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Analysis of the Main Factors Inducing Mortality in Artificial Ponds for Australian Freshwater Lobsters

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Abstract

With rising demand for Cherax quadricarinatus due to advancing aquaculture technology and consumer preference, this imported species faces strict environmental needs and technical challenges. Poor water quality and temperature extremes during breeding often cause mortality and yield losses, impacting farmers economically. This study analyzes primary mortality causes in artificial ponds through breeding experiments, collecting data on temperature, dissolved oxygen, ammonia nitrogen, nitrate nitrogen, and Vibrio infection. Key factors identified include temperatures ≤10°C or ≥31°C, dissolved oxygen ≤1.0 mg/L, and ammonia nitrogen or nitrate nitrogen ≥0.5 mg/L. These findings enhance understanding of artificial pond conditions affecting C. quadricarinatus, offering a reference for improving aquaculture practices.

Keywords: Cherax quadricarinatus, artificial pond, Vibrio, breeding experiment

1. Introduction

Cherax quadricarinatus, commonly known as the Australian freshwater lobster or red-clawed crayfish, is a popular delicacy native to Australia, now cultivated globally. Farmers initially grew it to 100–200 g within a year using artificial ponds, but its rising demand has spurred diverse techniques, from extensive lake cultures to intensive greenhouse systems. As an aquaculture species, it offers high nutritional value and economic potential, yet its sensitivity to environmental conditions poses challenges. This study investigates mortality factors in artificial ponds to support sustainable farming practices. The Australian freshwater lobster that was first introduced in 1992[5]by China, Hubei was the first province to start its breeding and to this day, it has amassed a wealth of farming skills in the cultivation of these lobsters. The primary culture techniques in China for the Australian freshwater lobster are pond culture and rice field culture. Nevertheless, aquaculture of freshwater lobster in Guangdong is sparkled up the existence of the larval nursery in the Pearl River Delta. Additionally, the Huazhou City has employed a modern method of lobster raising which is the use of greenhouses and the plastic net cages act as "independent delivery rooms" for the gravid lobsters; meanwhile, in Chengtou Village in northern Guangdong, what is now a conventional single-species culture model was anciently changed to an environmentally friendly co-culture system with lotus shrimp, thus their lobsters becoming widely known[6] Not only is Zhongshan City introducing a "rice-fish co-culture" system which has its rice fields managed without the use of chemical fertilizers or pesticides, and so the fields are able to produce high-quality, healthy and green Australian freshwater lobsters and rice within the "double-green" cultivation system[7].

The Australian freshwater lobster is an aquatic animal fed on fresh water that, though of great nutritional value and a high proportion of environmental potential for aquaculture, is critically endangered in the waters of its natural environment. Naturally, so many problems occur, even the most reminiscent ones, such as numerous diseases and high mortality rates, when dealing with artificial breeding[8]. One of the primary causes of death in the cultivation process, according to the information obtained from previously existing practices, is the low level of dissolved oxygen in the water, the intensity of the temperature, as well as nitrate nitrogen and ammonia poisoning, along with the Vibrio infections. This research on Australian freshwater lobsters will scrutinize such variables as water oxygen, ammonia nitrogen, nitrate nitrogen, temperature, and Vibrio infection in the production of artificial ponds.

The aims are the identification of corresponding countermeasures, ensuring that the technique becomes a reliable reference and guide for farmers, and boosting the healthy development of the lobster aquaculture industry[9].

2. Materials and Methods

2.1 Construction of the Experimental Cultivation Pond

The Australian freshwater lobster culturing pond was set inside a greenhouse to make the environment quieter and cleaner from harmful pests. A canvas pond of 12m² area and with 1.2 m height was chosen. The canvas was placed flat on the ground and zip ties were used to fasten it to a stainless steel frame by passing them through the holes in the canvas. Great care was taken to ensure that the canvas edges were smooth so that the leaking was minimized. The suitable amount of water was added then to the canvas pond which gave a water depth of about 0.25-0.3 m neither too shallow nor too deep. The water initially added should not be directly used for the lobster culture. It needs to be disinfected first by adding saturated lime in the pond and the water's pH must be adjusted in the range of 7 to 8. Precautions must be taken to disinfect all the items (experimental setup) using a saturated lime water solution, and then these must be rinsed with clean water before they are introduced into the pond to prevent the contamination by microorganisms. Furthermore, every biological material used for introduction purposes (e.g., Elodea, Potamogeton crispus, duckweed) should be washed with water before putting it into the water.

2.2 Materials and Reagents

Australian freshwater lobster seedlings, provided by Huazhong Agricultural University.pH precision test paper (range 5.5–9.0), from Hangzhou Shisan Technology Co., Ltd.Water quality monitoring reagent kits (including reagents for detecting dissolved oxygen, ammonia nitrogen, and nitrite), from Guangdong HuanKai Biotechnology Co., Ltd.IxPBS buffer solution, purchased from Beijing SolaiBao Co., Ltd.Quicklime, from Sichuan Jinghe Tai Agricultural Technology Co., Ltd.Potassium ferrate, from Shandong Wuchuang Yufeng Bio Co., Ltd.Yeast sediment, from Jianjiang Distillery Development Co., Ltd., Duyun City, Guizhou Province.TSB liquid culture medium, TCBS agar, and a Gram staining reagent kit, from Qingdao Haibo Biotechnology Co., Ltd.Sand and red duckweed from Jianjiang River in Duyun City, Guizhou Province; Azolla imbricata (Roxb.) Nakai; Elodea nuttallii; and Potamogeton crispus L.

2.3 Instruments and Equipment

Autoclave: GR110DR, from Zhiwei (Xiamen) Instruments Co., Ltd.Electronic balance: YP5002, from Shanghai Youke Instrument & Apparatus Co., Ltd.Vacuum filtration apparatus: SHB-IIIS, from Zhengzhou Great Wall Science and Technology Trade Co., Ltd.Water pump: DLXPH6594, from Hangzhou Delixi Group Co., Ltd.Drying oven: Model, from Shanghai Boxun Industrial Co., Ltd. Medical Equipment Factory.Alcohol thermometer: WNY-11, from Changzhou Xinwang Instrument Co., Ltd.Clean workbench: SW-CJ-2FD, from Suzhou Antai Air Technology Co., Ltd.Constant temperature incubator: ZDP-9902, from Shanghai Zhetu Scientific Instrument Co., Ltd.

2.4 Experimental Methods

180 of the healthiest Australian freshwater seed lobsters were narrowed down. Three ponds with 60 lobsters each were used for a 60-day testing period in each of the ponds whose growth conditions differed:Pond Condition 1: The pond, with duckweed (chiefly for the removal of nitrogen compounds and without adding oxygen), is also adorned with Elodea and Potamogeton crispus(planted at the bottom to respire).Pond Condition 2: The pond, with only the cover of duckweed (for the removal of ammonia and nitrate nitrogen only), exists in this case.Pond Condition 3: The pond contained Elodea and Potamogeton crispus only (planted at the bottom to supply oxygen).

2.4.1 Effect of Temperature on Lobster Mortality

In the greenhouse, temperature rises quickly and drops slowly; therefore, the highest water temperature recorded during the day was used.

2.4.2 Determination of Dissolved Oxygen: Method and Principle

Dissolved oxygen was determined using the iodometric method (GB/T7489—87)[10-13] in combination with colorimetry[13]. The procedure involves adding a specified amount of $MnSO_4$ and NaOH-KI to the water sample. Dissolved oxygen oxidizes $MnSO_4$ to form $Mn(OH)_4$ precipitate. After acidifying with H_2SO_4, the precipitate dissolves, and Mn^{4+} oxidizes KI to generate I_2. The released I_2 is then titrated with a standard $Na_2S_2O_3$ solution, allowing for the calculation of the dissolved oxygen concentration within a range of 0.5–20 mg/mL.

2.4.3 Determination of Ammonia Nitrogen and Nitrate Nitrogen: Method and Principle

Ammonia nitrogen and nitrate nitrogen poisoning in lobsters are indicated by which the animals float on the water surface, they react sluggishly, and the dead ones have empty intestines. Ammonia Nitrogen: Determined using the Nessler reagent colorimetric method (HJ533-2009)[14]. In this technique, a mixture of mercuric iodide and potassium iodide with a small amount of ammonia/ammonium ions is added. The mixture then turns a pale reddish-brown color. This solution of mercury iodide is characterized by intense absorption at a wide spectrum of wavelengths, of which 410–425 nm is the most frequent with the response range of 0.01–1.0 mg/mL.Nitrate Nitrogen: Determined using the diazotization-coupling spectrophotometric method (GB/T8538-2008)[15]. In this method, water with nitrite content reacts with p-aminobenzenesulfonamide to become a diazo compound that later couples with N-(1-naphthyl) ethylenediamine resulting in the purplish-red azo dye[15]. The nitrite concentration is directly proportional to the intensity of the color, which is mostly detected at 540 nm with a response range of 0.01–0.5 mg/mL.

2.4.4 Vibrio Detection Method

According to research[16], the TCBS agar plate method was the one chosen to be used. Samples were taken from Australian freshwater lobsters which had died during rearing. At first, the surface of the lobsters was wiped with 75% alcohol and was dried using a clean paper towel. Unserkaren Bedingungen in a clean bench, the hard part of the lobsters at the head was opened (the hepatopancreas, which is situated next to the ovaries and looks like a yellow, antler-like structure, was exposed), and 0.5 g of hepatopancreas tissue was taken. This tissue was then homogenized with 0.5 mL of sterile PBS and ground with a mortar. Later, 0.1 mL of the homogenate was evenly spread onto TCBS agar medium[17]. The plates were kept upside down and were put into a climatic chamber at 37°C for 16 hours. Continuously, the crystals were watched to see the size, the way they were, and the color to decide as a result if the Vibrios were present. (Certain pathogens are Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio cholerae, Vibrio alginolyticus, and Vibrio fluvialis.) On TCBS agar, these Vibrios are typically recognizable as below:

Green colonies indicate Vibrio vulnificus and Vibrio parahaemolyticus.

Yellow colonies indicate Vibrio cholerae, Vibrio alginolyticus, and Vibrio fluvialis.

The colonies, in general, have a diameter of 2-3 mm, and the bacterial cells are round, transparent, and smooth. After the colony's growth, the same size, and the same shape of the colonies are choose for further purification and culture. Gram staining is done next, and the bacteria are exactly observed under the oil immersion lens to see their morphology and staining properties[17].

2.4.5 Data Statistical Analysis

Data were processed using Microsoft Excel 2019, and statistical analyses were performed with Origin 2021 and SPSS 24.0. One-way analysis of variance (ANOVA) and Duncan’s multiple range test were used for multiple comparisons. Results are expressed as “mean ± standard deviation,” with differences considered statistically significant at $P < 0.05$.

3. Results and Analysis

3.1 Effect of Temperature on Lobster Mortality

Temperature significantly affects the mortality of Australian freshwater lobsters. When the temperature is below 13°C or above 31°C, mortality increases. In particular, when the temperature drops below 10°C or rises above 34°C, mortality rates reach 30% and 26.7% of the total lobsters, respectively. In contrast, a temperature range between 16°C and 28°C is most favorable for their survival. Therefore, temperature is one of the primary factors contributing to the mortality of one-year-old lobsters (see Figure 1).

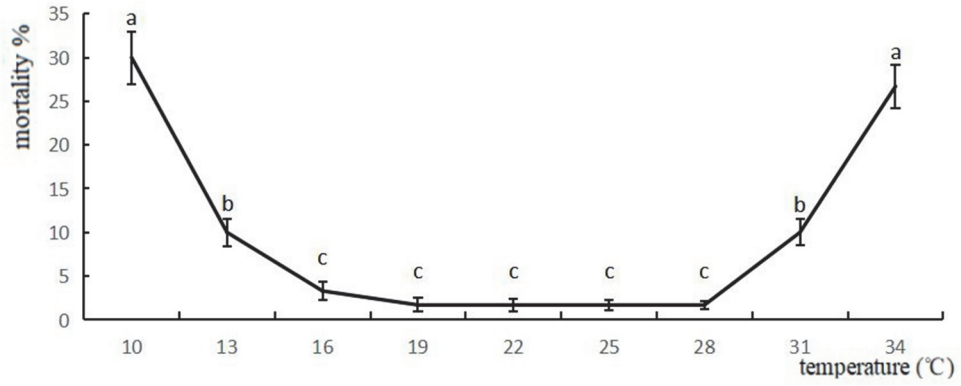

Figure 1. Temperature and mortality

3.2 Effect of Dissolved Oxygen on Lobster Mortality

Dissolved oxygen (DO) levels have a significant impact on lobster survival. When the DO level is ≤2.0 mg/mL, the mortality of Australian freshwater lobsters increases considerably, with the most severe impact observed when DO is ≤1.0 mg/mL. Conversely, a DO level of ≥3.0 mg/mL is optimal for their growth. Thus, dissolved oxygen is also a major factor influencing lobster mortality (see Figure 2).

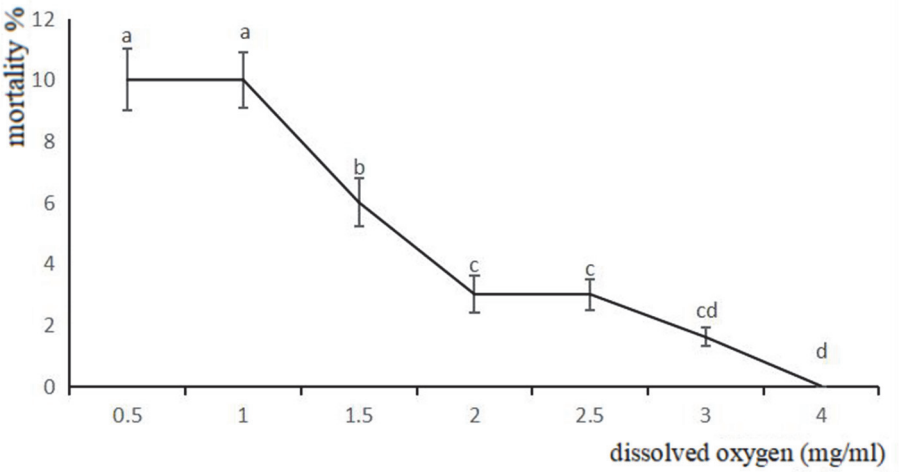

Figure 2. Dissolved oxygen and mortality

3.3 Effect of Ammonia and Nitrate Nitrogen on Lobster Mortality

The mortality of Australian freshwater lobsters is greatly affected when the levels of ammonia nitrogen and nitrate nitrogen are ≥0.4 mg/mL, with the highest mortality observed at levels ≥0.6 mg/mL. Only when the levels are controlled at ≤0.1 mg/mL is the impact on the lobsters minimized. Therefore, the concentrations of ammonia nitrogen and nitrate nitrogen are among the primary factors affecting the mortality of these lobsters (see Figure 3).

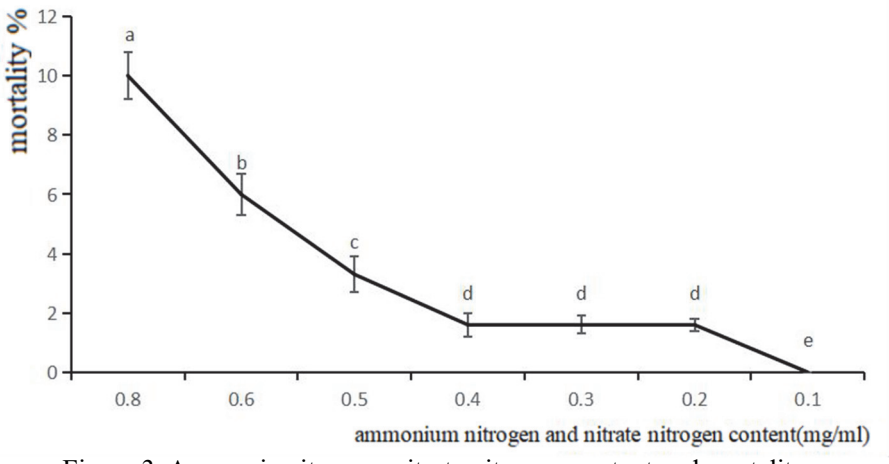

Figure 3. Ammonia nitrogen, nitrate nitrogen content and mortality

3.4 Effect of Vibrio Infection on Lobster Mortality

The source of the dead Australian freshwater lobsters was killed and analyzed on TCBS agar, the same as when they were alive. The plates were turned upside down, incubated at 37°C for 16 hours, and Vibrio colonies happened on the TCBS medium (see Figures 4–6). Nevertheless, there were not many colonies of Vibrio found in the brood, which told us that the death of the lobsters was hardly due to the Vibrio infection. In other words, the Vibrio infection was not a significant factor among other causes for the freshwater lobsters die-off[18].

Figure 4. Dead Australian freshwater lobster

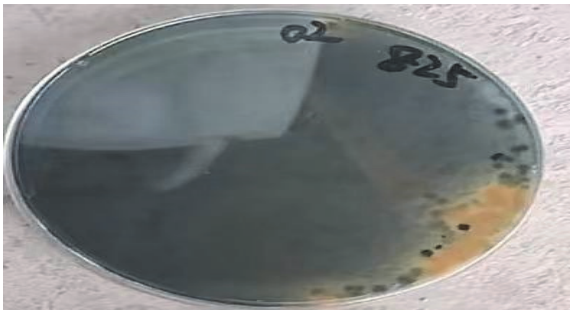

Figure 5. TCBS medium with vibr

4. Conclusions and Discussion

4.1 Effect of Temperature on Lobster Mortality

Studies show that the ideal temperature band for the life of Australian freshwater lobsters is 22°C to 28°C. Initially, at the critical temperature level of even 18°C, the lobsters are significantly small and the temperature of 12°C or less makes them die. Freshwater lobsters can naturally spawn in the temperatures above 25°C; broodstock, however, is a prerequisite[19]. In fact, the experimental culture we carried out pointed out the best suitable temperature is rather from 19°C up to 30°C with the growth of lobsters almost not being possible at those below 16°C as well as the mortality of them initiating when the temperature falls below 13°C, and deaths in excess of 10°C. The inconsistency in the findings from the experimental and past data might be due to the differences in the methods, such as the ponds with an artificial water body which have a smaller volume and thus, offer little buffering capacity to the lobsters, thus making the temperature range for the lobsters more narrow.

4.2 Impact of Ammonia Nitrogen and Dissolved Oxygen on Lobster Survival

According to existing research, when the levels of ammonia nitrogen and nitrate nitrogen are ≤0.1 mg/mL, the survival rate of Australian freshwater lobsters can reach 100%, and a dissolved oxygen concentration (ρ) of 4.0–6.0 mg/L is optimal for their growth. This experiment showed that when the ammonia nitrogen and nitrate nitrogen levels were controlled at ≤0.1 mg/mL, virtually no lobster mortality occurred; similarly, when ρ (dissolved oxygen) was maintained at ≥4.0 mg/mL, the mortality rate was zero. These results are consistent with the findings in the

literature[20]. It can thus be concluded that for the successful cultivation of Australian freshwater lobsters, the water must maintain ammonia nitrogen and nitrate nitrogen levels below 0.1 mg/mL and a dissolved oxygen level of at least 4.0 mg/mL.

4.3 Impact of Vibrio on Lobster Survival

According to these findings, in the dead Australian freshwater lobsters, that is, pre- Vibrio detection, the number of colonies of vibrio on TCBS plates was less than 10^3–10^4 cfu/cm², that is in line with the Yu studies from Yu et al. This then means that Vibrio was not found to be the main cause of death in this examination and freshwater lobsters were thus safe to use for experiments.

5. Conclusions

This experiment identifies temperature, dissolved oxygen, ammonia nitrogen, and nitrate nitrogen as the primary factors driving mortality in Australian freshwater lobsters reared in artificial ponds. Extreme temperatures (≤10°C or ≥31°C), low dissolved oxygen (≤1.0 mg/L), and elevated ammonia or nitrate nitrogen (≥0.5 mg/L) significantly increase death rates. Although Vibrio was detected in dead lobsters, its low prevalence suggests a negligible role in mortality. These insights guide optimal pond management for sustainable aquaculture.

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