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Comparative analysis between *Artemia parthenogenetica* and *Artemia franciscana* size from China, Vietnam and United States of America Sources

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Abstract *Artemia*, also known as brine shrimp, are important organisms in the aquaculture industry due to their ability to adapt to high salinity environments and high nutritional value, making them suitable live food for various aquatic species. Our research is focused on two distinct species of *Artemia franciscana* and *Artemia parthenogenetica* from various geographical regions. Because of the current demand, *Artemia* is the preferred the choice for small-sized live feed for aquatic larvae. Hence, it is crucial to choose suitable artemia sources that correspond with the dietary requirements of aquatic larvae. The results were significantly differed between the species, suggesting potential for optimizing specific strains tailored to distinct aquaculture applications. The findings revealed notable disparities across the species, suggesting the possibility of enhancing certain strains customized for specific aquaculture purposes. The sizes of cysts and the Instar I stage in *Artemia franciscana* from Vietnam were 31.7 ± 7.25 µm smaller than those of *A. franciscana* from United States of America and *A. parthenogenetica* from China. The gathered statistics provided preliminary criteria for choosing strains that are well-suited size of live feed to certain aquaculture hatcheries.

Keywords: Artemia, Hatching efficiency, Live feed, Nursery, Aquaculture, Brine shrimp

Introduction

Artemia spp. commonly known as brine shrimps are anostracan crustaceans found in harsh hypersaline environments worldwide, showcasing remarkable adaptation abilities. These organisms can thrive in extreme saline conditions, tolerating significant fluctuations in salinity, ionic composition, temperature, and oxygen levels (Machado Cardoso, 2023). Inland water *Artemia* spp. populations exhibit unique biological characteristics shaped by their habitat's isolation and varying ionic compositions, resulting in distinct

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morphological variations and adaptations (Mejía *et al.*, 2013). *A. franciscana* and *A. parthenogenetica* exhibit differences in morphology and habitat preferences. *A. franciscana*, a highly invasive species (Biju *et al.*, 2023). Meanwhile, *A. parthenogenetica* is a parthenogenetic population within the Artemia genus (Criel, 2018). *A. franciscana* thrives in hypersaline environments in the United States of America (USA), producing diapause cysts for survival, while *A. parthenogenetica* is adapted to different ecological niches, with variations in temperature salinity tolerances and genetic shifts in temperature optima (Lenz and Browne, 2018). The external morphology of Artemia reveals an extremely thin exoskeleton covering the entire body, with specific details on the larval stages and nervous system structure (Parraguez, 2022). These differences in morphology and habitat adaptations contribute to the ecological success and distribution patterns of these Artemia species (Gajardo and Beardmore, 2012).

Artemia spp. feed size is crucial in aquaculture due to its impact on the growth and development of fish larvae. Small-sized artemia are preferred for feeding fish larvae because they match the size of the larvae's feeding organs, leading to efficient consumption and digestion (Madkour *et al.*, 2022). Additionally, small *Artemia* spp. are essential during the weaning process of fish larvae, as they can be easily consumed by smaller fish sizes, aiding in their growth and survival rates (Planas *et al.*, 2017). Enriching artemia with essential elements further enhances their nutritional value, promoting better growth performance and survivability of various fish species (Kandathil Radhakrishnan *et al.*, 2020). Challenges persist when utilizing artemia nauplii for early feeding of shrimp, pelagic fish, and crab larvae due to prey size limitations, resulting in decreased survival rates. This underscores the need to explore alternative approaches, such as developing smaller artemia cysts, to meet the demands of the aquaculture industry. The global market demands small artemia cysts to enhance feeding strategies, offering advantages like improved digestibility and utilization, potentially leading to better growth and overall health. Additionally, their smaller size benefits production and transportation, increasing their attractiveness to aquaculture producers. Despite the success of selective breeding in other aquatic species, limited attention has been given to artemia selection, presenting a significant opportunity for further research and development in artemia genetics and breeding.

Brine shrimp (*A. franciscana*) is a native strain from USA (Vanhaecke and Sorgeloos, 1980), play a significant role in the unique aquatic habitat of the Great Salt Lake (GSL) in Utah. These invertebrates are able to thrive in the hypersaline conditions of the GSL, where the salinity ranges from approximately 75 to 150 g/L, depending on annual rainfall (Null and Wurtsbaugh, 2020). The lake's status as a terminal lake with limited freshwater input contributes to its high salinity levels. Additionally, the GSL has high dissolved organic carbon concentrations, ranging from 15 to 55

mg/L. The extreme salinity of the GSL severely restricts its biological diversity, making it uninhabitable for fish. However, brine shrimp have substantial commercial value due to their cysts, which are harvested and sold as tropical fish food (Camargo *et al.*, 2005). Furthermore, the global aquaculture industry relies heavily on Artemia production in Vietnam's Mekong Delta. However, this industry faces environmental and technical challenges that impact its productivity and profitability (Nguyen *et al.*, 2020). *A. franciscana* from the GSL is widely used in hatcheries worldwide, with late-hatched cysts and unconsumed nauplii often entering the environment through hatchery effluents. This has led to the species appearing in new coastal areas worldwide, complicating the species status of cyst products from these regions. For instance, artemia products from Bohai Bay, China, may contain a mix of local parthenogenetic strains, inland Chinese *A. sinica*, and introduced *A. franciscana*. Market artemia comes from feral strains or populations that have adapted to new environments introduced by humans, such as those in the Vietnamese saltworks (Dhont and Van Stappen, 2003; Van Stappen *et al.*, 2020). Brine shrimp (*A. parthenogenetica*) is a parthenogenetic lineage of the brine shrimp Artemia, has been extensively studied in various regions, including China. Lin *et al.* (2006) has identified ten strains of *A. parthenogenetica* collected from inland salt lakes and coastal salterns in China, revealing significant genetic variation among these populations. This highlights China as a key region for the presence and study of *A. parthenogenetica*.

The research aimed to determine the embryology of two forms of *Artemia* spp.; *A. parthenogenetica* and *A. franciscana* from different sources. It included a comparative analysis of *A. franciscana* sourced from Vietnam and the USA, focusing on their unique and distinctive biological traits and potential in hatchery live feed. The study was compared with Artemia's hatching performance and size of each stage from cyst nauplii to adults.

Materials and methods

Experimental setup

In this section, *Artemia* spp. samples originating from three different locations: *A. parthenogenetica* was designated as representative of Chinese artemia cysts (CN) (Lin *et al.*, 2006; Pang *et al.*, 2024), Meanwhile, *A. franciscana* was selected as the representative species for Artemia cysts from Vietnam (VN), specifically from the Vinh Chau salt fields in the Research Station for Artemia, as well as from the USA, sourced from the Great Salt Lake, North America. Which has been designated as a geographic indication Shown by Artemia Vietnam Cysts (425 g/can) were carefully prepared for the experiment according to a specific protocol. The experimental design included important parameters such as tank size (test glass cylinder tube with

a volume of 1,000 mL) and water volume with aeration. Meticulous care was taken to maintain consistent conditions throughout the entire experimental setup. The temperature in the laboratory is controlled within a range of 27-29 °C to simulate an optimal environment for Artemia cultivation. Each Artemia sample is weighed precisely to 0.25 grams to ensure consistency and reliability throughout the testing process. Three replications were performed to validate the findings. Additionally, the water's salinity level was 30 ppt, which included a pH level of 8.0, dissolved oxygen (DO) of more than 4 mg/l, and an alkalinity of 1,200 mg/l as $CaCO_3$ was meticulously monitored and controlled throughout the 24-hour experiment to ensure accuracy and reliability of the results thus provide a strong foundation for analysis and interpretation in later studies.

Artemia collection and feeding protocol

Artemia nauplii were collected using a fine mesh net with a 100-µm mesh size to effectively capture the nauplii while allowing water to flow freely. The mesh size was chosen to ensure efficient collection based on Artemia size. Sampling from each experimental group was conducted to assess hatching performance after 24 hours.

Following the hatching performance assessment, aeration was discontinued to continue culturing the Artemia for an additional three weeks. The Artemia were fed *Chaetoceros* sp. phytoplankton at a concentration of approximately 2.5×10^5 cells/mL. Microalgae density was monitored and quantified using a hemocytometer with a depth of 0.1 mm. At the end of the three-week culture period, artemia from each group were imaged and their size measured to evaluate growth.

Image analysis size of artemia

For the image analysis of Artemia, researchers employed a 10x and 40x magnification microscope equipped with the USB Microscope Camera Dino-Lite AM4025X. Utilizing the dinoXscope image analysis software, they were able to precisely measure the size of the artemia cyst and artemia Instar I (n=100 per treatment). This magnification level facilitated detailed inspection and measurement of Artemia samples, enables researchers to gather accurate data for analysis.

Assessment of Artemia hatching performance

In evaluating the hatching performance of Artemia, key metrics include the percentage of potential hatching (%PH), the percentages of absolute hatching (%AH), and the percentage of cyst purity (%Cyst Purity) (Asem, 2011).

Percentage of Potential hatching (%PH): the number of larvae harvested from 100 shells, perfect and cracked cysts.

$$\%PH = \frac{Nauplii}{Total\ Cysts} \times 100$$

Percentages of Absolute Hatching (%AH): is the number of larvae harvested from 100 embryo-bearing cysts whether perfect or cracked

$$\%AH = \frac{Nauplii}{Total\ Decapsulated\ Cycst} \times 100$$

Percentage of Cyst Purity (%Cyst Purity): percentage of perfect and perfect embryo-bearing cysts out the total embryo-bearing cysts whether perfect or cracked.

$$\%Cycst\ Purity = \frac{Nauplii + Umbrella + Embryo}{Total\ Decapsulated\ Cysts} \times 100$$

Data statistical analysis

The data, which includes the hatching performance and size of artemia, will be subjected to statistical analysis. Prior to performing one-way ANOVA, data normality was assessed using the Shapiro-Wilk test. The data are presented as mean ± SD. Statistical analysis was performed using Ordinary one-way ANOVA, Tukey's multiple comparisons test ($p < 0.05$) for identify variations in the hatching performance and size of artemia specimens under various conditions.

Results

Size of Artemia cyst and nauplius

The results of Artemia cyst observations under the microscope are shown in Figure 1. Measurements of cyst diameters revealed that the mean diameter of *A. franciscana* cysts from VN was 224.3 ± 21.81 µm. This was significantly smaller than that of cysts from the USA by approximately 34.28% ($p < 0.01$), with USA cysts measuring 341.3 ± 21.88 µm. Additionally, the diameter of *A. franciscana* cysts from VN was significantly smaller than that of *A. parthenogenetica* cysts from CN by approximately 22.41% ($p < 0.01$). The mean diameter of CN cysts was 289.1 ± 23.37 µm.

A comparison between *A. franciscana* cysts from the USA and *A. parthenogenetica* cysts from CN revealed that USA cysts were 18.06% larger than CN cysts ($p < 0.01$). Therefore, it can be concluded that the cysts from Vietnam are the smallest among the groups analyzed (Figure 2).

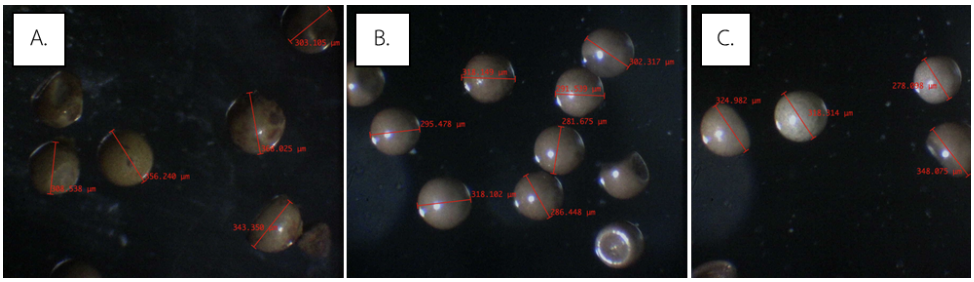

Figure 1. Cyst diameter of Artemia from different sources: China (A), Vietnam (B), and the United States of America (C); This image was taken using a dark-field stereomicroscope at 10x magnification

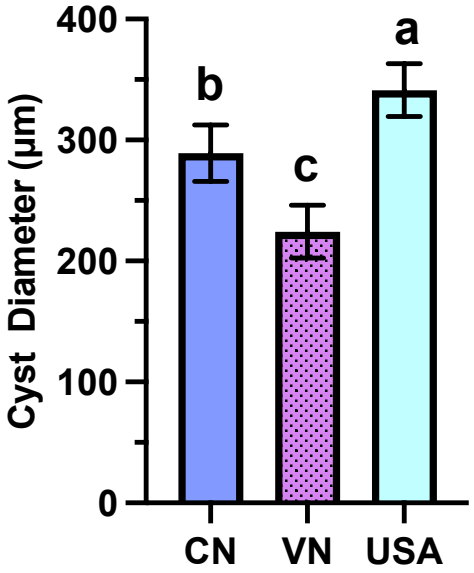

Figure 2. Cyst diameter of Artemia from different sources: China, Vietnam, and the United States of America; Data are presented as mean ± SD; Statistical analysis was performed using ordinary one-way ANOVA followed by Tukey's multiple comparisons test. Different letters indicate significant differences ($p < 0.01$)

After 24 hours under the same controlled environment (Figures 3 and 4), the samples were examined. The results showed that the size of the Instar I stage of *A. franciscana* from VN was 437 ± 26.2 µm, which was significantly smaller than that of the Instar I stage from the USA by approximately 10.45% ($p < 0.01$), with USA nauplii measuring 488 ± 25.4 µm. Additionally, the size of the Instar I stage of *A. franciscana* from VN was significantly smaller than that of *A. parthenogenetica* from CN by approximately 6.62% ($p < 0.01$), with CN nauplii having a mean size of 468 ± 17.1 µm.

The comparison between *A. franciscana* nauplius Instar I from the USA and *A. parthenogenetica* Instar I from CN showed that the USA nauplii were 4.27% larger than those from CN (p = 0.017). These findings indicate that the naupliar size at the Instar I stage follows the same trend as the cyst size, with A. franciscana from VN being the smallest.

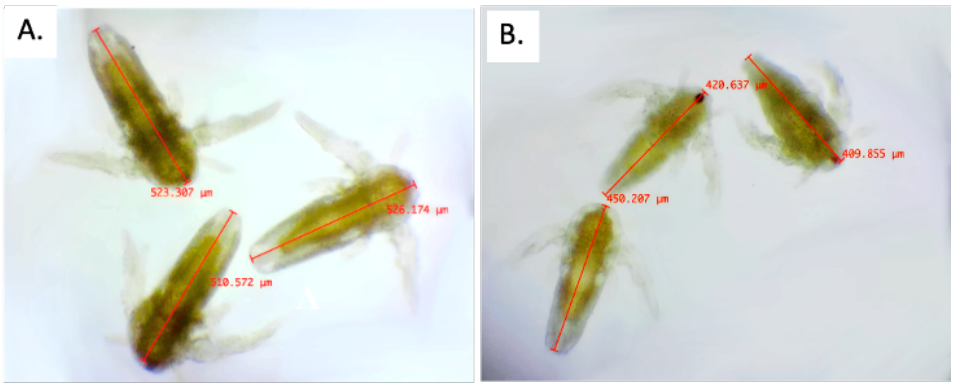

Figure 3. Artemia nauplius Instar I from different sources: United States America (A) and Vietnam (B); This image was taken using a light-field compound microscope at 40x magnification

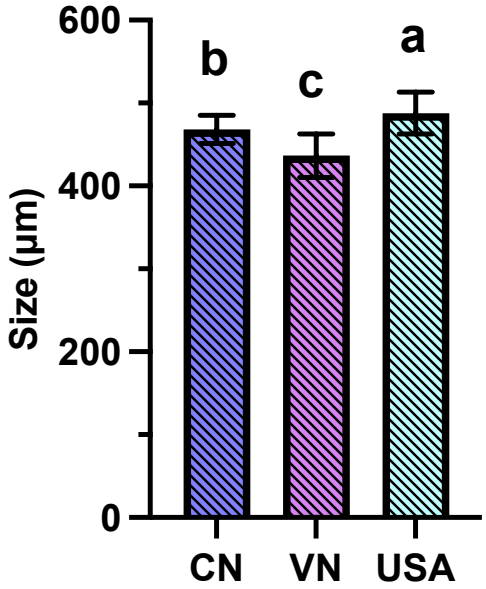

Figure 4. Hatch size of Artemia nauplius Instar I from different sources: China, Vietnam, and the United States; Data are presented as mean ± SD; Statistical analysis was performed using ordinary one-way ANOVA followed by Tukey's multiple comparisons test; Different letters indicate significant differences (p < 0.01)

Hatching efficiency

The Percentage of Potential Hatching (%PH), Percentage of Absolute Hatching (%AH), and Percentage of Cyst Purity (%Cyst Purity) were not significantly different ($p > 0.05$), indicating that the purity of the cysts from each source was similar (Figure 5). Additionally, the controlled environmental conditions temperature 27-29 °C, salinity 30 ppt, pH 8, DO 4 mg/l, and alkalinity 1200 mg/l as $CaCO_3$ had no effect on the hatching rate of the cysts.

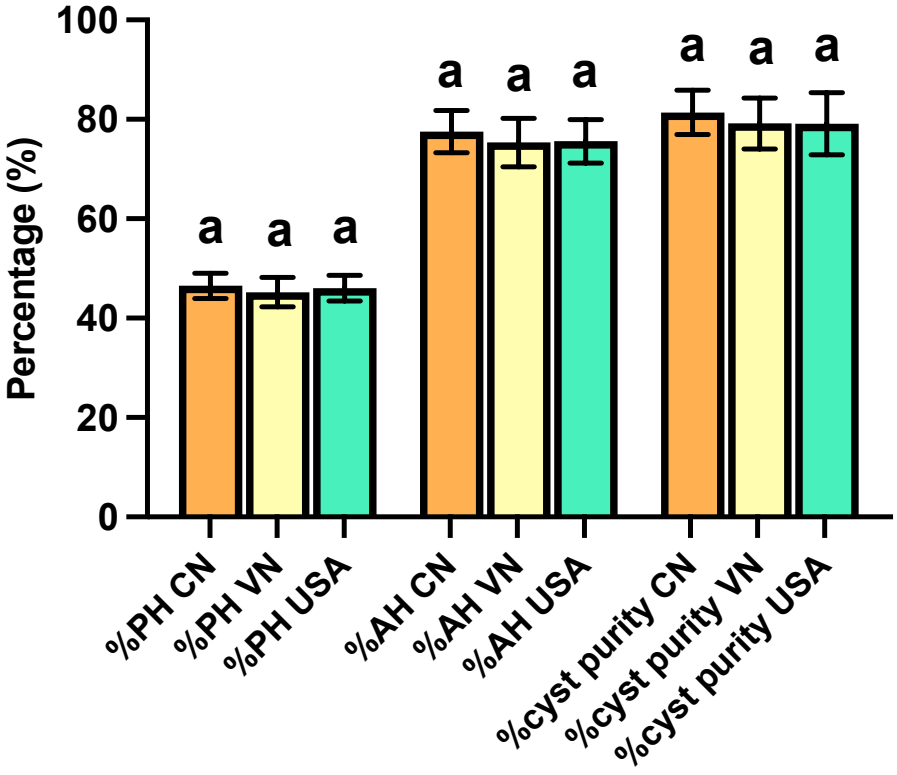

Figure 5. The percentage of potential hatching (%PH), percentage of absolute hatching (%AH), and percentage of cyst purity (%Cyst Purity) from different sources: China, Vietnam and United States America; Data are presented as mean ± SD; Statistical analysis was performed using ordinary one-way ANOVA, followed by Tukey's multiple comparisons test; Different letters indicate significant differences ($p < 0.01$).

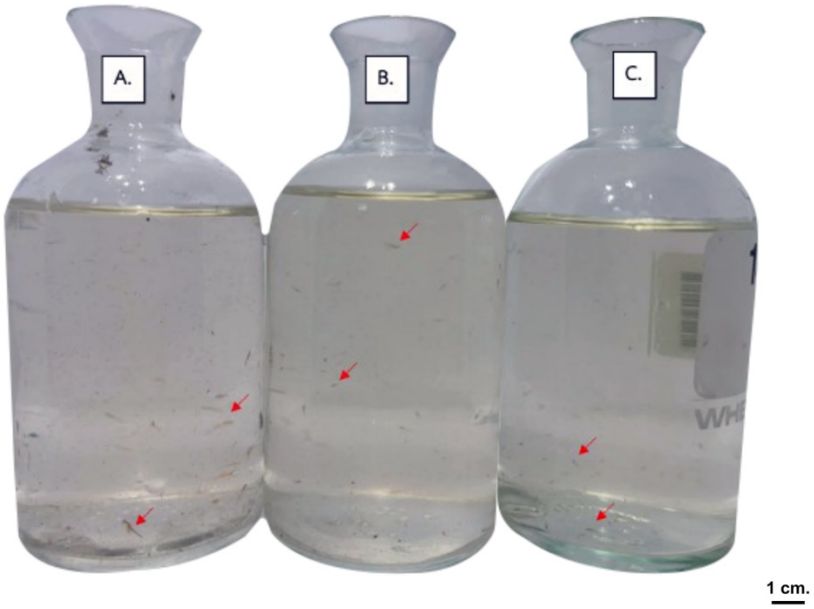

Figure 6. Adult artemia from different sources cultured for 21 days: United states America (A.), China (B.) and Vietnam (C.)

Discussion

A. franciscana cysts range from the Virrila population in Mexico average around 206.07 µm (Castro *et al.*, 2013). Conversely, *A. parthenogenetica* cysts in the Aral Sea Basin range from 210-310 µm on the eastern coast, while those from the southern coast of the Aral Sea, average around 198-330 µm (Utemuratova *et al.*, 2022). These size differences are attributed to both genetic and environmental factors. *A. franciscana* from Vietnam known for its small cyst size, is an important species of the global aquaculture industry, particularly in Vietnam's Mekong Delta. However, this industry faces environmental and technical challenges that impact its productivity and profitability (Nguyen *et al.*, 2020). The previous studies indicate that natural selection for high temperature tolerance plays the dominant role for artemia adaptation in hot conditions (Clegg *et al.*, 2000).

In Vietnam, the technique of integrated seasonal artemia production was widely adopted among artisanal salt farmers, especially in the Mekong Delta, with a dry season which resulted in a substantial upgrade of their economic situation. *A. franciscana* from San Francisco Bay, USA, was introduced because of its high tolerance to elevated water temperatures, its high productivity and its favorable cyst characteristics for aquaculture application. This species has been raised for over 30 years in Vinh Chau salt fields in the Mekong Delta and has adapted to the hot conditions of Vinh Chau (Le *et al.*, 2019). Differences in terms of heat tolerance as well as some

genetic characteristics with the original strain have been demonstrated (Clegg *et al.*, 2000; Kappas *et al.*, 2004). Market artemia, including *A. franciscana*, often originates from feral strains that have adapted to new environments, such as those found in Vietnamese saltworks (Van Stappen *et al.*, 2020). This implies that *A. franciscana* cysts, when dispersed, can introduce various genetic strains into different habitats, influencing the diversity and adaptation of the species in those locations. Moreover, Vietnam used unidirectional mass truncation selection to produce smaller cysts. Researchers achieved this by sieving and culturing selected specimens. As a result, they observed that the cyst size of the selected line decreased, Van *et al.* (2014) reported a gradual reduction in cyst size, with an approximate decrease of 3% per generation. By the end of the selection process, the cumulative reduction in cyst size ranged between 5.82% and 6.07% compared to the original population (Van *et al.*, 2014). This finding is consistent with our results, indicating that the cysts of *A. franciscana* cultured in Vinh Chau have significantly decreased in size compared to the original commercially available USA strain. Therefore, our study suggests that the selection process through sieving could contribute to the production of smaller cysts. This is further supported by the fact that researchers at the Vinh Chau Research Station have not introduced a new Artemia population to their farm since then.

Artemia adult size and growth after 21 days (Figure 6), was observed that the size of adult Artemia varied noticeably between different countries. When raised in the same environment, adult Artemia from Vietnam were the smallest, while those from America and China were similar in size. Consistent with other experimental results, both the diameter of the cysts and the size of Instar I Artemia from Vietnam were the smallest. This difference is attributed to them being different species with different origins (Triantaphyllidis *et al.*, 1994). After 21 days of culture, we continued rearing the Artemia for several months. However, by the second or third propagation cycle, we could no longer differentiate Artemia sources based on adult size. Nevertheless, the Artemia from the VN tank exhibited a higher number of nauplii at Instar I and II, as well as more juveniles, compared to those from the USA (unpublished data).

The morphological variations in Artemia, including cyst and decapsulated cyst diameter, as well as nauplii and adult length, have been studied based on samples collected from various geographical locations (Mejía *et al.*, 2013). They can be found in isolated habitats ranging from temperate to tropical climates, each with unique ecological, physical, and chemical characteristics. This isolation has led to distinct biological traits, influencing their evolution, morphology, and reproduction, and resulting in speciation (Stella, 1933; Vanhaecke *et al.*, 1987; Mejía *et al.*, 2013). Additional research is needed to assess the nutritional implications of size reduction and its impact on larval survival rates. Future studies should focus

on refining Artemia production strategies to enhance its applicability as an optimal live feed in aquaculture hatcheries.

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