

# Intentional introduction of *Artemia sinica* (Anostraca) in the high-altitude Tibetan lake Dangxiong Co: the new population and consequences for the environment and for humans\*

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**Abstract** The imbalance between supply and demand of *Artemia* cysts in China and around the world is increasing now. Salt lakes in Tibet may contribute to the solution of the problem. In Northern Tibet there are 26 saline lakes whose salinity and temperature may support *Artemia* survival at an altitude of 4 000–5 100 m. We found *Artemia* in 15 of these lakes. The saline lakes with *Artemia* populations mainly belong to the shallow basin lakes, and the majority of these lakes are small in area. The total area of lakes without *Artemia* is more than 1 000 km<sup>2</sup>. Lake Dangxiong Co (Co means lake in Tibet) was chosen for the intentional introduction of *Artemia sinica*. In 2004, 850 g of *A. sinica* cysts, originating from Qinghai, were introduced in the lake. Surveys in 2006–2014 showed that the average abundance of *Artemia* adults in the lake gradually increased from 20 ind./m<sup>3</sup> in 2006 to 1950 ind./m<sup>3</sup> in 2013. We assume that two subpopulations of *A. sinica*, separated by depth, may exist in the lake. The new *Artemia* population caused an increase in the number of species of phytoplankton and heterotrophic protozoa with a decrease of their total abundance. Water transparency also increased. Dominance in phytoplankton passed from cyanobacteria to diatoms. Changes occurred not only in the lake ecosystem; the number of water birds using the lakes also dramatically increased. Preliminary calculations showed that it is possible to harvest at least about 150 t cysts per year from the lake as well as 3.2 thousand tons of frozen or 350 t of dried biomass of adult *Artemia*.

**Keyword:** *Artemia*; phytoplankton; water birds; Tibet; alien species

## 1 INTRODUCTION

The human population is projected to reach 9 200 million by 2050 (UN, 2007). A fundamental question for science is whether it is possible to increase food production to meet the demands of a human population of that magnitude; it is possible only by increasing aquaculture production (Duarte et al., 2009). Cultivation of larvae is a bottleneck in the cultivation of different commercial organisms. The supply of live food organisms as feed for developing larvae is the weak link in the development of larviculture. *Artemia* nauplii are the main living food for fish larvae now (Lavens and Sorgeloos, 2000). Fluctuations in the harvest of *Artemia* cysts may lead to a rise or fall of production in the aquaculture industry; a sharp

decrease in a harvest of cysts from Great Salt Lake (USA), a leading producer of *Artemia* cysts, in 1994 and 1998 had a pronounced negative effect on aquaculture industry worldwide (Lavens and Sorgeloos, 2000). Additionally, biomass of adult *Artemia* may serve as good protein food for humans and as a resource for the cosmetic industry.

China is one of the countries richest in *Artemia* locations. To date, at least 274 *Artemia* sites have been recorded from China, both of continental and

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marine origin (Zheng and Sun, 2013). In the world there are four main areas of *Artemia* cyst resources, including Great Salt Lake (USA), Russia, Kazakhstan and China; China has 30% of the total world *Artemia* cyst production (Zhang and Zhao, in press). A serious decrease of *Artemia* cyst production in coastal and inland salt lakes in China is now observed due to global climate changes and human interventions (Liu et al., 2014). The demand for *Artemia* cysts in aquaculture continues to increase. Indoor intensive cultivation of *Artemia* is developing (Anh, 2009; Sultana et al., 2011), but this cannot solve the problem now. Rational exploitation and creation of new *Artemia* populations in salt lakes and ponds is one of the keys to overcome the imbalance between supply and demand of *Artemia* cysts in China and around the world (Liu et al., 2014). The salt lakes in Tibet may contribute to a solution of the problem.

The Qinghai-Tibet (Qingzang) Plateau is the world's highest and largest plateau covering an area of 2 500 000 km<sup>2</sup> with an average elevation exceeding 4 500 m. On the plateau there are more than 1 600 lakes; most of these are saline, and they cover 26 912 km<sup>2</sup> (Zheng, 1997). Our survey showed that in northern Tibet alone there are 26 saline lakes whose salinity and temperature may support *Artemia* survival at an altitude of 4 000–5 100 m. We found *Artemia* in 15 out of these lakes (Jia et al., 2014). The Tibetan saline lakes with *Artemia* populations mainly belong to the shallow basin lakes and most are small in area. The total area of lakes without *Artemia* exceeds 1 000 km<sup>2</sup>; they generally are deep and large. The question must be asked why these saline lakes are free from *Artemia*. In northern Tibet there are three hydrochemical types of saline lakes (carbonate, sulfate and chloride) (Zheng, 1997). In these three types of lakes, there are lakes with *Artemia* and lakes without them; in other regions different authors noted similar *Artemia* distribution (Litvinenko et al., 2009; Shadrin and Anufrieva, 2012; Shadrin et al., 2012). Therefore, the difference in *Artemia* presence or absence in some saline lakes as assume is not necessarily determined by the lake hydrochemical type, either in Tibet or elsewhere. Some other factors, perhaps biological, may play more important roles. Different causes may include natural isolation and remoteness from the routes of migratory birds that may transport cysts, scarceness of zooplankton and zoobenthos that might attract migratory birds to stop for foraging, presence of certain predators, etc. We do not have sufficient data yet to understand the causes.

However, we know that some Ostracoda species such as *Eucypris elliptica* (Baird, 1846) inhabit Tibetan lakes. Field and experimental studies showed that *Eucypris mareotica* (Fischer, 1855) in the Crimean lakes lives at a salinity up to 300 g/L and feeds on *Artemia* nauplii (Anufrieva and Shadrin, unpublished data).

Why is there a necessity of *Artemia* acclimatization in Tibetan saline lakes? This is dictated by several reasons: 1. The gap between supply and demand of global *Artemia* cysts resources is currently widening; 2. There is sparse vegetation in northern Tibet (coverage generally below 5%), and the vegetation is mainly small grass, which makes a fragile ecological environment. The industrial structure is very poorly developed in the northern Tibet; the main branch, which generates only low income, is pastoral animal husbandry. The current burden of grazing (average 9–10 sheep and 1–1.15 yaks/ha) leads to desertification. Vegetation destruction and desertification problems caused by livestock overgrazing is becoming more prominent. Therefore, creation of a new sustainable, environmental friendly alternative industry can promote a reduction in overgrazing and provide a restoration of vegetation, which is of great significance for the region.

We have chosen Lake Dangxiong Co (Co means lake in Tibet) for the introduction of *Artemia sinica* Cai, 1989. We decided to introduce *A. sinica* and not *A. tibetiana* Abatzopoulos, Zhang et Sorgeloos, 1998 because *A. tibetiana* cysts are big in size, have a thick shell and a low hatching rate, and its nauplii are big in size; those are disadvantages for aquaculture. In 2004, 850 g of *A. sinica* cysts, originating from the Xiao Qaidam sulfate saline lake, situated in Qinghai Province (Zheng, 1997), were introduced in the lake. Some data on results of this introduction were published earlier (Liu et al., 2014; Jia et al., 2014). The purpose of this general review is to summarize our published and unpublished data and provide a general discussion of the multiannual experimental and field data. This analysis is important not only for aquaculture development, but also for ecosystem theory.

## 2 MATERIAL AND METHOD

### 2.1 Site description

Terminal Lake Dangxiong Co is located in the southwestern part of the northern Tibetan plateau, the Nagqu Prefecture of the Tibet Autonomous Region (31°32'–31°36'N, 86°40'–88°48'E) at an altitude of

**Table 1 Ionic composition of water in Lake Dangxiong Co (compiled from Wu et al., 2012)**

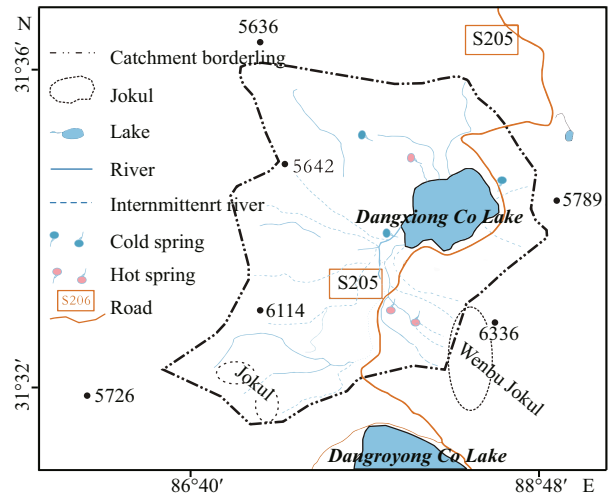
Total salinity (g/L)	Ion, part in total (%)							
	Li <sup>+</sup>	Na <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	CO <sub>3</sub> <sup>2-</sup>	HCO <sub>3</sub> <sup>-</sup>	B <sub>4</sub> O <sub>7</sub> <sup>2-</sup>
151.08	2.4	35.8	4.8	44.8	4.7	7.1	0	1.9
187.55	2.9	33.7	6.4	44.0	4.4	7.8	1.9	2.1
Average	2.7	34.8	5.6	44.4	4.6	7.4	1.0	2.0

4 475 m a.s.l. (Fig.1). The lake is surrounded by mountain ranges on three sides; it is not much affected by air currents due to the mountain blocking effect. There is only small mixing effect, which is generated by the breeze. The northwestern part of the lake, where the mounting blocking effect is relatively weak, is partly affected by the northwestern monsoon (Liu et al., 2014). The climate of the area is arid. Annual precipitation is about 150 mm, and the rainy season is from July to September; air temperature fluctuates between -30°C and +25°C and lake water temperature between -5°C and +22°C. The lake area is about 55.53 km<sup>2</sup>, and the maximum and average depths are 16 m and 12.4 m, respectively. Salinity in the lake fluctuates between 120 and 180 g/L. It is a carbonate-type hypersaline lake. Its ionic composition is given in Table 1 (Wu et al., 2012).

**2.2 Methods**

Quantitative phytoplankton and zooplankton samples were collected by a 2.5 or a 10 L Plexiglas bathometer in 2001–2013; abiotic parameters of water were measured during sampling or analyzed in the laboratory using standard methods (Wu et al., 2012; Liu et al., 2014). Zooplankton and phytoplankton were collected respectively at the south-west estuary, the lake center and near the north-east shore (3 points) in 2003–2005, at 10 points from center to east estuary in 2009–2010, and on 6 points in 2011–2012. A special *Artemia* survey was done at 26 points, including the points sampled in the plankton survey, in 2011 and 2013, at 3 points in 2014. Primary productivity (light and dark bottles) was measured at 6 points in the central part in 2003, two points in 2006, 2008, and 2013–2014. We collected and counted separately protozoans with depth range 1 m using the CFU method (Kemp et al., 1993). Sediment samples were taken using 1/25 m<sup>2</sup> Peterson grabs and analyzed under microscope; we present these data in Fig.3. Visually we counted birds on the lake surface.

The numbers of purple bacteria, algae, protists and



**Fig.1 The Lake Dangxiong Co map**

animals were determined by direct counting under an Olympus SZ-PT stereomicroscope with subsequent recalculation using the volume of water. To measure *Artemia* size we used an ocular-micrometer. Based on the length we calculated wet and dry mass (weight) of males and females based on Eqs.1–2 (Khmeleva, 1968).

$$W_d = 0.00103L^{2.66}, \tag{1}$$

where  $W_d$  is the dry weight, mg;  $L$ , length, mm.

$$W_d = 0.01083W_w + 0.0016, \tag{2}$$

where  $W_w$  is the wet weight.

*Artemia* cysts collected from the different water bodies were used in experiments (Table 2) to assess temperature influence on the development and the reproduction of *Artemia*. Hatched larvae and adult animals were kept 60 days in vessels with different temperature at salinity 60 with microalgae *Dunaliella* sp., *Phaeodactylum tricornutum* and *Chlorella* sp.; more details on experimental design and calculations are given in (Jia et al., 2009). Effective accumulative temperature as sum of growing degree days (GDD) was calculated:

$$GDD = D(T - M), \tag{3}$$

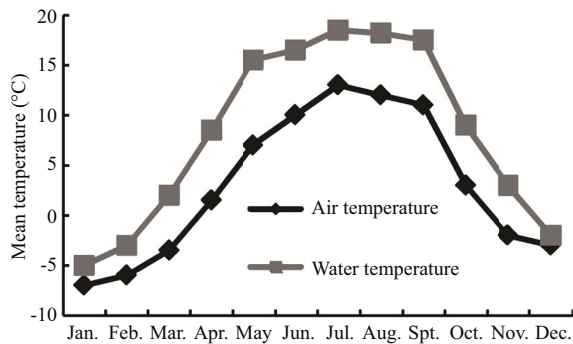
where  $T$  is the temperature, °C;  $M$ , developmental threshold temperature, °C;  $D$ , duration of development, days.

The experiments on competitiveness of introduced *A. sinica* and local strains of *Artemia* spp. were carried out in the experimental vessels as in (Barata et al., 1996). In the field, *A. sinica* and local *Artemia* strains were cultivated in a 10 L container with same initial density of nauplius (100 ind.) during 60 days. We evaluated survival rate and cumulative number of

**Table 2** Experimental results on the optimum temperature for development and reproduction of *Artemia* spp. from cysts of different origin

Place of cyst origin	Type of water body	Development (from cyst to adult)				Reproduction	
		T <sub>01</sub> (°C)	D <sub>1</sub> (Day)	M <sub>1</sub> (°C)	GDD <sub>1</sub> (°C day)	M <sub>2</sub> (°C)	T <sub>02</sub> (°C)
Xiao Chaidam salt lake, Qinghai Province, China	Sulfate	28.14	11.58	12.07	216.05	7.97	19.89
Marine saltpan, Putian, Fujian Province, China	Chloride	28.56	10.81	12.99	247.58	9.06	24.54
Great Salt Lake, USA	Chloride	28.61	11.11	12.80	261.75	7.11	24.66
Marine saltpan Jiangsu Province, China	Chloride	29.02	12.06	13.59	227.91	8.60	25.48
Marine saltpan, San Luis Potosi, Mexico	Chloride	29.10	11.61	12.77	229.01	8.75	25.49
Salt lake, Inner Mongolia, China	Carbonate	29.84	13.69	12.27	271.09	8.62	25.50
Salt lake, Ningxia Province, China	Sulfate	30.08	12.97	14.74	216.32	9.92	25.83
Gahai salt lake, Qinghai Province, China	Sulfate	30.83	11.07	14.48	227.05	8.17	26.32
Marine saltpan, Chenkou, Shandong Province	Chloride	31.08	12.80	12.79	254.72	8.14	26.53
Marine saltpan, San Francisco Bay, USA	Chloride	31.11	12.15	13.18	239.55	8.80	26.81
Yuncheng salt lake, Shanxi Province, China	Sulfate	31.24	12.50	14.16	200.89	9.27	26.95
Marine saltpan, Yangkou, Shandong Province	Chloride	31.56	13.83	14.35	205.34	9.29	27.38
Marine saltpan, Texcoco, Mexico	Chloride	31.41	12.00	14.01	214.15	7.89	27.47
Marine saltpan, Tianjin, China	Chloride	31.51	13.26	13.65	220.17	8.35	27.68
Salt lake, Xinjiang Province, China	Chloride	31.68	14.13	13.63	240.65	8.03	28.12
Marine saltpan, Sonora, Mexico	Chloride	31.69	13.44	13.37	209.55	7.18	28.51

T<sub>01</sub>: optimal temperature for development; D<sub>1</sub>: developmental duration under optimal temperature; M<sub>1</sub>: developmental threshold temperature; GDD<sub>1</sub>: effective accumulative temperature (from cyst to adult); M<sub>2</sub>: threshold temperature for reproduction (egg/cyst forming); T<sub>02</sub>: optimal temperature for reproduction.

**Fig.2** Seasonal fluctuations of air and water temperature in Lake Dangxiong Co (2008)

eggs of both species.

Data were subjected to standard statistical processing. Parameters of regression equations and correlation coefficients were calculated in Excel; the confidence level of the correlation coefficients was determined from the table of Müller et al. (1979).

### 3 RESULTS AND DISCUSSION

#### 3.1 Abiotic parameters

Salinity in the main part of the lake ranged between 120 and 180 g/L. The pH fluctuated between 9 and 10.

The maximum observed temperature (in 2011) was +19.4°C, the lowest -5°C. The seasonal changes of temperature (monthly average for the years 2011–2012) are shown in Fig.2. The water column in the lake is stratified. Field data collected during October–November 2011 showed that the surface, middle and bottom water layers temperatures were 0.0–9.0°C, 1.0–13.0°C, and 4.5–8.5°C, respectively. Average chemical parameters measured monthly during 2011–2012 are given in Table 2. The salinity change with depth from 0 to 8 m may be approximated by equation ( $R=0.981$ ,  $P=0.001$ ):

$$S=0.966h+148.26, \quad (4)$$

where  $S$  is the salinity, g/L;  $h$ , depth, m.

There is a halocline between 8 and 12 m; therefore the salinity near the bottom (15–16 m) was 191 g/L, not 163 g/L as predicted from Eq.4. Dissolved oxygen content was close to zero below 9–10 m depth. Methane gas bubbles were collected below 10 m, and the sampled water had a strong smell of hydrogen sulfide.

#### 3.2 The *Artemia* population

This lake was chosen for the introduction as our previous studies had shown that its salinity,

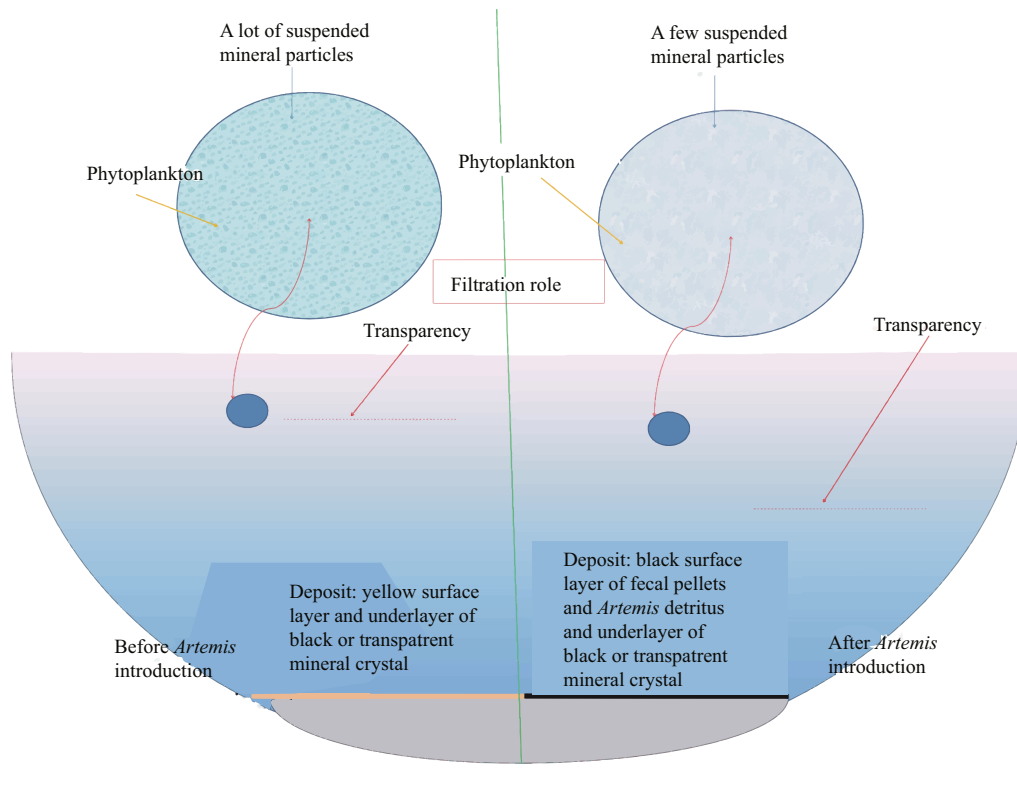


Fig.3 Changes in the Lake Dangxiong Co ecosystem after *Artemia sinica* intentional introduction

Table 3 Average concentrations (g/L) of chemical components of water in Lake Dangxiong Co (2011–2012) at different depths

Depth (m)	COD	K <sup>+</sup>	Na <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	CO <sub>3</sub> <sup>2-</sup>	HCO <sub>3</sub> <sup>-</sup>	Total solids
0–0.25	6.1	10.7	46.1	0.006	0.094	66.7	5.85	11.2	2.06	148.8
1	5.7	10.8	46.3	0.006	0.093	67.5	5.77	11.8	0.98	148.9
4	6.3	10.9	48.1	0.006	0.098	68.6	6.29	12.3	0.08	152.6
6	17.6	10.1	44.5	0.006	0.092	63.4	5.76	11.2	0.48	153.1
8	13.8	10.6	46.4	0.006	0.091	67.8	5.72	11.9	0.13	156.5
Near bottom	16.8	13.4	59.3	0.006	0.064	75.3	9.48	14.4	2.23	191.0

temperature (year GDD for selected *Artemia* strain in the lake was 1 400–1 455°C·days) and level of gross primary production (0.6–1.0 g O<sub>2</sub>·/(m<sup>2</sup>·days)) are suitable for development of an *Artemia* population. For the introduction we selected *A. sinica* originating from Qinghai province. Experimental culture in the lake water (high pH, high Li concentration) were conducted by the first author to evaluate the influence of temperature on cysts from 16 bisexual and parthenogenetic populations from different Chinese provinces, and found that *A. sinica* originating from the Qinghai province (the Xiao Qaidam sulfate saline lake) had optimal parameters for successful introduction (Table 2). We evaluated the duration of

development from cysts to adults and the fecundity of the females. *A. sinica* demonstrates optimal characteristics at low temperature. The survival rate of *A. sinica* was 42% at 19°C, decreasing to 14% at 34°C. The developmental threshold temperature for complete development and reproduction was 8°C, with sum of growing degree days (GDD) equaling 666°C·day. The optimum temperature was lowest among all tested *Artemia* strains: for larvae stages 28.1°C and 19.9°C for adults. Calculated GDD for the lake was 1 420°C·day; taking into account seasonal temperature changes (Fig.2) is supposed, *Artemia* may produce two generations annually. Fecundity was satisfactory: about 200 eggs per female at 19°C

**Table 4 Average densities of different stages of *Artemia* in two layers of Lake Dangxiong Co in August 2013**

Layer (m)	Average density (ind./m <sup>3</sup> ) ±SD					
	Cysts	Nauplii	Metanauplii	Juveniles	Adult females	Adult males
August 2013						
0–7	46 000±7 350	5 800±4 385	2 800±2 487	300±287	500±387	900±437
7–14	188 333±8 235	3 500±2 465	2 667±2 228	167±128	1 000±398	1 500±547
Average	117 167±6 937	4 650±3 535	2 739±2 352	234±179	750±398	1 200±520
November 2014						
0–2	-	-	30±29	133±150	685±332	292±190
8–10	-	-	75±31	275±96	245±50	200±27
14–16	-	-	300±71	400±141	80±110	45±75
Average	-	-	135±144	269±133	337±312	179±125

**Table 5 Average sizes and mass of adult females and males in Lake Dangxiong Co in August 2013**

Layer (m)	Females			Males		
	Length (mm±SD)	Wet mass (mg)	Dry mass (mg)	Length (mm±SD)	Wet mass (mg)	Dry mass (mg)
0–7	12.12±0.610	7.136	0.785	9.14±0.448	3.373	0.371
7–14	10.85±0.521	5.318	0.585	9.54±0.427	3.773	0.415

**Table 6 Average biomass of *Artemia* cysts and adults in Lake Dangxiong Co in August 2013**

Layer (m)	Cysts	Females	Males	Total adult
	Wet biomass (mg/m <sup>3</sup> )	Wet biomass (mg/m <sup>3</sup> )	Wet biomass (mg/m <sup>3</sup> )	Wet biomass (mg/m <sup>3</sup> )
0–7	681	3 568	3 036	6 604
7–14	2 712	5 318	5 660	1 208
Average	1 696	4 443	4 348	10 978

**Table 7 Approximate average values of total biomass of *Artemia* in Lake Dangxiong Co in August 2013**

	Cysts	Female	Male	Total adult
Wet weight (t)	1 356	3 059	2 994	6 053
Dry wet (t)	338.9	336.5	329.3	665.8

and about 300 eggs per female at 25–28°C. Special experiments and field study were performed to assess risk factors from the introduction of alien species (detailed results to be reported elsewhere). In long-term experiments no risk of cyst diffusion from the lake by wind or birds was found. In mixed cultures of the selected *A. sinica* with different Tibetan native strains we found that the reproductive competitiveness of *A. sinica* is weaker than that of the native strains. Cumulative number of eggs of local strains after 60 days was 790–820 and 680–710, for *A. sinica*. After 60 days about 55% of adults belonged to local strains. Our results strongly suggest that the introduction of allochthonous *Artemia* may create only a low risk of species diffusion into other water bodies.

Cysts (850 g) were grown in culture, gradually passing them from a small vessel to an experimental pond (May to July, 2004). Then about  $5 \times 10^6$  adults (63% females) were transported to the lake. Surveys of *Artemia* abundance were made in 2006, 2008, and

2011–2014. The results of the 2011 and 2013 surveys were published (Liu et al., 2014; Jia et al., 2014). The average abundance of *Artemia* adults in the lake gradually increased from 20 ind./m<sup>3</sup> in 2006 to 1 950 ind./m<sup>3</sup> in August 2013. This increase may be approximated by the equation ( $R=0.979$ ,  $P=0.001$ ):

$$N=2.8e^{0.786t}, \quad (5)$$

where  $N$ : total density of males and females, ind./m<sup>3</sup>;  $t$ : time after the introduction, years.

An exponential population growth after a lag stage is a common feature of all new successfully introduced populations (Karpevich, 1975; Crooks and Soulé, 1999; Shadrin, 2013). In 2014 we observed some decrease in *Artemia* abundance, perhaps due to the increase of bird abundance (Table 8). The percentage of females in the total adult density was 41.3% in 2012, 38.5% in 2013, and 65.3% in 2014. Tables 3–6 show the structure of the *Artemia* population during the last surveys in August 2013 (Jia et al., 2014) and November 2014. The abundance of cysts in 2013 was much higher than that of other stages; it was 117 167/m<sup>3</sup> on the average. The structure of the population varied very much from year to year and

**Table 8 Long-term changes of the ecosystem elements at one station in Lake Dangxiong Co**

Parameters, average value	Year				
	2003	2006	2008	2011	2014
	Phytoplankton				
Density (ind./L)	231 000	9 000	56 140	109 500	-
Biomass (mg/L)	0.004	0.061	0.094	0.153	-
Number of species	3	6	8	12	-
Dominant group	Cyanobacteria	Euglena	Diatoms	Diatoms	-
	Protozoa				
Density (ind./L)	1 800	750	1 086	1 650	-
Biomass (mg/L)	0.900	0.371	0.364	0.430	-
Number of species	2	3	8	7	-
	<i>Artemia</i>				
Density (ind./m <sup>3</sup> )	0	20.25	90.91	2 142.9	1 373
	Birds				
Number on observed aquatic area (ind.)	0	1–2	50–70	6 000–8 500	30 000–33 000

from season to season (Liu et al., 2014; Jia et al., 2014). The population produced at least two generations per year: the first in March–May (June), and the second in July–September. In 2012 we also observed a small peak in density of all stages from September until the beginning of November. In 2012 the proportions between different age stages changed from summer to autumn; part of the larval stages decreased but the percentages of adults and cysts increased. During the summer survey the density of larval stages (3 211 ind./m<sup>3</sup> on average) was 25 times higher than the density of adults (125/m<sup>3</sup> on average); the density of cysts (389/m<sup>3</sup> on average) was also much less than for the larval stages. In autumn a difference between the density of larval stages (2 006/m<sup>3</sup> on average) and adults (1 222/m<sup>3</sup> on average) was not so big, but the density of cysts was much higher (39 567/m<sup>3</sup> on average). The total density of cysts increased two orders of magnitude from summer to autumn.

The vertical distribution of different *Artemia* stages was studied in 2011–2013. Partial data for 2011 and 2013 were presented previously (Liu et al., 2014; Jia et al., 2014). In 2012 the vertical distribution in summer and autumn differed. It is important to note that in summer the maximal density of larvae occurred at 4 m depth (5 300 ind./m<sup>3</sup>), in autumn at 0.25 m (6 400/m<sup>3</sup>). We observed different distributions for cysts and adults; they both had a main maximum at 0.25 m and gradually decreased with increasing depth, but they showed a second maximum at 10–14 m in summer and in autumn. In autumn all stages

had a maximum at 0.25 m with a gradual decrease to 10 m, but at depth 12–14 m there was a pronounced increase in density. Taking into account that there also is a significant difference ( $P=0.005$ ) in length of adults taken from different depths (Table 5), we consider most likely that two subpopulations of *A. sinica* now exist in the lake. They had very similar proportions between age stages. Another explanation of those two peaks is also possible; it is possible that they resulted from dividing of the water column into two strata with a chemocline between them. We need to note that in the second case it cannot be explained why in the two aggregations of *Artemia* had significantly different average sizes.

In experiments with *Artemia* in the water column with oxic and anoxic layers, similar to the situation in Lake Dangxiong Co, it was shown that the brine shrimp in the stratified columns “were distributed throughout the water column, but with a preference for the deeper portion where food levels were higher and where they were shielded from bright light. Shrimps in the simulated deep brine columns were primarily located at the interface between the two layers, and they would briefly enter the anoxic upper part of the deep brine layer. Isotopic analyses of the shrimp indicated that they had fed on some of the organic material at the interface” (Wurtsbaugh and Jones, 2012). Taking into account that there was a peak of primary production at depth 0–2 m (our data), it is likely that the upper *Artemia* subpopulation in the lake consumes mostly phytoplankton, but that the subpopulation in the interface zone feeds more on

detritus, heterotrophic protozoa and/or purple bacteria. Deeper (10–11 m) purple sulfur bacteria had densities up to  $5 \times 10^7$  cells/L; this created a second peak of primary production formed by anoxygenic photosynthetic purple bacteria. We hypothesized that deeper *Artemia* subpopulation may consume purple bacteria as main food. The presence of two subpopulations at different depths in the lake is now only an assumption; more data are needed to verify this. Looking at all data we may conclude that a new sustainable population of *A. sinica* has established itself in the lake. We may also predict that the population will further increase its density in next years because much higher densities of *Artemia* populations are observed in other lakes (Rudneva, 1991; Litvinenko et al., 2009; Shadrin and Anufrieva, 2013).

### 3.3 The lake ecosystem

During surveys in different years we had only one common sampling station approximately in the center of the eastern part of the lake. Due to lack of more extensive data sets the data were not analyzed in detail. Data on changes in different ecosystem components collected at that station are summarized in Table 8 and Fig.3. Data from other sampling points support this. The data strongly suggest that the new *Artemia* population in the lake has caused major changes in the lake ecosystem; including an increase in species number of phytoplankton and heterotrophic protozoa with a decrease in total abundance. Water transparency also increased. Dominance in phytoplankton passed from cyanobacteria to diatoms. Changes occurred not only in the lake ecosystem; the number of water birds using the lake also dramatically increased. This is likely to have led also to changes in nutrient dynamics. We may predict that the lake ecosystem will further reorganize and reach a new alternative stable state (De Tezanos Pinto and O'Farelli, 2014). The chance for *Artemia* cysts to be transported by birds to other Tibetan lakes may also increase. New studies on these issues are needed.

Such environmental impact of a sharp increase of *Artemia* population on a lake ecosystem is not unique. Development of *Artemia* populations in hypersaline lakes correlates with a decrease of phytoplankton abundance worldwide (Wurtsbaugh and Berry, 1990; Balushkina et al., 2009; Mohebbi, 2010). A cascading effects of decreased phytoplankton abundance as a result of *Artemia* population development in Great Salt Lake has been shown: decreased chlorophyll

levels and increased Secchi depths, an increase in soluble reactive phosphorus and inorganic nitrogen in the mixolimnion, reducing shading by algae and purple sulfur bacteria in the chemocline, increased light penetration so that in the monimolimnion temperature started to fluctuating more (Wurtsbaugh and Berry, 1990). From this it follows that *Artemia* population development may affect phytoplankton, nutrients and thermal stratification in hypersaline lakes. *Artemia* is not exclusive in having such effects; successful invasive species in general may lead to the dramatic changes in the recipient ecosystems (Karpevich, 1975; Crooks and Soulé, 1999).

Laboratory experiments have shown diurnal vertical migrations of *Artemia* aggregations in an unstratified water column through the hydrodynamic interactions between neighboring swimmers may establish an alternate energy transfer route from the small scale of individually migrating plankton to significantly larger scales (Wilhelmus and Dabiri, 2014). The authors of that study proposed that *Artemia* aggregation may affect water body circulation to a degree comparable to winds. Establishment of the new *Artemia* population is supposed to result in an increase of water mixing in the lake, leading to a rise of primary productivity. This is a hypothesis now; new data are needed to verify it. In many lakes it has been shown that changes in *Artemia* density positively correlates with diversity and abundance of water birds (Khomenko and Shadrin, 2009; Varo et al., 2011; Shadrin and Anufrieva, 2013). An increase in the numbers of water birds using the lake may lead to intensification of nutrient cycling and an increase of primary production (Kulakov et al., 2011).

### 3.4 The potential social and economic results of *Artemia* introduction in the lake

Development of an *Artemia* harvesting industry may contribute to the solution of a number of social, economic, and environmental problems in Tibet. Preliminary evaluation of a possible sustainable harvesting of cyst and adult biomass in the lake was made using a method available for this purpose (Litvinenko et al., 2002). It is possible to harvest at least about 150 tonnes cysts per year from the lake as well as 3.2 thousand tonnes of frozen or 350 tonnes of dried biomass of adult *Artemia*. The Tibetan salt lakes have high potential for an *Artemia* industry development as a part of “saline lake agriculture” (Zheng, 1995). *Artemia* cysts are already exploited in Tibet. As example, on Lake Qixiang Cuo, now the



largest *Artemia* lake in the Tibet Autonomous Region (area is about 142 km<sup>2</sup>, altitude: 4 615 m a.s.l.), 2 100 local people using hand-operate nets harvest 310 tonnes (autumn 2010) of *Artemia* cysts. In perspective, if *Artemia* would be introduced in most part of the northern Tibetan saline lakes, 20 000–30 000 local people could then be involved in the industry. Taking into account experience from other places in the world (Russia, Kazakhstan), it is possible to think that the companies, which are active in other regions, might expand their action radius to the ‘new’ Tibetan lakes with use/import of trained labor forces from elsewhere, and not necessarily invest in the training of local people to join the new industry. Such strategy is unlikely to be used in Tibet, however. It is very difficult for people from lowland regions to adapt to the harsh high altitude environment. As a result, in development of *Artemia* harvesting industry in Tibet it is likely that mostly local people would be employed.

Diversification of the local economy resulting from the newly created industry may make the local economy more sustainable, decrease financial risks, create new job positions, increase the level of welfare, and this, inevitably, decrease social tension in the region. Furthermore, there is only sparse vegetation in northern Tibet, which is seriously degraded by livestock overgrazing. Some people may shift from livestock to such a new industry; and this may reduce the scale of the livestock industry and its pressure on vegetation and soil. As a result, the vegetation cover might be restored decreasing wind and water soil erosion and reducing dust storms. To develop a strategy and technologies for this we need new deeper studies in lake-watershed ecology, *Artemia* biology, and “saline lake agriculture”.

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#### References

- Anh N T N. 2009. Optimisation of *Artemia* Biomass Production in Salt Ponds in Vietnam and Use as Feed Ingredient in Local Aquaculture. Ph. D. thesis, Ghent University, Belgium. 250p.
- Balushkina E V, Golubkov S M, Golubkov M S, Litvinchuk L F, Shadrin N V. 2009. Effect of abiotic and biotic factors on the structural and functional organization of the saline lake ecosystems. *Zhurnal Obshchei Biologii*, **70**(6): 504-514. (in Russian)
- Barata C, Hontoria F, Amat F, Browne R. 1996. Demographic parameters of sexual and parthenogenetic *Artemia*: temperature and strain effects. *J. Exp. Mar. Biol. Ecol.*, **196**(1-2): 329-340.
- Crooks J A, Soulé M E. 1999. Lag times in population explosions of invasive species: causes and implications. In: Sandlund O T, Schei P J, Viken A eds. *Invasive Species and Biodiversity Management*. Kluwer Academic Publishers, New York. p.103-125.
- De Tezanos Pinto P, O’Farrell I. 2014. Regime shifts between free-floating plants and phytoplankton: a review. *Hydrobiologia*, **740**(1): 13-24.
- Duarte C M, Holmer M, Olsen Y, Soto D, Marbà N, Guiu J, Black K, Karakassis I. 2009. Will the oceans help feed humanity? *BioScience*, **59**(11): 967-976.
- Jia Q X, Anufrieva E, Liu S S, Liu X F, Kong F J, Zheng M P, Shadrin N. 2014. Results of an intentional introduction of *Artemia sinica* in the high-altitude Tibetan Lake Dangxiong Co: on a base of Surveys in 2011 and 2013. *Acta Geol. Sin.* (English Edition), **88**(Supp. 1): 74-76.
- Jia Q X, Wang H P, Kong F J, Zhen M P. 2009. Some ecological parameters of *Artemia parthenogenetica* Gahai and their use in resource exploitation. *Front. Biol. China*, **4**(3): 358-363.
- Karpevich A F. 1975. Theory and Practice of Acclimatization of Aquatic Organisms. Pishchevaya Promyshlennost, Moscow. 404p. (in Russian)
- Kemp P F, Sherr B F, Sherr E B, Cole J J. 1993. Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, New York, USA. 781p.
- Khmeleva N N. 1968. The expenditure of energy on respiration, growth and reproduction in *Artemia salina* (L.). *Biologiya Morya*, **15**: 71-98. (in Russian)
- Khomenko S V, Shadrin N V. 2009. Iranian endemic *Artemia urmiana* in hypersaline Lake Koyashskoe (Crimea, Ukraine): a preliminary discussion of introduction by birds. *Branta: Sbornik nauchnich trudov Azovo-Chernomorskoj ornitologicheskoi stancii*, **12**: 81-91. (in Russian)
- Kulakov D V, Ivanchev V P, Krylov A V. 2011. Influence of metabolites of water birds on zooplankton in small lake littoral (basin of River Oka). *Povolzhskiy Ekologicheskii Zhurnal*, **3**: 344-351 (in Russian)
- Lavens P, Sorgeloos P. 2000. The history, present status and prospects of the availability of *Artemia* cysts for aquaculture. *Aquaculture*, **181**(1-4): 397-403.
- Litvinenko A I, Litvinenko L I, Solovov V P, Yasuchenyha T L, Vesnina L V. 2002. Guidelines for the Determination of Total Allowable Catches of *Artemia* cysts. SibrybNIIproekt, Tyumen. 26p. (in Russian)
- Litvinenko L I, Litvinenko A I, Boyko E G. 2009. Brine Shrimp *Artemia* in Western Siberia Lakes. Nauka, Novosibirsk. 304p. (in Russian)
- Liu S S, Liu X F, Jia G X, Kong F J, Zheng M P, Lv G J. 2014. Assessment of spatial distribution and cysts resources of *Artemia* in late Autumn in Dangxiong Co salt lake. *Acta*

- Ecol. Sin.*, **34**(1): 26-33.
- Mohebbi F. 2010. The brine shrimp *Artemia* and hypersaline environments microalgal composition: a mutual interaction. *Int. J. Aquat. Sci.*, **1**(1): 19-27.
- Müller P H, Neuman P, Storm R. 1979. Tafeln Der Mathematischen Statistik. VEB Fachbuchverlag, Leipzig. 272p.
- Rudneva I I. 1991. *Artemia*. Prospects for the Use in the National Economy. Naukova Dumka, Kiev. 144p. (in Russian)
- Shadrin N V, Anufriieva E V. 2012. Review of the biogeography of the genus *Artemia* Leach, 1819 (Crustacea, Anostraca) in Russia. *Int. J. Artemia Biol.*, **2**(1): 51-61.
- Shadrin N V, Anufriieva E V. 2013. Climate change impact on the marine lakes and their Crustaceans: the case of marine hypersaline Lake Bakalskoye (Ukraine). *Turkish J. Fish. Aquat. Sci.*, **13**: 603-611.
- Shadrin N, Anufriieva E, Galagovets E. 2012. Distribution and historical biogeography of *Artemia* leach, 1819 (Crustacea: Anostraca) in Ukraine. *Int. J. Artemia Biol.*, **2**(2): 30-42.
- Shadrin N. 2013. *Acartia tonsa* (Copepoda) in the Black and Caspian Seas: Review of its success and some lessons. *J. Biosafety*, **22**(4): 229-236.
- Sultana R, Ameer F, Ali W, Nasir M. 2011. Culture of Vinhchau strain of *Artemia franciscana* Kellogg, 1906 (Crustacea: Anostraca) in Pakistan. *Int. J. Artemia Biol.*, **1**(1): 41-48.
- UN (United Nations). 2007. World Population Prospects: The 2006 Revision Population Database. 27 October 2009, esa.un.org/unpp.
- Varo N, Green A J, Sánchez M I, Ramo C, Gómez J, Amat J A. 2011. Behavioural and population responses to changing availability of *Artemia* prey by moulting black-necked grebes, *Podiceps nigricollis*. *Hydrobiologia*, **664**(1): 163-171.
- Wilhelmus M M, Dabiri J O. 2014. Observations of large-scale fluid transport by laser-guided plankton aggregation. *Phys. Fluids*, **26**(10): 101302.
- Wu Q, Zheng M P, Nie Z, Bu L Z. 2012. Natural evaporation and crystallization regularity of Dangxiongcuo carbonate-type salt lake brine in Tibet. *Chin. J. Inorg. Chem.*, **28**(9): 1 895-1 903. (in Chinese with English abstract)
- Wurtsbaugh W A, Berry T S. 1990. Cascading effects of decreased salinity on the plankton chemistry, and physics of the Great Salt Lake (Utah). *Can. J. Fish. Aquat. Sci.*, **47**(1): 100-109.
- Wurtsbaugh W A, Jones E F. 2012. The Great Salt Lake's deep brine layer and its importance for mercury bioaccumulation in brine shrimp (*Artemia franciscana*). *Watershed Sciences Faculty Publications*. Paper 551. [http://digitalcommons.usu.edu/wats\\_facpub/551](http://digitalcommons.usu.edu/wats_facpub/551).
- Zhang B, Zhao G. 2014. The distribution of *Artemia* and its commercial application in China. *J. Ocean University of China* (in press).
- Zheng B, Sun S C. 2013. Review of the biogeography of *Artemia* Leach, 1819 (Crustacea: Anostraca) in China. *Int. J. Artemia Biol.*, **3**: 20-50.
- Zheng M P. 1995. On "Saline lake agriculture". *Acta Geosci. Sin.*, (4): 404-418.
- Zheng M P. 1997. An Introduction to Saline Lakes on the Qinghai-Tibet Plateau. Kluwer Academic Publishers, Dordrecht. 294p.