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# Distribution and Management of Residual Antibiotics in the *Litopenaeus vannamei* Shrimp Farming Environment: Recommendations for Effective Control

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**Abstract:** This study specifically focused on *Litopenaeus vannamei* and examined the distribution of residual antibiotics in various components of shrimp ponds throughout an aquaculture cycle. The findings revealed that aquaculture feed served as the primary source of antibiotics, continuously introducing them into the ponds throughout the entire production cycle. A multimedia distribution model for antibiotics in the ponds was established based on the principle of mass balance. The distribution characteristics of six antibiotics with higher levels in the feed, namely, sulfamethoxazole (SMX), norfloxacin (NOF), levofloxacin (LEOF), tetracycline (TC), oxytetracycline (OTC), and chlortetracycline (CTC), were investigated in the pond water, sediment, and shrimp. At the end of the cultivation period, the total antibiotic residues accounted for 65~80% in various media, with the sediment containing 50~60% of the distribution proportion ($p < 0.01$), which was identified as the primary reservoir for most antibiotics, with LEOF and NOF accounting for the highest proportions (45.78% and 50.29%, respectively). Based on the model's findings and the allowable daily dosage of antibiotics, recommendations were made for the effective control of antibiotic residues in shrimp farming management. To address the significant net loss of sulfonamides (SAs) and tetracyclines (TCs) in aquaculture production, it is crucial to carefully regulate their dosages and administration methods. Implementing eco-friendly additives and regularly cleaning surface sediments can aid in reducing antibiotic residue levels in various environmental media, thereby mitigating the environmental impact on aquaculture production activities.

**Keywords:** antibiotics; aquaculture ponds; environmental media; distribution; management

**Key Contribution:** This study developed a multimedia distribution model to analyze the distribution characteristics of six antibiotics widely used in aquaculture across various environmental media. The findings indicate that, while sulfamethoxazole (SMX) and tetracycline (TC) can be utilized in aquaculture production, their usage must be strictly regulated.



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## 1. Introduction

Aquaculture serves as a high-quality source of essential nutrients for the human body, such as protein and vitamins, and has experienced rapid development worldwide [1–3]. In 2012, China dominated aquaculture production, accounting for 61.7% of the global output [4,5]. Carp, tilapia, and shrimp farming contributed to more than half of the total production [6]. Vietnam also achieved an annual aquaculture production of 6 million tons in the same year [7]. In Africa, Egypt ranked as the second largest tilapia-producing and -breeding country after China, with an output of 900,000 tons [8]. The extensive use of antibiotics as feed additives [9], especially tetracyclines (TCs), sulfonamides (SAs), and quinolones (QNs), has contributed to this growth [1,4,7,10]. Continuous low-dose antibiotic treatment effectively promotes animal growth [8], and also acts as a means of killing or inhibiting bacterial and fungal reproduction [11,12]. For example, QNs, a class of synthetic chemotherapeutic compounds, are commonly used to treat shrimp diseases, exhibiting particularly good bactericidal effects on Gram-negative bacteria [13]. The combination of SAs and sulfonamide synergists can enhance antibacterial activity by several to tens of times [14]. However, the continuous input of these drugs into the aquaculture environment has resulted in the detection of varying levels of drug residues in both the aquaculture water and aquatic products, which negatively impacts biodiversity and consumer health [5]. The excessive use of antibiotics leads to bacterial resistance and toxicity [15,16], rendering previously effective drugs ineffective against microorganisms [17]. Within the realm of aquaculture, more than 80% of antibiotic drugs can infiltrate the aquaculture environment via feed or animal waste [18], thereby inhibiting cell proliferation and growth [19], damaging aquatic ecosystems, and posing potential health risks to humans [20]. Research indicates that consuming aquatic products containing antibiotic residues can potentially cause allergic reactions, which, in severe cases, can lead to shock and even death [9]. Moreover, chloramphenicol (CLP) residues increase the risk of cancer [15], erythromycin (ETM) disrupts gastrointestinal stability [21], and QNs and TCs affect tooth development [22].

The majority of the existing research primarily focuses on identifying the types and concentrations of antibiotic residues in individual media within aquaculture areas, such as water, sediment, or aquatic products [23–25]. Thiang et al. detected twenty-three antibiotics in water samples from seven aquaculture areas in the Malaysian Peninsula, with a detection rate of 83% for TCs [26]. Chen et al. investigated sediments from six aquaculture areas in Hailing Island and found enrofloxacin (EFX) with a detection rate of 75% [1]. The concentrations of oxytetracycline (OTC) and chlortetracycline (CTC) in Nile tilapia were 658.5 μg/kg and 109.76 μg/kg, respectively [8]. Furthermore, the addition of antibiotics to aquaculture feed as a preventive measure against animal diseases was another significant source of residual antibiotics in the aquaculture environment [27,28]. Li et al. [29] analyzed the feed from the Hangzhou Bay aquaculture area and found nine antibiotics, with sulfamethoxazole (SMX), levofloxacin (LEOF), OTC, and CTC having the highest detection rates, reaching 100%. However, there is a lack of systematic analysis concerning the distribution of residues in each medium following the introduction of antibiotics into the aquaculture environment. This deficiency in the research has limited the practical implications for the development of sustainable aquaculture practices.

South American white shrimp (*Litopenaeus vannamei*), a prominent species in the aquaculture industry, is gaining increasing global significance due to its rapid growth, high meat yield, and ability to tolerate variations in salinity during cultivation [30,31]. However, the widespread adoption of shrimp aquaculture and high-density farming practices has led to concerns regarding antibiotic residues in the aquaculture environment and aquatic products [32–34]. Antibiotics are administered to shrimp both in the form of drugs [15] and feed additives [9] and enter the water and sediment through metabolism, accumulation, etc., or remain directly in the shrimp [18]. Palaniyappan et al. found 11 SAs with residual concentrations ranging from 12 μg/kg to 124 μg/kg in shrimp samples collected from coastal aquaculture areas in south India, exceeding the maximum residual limit specified by the EU [35]. It has become crucial to adopt sustainable practices in the utilization of antibiotics and other drugs in aquaculture to minimize the risks to both aquatic products and the aquaculture environment. To gain a comprehensive understanding of the types and distribution of antibiotic concentrations in various environmental media, researchers previously developed mathematical models based on mass balance differential equations to evaluate the residual antibiotic concentrations in aquatic products [36]. However, current research in the aquaculture environment primarily focuses on optimizing the use of mathematical models to better understand the distribution characteristics of antibiotics and to propose corresponding management measures in a clear and intuitive manner.

In recent years, research in China has primarily focused on antibiotic residues in aquaculture areas in the Pearl River Delta [1,3]. However, with the expansion of aquaculture in the Yangtze River Delta region, there is a relative scarcity of research on the content levels, distribution characteristics, and environmental risks of residual antibiotics in the aquaculture process. Cixi city, located on the southeast coast of Zhejiang Province in China, boasts abundant mudflat resources and serves as an essential breeding area for *Litopenaeus vannamei* [37]. Our preliminary investigations revealed the presence of antibiotics such as norfloxacin (NOF), levofloxacin (LEOF), and ETM in the water, sediment, shrimp, and feed within the farming areas, with a significant number of cases exceeding the standard [29]. To cover the antibiotics commonly used in shrimp culture [38], in addition to NOF, LEOF, and ETM, we also selected four SAs (sulfadiazine (SDZ), sulfaxourazine (SMD), SMX, sulfadiazine (SMZ)), one sulfonamide synergist (trimethoprim (TMP)), and three TCs (tetracycline (TC), OTC, CTC) as our targeted substances. Additionally, we incorporated tests for roxithromycin (RTM), since ETM was banned for use in aquaculture in 2002 [39], to assess the contamination levels of different antibiotics in the area. In light of these findings, this study focused on one aquaculture cycle of *Litopenaeus vannamei* and systematically analyzed the residual sources of antibiotics in the water, sediment, shrimp, and feed within aquaculture ponds. To further clarify the distribution characteristics of the antibiotics, a multimedia distribution model of the antibiotics with high usage in the ponds was constructed using the principle of mass balance. Additionally, recommendations for shrimp farming management were proposed, aimed at the effective control of antibiotic residues in the aquaculture process.

## 2. Materials and Methods

### 2.1. Aquaculture Pond

Three shrimp farming ponds of different ages (labeled as ponds 1, 2, and 3) were selected as experimental models for sampling in Cixi city, Zhejiang Province, China (Figure 1). The aquaculture modes and feeding measures adopted in all three ponds were the same (Table 1). The effective aquaculture areas of ponds 1–3 were 2634 $m^2$, 2584 $m^2$, and 2670 $m^2$, respectively, with a central water depth of approximately 0.8–1.2 m. The water depth along the pond was only 0.1–0.2 m, with an average water depth of 0.5 m. These ponds were primarily closed systems, with minimal external water exchange, limited to evaporation and a small amount of rainfall. Aeration devices were installed in the ponds to address hypoxia conditions if they arose. The aquaculture period spanned from June to September, during which the water temperature, salinity, and pH of the three shrimp ponds remained within the appropriate range (Table S1). No significant climate changes were observed during this period.

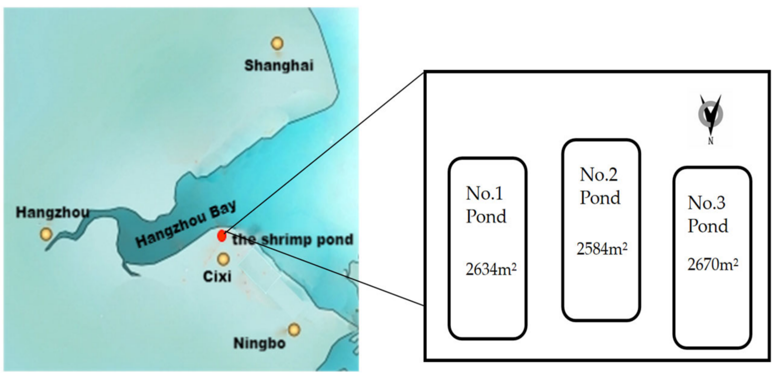


**Figure 1.** The study area location and experimental model diagrams for ponds 1–3.

**Table 1.** Detailed information of each aquaculture pond.

| Aquaculture Pond | Pond Age (Year) | Breeding Mode | Notes |
|---|---|---|---|
| 1 | 3 | Single culture of *Litopenaeus vannamei* | Track and study a breeding cycle |
| 2 | 5 | Single culture of *Litopenaeus vannamei* | Track and study a breeding cycle |
| 3 | 7 | Single culture of *Litopenaeus vannamei* | Track and study a breeding cycle |

### 2.2. Breeding Feed

The shrimp farming period for this study occurred between June and September 2022 and was divided into five stages. During the fry stage, post-larvae were fed, while juvenile shrimp in the subsequent stage were provided with juvenile 0 and 1 feeds. The adult shrimp in the final stage were fed with feed 1. Feeding was conducted by evenly sprinkling the feed at designated times, and the frequency of the feedings per day was determined based on its correlation with shrimp growth. The specific feed regimen is described in Table S2.

### 2.3. Chemical Reagents

This study specifically examined 12 antibiotics commonly used in aquaculture. These antibiotics included SDZ, SMD, SMX, SMZ, NOF, TC, OTC, CTC, ETM, and RTM, all of which were procured from the Dr. Ehrenstorfer company in Germany. Additionally, TMP and LEOF were purchased from Sigma-Aldrich in the United States of America (Burlington, MA, USA) and the Tokyo Chemical Industry (TCI, Tokyo, Japan), respectively.

The recovery indicator $^{13}$C-trimethoprim ($^{13}$C-TMP) was obtained from Sigma-Aldrich. All standard substances were in solid powder form with a purity of $\geq$97%. The reagents used in the experiment are shown in Table S3.

### 2.4. Sample Collection

The method of collecting the shrimp during the culture period was based on the method described by Li et al. [29]. A total of five sampling events were conducted on 11 June, 24 June, 14 July, 2 August, and 9 September 2022, respectively. The corresponding pond water samples (from a depth of approximately 15 cm) and surface sediment samples (5–10 cm from the top) were collected before feeding the seedlings, during the period of feed opening, while feeding No. 0 feed, while feeding No. 1 feed, and during the period of adult shrimp fishing, respectively. Each pond was designed with 5 sampling points, including 1 center point and 4 vertices. A total of 25 water and sediment samples were collected from each pond during each sampling event and stored in a refrigerator at 4 °C. The shrimp samples were collected while feeding No. 0 feed, while feeding No. 1 feed, and during the period of adult shrimp fishing. A total of 20 shrimp were collected from each pond during each sampling event. The shells were removed from the shrimp, and the edible parts were freeze-dried using SCIENTZ-12 N (Xinzhi, Ningbo, China). The dried shrimp samples were then thoroughly ground. The feed samples were collected during the feeding of feed opening, No. 0 feed, and No. 1 feed, and were stored after drying for antibiotic detection.

### 2.5. Extraction Method

In this experiment, the sample extraction was conducted according to the methods described by Gu [40], with the specific operations outlined as follows.

For water samples, 1 L of filtered water was taken and 0.2 g of $Na_2EDTA$ was added to chelate the metal ions. The water sample was thoroughly mixed, and its pH was adjusted to 2–4. Subsequently, 50 ηg/g of $^{13}$C-TMP was added. After performing solid-phase extraction (SPE), purifying, and concentrating the sample, the water was filtered through a 0.22 μm needle-type filter and stored in a refrigerator at 4 °C until detection.

For sediment samples, exactly 4.00 g of the sieved sample was transferred into a 50 mL plastic centrifuge tube. Then, a mixture of methanol and citric acid (10 mL) in a 1:1 volume ratio was added and the sample was sonicated for 15 min. The mixture was centrifuged at 4000 rpm for 10 min, and the resulting supernatant was transferred to a round-bottom flask. This extraction process was repeated three times, and the extracts were combined in the same flask. The organic solvents were evaporated under rotary evaporation at 40 °C until a constant volume was achieved. The volume was then adjusted to 250 mL with ultrapure water, thoroughly mixed, and purified using a C18 cartridge (Supelco, Burlington, MA, USA). The subsequent steps were identical to those for the water sample.

For the shrimp and feed samples, 2.00 g of each sample was weighed into a 50 mL centrifuge tube. Then, the recovery indicator $^{13}$C-TMP was added. The mixture was allowed to stand for 24 h in an EDTA–McIlvaine buffer and acetonitrile solution with a volume ratio of 1:4 (10 mL). The mixture was thoroughly blended and sonicated for 10 min. Next, the solution was centrifuged at 5000 rpm for 5 min, and the resulting supernatant was transferred to a separate centrifuge tube. The residue underwent the extraction process twice, and each extraction was combined. Then, n-hexane saturated with acetonitrile was added to remove the lipids. The mixture was vortexed, centrifuged, and left to stand. The resulting lower layer solution was evaporated at 40 °C until it reached a constant volume. Following this, it was diluted with 200 mL of ultrapure water and subjected to solid-phase extraction using a C18 cartridge (Supelco, Burlington, MA, USA). Before loading, the sample underwent SPE purification and concentration. Subsequently, it was filtered through a 0.22 μm needle-type filter and transferred to a vial for analysis.

### 2.6. Test Method

All samples underwent pre-treatment methods to obtain sample solutions and were analyzed using high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS, Thermo TSQ Quantum Access MAX, Waltham, MA, USA). Prior to analyzing the actual samples, spiked recovery experiments were conducted using ultrapure water and quartz sand as representative water samples, and blank sediment matrices were carried out [41]. The accuracy of the method was evaluated through spiked recovery experiments, and the relative standard deviations (RSDs) of the results were found to be less than 13%. To ensure instrument stability and result accuracy, solvent blank samples and mixed antibiotic standard samples with known concentrations were periodically analyzed.

#### 2.6.1. Liquid Chromatography Conditions

Chromatographic column: Agilent ZORBAX Eclipse XDB-C18 (Santa Clara, CA, USA, 150 mm × 2.1 mm, 5 μm), column temperature 30 °C. The mobile phase consisted of 0.1% acetic acid solution (A) and methanol (B). The flow rate was set at 0.35 mL/min, and the injection volume was 10 μL.

#### 2.6.2. Mass Spectrometry Conditions

A multi-ion reaction monitoring (MRM) positive ion scanning mode was utilized. The ion transfer capillary temperature was set at 350 °C, sheath gas flow rate ($N_2$) at 30 arb, auxiliary gas flow rate ($N_2$) at 10 arb, collision gas pressure (Ar) at 0.2 torr, capillary voltage at 4000 V, and nebulizer pressure at 40 psi. The mass spectrometry condition parameters for each target antibiotic are provided in Table S4.

### 2.7. Data Analysis

The measured data results were presented as the mean ± standard deviation (SD) based on three independent measurements. The measurement results were plotted using the Origin 2022 software (OriginLab Corporation, Northampton, MA, USA). Statistical analysis was conducted using SPSS 25, including *t*-test and Pearson analysis, with significance levels set at $p < 0.05$ and $p < 0.01$.

### 2.8. Model Construction

To visually analyze the impact of external sources of antibiotics on the shrimp farming ecosystem, we assumed that antibiotics were only introduced through the feed. Following the principle of mass balance, we established the relationship between the consumption of a target antibiotic in a shrimp pond and its net residue in each medium (including water, sediment, shrimp, and other items), as shown in Equation (1):

$$G_f = G_w + G_s + G_{P.v} + G_{others} \quad (1)$$

In the equation, $G_f$ represents the total usage amount of a specific antibiotic throughout the aquaculture process, corresponding to the total content of the antibiotic in the feed (μg); $G_w$, $G_s$, and $G_{P.v}$, respectively, represent the net residual quantities of the antibiotic in the water, sediment, and shrimp at the end of the farming activity. These values were obtained by subtracting the initial residual quantity before farming (similar to the concept of the gray box) (μg); and $G_{others}$ represents the net loss of the target antibiotic (μg), primarily including the portion of the antibiotic eliminated from the aquaculture environment through various means such as degradation by organisms, photolysis, and microbial degradation.

Based on the basic information of the aquaculture pond (area), shrimp farming information (feed feeding amount, yield), and sample detection results, the calculation formulas of each total amount were given, respectively:

(1) Total amount of an antibiotic in the feed (Equation (2):

$$G_f = \sum \left(C_{f,i} \cdot Q_{f,i}\right) \tag{2}$$

In the equation, $C_{f,i}$ represents the concentration level of the antibiotic in the i-th feed used for breeding (μg/kg); $Q_{f,i}$ represents the dosage of the i-th feed (kg), and i = 1, 2, and 3, respectively, refer to feed opening, feed No. 0, and feed No. 1. In one culture cycle of each shrimp pond in this study, $Q_{f,1}$ = 94 kg, $Q_{f,2}$ = 300 kg, and $Q_{f,3}$ = 2781 kg.

(2) Total net residue of an antibiotic in the water (Equation (3)):

$$G_w = (C_w - C_0) \cdot V_w \cdot 10^{-3} \tag{3}$$

In the equation, $C_w$ denotes the residual concentration of the antibiotic in the water sample during the adult shrimp fishing period (ηg/L); $C_0$ denotes the initial concentration of the antibiotic in the water sample after influent in the aquaculture pond and before seedling release (ηg/L); and $V_w$ denotes the volume of the aquaculture pond water (L). The $V_w$ of ponds 1–3 were 1317 $m^3$, 1292 $m^3$, and 1335 $m^3$, respectively.

(3) Total amount of antibiotic residues in the surface sediments (Equation (4)):

$$G_s = (C_s - C_0) \cdot m_s \tag{4}$$

In the equation, $C_s$ represents the residual concentration of the antibiotic in the sediments of adult shrimp during fishing (μg/kg, dry weight); $C_0$ represents the initial concentration of the antibiotic in the sediment (μg/kg, dry weight); and $m_s$ represents the total dry weight of the surface sediment (kg). Considering that antibiotics in sediments are primarily derived from residual bait and shrimp excreta, $m_s$ was calculated based on the total dry weight of the sediments accumulated during a culture period. According to Reference [42], the cumulative dry weight of the sediment in the water area of *Litopenaeus vannamei* aquaculture (150 individuals per square meter, aquaculture for 4 months) was reported as 2.29 kg/$m^2$. Based on the effective aquaculture density of 100 individuals per square meter and the 3-month aquaculture period of ponds 1–3, the estimated cumulative dry weight of the sediment was approximately 1.13 kg/$m^2$. Therefore, the estimated cumulative dry weights of the sediment for ponds 1, 2, and 3 were 2985 kg, 2927 kg, and 3026 kg, respectively.

(4) Total amount of antibiotic residues in the shrimp (Equation (5)):

$$G_{P.v} = C_{P.v} \cdot Q_{P.v} \tag{5}$$

In the equation, $G_{P.v}$ represents the residual concentration of the antibiotic in the whole shrimp (μg/kg, dry weight), and $Q_{P.v}$ represents the yield of *Litopenaeus vannamei* in the corresponding culture pond (dry weight kg). The moisture content of the adult shrimp averaged 75%. According to the statistics from the shrimp pond farmers, the wet weight output of shrimp ponds 1–3 was 1255 kg, 1201 kg, and 1591 kg, so the dry weight output of these ponds was 314 kg, 300 kg, and 398 kg, respectively.

The distribution of the antibiotics in the feed in the corresponding water, sediment, shrimp, and other items was expressed as a percentage using the results of the equation $\frac{G_w}{G_f}, \frac{G_s}{G_f}, \frac{G_{P,v}}{G_f}, \frac{G_{others}}{G_f}$.

*2.9. Estimation of Antibiotic Addition*

The maximum average daily addition of each antibiotic to the feed during the South American white shrimp culture cycle (based on 100 days) was estimated using calculations from the multimedia distribution model of antibiotics. This estimation was based on the promulgated standards for the relevant antibiotic limits (MRLs) in aquatic products.

## 3. Results and Discussion

*3.1. Residual Antibiotics in Various Media in the Aquaculture Pond*

Antibiotic detection was performed on the water, sediment, shrimp, and feed collected from ponds 1–3 (Figure 2, Table S5). As shown in Figure 2a–d, OTC exhibited the highest detected concentration in the water, ranging from 16.59 to 54.37 ηg/L, indicating its dominance in aquaculture water bodies. This may be attributed to the formation of complexes between OTC and metal ions in the water, leading to an increased residual time [1]. However, in the sediment, shrimp, and feed, the highest detected concentration was LEOF, with concentrations ranging from not detected (n.d.) to 21.72 µg/kg, 21.67 to 78.02 µg/kg, and 5.45 to 46.45 µg/kg, respectively. Although, LEOF was banned from use in aquatic products in 2017 [29], its presence suggests the potential illegal addition of antibiotics into shrimp feed. Additionally, the detection of RTM in the feed and sediment may be due to the lower absorption of RTM by shrimp. Non-edible antibiotics were excreted into the water through feces [18] and subsequently accumulated in the sediment. Except for LEOF, the antibiotics SMX, TC, OTC, and CTC were detected in the water, sediment, shrimp, and feed in this study. The residual antibiotics in each medium were roughly the same as the antibiotic components in the feed.

A Pearson correlation analysis indicated a significant correlation between the residual antibiotics in the sediment and shrimp and the antibiotic components in the feed ($R_1$ = 0.953, $R_2$ = 0.851, Table 2). The concentration of SMX detected in this study was much lower than the values reported in previous studies (12–124 µg/kg) [35,43], suggesting variations in the dosage and frequency of administration of the same drug among major producing countries. Additionally, the minimum and maximum residual concentrations of antibiotics detected in the water and sediment may be related to the amount of feed added. The minimum residual concentration mainly originated from samples collected before seedling release when a small amount of feed was added, about 0.021 g/g per day. At the adult shrimp stage, a larger amount of feed was added, about 3 g/g per day, which is approximately 200 times that provided at seedling release, resulting in the maximum residual concentration mostly occurring at the adult shrimp stage (Table S2). This finding aligned with the study conducted by Li et al. [29]. Furthermore, during on-site visits and investigations, the shrimp pond farmers reported that antibiotics were not used in the aquaculture process. Consequently, the antibiotic residues identified in the shrimp ponds mainly originated from the feed.

**Table 2.** Pearson correlation coefficients for total residual concentrations of each antibiotic in various media.

| Correlation | Feed | Sediment | Shrimp | Water |
|---|---|---|---|---|
| Feed | 1 | 0.953 ** | 0.851 * | 0.398 |
| Sediment | 0.953 ** | 1 | 0.676 | 0.303 |
| Shrimp | 0.851 * | 0.676 | 1 | 0.671 |
| Water | 0.398 | 0.303 | 0.671 | 1 |

Note: The symbol of "*" referred to $p < 0.05$ and the symbol of "**" referred to $p < 0.01$.

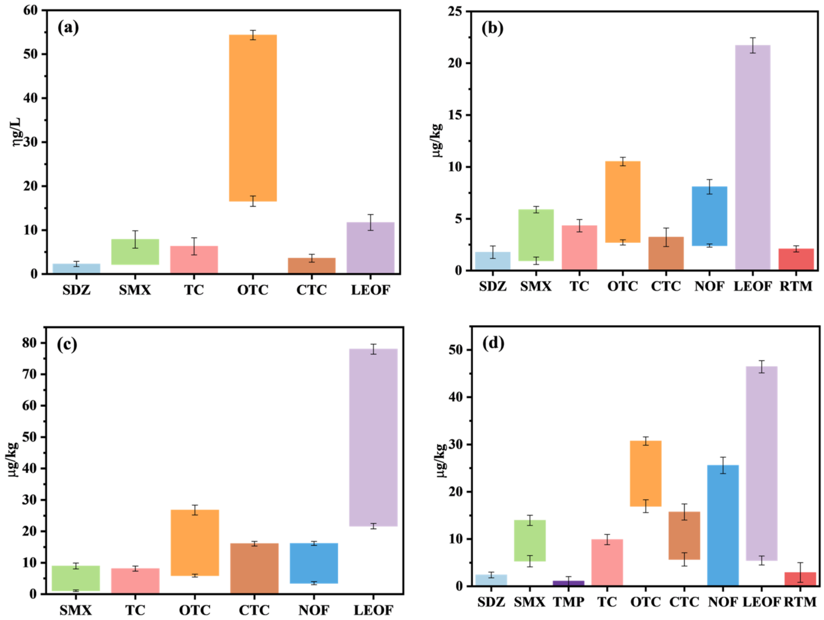


**Figure 2.** Content levels of antibiotics in (**a**) water; (**b**) sediment; (**c**) shrimp; and (**d**) feed in an aquaculture cycle.

*3.2. Distribution of Antibiotics in Various Media*

To mitigate potential calculation deviations, this study specifically examined six antibiotics widely used in aquaculture and frequently detected in feed. The selected antibiotics were SMX, NOF, LEOF, TC, OTC, and CTC. Given the consistent stocking density, feeding practices, and breeding methods employed in ponds 1–3 within the research area, as well as the similar distribution patterns of antibiotics in the various media of each pond, the average values of the three ponds were utilized for analysis purposes, as illustrated in Table 3 and Figure 3.

**Table 3.** Total amount of antibiotics in the feed, water, sediment, and others.

| | SMX | NOF | LEOF | TC | OTC | CTC |
|---|---|---|---|---|---|---|
| $G_f$ | 32.89 | 19.44 | 132.36 | 28.12 | 60.49 | 23.91 |
| $G_w$ | 5.99 | 0.00 | 10.54 | 6.25 | 20.76 | 4.35 |
| $G_s$ | 12.22 | 9.77 | 60.60 | 12.07 | 18.44 | 9.23 |
| $G_{P,v}$ | 2.56 | 5.19 | 24.37 | 2.65 | 8.30 | 1.74 |
| $G_{others}$ | 12.12 | 4.47 | 36.85 | 7.16 | 12.99 | 8.59 |

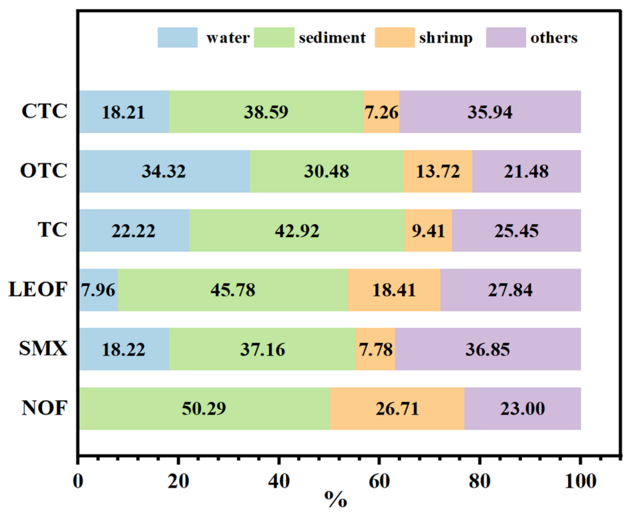


**Figure 3.** Proportional distribution of feed antibiotics in each media of shrimp ponds.

According to Table 3, the total amount of $G_f$ of the six major antibiotics present in the aquaculture feed was calculated. The antibiotics were ranked in the following order based on their total amounts: LEOF > OTC > SMX > TC > CTC > NOF, with quantities of 132.36 μg, 60.49 μg, 32.89 μg, 28.12 μg, 23.91 μg, and 19.44 μg, respectively. The results indicated that QNs and TCs were the most prevalent antibiotic residues found in the aquaculture, consistent with previous findings [44]. Figure 3 illustrates the proportional distribution of feed antibiotics in the corresponding water, sediment, shrimp, and other component samples, represented by the values of $\frac{G_w}{G_f}$, $\frac{G_s}{G_f}$, $\frac{G_{P,v}}{G_f}$, $\frac{G_{others}}{G_f}$ (expressed as %). It can be observed that the target antibiotics in the feed continuously entered the shrimp pond. At the end of each breeding cycle, the residual proportion in each medium, $\frac{G_w}{G_f}$, $\frac{G_s}{G_f}$, and $\frac{G_{P,v}}{G_f}$, ranged from 7% to 35%, 30% to 50%, and 7% to 27%, respectively. The proportion of the net loss part, $\frac{G_{others}}{G_f}$, ranged from 23% to 37%, with an average proportion of approximately 28%. These results indicated that antibiotics are continuously administered through feed [45], and a considerable portion ultimately exists in aquaculture water, sediment, and aquatic animals [46]. Only a small fraction of the antibiotics is removed from the aquaculture environment through degradation [47], metabolism [48], and other pathways. This highlights the high susceptibility of the aquaculture water environment and aquatic products to antibiotic contamination through feed administration. Regarding the specific antibiotics permitted for use in aquaculture in China, SMX, TC, OTC, and CTC were distributed in $\frac{G_{P,v}}{G_f}$ at proportions of 7.78%, 9.41%, 13.72%, and 7.26%, respectively, all below 15%. However, the proportion of the two prohibited aquatic drugs (NOF and LEOF) distributed in $\frac{G_{P,v}}{G_f}$ was relatively high, especially the proportion of NOF in shrimp, which was nearly 30%. In comparison, the banned antibiotics have a more pronounced impact on drug residues in aquatic products. Additionally, the total residue amounts of several antibiotics in both $\frac{G_w}{G_f}$ and $\frac{G_s}{G_f}$ exceeded 50%. To some extent, this finding reflects the lower absorption and utilization rate of antibiotics from the feed in *Litopenaeus vannamei*. A substantial portion of the antibiotics were excreted in their original form, entering the water environment and persisting for a certain period in both the water and sediment [49].

The $\frac{G_{others}}{G_f}$ of OTC was comparable in both the water and sediment, accounting for 34.32% and 30.48% of the drug dosage, respectively. However, the distribution of the other two TCs (TC and CTC), SAs (SMX), and QNs (NOF and LEOF) in the sediments was significantly higher than in the water. Among them, the QNs exhibited the highest proportion in the sediments (48%), followed by the two TCs (41%) and SMX (37%). These variations in the antibiotic distribution in the same environmental media may be attributed to differences in their structure and physical and chemical properties [13,50]. During the adsorption process, antibiotics can form low-soluble and stable complexes by neutralizing metal ions ($Ca^{2+}$, $Mg^{2+}$, $Fe^{3+}$) in the environmental media, which limits their distribution in the water phase [51,52]. However, as a type of TCs detected in all feed samples and added at relatively high levels, OTC exhibited similar distribution proportions in both the water and sediments. It is worth noting that differences in the dosage also influenced the antibiotic distribution. To further explore this, a correlation analysis was conducted between the usage amount of TCs and their distribution proportions in the water. The results revealed a strong correlation coefficient (R = 0.884, $p < 0.05$, Table S6), indicating that a significant relationship exists between the dosage and the variations in the multimedia distribution of antibiotics within the same class. Notably, the proportion of antibiotics in the water phase increased with a higher dosage.

The detection of antibiotics in the sediment of shrimp ponds of different ages is depicted in Figure 4. There was no significant increasing trend in the content of each antibiotic with the increase in pond age, and no discernible interannual variability was observed. Shrimp farmers adopt practices such as cleaning, sun-drying, and removing excess sediment at the end of each year and the beginning of the next to improve the bottom environment of the pond and create favorable breeding conditions for the next crop [20]. This thorough pond breeding environment repair method might help to reduce the accumulation of antibiotic residues in the sediment. Additionally, the antibiotics detected in the sediment exhibited a short half-life in the environment, as mentioned in Reference [53]. This means that they can undergo degradation to some extent and do not accumulate in the sediment over time. A *t*-test analysis was conducted on the antibiotic concentrations in the sediment of the three shrimp ponds, and the results showed no statistical difference, with $p > 0.05$.

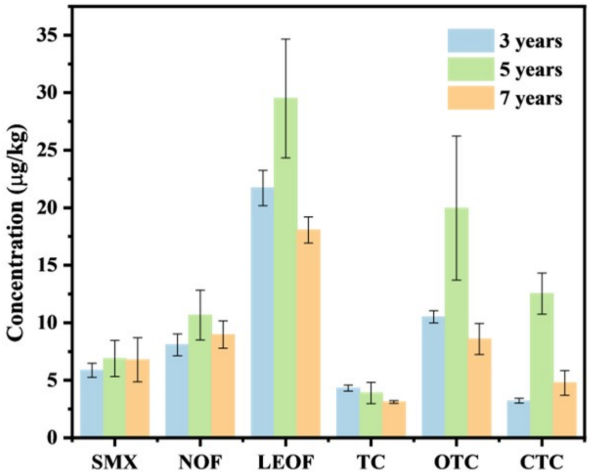


**Figure 4.** The content levels of antibiotics in the sediment of aquaculture ponds of different ages.

### *3.3. Estimation of the Amount of Available Antibiotics Added to the Aquaculture Feed*

Four antibiotics (SMX, TC, OTC, and CTC) were selected based on their high detection rates in aquaculture and the values of $\frac{G_W}{G_f}$, $\frac{G_s}{G_f}$, $\frac{G_{P,v}}{G_f}$, $\frac{G_{others}}{G_f}$ to estimate their average daily addition to aquaculture feed [32,54]. In the calculation process, the daily antibiotic dosage was normalized over a 100-day period, assuming a constant dosage throughout the cultivation cycle for practical applicability (Table 4). Taking OTC as an example, the residual limit requirement for OTC in aquatic products was set below 100 μg/kg, which corresponded to approximately 13.72 ± 0.67% of the total residual amount in the shrimp. This estimation suggests that the maximum allowable concentration of OTC in the feed should not exceed 162.54 μg/kg (Table 4). Similarly, the estimated maximum added concentrations of SMX, TC, and CTC were 527.68 μg/kg, 509.81 μg/kg, and 429.05 μg/kg, respectively (Table 4). Calculating the amount of antibiotics added to the aquaculture feed provides a basis for guiding the rational use of antibiotics in shrimp farming.

**Table 4.** Estimation of average daily addition of aquatic antibiotics in shrimp feed.

| | Residual Concentration in Aquatic Products * (μg/kg, Calculated by Fresh Weight) | Distribution Proportion in Aquatic Products (%) | Daily Feed Addition (μg/kg) |
|---|---|---|---|
| SMX | 100 | 7.78 | 527.68 |
| TC | 100 | 9.41 | 509.81 |
| OTC | 100 | 13.72 | 162.54 |
| CTC | 100 | 12.44 | 429.05 |

Note: *: refers to the maximum residue limit (MRL) of antibiotics in reclaimed water products in relevant standards.

### *3.4. Recommendations for Shrimp Farming Management*

At the end of a culture cycle, the shrimp ponds' environmental media contain varying levels of drug residues, especially the sediment, which not only affect the healthy development of the shrimp in this aquaculture area, but also pose a potential threat to the surrounding natural water. Therefore, there is a need for the proper treatment of the water, improvements in the drug residue status of the sediment, and regulation of the use of antibiotics. Based on the model results, SMX and TC, with a higher proportion of net losses, were selected for aquaculture production activities. Furthermore, to ensure the quality and safety of aquatic products, the average daily addition amount of TCs should be kept relatively low, and strict control of their usage in future aquaculture production is necessary. The aquaculture industry has the potential to further explore the utilization of modern biotechnology products, such as herbal preparations [55] and probiotics [56], as sustainable alternatives to antibiotics in feed additives. Aquaculture farmers should consider incorporating these green additives into their medication choices. Such alternatives will not only contribute to disease prevention and environmental improvements in aquaculture, but also play a crucial role in ensuring the safety of aquatic products and fostering the sustainable development of shrimp farming. To reduce drug residues in aquatic products, farmers must use antibiotic fishing drugs correctly by following established medication types, dosages, and administration routes. It is essential to avoid long-term, low-dose antibiotic consumption by aquaculture animals as much as possible. Additionally, farmers should strictly adhere to medication ban periods and selling aquatic products before the end of the ban period should be prohibited. Given that certain antibiotics can persist for extended periods, even at low concentrations, under natural lighting conditions [47], it is necessary to implement measures for treating aquaculture wastewater prior to its discharge into the external environment. Moreover, residual bait, shrimp excrement, and dead algae in the shrimp pond settle at the bottom, forming surface sediment with antibiotic residues [20]. Hence, after draining the pond water, practices such as plowing and exposing the surface sediment to sunlight, along with techniques like dry ponds and sun ponds to remove excessive sludge, could be highly advantageous in mitigating or even eradicating the antibiotic residues present in the sediment.

## 4. Conclusions

The antibiotic residues detected in the shrimp pond water, sediment, and shrimp samples in the study primarily consisted of SMX, TC, OTC, CTC, LEOF, and NOF, with small amounts of RTM found in the sediment and feed. These results indicate that $G_w$, $G_s$, and $G_{P,v}$ mainly originated from the feed, showing consistent patterns across different media. The calculation results from the antibiotic multimedia distribution model revealed that antibiotics continuously entered the shrimp pond through ongoing feed administration, with total residues accounting for 65–80% in each medium. This suggests that only a small fraction of antibiotics underwent elimination through absorption, degradation, and metabolism, while the majority of antibiotic residues persisted in the aquaculture environment. Among these residues, over 50% were distributed in the sediment, displaying a significant correlation with feed administration. By estimating the daily addition of antibiotics, it became evident that the strict control of OTC usage in shrimp farming is necessary, and SMX and CTC are suitable options for achieving low residual concentrations. Alternatively, the use of green feed additives instead of antibiotics or the regular cleaning of aquaculture ponds can reduce the antibiotic levels to effectively control antibiotic residues in the farming environment.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes9030084/s1, Table S1: Temperature, salinity, and pH of water bodies in ponds 1–3; Table S2: Feeding situation in shrimp farming; Table S3: Reagents used in the experiment; Table S4: Mass spectrometry conditions for various target antibiotics; Table S5: Levels of various antibiotics in feed, pond water, sediment, and shrimp during a farming cycle; Table S6: Correlation analysis between the content of TCs antibiotics in feed and residual concentrations in water.

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**Institutional Review Board Statement:** This study did not involve experiments on live animals. All South American white shrimp used in this study came from commercial fishing and did not undergo any experimental procedures. None of the species used in this study are protected. Therefore, this study does not require ethical approval.

**Data Availability Statement:** The raw data supporting this article are available on request from the authors. The data are not publicly available due to privacy policy.

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## References

1. Chen, H.; Liu, S.; Xu, X.-R.; Liu, S.-S.; Zhou, G.-J.; Sun, K.-F.; Zhao, J.-L.; Ying, G.-G. Antibiotics in typical marine aquaculture farms surrounding Hailing Island, South China: Occurrence, bioaccumulation and human dietary exposure. *Mar. Pollut. Bull.* **2015**, *90*, 181–187. [CrossRef] [PubMed]
2. Zhang, R.; Pei, J.; Zhang, R.; Wang, S.; Zeng, W.; Huang, D.; Wang, Y.; Zhang, Y.; Wang, Y.; Yu, K. Occurrence and distribution of antibiotics in mariculture farms, estuaries and the coast of the Beibu Gulf, China: Bioconcentration and diet safety of seafood. *Ecotoxicol. Environ. Saf.* **2018**, *154*, 27–35. [CrossRef] [PubMed]
3. Chen, J.; Sun, R.; Pan, C.; Sun, Y.; Mai, B.; Li, Q.X. Antibiotics and Food Safety in Aquaculture. *J. Agric. Food Chem.* **2020**, *68*, 11908–11919. [CrossRef] [PubMed]
4. Mo, W.Y.; Chen, Z.; Leung, H.M.; Leung, A.O.W. Application of veterinary antibiotics in China's aquaculture industry and their potential human health risks. *Environ. Sci. Pollut. Res.* **2017**, *24*, 8978–8989. [CrossRef] [PubMed]
5. Lulijwa, R.; Rupia, E.J.; Alfaro, A.C. Antibiotic use in aquaculture, policies and regulation, health and environmental risks: A review of the top 15 major producers. *Rev. Aquac.* **2020**, *12*, 640–663. [CrossRef]
6. Cao, L.; Naylor, R.; Henriksson, P.; Leadbitter, D.; Metian, M.; Troell, M.; Zhang, W. China's aquaculture and the world's wild fisheries. *Science* **2015**, *347*, 133–135. [CrossRef] [PubMed]
7. Uchida, K.; Konishi, Y.; Harada, K.; Okihashi, M.; Yamaguchi, T.; Do, M.H.N.; Thi Bui, L.; Duc Nguyen, T.; Do Nguyen, P.; Thi Khong, D.; et al. Monitoring of Antibiotic Residues in Aquatic Products in Urban and Rural Areas of Vietnam. *J. Agric. Food Chem.* **2016**, *64*, 6133–6138. [CrossRef]
8. Ahmed, M.B.M.; Abdel-Rahman, G.N.; Salem, S.H.; Fouzy, A.S.M. Incidence, stability and risk assessment for sulfonamides and tetracyclines in aqua-cultured Nile Tilapia fish of Egypt. *Toxicol. Rep.* **2020**, *7*, 836–843. [CrossRef]
9. Liu, X.; Steele, J.C.; Meng, X.-Z. Usage, residue, and human health risk of antibiotics in Chinese aquaculture: A Review. *Environ. Pollut.* **2017**, *223*, 161–169. [CrossRef]
10. Chang, B.-V.; Chang, Y.-T.; Chao, W.-L.; Yeh, S.-L.; Kuo, D.-L.; Yang, C.-W. Effects of sulfamethoxazole and sulfamethoxazole-degrading bacteria on water quality and microbial communities in milkfish ponds. *Environ. Pollut.* **2019**, *252*, 305–316. [CrossRef]
11. Heuer, O.E.; Kruse, H.; Grave, K.; Collignon, P.; Karunasagar, I.; Angulo, F.J. Human Health Consequences of Use of Antimicrobial Agents in Aquaculture. *Clin. Infect. Dis.* **2009**, *49*, 1248–1253. [CrossRef] [PubMed]
12. Carrizo, J.C.; Griboff, J.; Bonansea, R.I.; Nimptsch, J.; Valdés, M.E.; Wunderlin, D.A.; Amé, M.V. Different antibiotic profiles in wild and farmed Chilean salmonids. Which is the main source for antibiotic in fish? *Sci. Total Environ.* **2021**, *800*, 149516. [CrossRef] [PubMed]
13. Pham, T.D.M.; Ziora, Z.M.; Blaskovich, M.A.T. Quinolone antibiotics. *MedChemComm* **2019**, *10*, 1719–1739. [CrossRef] [PubMed]
14. Cheng, S.; Wei, Z.; Zhiming, X.; Yang, L.; Xia, F. Trace analysis and identification of 33 sulfonamides and sulfonamide potentiators in eggs by ultrahigh-performance liquid chromatography coupled with quadrupole-high field orbitrap high-resolution mass spectrometry. *Anal. Methods* **2021**, *13*, 4452–4460. [CrossRef] [PubMed]
15. Okocha, R.C.; Olatoye, I.O.; Adedeji, O.B. Food safety impacts of antimicrobial use and their residues in aquaculture. *Public Health Rev.* **2018**, *39*, 21. [CrossRef] [PubMed]
16. Qiao, M.; Ying, G.-G.; Singer, A.C.; Zhu, Y.-G. Review of antibiotic resistance in China and its environment. *Environ. Int.* **2018**, *110*, 160–172. [CrossRef] [PubMed]
17. Cabello, F.C.; Godfrey, H.P.; Tomova, A.; Ivanova, L.; Dölz, H.; Millanao, A.; Buschmann, A.H. Antimicrobial use in aquaculture re-examined: Its relevance to antimicrobial resistance and to animal and human health. *Environ. Microbiol.* **2013**, *15*, 1917–1942. [CrossRef]
18. Imtiaz, N.; Anwar, Z.; Waiho, K.; Shi, C.; Mu, C.; Wang, C.; Qingyang, W. A review on aquaculture adaptation for fish treatment from antibiotic to vaccine prophylaxis. *Aquac. Int.* **2023**. [CrossRef]
19. Malchi, T.; Maor, Y.; Tadmor, G.; Shenker, M.; Chefetz, B. Irrigation of Root Vegetables with Treated Wastewater: Evaluating Uptake of Pharmaceuticals and the Associated Human Health Risks. *Environ. Sci. Technol.* **2014**, *48*, 9325–9333. [CrossRef]
20. Bondad-Reantaso, M.G.; MacKinnon, B.; Karunasagar, I.; Fridman, S.; Alday-Sanz, V.; Brun, E.; Le Groumellec, M.; Li, A.; Surachetpong, W.; Karunasagar, I.; et al. Review of alternatives to antibiotic use in aquaculture. *Rev. Aquac.* **2023**, *15*, 1421–1451. [CrossRef]

21. Hao, H.; Gokulan, K.; Piñeiro, S.A.; Williams, K.M.; Yuan, Z.; Cerniglia, C.E.; Khare, S. Effects of Acute and Chronic Exposure to Residual Level Erythromycin on Human Intestinal Epithelium Cell Permeability and Cytotoxicity. *Microorganisms* **2019**, *7*, 325. [CrossRef] [PubMed]
22. Kümmerer, K. Antibiotics in the aquatic environment—A review—Part II. *Chemosphere* **2009**, *75*, 435–441. [CrossRef] [PubMed]
23. Luu, Q.H.; Nguyen, T.B.T.; Nguyen, T.L.A.; Do, T.T.T.; Dao, T.H.T.; Padungtod, P. Antibiotics use in fish and shrimp farms in Vietnam. *Aquac. Rep.* **2021**, *20*, 100711. [CrossRef]
24. Song, C.; Zhang, C.; Fan, L.; Qiu, L.; Wu, W.; Meng, S.; Hu, G.; Kamira, B.; Chen, J. Occurrence of antibiotics and their impacts to primary productivity in fishponds around Tai Lake, China. *Chemosphere* **2016**, *161*, 127–135. [CrossRef] [PubMed]
25. Kingsbury, M.V.; Hamoutene, D.; Kraska, P.; Lacoursière-Roussel, A.; Page, F.; Coyle, T.; Sutherland, T.; Gibb, O.; Mckindsey, C.W.; Hartog, F.; et al. Relationship between in feed drugs, antibiotics and organic enrichment in marine sediments at Canadian Atlantic salmon aquaculture sites. *Mar. Pollut. Bull.* **2023**, *188*, 114654. [CrossRef] [PubMed]
26. Thiang, E.L.; Lee, C.W.; Takada, H.; Seki, K.; Takei, A.; Suzuki, S.; Wang, A.; Bong, C.W. Antibiotic residues from aquaculture farms and their ecological risks in Southeast Asia: A case Study from Malaysia. *Ecosyst. Health Sustain.* **2021**, *7*, 1926337. [CrossRef]
27. Rosa, J.; Leston, S.; Freitas, A.; Barbosa, J.; Rema, P.; Dias, J.; Lemos, M.F.L.; Pardal, M.Â.; Ramos, F. Tissue depletion of five antibiotic residues in farmed European seabass (*Dicentrarchus labrax*). *Aquaculture* **2019**, *498*, 413–421. [CrossRef]
28. González-Gaya, B.; García-Bueno, N.; Buelow, E.; Marin, A.; Rico, A. Effects of aquaculture waste feeds and antibiotics on marine benthic ecosystems in the Mediterranean Sea. *Sci. Total Environ.* **2022**, *806*, 151190. [CrossRef]
29. Li, F.; Huang, J.; Wang, M.; Chen, L.; Xiao, Y. Sources, distribution and dynamics of antibiotics in *Litopenaeus vannamei* farming environment. *Aquaculture* **2021**, *545*, 737200. [CrossRef]
30. Dai, P.; Li, D.; Sui, J.; Kong, J.; Meng, X.; Luan, S. Prediction of meat yield in the Pacific whiteleg shrimp *Penaeus vannamei*. *Aquaculture* **2023**, *577*, 739914. [CrossRef]
31. Wang, W.; Zhong, P.; Yi, J.-Q.; Xu, A.-X.; Lin, W.-Y.; Guo, Z.-C.; Wang, C.-G.; Sun, C.-B.; Chan, S. Potential role for microRNA in facilitating physiological adaptation to hypoxia in the Pacific whiteleg shrimp *Litopenaeus vannamei*. *Fish Shellfish Immunol.* **2019**, *84*, 361–369. [CrossRef] [PubMed]
32. Holmström, K.; Gräslund, S.; Wahlström, A.; Poungshompoo, S.; Bengtsson, B.-E.; Kautsky, N. Antibiotic use in shrimp farming and implications for environmental impacts and human health. *Int. J. Food Sci. Technol.* **2003**, *38*, 255–266. [CrossRef]
33. Devadas, S.; Zakaria, Z.; Shariff, M.; Bhassu, S.; Karim, M.; Natrah, I. Methodologies and standards for monitoring antimicrobial use and antimicrobial resistance in shrimp aquaculture. *Aquaculture* **2024**, *579*, 740216. [CrossRef]
34. Jin, X.; Liu, S.; Zhang, Z.; Liu, T.; Li, N.; Liang, Y.; Zheng, J.; Peng, N. Enrofloxacin-induced transfer of multiple-antibiotic resistance genes and emergence of novel resistant bacteria in red swamp crayfish guts and pond sediments. *J. Hazard. Mater.* **2023**, *443*, 130261. [CrossRef] [PubMed]
35. Palaniyappan, V.; Nagalingam, A.K.; Ranganathan, H.P.; Kandhikuppam, K.B.; Kothandam, H.P.; Vasu, S. Antibiotics in South Indian coastal sea and farmed prawns (*Penaeus monodon*). *Food Addit. Contam. Part B* **2013**, *6*, 196–199. [CrossRef] [PubMed]
36. Rico, A.; Jacobs, R.; Van den Brink, P.J.; Tello, A. A probabilistic approach to assess antibiotic resistance development risks in environmental compartments and its application to an intensive aquaculture production scenario. *Environ. Pollut.* **2017**, *231*, 918–928. [CrossRef] [PubMed]
37. Jiang, H.; Yu, T.; Yang, Y.; Yu, S.; Wu, J.; Lin, R.; Li, Y.; Fang, J.; Zhu, C. Co-occurrence of Antibiotic and Heavy Metal Resistance and Sequence Type Diversity of *Vibrio parahaemolyticus* Isolated from *Penaeus vannamei* at Freshwater Farms, Seawater Farms, and Markets in Zhejiang Province, China. *Front. Microbiol.* **2020**, *11*, 1294. [CrossRef]
38. Nadella, R.K.; Panda, S.K.; Madhusudana Rao, B.; Pani Prasad, K.; Raman, R.P.; Mothadaka, M.P. Antibiotic Resistance of Culturable Heterotrophic Bacteria Isolated from Shrimp (*Penaeus vannamei*) Aquaculture Ponds. *Mar. Pollut. Bull.* **2021**, *172*, 112887. [CrossRef]
39. Ministry of Agriculture of the PRC. *Pollution-Free Food—Rules for Application of Fishery Medicine*; The Ministry of Agriculture of the PRC: Beijing, China, 2002.
40. Gu, B.; Li, F.; Liu, Y.; Mao, L.; Tao, H. Effect of Vegetable Growth on Content and Composition of Antibiotics in *Litopenaeus vannamei* Pond Sediments in Crop/Aquacultural Rotation Process. *Sustainability* **2021**, *13*, 8400. [CrossRef]
41. He, X.; Wang, Z.; Nie, X.; Yang, Y.; Pan, D.; Leung, A.O.W.; Cheng, Z.; Yang, Y.; Li, K.; Chen, K. Residues of fluoroquinolones in marine aquaculture environment of the Pearl River Delta, South China. *Environ. Geochem. Health* **2012**, *34*, 323–335. [CrossRef]
42. Ni, M.; Yuan, J.; Liu, M.; Gu, Z. Assessment of water quality and phytoplankton community of *Limpenaeus vannamei* pond in intertidal zone of Hangzhou Bay, China. *Aquac. Rep.* **2018**, *11*, 53–58. [CrossRef]
43. Jayasinghe, G.D.T.M.; Szpunar, J.; Lobinski, R.; Edirisinghe, E.M.R.K.B. Determination of Multi-Class Antibiotics Residues in Farmed Fish and Shrimp from Sri Lanka by Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS). *Fishes* **2023**, *8*, 154. [CrossRef]
44. Gao, L.; Shi, Y.; Li, W.; Liu, J.; Cai, Y. Occurrence, distribution and bioaccumulation of antibiotics in the Haihe River in China. *J. Environ. Monit.* **2012**, *14*, 1247–1254. [CrossRef] [PubMed]
45. Xiong, J. Progress in the gut microbiota in exploring shrimp disease pathogenesis and incidence. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 7343–7350. [CrossRef] [PubMed]
46. Xing, G.; Lu, J.; Xuan, L.; Chen, J.; Xiong, J. Sediment prokaryotic assembly, methane cycling, and ammonia oxidation potentials in response to increasing antibiotic pollution at shrimp aquafarm. *J. Hazard. Mater.* **2022**, *434*, 128885. [CrossRef] [PubMed]

47. Wang, J.; Zhuan, R. Degradation of antibiotics by advanced oxidation processes: An overview. *Sci. Total Environ.* **2020**, *701*, 135023. [CrossRef]
48. Eheneden, I.; Wang, R.; Zhao, J. Antibiotic removal by microalgae-bacteria consortium: Metabolic pathways and microbial responses. *Sci. Total Environ.* **2023**, *891*, 164489. [CrossRef]
49. Yezli, S.; Li, H. Antibiotic resistance amongst healthcare-associated pathogens in China. *Int. J. Antimicrob. Agents* **2012**, *40*, 389–397. [CrossRef]
50. Chen, W.-R.; Huang, C.-H. Transformation kinetics and pathways of tetracycline antibiotics with manganese oxide. *Environ. Pollut.* **2011**, *159*, 1092–1100. [CrossRef]
51. Ahmad, F.; Zhu, D.; Sun, J. Environmental fate of tetracycline antibiotics: Degradation pathway mechanisms, challenges, and perspectives. *Environ. Sci. Eur.* **2021**, *33*, 64. [CrossRef]
52. Xu, Z.; Jia, Y.; Zhang, X.; Hu, S.; Luo, Y.; He, H.; Chen, B.; Huang, B.; Pan, X. Algal organic matter accelerates the photodegradation of tetracycline: Mechanisms, degradation pathways and product toxicity. *Chem. Eng. J.* **2023**, *468*, 143724. [CrossRef]
53. Gómez-Pacheco, C.V.; Sánchez-Polo, M.; Rivera-Utrilla, J.; López-Peñalver, J. Tetracycline removal from waters by integrated technologies based on ozonation and biodegradation. *Chem. Eng. J.* **2011**, *178*, 115–121. [CrossRef]
54. Chen, H.; Liu, S.; Xu, X.-R.; Diao, Z.-H.; Sun, K.-F.; Hao, Q.-W.; Liu, S.-S.; Ying, G.-G. Tissue distribution, bioaccumulation characteristics and health risk of antibiotics in cultured fish from a typical aquaculture area. *J. Hazard. Mater.* **2018**, *343*, 140–148. [CrossRef] [PubMed]
55. Guo, F.C.; Kwakkel, R.P.; Soede, J.; Williams, B.A.; Verstegen, M.W.A. Effect of a Chinese herb medicine formulation, as an alternative for antibiotics, on performance of broilers. *Br. Poult. Sci.* **2004**, *45*, 793–797. [CrossRef] [PubMed]
56. Alagawany, M.; Abd El-Hack, M.E.; Farag, M.R.; Sachan, S.; Karthik, K.; Dhama, K. The use of probiotics as eco-friendly alternatives for antibiotics in poultry nutrition. *Environ. Sci. Pollut. Res.* **2018**, *25*, 10611–10618. [CrossRef]