The JSFS 85th Anniversary-Commemorative International Symposium
"Fisheries Science for Future Generations"
Symposium Proceedings, No. 07012

Original article

Rearing Trial of Eel, *Anguilla japonica* in a Closed Recirculating System Using Hot Spring Water Welled up in Saito City, Miyazaki, Japan

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Keywords: Eel; *Anguilla Japonica*; Hot Spring Water; Closed recirculating system; *Edwardsiella tarda*.

Received: 19 July 2017 / Accepted: 18 September 2017

Introduction

Recently, University of Miyazaki concluded an agreement on partnership cooperation with Saito city, Miyazaki, Japan and is strengthening collaborations among industry, universities and the government sector. In Saito city, the natural hot spring water (HSW) called as TSUMA ONSEN in Japanese is welled-up, and the water temperature is approximately 40°C. At present, we are trying to utilize this HSW in the field of aquaculture. South Kyushu, Japan such as Miyazaki and Kagoshima prefectures are major producers of Japanese eels [1]. For stable eel culture, the rearing water should be heated by a boiler. However, the cost of fuel for boiler accounted for most of total cost. Therefore we focused on the utilization of the HSW as rearing water in eel culture to reduce the cost of water temperature maintenance with a boiler.

In this study, the preliminary rearing trial of eels with HSW was performed to evaluate the effect of HSW on the growth of eel and water quality in a closed recirculating system (CRS). Additionally, pathogen challenge test was carried out to confirm the influences on the resistance of eels to bacterial disease.

Materials and methods

Rearing condition and sample collection
Thirty eight Japanese eel, *Anguilla japonica* with a mean weight of 20 g were obtained from commercial eel farm in Shibushi city, Kagoshima, Japan. Eels were transported to University of Miyazaki and were conditioned in a CRS at 27°C for 2 weeks prior to the experiment. HSW was collected from TSUMA hot spring where located on Saito city, Miyazaki, Japan. The acclimatized eels to the rearing condition were divided into two CRSs at a density of 19 individuals per tank. One tank was supplied with the tap water aerated overnight of 150 L (tank A), and other tank was supplied with the HSW (tank B). Each CRS was equipped with an external filter unit (SV10000, Kotobuki Co. Ltd., Nara, Japan), and vigorous aeration was supplied by a bower. Water in each tank was heated by a heater, and water temperature was maintained at 27°C by a thermostat. Eels were fed with the commercial diet (Nosan corporation, Kanagawa, Japan) once daily at 1% of body for 28 days.

After 28 days of feeding, the body weight and the body length of eels were collectively measured. Growth performance indicators; survival rate, Fulton's condition factors, specific weight gain (SWG) and specific growth rate (SGR) were calculated.

Water quality analysis
Dissolved oxygen (DO) and water temperature were checked by a portable DO-pH meter (D-55, HORIBA Ltd., Kyoto, Japan). Salinity, total dissolved solids (TDS) and conductivity (COND) were measured by a salinity meter (AS710, AS ONE Corporation, Osaka, Japan). Ammonia-nitrogen (NH_4-N) and nitrite nitrogen (NO_2-N) were analyzed by the method of Strickland and Parsons [2].

Pathogen challenge test
The eels in each tank were challenged with the pathogenic bacteria *Edwardsiella tarda*. Nine eels in each tank were separately transferred into a 90 L aquaria with a filter unit (SV4500, Kotobuki Co. Ltd., Nara, Japan). The eels were anesthetized by 2-phenoxyethanol at 500 ppm, and *E. tarda* suspensions

of 100 µl (1×10^9 CFU/ml) was intraperitoneally injected into the eels and were reared for 10 days. During 10 days, the eels were fed with same diet with the rearing trial once daily at 1% of body weight, and fish mortality was monitored.

Results and discussion

Figure 1 shows result of growth trial. The average weight in tank A and tank B decreased and increased, respectively after 28 days of rearing without statistical support (Fig. 1A). In the case of body length, no significant difference was observed (Fig 1B). The total body weight in tank A decreased to 237 g from 281 g, and that in tank B increased to 284 g from 273 g after 28 days of rearing (Fig. 1C). Condition factor in tank A significantly decreased after 28 days of rearing. In case of tank B, no significant change was observed (Fig. 1D).

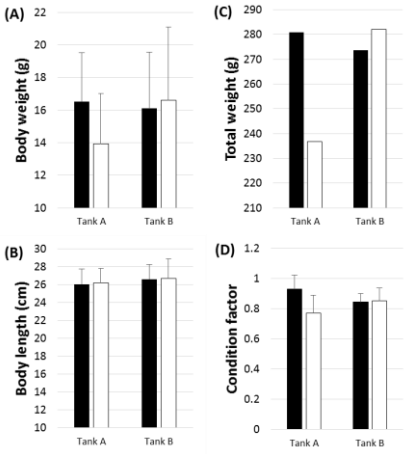

Fig. 1. Growth and feeding performance in *A. japonica* after 28 days of rearing. (**A**) mean body weight per fish, (**B**) mean body weight per fish, (**C**) total fish body weight per tank, (**D**) condition factor. Black bar, 0 day; White bar, 28 days.

Figure 2 showed change of water quality parameters. The HSW used in tank B contained salinity and showed higher pH than tap water in tank A. The TDS and COND were higher in tank B than tank A throughout rearing period. In the case of NH_4-N and NO_2-N, significant difference was not observed except for 3 days after start of rearing.

In Kagoshima region, the eel culture with HSW has been done for from 1950s, however, the information on the eel growth is scarce. Oyama described that rearing in HSW negatively impacted the quality of eel such as hardening of skin although use of HSW was effective to the cost reduction for heating [3].

Figure 3 showed survival rate of *A. japonica* in pathogen challenge test. The survival rate in tank A and tank B decreased to 62.5% and 87.5%, respectively, at 10 days after injection of *E. tarda* suspensions.

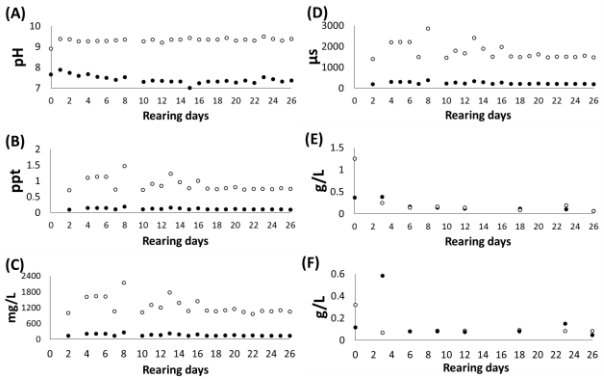

Fig. 2. Change of water quality parameters in a CRS after 28 days of culture. (A) pH, (B) salinity, (C) TDS, (D) COND, (E) NH_4-N, (F) NO_2-N. ● Tank A, ○ Tank B.

The result indicated the rearing with HSW enhanced the resistance of eel to *E. tarda* infection. Recently, the aquaculture using HSW has attracted interests such as culture of tiger puffer fish in a CRS [4]. However, there was no report about effect of HSW on fish immune responses or disease resistance. The elucidation of mechanism for enhancement of disease resistance by HSW is needed to clarify the usefulness of HSW for eel culture.

This study indicated that HSW enhanced the growth of eels and improved the disease resistance to *E. tarda* infection. At present, we are planning the pilot scale-culture experiment of eel with HSW.

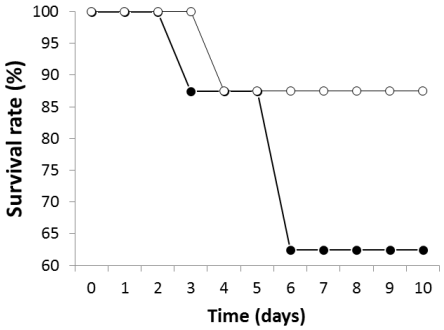

Fig. 3. Survival of *A. japonica* after injection of *E. tarda* suspensions. ● tank A, ○ tank B.

Acknowledgements

I'd like to express my gratitude to Yamada Suisan Co., Ltd., Shibushi, Kagoshima, Japan and for providing eels for this experiment and Wint Co. Ltd., Saito, Miyazaki, Japan for supplying HSW.

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