MINISTRY OF EDUCATION AND TRAINING
NHA TRANG UNIVERSITY

NGUYEN DINH HUY

USE OF ARTEMIA FOR WASTEWATER TREATMENT IN INTENSIVE WHITELEG SHRIMP (*Litopenaeus vannamei* Boone, 1931) FARMING SYSTEMS

Major: Aquaculture
Major code: 9620301

SUMMARY OF DOCTORAL THESIS

KHANH HOA – 2026

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INTRODUCTION

Over the past two decades, global aquaculture has expanded rapidly to meet the increasing demand for aquatic food products (Naylor et al., 2021). Among cultured species, farmed shrimp production has shown particularly strong growth, increasing from approximately 3.2 million tons in 2010 to 6.8 million tons in 2022, and is projected to continue rising in the coming years (FAOSTAT, 2024). The rapid expansion of shrimp farming toward intensive and super-intensive systems has substantially increased water use as well as the generation of wastes discharged into the environment.

In natural aquatic environments, dissolved inorganic nitrogen compounds and organic matter are utilized by microbial communities and microalgae as nutrient sources for biomass production (Khatoon et al., 2016; Santanumurti et al., 2022). This primary biomass is subsequently transferred as food to filter-feeding zooplankton such as rotifers, copepods, and *Artemia* (Kumar et al., 2016; Nguyen Van Hoa et al., 2007). These zooplankton groups then serve as food for larger organisms at the juvenile stage, including marine fish and crustaceans (Nguyen Thi Ngoc Anh, 2011; Sorgeloos et al., 1998), forming natural food webs that transform nitrogen in water into the biomass of high-value organisms.

Although numerous wastewater treatment solutions for intensive shrimp farming have been developed and applied, most existing approaches remain fragmented, focusing on individual waste components (e.g., suspended solids, TAN, NO_2^-, NO_3^-) rather than restructuring the entire material flow within the culture system. Even in integrated multi-trophic aquaculture (IMTA) systems, a fundamental limitation persists in terms of imbalance among material-transforming organism groups. Microbial-based systems (biofloc, RAS) are highly effective in converting TAN but often result in the accumulation of microbial biomass and nitrate (NO_3^-), whereas autotrophic groups (seaweeds and microalgae) are capable of assimilating dissolved nutrients but are constrained by environmental conditions, space requirements, and treatment time.

Artemia is one of the most preferred live feeds for marine fish and crustacean larvae due to its wide size range (0.5–12 mm) and high nutritional value, which meet the requirements of most developmental stages of shrimp, from larvae to juveniles (Nguyen Van Hoa, 2007). In addition, owing to its passive filter-feeding behavior on

organic particles smaller than 50 µm, *Artemia* has been used to reduce organic matter in water. Its organic matter assimilation capacity has been estimated to exceed 60% of body weight per day, with filtration rates of up to 64 mL h^{-1} (Ohbum et al., 2023). Together with a short reproductive cycle of 12–15 days, high fecundity of up to 600 nauplii per individual, and rapid biomass accumulation of 2–4 g L^{-1}, *Artemia* exhibits strong potential for efficient removal of organic wastes from water.

This study was conducted to evaluate the capacity of *Artemia* to remove and recover nitrogen from intensive whiteleg shrimp (*Litopenaeus vannamei*) farming wastewater, with the aim of reducing adverse environmental impacts and clarifying the feasibility of using *Artemia* biomass as an intermediate trophic link in nutrient-circulating shrimp-farming systems.

Objectives of the Study

The specific objectives of this study were to: (1) Determine the wastewater treatment efficiency of *Artemia* as influenced by size, stocking density, and treatment duration; (2) Evaluate the adaptability of *Artemia* at different initial stocking densities in various shrimp-farming wastewater sources; (3) Assess the feasibility of applying *Artemia* for industrial-scale wastewater treatment and reusing the harvested *Artemia* biomass as a supplementary feed for shrimp.

Significance of the Study

Scientific significance: The results of this study provide important scientific and practical evidence regarding the capacity of *Artemia* for wastewater treatment in intensive shrimp-farming systems. The findings serve as valuable reference material for teaching and scientific research in the field of aquaculture at both national and international levels.

Practical significance: The application of the study outcomes contributes significantly to solutions for nutrient treatment and recovery in shrimp-farming systems by mimicking natural food-web processes through the integration of autotrophic organisms and heterotrophic organisms (*Artemia*), thereby supporting circular shrimp aquaculture and responsible water resource use.

CHAPTER 1: OVERVIEW

1.1. Current status of the global shrimp farming industry and development trends

1.1.1. The role of shrimp farming in global aquaculture

Within the global aquaculture sector, shrimp farming is regarded as one of the highest-value production industries, characterized by a high degree of intensification and a pronounced role in driving technological innovation. Shrimp has long been among the aquatic products with the highest international commercial value, typically accounting for approximately 15–20% of the total global seafood trade value, and for many decades has been considered the most widely traded aquatic commodity in terms of value (FAOSTAT, 2024).

1.1.2. Global development of whiteleg shrimp

Over the past two decades, whiteleg shrimp (*Litopenaeus vannamei*) has become the dominant farmed shrimp species worldwide. Initially cultured primarily in the Americas, this species was introduced to Asia in the late 1990s and rapidly replaced many indigenous shrimp species in commercial production systems (Briggs et al., 2004). Currently, whiteleg shrimp is estimated to account for approximately 80–90% of total global farmed shrimp production, far exceeding that of black tiger shrimp (*Penaeus monodon*) and other species (Emerenciano et al., 2022; Jory, 2023). Global farmed shrimp production trends during the period 2010–2024 clearly reflect the dominant contribution of whiteleg shrimp to total output. Following a period of stagnation caused by early mortality syndrome (EMS) during 2012–2013, shrimp production recovered strongly from 2014 onward and has remained at high levels to the present (Jory, 2023).

1.2. Characteristics of intensive shrimp farming systems and commonly applied technologies

1.2.1. Classification of shrimp farming systems by intensification level

1.2.1.2. Intensive shrimp farming systems

Intensive systems involve substantially higher levels of investment in technology and management compared with semi-intensive systems. Stocking densities in intensive shrimp farming commonly range from 35 to 100 postlarvae (PL) m^{-2}, depending on shrimp species and pond design (FAO, 2018; Boyd & Tucker, 2012).

Intensive ponds are usually relatively small in size (0.1–1.0 ha and rarely larger than 2–5 ha) to facilitate management and environmental control (Boyd, 2003).Management intensity is markedly increased in these systems, with farmers applying strict technical protocols and conducting daily monitoring of environmental parameters such as temperature, pH, dissolved oxygen, alkalinity, and shrimp health. Feed inputs consist entirely of formulated commercial feeds, typically containing 30–40% crude protein, and feeding rates are calculated based on estimated shrimp biomass. Shrimp no longer rely on natural food sources; therefore, precise feeding management is critical to optimize FCR and minimize water pollution (Boyd & Tucker, 2012; FAO, 2018). According to FAO, FCR values in intensive whiteleg shrimp farming commonly range from 1.4 to 1.8.

1.2.1.4. Common shrimp farming models in Vietnam

Research by Cao et al. (2025) in Vietnam indicates that for every 1,000 kg of intensively farmed shrimp harvested, approximately 263.6 kg of nitrogen, 5.0 kg of phosphorus, and 12,175 L of nutrient-rich wastewater may be discharged. Clearly, as feed input levels increase to achieve higher production, waste outputs—particularly excess nutrients—increase exponentially. If not properly treated, these wastes lead to water eutrophication, stimulate the proliferation of harmful algae and bacteria, and result in the degradation of both pond water quality and surrounding environments. This explains why high-density intensive shrimp farming areas frequently experience algal blooms, nocturnal oxygen depletion, and disease outbreaks.

1.3. Characteristics of wastewater in intensive shrimp farming systems and environmental impacts

1.3.3. Environmental impacts of intensive shrimp farming wastewater

1.3.3.1. Risk of eutrophication in receiving water bodies

Wastewater from intensive and super-intensive shrimp farming systems contains large quantities of nitrogen (N) and phosphorus (P) originating from uneaten feed, shrimp excretion, and the mineralization of organic matter within culture ponds. Nutrient budget studies have shown that only about 20–30% of feed-derived nitrogen is retained in shrimp biomass, whereas the remaining 70–80% is released into wastewater and sediments in both dissolved and particulate forms (Boyd & Massaut, 1999; Piedrahita, 2003; Verdegem, 2013).

1.3.3.4. Increased risk of pathogen dissemination

Shrimp-farming wastewater enriched with organic matter and nutrients also creates favorable conditions for the proliferation of opportunistic microorganisms, particularly *Vibrio* spp. Numerous studies have demonstrated a positive correlation between *Vibrio* abundance in aquatic environments and organic and nitrogen loads, thereby increasing the risk of disease outbreaks and pathogen transmission among shrimp ponds and between aquaculture systems and natural ecosystems (Tseng et al., 2004; Thornber et al., 2020). Global statistics indicate that crustacean aquaculture discharges up to 3.74×10^{10} m³ of wastewater annually, with shrimp farming alone releasing approximately 5,345–7,157 m³ of wastewater per ton of shrimp produced, significantly increasing the risk of pathogen dissemination at regional and national scales (Ng et al., 2018; Badraeni et al., 2020).

1.4. Current approaches to wastewater treatment in intensive shrimp farming

1.4.4. Integrated Multi-Trophic Aquaculture (IMTA)

IMTA represents an ecological approach to aquaculture in which wastes generated by one cultured species are utilized as nutrient sources by other species occupying lower trophic levels, thereby restructuring material flows within the farming system toward greater closure and efficiency. The theoretical core of IMTA lies not in “wastewater treatment” per se, but in the management and transformation of material flows—particularly nitrogen and phosphorus—following principles analogous to those of natural ecosystems (Troell et al., 2009; Chopin et al., 2012).

1.5. *Artemia* and its applications in aquaculture

1.5.2. Applications of Artemia in aquaculture

1.5.2.2. Application of Artemia in wastewater treatment

Within the context of integrated multi-trophic aquaculture (IMTA), the combination of fed species with extractive organisms capable of assimilating and transforming both dissolved and particulate wastes is regarded as an effective strategy to reduce nutrient emissions and enhance system sustainability (Troell et al., 2009; Chang et al., 2020). In parallel, the concept of circular aquaculture emphasizes closing nutrient loops through biological recovery and reuse of nitrogen-rich wastewater streams, thereby converting wastes into biomass with practical value (Turcios & Papenbrock, 2014; Lothmann & Sewilam, 2023).

CHAPTER 2 – MATERIALS AND RESEARCH METHODS

2.1. Time, location, and research objects

The study was conducted from January 2021 to December 2024. The duration of each experiment ranged from 20 to 90 days, depending on the specific experimental conditions, including preparation time. All experiments were carried out at the Marine Aquaculture Experimental Farm in Cam Ranh, Khanh Hoa Province, Vietnam. After collection, samples were preserved and analyzed at the Experimental and Practical Center, Nha Trang University.

2.2. Research contents

The study comprised three main components focusing on evaluating the effects of stocking density, initial Artemia size, and wastewater sources from different shrimp-farming models on the adaptability and wastewater treatment capacity of *Artemia*. In addition, the feasibility of reusing *Artemia* harvested from shrimp wastewater treatment as a supplementary feed for shrimp was investigated to assess its applicability in industrial-scale farming systems.

Content 1: Effects of *Artemia* stocking density and initial size on the efficiency of shrimp-farming wastewater treatment over time.

- **Experiment 1:** Effects of initial *Artemia* stocking densities (0 – 10 – 50 – 100 individuals L^{-1}) on the efficiency of shrimp wastewater treatment (TN1).
- **Experiment 2:** Effects of different *Artemia* body length classes (0.5 – 3 – 5 – 7 mm $individual^{-1}$) on the efficiency of shrimp wastewater treatment (TN2).

Content 2: Effects of shrimp wastewater sources on growth, survival rate, and biomass of *Artemia* over time.

- **Experiment 3:** Effects of initial stocking densities on growth, survival rate, and biomass of *Artemia* (TN3).
- **Experiment 4:** Effects of wastewater from different shrimp-farming models on growth, survival rate, and biomass of *Artemia* (TN4).
- **Experiment 5:** Evaluation of the wastewater treatment efficiency of *Artemia* in different wastewater sources (autotrophic, mixotrophic) over time (TN5).

Content 3: Feasibility of applying *Artemia* in experimental shrimp farming systems.

- **Experiment 6:** Use of wastewater from industrial shrimp ponds to produce *Artemia* biomass at experimental scale (TN6).
- **Experiment 7:** Utilization of *Artemia* harvested from wastewater treatment as a supplementary feed for shrimp (TN7).

2.3. Research approach

2.3.1. Historical approach

The dissertation reviewed and analyzed previous studies on wastewater characteristics in intensive shrimp farming systems, growth performance of *Artemia* cultured using shrimp wastewater, and the wastewater treatment capacity of *Artemia*. Through systematic synthesis and evaluation of published findings, the dissertation identified existing knowledge gaps and proposed new research directions to address these gaps.

2.4. Research materials

2.4.1. Seawater source for experiments

The seawater used in the experiments was natural seawater stored and treated in a 100 m^3 pond system for experimental use. Seawater was treated following standard procedures at the Cam Ranh Aquaculture Experimental Center using chlorine at 10 ppm (powder form, 70% active ingredient), followed by aeration for 48 h under natural light conditions. Seawater was pumped directly from the sea, and inlet water quality parameters were highly stable during February–September: salinity 31–33 ppt; pH 8.2–8.4; alkalinity 120–140 mg L^{-1}; transparency > 2 m; water temperature 29–31°C; and TAN < 0.1 mg L^{-1}. In addition, freshwater for domestic use was used to adjust salinity when necessary.

2.4.2. *Artemia* source for experiments

Artemia were hatched from commercially available *Artemia franciscana* cysts (Vinh Chau strain). After 24 h of hatching following the manufacturer's instructions, *Artemia* were cultured in treated seawater in 200-L glass tanks at a density of 500 individuals L^{-1} and fed the microalga *Chaetoceros muelleri* as feed, with daily feeding rates per individual by age referenced from Naegel et al. (1999). Depending on experimental requirements, *Artemia* were acclimated to wastewater and fed *C. muelleri* until they reached the target size required for experimental setup.

2.5. Research methods

2.5.1. Effects of initial stocking density and size of *Artemia* on shrimp wastewater treatment efficiency over time

2.5.1.1. Effect of initial stocking density on the wastewater treatment capacity of Artemia *(TN1)*

Experimental *Artemia***:** *Artemia* used in the experiment had a body length of 7.1 ± 0.18 mm and were prepared as described in Section 2.4.2. Prior to experimental setup, *Artemia* were acclimated for 5 days in 250-L tanks at a density of 100 individuals L^{-1} using shrimp wastewater exclusively to adapt them to wastewater conditions.

Experimental design: The experiment was arranged in a completely randomized design (CRD) with four treatments corresponding to four initial stocking densities: 0 – 10 – 50 – 100 individuals L^{-1}, with three replicates per treatment, resulting in a total of 12 plastic containers (10 L per container). The experimental setup followed the method described by Marinho et al. (2011), using *Artemia* with a body length of 7.1 ± 0.18 mm over a 24-h period. The treatments were as follows: Treatment 1: No *Artemia* added (NT1 – Control); Treatment 2: *Artemia* added at 10 individuals L^{-1} (NT2); Treatment 3: *rtemia* added at 50 individuals L^{-1} (NT3); Treatment 4: *Artemia* added at 100 individuals L^{-1} (NT4).

2.5.1.2. Effect of initial Artemia size on wastewater treatment efficiency (TN2)

The experiment was arranged in a completely randomized design (CRD) with four treatments corresponding to four initial *Artemia* body length classes: 0.5 – 3.1 – 5.3 – 7.1 mm individual^{-1}. Each treatment was replicated three times, resulting in 12 plastic containers with a volume of 10 L per container. The experimental setup followed Marinho et al. (2011), using a uniform initial *Artemia* density of 100 individuals L^{-1} over a 24-h experimental period. The treatments were as follows: Treatment 1: *Artemia* with a body length of 0.5 mm individual^{-1} (NT1); Treatment 2: *Artemia* with a body length of 3.1 mm individual^{-1} (NT2); Treatment 3: *Artemia* with a body length of 5.3 mm individual^{-1} (NT3); Treatment 4: *Artemia* with a body length of 7.1 mm individual^{-1} (NT4).

The wastewater used in this experiment was collected from shrimp ponds and prepared in the same manner as described for Experiment 1. *Artemia* used in the experiment were acclimated in advance as described in Experiment 3, with three staggered culture

phases spaced 7 days apart to obtain the target average body sizes of 3.1, 5.3, and 7.1 mm. *Artemia* of 0.5 mm were newly hatched individuals at 24 h post-hatching.

2.5.2. Effects of shrimp wastewater sources on growth, survival rate, and biomass of *Artemia* over time

2.5.2.1. Effects of initial *Artemia* stocking density on growth, survival rate, and biomass (TN3)

The effects of initial *Artemia* stocking density and supplemental feed on body length, survival rate, and biomass of *Artemia* were evaluated using a two-factor experimental design comprising five initial stocking densities (100 – 200 – 300 – 400 – 500 individuals L^{-1}) and two microalgal feed sources.

Shrimp wastewater: Shrimp wastewater was collected from daily effluent of an intensive whiteleg shrimp pond operated under a 10–30% daily water exchange regime. The commercial pond had a bottom liner, an area of 200 m^2, and a stocking density of 250 individuals m^{-2}. At the time of wastewater collection, shrimp had an average body weight of 15.8 g $individual^{-1}$ and were fed commercial feed at a feeding rate of 4% body weight per day. Intensive shrimp wastewater was stored in a 5 m^3 composite tank under gentle aeration and fully covered with shading nets reducing light intensity by 75%. Physicochemical parameters of the wastewater (NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, alkalinity, pH, temperature, and salinity) were determined using Sera rapid test kits; temperature was measured using a mercury thermometer, and salinity (‰) was measured using a hydrometer prior to experimental use.

Microalgal feed sources: Two microalgal feed sources were used for *Artemia*: (1) microalgae cultured entirely in shrimp wastewater supplemented with 30 ppm silicate, and (2) microalgae cultured in treated natural seawater supplemented with f/2 medium as a control. Microalgae cultured in shrimp wastewater were produced as follows: microalgae were cultured in 20-L containers under natural temperature and light conditions, with shading nets reducing light intensity by 75% from 07:00 to 14:00 h daily, corresponding to light intensities ranging from 2,480 to 15,294 lux. Air temperature ranged from 28.5°C to 31.5°C. The culture medium consisted of shrimp wastewater collected from the 5 m^3 storage tank and filtered through a 5 µm filter bag before being supplied to the 20-L containers, which were continuously aerated. Powdered silicate (Na_2SiO_3, 90%) was added at a dose of 30 ppm, and after 60 min,

Chaetoceros muelleri prepared as described in Section 2.4.3 was inoculated at an initial density of 50,000 cells mL^{-1}. After 48 h, when microalgae were in the exponential growth phase, they were used as supplemental feed for *Artemia* over a 24-h feeding period. Each day, 2–4 new microalgal cultures were initiated to ensure a continuous feed supply. Control microalgae were cultured in treated natural seawater (Section 2.4.1) supplemented with f/2 medium in 20-L containers. Culture conditions (temperature, natural light, initial algal density, harvest time, and feeding schedule) were maintained equivalent to those of the wastewater-grown microalgae to allow comparison of microalgal growth under nutrient-replete (f/2) versus wastewater conditions.

Experimental *Artemia***:** *Artemia* used in the experiment were prepared as described in Section 2.4.2. Three-day-old *Artemia* were further acclimated for 2 days in shrimp wastewater to reach an initial body length of 2.18 ± 0.06 mm prior to stocking.

Experimental setup: The experiment was conducted in 30 glass containers (10 L each, with 5 L working volume) arranged in a factorial design of five stocking densities × two microalgal feed sources × three replicates. Experiments were performed under natural temperature conditions with shading nets reducing light intensity by 75%, with light intensity ranging from 2,480 to 15,294 lux between 07:00 and 14:00 h daily. Air temperature ranged from 28.2°C to 31.1°C. Each container was equipped with one air stone providing gentle aeration to ensure continuous oxygen supply during the 15-day culture period.

2.5.2.2. Effects of different shrimp wastewater sources on growth, survival rate, and biomass of *Artemia*

The experiment was arranged in a completely randomized design (CRD) with four wastewater source treatments in 20 plastic containers (five replicates per treatment), each with a total volume of 10 L (8 L working volume). The treatments were as follows: Treatment 1: Wastewater collected from an industrial shrimp pond at the Cam Ranh Experimental Farm (Control). Treatment 2: Wastewater from an industrial shrimp pond treated by filtration (300 mesh) and supplemented with microalgae and silicate. Treatment 3: Wastewater from an autotrophic shrimp farming system. Treatment 4: Wastewater from a mixotrophic shrimp farming system.

Culture containers were placed indoors to avoid direct sunlight and to maintain stable temperature conditions (28.2–29.7°C) throughout the experiment. Initial *Artemia* stocking density was 100 individuals L^{-1} (6 days old) with a body length of 2.6 ± 0.13 mm, prepared in advance following the procedures described in Section 2.4.2. Each container was equipped with a small air stone to provide continuous oxygen supply.

Wastewater preparation and supply: Initially, wastewater corresponding to each treatment was prepared in 20-L containers and then supplied to *Artemia* culture containers at a volume of 8 L per container. Acclimated *Artemia* (Section 2.4.2) were measured for body length and wet biomass, and exactly 800 individuals were stocked in each container.Prior to supply, wastewater for each treatment was filtered through a 50 µm filter bag. The volume of wastewater supplied to each *Artemia* container was estimated based on the filtration rate equation proposed by Li et al. (2024):

$$FFR = 0.487 \times BL + 0.067 \times T - 0.01 \times D - 0.064 \times PS - 1.508$$

where BL is *Artemia* body length (mm), T is culture water temperature (°C), D is *Artemia* density (individuals L^{-1}), and PS is particle size (µm). Adjustments were made based on water transparency and visual observation of *Artemia* gut fullness following the method of Nguyen Van Hoa (2007). Prior to each wastewater addition, an equivalent volume of water was removed from each container to maintain a constant volume of 8 L. Each *Artemia* culture container was siphoned once daily to remove wastes.

2.5.2.3. Evaluation of shrimp wastewater treatment efficiency of** Artemia **over time in an experimental model (TN5)

Shrimp farming systems following autotrophic and control protocols were established in 12 circular composite tanks with a volume of 1 m^3 per tank, comprising two treatments. Treatment 1 consisted of supplementation with *Chaetoceros muelleri* and silicate (autotrophic system), while Treatment 2 involved supplementation with silicate only (control). Each treatment was replicated six times and operated for 60 days. Tanks were placed indoors with shading from direct sunlight between 09:00 and 16:00 h and continuously aerated 24 h day^{-1}.Initial shrimp stocking density was 180 individuals per tank, with an average body weight of 1.22 ± 0.08 g $individual^{-1}$. Shrimp were fed commercial feed containing 40% crude protein at feeding rates of 4–8% body weight per day, divided into four equal meals (07:00, 11:00, 16:00, and 21:00

h). Wastes and uneaten feed were siphoned twice daily (09:00 and 18:00 h), and water exchange was conducted evenly across all tanks at 10–30% per day.

Shrimp wastewater used for *Artemia* culture:Wastewater was collected on culture day 32, when shrimp reached 7.7 ± 0.46 g individual^{-1} and were fed 76 g of commercial feed per tank. All wastewater from the two shrimp culture treatments was filtered through filter bags with mesh size < 50 µm and stored in separate circular composite tanks with a volume of 2,000 L each, equipped with aeration at each corner. Wastewater environmental parameters, including salinity, alkalinity, pH, temperature, dissolved oxygen, NH_4^+, NO_2^-, total Kjeldahl nitrogen (TKN), microalgal composition and density, and total suspended solids (TSS), were measured every 7 days during the 21-day *Artemia* culture period.

Experimental *Artemia* **culture:***Artemia* culture was conducted in 24 plastic containers with a working volume of 8 L per container, comprising two treatments: (1) wastewater from the autotrophic shrimp farming system and (2) wastewater from the shrimp farming system without microalgal supplementation (control). Each treatment included 12 replicate containers. Initial *Artemia* stocking density was 100 individuals L^{-1}, with an initial body length of 3.1 ± 0.04 mm across all treatments. Containers were placed indoors with shading from direct sunlight, and each container was equipped with an air stone to provide continuous aeration 24 h day^{-1}.Wastewater from the two shrimp culture systems was supplied 2–6 times per day depending on increases in *Artemia* biomass over time. The daily volume of shrimp wastewater supplied to each container was equal among containers and determined based on the filtration rate of one *Artemia* individual per hour using the equation:

$$FFR = 0.487 \times BL + 0.067 \times T - 0.01 \times D - 0.064 \times PS - 1.508$$

(Li et al., 2024),where BL is *Artemia* body length (mm), T is culture water temperature (°C), D is *Artemia* density (individuals L^{-1}), and PS is organic particle size (µm). Adjustments were made based on water transparency and visual observation of *Artemia* gut fullness (Huy et al., 2024; Thong et al., 2024). Prior to each wastewater addition, an equivalent volume of water was removed from each container to maintain a constant volume of 8 L. All effluent from *Artemia* culture containers for each treatment was collected separately in 2,000-L tanks, and wastewater quality parameters were measured on culture days 2, 8, 15, and 21.

2.5.3. Application of *Artemia* in intensive shrimp farming systems (TN6–TN7)

2.5.3.1. *Artemia*-based wastewater treatment at industrial scale

Wastewater source for Artemia biomass production:The wastewater used for *Artemia* biomass production was collected from daily water exchange (10–30% day^{-1}) of an industrial shrimp pond with an area of 500 m^2. Shrimp were cultured in phase 3 of the production cycle, corresponding to 76 days of culture, with an average body weight of 16.7 ± 0.34 g $individual^{-1}$ and an estimated stocking density of 180 individuals m^{-2}. Shrimp were fed commercial feed at a daily feeding rate of 62 kg. The technical farming procedure followed the mixotrophic shrimp farming protocol (silicate supplementation at 10 ppm $week^{-1}$) described in Section 2.4.3.

The wastewater was filtered through 50 µm mesh filter bags and stored in 8 m^3 tanks under aeration and 70% light shading for 3–5 days. Prior to being supplied to *Artemia* culture tanks, environmental parameters including total Kjeldahl nitrogen (TKN), total ammonia nitrogen (TAN), NO_2^-, NO_3^-, PO_4^{3-}, microalgal composition, and water transparency were determined to calculate appropriate wastewater loading rates for *Artemia* culture. Each wastewater storage tank was used for 24–48 h.

Artemia biomass production:*Artemia* were pre-acclimated for 5 days using shrimp wastewater supplemented with *Chaetoceros muelleri* as additional feed at a density of 1,500 individuals L^{-1} in 3 m^3 tanks. Subsequently, *Artemia* were evenly distributed into three 3 m^3 culture tanks at an initial density of 300 individuals L^{-1} under 70% light shading. Dissolved oxygen was continuously supplied throughout the culture period using 80-cm nano aeration diffusers installed in each tank.

Artemia were further cultured for 15 days using only shrimp wastewater supplied from the pre-treated 8 m^3 tanks. Wastewater was added 3–5 times per day, with incremental volumes ranging from 5–40% tank volume per addition, depending on *Artemia* age and biomass. In cases where wastewater quality could not be adjusted in time to meet nutritional requirements, baker’s yeast and rice bran fermented for 24 h at a ratio of 2:1000 were supplemented following the feeding ration of individual *Artemia* as described by Nguyen Van Hoa (1994).Solid wastes were removed daily by siphoning, and 70% of the tank water was replaced every 3 days using wastewater from the 8 m^3 storage tanks.

After 15 days of culture, *Artemia* were harvested to determine survival rate, body length, and wet biomass. Basic biochemical composition, including protein, lipid, carbohydrate, moisture, ash, and crude fiber contents, was analyzed at the Analysis Center of Nha Trang University. Samples were rapidly rinsed with freshwater, drained for 1 min, divided into subsamples, and stored at −20°C for subsequent experiments.

2.5.3.2. Utilization of *Artemia* as supplementary feed for shrimp (TN7)

The experiment consisted of five treatments corresponding to five *Artemia* biomass inclusion levels in the shrimp diet: 0% (control, no *Artemia* supplementation), 10% *Artemia*, 20% *Artemia*, 30% *Artemia*, and 40% *Artemia*.

Whiteleg shrimp (*Litopenaeus vannamei*) used in the experiment had an initial average body length of 6.41 ± 0.31 cm and body weight of 1.42 ± 0.13 g individual^{-1}. Shrimp were nursery-reared in 100 m² ponds at the Cam Ranh Experimental Farm following the protocol described in Section 2.4.2 and exhibited stable growth prior to the experiment.

Experimental tank system: The experimental system consisted of 20 composite tanks, each with a volume of 240 L (dimensions: 80 × 60 × 50 cm), operated under a recirculating aquaculture system (RAS) and placed indoors. Each tank was equipped with four air stones positioned at the four corners to ensure continuous dissolved oxygen concentrations above 5 ppm throughout the experiment.

The commercial feed used in the experiment had the following nutritional composition as declared by the manufacturer: crude protein > 36%, lipid > 4%, moisture < 10%, and carbohydrates < 40%.

Frozen *Artemia* biomass was thawed naturally for 30 min prior to feeding, then weighed according to the assigned treatment level. Supplemented *Artemia* consisted of wet biomass and was included as a percentage of the daily amount of commercial feed. Shrimp were fed commercial feed four times per day (07:00, 10:00, 15:00, and 18:00 h) at feeding rates of 4–8% body weight day^{-1}. *Artemia* biomass was supplemented once daily at 21:00 h (except in the control treatment). Stocking density was 60 shrimp per tank. Each treatment was replicated four times, and the experimental period lasted 40 days. Feed amounts were adjusted based on shrimp feeding behavior and residual feed. Culture tanks were siphoned four times daily, conducted 2 h after each feeding.

Uneaten feed was collected separately from each tank, air-dried, and stored for calculation of feed utilization efficiency at the end of the experiment.

After 40 days of culture, shrimp were harvested to determine growth performance, survival rate, and feed utilization efficiency.

2.5.4. Methods for determination of environmental parameters and biological indices of *Artemia* and shrimp

2.5.4.1. Environmental parameters

In Research Components 1 and 3, environmental parameters including dissolved oxygen (DO), temperature, and pH were measured using portable multi-parameter meters (Hanna HI9147 and ENZO 7011). Alkalinity, NH_4^+, and NO_2^- were determined using a TOMOTA A3 digital colorimeter, while NO_3^- and PO_4^{3-} were measured using commercial test kits (Sera, Germany).

In Research Component 2, environmental parameters including DO, temperature, and pH were measured using Hanna HI9147 and ENZO 7011 meters. Total suspended solids (TSS) were determined using the gravimetric method (SMEWW 2540D). Ammonium nitrogen ($N–NH_4^+$) was analyzed using the phenate method (SMEWW 4500-NH_3-F); nitrite nitrogen ($N–NO_2^-$) using the colorimetric method (SMEWW 4500-NO_2^- B); total nitrogen (TN) using the Kjeldahl method (SMEWW 4500-Norg C); chemical oxygen demand (COD) using SMEWW 5220C:2023; and chlorophyll-a using the fluorometric method (APHA, 2017). Turbidity was measured using a digital turbidity meter (Sper Scientific 860040) with an accuracy of 0.01 NTU and a measurement range of 0–50 NTU.

2.6. Data analysis

Data normality and homogeneity of variances were tested using the Shapiro–Wilk test and Levene's test [227]. All experimental data from Experiments 2–6 were statistically analyzed using SPSS software version 26.0 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was applied to compare differences among treatments, followed by Duncan's multiple range test to identify statistically significant differences between treatment means ($p < 0.05$). All results are presented as mean ± standard error.

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Effects of initial density and body size of *Artemia* on shrimp wastewater treatment efficiency over time

3.1.1. Effects of Artemia density on shrimp wastewater treatment efficiency (TN1)

3.1.1.1. Effects of Artemia density on organic matter removal in shrimp wastewater

Table 3.1. Removal efficiency of organic matter in shrimp wastewater by *Artemia* at different stocking densities over time.

Parameter	**Initial**	**Density (ind./L)**			
		0	10	50	100
TSS (mg/L)	41.35 ± 0.56[a]	39.64 ± 1.62[a]	31.83 ± 2.02[b]	13.67 ± 1.02[c]	10.66 ± 1.01[c]
COD (mg/L)	27.30 ± 0.66[a]	26.14 ± 1.08[a]	21.09 ± 2.02[b]	10.82 ± 1.21[c]	7.31 ± 0.81[c]
BOD (mg/L)	21.80 ± 0.26[a]	19.20 ± 1.80[a]	15.71 ± 1.81[b]	7.72 ± 0.98[c]	5.34 ± 0.81[c]
Turbidity (NTU)	12.05 ± 0.52[a]	11.37 ± 0.81[a]	8.83 ± 0.62[b]	2.67 ± 0.42[c]	2.13 ± 0.31[c]

Data are presented as Mean ± SD (n = 3). Mean values with different superscript letters within the same row indicate significant differences ($p < 0.05$).

Results presented in Table 3.1 indicate that total suspended solids (TSS) concentrations in shrimp wastewater decreased markedly with increasing *Artemia* density over time. In the control treatment (0 ind./L), TSS concentration showed only a slight reduction from the initial value of 41.35 ± 0.56 mg/L to 39.64 ± 1.62 mg/L, with no statistically significant difference observed ($p > 0.05$).

3.1.1.2. Effects of Artemia density on nitrogen removal efficiency in shrimp wastewater

The results indicate that *Artemia* stocked at higher densities (50–100 ind./L) contributed to a reduction in total nitrogen (TN) concentrations in shrimp wastewater, whereas inorganic nitrogen forms such as NO_2^- and NO_3^- showed no significant variation among treatments. An increase in NH_4^+ concentration was observed at higher *Artemia* densities, reflecting differences in nitrogen partitioning within the experimental system.

Table 3.2. Nitrogen removal efficiency in shrimp wastewater by Artemia at different stocking densities over time.

Parameter	**Initial**	**Density (ind./L)**			
		0	10	50	100

Parameter	Initial	Density (ind./L)			
TN (mg/L)	2.61 ± 0.21[a]	2.53 ± 0.16[a]	2.31 ± 0.22[a]	2.16 ± 0.19[b]	1.98 ± 0.08[b]
NH_4^+ (mg/L)	1.32 ± 0.13[a]	1.27 ± 0.17[a]	1.31 ± 0.16[a]	1.53 ± 0.09[b]	1.64 ± 0.21[b]
NO_2^- (mg/L)	1.88 ± 0.09[a]	1.23 ± 0.11[a]	1.28 ± 0.23[a]	1.17 ± 0.10[a]	1.39 ± 0.28[a]
NO_3^- (mg/L)	6.80 ± 0.37[a]	7.12 ± 0.26[a]	7.05 ± 0.31[a]	6.44 ± 0.28[a]	6.05 ± 0.19[a]

Data are presented as Mean ± SD (n = 3). Mean values with different superscript letters within the same row indicate significant differences (p < 0.05).

As shown in Table 3.2, total nitrogen (TN) concentration in shrimp wastewater decreased with increasing *Artemia* density. The initial TN concentration was 2.61 ± 0.21 mg/L. In the control treatment (0 ind./L), TN decreased slightly to 2.53 ± 0.16 mg/L, with no statistically significant difference compared to the initial value ($p > 0.05$).

3.1.2. Effects of Artemia body size on shrimp wastewater treatment efficiency (TN2)

3.1.2.1. Effects of Artemia body size on organic matter removal in shrimp wastewater

During the experimental period, environmental parameters remained relatively stable among treatments. Water temperature ranged from 28.4 to 30.1°C, and salinity varied between 30.7 and 32.8‰, which are suitable for *Artemia* growth and development. pH values were maintained between 7.8 and 8.2, alkalinity ranged from 119 to 133 mg/L, and dissolved oxygen concentrations varied from 4.7 to 5.6 mg/L.

Table 3.3. Removal efficiency of organic matter in shrimp wastewater by Artemia at different body sizes.

1. Parameter	2. Initial	3. Body length (mm/ind.)			
		0.5	3.1	5.3	7.1
TSS (mg/L)	38.31 ± 0.43[a]	35.67 ± 0.36[a]	28.31 ± 0.78[a]	17.31 ± 0.76[a]	10.96 ± 0.81[c]
COD (mg/L)	21.74 ± 0.55[a]	21.13 ± 0.44[a]	17.74 ± 0.35[a]	9.84 ± 0.55[a]	6.31 ± 0.48[c]
BOD (mg/L)	16.63 ± 0.36[a]	16.08 ± 0.26[a]	12.63 ± 0.51[a]	6.33 ± 0.24[a]	4.34 ± 0.41[c]
Turbidity (NTU)	11.14 ± 0.27[a]	11.02 ± 0.19[a]	7.39 ± 0.43[a]	3.86 ± 0.26[a]	1.92 ± 0.13[c]

Data are presented as Mean ± SD (n = 3). Mean values with different superscript letters within the same row indicate significant differences (p < 0.05).

3.1.2.2. Effects of Artemia body size on nitrogen removal in shrimp wastewater

Overall, the results indicate that larger *Artemia* exhibited a higher capacity to reduce total nitrogen concentrations in shrimp wastewater, whereas the increase in

NH_4^+ reflects dissolved nitrogen excretion associated with *Artemia* growth and metabolic processes.

Table 3.4. Nitrogen removal efficiency in shrimp wastewater by Artemia at different body sizes.

Parameter	Initial	Body length (mm/ind.)			
		0.5	3.1	5.3	7.1
TN (mg/L)	2.49 ± 0.21^a	2.53 ± 0.16^a	2.31 ± 0.22^a	2.16 ± 0.19^b	1.98 ± 0.08^b
NH_4^+ (mg/L)	0.83 ± 0.13^a	0.87 ± 0.17^a	0.97 ± 0.16^{ab}	1.04 ± 0.09^b	1.21 ± 0.21^b
NO_2^- (mg/L)	1.36 ± 0.09^a	1.23 ± 0.11^a	1.38 ± 0.23^a	1.17 ± 0.10^a	1.19 ± 0.28^a
NO_3^- (mg/L)	4.34 ± 0.37^a	4.12 ± 0.26^a	5.10 ± 0.31^a	5.44 ± 0.28^a	5.63 ± 0.19^a

Data are presented as Mean ± SD (n = 3). Mean values with different superscript letters within the same row indicate significant differences ($p < 0.05$).

Results presented in Table 3.4 demonstrate that *Artemia* body size significantly influenced the dynamics of several nitrogen forms in shrimp wastewater, particularly total nitrogen (TN) and ammonium (NH_4^+).

3.2. Effects of wastewater characteristics on the adaptability of *Artemia*

3.2.1. Effects of *Artemia* stocking density on growth, survival, and biomass of *Artemia* cultured in shrimp wastewater

3.2.1.1. Environmental parameters of shrimp wastewater

3.2.1.2. Growth, survival rate, and biomass of Artemia

Stocking density significantly affected the survival rate of *Artemia*. The highest survival rate (81.33 ± 15.5%) was recorded in the treatment stocked at 100 ind./L. In contrast, survival decreased progressively and reached only 61.33 ± 6.87% and 66.58 ± 5.30% at the higher densities of 400 and 500 ind./L, respectively ($p < 0.05$).

Figure 3.1. Survival rate, body length, and biomass of *Artemia* at different stocking densities.

Stocking density also influenced the length growth of *Artemia*. At the end of the experiment, the greatest body length was observed in the treatment stocked at 100 ind./L. Body length gradually decreased in treatments stocked at higher densities (200, 300, and 400 ind./L). Conversely, the lowest body length occurred at the highest density (500 ind./L), showing a statistically significant difference compared to the 100 ind./L treatment (Figure 3.1).

3.2.2. Effects of different wastewater sources on growth, survival, and biomass of

Artemia

3.2.2.1. Characteristics of wastewater sources used for** Artemia **biomass culture

Environmental parameters of the wastewater sources used for *Artemia* culture are presented in Table 3.x. The results indicate that water quality conditions across treatments were relatively stable and generally within suitable ranges for *Artemia* growth.

Table 3.5. Water quality parameters of wastewater sources used for Artemia culture.

Parameter	**Wastewater source**			
	Shrimp pond (NT1)	Microalgae-supplemented (NT2)	Autotrophic (NT3)	Mixotrophic (NT4)
Temperature (°C)	29.16 ± 1.02	28.77 ± 0.80	28.90 ± 0.80	29.20 ± 0.80
Salinity (ppt)	32.7 ± 1.72	33.6 ± 1.12	33.2 ± 1.87	33.4 ± 1.37
DO (mg/L)	5.48 ± 0.21	6.31 ± 0.18	6.01 ± 0.23	5.78 ± 0.20
Alkalinity (mg/L)	127.6 ± 23.6	101.8 ± 33.83	104.2 ± 23.60	112.4 ± 26.1
pH	8.08 ± 0.10	7.87 ± 0.12	7.82 ± 0.15	8.06 ± 0.11
TAN (mg/L)	1.10 ± 1.05^{b}	0.29 ± 0.84^{c}	0.53 ± 0.36^{a}	0.99 ± 0.97^{a}
NO_2^- (mg/L)	1.65 ± 2.35^{a}	0.57 ± 1.00^{a}	0.93 ± 2.25^{b}	1.10 ± 0.10^{b}

Data are presented as Mean ± SD (n = 3). Mean values with different superscript letters within the same row indicate significant differences (p < 0.05). (*Note: the Vietnamese source line "Các giá trị trung bình mang ký tự chữ cái …" was incomplete; the standard statement above is used consistently.*)

3.2.2.2. Growth, survival rate, and biomass of Artemia cultured in different wastewater sources

The survival rate of *Artemia* decreased over time in all treatments; however, the magnitude of decline differed markedly among wastewater sources (Table 3.7).

Table 3.7. Effects of different wastewater sources on Artemia survival rate over time.

Thời gian	**Tỉ lệ sống (%)**			
	Ao nuôi tôm	**Nước thải +**	**Autotrophic**	**Mixotrophic**

	(NT1)	*C.muelleri* (NT2)	(NT3)	(NT4)
Ban đầu	100	100	100	100
Ngày 5	$73,2 \pm 4,97^{b}$	$88,2 \pm 2,77^{a}$	$84,8 \pm 4,66^{a}$	$77,4 \pm 3,21^{ab}$
Ngày 10	$58,2 \pm 5,02^{c}$	$81,4 \pm 1,95^{a}$	$74,2 \pm 5,17^{ab}$	$69,2 \pm 6,61^{b}$
Ngày 15	$43,2 \pm 4,92^{c}$	$74,8 \pm 3,70^{a}$	$68,2 \pm 6,69^{a}$	$57,2 \pm 5,76^{b}$

Data are presented as Mean ± SD (n = 5). Mean values with different superscript letters within the same row indicate significant differences (p < 0.05).

Table 3.8. Effects of different wastewater sources on Artemia body length over time.

Thời gian	Kích thước Artemia (mm)			
	Ao nuôi tôm (NT1)	Nước thải + *C.muelleri* (NT2)	Autotrophic (NT3)	Mixotrophic (NT4)
Ban đầu	$2,18 \pm 0,13^{a}$	$2,18 \pm 0,13^{a}$	$2,18 \pm 0,14^{a}$	$2,18 \pm 0,12^{a}$
Ngày 5	$3,9 \pm 0,16^{a}$	$4,1 \pm 0,14^{b}$	$4,1 \pm 0,1^{b}$	$4,1 \pm 0,12^{b}$
Ngày 10	$6,1 \pm 0,1^{a}$	$6,3 \pm 0,1^{b}$	$6,3 \pm 0,07^{b}$	$6,2 \pm 0,07^{ab}$
Ngày 15	$7,8 \pm 0,25^{a}$	$7,6 \pm 0,16^{a}$	$7,7 \pm 0,16^{a}$	$7,7 \pm 0,25^{a}$

Data are presented as Mean ± SD (n = 5). Mean values with different superscript letters within the same row indicate significant differences (p < 0.05).

After 15 days of culture, *Artemia* reached the adult size range, with mean body length varying from 7.6 ± 0.04 mm to 7.8 ± 0.04 mm. Differences in *Artemia* body length among treatments at this time point were negligible.

Table 3.9. Effects of different wastewater sources on Artemia wet biomass over time.

Thời gian	Sinh khối ướt (mg/l)			
	Ao nuôi tôm (NT1)	Nước thải + *C.muelleri* (NT2)	Autotrophic (NT3)	Mixotrophic (NT4)
Ban đầu	$138 \pm 18,44^{a}$	$138 \pm 18,44^{a}$	$138 \pm 18,44^{a}$	$138 \pm 18,44^{a}$
Ngày 15	$288,6 \pm 32,25^{a}$	$487,6 \pm 23,08^{c}$	$448,8 \pm 35,81^{c}$	$376,2 \pm 32,73^{b}$

Data are presented as Mean ± SD (n = 5). Mean values with different superscript letters within the same row indicate significant differences (p < 0.05).

Results in Table 3.x show that *Artemia* wet biomass increased substantially after 15 days in all treatments; however, the magnitude of increase differed markedly among wastewater sources. Starting from the same initial biomass (138 ± 18.44 mg/L), *Artemia* cultured in the microalgae-supplemented wastewater (NT2) achieved the highest biomass (487.5 ± 18.62 mg/L), followed by the autotrophic treatment (NT3) (448.8 ± 19.64 mg/L), the mixotrophic treatment (NT4) (376.2 ± 17.31 mg/L), while the shrimp pond wastewater (NT1) produced the lowest biomass (288.65 ± 27.29 mg/L).

3.2.3. Evaluation of shrimp wastewater treatment performance of *Artemia* over time

3.2.3.1. Water quality parameters

Water quality parameters of influent and effluent from the two treatments (Autotrophic and Control) during days 1–21 of *Artemia* culture are presented in Table 3.10.

Table 3.10. Influent and effluent water quality parameters of shrimp wastewater in Artemia culture treatments.

Parameter	**Autotrophic**		**Control**	
	Input	Output	Input	Output
Temperature (°C)	28.3 ± 0.63^{a}	28.4 ± 0.65^{a}	28.5 ± 0.53^{a}	28.4 ± 0.36^{a}
Salinity (ppt)	32.7 ± 0.21^{a}	33.9 ± 0.31^{a}	33.2 ± 0.19^{a}	34.1 ± 0.35^{a}
DO (mg/L)	6.2 ± 0.47^{a}	6.0 ± 0.56^{a}	5.95 ± 0.66^{a}	5.78 ± 0.62^{a}
pH	7.9 ± 0.18^{a}	7.8 ± 0.21^{a}	8.1 ± 0.18^{a}	7.9 ± 0.22^{a}
Alkalinity (mg/L)	112.0 ± 8.4^{a}	105.5 ± 12.6^{a}	121.3 ± 9.5^{a}	107.0 ± 12.8^{a}
NH_4^+ (mg/L)	0.47 ± 0.16^{a}	0.73 ± 0.17^{bc}	0.68 ± 0.09^{b}	0.85 ± 0.13^{c}
NO_2^- (mg/L)	0.83 ± 0.25^{a}	0.90 ± 0.21^{a}	0.73 ± 0.34^{a}	0.93 ± 0.42^{a}
TKN (mg/L)	2.45 ± 0.26^{b}	1.95 ± 0.24^{a}	2.75 ± 0.38^{b}	2.12 ± 0.36^{a}
Chlorophyll-a (µg/L)	138.3 ± 8.96^{c}	39.6 ± 14.43^{a}	117.8 ± 12.04^{b}	36.3 ± 10.31^{a}
COD (mg/L)	27.5 ± 4.66^{b}	18.8 ± 3.30^{a}	29.0 ± 3.92^{b}	19.2 ± 4.08^{a}
TSS (mg/L)	54.8 ± 7.14^{b}	28.5 ± 4.5^{a1}	50.5 ± 8.51^{b}	27.8 ± 9.22^{a}

Data are presented as Mean ± SD. Different letters indicate significant differences ($p < 0.05$).

The water quality characteristics of the two wastewater sources were suitable for *Artemia* biomass production (Hoa & Trinh, 2016; Thong et al., 2024; Huy et al., 2024).

***3.2.3.1. Length growth, survival rate, and biomass of** Artemia*

Growth performance and nitrogen content of *Artemia* cultured in autotrophic wastewater were higher than those cultured in the control wastewater. Biomass and accumulated nitrogen content of *Artemia* on day 14 were the highest compared with day 7 and day 21, with statistically significant differences ($p < 0.05$), as presented in Table 3.12.

Table 3.12. Body length, survival rate, dry biomass, and nitrogen content of Artemia cultured in two wastewater sources over time.

Chỉ tiêu	Nước thải	Ban đầu	Ngày 7	Ngày 14	Ngày 21
Chiều dài (mm)	Autotrophic	3,1 ± 0,12[a]	6,6 ± 0,18[a]	8,1 ± 0,01[b]	9,2 ± 0,09[a]
	Mixotrophic	3,1 ± 0,16[a]	6,4 ± 0,17[a]	7,9 ± 0,15[a]	9,1 ± 0,15[a]
Tỉ lệ sống (%)	Autotrophic	100	82,3 ± 3,86[a]	68,7 ± 4,03[a]	32,5 ± 4,65[b]
	Mixotrophic	100	79,8 ± 2,98[a]	60,2 ± 5,37[a]	25,5 ± 2,64[a]
Sinh khối khô *Artemia* (mg/bình)	Autotrophic	71,2 ± 0,95[a]	280,8 ± 3,59[a]	359,0 ± 9,30[b]	230 ± 23,94[b]
	Mixotrophic	71,5 ± 1,29[a]	276,0 ± 5,44[a]	310,5 ± 5,26[a]	191,8 ± 4,27[a]
Tổng lượng Nitơ trong sinh khối *Artemia* (mg/bình)	Autotrophic	5,46 ± 0,25[a]	22,3 ± 0,42[b]	29,1 ± 0,92[b]	22,8 ± 2,43[b]
	Mixotrophic	5,46 ± 0,02[a]	20,5 ± 0,41[a]	25,2 ± 0,77[a]	18,9 ± 1,25[a]
Lượng Nitơ tích lũy trong sinh khối *Artemia* (mg/bình)	Autotrophic	-	16,8 ± 0,43[b]	23,6 ± 0,93[b]	17,4 ± 2,44[b]
	Mixotrophic	-	15,1 ± 0,41[a]	19,7 ± 0,77[a]	13,5 ± 1,25[a]

3.2.3.2. Nitrogen recovery efficiency of Artemia

Nitrogen recovery efficiency was significantly higher in the autotrophic wastewater than in the control wastewater ($p < 0.05$), as shown in Table 3.13. In addition, nitrogen

recovery efficiency differed among *Artemia* developmental stages within the same wastewater treatment.

Table 3.13. Nitrogen accumulation efficiency of Artemia from wastewater over time under two wastewater sources.

Chỉ tiêu	Nguồn nước thải	Ngày 7	Ngày 14	Ngày 21
Thể tích nước thải sử dụng để thu hồi 1 mg N (L/mg N)	Autotrophic	4,7 ± 0,18[a]	9,8 ± 0,39[a]	20,3 ± 2,76[a]
	Mixotrophic	5,3 ± 0,14[b]	11,8 ± 0,47[b]	26,0 ± 2,57[b]
Lượng N đầu vào để thu hồi 1 mg N *Artemia* (mg N/mg N)	Autotrophic	10,7 ± 0,27[a]	23,3 ± 0,93[a]	49,8 ± 6,76[a]
	Mixotrophic	14,1 ± 0,08[b]	32,2 ± 1,29[b]	71,6 ± 7,08[b]
Hiệu suất thu hồi N vào sinh khối *Artemia* (%)	Autotrophic	9,3 ± 0,25[b]	4,3 ± 0,18[b]	2,0 ± 0,29[b]
	Mixotrophic	7,1 ± 0,21[a]	3,1 ± 0,14[a]	1,4 ± 0,14[a]

3.3. Application of *Artemia* in an experimental shrimp farming system

3.3.1. Biomass production of *Artemia* using wastewater from the experimental shrimp farming system

The results demonstrated that *Artemia* biomass culture using effluent from a whiteleg shrimp (*Litopenaeus vannamei*) farming system was successfully achieved (Table 3.14). The mean *Artemia* biomass yield reached 56.43 ± 3.66 g/m³ of wastewater (fresh weight) and 4.93 ± 0.26 g/m³ of wastewater (dry weight).

Table 3.14. Results of Artemia culture using wastewater as a nutrient source and reusing biomass as shrimp feed in the experiment (n = 3).

No.	Parameter	Value
	Size and biomass of *Artemia*	
1	Body length (mm)	6.72 ± 0.84
2	Wet weight (mg/individual)	5.94 ± 0.61
3	Survival rate (%)	42.13 ± 2.41
4	Fresh biomass (g/m³ wastewater)	56.43 ± 3.66
5	Dry biomass (g/m³ wastewater)	4.93 ± 0.26
	Biochemical composition	

No.	Parameter	Value
1	Moisture (% fresh weight)	91.13 ± 0.79
2	Crude protein (% dry weight)	51.60 ± 0.82
3	Crude lipid (% dry weight)	12.33 ± 0.40
4	Carbohydrate (% dry weight)	17.13 ± 0.25
5	Ash (% dry weight)	18.23 ± 0.60

Data are presented as mean ± SD (n = 3).

3.3.2. Utilization of *Artemia* as a supplemental feed for shrimp

Water quality during the feeding trial

Water quality parameters throughout the trial were maintained within suitable ranges for the growth and development of whiteleg shrimp (Table 3.15). Mean temperature, salinity, dissolved oxygen, pH, and alkalinity ranged from 28.07–29.02°C, 31.4–33.1‰, 5.81–6.20 mg O_2/L, 8.08–8.32, and 139.7–152.5 mg $CaCO_3$/L, respectively.

Table 3.15. Water quality parameters throughout the experimental period.

Ngày	Thông số						
	Nhiệt độ (ºC)	**Độ mặn (‰)**	**DO (mg/L)**	**pH**	**Độ kiềm (mg $CaCO_3$/L)**	**TAN (mg/L)**	**NO_2^- (mg/L)**
10	28,2 ± 0,32	31,4 ± 0,51	6,20 ± 0,12	8,08 ± 0,10	146,8 ± 11,21	0,53 ± 0,16	0,85 ± 0,25
20	28,1 ± 0,50	32,3 ± 0,62	5,81 ± 0,11	8,10 ± 0,11	139,7 ± 12,23	0,60 ± 0,21	1,21 ± 0,31
30	28,5 ± 0,69	32,8 ± 0,66	6,12 ± 0,13	8,17 ± 0,15	142,8 ± 10,91	0,82 ± 0,33	1,41 ± 0,34
40	29,0 ± 0,47	33,1 ± 0,71	5,91 ± 0,18	8,32 ± 0,21	152,5 ± 16,18	0,97 ± 0,41	1,87 ± 0,45

Data are presented as mean ± SD (n = 5).

Supplementation of *Artemia* in the diet significantly affected the growth of whiteleg shrimp (Table 3.16). Shrimp fed the diet containing **40%** *Artemia* showed significantly higher final body weight (W_2) and specific growth rate in weight (SGRW) compared with the **10%** *Artemia* and control (0% *Artemia*) treatments ($p < 0.05$).

Table 3.16. Growth performance and survival of whiteleg shrimp at different Artemia inclusion levels.

Chỉ tiêu	Các mức bổ sung *Artemia* vào khẩu phần ăn				
	NT1 : ĐC (0%*Artemia*)	**NT2: Bổ sung (10%*Artemia*)**	**NT3: Bổ sung (20%*Artemia*)**	**NT4: Bổ sung (30%*Artemia*)**	**NT5: Bổ sung (40%*Artemia*)**
L1 (cm)	6,41 ± 0,31	6,41 ± 0,31	6,41 ± 0,31	6,41 ± 0,31	6,41 ± 0,31
W1 (g)	1,42 ± 0,13	1,42 ± 0,13	1,42 ± 0,13	1,42 ± 0,13	1,42 ± 0,13
L2 (cm)	13,68 ± 0,08^{a}	13,18 ± 0,31^{a}	13,27 ± 0,14^{a}	13,16 ± 0,13^{a}	13,67 ± 0,17^{a}
W2 (g)	14,64 ± 0,11^{ab}	14,1 ± 0,15^{a}	15,16 ± 0,31^{ab}	13,96 ± 0,16^{a}	15,68 ± 0,28^{b}
SGRL (%/day)	1,81 ± 0,02	1,80 ± 0,06	1,84 ± 0,03	1,84 ± 0,03	1,89 ± 0,03
SGRW (%/day)	5,83 ± 0,02^{a}	5,82 ± 0,03^{a}	5,92 ± 0,05^{ab}	5,91 ± 0,03^{ab}	6,00 ± 0,05^{b}
BM (g/L)	2,24 ± 0,06^{a}	2,42 ± 0,01^{ab}	2,42 ± 0,07^{ab}	2,49 ± 0,09^{ab}	2,64 ± 0,10^{b}
SR (%)	63,75 ± 1,08^{a}	71,67 ± 1,08^{ab}	67,08 ± 1,25^{ab}	74,6 ± 2,04^{b}	68,75 ± 1,85^{ab}

Data are presented as mean ± standard error (SE) (n = 4).

Table 3.17. Feed utilization efficiency of whiteleg shrimp at different Artemia inclusion levels.

Chỉ tiêu	Nghiệm thức				
	0% SA	**10% SA**	**20% SA**	**30% SA**	**40%SA**
FI (g/tôm)	11,51 ± 0,46^{a}	12,59 ± 0,43^{b}	12,18 ± 0,53^{ab}	12,97 ± 0,7^{b}	12,86 ± 0,71^{b}
FCR	1,45 ± 0,02^{b}	1,42 ± 0,02^{ab}	1,39 ± 0,02^{a}	1,39 ± 0,03^{a}	1,38 ± 0,03^{a}

CHAPTER 4. CONCLUSIONS AND RECOMMENDATIONS

4.1. Conclusions

This study demonstrated the potential of Artemia for shrimp wastewater treatment, nutrient recovery, and the production of reusable biomass in intensive shrimp farming systems.

- Artemia effectively reduced organic matter and suspended solids in shrimp farming wastewater. Treatment efficiency was influenced by Artemia density and

body size, with densities of 50–100 individuals/L and body lengths of 5.3–7.1 mm providing high treatment efficiencies. At a body length of 7.1 mm, the removal efficiencies of TSS, COD, and BOD reached approximately 71.4%, 71.0%, and 73.9%, respectively.

- Artemia was capable of reducing total nitrogen in shrimp farming wastewater at appropriate stocking densities and body sizes. However, inorganic nitrogen compounds showed different patterns of variation: NO_2^- and NO_3^- remained relatively stable, whereas NH_4^+ tended to increase.

- Artemia was able to recover a proportion of nitrogen from shrimp farming wastewater. In wastewater from the autotrophic system, nitrogen recovery efficiencies reached 9.3%, 4.3%, and 2.0% on days 7, 14, and 21, respectively, which were higher than those recorded in wastewater from the mixotrophic system (7.1%, 3.1%, and 1.4%, respectively).

- Artemia biomass could be produced directly using wastewater from shrimp farming systems at the experimental scale and exhibited substantial nutritional value, with crude protein and crude lipid contents of 51.60% and 12.33% of dry matter, respectively. This biomass could potentially be reused as a supplementary feed source for Pacific white shrimp (Litopenaeus vannamei). Dietary inclusion of Artemia at 20–40% improved several growth performance and feed utilization parameters in shrimp.

- Overall, the findings provide a scientific basis for integrating Artemia into intensive shrimp farming systems for wastewater treatment and valuable biomass production, while providing a foundation for further development of nutrient-cycling and circular shrimp farming systems.

4.2. Recommendations

- Further studies should investigate nitrogen balance and nitrogen transformation pathways throughout the entire food–production chain: feed → shrimp → wastewater → microalgae/organic matter → *Artemia* → shrimp. The use of the stable isotope ^{15}N is recommended to accurately quantify nitrogen transformation and recovery efficiencies at each stage of the system.

- Further research should focus on integrating *Artemia* into shrimp wastewater treatment systems at pilot and commercial scales, with particular emphasis on continuous operation, treatment loading capacity, water reuse efficiency, and overall economic feasibility.

- The biosafety of *Artemia* biomass produced from shrimp farming wastewater should be thoroughly evaluated before its reuse as a feed ingredient, particularly with regard to the potential accumulation and transmission of pathogens. The consistency of the nutritional quality of *Artemia* biomass among different wastewater sources and production periods should also be assessed.

LIST OF THE AUTHOR'S PUBLICATIONS

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