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Quality assessment of *Litopenaeus vannamei* postlarvae produced in some commercial shrimp hatcheries of Choubdeh Abadan, Iran

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ABSTRACT

In the present study, the quality of *Litopenaeus vannamei* postlarvae (PL) produced in some commercial hatcheries evaluated in three different hatcheries with different management in Chouebdeh Abadan, Iran. For evaluating management effects on PL quality, 180 postlarvae (30 postlarvae for each quality index) sampled from three hatcheries during the two sampling periods. Then morphometric indices (including body weight, total length, carapace length, length of the intestine, diameter of the sixth abdominal segment), behavioral indices (swimming activity), stress test (salinities of 0%, and 50%, formalin 100 and 200 ppm), carcass biochemical compositions, microbial indices, and microscopic observations) were analyzed and recorded. Management features assessed using a questionnaire. Based on the results, the highest morphometric indices were attributed to hatcheries 2 & 3. Also, the hepatopancreas index in hatchery no. 3 was better than other hatcheries. Similarly, the highest survival rate in the stress test and behavioral index were observed in the PLs of hatchery no. 3. The results of carcass biochemical composition also showed that carcass lipid decreased by increasing carcass protein content. The highest bacterial load was observed in the PLs of hatchery no. 2. The results of microscopic observations indicated that the post-larvae in the third hatchery had a completely dilated intestine and contained more food. The hepatopancreatic tissue was larger and consisted of multiple B cells with large vesicles. Based on the questionnaire, all PL samples were in PL 12 stage. The main differences between hatchery no. 3 and other hatcheries were as follows: The broodstocks weight was higher than other hatcheries; fresh and formulated diet was used in hatchery no. 3; larval growth assay, nauplius, and postlarval survival assay was carried out on a regular basis, and no sludge accumulation at the bottom of the tanks was observed in hatchery no. 3. The results of this research show that appropriate management, in hatchery no. 3 in comparison to other hatcheries, is considered as the main factor resulting in better quality for postlarvae production.

1. Introduction

Nowadays, many species of shrimp introduced for breeding in various hatcheries overseas. One of the most important of these species is *Litopenaeus vannamei*. This species was initially introduced to shrimp farms in Iran by the Iranian Fisheries Research Institute in 2004 (Afsharnasab et al., 2008). *L. vannamei* has various advantages including: better growth rate, high tolerance to the wide range of temperature and salinity, high survival rate and high production efficiency in larval and growing stages, needs low protein diet and the possibility of producing specific pathogen-free (SPF) and specific pathogen resistant (SPR) broodstocks. These advantages are known to reduce production costs. Furthermore, due to the relative ease of breeding, it has spread to all parts of the world (Wyban and Sweeney, 1991). Due to the outbreak of white spot disease in *Penaeus indicus* in Iranian shrimp farms in 2002 and extensive losses in the said species, *Litopenaus vannamei* considered as a suitable alternative for breeding and culture in the southern parts of Iran and at present is the main culture species in the region (Khademzade et al., 2020). The high development rate of shrimp farms in coastal areas has led to an increase in demand for high-quality PLs. PL quality is one of the critical factors, affecting the whole process of growing and producing farmed shrimps.

Despite the high importance of this factor, few scientific studies have been conducted in this regard. One of the best ways to evaluate

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the quality of postlarvae in the Penaeidae shrimp, which can easily perform in the hatcheries, is to apply environmental stressors, including salinity stress (Racotta et al., 2004; Ruff et al., 2001). Dhert (1996) suggested using stress tests as a tool in assessing the quality of fish larvae and crustaceans. Researchers have also reported that PLs that exhibit more top survival conditions under stress tests have higher quality (Racotta et al., 2004; Gallardo et al., 1995).

The quality of the larvae produced in the hatcheries is evaluated based on standard indicators (Racotta et al., 2003). Some of the indicators include: growth rate and size, nutritional status, general condition, and biochemical composition of the body, resistance to stress and symptoms of the disease. In recent years, one of the main procedures for larval quality assessment in the farms is using molecular methods for identifying viral contamination (Gholamhosseini et al., 2020; Hakami, 2018; Pazir et al., 2011; Maralit et al., 2011). Chapman et al. (2004) used molecular diagnostics and bioassay techniques to investigate the presence of WSSV in a community of wild shrimps in US coastal waters. However, the use of molecular methods to determine the postlarval quality is not always available on the farm and, in many cases, resulted in a false positive or false negative result (Gholamhosseini et al., 2020). The primary purpose of quality control of postlarval production in shrimp hatchery is to try to predict their survival and growth rate in grow-out ponds. In recent years, due to the occurrence and prevalence of shrimp diseases, the quality control of PLs production has been given special consideration (Farabi et al., 2017). Due to poor PL stocking in shrimp ponds, shrimp farmers ultimately face with low survival of cultured shrimps. So to overcome the declining economic revenues due to low shrimp growth rate and also reduced survival of stocked PL, the use of quality control for assessing shrimp PL is critical (Racotta et al., 2004). In Iran's shrimp hatchery industry, there is no comprehensive information about PL quality. Considering the critical role of PL quality in production efficiency, special attention should pay to the PL quality. Therefore, the primary purpose of this study is to evaluate the quality of shrimp PL produced in some commercial shrimp hatcheries in Chooebdeh Abadan, Iran, and compare the results with national and international standards and provide suggestions and strategies to improve the situation in the study area.

2. Material and method

2.1. PL sampling

PL samples were taken from three shrimp hatcheries with different management during two sampling periods with 15 days interval. At least 30 PLs from each tank and a totally 180 PLs from each hatchery were sampled. All the PLs samples were in PL 12 stage.

2.2. PLs biometrics evaluation

PLs biometry was measured and recorded using standard methods (Madhukiran et al., 2009). The total length of PLs (distance from the beginning of the rostrum to the end of the telson in mm), measured using an optical micrometer and a caliper. Also, the total weight recorded on a mass scale using a 0.001 g digital scale (Schulte, 1975). The ratio of the diameter of the sixth abdominal segment to the width of the intestine, the length of the carapace, the total length of the intestine, and the percentage of gut fullness rate also calculated using an optical micrometer (Bauman and Jamandre, 1990).

To assess swimming activity, 30 PLs placed in a shallow circular pan with the circular flow, and their movements examined. The number of larvae that were able to swim against the direction of water flow recorded as a percentage (National Veterinary Organization, 2019).

2.3. Hepatopancreatic and intestinal status

The status of the hepatopancreas and intestine assessed by preparing a wet slide of post-larval specimens on a microscopic slide. Then the wet mounts observed by an optical microscopy with a magnification of ×40. Healthy PLs showed active nutrition and digestion, and the hepatopancreas and midgut were full. Also, the small vacuoles inside the hepatopancreas were easily visible. To observe the status of hepatopancreas and intestine, 30 PLs analyzed, and their results recorded based on the percentage (National Veterinary Organization, 2019).

2.4. Resistance and survival tests

Both salinity and formalin stress tests are practical and relatively easy to perform. Thus, these two tests often use to evaluate PL quality (Bauman and Jamandre, 1990).

30 PLs exposed to two different salinity levels, equal to 50% of reservoir tanks water salinity and freshwater to investigate the larval resistance to severe salinity changes. Also, 30 PLs exposed to two different formalin concentrations (100 and 200 ppm). The postlarval survival checked and recorded after one hour of exposure (Samocha et al., 1998; Joseph et al., 2000; Yahyavi et al., 2007).

2.5. Microbial examination

For microbial analysis, 30 PLs were randomly selected from each tank and immediately transferred to the laboratory using ice. In the laboratory, samples washed, homogenized, and diluted (1 to 10 *W/V*). Then, 100 μL of the diluted homogenate poured on marine agar (Zobell Marine Agar 2216, Code: M384, Himedia®, India) as a pour plate method and incubated for 48 h at 25 °C. Then the number of colonies counted and presented on log10 CFU/g (Egan et al., 1997).

2.6. Histopathological examination

Tissue sections used to examine anatomical abnormalities and deformities, as well as the presence or absence of intracellular inclusions to investigate the possible viral contamination. For this purpose, 20 PLs collected from each tank and immediately transferred to containers containing Davidson fixation. After fixation, they processed as routine procedures, embedded in paraffin blocks, and sectioned on 0.5 μm thickness sections. The sections stained with Hematoxylin and Eosin (H &E) and examined by light microscopy (Fig. 2).

2.7. Carcass biochemical analysis

Standard methods used to analyze the larval carcass biochemical composition. Moisture measured by drying PLs in an oven for 18 h at 105 °C (AOAC, 2005). Moisture reduction method was used as a standard method for measuring total lipid. For this purpose, each sample was completely homogenized, and 0.05 g of the sample transferred into a 500 L microtube, and 100 μL of distilled water added. Then, the microtubes immersed in a bain-marie at 85 °C for 60 min. The solution was stirred every 10 min and shook carefully. The crude oil extracted by centrifuging at 5000*g* for 10 min at 4 °C (Hao et al., 2015). To extract and evaluate the protein of the sample, 10 mL of 5% sodium hydroxide added to 0.5 g PL sample and placed in a bain-marie at 95 °C for 2.5 h (Setoguchi et al., 2012). Then, It centrifuged at 5000 rpm for 10 min. The supernatant used to measure protein concentration using the Bradford method (Bradford, 1976).

Ash content of the samples measured by drying in an oven, and heated in an electric furnace at 500 °C for 3 h (AOAC, 2005).

Carbohydrate levels calculated according to the following formula (AOAC, 2005).

Carbohydrate percentage: 100 - (moisture% + fiber% + ash % + protein% + lipid%).

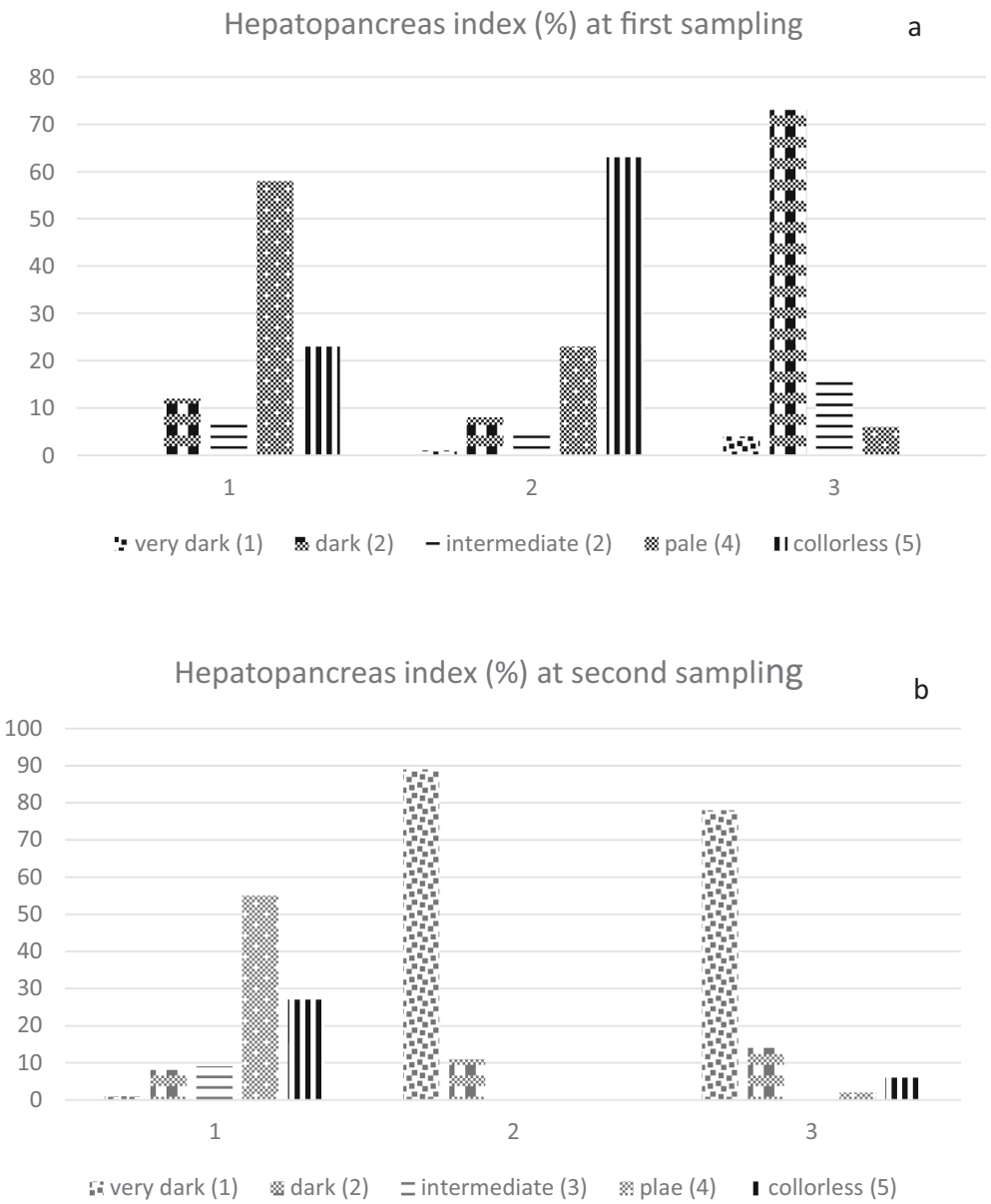

Fig. 1. The hepatopancreas status index, during the first (Fig. 1a) and second (Fig. 1b) period of sampling.

To calculate the energy of the carcass: the total energy obtained from the protein, and lipid energy content of the carcass calculated as the sample energy level according to the following equation:

energy level (Kilojoules) = 39.8% × lipid content % + 23.6% × protein content%).

2.8. Hatchery management assay and measurement of water physicochemical parameters

For assessment of the management status of shrimp hatcheries, a questionnaire prepared and hatcheries management information recorded.

The water quality parameters, including water temperature, pH, salinity, and dissolved oxygen, were measured during sampling by a multiparameter analyzer (model 556 MPS, YSI Inc., USA). During the study period, the standards, such as using optimum water temperature, the optimal physicochemical conditions of the reservoir water, and the balanced diet for all sampled PLs, assessed. The range of water temperature fluctuation during the hatchery operation was between 20 and 30 °C. The pH of the water recorded was 8–9. The salinity range of reservoir water measured between 20 and 30 ppt. Dissolved oxygen of the PL rearing tanks, which is one of the most critical factors in water quality were between 5 and 10 mg/L during the study period.

2.9. Data analysis

The results of this study reported as Mean ± Standard error. One-way analysis of variance used to compare different parameters among hatcheries. Duncan's post-test used for multiple comparisons. Also, an independent samples t-test used to compare these parameters in two sampling times. The significance level in this study considered at 95%. All the statistical analyzes performed in SPSS software version 16.

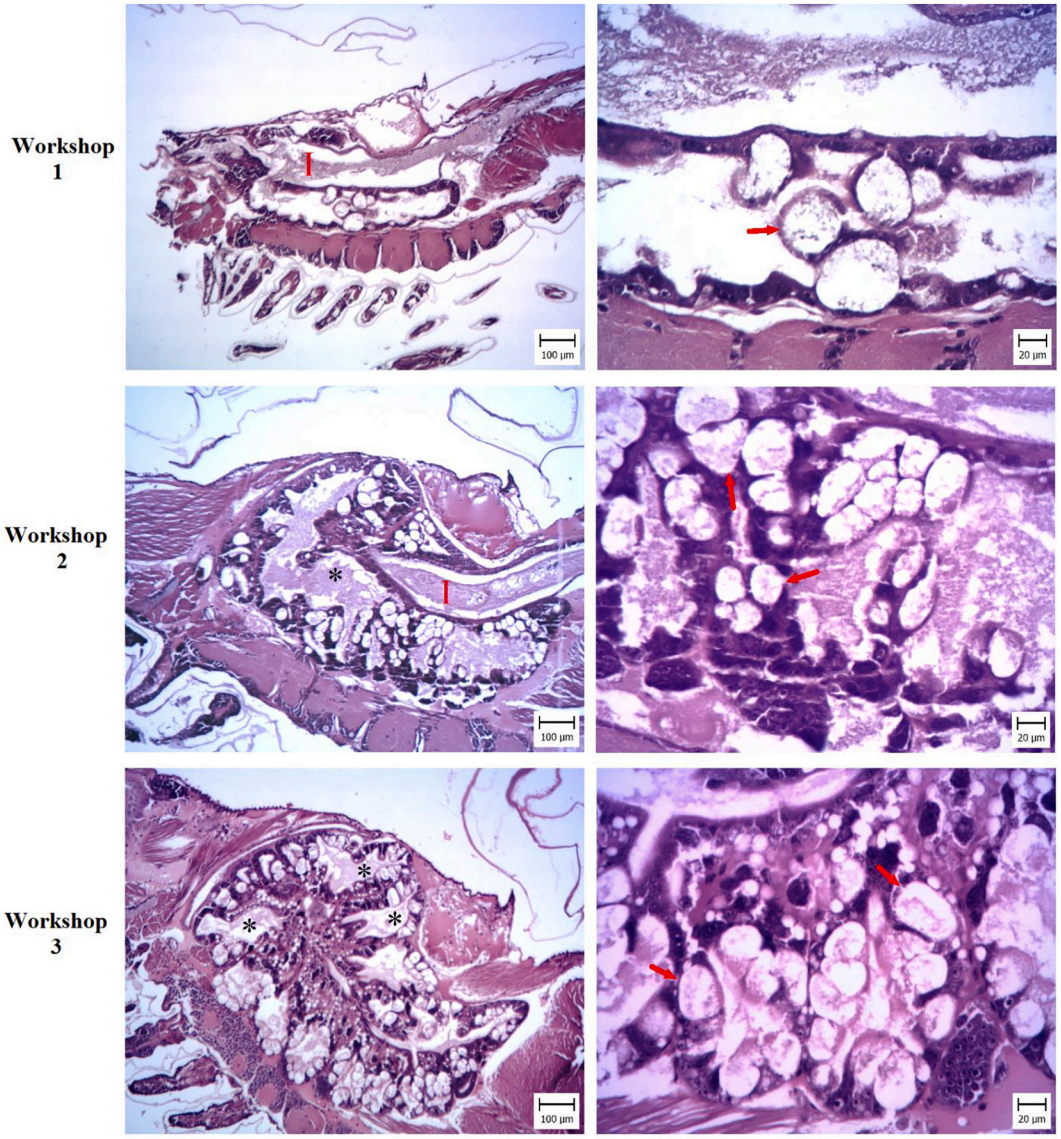

Fig. 2. Photomicrograph of longitudinal section of Post larvae in different workshops (H&E, Bar: 20 & 100 µm). Workshop 1: Note to small size of hepatopancreas and low number of B cells (red arrow). Workshop 2: Increased numbers of B cells with vesicles are obvious (red arrows). Also the central tubule is dilated. Workshop 3: Note to larger size of hepatopancreas and multiple B cells (arrows) with big vesicles. They covered the blind end tubules. The dilation of tubules is obvious (asterisks). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. Management status assay and water physicochemical parameters measurement

The summary of the results of the management assay shown in Table 1. The main differences between hatchery no. 3 and other hatcheries as follows: The weight of broodstocks in hatchery no. 3 weight is higher than other hatcheries; using from animal and formulated diet in hatchery no. 3; larval growth assay, nauplius, and postlarval survival assay, and no sludge accumulation in the bottom of the tanks in hatchery no. 3.

3.2. PLs biometrics

The results of the L. *vannamei* PLs quality based on morphological indicators during the two sampling periods shown in Table 2. In the first sampling period, the highest total weight (0.13 ± 0.02 g), length of carapace (1.96 ± 0.03 mm), the total length of the intestine (4.30 ± 0.20 mm), intestinal fullness rate (33.95 ± 4.8%), and sixth segment diameter (0.47 ± 0.01 mm), were observed in hatchery no. 3 ($p < .05$). The highest total length was recorded in hatchery no. 2 (8.06 ± 0.08 mm), and hatchery no. 3 (7.70 ± 0.25 mm), respectively.

In the second sampling period, the highest total weight (0.12 ± 0.00 g), total length (7.10 ± 0.05 mm), length of carapace (1.86 ± 0.03 mm), the total length of the intestine (4.30 ± 0.05 mm), intestinal fullness (49.53 ± 6.5%), and sixth abdominal segment diameter (0.45 ± 0.00 mm), were observed in hatchery no. 2 ($p < .05$).

3.3. Hepatopancreas index

As shown in Fig. 1a,b, during the first and second period of sampling, the highest (73%), and lowest (1%) percentages of the hepatopancreas status index observed and recorded in hatcheries 3 and 2, respectively.

Table 1
Summary of the management information of shrimp hatcheries in this study. All data obtained by questionnaire.

Management characteristics	Hatchery 1	Hatchery 2	Hatchery 3
Broodstocks			
Broodstocks weight (g)	35–50	35–50	50–60
Socking tanks	Special for broodstocks	Special for larval stage	Special for broodstocks
Water quality of resources tanks			
Temperature (°C)	20–30	20–30	20–30
pH	8–9	8–9	8–9
Salinity (ppt)	20–30	20–30	20–30
Dissolved Oxygen (ppm)	5–10	5–10	5–10
Kind of feeding for broodstocks	Formulated diet + Fresh food	Formulated diet + Fresh food	Formulated diet + Fresh food
Feeding rate (per day)	3	4	4
Feeding percentage (per biomass)	10–15	5–10	5–10
Type of Eggs disinfection	Betadine	Formalin	Formalin
Stocking Tank colour used for larval stage	White	White	Brown
Shape of stocking tank	Rectangle	Rectangle	Rectangle
Larval feeding	Herbal, animal and formulated diets	Herbal, animal and formulated diets	Animal and formulated diets
Larval growth assay	No	No	Yes
Nauplius and postlarval survival diagram	No	No	Yes
Light in hatchery (hour)	16 light, 8 dark	12 light, 12 dark	16 light, 8 dark
Lighting rate (lux)	1250	1250	2000
Technical staff	Labor, Technician	Labor	Labor, Technician
Larval status			
Sludge accumulation in the bottom of the tank	Yes	Yes	No
Gut fullness index	1	2	2
Swimming leg and muscle transparency	2	1	1
presence of foreign particles on body appendages	2	2	1
presence of structural and refractive abnormalities	2	2	1
algal and protozoan contamination on larval shells	Yes	No	No
size difference of more than 20%	No	Yes	No
Physico-chemical parameters during postlarvae sampling			
Dissolved Oxygen	6.5 ± 0.7	7 ± 0.4	6.8 ± 0.8
pH	8 ± 0.7	8 ± 0.5	8 ± 1
Temperature (°C)	28 ± 3	30 ± 2	29 ± 3

3.4. Behavioral indicators (resistance tests)

The results of behavioral indicators of L. *vannamei* postlarvae, during the two sampling periods, shown in Table 3. Based on the results, in both periods, there was no significant difference in survival rate between different hatcheries in the salinity 0% and formalin 50 ppm tests and the percentage of swimming activity ($P > .05$). Also, there was no significant difference in the results of the 50% salinity stress test between hatcheries in the first sampling period. In the first sampling period, the survival rate significantly decreased in the hatchery no. 1 in the formalin 200 ppm stress test ($P < .05$). The survival rate in the 50% salinity stress test was significantly better in hatchery no. 2 during the second sampling period ($P < .05$). There was no significant difference in the other resistance stress assays between hatcheries during the second sampling period ($P > .05$). The hatchery no. 3 had the best survival rate among three hatcheries concerning stress assays and swimming activity.

3.5. Carcass biochemical composition

The results of the biochemical composition of carcasses during the two sampling periods shown in Table 4. The amount of protein, moisture, and energy in both periods did not show a significant difference between different hatcheries ($P > .05$). The lipid percentage in both periods showed a significant difference between different hatcheries ($P < .05$). In the first period, the highest lipid percentage observed in Hatchery no. 3 (31.23 ± 0.04) and the lowest rate observed in Hatchery no. 1 (29.17 ± 0.68). In the second period, the highest percentage of lipid observed in Hatchery no. 2. (30.35 ± 0.41) and the lowest amount observed and recorded in Hatchery no. 3 (29.29 ± 0.67). Also, only Hatchery no. 3 had significant differences between the two periods ($P < .05$). The percentage of ash in both periods showed a significant difference between the three hatcheries ($P < .05$). In the first period, the highest percentage of ash was observed in Hatchery no. 2 (2.65 ± 0.43) and the lowest rate observed and recorded in Hatchery no. 1 (0.95 ± 0.15). In the second period,

Table 2
Morphological characteristics of PL12 specimens during two sampling periods in shrimp postlarvae in different hatcheries (Mean ± SE).

Morphometric parameters	Sampling period					
	1st sampling period			2nd sampling period		
	Hatchery 1	Hatchery 2	Hatchery 3	Hatchery 1	Hatchery 2	Hatchery 3
Total weight (g)	0.02 ± 0.00^{b}	0.06 ± 0.00^{b}	0.13 ± 0.02^{a}	0.04 ± 0.00^{b}	$0.12 \pm 0.00^{a*}$	0.03 ± 0.00^{b}
Total length (mm)	5.86 ± 0.12^{b}	8.06 ± 0.08^{a}	7.70 ± 0.25^{a}	5.73 ± 0.08^{b}	$7.10 \pm 0.05^{a*}$	$4.73 \pm .08^{c*}$
Carapace length (mm)	1.26 ± 0.03^{c}	1.66 ± 0.06^{b}	1.96 ± 0.03^{a}	$1.46 \pm 0.03^{b*}$	$1.86 \pm 0.03^{a*}$	$1.43 \pm 0.18^{b*}$
Intestinal length (mm)	2.70 ± 0.10^{b}	3.86 ± 0.03^{a}	4.30 ± 0.20^{a}	$3.36 \pm 0.06^{b*}$	$4.30 \pm 0.05^{a*}$	$2.60 \pm 0.05^{c*}$
Intestinal fullness rate (%)	16.66 ± 5.2^{b}	21.50 ± 3.6^{b}	33.95 ± 4.8^{a}	23.80 ± 2.6^{b}	$49.53 \pm 6.5^{a*}$	44.61 ± 5.3^{a}
6th intestinal segment diameter (mm)	0.30 ± 0.01^{c}	0.42 ± 0.01^{b}	0.47 ± 0.01^{a}	0.32 ± 0.00^{b}	0.45 ± 0.00^{a}	$0.26 \pm 0.00^{b*}$

* The presence of dissimilar letters in each row indicates a significant difference in each sampling period between the studied hatcherys ($P < .05$). * The star sign in each row indicates a significant difference in each hatchery between the two sampling periods ($P < .05$).

Table 3
Behavioral Indicators (Resistance Tests) of PL12 specimens during two sampling periods in shrimp postlarvae in different hatcherys (Mean ± SE), all of resistance tests are based on survival percentage.

Morphometric parameters	Sampling period					
	1st sampling period			2nd sampling period		
	Hatchery 1	Hatchery 2	Hatchery 3	Hatchery 1	Hatchery 2	Hatchery 3
Salinity 0%	2.66 ± 1.20^{a}	2.33 ± 0.88^{a}	5.50 ± 2.50^{a}	3.33 ± 0.88^{a}	4.50 ± 0.50^{a}	3.33 ± 0.88^{a}
Salinity 50%	94.33 ± 2.33^{a}	96.00 ± 3.05^{a}	94.00 ± 3.05^{a}	89.33 ± 0.66^{b}	96.00 ± 0.57^{a}	88.33 ± 1.66^{b}
Formalin (100 ppm)	96.50 ± 0.50^{a}	94.00 ± 2.08^{a}	97.66 ± 0.88^{a}	96.33 ± 1.66^{a}	96.66 ± 1.33^{a}	97.33 ± 0.33^{a}
Formalin (200 ppm)	55.00 ± 5.00^{b}	83.33 ± 7.26^{a}	93.33 ± 3.28^{a}	89.00 ± 2.08^{a*}	91.00 ± 1.52^{a}	93.66 ± 0.66^{a}
Swimming rate (%)	91.66 ± 3.92^{a}	92.00 ± 1.15^{a}	94.00 ± 1.52^{a}	90.66 ± 1.76^{a}	90.66 ± 2.96^{a}	94.66 ± 1.45^{a}

* The presence of dissimilar letters in each row indicates a significant difference in each sampling period between the studied hatcherys ($P < .05$). * The star sign in each row indicates a significant difference in each hatchery between the two sampling periods ($P < .05$).

Table 4
Post-larval bacterial colony count of PL12 of L. *vannamei*, in the different hatcherys over two periods (Mean ± SE).

Parameter	Sampling period					
	1st sampling period			2nd sampling period		
	Hatchery 1	Hatchery 2	Hatchery 3	Hatchery 1	Hatchery 2	Hatchery 3
Bacterial Colony Count	6.53 ± 0.93^{a}	8.88 ± 0.34^{a}	6.37 ± 1.10^{a}	2.00 ± 2.00^{b}	7.81 ± 0.81^{a}	7.57 ± 0.84^{a}

* The presence of dissimilar letters in each row indicates a significant difference in each sampling period between the studied hatcherys ($P < .05$).
* The star sign in each row indicates a significant difference in each hatchery between the two sampling periods ($P < .05$).

the highest ash rate observed in the hatchery no. 2 (2.73 ± 0.12), and the lowest amount observed in hatcheries no. 1 and no. 3 (0.60 ± 0.00). Also, only the hatchery no. 3 had significant differences in the two periods ($P < .05$). The percentage of carbohydrates in both periods showed a significant difference between different hatcheries ($P < .05$). In the first period, the highest percentage of carbohydrates was observed in hatchery no. 2 (18.88 ± 1.35), and the lowest rate observed in Hatchery no. 3 (14.43 ± 0.33). In the second period, the highest percentage of carbohydrates observed in Hatchery no. 2 (17/30 ± 2.17), and the lowest amount observed and recorded in Hatchery no. 3 (11/07 ± 0.77). Also, only Hatchery no. 3 had significant differences in the two periods ($P < .05$).

3.6. Microbial analyses

The results of the study of post-larval quality of L. *vannamei*, based on microbial characteristics during two sampling periods, shown in Table 5. The number of bacteria counted in both periods did not show a significant difference between different hatcheries ($P > .05$). In the first period, the highest number of colonies observed in Hatchery no. 2 (8.88 ± 0.34 log 10 CFU/g) and the lowest amount observed and recorded in Hatchery no. 3 (6.37 ± 1.10 log 10 CFU/g). In the second period, the highest rate observed in the hatchery no. 2 (7.81 ± 0.81 log 10 CFU/g), the amount of which did not significantly differ from Hatchery no. 3, and the lowest rate observed and recorded in Hatchery no. 1 (2.00 ± 2.00 log 10 CFU/g). Also, neither of the hatcheries showed significant differences in the two periods ($P > .05$).

3.7. Histopathological study

According to the microscopic study of PLs in hatchery no. 1, the hepatopancreas was relatively small and have not yet developed much. However, there were amounts of food in the intestine (). The intestinal wall was thin and covered by small epithelial cells.

In hatchery no. 2, hepatopancreatic tissue more developed in comparison with hatchery no. 1. The hepatopancreatic tissue had more B cells with different vesicles. Also, the intestine is dilated and contains food particles ().

The larvae in the third hatchery have a completely dilated intestine and contain food. The hepatopancreatic tissue was larger and consists of multiple B cells with large vesicles. Also, blind end tubules that connect to the intestine, was dilated. This issue is due to an increase in hepatopancreas secretion.

Table 5
Carcass biochemical composition of PL12 of *L. vannamei* during two sampling periods in different hatcherys (Mean ± SE).

Carcass biochemical composition	Sampling period					
	1st sampling period			2nd sampling period		
	Hatchery 1	Hatchery 2	Hatchery 3	Hatchery 1	Hatchery 2	Hatchery 3
Protein (%)	41.27 ± 1.99^{a}	40.35 ± 1.06^{a}	39.24 ± 0.38^{a}	40.28 ± 0.85^{a}	39.06 ± 0.24^{a}	38.70 ± 0.04^{a}
Lipid (%)	29.17 ± 1.99^{b}	30.28 ± 0.53^{ab}	31.23 ± 0.04^{a}	29.58 ± 0.68^{a}	30.35 ± 0.41^{a}	29.29 ± 0.67^{a*}
Moisture (%)	46.06 ± 2.88^{a}	45.67 ± 0.72^{a}	40.02 ± 1.62^{a}	42.98 ± 0.98^{a}	45.15 ± 2.28^{a}	42.65 ± 1.46^{a}
Ash (%)	0.95 ± 0.15^{b}	2.56 ± 0.43^{a}	2.06 ± 0.29^{a}	0.60 ± 0.00^{b}	2.73 ± 0.12^{a}	0.60 ± 0.20^{b*}
Carbohydrate (%)	17.94 ± 0.96^{ab}	18.88 ± 1.35^{a}	14.43 ± 0.33^{b}	13.31 ± 1.46^{ab}	17.30 ± 2.17^{a}	11.07 ± 0.77^{b*}
Energy (KJ)	2135 ± 58.38^{a}	2157 ± 14.18^{a}	2169 ± 7.25^{a}	2128 ± 39.79^{a}	2130 ± 26.69^{a}	2079 ± 11.95^{a}

* The presence of dissimilar letters in each row indicates a significant difference in each sampling period between the studied hatcherys ($P < .05$).
* The star sign in each row indicates a significant difference in each hatchery between the two sampling periods ($P < .05$).

4. Discussion

The larval quality management in shrimp hatcheries directly impacts L. *vannamei* farming management in the grow-out systems. Also, both play a critical role in the sustainable production of farmed shrimp. The production of L. *vannamei* in shrimp farms is often influenced by the quality of the postlarvae (Ferreira et al., 2011). Therefore, the present study examined the quality of L. *vannamei* postlarvae from different hatcheries with different management. The morphological indices determine the situation of aquatic animals' growth and monitor the availability of food resources. Also, these indices identify possible differences between the environmental conditions of aquatic species (King et al., 2007; Isa et al., 2012). According to the results of the present study, in general, the best growth indices (total length, total weight, carapace length, intestinal length, intestinal fullness rate, and sixth abdominal segment diameter), in two sampling periods, were observed in hatcheries No. 3 and 2. All the values were less than the values measured and recommended by Hernandez et al. (2001) on the PL12 of *L. vannamei*. The fluctuation in weight and length of the postlarvae in hatchery no. 3, in the second period of the sampling, could be due to the reuse of broodstocks during the two periods in the said hatchery (Ouraji et al., 2011). Also, based on management parameters that obtained using a questionnaire, using diet including algae, artemia, and formulated food, and assessment of larval growth and survival and light intensity in hatcheries 2 and 3, maybe the most critical reasons affecting the postlarval growth parameters (Wang et al., 2004; Zhang et al., 2006; Ricardo et al., 2008). Moreover, the basic need for energy and protein depends on the weight of the shrimp. This need is higher in high-weight shrimp than in low-weight shrimp (Li et al., 2015). Various studies show that large shrimps are more efficient in storing energy and protein in the body than smaller shrimps. As a result, the weight of the shrimp or its size is an important parameter affecting the body's metabolic processes and can affect the growth rate of the shrimp (Hossain et al., 2009). It is notable that there is a direct relationship between the growth status and survival of the larvae. Thus, with the increase in growth factors, the survival rate also improved is consistent with the results of the present study.

According to the results obtained in the first sampling period, the highest percentage of survival, in salinity of 0%, was related to hatchery no. 3. In the second period of sampling, it associated with hatchery no. 2. This survival rate in both periods was lower than the rate reported by the National Veterinary Organization (2019). Also, considering that similar results obtained in all three hatcheries during both periods, it can be said that this test is not suitable for evaluating the quality of postlarvae. Because this species is compatible with saltwater, and it will not be very resistant to freshwater. Therefore this stress test cannot be a good indicator for measuring the quality of the postlarvae. According to the results of 50% salinity test, in both periods, hatcheries no. 2 and no. 3, had the highest survival rate. These results were consistent with the standard reported by the State Veterinary Organization (2009). According to the information collected in the questionnaire, an increase in the resistance of postlarvae in hatcheries no. 2, and no. 3, can be attributed to their proper management status (health management, water management, and nutrition management). Probably, the main factors for resistance to the stress test are using different diet sources, lighting intensity (1250 lx), and no fungal or protozoan contamination in postlarval tanks. The results of the formalin test also showed that among the three hatcheries, as well as in the two sampling periods, the postlarvae of hatchery no. 3 showed the highest percentage of survival. According to studies conducted on the survival rate with salinity and formalin stress tests in the postlarval stages of PL15, PL5, PL1 of Indian white shrimp (*Fenneropenaeus indicus*); larvae that had a better growth status compared to formalin tests, showed better resistance (Azari Takami et al., 2004) similarly in the present study, hatchery no. 3 had the best growth rate and survival rate among three hatcheries during the two sampling periods. In 2014, Zare et al. stated that salinity is one of the most important environmental factors that affect the growth and survival of shrimp in the Panaeidae family, especially in nursery ponds areas that may expose to rapid saline changes and environmental conditions (Kumlu et al., 2000). The increase in environmental salinity leads to increased disturbance in the body physiological processes, and it will lead to the animal death. The tolerance range of each shrimp varies according to these changes (Shakori, 1994); therefore the postlarvae, which were in the 0% salinity, had less survival. The previous researches reported that the growth and survival of aquatic animals decreased outside their salinity tolerance; and even led to mortality at higher or lower salinity levels (*Penaeus indicus* (Abedian Kennari and Pagheh, 2007; Vijayan and Diwan, 1995), *Cherax destructor* (Mills and Geddes, 1980), *Penaeus monodon* (Hajra et al., 1988), *Cherax quadricarinatus* (Meade et al., 2002)). Furthermore, Ahmed et al. (1999) showed that water quality management and fertilization have a positive impact on production performance of Abedian Kennari and Pagheh, 2007*P. monodon*. Formalin and decreased salinity resistance vary with age. Stress tests, which are fast, cheap, and simple, can be used in shrimp hatcheries as a quality control method (Samocha et al., 1998). Studies have shown that the size and quality of the larvae depends on the diet.

The biochemical composition of the body of a penaeid shrimp varies widely, depending on several factors, including species, size, sex, season, and sexual orientation (Viswanathan and Suseela, 2000). Because there was no report on the percentage of carcass biochemical compounds in the postlarval stage of this species. The comparison made with the rates obtained from the adult species. The percentages of protein and lipid recorded in the present study were higher than Gunalan et al. (2013). Based on the information collected in the questionnaires during the two sampling periods, the reason for the higher percentage of postlarvae proteins in hatchery no. 1, can be considered as an increase in the percentage of nutrition, which increases the percentage of protein in post-larval carcass protein content. According to the results of the present study, the percentage of moisture in two sampling periods was less than the percentage reported by Gunalan et al. (2013) for the adult species of L. *vannamei*, which certainly has a higher percentage of moisture during the adult stage. The ash content in two sampling periods was the highest in the postlarvae of the hatchery no. 2, although not significantly different from hatchery no. 3, which was higher than the Gunalan et al. (2013). Growth in crustaceans is an increase in body dry weight that usually occurs during molting when the adsorbed water replaced by protein (Thomas, 1993). The difference in the body's biochemical composition of aquatic species depends on factors such as age, sex, environmental conditions, and season, without a doubt, the main difference in the biochemical composition is related to food quality and even the percentage and amount of daily food (Razavi Shirazi, 2000). According to the information collected via questionnaire from hatchery no. 1, it had the highest feeding percentage, as a result of which the highest percentage of protein observed. Fatty compounds are the most important aspect of the quality of aquatic food depending on the type of nutrition (Medina et al., 1995). In general, the body's biochemical composition is always affected by the diet composition, the amount of food, and the percentage of daily food intake (Gawlicka et al., 2002). The dietary quality of various studies shows that the physical and morphological conditions of living organisms and environmental and nutritional factors are among the factors that affect the biochemical composition of the shrimp (Weatherley and Gill, 1987). The composition of the body in penaeid shrimp depends on factors such as the quantity and quality of food, storage density, and water quality (Cruz-Suarez et al., 1993). Indeed, the most important reason for the difference in chemical composition is the amount and type of food received by living organisms (Razavi Shirazi, 2000).

According to the results obtained in the first sampling period, the lowest number of bacterial colonies observed in hatchery no. 3, which shows the better condition of this hatchery than other hatcheries. Sivasankar and Jayabalan (1995), consider salinity to be one of the

main causes of *V. harveyi* release and find the salinity factor to be more effective in the release of this bacterium than other factors such as oxygen, temperature, and pH. Under stressful conditions and especially poor farm management, it can cause disease in farmed shrimp. Among the pathogens reported in different life stages of Penaeid shrimp, bacterial agents have caused the most infection and disease after viruses. Bacterial genus, including vibrio, identified as part of the natural microflora of water and shrimp, which can cause vibriosis (Fulks and Main, 1992). These bacteria are commonly known as opportunistic bacteria and have even been isolated from seemingly healthy shrimp hemolymph. Therefore, it is difficult to separate them for the interpretation of disease (Gomez-Gil et al., 1998). Weaker shrimps are more susceptible to contamination, and malnutrition, increased organic matter load, and lack of hygiene and management, bacterial contamination of pools, and consequently increase the microbial load of post-larvae. According to the results obtained in the present research, most of the bacteria isolated from shrimp ponds were vibrio, although these bacteria considered as flora, they considered as pathogenic bacteria in shrimp. Also, a high microbial load as a stressor can prevent the proper growth of postlarvae.

According to the microscopic study of PLs in hatchery no. 1, the hepatopancreas was relatively small and have not yet developed much. However, there were some amounts of food in the intestine. The intestinal wall was thin and covered by small epithelial cells. In hatchery no. 2, hepatopancreatic tissue more developed in comparison with hatchery no. 1. They had more B cells with different vesicles. Also, the intestine is dilated, and contains food particles. The larvae in the third hatchery have a completely dilated intestine, and contain food. The hepatopancreatic tissue was larger and consists of multiple B cells with large vesicles. Also, blind end tubules, which connected to the intestine, were dilated. This issue is due to an increase in hepatopancreas secretion. Postlarvae in the hatchery no. 3, had larger hepatopancreas and more large intestines, and mostly full of food. The postlarvae of this hatchery were in a better state of growth and resistance than other hatcheries. Also, according to the information provided by the questionnaires from the management of the hatcheries, during the two sampling periods, it can be said that one of the reasons for the better quality of the postlarvae in hatchery no. 3, is the tanks separation. Cultivation of PLs is one of the productive reservoirs that can be a reason for the ease of management and production of healthier and better quality larvae. The increase in hepatocellular vacuolated cells is due to high activity against environmental factors, high metabolism, and increased cellular immunity. The researchers believe that hepatopancreas size, shape, and the cell types are affected by environmental factors such as satiation, toxins, chemicals, and internal or physiological factors (Sanchez, 2006; Saravana and Gerdaline, 2000).

5. Conclusion

Based on the results of the present study, the quality of postlarvae was comparable with the national and international standards. The appropriate management in hatchery no. 3, considered as the main factor resulting in better quality for postlarvae production. Also, the results of the present study showed that the histopathological method is a reliable diagnostic technique for diagnosing postlarval quality in the early stages (postlarval stage) and before introducing postlarvae into the farms. Also, morphological indicators and formalin test (200 ppm) can be considered as the most appropriate indicators for evaluating post-larval quality among the indicators of postlarval quality assessment.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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