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Quality of the cysts of *Artemia franciscana* collected at Tuticorin with observations on growth, maturity and sex ratio of the offspring

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ABSTRACT

The cysts of the exotic species of brine shrimp *Artemia franciscana* collected from the natural ecosystem at Tuticorin during January 2001 were processed, stored and their quality evaluated. A total of 22 experiments were conducted on hatching and it was observed that the time taken for first hatching ranged from 13 hours and 10 minutes to 17 hours. The maximum hatching percentage recorded at 24 hours and 48 hours were 52.0 and 79.9 respectively. The I instar obtained from the hatching experiments were reared in outdoor culture tanks at 30, 50, and 70 ppt with two different types of feed and the maximum size of 10.04 ± 0.3 mm was recorded on 18th day in the animals fed with rice bran at 70 ppt. The earliest first pairing and first spawning were recorded on 6th and 12th days respectively in the animals fed with mixed phytoplankton at 50 ppt.

Introduction

In India, occurrence of the exotic species of brine shrimp *Artemia franciscana* from the natural ecosystem was reported for the first time from the salt pans of Karapad at Tuticorin (Rajamani *et al.*, 1998). Subsequent survey of the salt pans in and around Tuticorin showed that it occurred in the salt pans of Veppalodai, north of Tuticorin also. However, in the salt pans of Alangarathittu, east of Tuticorin only the native species *A.parthenogenetica* was found (Rajamani *et al.*, 2001). Itwas observed that the native species occurred in the salt pans of Alangarathittu only till November 2000 after which it was completely replaced by the exotic species, *A.franciscana*. Interestingly, during January 2001, large quantities of cysts of brine shrimp were collected from the shores of a salt water pond at Alangarathittu. The cysts were processed stored and their qualities in terms of time taken for first hatching, size of I instar, hatching percentage and hatching efficiency were evaluated. Some aspects of the biology of their offspring viz. rate of growth, age and size at first maturity and first spawning and sex ratio were also studied.

Materials and methods

The cysts of brine shrimp were collected from the shores of a large shallow water pond adjoining the salt pans in Alangarathittu region in the out-skirts

of Tuticorin, for a seven days from 18.01.2001 to 24.01.2001. As the cysts were mostly scooped along with sand particles, dust materials etc., they were cleaned and processed following the biphase floatation technique(Sorgeloos and Kulasekarapandian, 1984). The processed cysts were packed in polythene bags, each bag containing approximately 100 g of cysts and stored in plastic containers at room temperature. In order to find out the actual number of cysts present in one gram, 2 mg of cyst was weighed,counted. The average number present in five replicates was recorded. The diameter of the cysts before and after drying and the size of freshly hatched nauplii were measured with the help of an ocular micrometer fitted microscope. For this a minimum of 15 cysts were measured in the beginning. In order to find the total length of the I instar, a minimum of five freshly hatched nauplii were measured in each hatching experiment.

The hatching experiments were carried out at two salinities viz. 25 and 30 ppt and the quantity of cysts taken for the experiments varied from 0.250 g to 5.000 g. The hatching rate, hatching efficiency and hatching percentage were worked out following the standard methods. The nauplii obtained from some of the hatching experiments were reared in outdoor culture tanks of 300 l capacity at three different salinity levels viz. 30 50 and 70 ppt. with two different types of feed namely an inert feed of rice bran and mixed phytoplankton and the rate of growth, age at first maturity and age at first spawning were studied. The composition of males, females and riding pairs in the population were recorded at weekly intervals. Salinity and temperature in both hatching experiments and in culture experiments were monitored daily. The pH in the culture tanks was monitored at weekly intervals. In the hatching experiments fluctuation in the light intensity was monitored with the help of a digital lux meter.

Results

The cysts of brine shrimp were collected for seven days at a stretch, after cleaning and processing the total quantity of dry cysts collected amounted to 2.660 kg. The cysts were packed in 28 polythene bags by heat sealing, each bag containing 76.800 g to 107.400 g of dry cysts. During storage eighteen packets got damaged and the cysts in these packets were transferred to a plastic container and kept in loose condition from which cysts were taken periodically for hatching experiments. The changes in the weight of the packets were recorded after 73, 117, 148, 220,325 and 406 days and the total loss in the weight after a period of 406 days was found to vary between 1.1 and 2.7 % with an average loss of 1.9 %(Table 1). The diameter of the cyst before and after drying was found to vary between 263 ± 12 m and 212 ± 11 m respectively. The number of cysts present in 1 mg was found to vary between 313 and 395 (Table 2). The time taken for first hatching of the cyst (T0) was noted and is very important as it primarily determines the quality of the cyst (Table 3).

In the entire series of experiments the size of the first hatched nauplii ranged between 462 and 490µ with an average of 478 ± 6µ. As the I instar moults to second stage in about 8 hours the hatching percentage at 24 hours is very important which varied from 2.1 to 52.0 %. The hatching percentage at 48 hours exceeded 40 in most of the experiments with four experiments showing higher values exceeding 70 % (Table 3 ; Figure 1a to 1d).

TABLE 1: *Variations in weight of the cysts of* Artemia franciscan*a packed in polythene bags during storage*

Sl. No.	Packet No.	Initial Weight (Gms)	Periodic Weight after 73 days	117 days	148 days	220 days	325 days	406 days	Wt. Loss after after 406 days	Total weight loss % after 406 days
1	3	96.250	95.990	95.440	95.090	94.990	94.260	94.030	2.220	2.31
2	7	92.090	91.680	91.220	90.850	90.660	89.990	89.640	2.450	2.66
3	8	87.560	87.430	87.230	86.960	86.890	86.280	85.860	1.700	1.94
4	9	88.730	88.250	87.800	87.450	87.400	87.120	86.920	1.810	2.04
5	10	103.130	103.090	102.680	102.430	102.330	102.110	101.980	1.150	1.12
6	12	94.230	93.730	93.230	92.940	92.850	92.050	91.760	2.470	2.62
7	14	93.630	93.250	92.860	92.500	92.270	92.010	91.800	1.830	1.95
8	21	100.120	99.370	99.050	100.090	100.030	99.710	98.730	1.390	1.39
9	22	106.400	105.200	105.020	106.070	106.000	105.080	104.970	1.430	1.34
10	26	90.050	89.300	88.950	89.970	89.760	88.790	88.590	1.460	1.62
									Average	1.899

TABLE 2: Size of cysts and changes in the numbers of cysts per unit weight during storage

Sl. No.	Date of counting	No. of cysts per gram	Wt. of individual cyst (µg)	Date of measurement	Size of the cyst (µ) Min	Max	Mean ± SD
1	30.01.2001	3,13,000	3.195	18.01.2001	0.210	0.252	0.244±0.021*
2	29.03.2001	3,86,000	2.591	19.01.2001	0.238	0.280	0.258±0.016*
3	12.07.2001	3,95,000	2.532	21.01.2001	0.252	0.280	0.263±0.012*
4	05.12.2001	3,42,500	2.920	24.01.2001	0.252	0.280	0.258±0.013*
5	22.04.2002	3,27,500	3.053	30.01.2001	0.210	0.224	0.215±0.008^
6	10.07.2002	3,20,500	3.120	27.03.2001	0.200	0.220	0.212±0.011^

* Size of hydraded cysts ; ^ Size of dried cysts

The time taken for hatching of 90 % cysts (T90) is very important for assessing the quality of cysts and it should be less than 30 hours. However, in the present investigation even though the observation was continued till 48th hour the maximum hatching percentage recorded was only 79.7 %.

A knowledge on the hatching efficiency of the cysts is important as it indicates the actual number of nauplii that can be harvested from 1g of cysts. In the entire series of experiments the hatching efficiency showed wide variation ranging from 6.4 x 10^3 to 205.4 x 10^3 at 24 hours and from 54.1 x 10^3 to 314.7 x 10^3 at 48 hours. At 24 hours the hatching efficiency exceeded 100 x 10^3 in 13 out of 22 experiments conducted, whereas at 48 hours the hatching efficiency exceeded 100 x 10^3 in all the experiments except two and it exceeded 200 x 10^3 in six experiments. In one of the experiments the hatching efficiency was very high exceeding 300 x 10^3 (Table 3).

pH was found to range between 7.6 and 7.9 only. However, there was wide variation in ambient temperature (23.0 - 30.0 ^{0}C). Also, there was wide fluctuation in the light intensity, ranging between 480 and 1612 Lux, which is attributed to fluctuation in atmospheric temperature and variation in the intensity of the natural light.

TABLE 3: *Time taken for first hatching, size of I instar , hatching percentage and hatching efficiency of the cysts of* Artemia franciscana *in different experiments*

Expt. No.	Quantity of cyst taken (gms)	Time taken for I hatching (Hr)	Size of the I instar(mm)	Hatching percentage		Hatching efficiency	
				24 Hours	48 Hours	24 Hours	48 Hours
1	1.000	14.10	0.490 + 0.01	7.16(23.50)*	75.07	22,411(23.50)*	2,34,969
2	1.000	17.00	0.481 + 0.01	2.06	73.48	6,448	2,29,992
3	1.000	14.00	0.476 + 0.00	10.91	_	34,148	_
4a	3.000	14.30	0.474 + .01	33.76	41.97	1,30,314	1,62,004
4b	3.000	14.30	0.478 + 0.004	34.2	46.98	1,32,012	1,81,343
5a	3.000	14.15	0.470 + 0.01	30.4	46.52	1,17,344	1,79,567
5b	3.000	14.15	0.478 + 0.004	30.4	49.05	1,17,344	1,89,333
6a	5.000	14.30	0.474 + 0.01	33.20(23.00)*	_	1,28,152(23.00)*	_
6b	5.000	14.30	0.476 + 0.01	29.35(23.00)*	_	1,13,291(23.00)*	_
7	0.250	13.45	0.470 + 0.01	33.82(23.00)*	51.78(41.00)*	1,33,589(23.00)*	2,04,531(41.00)*
8	0.100	13.05	0.462 + 0.004	46.08	79.67	1,82,016	3,14,657
9	0.025	13.45	0.474 + 0.01	51.99	_	2,05,361	_
10	0.500	13.30	0.486 + 0.01	33.50(21.00)*	52.7	1,32,325(21.00)*	2,08,165
11	3.000	15.30	0.476 + 0.01	30.87(22.30)*	42.1	1,21,937(22.30)*	1,66,295
12	0.025	14.00	0.478 + 0.004	29.37	71.9	1,16,012	2,84,005
13	0.500	14.10	0.478 + 0.004	22.58	_	89,191	_
14	0.250	16.00	0.480 + 0.00	9.72	34.16	38,394	1,34,932
15	0.250	14.10	0.482 + 0.004	13.47	39.32	46,135	1,34,671
16	0.250	14.20	0.484 + 0.005	43.52	53.49	1,49,056	1,83,203
17	5.000	13.40	0.486 + 0.01	14.99	39.61	51,341	1,35,664
18a	5.000	16.30	0.484 + 0.005	_	16.53	_	54,136
18b	5.000	16.30	0.484 + 0.01	_	17.55	_	57,476

* Actual time of observation

The rate of growth of the brine shrimp from I instar was studied for 18 days at 30, 50 and 70 ppt with two different types of food namely, rice bran and mixed phytoplankton. Maximum size of 10.04 ± 0.30 mm was recorded at 70 ppt for rice bran fed animals (Figure 2a and 2b & 3a, 3b and 3c). The earliest first pairing was recorded on the 6th day at 50 ppt, for the animals fed with mixed phytoplankton, when the animals were just 4.8 mm in total length. In the case of animals fed with rice bran, first pairing was recorded on the 8th day at 70 ppt when the animals measured 5.90 ± 0.36 mm in total length. The earliest first spawning was recorded on 12th day at 30 and 50 ppt in the animals fed with mixed phytoplankton and on 13th day at 30 and 50 ppt in the animals fed with rice bran.

The overall sex ratios and composition of riding pairs recorded in the population maintained at the three different salinity levels are given in Table 4. Males were dominant in all the experiments except one where the sex ratio was equal. The maximum composition of males was recorded at 30 ppt in the population maintained with rice bran, whereas the composition of the riding pairs were high in the population maintained with mixed phytoplankton at 30 and 50 ppt. The hydrographical conditions recorded in the

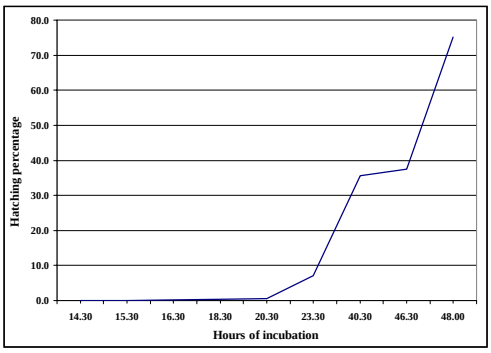

Figure 1a. Periodic observation on cyst hatching (Experiment No. 1)

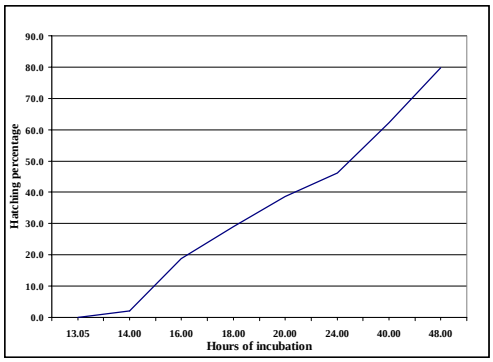

Figure 1b. Periodic observation on cyst hatching (Experiment No. 2)

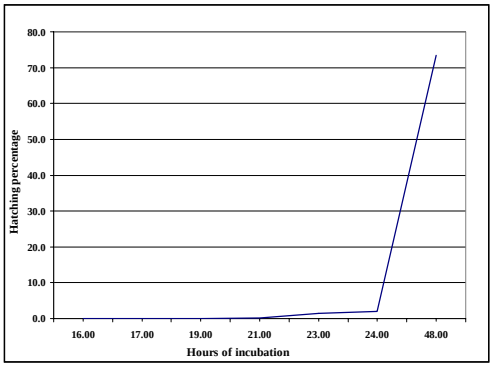

Figure 1c. Periodic observation on cyst hatching (Experiment No. 8)

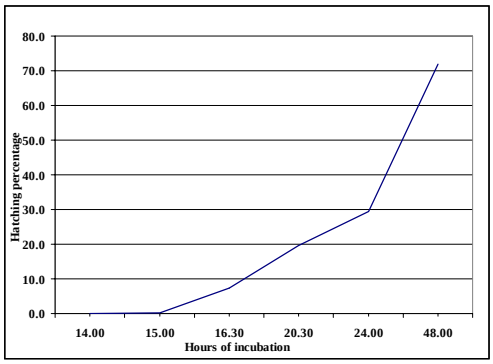

Figure 1d. Periodic observation on cyst hatching (Experiment No. 12)

culture experiments are given in Table 5.

Discussion

The collection of large quantities of cysts of *A.franciscana* for the first time from the natural ecosystem at Tuticorin assumes great significance in view of the better adaptability of this species to wide fluctuations in environmental conditions. The occurrence of *A.parthenogenetica* till November 2000 in the same region from where the cysts of the exotic species were collected in January 2001 and the disappearance of the native species from that region thereafter is a clear indication that the entry of the exotic species into the natural ecosystem have disastrous effect on the native population in near future. The earlier observation carried out by Rajamani *et al.*, (2001) on the distribution of the exotic species in and around Tuticorin further confirms this view.

In the present investigation the cysts of brine shrimp were found to have been deposited along the shores of a salt water pond in Alangarathittu region during one of our routine observations which resulted in the collection of cysts for a period of seven days at a stretch. Although it is possible to some extent to distinguish the cysts of sexual species from that of asexual species based on the size of the cysts, in the present study further observations were carried out to understand the time taken for first hatching and also the composition of male and female in the populations by rearing the

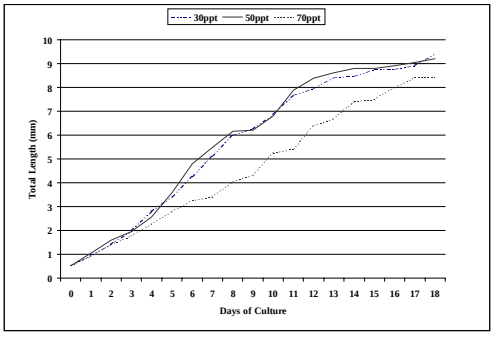

Figure 2a. Growth of *Artemia franciscana* fed with mixed phytoplankton

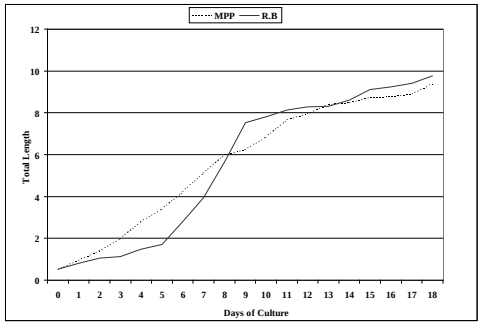

Figure 2b. Growth of *Artemia franciscana* fed with rice bran

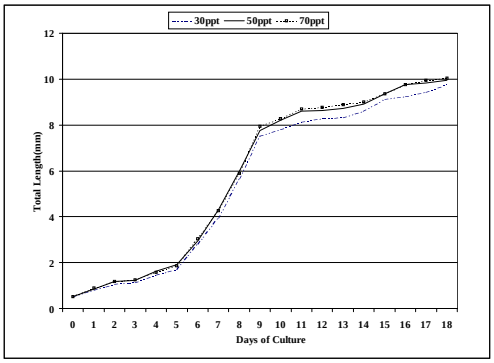

Figure 3a. Growth of *Artemia franciscana* reared at 30 ppt.

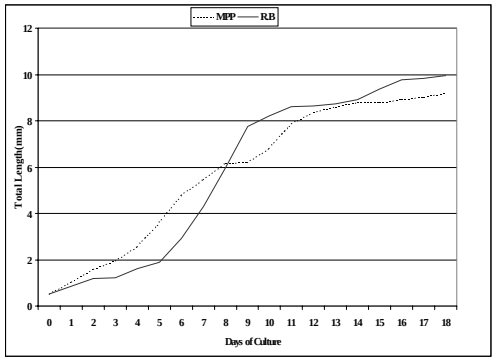

Figure 3b. Growth of *Artemia franciscana* reared at 50 ppt.

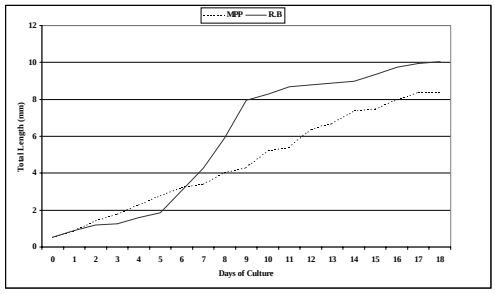

Figure 3c. Growth of *Artemia franciscana* reared at 70 ppt.

nauplii obtained from the hatching experiments to further confirm that the cysts belonged to the sexual species only.

The diameter of the dried cysts varied between 212 ± 11 and 215 ± 8µ only, which indicated that the cysts belong to a sexual species as the cysts of asexual species are known to be larger in size. The time taken for first hatching was less than 15 hours in most of the experiments, which is another characteristic feature of the cysts of sexual species. According to Liao *et al.*, (1993) the time taken for first hatching (T0) for the cysts of Great salt lake was only 14.10 hours which is in agreement with the present investigation. The time taken for the first hatching is normally longer in most of the parthenogenetic strains (Vanhaecke and Sorgeloos, 1982). The average size of the I instar recorded in the entire series of experiments was only 478 ± 6µ which is comparable to the size of the I instar of sexual species (Dhont *et al.*, 1993).

The hatching experiments conducted under laboratory conditions have shown that the quality of the cysts were high

TABLE. 4: *Sex ratio and composition of riding pairs in Artemia franciscana cultured in cylindro-conical tank.*

Source of nauplii (Hatching Expt.No)	Culture Expt.No.	Salinity (ppt)	Feed	Day on which maximum riding pairs were observed	Estimated No. of riding pairs	Estimated No. of unpaired males	Estimated No. of unpaired females	Total No. of males	Total No. of females	Percentage composition	
										Male	Female
3	8	30	Mixed phyto-plankton	17	3733	3266	2466	6999	6199	53	47
3	9	50	Mixed phyto-plankton	17	5066	3600	2932	8666	7998	52	48
3	10	70	Mixed phyto-plankton	24	266	132	132	398	398	50	50
6a	16	30	Rice bran	21	133	266	_	399	133	75	25
6a	17	50	Rice bran	13	533	1332	800	1865	1333	58	42
6a	18	70	Rice bran	13	800	1066	600	1866	1400	57	43

TABLE. 5: *Hydrographical conditions in* Artemia franciscana *rearing tanks.*

Culture Expt.No.	Temperature (`C) min.	max.	Initial Salinity	Salinity range (ppt) min.	max.	Feed(cells/ml) min.	max.	Total quantity of rice bran used (g)
8	23.0	33.0	30.0	27.0	32.0	10000	25000	_
9	23.0	33.0	50.0	44.0	53.0	5000	34000	_
10	23.0	33.5	70.0	68.0	72.0	5000	34000	_
16	24.0	33.0	30.0	30.0	35.0	_	_	28.000
17	24.0	33.0	50.0	50.0	54.0	_	_	90.300
18	24.0	33.0	70.0	70.0	75.0	_	_	98.800

indicating that the cysts were deposited by the animals very recently. Also, the storage of the cysts up to one year has not affected the quality of the cysts significantly as the hatching percentages at 24 and 48 hours were good with values of 43.5 and 53.5 % respectively. However, a remarkable reduction in the hatching percentage was observed thereafter suggesting that the cysts must be utilized within a period of one year when the cysts are dried and stored in plastic containers at room temperature. The hatching efficiency exceeded 100 x 10^3 in all the experiments except two, which were carried out after storing the cysts for more than one year, thus indicating that the cysts are of high quality which can successfully meet the requirement of shrimp seed production (Sorgeloos *et al.,* 1997).

The larvae were reared in outdoor culture tanks to study mating behaviour

and composition of males and females in the population. The earliest sexual maturity was recorded on the 6th day at 50 ppt when the animals were fed with mixed phytoplankton, whereas at the same salinity sexual maturity was delayed by four days when fed with rice bran indicating that feed plays a major role in attaining the sexual maturity. It is interesting to note that in the animals fed with mixed phytoplankton the size at first maturity was only 4.8 mm, whereas in the animals fed with rice bran the size was 8.2 mm at 50 ppt.

In the present investigation the age at the first spawning was 12 days at 50 ppt when fed with mixed phytoplankton, whereas it was 13 days when fed with rice bran. In other words, the interval between first mating and first spawning was five days in the former group whereas, it was only 2 days in the latter suggesting that rice bran is a better feed during the period of egg development particularly at 50 ppt. However, further experiments are required to confirm this hypothesis. Almost similar trends were observed when the animals were reared at 30 ppt. But in contrast the experiments conducted with rice bran at 70 ppt showed an interval of four days between first mating and first spawning which could not be compared with the results obtained for mixed phytoplankton fed animals at this salinity due to the growth of filamentous algae in the culture tanks affecting the growth and maturity of the animals.

The days on which maximum riding pairs were recorded and the percentage composition of males and females in the population in the experiments are given in Table 6. The composition of male was high in all the experiments except one where the sex was equally distributed. The percentage composition of riding pairs in the population indicates the reproductive performance of the animals. In the present investigation the maximum number of 5066 riding pairs, (54% of the total population) were recorded on 17th day in population fed with mixed phytoplankton at 50 ppt followed by 3733 numbers (44% of the total population) at 30 ppt fed with the same feed. The composition of riding pairs was low in rice bran fed population at all the three salinity levels indicating the superiority of the mixed phytoplankton on the reproduction of *A.franciscana*.

Salinity at Langarathittu from where the cysts of *A.fanciscana* were collected, increased from 120 ppt in September 2000 to 180 ppt in October 2000 and then to 210 ppt in November 2000. Thereafter due to the influx of rain water the salinity came down to 95 ppt. *A.parthenogenetica* which was recorded in the salt water pond from September 2000 to November 2000 was not found thereafter indicating that the species could not tolerate extreme saline conditons. It has been observed by Brown and Halanych (1989) and Triantaphyllidis *et al*. (1995) that the sexual species of *A.parthenogenetica* is a superior competitor as it is more adapted to higher salinities when compared to the asexual species. As the cysts of brine shrimp are known to be dispersed by wind and birds there is every chance of the exotic species spreading to other places also. Such a natural dispersal may result in the successful proliferation of the exotic species in the natural ecosystem in near future affecting the native species. Hence measures must be taken to preserve the cysts of *A.parthenogenetica* and also culture of the native species under controlled conditions may be taken up to prevent extinction.

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