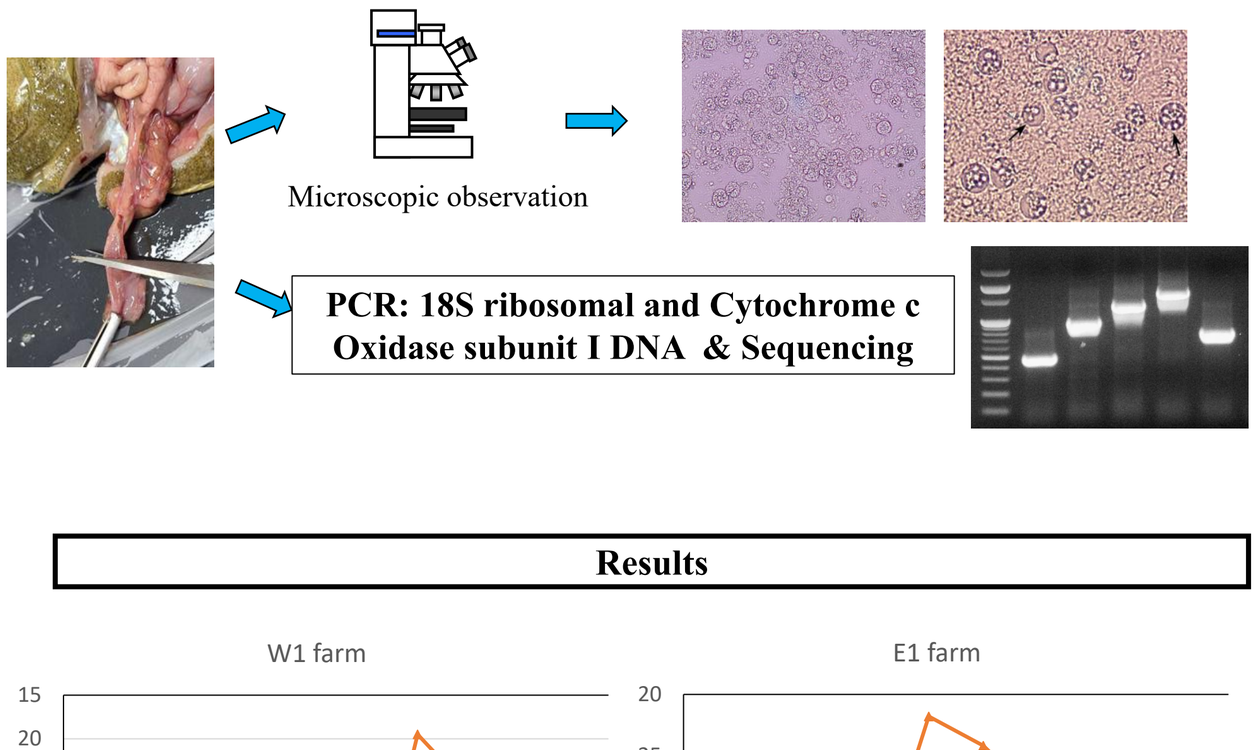
Fig 1. Sampling area of inflow and rearing water in Jeju island

Examination of *E. leei* in seawater



Flounder fish farm intake and rearing seawater → Sampled seawater filtering(2L) → DNA extraction → Real-time PCR analysis

Diagnosis of *E. leei* in fish intestines



Microscopic observation

PCR: 18S ribosomal and Cytochrome c Oxidase subunit I DNA & Sequencing

Results

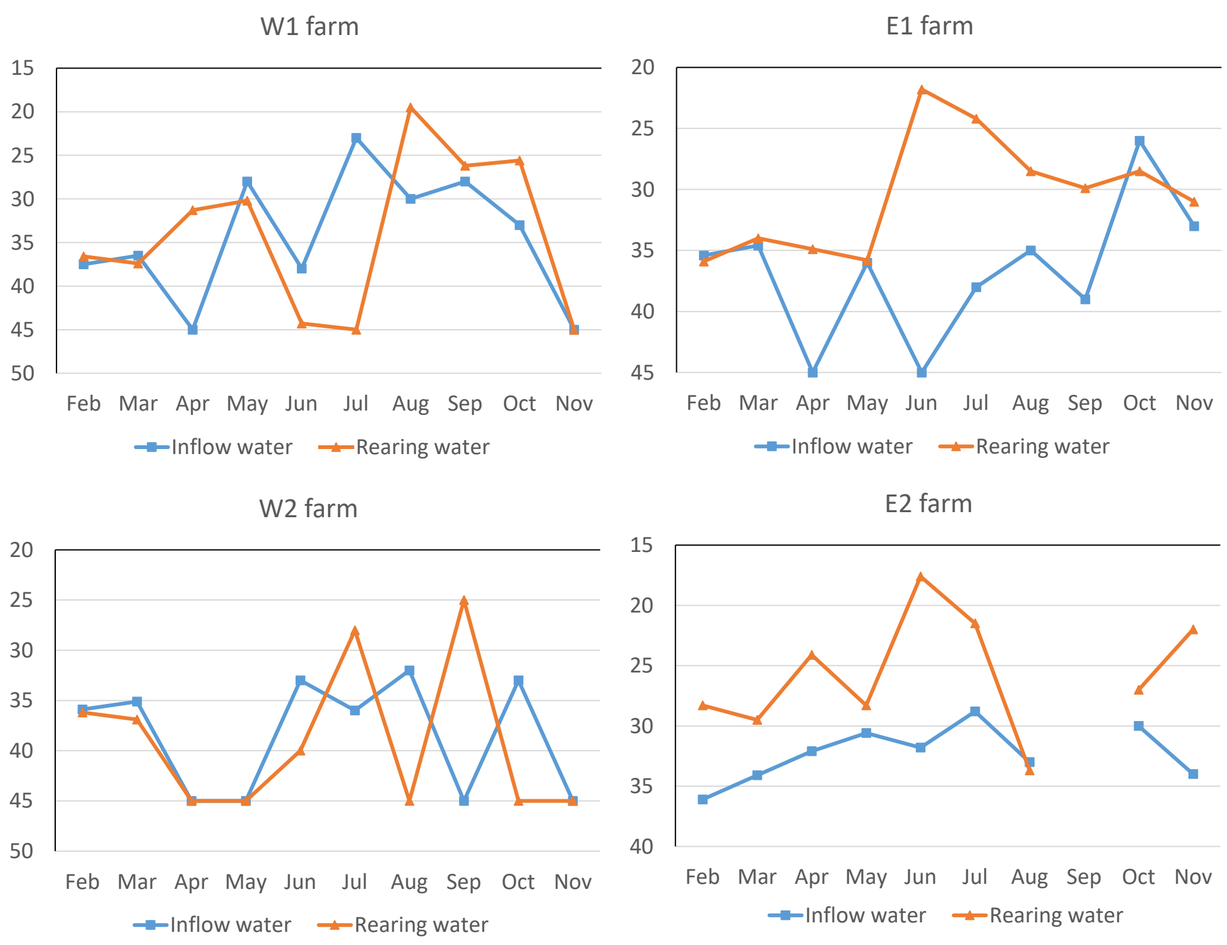


Fig 2. Monthly *E. leei* Cq value of intake and rearing seawater from Jeju island fish farm

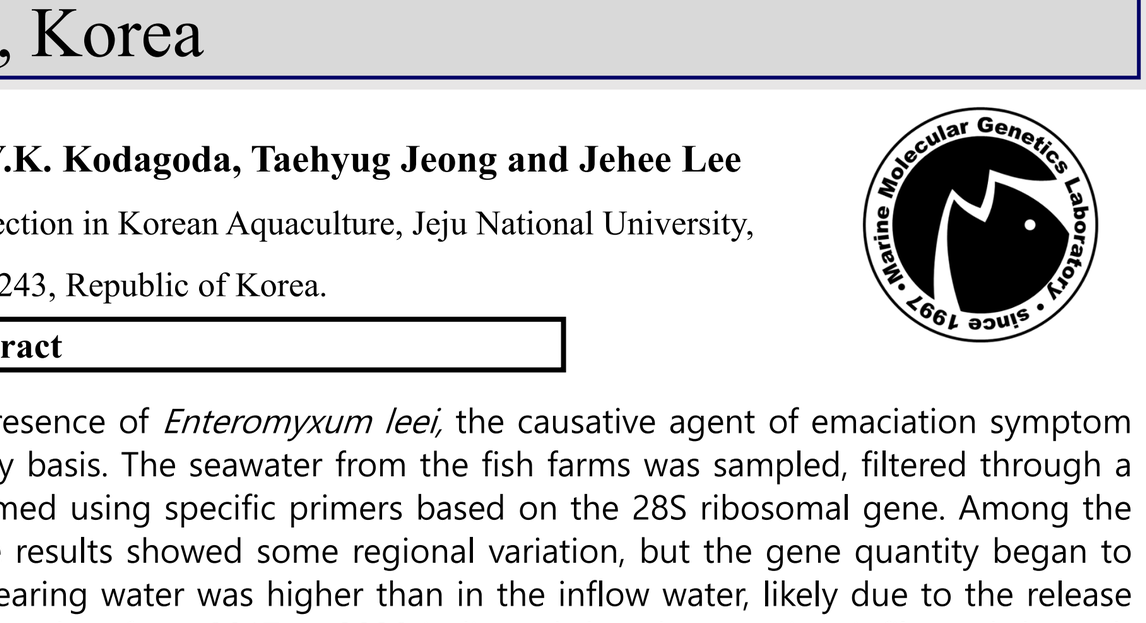


Fig 3. *E. leei* infection sort by size of the flounders

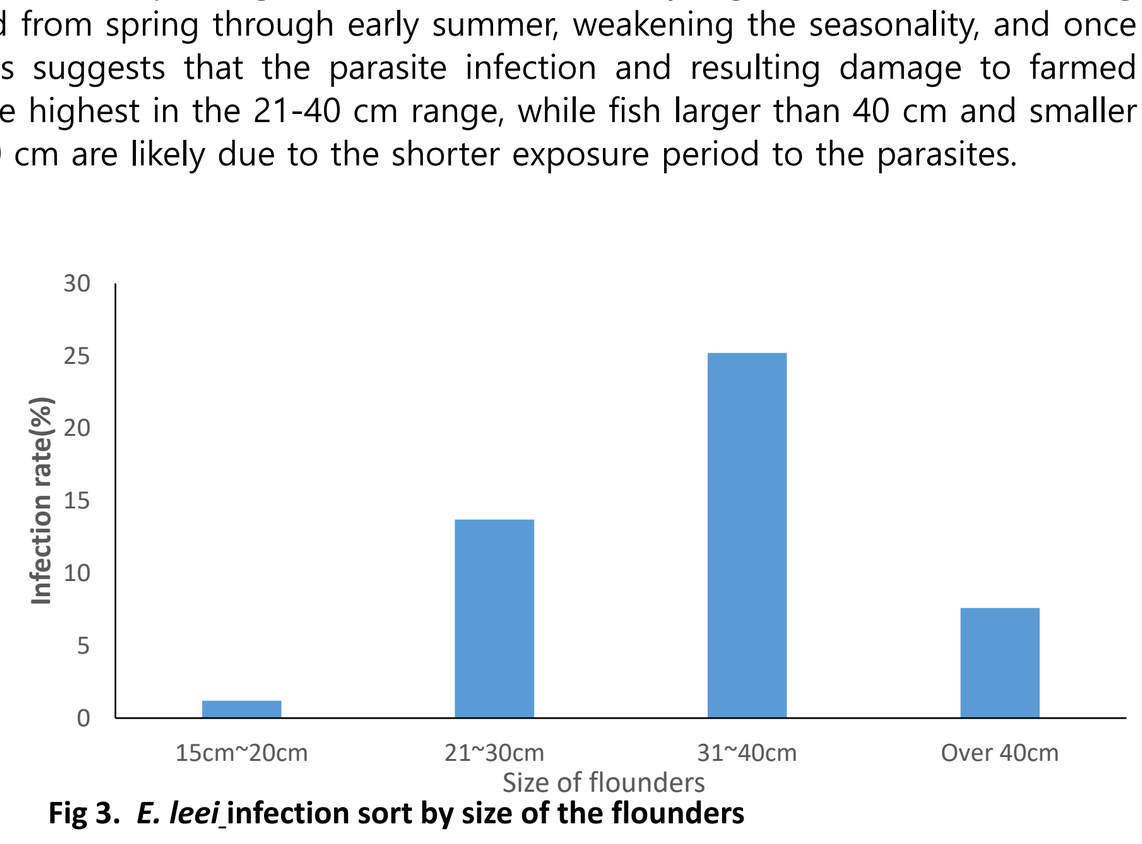


Fig 4. Monthly Trends of Enteromyxosis in Jeju Island (2023)

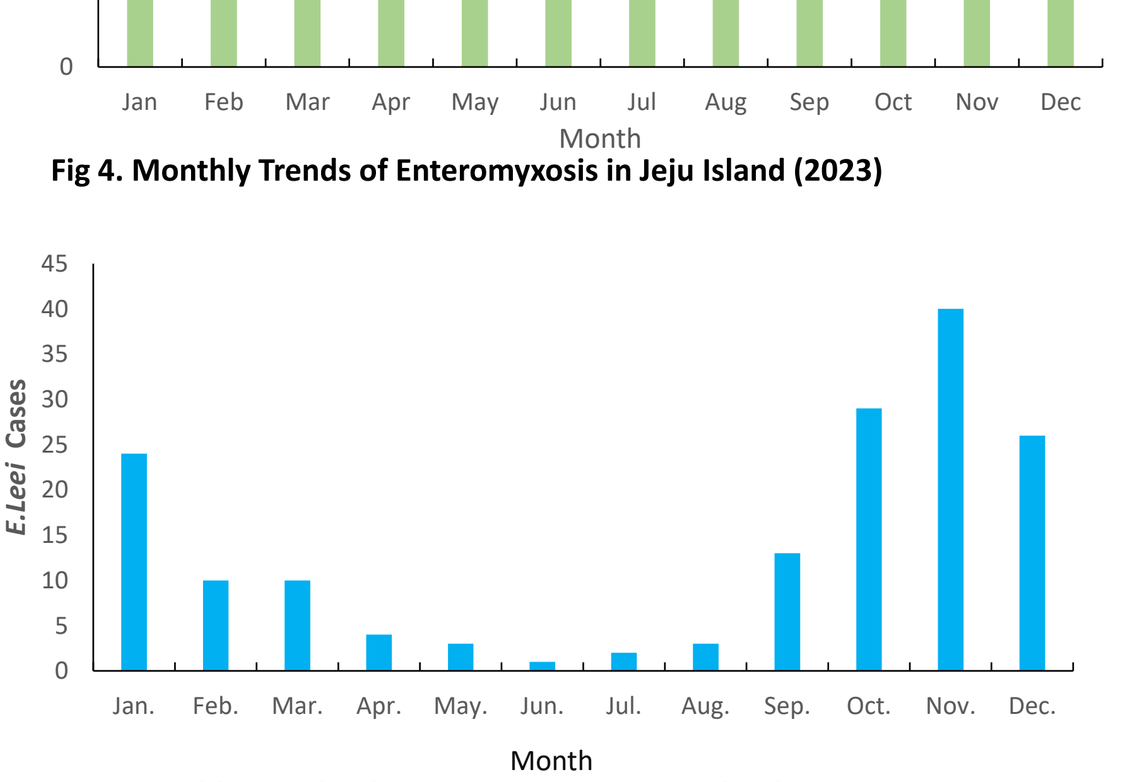


Fig 5. Monthly Trends of Enteromyxosis in Jeju Island (2017 to 2020)

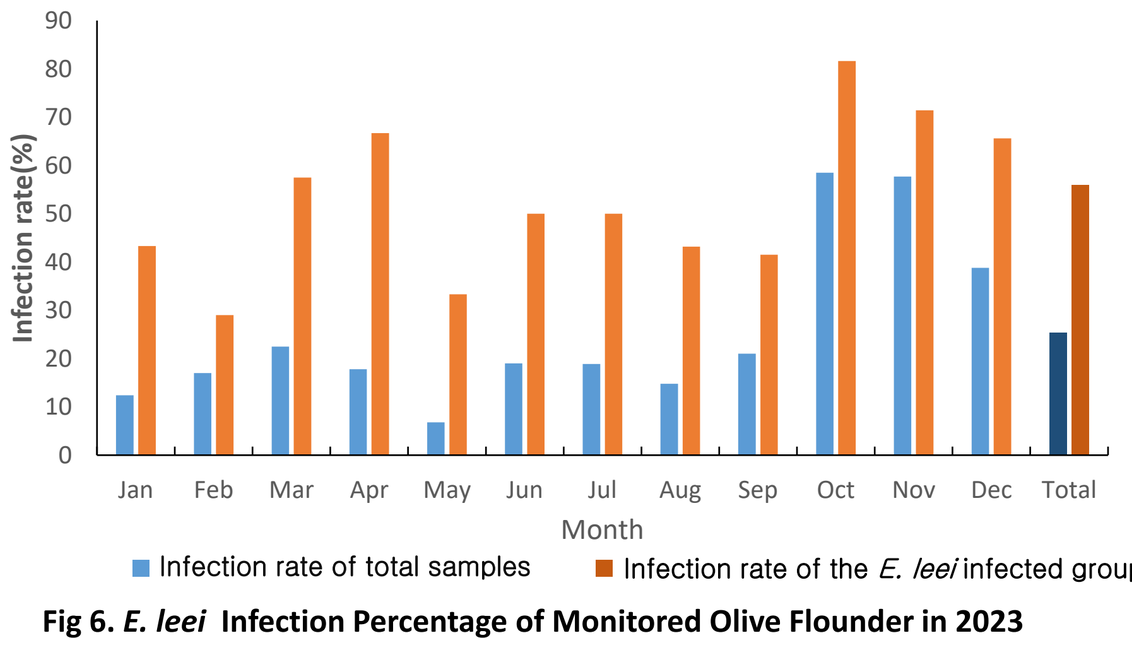


Fig 6. *E. leei* Infection Percentage of Monitored Olive Flounder in 2023

Conclusion

Analysis of the *E. leei*, the causative agent of emaciation symptom, in seawater from flounder farms revealed that the parasite primarily enters the farms through natural seawater between May and October. The parasites infiltrate during this period, leading to symptoms of emaciation and mass mortality from September onwards, with the highest outbreak occurring each year in November. However, the 2023 monitoring results indicated a weakening of the seasonal pattern, with the disease showing a tendency to occur year-round. This suggests that there is a higher likelihood of increased outbreaks and damage from this disease in the future.