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## Abstract

This paper reported general information and *Artemia* population characterization in small, medium and large-sized solar saltworks along Bohai Bay coastal region in China. The survey was conducted in September and October, 2017. The *Artemia* population in Bohai Bay saltworks are mostly mixed with *A. parthenogenetica* and *A. franciscana*, except for the Chengkou Saltworks where *A. sinica* was also detected. Despite the closer locality and similar climate conditions, the dominant *Artemia* reproductive mode (oviparity vs viviparity), ratio of female/male (parthenogenetic population vs bisexual species), cyst diameter and chorion thickness varied remarkably in different saltworks. This should relate to the local pond conditions, such as food availability and change of salinity. The physiochemical and biological diversity in the brine are need to further studied, to provide more information for better biological management in saltworks.

**Keywords:** *Artemia*, solar saltworks, Bohai Bay

China is the world's biggest salt producer, with the Bohai Bay coastal area in north-eastern China being one of the main production areas, producing about 12 million tons of salt per year. *Artemia* is a main zooplankton population and almost exclusive in the intermediate and high salinity ponds. *Artemia* plays an important role in the saltpond ecosystem and salt production. As a secondary producer, proper *Artemia* density in the saltpond reduce the nitrogen and phosphorous levels in the brine by taking up the phytoplankton and organic detritus. The dead *Artemia* in high salinity pond provide organic substrates for red halophilic bacteria and archaea, which favor the brine

## Current Situation of *Artemia* Resources in Bohai Bay Solar Saltworks, China

evaporation and viscosity reduction (Tackaert and Sorgeloos, 1993).

China is a leading aquaculture country in the world, with an annual aquaculture production of 45 million tons (data of 2014, FAO). As an important live food for marine fish and crustacean larviculture, the demand on *Artemia* cysts has been increased (about 1000 tons in 2016). *Artemia* cysts from the Bohai Bay saltponds are known for higher hatchability and better nutritional value and are thus sold in China at higher price. However, *Artemia* cyst harvest has been decreased dramatically due to the reduced salt production area, unbalanced ecosystem, etc.

In September and October, 2017, we investigated ten solar saltworks along the Bohai Bay coastal region with focusing on characterization of *Artemia* population. The purpose of the study is to better understand the *Artemia* population in the Bohai Bay saltworks, and provide basic information for biological management of saltponds.



**Fig. 1 Distribution of ten solar saltworks along Bohai Bay coastal region**

The saltworks locate in Liaoning Province, Hebei Province, Tianjin Municipal City and Shandong Province, respectively (Fig. 1). The covering area of the saltworks

## Current Situation of *Artemia* Resources in Bohai Bay Solar Saltworks, China

ranged 43-300 square kilometers and their annual salt production in 2017 ranged from less than  $10 \times 10^4$ - $160 \times 10^4$  tons. Among them, Yangkou, Nanpu and Chengkou Saltworks are the top three in terms of area; Yangkou, Nanpu and Chengkou Saltworks are the top three in terms of salt yield (Table 1).

**Table 1 Information of ten solar saltworks**

| No. | Name  | Location                     | Covering area<br>( km <sup>2</sup> ) | Annual salt yield in<br>2017 ( $\times 10^4$ ton ) |
|-----|---|------------------------------|--------------------------------------|--|
| 1   | Yangkou Saltworks                               | N37°16'29'',<br>E118°54'59'' | 300                                  | 160  |
| 2   | Wangdao Saltworks                               | N38°02'36'',<br>E118°51'04'' | -                                    | <10  |
| 3   | Chenghou Saltworks (Huitai Group)               | N38°12'22'',<br>E117°56'46'' | 200                                  | 120  |
| 4   | Wudi Second Saltworks (Fengyuan Salt Chemicals) | N38°14'42'',<br>E117°50'27'' | 43                                   | <10  |
| 5   | Tanggu Saltworks                                | N38°51'37'',<br>E117°35'55'' | 170                                  | 110  |
| 6   | Hangu Saltworks                                 | N39°13'45'',<br>E117°57'37'' | 100                                  | 75   |
| 7   | Nanpu Saltworks                                 | N39°11'11'',<br>E118°10'34'' | 270                                  | 140  |
| 8   | Luannan Saltworks                               | N39°09'29'',<br>E118°20'20'' | -                                    | <10  |
| 9   | Yingkou Saltworks                               | N40°36'13'',<br>E122°12'53'' | 80                                   | 28   |
| 10  | Fuzhouwan Saltworks                             | N39°22'33'',<br>E121°36'09'' | 140                                  | 60   |

*Artemia* were sampled in the intermediate evaporation ponds with salinity range of 10.0-12.3%, where *Artemia* population are mostly condensed in the local saltworks (Table 2). The observed number of *Artemia* in each saltworks was 305-657 individuals. The ratio of female and male (F/M) adults ranged 1.2-2.0, except for Nanpu Saltworks where the F/M was 4.2. In general the female and male adults in bisexual *Artemia* population is approximately equal, and no male *Artemia* exists in parthenogenetic *Artemia* population. Thus we estimate that the F/M ratio is approximately 3 when both bisexual and parthenogenetic population exist in the same water column and in the equal density. F/M>3 should indicate the potential of parthenogenetic population being dominant, whilst F/M<3 indicates dominant bisexual population. Therefore we

## Current Situation of *Artemia* Resources in Bohai Bay Solar Saltworks, China

proposed that parthenogenetic *Artemia* population dominated in Nanpu saltworks, while bisexual *Artemia* dominated in other saltworks.

Mature female *Artemia* has two reproduction modes, being oviparous (producing cysts) and viviparous (producing nauplii). In general, lack of food and sudden change of salinity often result in the oviparous reproduction. Ratio of oviparity and viviparity  $<1$  indicates that females tend to produce nauplii, whilst ratio of oviparous and viviparous  $>1$  indicates that females tend to produce cysts. The ratio of oviparous and viviparous reproduction varied remarkably from 0.7 and 0.8 in Chengkou and Wudi Second Saltworks to 6.5 in Yangkou Saltworks, meaning that *Artemia* produce more nauplii in Chengkou saltworks and Wudi second saltworks, while more cysts production could be obtained in Yangkou Saltworks and other saltworks. Since the investigated saltworks locating along Bohai Bay coastal region and having similar climate conditions, the shift of the reproductive mode should relate to the varied environmental conditions, such as food availability and salinity fluctuation in different saltworks.

Both cysts size and chorion thickness are species and strain dependent. The cysts diameters in the saltworks ranged 225  $\mu\text{m}$  to 242  $\mu\text{m}$ . The maximum value was observed in Chengkou Saltworks and the minimum value was observed in Yingkou Saltworks. The chorion thickness of the cysts ranged from 5.35  $\mu\text{m}$  to 9.76  $\mu\text{m}$ .

**Table 2 Characterization of *Artemia* population in ten solar saltworks**

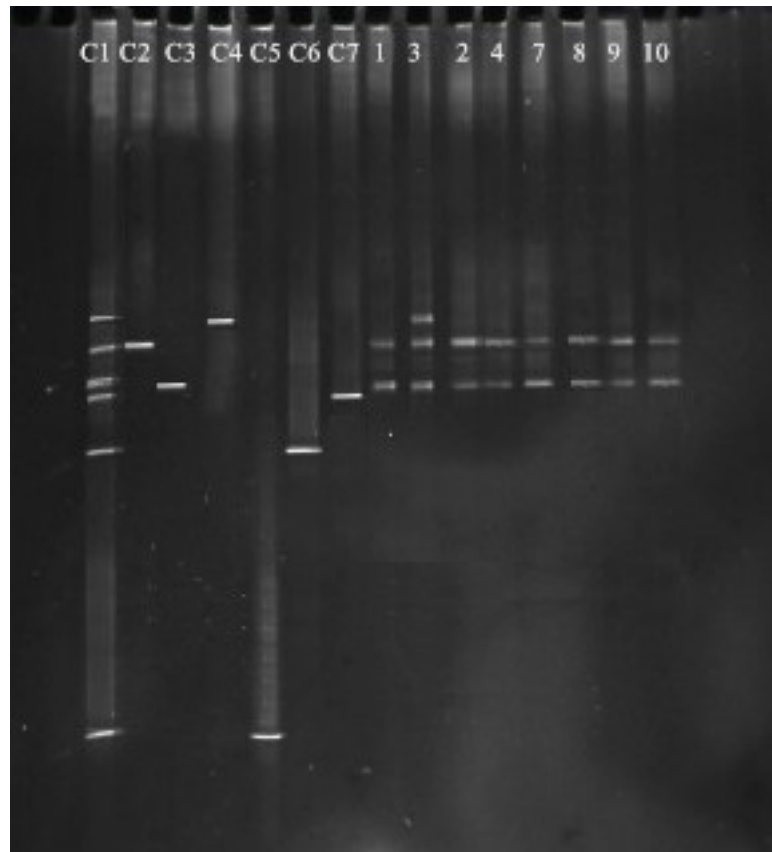
| No. | Name   | Salinity<br>( % ) | Number<br>observed<br>of <i>Artemia</i> | Ratio of<br>Female<br>and<br>male | Ratio<br>of<br>oviparo<br>us and<br>vivipar<br>ous | Cyst<br>diameter<br>( $\mu\text{m}$ ) | Chorion<br>thickness<br>( $\mu\text{m}$ ) | Species<br>composition  |
|-----|--|-------------------|---|-----------------------------------|--|---------------------------------------|---|---|
| 1   | Yangkou<br>Saltworks   | 11.0              | 333                                     | 2.0                               | 6.5  | 233.3 $\pm$ 10.7                      | 5.6 $\pm$ 0.3                             | <i>A. parthenogenetica</i><br><i>A. franciscana</i>                     |
| 2   | Wangdao<br>Saltworks   | 11.2              | 306                                     | 1.2                               | 3.2  | 237.2 $\pm$ 9.8                       | 6.4 $\pm$ 0.3                             | <i>A. parthenogenetica</i><br><i>A. franciscana</i>                     |
| 3   | Chengkou<br>Saltworks<br>(Huitai<br>Group)                     | 10.0              | 603                                     | 1.2                               | 0.7  | 242.5 $\pm$ 16.3                      | 9.8 $\pm$ 0.7                             | <i>A. parthenogenetica</i><br><i>A. franciscana</i><br><i>A. sinica</i> |
| 4   | Wudi<br>Second<br>Saltworks<br>(Fengyuan<br>Salt<br>Chemicals) | 11.5              | 305                                     | 1.2                               | 0.8  | 236.0 $\pm$ 10.7                      | 5.8 $\pm$ 0.4                             | <i>A. parthenogenetica</i><br><i>A. franciscana</i>                     |

## Current Situation of *Artemia* Resources in Bohai Bay Solar Saltworks, China

|    |                     |      |     |     |     |            |         |   |
|----|---------------------|------|-----|-----|-----|------------|---------|---|
| 5  | Tanggu Saltworks    | 12.0 | 621 | 4.4 | 1.4 | -          | -       | -   |
| 6  | Hangu Saltworks     | 12.3 | 598 | 1.6 | 1.7 | -          | -       |   |
| 7  | Nanpu Saltworks     | 11.5 | 315 | 1.5 | 4.2 | 225.1±11.8 | 5.4±0.5 | <i>A. parthenogenetica</i><br><i>A. franciscana</i> |
| 8  | Luannan Saltworks   | 11.0 | 657 | 1.6 | 2.3 | 230.5±11.2 | 6.7±0.4 | <i>A. parthenogenetica</i><br><i>A. franciscana</i> |
| 9  | Yingkou Saltworks   | 11.5 | 495 | 1.5 | 3.9 | 229.8±11.4 | 6.9±0.6 | <i>A. parthenogenetica</i><br><i>A. franciscana</i> |
| 10 | Fuzhouwan Saltworks | 11.0 | 540 | 1.8 | 2.2 | 229.3±10.9 | 5.5±0.3 | <i>A. parthenogenetica</i><br><i>A. franciscana</i> |

\*The cysts diameter and chorion thickness were the average of 100 cysts measurement.

According to our previous study, *Artemia* species in a mixed population can be determined through DGGE techniques using COI gene or 16S-12S ribosomal RNA gene as markers. In this study, the DGGE profile of 16S-12S gene fragment of *Artemia* cysts collected from eight solar saltworks showed that both *Artemia parthenogenetica* and *A. franciscana* existed in most of Bohai Bay saltworks, except for the Chengkou Saltworks where *A. parthenogenetica*, *A. franciscana* and *A. sinica* were detected (Fig. ). The indigenous *Artemia* population in Bohai Bay saltworks is parthenogenetic population, which shifted to the mixed population of bisexual strains, mainly *Artemia franciscana* originated from San Francisco Bay, USA as well as *A. sinica* originated from inland salt lakes in China since earlier 1990s (Guan et al., 2005; Zhou et al., 2006; Zhao et al., 2007). This notable change is due to the artificial inoculation of exotic *A. franciscana* (Tackaert and Sorgeloos, 1991; Zhang & Guo, 1993) and extensive use of commercial *Artemia* cysts in local shrimp and fish hatcheries in Bohai Bay coastal region.



**Fig. 2 DGGE profile of 16S-12S gene fragment of *Artemia* cysts collected from solar saltworks.** C1-C7 are the reference of *Artemia* species obtained in Asian Regional Artemia Reference Center. C1 is the DNA mixture of C2 to C7. C2: *A. parthenogenetica*; C3: *A. franciscana*; C4: *A. sinica*; C5: *A. salina*; C6: *A. persimilis*; C7: *A. tibetiana*

In conclusion, this paper reported general information and *Artemia* population characterization in small, medium and large solar saltworks along Bohai Bay coastal region in China. The *Artemia* population in Bohai Bay saltworks are mostly mixed with *A. parthenogenetica* and *A. franciscana*, except for the Chengkou Saltworks where *A. sinica* was also detected. Despite the closer locality and similar climate conditions, the differentiation on dominant reproductive mode (oviparity vs viviparity), ratio of female/male (parthenogenetic population vs bisexual species), cyst diameter and chorion thickness of *Artemia* in different saltworks should relate to the local pond conditions, such as food availability and change of salinity. Further study is needed in aspect of physiochemical and biological diversity in the brine and to provide more

information for better biological management in saltworks.

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