aquaculture journal

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Individual Shrimp Rearing Increases the Power of Experimental Trials

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Abstract: Comparable and reproducible research is needed to improve Pacific white shrimp (PWS) aquaculture. These experiments typically involve before-and-after measurements of the same individual for paired statistical testing. However, marking shrimp with external or internal tags is challenging, especially for juveniles. A possible alternative is to rear shrimp individually in single-shrimp systems. While such systems may also prevent competitive interactions, PWS are considered social animals and individual rearing may negatively affect social behavior. Therefore, the general goal of this study was to evaluate the effects of single-shrimp versus multi-shrimp systems on the survival, growth, and welfare of juvenile PWS using a randomized controlled trial with group sizes of one, three, and five individuals. We found that shrimp kept individually had a higher survival rate, higher final body weights and lengths, and longer antennae than shrimp kept in groups of three or five. The incidence of eye cataracts was not significantly different among groups. Based on these results, we conclude that the individual rearing of juvenile PWS has no negative effects on survival, growth, or welfare. Therefore, a single-shrimp system could be an alternative to individually marked shrimp to allow paired statistical testing in experimental trials, especially when using post-larvae or juvenile specimens.

Keywords: Pacific white shrimp; aquaculture; single-shrimp system; group-size trial; survival rate; growth rate; welfare

Academic Editor: Aires Oliva-Teles

Received: 7 December 2024
Revised: 1 January 2025
Accepted: 9 January 2025
Published: 20 January 2025

Citation: Wilke, T.; Bendag, S.; Barth, A.; Reinold, T.; Schubert, P. Individual Shrimp Rearing Increases the Power of Experimental Trials. *Aquac. J.* **2025**, *5*, 2. https://doi.org/10.3390/aquacj5010002

1. Introduction

The Pacific white shrimp (PWS, *Penaeus vannamei* Boone, 1931) is the most commercially important shrimp species worldwide, with aquaculture accounting for the majority of production [1]. The industry has experienced significant growth in recent decades, with major production centers in Asia and Latin America. However, the environmental footprint of PWS aquaculture may be high [2]. Therefore, several challenges need to be addressed to strengthen the environmental, economic, and social sustainability of shrimp farming. These include, but are not limited to, reducing fishmeal and fish oil in PWS feeds [3–5], improving the disease resistance and welfare of PWS [6,7], enhancing breeding stock quality and genetic selection [3,8], and utilizing more efficient and environmentally friendly rearing practices [9].

To address these challenges, comparable and reproducible research is needed [10], such as feed benchmarking experiments, disease management and behavioral investigations, and controlled environmental studies. Many of these experiments involve before-and-after

measurements of the same individual (i.e., paired statistical tests). This, in turn, would require individual marking [11,12].

However, marking shrimp is challenging, especially for larval or juvenile shrimp. While most external electronic tags commonly used in experiments with fish and crustaceans, such as electronic transmitters [12], are normally effective for individual identification, for shrimp they may be too large and heavy, interfere with the shrimp's molting process, be lost during ecdysis [13], or cause stress, tissue damage, or death to the shrimp [11]. Some or all of these obstacles may also apply to non-electronic tags, such as nylon filaments, adhesive tags [14], or rings attached to the shrimp's eyestalk [15,16].

Internal tags, such as Visible Implant Elastomer (VIE) tags, Visible Implant Alpha (VI Alpha) tags, and Passive Integrated Transponder (PIT) tags, are also commonly used on shrimp. VIE tags are injected into the shrimp's tissue and are visible through the exoskeleton. However, they are typically only used for batch coding or for tagging a small number of individuals [17]. VI Alpha tags are also small, alphanumeric tags implanted in the shrimp's transparent tissue. They are externally readable and allow individual identification. However, readability may deteriorate rapidly in juvenile shrimp and there may be problems with retention times [18]. PIT tags are microchips implanted in the shrimp's body that can be read by an external scanner. They allow the unique identification of each shrimp but are relatively expensive, time-consuming to implement, and require special equipment to read [19].

An alternative to the individual marking of shrimp to facilitate paired statistical testing, especially for post-larvae and juvenile shrimp, would be to rear them individually in single-shrimp systems. Such systems not only allow paired statistical testing, but also offer several other advantages. Behavioral changes and welfare deterioration in shrimp due to tagging can be excluded, experimental results are not biased by adverse shrimp interactions [20,21], such as eye injuries (cataracts), antenna damage, or cannibalism, and problems with maintaining "social distance" (i.e., the distance that prevents fight or flight reactions) can be avoided [20].

Although adverse behavior may occur among shrimp, they are considered social individuals [20,22], especially post-larvae and juvenile PWS that occur naturally in coastal waters such as estuaries, lagoons, and mangrove forests [23,24]. This presumed complex interplay of intraspecific antagonistic and synergistic factors may also be the reason why scientific studies [25] and some practical guides to shrimp farming [26,27] suggest intermediate PWS group sizes to balance feeding efficiency and welfare.

Thus, while single-shrimp systems have many advantages in terms of statistics and experimental design, it remains unclear whether individual shrimp rearing has negative social effects (e.g., increased mortality, reduced weight gain, reduced welfare) that negate the various positive effects.

Therefore, the general goal of this study was to evaluate the effects of single-shrimp versus multi-shrimp systems on the survival, growth, and welfare parameters of PWS. Using a randomized controlled trial with group sizes of one, three, and five juveniles, we specifically undertook the following:

1. Calculated differential survival rates;
2. Evaluated growth performance parameters (i.e., final body weight and length);
3. Determined welfare parameters (i.e., final antenna length and frequency of cataracts).

Our working hypothesis is that rearing social juvenile shrimp individually has significant negative effects on survival, growth, and welfare characteristics.

2. Materials and Methods

2.1. Population Studied and Pre-Treatment

The main characteristics of the studied PWS population are presented in Table 1, following the guidelines for reporting experimental studies in shrimp [10]. PWS post-larvae (21 days old) were purchased from Suburban Seafood Germany UG (Nebelschütz, Germany). They were acclimated and grown in glass aquaria for 50 days to an average length of 2.5 cm. The two glass aquaria (60 × 35 × 43 cm) were attached to a coral rearing system equipped with a PowerCone 250 skimmer (ATI, Hamm, Germany) and a type 391 UV unit (Deltec, Delmenhorst, Germany). Dimmed ambient light was provided for 13 h per day. Water conditions were as follows: salinity 35, temperature 27 °C, pH 8.1 ± 0.1, oxygen saturation 98–103% (about 6.5 mg L^{-1} dissolved oxygen), nitrite content not detectable, nitrate content < 10 mg L^{-1}, and phosphate content 0.02 mg L^{-1}.

Table 1. Characteristics of the studied Pacific white shrimp (PWS).

Characteristic	PWS
Species name	Pacific white shrimp, *Penaeus vannamei* Boone, 1931
Origin	Suburban Seafood Germany UG (Nebelschütz, Germany)
Genetic strain	For DNA barcoding information (COI) on the strain used see GenBank accession numbers PQ465998 and PQ465999
Life stage	Juvenile specimens (71 days old)
Weight	0.10 ± 0.07 g
Length	2.48 ± 0.46 cm

During the first five days, they were fed *Artemia* nauplii ad libitum three times a day, then the compound feed "Larviva 0.5 mm" (BioMar, Brande, Denmark) six times a day with an automated feeder. Leftover feed and feces were removed three times a week.

DNA barcoding of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene in two individuals was performed according to [10].

2.2. Experimental Design of Group-Size Trial

The group-size trial was conducted in a climatic chamber at the Biotechnikum of the Justus Liebig University Giessen, Germany. The experiment was carried out in an indoor recirculating clear water system (Figure 1) using 45 plastic tanks (21 cm high, 35 cm deep, 20 cm wide; capacity 9.2 L; area 700 cm^2). We studied 3 group sizes (GS): specimens held individually (GS1), in groups of 3 (GS3), and in groups of 5 (GS5). For each group size, 15 replications were used, i.e., 15 × 1 (15) specimen for GS1, 15 × 3 (45) specimens for GS3, and 15 × 5 (75) specimens for GS5.

Key information on the experimental conditions and water parameters for the main experiment are listed in Table 2. The water parameters of salinity (HI98319 salinity tester, Hanna Instruments Deutschland GmbH, Vöhringen, Germany), temperature, and pH (WTW 3620 IDS set G multiparameter, Xylem Analytics Germany GmbH, Weilheim, Germany) were monitored daily. The values for nitrite and nitrate (test strips QUANTOFIX Peroxid 25, Macherey-Nagel GmbH & Co. KG, Düren, Germany) were recorded weekly; the values for phosphate (Spectroquant test kit, Merck KGaA, Darmstadt, Germany) were recorded at day 1 and day 15. The water parameters conformed to the ideal standards proposed by [7].

Figure 1. Photograph of the experimental system used for the group-size trial with juvenile Pacific white shrimp (photo: T. Wilke).

Table 2. Experimental conditions and water parameters (mean ± standard deviation) for the Pacific white shrimp (PWS) group-size trial.

Characteristic	PWS
Duration of experiment	30 days (20 July 2023–18 August 2023)
Rearing system	Indoor recirculation clear water system with plastic tanks (capacity 9.2 L each)
Group size	1, 3, or 5 shrimp per tank
Replications	15 replications each for group sizes of 1, 3 and 5 shrimp
Feed	Daily at 8.00 a.m., 12:00 a.m., 4:00 p.m., and 8:00 p.m.: 0.003 g per shrimp "Vanna Starter PL3" (Le Gouessant Aquaculture, Lamballe, France) Daily at 8:00 p.m.: 0.004 g (days 1–18) or 0.008 g (days 19–30) per shrimp *Spirulina* (Spirulina Flakes, Ocean Nutrition, Essen, Belgium)
Water filtration	Vlies Dreambox 3.0 with fleece filter and protein skimmer (Royal Exclusiv Christian Walter GmbH & Co. KG, Wesseling, Germany), and a flow-through biofilter with media (Aqua-Light GmbH, Bramsche, Germany)
Lightning	LED light (ca. 12 h light/12 h dark)
Water parameters	
Salinity	26.8 ± 0.53
Temperature (°C)	27.5 ± 0.36
pH	8.0 ± 0.1
Dissolved oxygen content (mg L^{-1})	6.30 ± 0.05
Nitrite content (mg L^{-1})	2–10
Nitrate content (mg L^{-1})	25–50
Phosphate content (mg L^{-1})	0.2–0.8

Shrimp were weighed, measured, and photographed on graph paper (digital camera DMC-FZ1000, Panasonic Holdings Corporation, Osaka, Japan) at the beginning of the 30-day trial. At the end of the trial, shrimp were reweighed, measured, examined for

cataracts, and photographed using the same camera position. They were then chill-killed in ice water, placed in labeled bags, and stored in a freezer at –20 °C.

2.3. Statistical Analyses

All statistical analyses and data visualization were performed in the R statistical environment, version 4.3.1. [28].

The following parameters were analyzed:

- Survival rate (%) = 100 × final number of shrimp/initial number of shrimp;
- Final body weight (g);
- Final body length (cm);
- Total antenna length (cm) = length left antenna + length right antenna;
- Cataract rate (%) = 100 × number of shrimp/(number of cataracts in left eye + number of cataracts in right eye).

The homogeneity of variance was confirmed using the Levene test to ensure that the assumptions of the model tests were met. Data were also tested for normality using the Shapiro–Wilk test. When residuals were normally distributed ($p > 0.05$), the overall differences among the three groups were evaluated using an ANOVA and pairwise differences with the Tukey HSD test. When data were not normally distributed ($p \leq 0.05$), the overall differences among the three density groups were analyzed with a Kruskal–Wallis test and pairwise comparisons were made with a Dunn test. Fisher's exact test was used to calculate differences in survival rates among the density groups, as it is appropriate for small sample sizes and categorical data.

Box plots were generated for each parameter to visually compare the distributions between treatments. These plots were supplemented with scatter plots where appropriate to display individual data points. All plots were generated using the R package ggplot2 v3.5.1. [29], with adjustments for aesthetic presentation. Raw data are provided in Table S1.

3. Results

3.1. PWS Survival Rate

At the end of the 30-day group-size trial, shrimp survival was 100% (15 of 15 shrimp) for group size 1 (GS1), 84.4% (38 of 45 shrimp) for GS3, and 82.6% (62 of 75 shrimp) for GS5 (Figure 2A, Table S2). However, the differences among the density groups were not statistically significant ($p = 0.259$; Table S2).

3.2. PWS Growth Parameters

The mean final body weights of the GS1, GS3, and GS5 specimens at the end of the trial were 0.59 ± 0.28 g, 0.37 ± 0.27 g, and 0.42 ± 0.34 g, respectively (Table S2, Figure 2B). The corresponding final body sizes were 4.62 ± 0.69 cm, 3.80 ± 0.84 cm, and 3.94 ± 1.04 cm, respectively (Table S2, Figure 2C). Both the mean body weight and mean body size of the GS1 specimens were significantly higher than those of the GS3 and GS5 specimens (Table S2).

3.3. PWS Welfare Parameters

At the end of the trial, the total antenna length of the GS1, GS3, and GS5 specimens was 12.80 ± 2.84 cm, 8.70 ± 3.47 cm, and 9.08 ± 3.34 cm, respectively (Table S2, Figure 2D). The antennae of the GS1 specimens were significantly longer than those of the GS3 and GS5 specimens (Table S2).

The cataract rate in surviving shrimp after 30 days was 20% (3 cataracts in 15 shrimp) for GS1, 18.4% (7 cataracts in 38 shrimp) for GS3, and 27.4% (17 cataracts in 62 shrimp) for

GS5 (Table S2, Figure 2E). However, the differences between and among the density groups were not statistically significant (Table S2).

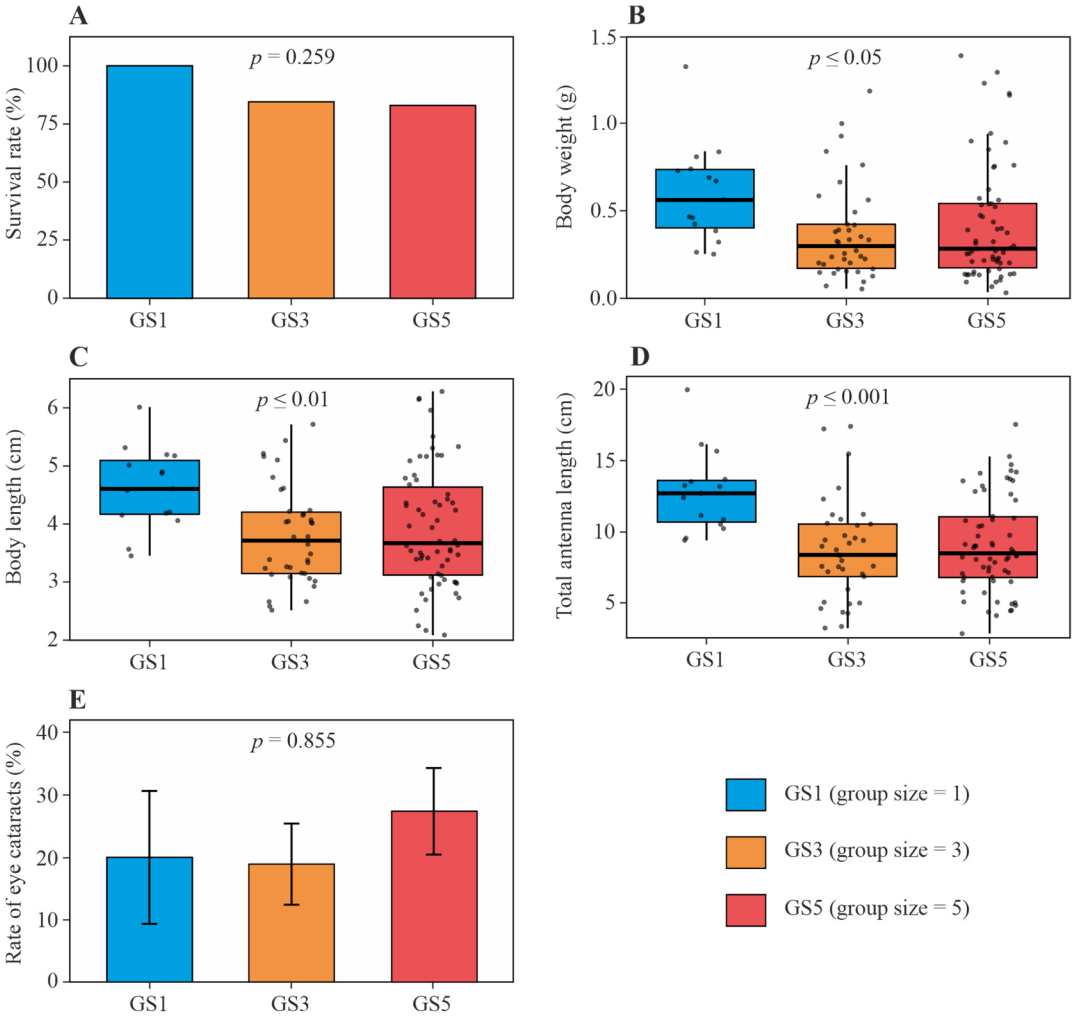

Figure 2. Differential effects of group size (1, 3, and 5 individuals) on survival (**A**), growth ((**B**): body weight; (**C**): body length), and welfare ((**D**): total antenna length; (**E**): rate of cataracts) parameters of juvenile Pacific white shrimp at the end of the 30-day controlled trial. For detailed statistical comparisons, see Table S2; *p*-values refer to overall differences among treatments.

4. Discussion

4.1. High Survival Rate of PWS in Single-Shrimp System

Based on our randomized, controlled group-size trial, we found a 100% survival rate in our single-shrimp system (Figure 2A), compared to 82.7–84.4% for specimens kept in groups. Although the differences are not statistically significant, probably due to the small sample size, the first part of our working hypothesis—that rearing social juvenile shrimp individually has significant negative effects on survival—is clearly rejected. Apparently, antagonistic competitive effects in groups of shrimp [21] outweigh potential synergistic social effects.

During the trial, we observed several forms of adverse behavior in the shrimp kept in groups, such as feeding competition (especially between individuals of different sizes), aggressive behavior, and cannibalism. In particular, the observed feeding interactions may have important implications for future feeding trials, where attractive feed or larger diet sizes may lead to dominance behavior and increased mortality, especially in populations with individuals of different sizes [15,30]. Although these antagonistic effects

between specimens may bias the results of group feeding trials, such group experiments are likely to provide more realistic behavioral responses applicable to commercial aquaculture conditions [31].

4.2. Increased Growth Performance of PWS in Single-Shrimp System

Our group-size trial also showed that rearing juvenile shrimp individually resulted in significantly higher mean body weight and body length compared to the shrimp reared in groups (Figure 2B,C). Thus, the second part of our working hypothesis—that rearing juvenile shrimp individually has significant negative effects on growth characteristics—can also be rejected. Similar to the high survival rate (see Section 4.1), the absence of adverse behavior in the single-shrimp system may play a role in this good growth performance. In addition, keeping shrimp in groups may not have strong beneficial social effects on feeding behavior. The latter may be generally true for most aquatic taxa, except for species that show strong intraspecific cooperative predatory behavior, such as the gag grouper (*Mycteroperca microlepis*) [32].

Our results are also consistent with other PWS studies showing that growth performance increases with decreasing group size [33].

4.3. No Reduced Welfare of PWS in Single-Shrimp System

Finally, our results show that total antenna length was significantly higher in shrimp kept individually than in shrimp kept in groups of three or five (Figure 2D). Furthermore, the frequency of cataracts was slightly (but not significantly) higher in the shrimp reared individually than in shrimp kept in groups of three, but lower than in shrimp kept in groups of five (Figure 2E). Thus, the last part of our working hypothesis—that rearing social shrimp individually has significant negative effects on welfare characteristics—is also rejected. In fact, our data for shrimp kept in groups may even be biased, as they showed mortality rates of 15–17%. Specimens that died in the group trials may have had a higher incidence of cataracts and shorter antennae due to competitive interactions. However, they had to be excluded from the calculation of welfare characteristics because only surviving individuals were scored to avoid potential bias due to post-mortem scavenging by other specimens. Therefore, the actual cataract rate in shrimp kept in groups may be higher and total antenna length lower than shown in Figure 2D,E.

4.4. Rearing PWS in Single-Shrimp Systems Does Not Bias Experimental Trials

The results of our group-size trial with juvenile PWS are unequivocal: single-shrimp rearing has no significant negative effects on any survival, growth, or welfare characteristic. Therefore, we consider our single-shrimp system (Figure 1) to be a suitable alternative to individually marked shrimp to allow paired statistical testing in experimental trials. This is especially true for post-larvae and juvenile specimens, where tagging may cause behavioral changes and/or welfare deterioration due to their small size [11]. In addition, the adverse behavior of shrimp [21], which potentially confounds experimental trials, can be excluded in our single-shrimp system.

The reasons for the good performance of the single-shrimp system have already been briefly mentioned in Sections 4.1–4.3. In particular, antagonistic competitive effects [21] in groups of shrimp, such as stress and injuries caused by aggressive behavior, competition for feed, and cannibalism, are effectively prevented. Moreover, potential synergistic social effects in shrimp groups may be low, and the lack of social interactions in the single-shrimp system is likely more than compensated by the lack of adverse behavior. Another explanation could arise from the fact that we used a flow-through design without an ozone filter in our single-shrimp system. This setup may have allowed chemical communication among individuals, possibly mitigating the negative social effects of physical isolation. Chemical

cues play an important role in many aquatic animals, especially in short-term interactions [34]. In fact, chemical signaling is probably the most common form of communication among decapod crustaceans [34,35].

Furthermore, the results of our study raise general concerns about the sociality of (juvenile) PWS. On the one hand, the temporal grouping of juvenile shrimp has been proposed [23]. On the other hand, even young PWS require social distancing [20] to avoid adverse behavior. Indeed, surprisingly little is known about the interplay between adverse and beneficial social behaviors in PWS, both in the wild and in captive populations. Possibly, they require both social distancing and social communication. Until these complex interactions and the role of chemical communication in PWS are better understood, we recommend using only single-shrimp systems in experimental trials where chemical signaling between individuals is possible. Moreover, although we strongly discourage the practice of keeping shrimp individually in commercial aquaculture conditions, the results of our study may support the need for increased environmental enrichment in shrimp farms to reduce competitive interactions, for example by providing hiding places in recirculation systems [6].

4.5. Limitations and Outlook

To minimize "tank effects" in aquatic animal experiments, especially density studies, it is necessary to ensure comparable experimental conditions for all treatments. This concerns, for example, water flow and lighting conditions, water depth, stocking density, and the availability of hiding and resting places. However, providing such comparable conditions in experimental setups with different group sizes is very challenging due to constructional limitations. For example, in our experimental setup with a group size of one individual (GS1), the density was 14 shrimp m^{-2}; for GS3, 43 shrimp m^{-2}; and for GS5, 71 shrimp m^{-2}. However, these densities are scattered around a density of 50 shrimp m^{-2}, which is considered optimal to ensure the growth and welfare of juvenile PWS [20]. Therefore, we believe that a setup with equal densities (where differences in other parameters may occur) would not change the very clear result of the current study, i.e., that keeping shrimp individually does not have a negative effect on survival, growth, or welfare parameters.

Our study also revealed a considerable lack of behavioral information on PWS in both wild and captive populations. We therefore recommend that future studies focus on the interplay of potentially adverse and beneficial behaviors, for example in the context of chemical signaling.

5. Conclusions

Based on a randomized, controlled group-size trial, we found that shrimp kept individually (GS1) had a higher survival rate and a higher final body weight and length than shrimp kept in groups of three (GS3) or five (GS5). Total antenna length was also higher in GS1 individuals than in GS3 and GS5. The frequency of cataracts was slightly higher in GS1 specimens than in GS3, but lower than in GS5. Based on these results, our working hypothesis—that rearing social juvenile shrimp individually has significant negative effects on survival, growth, and welfare characteristics—can be rejected in all parts. Therefore, a single-shrimp system could be an alternative to the individual tagging of shrimp to allow paired statistical testing in experimental trials, especially for post-larvae and juvenile shrimp. However, the potential role of social interactions in single-shrimp systems remains ambiguous. Future behavioral studies are therefore recommended. Until then, it is suggested to enable chemical communication between individuals in single-shrimp systems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/aquacj5010002/s1, Table S1: Raw data from a 30-day controlled group-size trial investigating the effects of group sizes of 1, 3, and 5 juvenile individuals on survival, growth, and welfare parameters of Pacific white shrimp; Table S2: Effects of group sizes of 1, 3, and 5 juvenile individuals on survival, growth and welfare parameters of Pacific white shrimp.

Author Contributions: A.B. and P.S. designed the study. A.B., S.B. and T.R. constructed the single-shrimp system with the help of P.S., A.B. and S.B. performed the experiments with assistance from P.S., S.B. and T.W. analyzed the data. T.W. drafted the manuscript and prepared the final figures and tables. All authors have read and agreed to the published version of the manuscript.

Funding: The study was funded by the German Federal Ministry of Education and Research (BMBF) under grant numbers 031B0938C, 031B1291A, and 01DU20004A.

Institutional Review Board Statement: In Hesse, Germany, experiments with shrimps (order Decapoda) are not subject to authorization. All applicable regulatory requirements were met.

Data Availability Statement: Raw data from this study are available in the Supplementary Materials (Table S1).

Acknowledgments: We acknowledge Valeria Schaub, Laura Schmid-Kapfenburg, and Monique Wilde for their help with the experimental setup of the single-shrimp facility and the shrimp trials. We thank Björn Stelbrink (Justus Liebig University Giessen, Germany) for providing us with the DNA barcoding information for the studied PWS. We also thank three anonymous reviewers for their comments on an earlier version of our paper.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in writing of the manuscript; or in the decision to publish the results.

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