Hydrobiologia **194**: 13–22, 1990.

Factors affecting the hatching of *Streptocephalus macrourus* Daday (Crustacea; Eubranchiopoda) eggs

S.A. Mitchell
Unit for Limnology, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300, Bloemfontein 9300, South Africa

Received 25 August 1988; accepted 20 December 1988

Key words: anostracan, egg hatching, light, temperature, desiccation, dissolved oxygen

Abstract

A major drawback to the use in aquaculture of members of the Eubranchiopoda from temporary pool environments is that their eggs do not hatch readily. An investigation of the factors influencing the hatching of eggs of the fairy shrimp *Streptocephalus macrourus*, showed that light was the only factor of those investigated that was obligatory for hatching. It was found that eggs which had not been desiccated hatched successfully in the presence or absence of adults, while those which had been desiccated showed a block in hatching initially, although this block deteriorated with time and after approximately two months the eggs which had been desiccated showed a similar hatching success to that of the non-desiccated eggs. Exposure of eggs to extremes of heat or cold before incubation did not influence the hatching success of the eggs significantly, but the temperature at which incubation took place was important. The optimal range lay between 14 °C and 20 °C. Eggs hatched and nauplii survived at dissolved oxygen tensions of below 0.5 mg l^{-1}

Introduction

The fairy shrimp *Streptocephalus macrourus* Daday is apparently typical of freshwater anostracans in that it produces only one type of egg, and this is capable of resisting desiccation. Although desiccation is not essential for hatching, the difficulty of stimulating eggs to hatch has been identified by Dinges (1982) as a major drawback to their use in aquaculture. In contrast to this, the brine shrimp *Artemia* is able to reproduce either ovoviviparously or by means of resting eggs, and so this problem does not occur in *Artemia* cultures. In the opinion of Persoone & Sorgeloos (1980), the inability of the freshwater anostracans to reproduce ovoviviparously is the reason that dense populations of these organisms are seldom found in nature. These authors consider the production of resting eggs solely as an adaptation to the temporary nature of the habitat occupied by the anostracans.

The natural environment of these organisms are pans (playas) which hold water for varying periods after rain, but are dry for the greater proportion of the year. The organisms survive the dry period as eggs in the pan sediment, and during this time are exposed to temperature extremes. In mid-summer a maximum temperature of 66 °C was recorded at a depth of 2 mm in the dry sediment, with the temperature remaining over 60 °C for 2.75 hours. It is estimated that dried eggs in the surface sediments of pans in the western

Orange Free State could be exposed to these high midday temperatures for 30–45 days consecutively during mid summer. During winter, ground frosts occur nightly for four to five months, with the minimum temperatures reaching approximately – 10 °C during mid winter. Thus it is apparent that the eggs in the dry sediment are exposed to extreme temperatures. During wet periods, the turbidity of the water in the pans was high, averaging between 12000 and 15000 NTU's (Seaman & Kok, 1987). Calculation of the extinction coefficient (Wetzel, 1983) estimated that 1% of the incident light would penetrate to 6 cm and 0.1% of the incident light would penetrate to 9 cm. Thus light attenuation was very rapid, and under normal conditions light would only reach the bottom mud in the shallow areas of the waterbody.

Various studies have shown that not only are the factors affecting the hatching of anostracan eggs complex, but that various investigators have reached different conclusions as to the precise role of individual factors in stimulating or inhibiting the hatching of eggs. Hall (1959) and Ivleva (1973), working with *Chirocephalus diaphanus* and *Streptocephalus torvicornis* respectively, concluded that the presence of adults inhibited the hatching of eggs.

Moore (1963), working in Louisiana with *Streptocephalus seali* and *Eubranchipus holmani*, found that although the two species occurred in the same habitat, they were seldom present at the same time. He attributed this to temperature, as if the pools filled in the summer or autumn, then only *S. seali* hatched, but if they filled in the winter, then only *E. holmani* hatched. Laboratory studies revealed that the former species would not hatch at temperatures below 10 °C, and observation in the field showed that the reverse was true for *E. holmani*. However, he observed that if the water temperature rose to above 10 °C within 7–10 days of the habitat filling, the *S. seali* eggs remained unhatched. He attributed this to the low dissolved oxygen tensions at the bottom of the pool at this time.

Eubranchipus vernalis eggs will hatch soon after the spring thaw in Ohio, but will not hatch in the water in which they are laid. Weaver (1943) concluded that eggs of this species need to be dried and frozen before they will hatch. Dexter & Kuehnle (1951) found that eggs would only hatch when the pools filled initially in the spring.

Branchinecta mackini is an anostracan from the arid south-western regions of the United States of America. Brown & Carpelan (1971) found very complex relationships between the hatching of eggs and environmental factors. They found that the eggs of this species hatched readily in the absence of inhibition, and they postulated that the main source of inhibition to immediate hatching was the low dissolved oxygen content at the bottom of the waterbody. They also found that once the eggs had been desiccated, hatching was more or less confined to times of low salinity, although an influx of fresh water would stimulate further hatching even after the initial hatch was over. They found that not only did the desiccated and non-desiccated eggs respond differently to the various stimuli, but the response of the eggs also varied enormously. This variability ensured that the species had a better chance of survival, as not all of the eggs would hatch at the same time.

Wiggins *et al.* (1980) noted that *Chirocephalus bundyi* hatched when pools filled in the spring, but very rarely when pools filled in the autumn. Broch (1965) outlined the succession of environmental conditions to which the eggs must be exposed before they will hatch. The summer phase of the development needs some soil moisture, high temperatures and high oxygen tensions such as occur in shallow receding water or in the air. This phase of development is inhibited by total desiccation or by the low dissolved oxygen tensions associated with inundated bottom mud. The autumn developmental phase can only succeed once this phase is complete, and the autumn requires low temperatures, aerobic conditions and humidity. At the end of this phase, the metanauplius is fully formed within the egg and ready to hatch when flooded by suitably warm water.

Both continual darkness and dissolved oxygen tensions of less than 2 mg l^{-1} inhibited the hatching of *Limnadia stanleyana* (Eubranchiopoda; Conchostraca) eggs (Bishop, 1967).

Light was also shown to be essential for the hatching of both *Artemia* and *Brachionus plicatilis* (Rotifera) resting eggs (Sorgeloos, 1980; Minkoff *et al.*, 1983).

This study was designed to investigate factors which may influence the hatching of *S. macrourus* eggs to gain a greater insight into the stimulatory or inhibitory influences acting on the eggs in the environment. The understanding gained from this study could then be applied to a mass culture situation in an effort to overcome the problem of eggs not hatching readily when they are first laid.

Materials and methods

Eggs from a single collection were used for each of the experiments described in order to minimize variation amongst hatching of eggs. As all the experiments were not conducted simultaneously, the absolute hatching success between different experiments may not be directly comparable, but each experiment is considered as a separate entity, and the effects of the different treatments within the experiment are compared.

All but one of the experiments were carried out in 100 mm diameter glass petri dishes containing water to a depth of 5 mm. Each treatment consisted of five replicates of 25 eggs. Petri dishes were examined daily and nauplii counted and removed. Eggs hatched in the presence of adults were incubated in baskets made of 200 μm mesh suspended in 3 l of culture containing adults at the appropriate density. In this experiment each treatment consisted of three replicates of 100 eggs each. As these eggs were suspended off the bottom and it has been suggested that the faeces of the adults contain an inhibitory substance (Ivleva, 1973), the eggs laid during the treatment were examined. In all cases empty shells were found, and in most cases nauplii were seen. The percentage hatch amongst the eggs on the bottom of the containers was lower than that of the eggs in the baskets but this was to be expected, as the eggs in the basket had all been incubated for 10 days, while those on the container bottom had only been incubated for a part of the duration of the treatment. At the end of each treatment the number of empty shells was counted under a microscope to confirm the number of nauplii counted during the treatment.

All eggs except those in the experiment investigating the effect of temperature on hatching were incubated at 20 °C and exposed to a 12 hour diel light cycle.

Non-desiccated eggs were incubated both in the presence and in the absence of adults. The adults were fed microalgae (*Chlorella, Scenedesmus*) during the incubation period. At the lowest level (150 mg per 10 days), the adults were at the level of incipient starvation, and the females ceased to produce eggs during the treatment. However, at the higher levels (280–370 mg per 10 days) egg production continued throughout the treatments.

Eggs which had been desiccated were used in the following experiments. Eggs desiccated for between 1 and 65 days were incubated either with or without pre-incubation exposure to either − 18 °C, 45 °C or both for a 24 hr period. This was done to investigate the possibility of the extreme temperatures to which the eggs are exposed under natural conditions being necessary for hatching. Eggs which had been desiccated for 30 days were incubated at a range of temperatures between 6 and 30 °C, in water with dissolved oxygen content ranging between 0.4 and 6 mg l^{-1} and exposed to varying light treatments in various experiments. The treatments incubated at low dissolved oxygen tensions were exposed to continuous light as this was shown to stimulate hatching.

The following test for the difference between two sample proportions was used where the Null hypothesis is that there is no difference between the proportion of eggs responding to the stimulus from either of the treatments:

$$p = (N_A \cdot P_A + N_B \cdot P_B)/(N_A \cdot N_B),$$

where p is the mean proportion responding to the stimulus, N_A is the number off eggs subjected to the stimulus in treatment A and N_B the number of eggs subjected to the stimulus in treatment B.

The standard deviation is calculated as follows:

$$SD = (p * p(1 - p) * (1/N_A + 1/N_B))^{0.5} .$$

Z, the relationship between the proportions, is calculated as follows:

$$Z = (P_A - P_B)/SD .$$

A Z value of ± 1.96 gives a probability of < 0.05, and a Z value of ± 2.58 gives a probability of < 0.01.

All experiments held at 20 °C were terminated after 10 days, as observation showed that hatching had ceased by day 5 or 6 in all treatments. Results are given as the mean percentage of eggs hatched per treatment $\pm$ the 95% confidence limits. Eggs which did not hatch were not necessarily non-viable, as if these eggs were again desiccated and reincubated at a later stage, further nauplii would hatch.

Results

The incubation of non-desiccated eggs in the absence of adults

Non-desiccated eggs incubated in the absence of adults showed a mean hatching success of 70% ($\pm$ 5.5). The first of these eggs had hatched within 48 hr of being laid.

The incubation of non-desiccated eggs in the presence of adults

The first treatments tested the mean hatching success of eggs in the presence of monosex or mixed sex cultures at a density of six organisms l^{-1} (Table 1). It is apparent that the hatching success in the presence of males only is similar to the hatching success of non-desiccated eggs in the absence of adults. It is also apparent that the females exert a significantly greater ($p = < 0.01$) influence than males. While the difference between the hatching success of eggs incubated in the presence of females only and the mixed culture was not significant, that between the mixed culture and the males was significant ($p = < 0.05$).

Table 1. The influence of male and female *Streptocephalus macrourus* adults on the hatching success of eggs.

Sex of adults	Mean hatching success (%)	Range of 3 replicates (%)
Females only	40.67%	39–43
Mixed (ratio 1 : 1)	51.67%	27–71
Males only	69.3%	50–84

The adults used in all further treatments were in the sex ratio of 1 : 1.

The variation of hatching success of eggs incubated in the presence of adults at five densities, with the adults being fed algae at three different rations is shown in Fig. 1. The highest hatching success (> 60%) was obtained at the highest density of organisms and at the lowest algal ration tested. Conversely, the lowest hatching success (< 20%) was obtained from the treatments containing the smallest number of organisms and fed the highest ration.

The results obtained in this experiment would argue against the adults exerting an inhibitory effect on the hatching of the eggs. In the first place, if there was direct inhibition by adults, then the hatching success should be inversely related to the density of adults in the culture. However, this was not the case, with the hatching success increasing from ca. 50% at < 1 adult l^{-1} to > 60% at 10 adults l^{-1} in cultures fed the lowest ration, and from < 20% to > 40% over the same range of adult densities in cultures fed the highest ration.

The influence of the period of desiccation on incubation

There was a check in the hatching shortly after desiccation which decayed with time to a point where the desiccated eggs showed a similar hatching success to that of the non-desiccated eggs (Fig. 2). Although the hatching success was low after 24 hr desiccation, this decreased to its

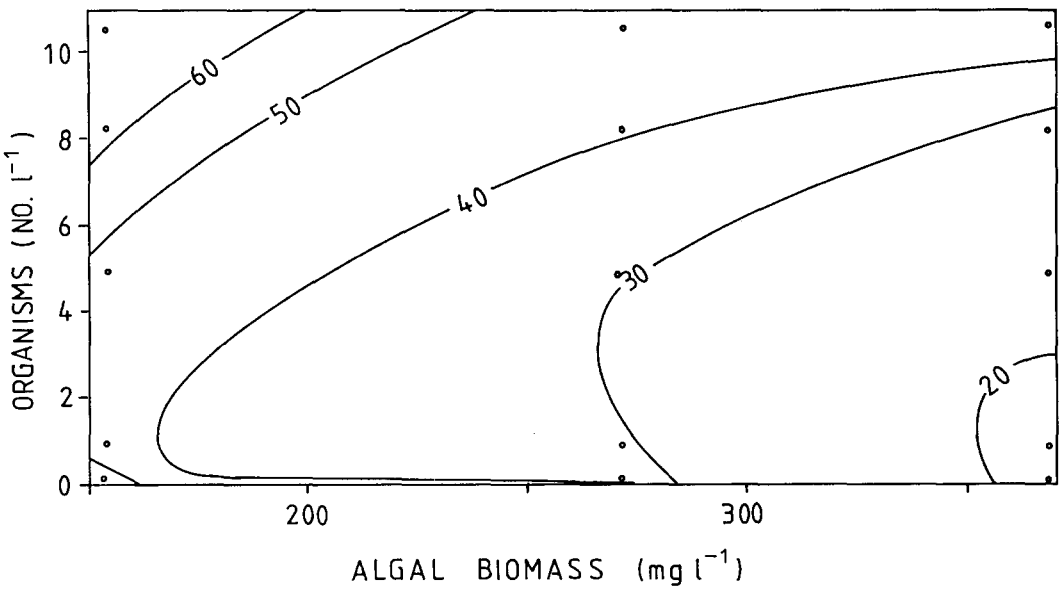

Fig. 1. The combined effects of the density of *Streptocephalus macrourus* adults and algal biomass on the hatching success of non-desiccated eggs. The figures 20 through 60 indicate percentage hatching success.

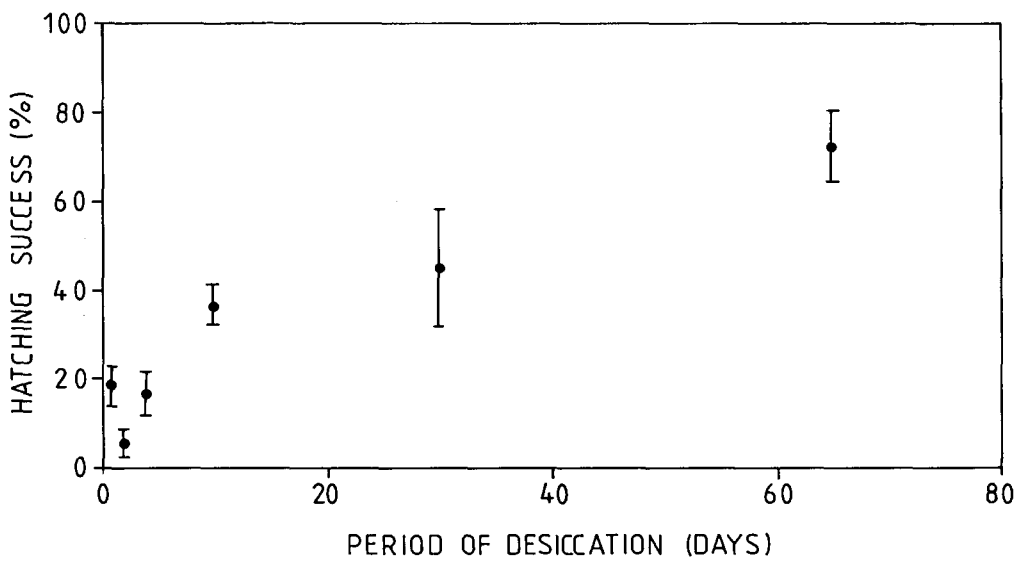

Fig. 2. The hatching success of *Streptocephalus marcrourus* eggs (±95% confidence limits) desiccated for periods ranging from 1 to 65 days before incubation.

lowest (5%) three days after the eggs had been removed from the water. After this period the hatching success increased again.

The influence of exposure to extremes of temperature before incubation

There was no significant difference between the hatching success of eggs exposed to −18 °C, 45 °C or a combination of the two, and the hatching success of the eggs in the control (held

at room temperature with no exposure to extreme temperatures before incubation) after any period of desiccation. It is thus apparent that exposure to extreme temperatures does not serve to break down any block to hatching as shown by Bishop (1967) for the conchostracan *Limnadia stanleyana*. Eggs of this organism required exposure to a period of low temperature to break the diapause initiated by short daylength.

What was apparent from the treatments to extreme temperatures before incubation, however, was the health of the nauplii which hatched from the eggs. Up until 30 days desiccation, all the nauplii which hatched were swimming strongly. However, after 65 days of desiccation a percentage of the nauplii which hatched were unable to swim, and died within 24 hr. This percentage varied between 4.8 and 9.2 for single temperature treatments (room temperature included), but the treatment involving exposure to the both − 18 °C and 45 °C showed a naupliar mortality of 44.25% during the first 24 hr.

The influence of incubation temperature on hatching

The $E_{50\%}$ (time taken for 50% of the eggs which finally hatched to hatch) of eggs hatched at temperatures between 14 °C and 30 °C ranged from 6.45–3.4 days (Fig. 3). At 10 °C less than 1% of the eggs hatched, and at 6 °C no eggs hatched. The hatching success of eggs between 10 °C and 30 °C is shown in Fig. 4. These figures show that although the eggs may hatch more rapidly at temperatures over 20 °C, that the percentage of eggs which hatch successfully at the higher temperatures is significantly ($p = <0.01$) lower than that of the eggs incubated at lower temperatures. Therefore, the optimum temperature for the incubation of *S. macrourus* eggs, based on survival of the nauplii, lies between 14 °C and 20 °C.

The hatching success was low at 25 °C and 30 °C, but nauplii hatched from eggs incubated at 30 °C were perceptibly smaller and weaker than those incubated at lower temperatures.

The influence of light on the hatching success

The treatment exposed to continuous light gave the highest hatching success (84%) of the three treatments in the experiment (Fig. 5). Eggs exposed to the normal diel cycle during incubation showed a 66.6% hatching success, which is similar to the hatching success of non-desiccated eggs incubated in the absence of adults. While the

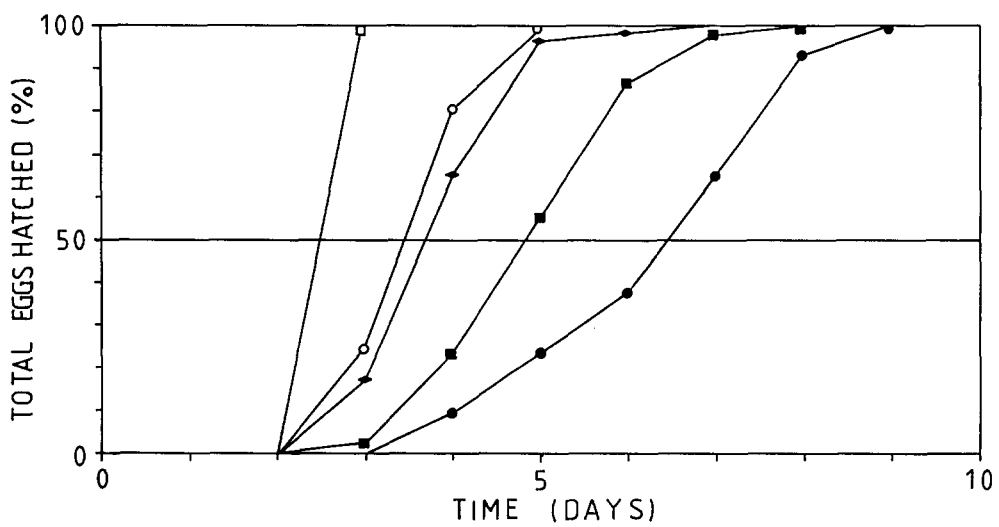

Fig. 3. The influence of temperature on the incubation period of *Streptocephalus macrourus* eggs over the range 14 °C to 30 °C. The $E_{50\%}$ is read from the time taken for 50% of the eggs to hatch. ●——● 14 °C ■——■ 17 °C ◆——◆ 20 °C ○——○ 25 °C □——□ 30 °C.

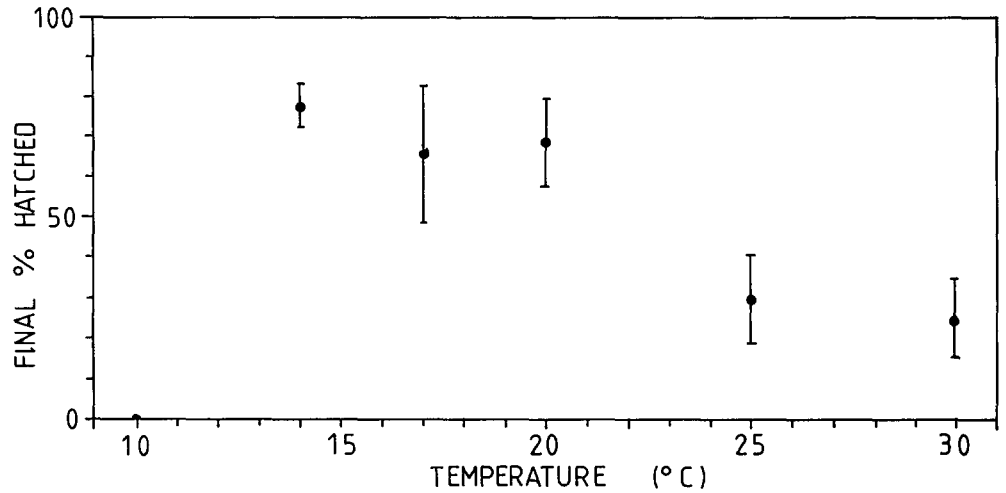

Fig. 4. The hatching success of eggs (±95% confidence limits) incubated at constant temperatures ranging from 10 °C to 30 °C.

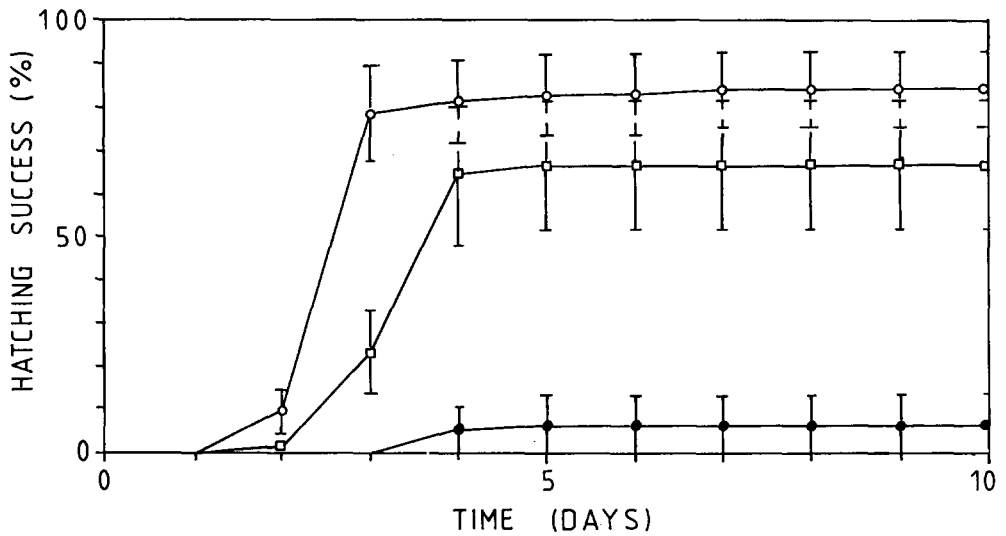

Fig. 5. The cumulative daily hatching success of *Streptocephalus macrourus* eggs (±95% confidence limits) exposed to different light regimes. o———o continuous light □———□ 12 hour daylight/24 hour cycle ●———● continuous darkness.

means of the two treatments are significantly ($p = <0.05$) different, there is substantial overlap in the 95% confidence limits. However, the treatment incubated in the dark showed a much lower (7%) hatching success. Not only are the means of this treatment and that incubated under the 12 h light cycle significantly ($p = <0.01$) different, but there is no overlap between the 95% confidence limits. Thus, while the two treatments exposed to light showed high hatching success, continuous light was a stronger stimulant to hatching than the light of the normal diel cycle. However, the mean final hatching success of the treatment incubated in the dark was approximately an order of magnitude lower than the treatments incubated in the light.

The influence of low dissolved oxygen on the hatching success

Eggs hatched more or less unpredictably in all the treatments (Table 2). Not only did some eggs

Table 2. The hatching success (%) of *S. macrourus* eggs incubated at low dissolved oxygen (DO) tensions.

DO at start ($mg\ l^{-1}$)	Percentage hatch (± S.D.)
6 (control)	62 (± 8.5)
2	33.3 (± 7.4)
1	47.8 (± 28.1)
0.5	22.2 (± 26.2)
0.0	12.0 (± 0.0)

hatch in the treatments which started at 0.0 mg l^{1-1} of dissolved oxygen, but the nauplii survived. From this experiment it was apparent that low dissolved oxygen tensions *per se* would not inhibit the hatching of eggs provided other conditions were suitable.

Discussion

Light was the only factor which was found to be obligatory for the hatching of *Streptocephalus macrourus* eggs. The eggs in the treatment incubated in continuous darkness showed a mean hatching success of 7%, while eggs in the treatment incubated in a diel cycle of 12 hours light showed a mean hatching success of 66.6%. The hatching success was further improved (84%) when eggs were incubated in continuous light. Exposure to continuous light has been shown to double the hatching success of *Artemia* cysts (Sorgeloos, 1980), who recommended this treatment for the production of *Artemia* nauplii for aquaculture purposes. Minkoff *et al.* (1983) investigated the influence of a number of factors on the hatching success of the salt-water rotifer (*Brachionus plicatilis*) resting eggs, and also found that light was the only factor essential to egg hatching. An exposure of 10 minutes was sufficient to stimulate some eggs to hatch, and maximal hatching was obtained after an exposure of 48 hours.

The eggs of *S. macrourus* sink when freshly laid, but when desiccated eggs are rehydrated, the majority float. Ivleva (1973) described the same response when the eggs of *S. torvicornis* were rehydrated, and noted that there was no difference in the hatching success of the eggs which floated or sank. The same observation was true of rehydrated *S. macrourus* eggs in the laboratory when incubated in clear water in glassware. Under these conditions of incubation, both the floating and the sinking eggs would receive sufficient light to stimulate hatching. However, in a field situation where the turbidity of the water would curtail light penetration severely, the eggs which sank would not receive sufficient stimulation from light to initiate embryogensis.

Light is possibly the key factor in the inhibition of hatching in newly laid eggs. When mature *S. macrourus* are cultured in glass or perspex containers in the laboratory, nauplii and juveniles are frequently observed with the adults. However, this situation does not occur in the field. The reason for this is apparently that in the laboratory the freshly laid eggs on the bottom of the container are exposed to light, and so are stimulated to hatch, while those in the field are in the dark and so do not receive the necessary stimulus to initiate embryogensis. The ability of rehydrated *S. macrourus* eggs to float is thus a prerequisite to successful hatching in the field.

Adult *S. macrourus* males exert no inhibition on the hatching of eggs, but the females do exert a partial inhibition. This effect, however, was overidden by some other inhibitory influence in the experiment. Hatching success was positively correlated with adult *S. macrourus* numbers, but negatively correlated with the concentration of algae in the culture medium. Thus the highest hatching success occurred at the highest adult density when the organisms were under conditions of incipient starvation, and *vice versa*. Ivleva (1973) postulated that inhibition of egg hatching by adults was an ecological adaption to prevent juveniles competing with the adults for the same food source, thereby reducing the adults chances of breeding successfully. From this it might be expected that adults under stress from food shortage would exert a stronger inhibitory influence than those with excess food. However, the reverse was found to be the case. The only two parameters which were varied in this experiment were the numbers of adults and the algal concen-

tration, so it is apparent that the algae is responsible for this inhibition. This is contrary to the findings of Minkoff *et al.* (1983), who found that algae in the medium enhanced the hatching success of the saltwater rotifer (*B. plicatilis*) resting eggs.

Incubation temperatures between 14 °C and 20 °C were optimal. Less that 1% of eggs incubated at 10 °C hatched and no eggs hatched in the treatment incubated at 6 °C. Eggs incubated at temperatures above the optimal range showed two deleterious effects. The first was a sharp decrease in the hatching success of eggs incubated at 20 °C and 25 °C. The second was that, while there was no significant difference in the hatching success of the eggs incubated at 25 °C or 30 °C, the nauplii of *S. macrourus* which hatched from eggs incubated at 30 °C were small and weak. Laughlin (1983) described a similar effect of temperature on newly hatched horseshoe crabs (*Limulus polyphemus*). He found that newly hatched *L. polyphemus* from eggs incubated at 35 °C had a lower ash free dry mass than those from eggs incubated at 30 °C, and this was a result of greater consumption of yolk by the embryo at the higher temperature. Dissolved oxygen tensions lower than 0.5 mg l^{-1} have been shown to inhibit hatching of the eggs of *Streptocephalus seali* (Moore & Burn, 1968) and *Branchinecta mackini* (Brown & Carpelan, 1973). Moore & Burn (1968) also showed that dissolved oxygen tensions of lower the 0.5 mg l^{-1} caused total mortality amongst *S. seali*. This was not the case with *S. macrourus*, as the eggs were hatched and the nauplii survived at dissolved oxygen tensions of less then 0.5 mg l^{-1}. The incubation technique employed in this study was chosen deliberately to stimulate hatching, as it was necessary to determine whether low dissolved oxygen tensions were inhibitory *per se*, or whether they strengthened inhibition caused by other factors. The results show that when suitably stimulated, eggs would hatch and nauplii survive in water with a dissolved oxygen tension of less than 0.5 mg l^{-1}.

The trend of hatching success with increasing periods of desiccation for *S. macrourus* is different from that shown by *S. torvicornis*. The hatching success of *S. macrourus* is initially low following desiccation, but increases over a period before decreasing again. Ivleva (1973) showed that initially the hatching success of *S. torvicornis* was high, showing a logarithmic decay curve from 87% five days after initial desiccation and decreasing to 1.6% hatching success 604 days after initial desiccation. Although the hatching success of *S. macrourus* eggs desiccated for more than 65 days was not investigated quantitatively during this study, observation showed that eggs desiccated for more than one year gave a very low hatching success.

Exposure of desiccated eggs to temperature extremes had no influence on the hatching success. However, the increased mortality of eggs exposed to pre-incubation temperature extremes after 65 days could have far reaching ecological implications regarding the distribution of the organisms. The eggs of organisms lying dormant in the dry pan sediment are exposed to temperature extremes for varying periods of time. The ability of eggs to withstand varying lengths of exposure and temperature extremes will be a factor in determining the harshness of the environment which the organisms may colonize. *S. macrourus* eggs were able to withstand a 24 hour exposure to temperature of either or both 45 °C or − 18 °C without jeopardising larval survival provided that the eggs had been desiccated for 30 days or less. However, a proportion (4.8–9.2%) of the nauplii which hatched from eggs desiccated for 65 days failed to survive, and when the eggs had been exposed to both 45 °C and − 18 °C before incubation, the proportion failing to survive rose to 44.25%. Thus, it is apparent that the eggs of this particular species would suffer heavy mortality in arid areas where the pans may remain dry for periods in excess of a year. This assumption fits in with the distribution recorded by Barnard (1929), who recorded *S. macrourus* from the semi-arid areas of South Africa and northern Namibia. These semi-arid areas have well defined dry seasons, but at the same time rainfall may be anticipated during the summer rainy season. From the data presented here, it is doubtful whether this species

would occur in the arid areas of the central Kalahari or Namib Desert. This remains to be investigated.

References

Barnard, K.H., 1929. Contributions to the crustacean fauna of South Africa – No 10. A revision of South African Branchiopoda (Phyllopoda). Ann. S. Afr. Mus. 29: 181–276.

Bishop, J.A., 1967. Some adaptations of *Limnadia stanleyana* King (Crustacea: Branchiopoda: Conchostraca) to a temporary freshwater environment. J. Anim. Ecol. 36: 599–609.

Broch, E.S., 1965. Mechanism of adaptation of the fairy shrimp *Chirocephalus bundyi* Forbes to the temporary pond. Cornell Univ. Agr. Exp. Sta. Mem. 392: 1–48.

Brown, L.R. & L.H. Carpelan, 1971. Egg hatching and life history of the fairy shrimp *Branchinecta mackini* Dexter (Crustacea: Anostraca) in a Mohave Desert playa (Rabbit Dry Lake). Ecology 52: 41–54.

Dexter, R.W. & C.H. Kuehnle, 1951. Further studies of the fairy shrimp populations of North eastern Ohio. Ohio Jour. Sci. 51: 73–86.

Dinges, R., 1982. Natural systems for water pollution control. Van Nostrand Rheinhold Engineering Series. New York.

Hall, R.E., 1959. Delayed development of eggs of *Chirocephalus diaphanus* Prevost. Hydrobiologia 14: 160–169.

Ivleva, I.V., 1973. Mass cultivation of invertebrates, biology and methods. Israel Program for Scientific Translations. Jerusalem.

Laughlin, R., 1983. The effects of temperature and salinity on larval growth of the horseshoe crab *Limulus polyphemus*. Biol. Bull. 164: 93–103.

Minkoff, G., E. Lubzens & D. Kahan, 1983. Environmental factors affecting hatching of rotifer (*Brachionus plicatilis*) resting eggs. Hydrobiologia 104: 61–69.

Moore, W.G., 1963. Some interspecies relationships in Anostraca populations of certain Louisiana ponds. Ecology 44: 131–139.

Moore, W.G. & B. Burn, 1968. Lethal oxygen thresholds for certain temporary pond invertebrates and their applicability to field situations. Ecology 49: 349–351.

Persoone, G. & P. Sorgeloos, 1980. General aspects of the ecology and biogeography of *Artemia* pp. 3–24. IN G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (eds). The Brine Shrimp *Artemia* Vol 3. Universa Press, Wetteren, Belgium.

Seaman, M.T. & D.J. Kok, 1987. Ecological diversity in Orange Free State pans. pp. 260–273. IN Symposium on ecology and conservation of wetlands in South Africa. Occasional report no. 28, Ecosystems programmes, Foundation for Research and Development, CSIR, Pretoria.

Sorgeloos, P., 1980. The use of the brine shrimp *Artemia* in aquaculture. pp. 25–46. In: G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (eds). The Brine Shrimp *Artemia* Vol 3. Universa Press, Wetteren, Belgium.

Weaver, C.R., 1943. Observations on the brine shrimp *Eubranchipus vernalis*. Ecology 24: 500–503.

Wetzel, R., 1983. Limnology. Second edition. Saunders College Publishing. Philadelphia.

Wiggins, G.B., R.J. Mackay & I.M. Smith, 1980. Evolutionary and ecological strategies of animals in temporary pools. Arch. Hydrobiol./Suppl. 58: 97–206.