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Reproduction, molting, and growth of two Mexican uniparental forms of the tadpole shrimp *Triops* (Branchiopoda: Notostraca) under a recirculating culture system

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Abstract

Two forms (here called short and long) of the tadpole shrimp *Triops* from the Baja California peninsula (Mexico) exhibit interesting features for aquacultural purposes; rapid growth, early maturation (six days) and uniparental reproduction via cysts (i.e. drought-resistant dormant eggs). The use of *Triops* for aquaculture depends on the standard production of viable cysts. Basic information on the reproductive potential of *Triops* is scarce. Using a recirculating system, we studied, through two culture tests, the cyst production, frequency of molting, and growth rate of the two Mexican forms. For each form, individual data were obtained from five specimens in Test 1 over 15 days, and from 10 specimens in Test 2 over 20 days. Hatching response of cysts produced in Test 1 was also studied. The short-form showed a high fecundity depositing groups of cysts from the ovisacs several times a day. The mean cyst production per day was 156–306 (Test 1 & 2), and the mean total cyst production was 2028 (range 728–3193) (Test 1), and 5821 (range 4136–7554) (Test 2). The maximum number of cysts deposited by one short-form individual in a day was 1231 (Test 2). The reproductive performance of the long-form was poor. The mean cyst production per day was only 4.2–7.9 (Test 1 & 2). The short-form molted every 2.5–2.8 days and the long-form molted every 2.8–3.7 days. The mean growth rate calculated from the standard length (mm d^{-1}) of the short-form was 0.43–0.84 (Test 1 & 2), and in the long-form the values were 0.84–1.25 (Test 1 & 2). The poor cyst production of the long-form may be explained by inadequate food resources that did not fulfill the nutritional requirements for reproduction. Given its prolific uniparental reproduction (vs. biparental reproduction), the short-form *Triops* appears as a good potential candidate for aquaculture.

Introduction

The tadpole shrimp *Triops* occurs in all continents except Antarctica. Its natural habitats are temporary freshwater bodies that are characterized by their extreme physical and chemical conditions. Through the production of drought-resistant dormant eggs (cysts), *Triops* populations survive the dry phase of the habitat. Tadpole shrimp are omnivorous and predominantly benthic; they feed on detritus or on live or dead organisms (Martin, 1992). Some forms of *Triops* have been reported as a pest in rice fields of California, U.S.A., Swaziland, Spain, France, Italy, India and Japan (Fox, 1949; Grigarick et al., 1961). Although in the past, attempts were made to eliminate this shrimp, more recently it has been used to control weeds in the rice fields (Takahashi, 1977a,b; Takahashi & Gohda, 1981). *Triops* has been proposed as a biological control agent of mosquitoes (Tietze & Mulla, 1989, 1990, 1991).

In Mexico, two *Triops* forms (here called short and long), which reproduce uniparentally, occur in the southern part of the Baja California peninsula (Maeda-Martínez et al., 2000). Males have not been

observed in the field. The absence of males in these populations has been confirmed through outdoor serial cultures made over the last three years. Besides the uniparental reproduction via cysts, both morphotypes exhibit some interesting biological characteristics for aquacultural purposes, such as a rapid growth and an early maturation (about six days). The uniparental reproduction in notostracans has been explained either by self-compatible hermaphroditism (given the presence of testicular lobes within the ovaries) (Bernard, 1891, 1895), or by automictic parthenogenesis (Zaffagnini & Trentini, 1980). The presence of ovariotestes in histological preparations indicates that both the short and the long *Triops* forms from the Baja California peninsula are anatomically hermaphrodites (unpub. data). However, the former explanations for this uniparental reproduction are still inconclusive given the absence of direct cytogenetic evidence on the mechanism of restoration of diploidy by the haploid oocytes (Sassaman, 1991).

The use of *Triops* for aquaculture, and for mosquito control, depends on the availability of viable cysts. Soil from natural habitats or from man-made pools could function as a source of cysts. Uncontrolled cyst production may result, however, in a limited quantity and a highly variable quality (e.g. variable hatching percentage and nutritional value). Obviously, the alternative is a mass-production technique, which has yet to be developed. For mass production, information on the reproductive attributes of *Triops*, such as the frequency of oviposition, number of cysts per oviposition, frequency of molting, and total reproductive potential is essential. Only few papers have focused on these topics. Takahashi (1977a) observed the daily oviposition of ten cultured individuals of *Triops* '*longicaudatus*' from Japan and found the maximum number of cysts deposited within 24 h was 246, and estimated the total number of cysts deposited during a life span was about 1850. Takahashi also reported the maximum number of cysts deposited within a day in individuals collected from the field was 430. This author concluded that an individual may produce several thousand cysts in one generation. Scott & Grigarick (1978) studied the fecundity of a uniparental form of *Triops* from the northern part of California (cited as *T. longicaudatus*). According to the characteristics the authors presented, this form is morphologically similar to our short form. These authors reported the individual fecundity in the laboratory was quite variable; in 24 h the mean cyst production was 81 (range 5–153), and the maximum number of cysts from a single shrimp in 72 h was 594. The cysts were carried from 19 h to several days, and later oviposited between molts. Weeks (1990) studied the life-history variation of four lines, three uniparentals (cited as selfing) and one biparental (cited as sexual) of *Triops 'longicaudatus'*. Within these lines, Weeks distinguished two morphological forms, short and long. The short form included two uniparental lines with 5 apodous segments (range 4–6), and the long form included the biparental line and one uniparental line with 8 apodous segments (range 7–9). Weeks studied the growth, cyst production, and survival of the four lines at three different densities (5, 10 and 16 animals). This author reported individual cyst production was severely affected by increased density. The shrimp line was also significant with the two short-form lines producing the most cysts, the uniparental long-form with an intermediate quantity, and the biparental long-form producing the fewest cysts (Weeks, 1990). Meintjes (1996) reported that biparental *Triops 'granarius'* exhibited a clutch size of 1–154 cysts, and a clutch frequency of 1–9 in 24 h. Other reproductive studies have focused on the gonadal anatomy of individuals from both uniparental and biparental populations of *Triops cancriformis*, *T. 'granarius'*, and *T. 'longicaudatus'* (Bernard, 1891, 1895; Longhurst, 1954, 1955; Akita, 1971; Zaffagnini & Trentini, 1980). Oogenesis in uniparental and biparental *Triops cancriformis* was studied by Trentini & Sabelli-Scanabissi (1978), Sabelli-Scanabissi & Trentini (1979), and Engelmann et al. (1996, 1997). Yolk protein synthesis in *Triops 'longicaudatus'* has been investigated by Riley & Tsukimura (1998).

In this paper, we present data on cyst production, frequency of molting and the growth rate of the short and long forms of the Baja California peninsula in a recirculating culture system. Additional data on hatching response are provided. In this study, we considered the following two points: (1) To make observations on animals kept individually; this prevents a possible cannibalism, and competition for the resources (see Weeks, 1990), therefore, the reproductive output observed can be a good estimate of the reproductive potential of these animals; and (2) to provide a culture water with physical, chemical and biological characteristics similar to the natural habitat.

Materials and methods

Soil containing short-form cysts was collected at km 64, Federal Highway No. 1, La Paz-San José del Cabo, Baja California Sur. Soil containing long-form cysts was collected at 76.5 km, Federal Highway No. 1, La Paz-Cabo San Lucas, Baja California Sur. Reproduction, molting, and growth of both *Triops* forms were studied through two culture tests. Test 1, made during May–June 1999, included individual observations to five animals per morphotype; soil from the short-form habitat was used as substrate. At the beginning of Test 1, the animals of both forms were adults with ovisacs full of cysts, however the age differed between the forms (see Test 1). Test 2, made during June–July 2000, included individual observations on 10 animals per morphotype; soil from the long-form habitat was used as substrate. At the beginning of Test 2, the animals of both forms were adults of the same age, just at the maturation stage of the first cyst production.

Culture system

The main parts of the system (Fig. 1) are: (1) Outdoor 2000-l fiber glass tank. In this tank, water conditions of a temporary pond were simulated. Water from this tank is transferred using an electric pump. The suction tip consists of a copper valve connected to a PVC tube. The suction tip is placed into a cage covered with a 2-mm mesh net. (2) Indoor 800-l plastic tank. This tank receives water from the outdoor tank. The water level (volume = 400 l) is controlled by an electric float. (3) Culture containers. Each plastic container receives water from the indoor tank by gravity. The inlet water flow for each container is controlled by a PVC tap. The water flow in the containers was ca. 1 l min^{-1}. In Test 1, 10 plastic containers (69 × 37 × 21 cm) were used, and the water column in the containers was ca. 10 cm. In Test 2, 20 plastic containers (47 × 29 × 14.5 cm) were used, and the water column in the containers was ca. 5 cm. (4) Fiber glass collector tank (50 l). This tank collects (by gravity) the outlet water from the culture containers. The collected water is returned to the outdoor tank by gravity.

Test 1

Cyst production, molting, and growth

Individuals of both short and long forms were obtained from outdoor static cultures. These cultures were established in 1200-l fiber glass tanks using tap water and 20 kg of soil of the original habitat as a source of cysts and as a culture substrate. Data of water temperature and total dissolved solids (TDS) were taken daily at 1000 with a Conductivity - TDS meter HACH-44600. The outdoor culture of the short form started 09 May 1999, and lasted 60 days with a mean temperature of 23.9 °C (*s* 2.31, range 20.6–27.2), and a mean TDS of 1.34 g l^{-1} (*s* 0.07, range 1.15–1.52). The outdoor culture of the long form was started 02 June 1999, and lasted 40 days with a mean temperature of 24.6 °C (*s* 2.10, range 21.9–30.0), and a mean TDS of 0.79 g l^{-1} (*s* 0.08, range 0.70–1.04). On 16 June 1999, five short-form individuals (ca. 38-days-old) and five long-form individuals (ca. 13-days-old) were taken from the outdoor cultures and used for the indoor study. All animals had ovisacs full of cysts. If an animal died during the study, it was replaced by another animal taken from the same outdoor culture. The culture test in the recirculating system lasted 21 days. Soil (20 kg) from the habitat of the short form was added as substrate in the outdoor 2000-l tank of the system. The tank was then filled with tap water (TDS = 0.6 g l^{-1}) on 09 May 1999. To determine the growth, the morphometry was taken at the beginning (culture day 0) and the end of the culture test (culture day 21) or when the animal died (Tables 4 and 5). The first six days were used as an acclimation period. From day 6, 1.2 g of commercial food pellets for shrimp (PIASA-40, México) was added daily to each container. Water temperature and TDS in the culture containers were taken daily at 1030. During the study the water had a mean temperature of 22.4 °C (*s* 1.01, range 20.9–24.4), and a mean TDS of 1.43 g l^{-1} (*s* 0.06, range 1.34–1.55). On the last culture day (day 22), the water had a pH of 8.4 (pH meter ORION-230A). Each day, the culture containers were cleaned after carefully removing the shrimp. Water and sediment were siphoned and passed through a 200-μm nylon gauze to separate cysts, exoskeletons, and food pellets. Observations to determine the frequency of molting started on day 6, and ended on day 21, and the evaluation of the cyst production started from the first molt observed in that period. Cysts collected from the culture containers were counted, grouped in a plastic flask with water from the containers, and stored in the dark.

Incubation method and hatching response

Cysts from short-form individuals produced on day 15 were used to study the hatching response. Eight groups of 100 cysts were formed. Four groups were

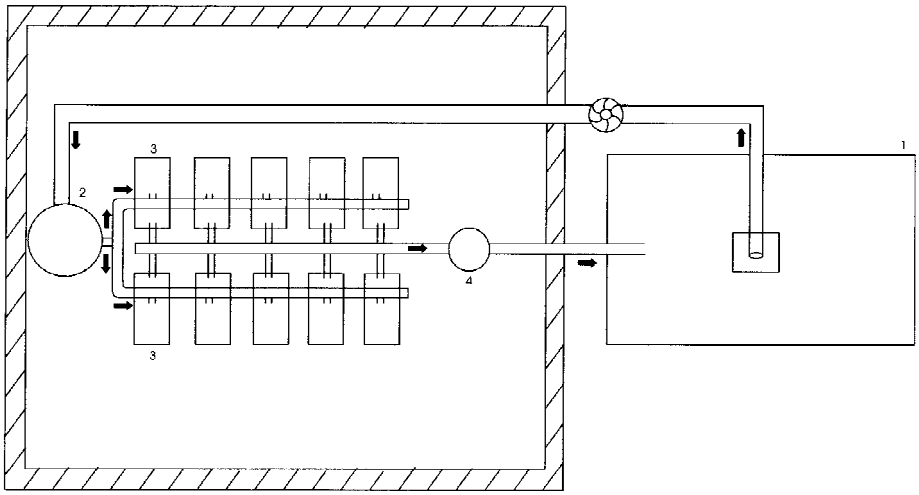

Figure 1. Re-circulating culture system. 1. outdoor 2000-l fiber glass tank, 2. indoor 800-l plastic tank, 3. plastic containers for culture, & 4. fiber glass collector tank (50 l).

Table 1. Cyst production and molting of the short-form *Triops* in Test 1. Sp = specimen; *s* = standard deviation; M = molt

Sp	Culture day																Total	Mean	*s*	Total
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	cysts			molts
1	–	–	M	10	75 M	3	83	119 M	182	112	46 M	37	23	21	17	0	728	56	55	4
2	–	–	M	200	167 M	181	164 M	319	266	217 M	320	268	219	350 M	404	118	3193	246	84	5
3	–	–	M	37	336 M	70	292 M	170	399 M	185	165	256 M	307	60	71	250 M	2598	200	118	6
4	M	2	52	50 M	187	92 M	215	132 M	315	150	247 M	133	371	476	43 M	19	2484	166	138	6
5	–	M	72	65 M	30	57	156 M	138	307	55 M	0	10	0	240 M	2	7	1139	81	96	5

dried in a Millipore oven for 7 days at 39 °C. The other four groups were not dried. For hatching, the eight groups were incubated at the same time and under same conditions. The few cysts produced by the long-form individuals were divided into three groups of 36 and first submitted to incubation without dehydration. The hatching response of cysts produced in the recirculating system was compared to the hatching response of cysts produced in outdoor static cultures. The last cysts were obtained as follows: individuals of both the short and the long shrimp were cultured in separate outdoor tanks using soil from their original habitats as substrate. From these cultures, 25 short-form adults were placed in one tank, and 21 long-form adults were placed in another tank. Before transferring the animals, 20 kg of soil (free of *Triops* cysts) from the campus of CIBNOR (El Comitán, La Paz, Baja California Sur) was placed in each tank. As a food supplement, 5 g of dry baker's yeast (Leviatan, Mexico), and 5 g of corn powder (Maseca, Mexico) were added once every two days to the tanks. The culture water remained in the tanks from 15 October 1998 to 16 December 1998. During that period, the water temperature varied and slowly decreased from 27.5 to 15.2 °C, and the TDS increased from 0.69 to 1.81 g l^{-1}. To obtain the cysts, the water in the tanks was allowed to evaporate for 30 days. From the dry mud, cysts were separated by hand under a stereomicroscope to form groups of 100 cysts each. The hatching percentage was determined from three replicates for the short form, and from four replicates for the long form. The incubation method was as follows: each cyst group (dehydrated or nondehydrated) was placed in a transparent polystyrene flask (60 ml) with cap, and bottom fixed with a 100-μm gauze. Each incubation flask was placed in a 600-ml container, submerged in potable water (TDS 0.25 g l^{-1}, pH 8.0), and incubated at 25.8 ± 0.2 °C. Scott & Grigarick (1979) reported *Triops 'longicaudatus'* cyst hatching occurred at a pH range of 3.1–10.0 (maximum hatching at 5.6) and a temperature range of 16.5–29.0 °C (maximum hatching at 22.0 °C). Constant aeration was supplied

to the container through Pasteur pipettes. Light was provided continuously by two 40-W fluorescent lamps installed 30 cm above the hatching flasks. Hatching was determined every 24 h, for 8 days.

Test 2

Cyst production, molting, and growth

On 6th June 2000, 200 cysts of each form were incubated using the method described above. On the next day, hatched nauplii were transferred to an outdoor tank. Eight days later, individuals of both forms started to produce cysts. On that day, 10 animals of each form were distributed into 20 culture containers. The culture under the recirculating system lasted 20 days. On 6th June 2000, soil (10 kg) from the long-form habitat was added as substrate in the outdoor 2000-l tank of the system. To determine the growth, morphometry was taken at the beginning (culture day 0) and at the end (culture day 20) of the test (Tables 9 and 10). From culture day 0, a feed composed of 1.2 g of commercial food pellets for shrimp (PIASA-40, México), 0.3 g of dry baker's yeast (Leviatan, México), and 0.5 g of frozen adult *Artemia* was added daily to each container. Water temperature and TDS in the culture containers were taken daily at 1030. During the study, the water had a mean temperature of 22.4 °C (*s* 1.01, range 20.9–24.4), and a mean TDS of 1.43 g l^{-1} (*s* 0.06, range 1.34–1.55). On the last culture day (day 20), the water had a pH of 8.4. Each day, the culture containers were cleaned after carefully removing the shrimp. Water and sediment were siphoned and passed through a 200-μm nylon gauze to separate cysts, exoskeletons, and food pellets. Observations to determine the cyst production and the frequency of molting started at culture day 1, and ended at culture day 20.

Morphometry

Using a vernier caliper and a binocular stereomicroscope, the following morphometric measurements were made: carapace length, taken from the anterior edge to the posterior edge on the middle line of carapace, and standard length, taken from the anterior edge of carapace to the posterior edge of the telson.

Morphological features of morphotypes

Individuals of the short form had an olive-green dorsal side of the carapace with irregular dark green spots, spinous carina, five to seven legless segments, and three to six spines on the dorsal posterior border of the telson. Individuals of the long form had a yellow-brownish dorsal side of the carapace, smooth carina, seven to eight legless segments, and no spines on the dorsal posterior border of the telson.

Table 2. Number of cysts produced by the short-form *Triops* between consecutive molts in Test 1. Sp = specimen; *s* = standard deviation

Sp	Intermolt					Total intermolts	Mean	*s*
	1	2	3	4	5			
1	85	205	340	–	–	3	210	128
2	367	345	802	1157	–	4	668	388
3	373	362	569	606	688	5	520	145
4	104	279	347	712	1023	5	493	370
5	137	243	500	250	–	4	283	154

Results

Test 1

Cyst production, molting, and growth

Short-form individuals normally produced cysts every day (Table 1). The maximum number of cysts deposited by one individual in 24 h was 476. The overall mean number of cysts produced per day was 156 (*s* 80, range 56 – 246). Shrimp molted four to six times in 15 days, i.e. about one molt every 2.8 days. During the culture period, the survival of short-form individuals was 100%. In comparison, reproduction of long-form individuals was poor (Table 3). Shrimp of containers 3 and 4 died on day 12 and 11. The overall mean number of cysts produced per day was 4.2 (*s* 4.3, range 0.2–12.4). The three shrimp that survived the whole observation period molted four times, i.e. about one molt in every 3.7 days. Long-form individuals grew faster than the short-form ones (Tables 4 and 5). The growth rate of long-form individuals was 0.46 mm d^{-1} in carapace length, and 0.84 mm d^{-1} in standard length, whereas in the short-form individuals they were 0.28 mm d^{-1} and 0.43 mm d^{-1} (Tables 4 and 5).

Hatching response

Hatching percentages are shown in Figure 2. Short-form cysts from the indoor culture did not show a significant difference in total hatching between the nondehydrated and dehydrated cyst groups (Student's *t*-test, $P > 0.05$), with a mean of 31% (*s* 4.6, range

Table 3. Cyst production and molting of the long- form *Triops* in Test 1. D = dead specimen; M = molt; Sp = specimen; *s* = standard deviation

Sp	Culture day																Total cysts	Mean	*s*	Total molts
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21				
1	–	–	M	0	1 M	0	0	6	0	1	0 M	0	0	0	16 M	1	25	1.9	4.53	4
2	–	–	M	0	2	0 M	0	0	0 M	1	0	0 M	0	0	0	0	3	0.2	0.59	4
3A	–	–	M	3	3 M	0	0 M D										6	1.5	1.73	3
3B								–	M	37	0	0	0 M	19	0	31 M	87	12.4	16.37	3
4A	–	–	M	2	4 M	0 D											6	2.0	2.00	2
4B							–	–	M	5	0	46 M	0	0	0	1	52	7.4	17.10	2
5	–	–	M	3	49 M	0	0	0 M	0	0	0	0	0 M	0	0	0	52	4.0	13.54	4

Table 4. Morphometry, growth and growth rate of the short-form *Triops* in Test 1. Sp = specimen; *s* = standard deviation

Sp	days	Carapace length (mm)				Standard length (mm)			
		initial	final	growth	growth rate (d^{-1})	initial	final	growth	growth rate (d^{-1})
1	21	11.9	18.0	6.1	0.29	23.4	33.2	9.8	0.46
2	21	12.2	19.2	7.0	0.33	24.4	35.1	10.7	0.50
3	21	12.1	18.9	6.8	0.32	22.7	33.4	10.7	0.50
4	21	12.5	17.9	5.4	0.25	25.0	33.2	8.2	0.39
5	21	12.0	16.6	4.6	0.21	23.6	30.3	6.7	0.31
Mean		12.1	18.1	6.0	0.28	23.8	33.0	9.2	0.43
s		0.2	1.0	1.0	0.05	0.9	1.7	1.7	0.08

Table 5. Morphometry, growth and growth rate of the long-form *Triops* in Test 1. Sp = specimen; *s* = standard deviation

Sp	days	Carapace length (mm)				Standard length (mm)			
		initial	final	growth	growth rate (d^{-1})	initial	final	growth	growth rate (d^{-1})
1	21	14.0	23.5	9.5	0.45	28.1	45.2	17.1	0.81
2	21	15.3	25.0	9.7	0.46	30.5	48.5	18.0	0.85
3A	12	12.4	18.2	5.8	0.48	24.0	35.9	11.9	0.99
3B	09	18.5	23.0	4.5	0.50	32.2	43.3	11.1	1.23
4A	11	14.6	19.2	4.6	0.41	29.4	38.3	8.9	0.80
4B	10	18.2	22.1	3.9	0.39	33.3	41.4	8.1	0.81
5	21	14.7	24.5	9.8	0.46	29.4	47.3	17.9	0.85
Mean [a]		14.7	24.3	9.6	0.46	29.3	47.0	17.7	0.84
s^a		0.6	0.7	0.1	0.01	1.2	1.6	0.4	0.02

[a] From specimens 1, 2 & 5.

25–35), and 23% (s 4.96, range 19–30). However, a significant difference in the hatching rate was observed. In the dehydrated cyst group, the maximum hatching was during the first 24 h, whereas in the nondehydrated cyst group there was a delayed hatching; the hatching started after 48 h, and the maximum hatching was between 72 and 96 h of incubation. The mean total hatching percentages of dehydrated cysts of both short and long forms from outdoor cultures were 84% (*s* 7.23), and 60% (*s* 5.85) (Fig. 2). Hatching response of nondehydrated long-form cysts from the indoor culture was zero.

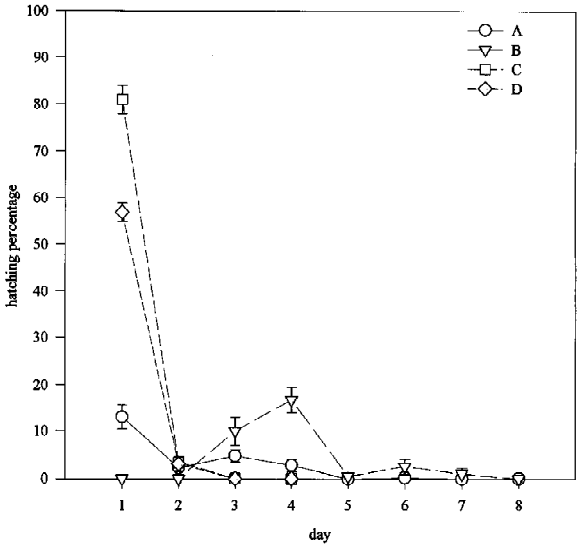

Figure 2. Hatching response of (A) dehydrated short-form cysts from indoor culture, (B) nondehydrated short-form cysts from indoor culture, (C) dehydrated short-form cysts from outdoor culture, (D) dehydrated long-form cysts from outdoor culture. The symbols show the mean and the standard error.

Test 2

Cyst production, molting, and growth

From culture day 2, short-form individuals produced cysts every day (Table 6). The maximum number of cysts deposited by one individual in 24 h was 1231. The overall mean number of cysts produced per day was 306 (*s* 70.4, range 217–397). Shrimp molted seven to nine times within the 20 culture days, i.e. about one molt every 2.5 days. Survival of short-form individuals was 100%. Again, reproduction of long-form individuals was poor (Table 8). Shrimp in containers 4, 5, 7, 8 and 9 died between culture days 12 and 17. The overall mean number of cysts produced per day was 7.9 (*s* 7.4, range 1.1–23.5). The five shrimp that survived the 20-day culture period molted seven times, i.e. about one molt in every 2.8 days. Again, long-form individuals tended to grow faster than the short-form ones. The mean growth rate of long-form individuals was 0.59 mm d^{-1} in carapace length, and 1.25 mm d^{-1} in standard length, whereas in short-form individuals they were 0.48 mm d^{-1} and 0.84 mm d^{-1} (Tables 9 and 10).

Discussion

As estimated by Takahashi (1977a), the reproductive potential of *Triops* is thousands of cysts produced during the life span of one individual. We observed from the short-form an overall mean production of 2028 cysts (range 728–3193) within 15 days (Table 1) in Test 1, and 5821 cysts (range 4136–7554) within 19 days (Table 6) in Test 2, where the maximum number of cysts produced by one individual in a day was 1231. Short-form shrimp had larger mean individual fecundity (150 cysts d^{-1} in Test 1, and 299 cysts d^{-1} in Test 2) than that (81 cysts d^{-1}) reported for a uniparental *Triops* from California studied by Scott & Grigarick (1978) under a static culture. Low values of 129 and 141 of mean individual cyst production in a period of 30 – 40 days were reported by Weeks (1990) for two short-form lines of *T. 'longicaudatus'*. The reproductive advantage of the uniparental forms over biparental entities appears obvious when comparing the short-form data with those presented by Meintjes (1996), who estimated about 206 cysts for a life span of 25 days in biparental *Triops 'granarius'*. But, how long is the reproductive period of *Triops*? Unfortunately, there are no precise data on this in the literature. At the end of the culture Test 1, the short-form shrimp were about 60-days-old. These animals started producing cysts at day 6 (in the outdoor culture), so the shrimp were probably in reproduction for at least 50 days. Therefore, 60 days is a conservative number for the length of the reproductive period of the short-form *Triops*. From laboratory-reared animals, Takahashi (1977a) reported *T. 'longicaudatus'* producing cysts during 15 days, and Takahashi & Gohda (1981) observed *T. 'cancriformis'* producing cysts for about 50 days.

To estimate the number of cysts per oviposition and the frequency of oviposition, we determined the number of cysts contained in both ovisacs of ten individuals of each form collected from the outdoor cultures. As expected, we found that there is a positive relation between the carapace length and the number of cysts in ovisacs (Tables 11 and 12). Short-form animals with a carapace length of 13.7–18.2 mm had from 62 to 124 cysts. Long-form animals with a carapace length of 17–20 mm had from 81 to 192 cysts. Of 69 cyst releases (Test 1) and 190 cyst releases (Test 2) of the short form, 21 (Test 1) and 111 (Test 2) had more than 200 cysts discharged within a day (Tables 1 and 6). This indicates that the short-form shrimp deposited groups of cysts from the ovisacs several times in a day.

In branchiopods of the order Anostraca, there is a direct relation between oviposition and molting, i.e. the molt occurs after every oviposition (Bowen, 1962; Murugan et al., 1996). Though Fox (1949) mentioned

Table 6. Cyst production and molting of the short-form *Triops* in Test 2. M = molt; Sp = specimen; *s* = standard deviation

Sp	Culture day																				Total	Mean	*s*	Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	cysts			molts
1	0	9 M	35	38 M	104	17	4 M	324	111 M	275	541	565	324	521 M	516	831	627 M	635	923	535 M	6935	365	293	7
2	0	62 M	8	18 M	0	340 M	84	423 M	1	410 M	347	406	426 M	508	377	362 M	934	1122	713 M	410	6951	365	310	8
3	0	29 M	7	4 M	273	268 M	276	170 M	150	125	232 M	276	253	334	204	105 M	420	660 M	340	10	4136	217	162	7
4	0	23 M	13	84 M	47	181 M	9	24 M	167	379 M	712	453 M	421	784	510 M	93	520	1231	1099	262 M	7012	369	369	8
5	0	102 M	4	349 M	506	50 M	291	58 M	92	255 M	215	458 M	198	405 M	265	472	272 M	221	207	226 M	4646	244	146	9
6	0	32 M	4	7 M	3	192 M	103	176 M	309	275 M	342	580	370 M	351	979 M	856	650	703	822 M	800	7554	397	323	8
7	0	70	100 M	99	102	253	293 M	447	313 M	466	274 M	423	279 M	360	286	339 M	682	560	297 M	109	5752	302	165	7
8	0	152 M	100 M	35	39 M	175	347 M	220	278	276 M	205	571 M	559	335	624 M	386	678	825 M	388	364	6557	345	221	8
9	0	10 M	45	277 M	113 M	72	315 M	106	560 M	470	63	97 M	198	50	29 M	532	747 M	178	230	192	4284	225	210	8
10	0	77 M	96	189 M	109	229 M	193	59 M	162 M	165 M	270	216 M	117	281 M	74	570	224 M	184	758	419	4392	231	178	9

Table 7. Number of cysts produced by the short-form *Triops* between consecutive molts in Test 2. Sp = specimen; *s* = standard deviation

Sp	Intermolt									Total intermolts	Mean	*s*
	1	2	3	4	5	6	7	8	9			
1	9	73	125	435	2226	1974	2093	–	–	7	990	1046
2	62	26	340	507	411	1179	1247	2769	–	8	817	911
3	29	11	541	446	507	1172	1080	–	–	7	540	454
4	23	97	228	33	546	1165	1715	3205	–	8	876	1120
5	102	353	556	349	347	673	603	1009	654	9	516	262
6	32	11	195	279	584	1292	1330	3031	–	8	844	1026
7	170	747	760	740	702	985	1539	–	–	7	806	407
8	152	100	74	522	774	776	1518	1889	–	8	725	673
9	10	322	113	387	666	630	277	1279	–	8	460	400
10	77	285	338	252	162	165	486	398	868	9	336	236

Table 8. Cyst production and molting of the long-form *Triops* in Test 2. D = dead specimen; M = molt; Sp = specimen; *s* = standard deviation

Sp	Culture day																				Total cysts	Mean	*s*	Total molts
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20				
1	12	0	0 M	0	10 M	0	0 M	16	27 M	110	31 M	0	25	9	0 M	40	6	12 M	9	39	346	17.3	25.5	7
2	25	0 M	0 M	15	11 M	0	0 M	0	0 M	0	1	0	0 M	0	0	0	0 M	0	0	0	52	2.6	6.6	7
3	22	0 M	0	0	0 M	15 M	0	2 M	0	0	0 M	0	0	3	0 M	0	0	9 M	0	0	51	2.5	6.9	7
4	9	0 M	0	0 M	0	0 M	0	2 M	0	0	0 M	3 D									14	1.1	2.6	5
5	45 M	0 M	0	0 M	0	0 M	0	0 M	0	20 M	15 M	0	10	0	2	0	50 D				142	8.3	15.9	7
6	33 M	0	0 M	0	3 M	0	0 M	0	15	37 M	40	300	0 M	0	0	0	5 M	0	20	18	471	23.5	66.4	7
7	14 M	0	0 M	0	0 M	24	0	0 M	0	0	0	0 M D									38	3.1	7.6	5
8	13 M	0	0 M	0	0 M	2 M	0	0	0 M	0	0	2	2	3	0 M	10 D					32	2.0	3.8	6
9	72 M	0	0 M	50 M	0	0 M	0	0	0 M	0	0	0 M	0	0 D							122	8.7	22.5	6
10	39	0 M	0 M	0	0 M	0	0 M	0	90 M	0	76	0	0 M	0	0	0	0	0 M	0	0	205	10.2	26.4	7

Table 9. Morphometry, growth and growth rate of the short-form *Triops* in Test 2. Sp = specimen; *s* = standard deviation

Sp	days	Carapace length (mm)				Standard length (mm)			
		initial	final	growth	growth rate (d^{-1})	initial	final	growth	growth rate (d^{-1})
1	20	12.2	21.4	9.2	0.46	23.2	37.8	14.6	0.73
2	20	11.4	21.1	9.7	0.48	21.4	37.2	15.8	0.79
3	20	11.7	22.0	10.3	0.51	21.5	39.8	18.3	0.91
4	20	11.5	20.7	9.2	0.46	21.8	35.9	14.1	0.70
5	20	9.8	21.6	11.8	0.59	18.9	37.5	18.6	0.93
6	20	11.6	21.4	9.8	0.49	22.6	37.7	15.1	0.75
7	20	9.5	20.2	10.7	0.53	18.0	36.0	18.0	0.90
8	20	9.6	19.0	9.4	0.47	18.2	35.4	17.2	0.86
9	20	10.1	20.0	9.9	0.49	19.0	35.4	16.4	0.82
10	20	9.2	20.4	11.2	0.56	17.7	38.3	20.7	1.03
Mean		10.8	20.7	10.1	0.48	20.2	37.1	16.9	0.84
s		1.0	0.9	0.8	0.04	2.0	1.4	2.0	0.10

Table 10. Morphometry, growth and growth rate of the long-form *Triops* in Test 2. Sp = specimen; *s* = standard deviation

Sp	days	Carapace length (mm)				Standard length (mm)			
		initial	final	growth	growth rate (d^{-1})	initial	final	growth	growth rate (d^{-1})
1	20	8.6	19.7	11.1	0.55	17.3	41.5	24.2	1.21
2	20	13.0	21.5	8.5	0.42	23.1	43.4	20.3	1.01
3	20	9.8	21.7	11.9	0.59	18.3	44.3	26.0	1.30
4	12	12.0	20.5	8.5	0.70	21.5	41.0	19.5	1.62
5	17	10.8	22.7	11.9	0.7	20.2	40.8	20.6	1.21
6	20	11.5	21.6	10.1	0.50	21.3	42.0	20.7	1.03
7	12	12.0	19.0	7.0	0.58	22.0	40.4	18.4	1.53
8	16	11.5	23.0	11.5	0.71	21.4	40.5	19.1	1.19
9	14	11.4	20.0	8.6	0.61	20.8	39.2	18.4	1.31
10	20	10.7	20.9	10.2	0.51	18.2	41.3	23.1	1.15
Mean		11.1			0.59	20.4			1.25
s		1.2			0.09	1.8			0.19

Table 11. Number of cysts contained in ovisacs of the short-form *Triops*

Specimen	Carapace length (mm)	Ovisac		Total cysts
		left	right	
1	13.7	40	22	62
2	14.2	29	34	63
3	14.6	43	52	95
4	15.1	33	48	81
5	15.2	56	40	96
6	15.3	44	31	75
7	15.3	46	46	92
8	15.4	49	53	102
9	15.6	48	31	79
10	18.2	78	46	124

Table 12. Number of cysts contained in ovisacs of the long-form *Triops*

Specimen	Carapace length (mm)	Ovisac		Total cysts
		left	right	
1	17	50	48	98
2	18	41	40	81
3	19.3	50	57	107
4	19.3	70	62	132
5	19.4	60	65	125
6	19.4	70	65	135
7	19.8	41	72	113
8	19.9	60	103	163
9	19.9	75	92	167
10	20	110	82	192

that *Triops cancriformis* carried the cysts for about one day and then shed them with the molt, Scott & Grigarick (1978) reported *T. 'longicaudatus*' carried the cysts from 19 h to several days and the cysts were usually oviposited between molts. Meintjes (1996) pointed out that oviposition by *Triops 'granarius'* occurred daily and independent of molting. We also found that oviposition and molting appear as two independent events. The short-form shrimp released groups of cysts from one to several times in a day and had several ovipositions between two consecutive molts. We determined the number of cysts produced between consecutive molts and found that this number normally increased in every next molt (Tables 2 and 7). This can be explained by the increase of the body size (Tables 11 and 12).

The hatching levels of 23% and 31% shown by both the dehydrated and nondehydrated cyst groups of the short form appear quite low if compared with the hatching response of dehydrated cysts of both short and long forms obtained from the outdoor cultures, where the mean percentages were 84% and 60% (Fig. 2). These results contrast with those obtained by Scott & Grigarick (1979) who studied *Triops 'longicaudatus'* from California rice fields and found the degree of desiccation prior to incubation had a negative influence on cyst hatching, where the maximum hatch of 78% observed during 18 days occurred in

cysts kept in water. Takahashi (1977a) reported that the hatching of *T. 'granarius'* cysts kept in water at 19 °C began on the eighth day after oviposition and continued intermittently during 100 days reaching about 52% of total hatching. It seems the lapse between laying of cysts and the moment when the desiccation occurs has a strong impact on hatching. Hempel-Zawitkowska & Klekowski (1968) found that the total hatchability of *Triops cancriformis* cysts after desiccation at different relative humidities (33%, 55% 77% and 85%) was from 0 to 36% when newly laid cysts remained in water from 1 to 3 days prior to desiccation, whereas cysts of the same type remaining in water for 6 days hatched from 76% to 84%. Under standard conditions of harvesting, cleaning, storing and incubating the cysts, the hatching response variation within the same population or species can be explained by differences in the life conditions of the reproducers (Lavens & Sorgeloos, 1987; Mura & Zarattini, 1999). According to Lavens & Sorgeloos (1987), hatching features in the brine shrimp *Artemia* are related to the amount of food available and the salinity. However, Scott & Grigarick (1979) and Mura & Zarattini (1999) have reported wide hatching variations even within cyst samples from the same parents of the branchiopods *Triops 'longicaudatus',* and *Chirocephalus ruffoi*. Cysts produced by individual pairs of fairy shrimp hatched continuously during several hydration-drying cycles (Hildrew, 1985; Simovich & Hathaway, 1997).

The poor reproduction exhibited by the long-form shrimp in both culture tests, can be explained by inadequate food resources that did not fulfill the nutritional requirements for reproduction, even though the animals molted and grew (Tables 5 and 10). In fact, the long form grew faster than the short form in both tests. Weeks (1990), comparing a long and a short form of *Triops* from the United States, also found the long form tended to grow larger but produced fewer cysts than the short form.

The reproductive maturation size (i.e. first time with cysts in ovisacs) in the short-form shrimp was 9.0–9.6 mm carapace length, and 16.2–19.0 mm standard length. Takahashi (1977a) reported that the oviposition in *T. 'longicaudatus'* and *T. 'granarius'* began when the carapace length exceeded 7 mm. From the growth data of the carapace length presented by this author, we calculated that, from a mean of 0.65 mm at day 4 up to a mean of 8.33 mm at day 22, *T. 'granarius'* showed a growth rate of 0.42 mm d^{-1}, whereas *T. 'longicaudatus*', from a mean of 7.17 mm at day 8 up to a mean of 11.01 mm at day 19, had a growth rate of 0.34 mm d^{-1}. Our long-form had a comparable growth rate of the carapace length (0.46 mm d^{-1} in Test 1, and 0.59 mm d^{-1} in Test 2).

The short and long forms of *Triops* from the Baja California peninsula exhibited significant differences in reproduction when reared under identical conditions. These data support the view that these forms are indeed two different species (Maeda-Martínez et al., 2000). Given its prolific uniparental reproduction (vs. biparental reproduction) via cysts, the short-form *Triops* appears as a good potential candidate for aquaculture, for example, through the use of its nauplii, similarly as the larvae of the branchiopod *Artemia* are used.

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