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The cryptobiotic state of *Artemia* cysts, its diapause deactivation and hatching : a review

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Introduction

The ability of an organism to create a cryptobiotic state during its lifespan is a major survival mechanism in populations inhabiting biotopes characterized by unstable environmental conditions which may preclude survival of the individual. As a representative of such a group *Artemia* ensures its survival during periods of *e.g.* desiccation, extreme temperatures, etc., by the production of dormant embryos. These so-called cysts can resist extremely unfavorable circumstances and will be the origin of a new population when abiotic and biotic parameters in the habitat become again favorable.

Although the formation of a dormant state during the reproductive part of the life cycle is not unique in crustaceans, brine shrimp seem to have developed a sort of flexibility towards the variety of 'unstable' habitats they inhabit. Unlike related phyllopods, *Artemia* females easily switch from cyst production (oviparity) to live nauplii birth (ovoviviparity), resulting in a fast increase of the population when environmental conditions are optimal. Moreover, there is no 'sexual' control over these modes of reproduction as in most cladocerans or rotifers where only fertilized, mictic females produce resting eggs. This ensures a fast response to fluctuating

circumstances which may prevail in a period of severe environmental conditions. In fact, this flexible life-history strategy may also explain why some strains inhabiting relatively stable biotopes appear to have lost this ability to produce dormant cysts, *i.e.* this second mode of reproduction has no adaptive value anymore (D'Agostino and Provasoli, 1968 ; Mitchell and Geddes, 1977 ; Dimentman and Spira, 1982 ; Lenz, 1987 ; Lenz and Dana, 1987).

The basic biochemical mechanisms involved in the switch of the reproduction mode in *Artemia* are not yet fully understood. Laboratory experiments showed that cyst production can be induced by applying oxygen stresses when Fe-EDTA is present in the medium (Versichele and Sorgeloos, 1980 ; Lavens and Sorgeloos, 1984). Under these conditions a specific haemoglobin is produced which may play a role in the formation of the outer cyst shell (Lavens and Sorgeloos, 1984). The triggering mechanism(s) for the induction of the state of diapause is (are), however, not yet known.

The unraveling of the processes involved in dormancy will help to better understand the ecological adaptations of this crustacean to its specific biotopes ; it will furthermore result in a more optimal use of *Artemia* as a study-object in many disciplines of biological sciences and as a live food source in fish and crustacean rearing : *i.e.* for the aquarium hobbyist as well as the aquaculturist the cysts are of great importance since they can be stored for years in a dry form to resume their development and produce free-swimming nauplii within a 24-h incubation period in seawater. However, not all sources of cysts yield maximal hatching outputs even when the cysts were harvested, processed, and dried following standardized techniques. Such 'inferior' products are not suitable for application in research, and, from an economical point of view, cannot be optimally used in larval rearing of aquatic organisms.

This review aims to characterize the state of dormancy, identify its role in *Artemia* embryos, and elucidate the impact of different environmental and genetical factors on this process. Specific treatments which may deactivate the diapause process resulting in improved hatchability of the cysts will be discussed, including strain-specific adaptations which may be related to the ecology of the natural biotope. Topics on fundamental and biochemical processes involved with the cryptobiotic state (*e.g.* anhydrobiosis), and its depressed metabolism are covered *in extenso* by Crowe *et al.* (1987).

Finally, we hope that this review will create a better basis for more directed research related to dormancy and its termination.

Definitions, occurrence, and functional role of cryptobiosis in the animal kingdom

Because of the confusion in the literature on cryptobiosis it is essential to first define the different states of arrested metabolism. The fact that different cryptobiotic life stages (eggs, larvae) have been described for widely different groups of plants and animals, *e.g.* protozoa, rotifers, tardigrades, nematodes, crustaceans, and insects (Lees, 1961 ; Crowe and Clegg, 1973, 1978 ; Crowe and Madin, 1974 ; Gilbert, 1974 ; Belk and Cole, 1975), is probably at the origin of the confusion in terminology. Furthermore, all definitions related to this subject (Keilin, 1959 ; Hinton, 1960 ; Sussman and Halvorson, 1966 ; Gilbert, 1974 ; Belk and Cole, 1975 ; Clegg, 1978a ; Grice and Marcus, 1981 ; Wommersley, 1981) deal with two characteristics which may be subjected to different interpretations, *i.e.* the degree of depressed metabolic activity of the resting stage, and the influence of the environment on this process.

The following terminology has been adapted in this review for the precise description of the different states of arrested or retarded development in cysts of *Artemia* and of other animals (species list in Table III, see further).

Cryptobiosis is a general term which, as indicated by Keilin (1959), comprises those states of an organism which show no visible sign of life and of which the metabolic activity becomes hardly measurable, eventually reaching a reversible standstill.

Dormancy is a specific form of cryptobiosis with an endogenous control of metabolism and development ; *i.e. Artemia* gastrulae enter the dormant state within the uterus independently of the prevailing environmental circumstances.

Quiescence, on the other hand, refers to the environmental (exogenous) control of metabolism and development, *e.g.* extremes of temperature, oxygen, desiccation, etc., inducing a state of retarded development ; further embryogenesis will only resume when the environmental conditions become favorable. Depending on the adverse factor, different types of quiescence can be considered, *i.e.* **anhydrobiosis** (lack of sufficient amounts of water), **cryobiosis** (low temperatures), **anoxybiosis** (lack of oxygen), etc. (Crowe *et al.*, 1987).

The denomination diapause is frequently used in relation to *Artemia* and indicates the state of dormancy where an arrest of development is initiated by internal factors before the environment has become unfavorable (Fig. 1). In the oviparous mode of reproduction embryonic development in *Artemia* is arrested at the gastrula stage within the uterus. This onset of dormancy is rigidly programmed into the development of *Artemia*, independent of geographical origin or environmental conditions (Olson and Clegg, 1976). The dormant embryos, composed of a partial syncytium of about 4 000 nuclei (Nakanishi *et al.*, 1962) surrounded by complex shells (Morris and Afzelius, 1967 ; Anderson *et al.*, 1970 ; Khalaf *et al.*, 1978) are released from the ovisac into the biotope. There is no measurable metabolic activity (Dutrieu, 1960 ; Finamore and Clegg, 1969 ; Clegg, 1974) and the cysts will not hatch, even when climatic conditions are favorable, *i.e.* they remain in a dormant state. The term diapause is correctly applied here since the induction for an ovigerous reproduction may have been initiated before the climatic circumstances turned unfavorable (Lenz, 1987 ; Lenz and Dana, 1987), or even without adverse environmental conditions occurring. Further development to a quiescent phase can only be achieved when the endogenous mechanism(s) responsible for the induction of diapause is (are) deactivated. In most cases this is realized by dehydration of the cysts, *i.e.* by air drying of the cysts which accumulate on the shore or by osmotic water removal in the highly saline waters where they are normally released. Once this dormancy has been terminated, the metabolism and embryonic development is controlled by external factors, and as soon as optimal conditions are restored (*e.g.* rehydration) cysts will eventually hatch. The cellular and developmental biology/biochemistry involved with these processes have been covered *in extenso* by Bagshaw (1980), and Clegg and Conte (1980).

To be complete we should also cite the reports of non-dormant *Artemia* cysts (also called 'subitaneous' or 'summer eggs') which hatch immediately after deposition (Mathias, 1937 ; Lochhead, 1941 ; Dutrieu, 1960 ; Versichele, 1983 ; Amat *et al.*, 1987). This rather uncommon phenomenon might be a malfunctioning of the diapause-inducing mechanism, resulting in the direct production of quiescent cysts or even embryos, *i.e.* when no thick shells are formed, and might only occur when the female brine shrimp switches its mode of reproduction.

Besides *Artemia* and other anostracans dormant cysts are also produced by some notostracan, conchostracan, cladoceran, and calanoid copepod species, as well as some rotifers (Monogononta) (Table III, see further).

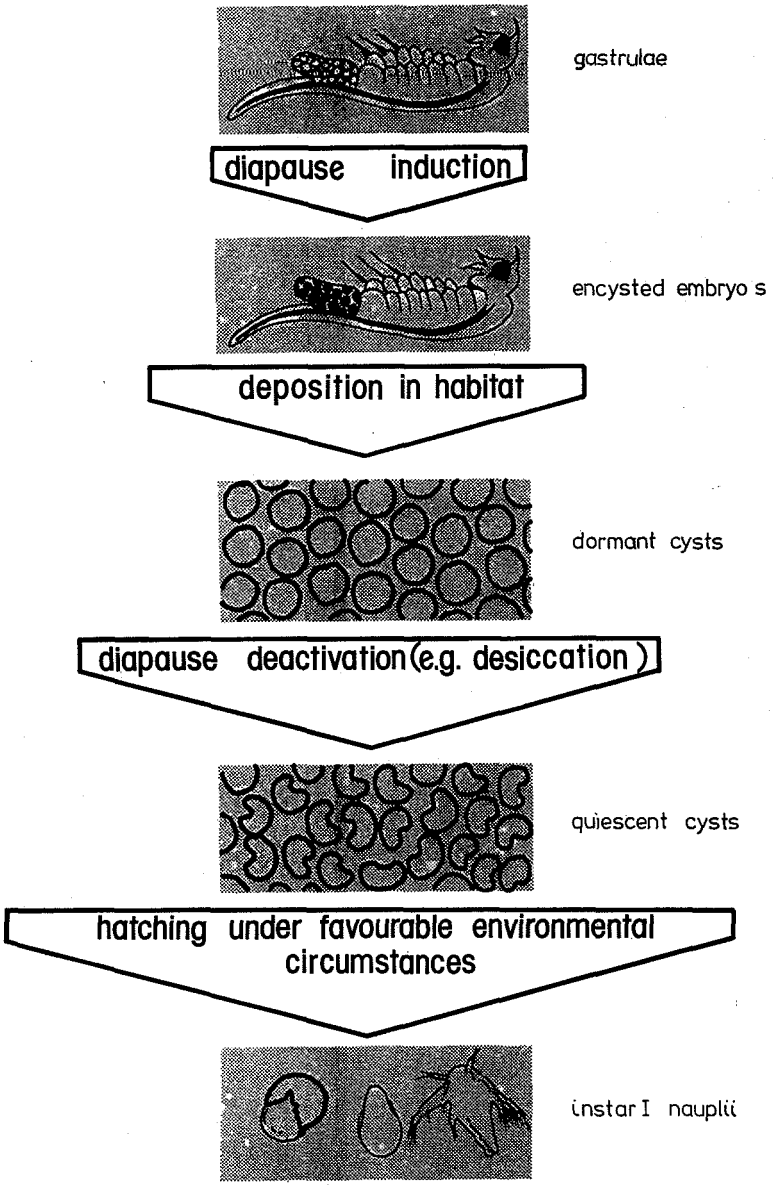

FIG. 1. Schematic diagram explaining the specific terminology used in relation with dormancy of *Artemia* embryos.

As already mentioned the major functional role of cryptobiosis is most likely the survival of the population under temporarily adverse conditions. From an ecological point of view, the most appropriate strategy in biotopes with more or less predictable cyclic environmental stresses would be dormancy, whereas quiescence would be optimal in non-cyclic circumstances (Belk and Cole, 1975). By the production of diapausing eggs the organisms may anticipate sub-optimal biotope conditions. Dormancy as a cryptobiotic process may also be relevant as a life-history strategy, *i.e.* by synchronising the life cycles to the variations that occur in the habitat. Endogenous control over metabolism and development ensures a synchronous hatching, resulting in a fast start and consequent development of the population shortly after the re-establishment of favorable environmental conditions. It is this synchrony that allows effective colonization in temporal biotopes. Last but not least dormancy also provides indirect ways to effectively overcome dispersion difficulties. The properties of the cysts to resist severe conditions without losing their viability make them ideal both for passive and active transportation by wind, waterfowl, and men into new suitable biotopes (Horne, 1966 ; MacDonald, 1980 ; Persoone and Sorgeloos, 1980).

Mechanisms for the induction and termination of diapause

The following hypotheses have been formulated on diapause mechanisms in freshly-released cysts of *Artemia.*

Anderson *et al.* (1970) suggested that the degree of permeability of the cyst shell regulates diapause. According to these authors this permeability may be controlled by the thickness of the shell, or by density, microstructure or chemical composition of particular layers in the shell. In this regard Morris and Afzelius (1967) considered an outer membrane to be the barrier for penetration ; *i.e.* they claimed that this 'sealing envelope' is built up after dehydration of the cysts in the uterus, and that resumption of development will only take place after destruction or removal of this membrane. Our observations on cysts freshly released from a controlled cyst-production system (Lavens and Sorgeloos, 1984) revealed, however, that cysts are not always anhydrobiotic nor that they have an intact outer membrane, but still remain in diapause. Even the complete removal of the tertiary envelope (*i.e.* decapsulation) of the latter cysts did not result in the resumption of their embryonic metabolism.

Dutrieu (1960) assumed that diapause is interrupted by the splitting of carotenoproteins into free carotenoids and proteins, as a result of which the cysts become quiescent. The splitting mechanism, however, has not been elucidated. The recent discovery of egg-specific *cis*-canthaxanthins (Nelis *et al.*, 1984ab, 1987a) might, however, increase the speculation of a possible function of these carotenoids in the cryptobiotic process in cysts, *i.e.* until now pigments with the unusual *cis*-configuration have only been isolated in *Artemia* and some related cyst-producing crustaceans (Nelis *et al.*, 1987a). Especially the divergence in relative abundance of *cis*- and *trans*-canthaxanthins in hydrated *versus* dehydrated cysts is remarkable (Nelis *et al.*, 1987ab). It is, however, not yet clear if the role of these egg-specific carotenoids is related to the basic diapause mechanism(s) or to the biochemical processes which protect the embryo's viability during *e.g.* anhydrobiosis by *e.g.* stabilizing the membrane structures (see also Crowe *et al.*, 1987).

Finally, the team of Crowe and co-workers detected intimate changes in the intra cyst pH of dormant and quiescent (anoxybiotic) cysts (Busa and Crowe, 1983 ; Drinkwater and Crowe, 1986 ; Crowe *et al.*, 1987), and revealed that depression of the internal pH (pH_i) can lead to

the breaking of dormancy. Since the pH_i affects respiration, metabolism, and development in anoxybiotic cysts, Crowe *et al.* (1987) assumed that dormancy might be induced by an internal pH which exceeds the physiological range ($pH_i > 7.9$). However, other data collected by the same team suggest that there may actually be two compartments in the dormant cysts that are separated in pH_i by about 0.5 pH units. The function of these compartments has still to be elucidated. As mentioned by Crowe *et al.* (1987), terminating dormancy "appears to be considerably more complex than acidification of a basic cytoplasm".

Factors affecting the hatchability of cysts

Fundamental research on potential mechanisms and processes involved in diapause induction and/or inhibition is seriously hampered by potential interferences, *e.g.* various mechanisms which may mask possible effects of eventual diapause deactivation mechanisms. We have tried to group the factors which operate at succeeding stages of the cyst-to-nauplius process into five distinct clusters (Fig. 2). Overlapping or interactions, however, may occur, especially with regard to the diapause terminating mechanisms (see further).

As a consequence, a high degree of interference exists between the different sets of parameters which are involved in cyst hatching, eventually resulting in misinterpretation of the experimental results. Since this might explain much of the confusion in the literature we have extended the present review on diapause to the other groups of factors which are involved in cyst hatching.

Genotypic factors

It is very likely that the hatching capability of *Artemia* cysts differs in function of the sibling species or the geographical origin. Genotypical differences may be the basis of varying hatching characteristics (hatching percentage and/or hatching rate) among brine shrimp populations (Vanhaecke and Sorgeloos, 1982 ; Tackaert *et al.*, 1987), as was found in rotifers (Gilbert, 1974). However, hitherto literature has not provided the decisive evidence. Browne *et al.* (1984) compared reproductive and life-span characteristics in 12 *Artemia* strains and found that hatching was strongly correlated with the environment and not with the genotype. Some indications of strain-specific adaptations with regard to diapause are given in Lavens *et al.* (1986b) ; (see further under diapause termination techniques).

Culture conditions

The recent development of laboratory systems for the controlled production of brine shrimp cysts (Versichele and Sorgeloos, 1980 ; Lavens and Sorgeloos, 1984) has provided the experimental tool to study the influence of abiotic and/or biotic factors on the hatchability of *Artemia* cysts. Indeed, the variation in hatching characteristics of cyst batches from the same origin (Vanhaecke and Sorgeloos, 1982) could never be fully explained due to lack of essential data on the antecedents of these dormant cysts produced by wild populations. Cunningham and Grosch (1978), Sarasquete Reiriz (1979), and Bohra (1980) claimed the existence of hatching differences depending upon the season in which the cysts were produced. Pond production tests in conditions which facilitated a better control over the environmental production parameters and over the harvesting conditions, revealed some indications that the biotope may have interfered

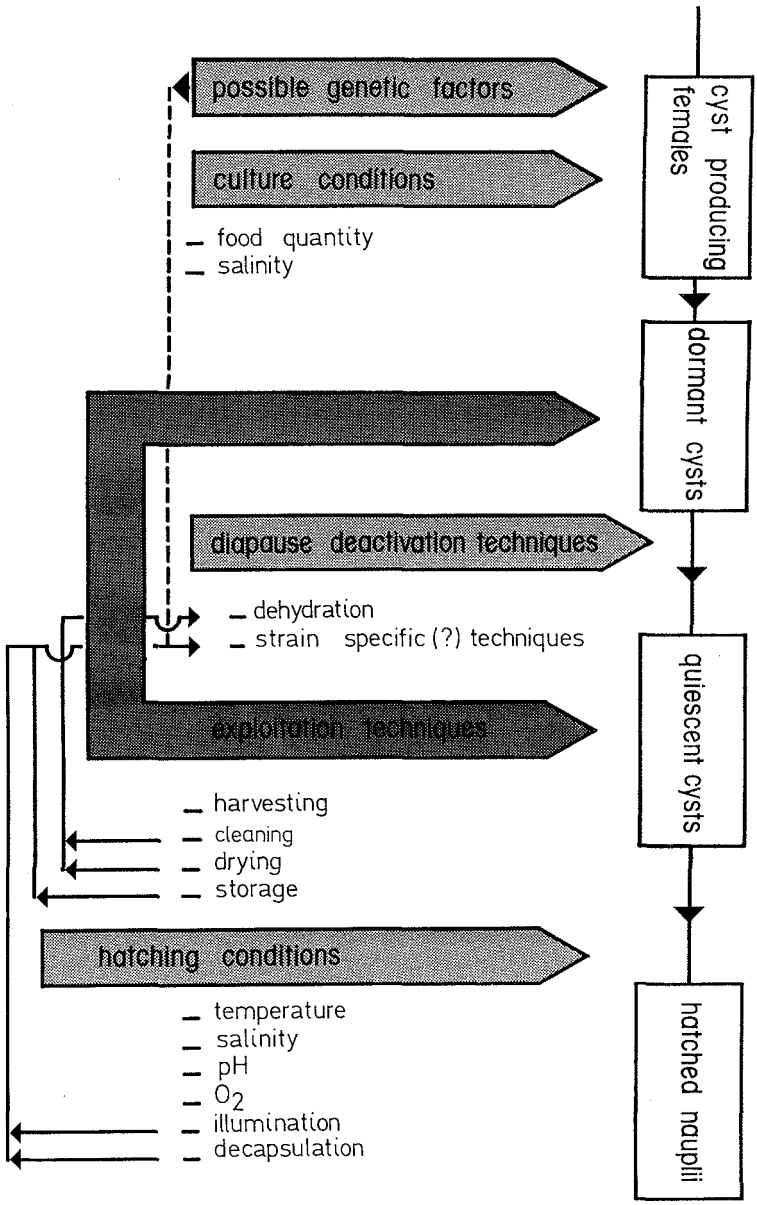

FIG. 2. Schematic diagram showing the (combined) interaction of various clusters of factors on the hatchability of *Artemia* cysts.

with the hatching quality. Vos *et al.* (1984) demonstrated varying hatching efficiencies (from 214 000 up to 339 200 nauplii/g of cysts) for cysts produced after inoculation with San Francisco Bay material in different regions in Southeast Asia. Precise detection of the parameter(s) which interfered was, however, not possible.

Decisive experimental evidence concerning the impact of parental culture conditions on the hatchability of the encysted offspring has been summarized in Table I. Production circumstances were maximally standardized and only one variable was tested at the same time.

These literature data reveal an obvious interaction between the amount of food available to the parental brine shrimp cultures or the salinity of their biotopes on the one hand, and the hatching characteristics of the produced cysts on the other. No direct influence of culture temperature or food quality was observed.

In rotifers, the hatching characteristics of their resting eggs appear also to be influenced by the culture parameters : *e.g.* food quantity (Gilbert, 1974), food quality (Gilbert, 1980 ; Pourriot and Snell, 1983), and salinity (Ito, 1960).

Food quantity

As proven by Lavens *et al.* (1986b), the food quantity available to the reproducing adults appears to be a parameter of primary importance in determining the hatching quality of the encysted offspring. Significant increases in hatching percentages were detected when two types of (sub-)optimal feeding regimes were applied in a standard recirculating culture system for the controlled production of cysts (Lavens and Sorgeloos, 1984). The most spectacular increase (27 %) was attained when Lavalduc adults received the rice bran/corn bran diet at a daily rate of 15 % of the population wet-weight biomass (Lavens and Sorgeloos, 1987). This correlation between food quantity and hatchability has recently been confirmed for other brine shrimp strains by Tackaert *et al.* (1987) using live algae as food source.

The interaction between food availability in adult brine shrimp populations and the hatchability of their encysted, dormant offspring remains unclear. Two possible pathways of interaction seem plausible. The first hypothesis involves a direct interference with the state of dormancy itself, resulting for example in a variable sensitivity for diapause deactivation treatments. This hypothesis is supported by Bogoslavsky (in Gilbert, 1974) who stated that the duration of dormancy may be determined by the physiological conditions of the reproducing rotifers. Another example is the observation by Versichele (1983) that hatchability (H %) and hatching rate (T_{90}) of the encysted *Artemia* offspring temporarily decrease (by 18 %, respectively 6 h) shortly after a change of the adult's diet. On the other hand Pourriot and Snell (1983) concluded from a compilation of hatching data that in rotifers resting egg formation conditions, *e.g.* food, do not modify the dormant state, but have an effect on the relation with external hatching factors (temperature, salinity, etc.). The second hypothesis entails an effect on the viability *sensu stricto* of the cysts produced, *e.g.* morphological differences, biochemical content and/or abnormal embryogenesis. However, no obvious differences have been found hitherto between cysts produced under optimal *versus* sub-optimal conditions. Even preliminary analysis of carbohydrate levels – which are known to be critical for an optimal functioning of the trehalose-glycerol hyperosmotic regulatory system (Conte *et al.*, 1977) – revealed no correlation between food availability for the parental brine shrimp, trehalose or glycerol concentrations in the cysts produced under laboratory conditions, and their hatchability (Lavens and Crowe, unpubl.).

Salinity

According to Versichele and Sorgeloos (1980) cysts produced at low salinity appear to have a low hatchability ; *e.g.* a 50 % difference in hatching percentage is noted in laboratory populations from San Francisco Bay origin kept in 35 and 90 ‰ respectively. This has recently been confirmed by Naessens (pers. commun.) who harvested cysts from 70 ‰ and over 100 ‰ saltponds in Kenya that had been inoculated with San Francisco Bay *Artemia.* These observations might be strain specific as only small improvements were observed for Chaplin Lake and Tientsin cysts produced at increased salinities (35 *versus* 90 ‰ ; Tackaert *et al.*, 1987). High salinity (> 150 ‰), on the other hand, also seems to negatively affect hatchability : *e.g.* hatching levels drop by 50 %, respectively 35 % in cysts produced at > 150 ‰ *versus* 90 ‰ from Mono Lake (Dana and Lenz, 1986) and San Francisco Bay origin (Versichele and Sorgeloos, 1980 ; Versichele, 1983). However, to interpret the different results one should take possible variations in food availability (qualitatively and quantitatively) into account. This parameter was carefully optimized in the culture tests by Tackaert *et al.* (1987) who observed the slightest interaction on hatching quality. Similarly, Great Salt Lake and Macau cysts produced at 35 ‰ S in intensive flow-through systems gave hatching levels comparable to commercial cyst batches (Lavens, unpubl. ; Tobias, pers. commun.).

Versichele (1983) furthermore detected a direct correlation between lower cyst hatchability and decreased concentration of haematine content in the shell of cysts produced at low salinity. This observation, however, could not be confirmed by Lavens (unpubl.) for cysts collected from a standard cyst-production system.

Finally, Ito (1960) stated that for *Brachionus plicatilis* the salinity at which cyst production took place, determines the optimal salinity level at which maximal hatching will prevail. This finding was confirmed for *Artemia* by Tobias (pers. commun.) who detected a 20 % increase in hatching success when incubating cysts produced at low salinity (35 ‰ culture system) in a 5 ‰ hatching medium (Table I). However, this could not be confirmed by Lavens (1981) and Versichele (1983) who performed cyst-production tests at the same salinity.

Other factors

Reduced hatchability of *Artemia* cysts has been reported in cyst-producing cultures exposed to non-lethal doses of toxic compounds or irradiation (Grosch, 1966, 1973 ; Squire, 1970 ; Cunningham and Grosch, 1978).

EXPLOITATION FACTORS

A third set of conditions that may control the hatchability of diapausing or quiescent *Artemia* embryos are those created by the techniques applied for harvesting, cleaning, drying, and storing of the cyst material. Processing methods are described in detail by Voronov (1973), Rakowicz (1975), and Sorgeloos *et al.* (1986). The major impact of most of these conditions can be related to effects of dehydration or combined dehydration and rehydration, as is summarized in Table II. As a result, these conditions may in the first place interfere with the cryptobiotic state and act as true diapause deactivation processes if the cyst material is dormant. For anhydrobiotic, quiescent cysts, on the other hand, insufficient dehydration levels, too long periods of hydration before a next dehydration, or too many hydration/dehydration cycles result in a serious drop in the viability of brine shrimp cysts (Morris, 1971 ; Rakowicz, 1975 ; Sorgeloos *et al.*, 1976 ; Benijts *et al.*, 1977 ; Vanhaecke and Sorgeloos, 1982 ; Sorgeloos *et al.*, 1986) (Fig. 3).

TABLE I

Experimental results on the influence of abiotic and biotic conditions on the hatchability of cysts, produced under controlled conditions, and processed under standard circumstances

Type of production system	Strain origin	Experimental abiotic and biotic parameter(s)	Hatching percentage	Standard deviation	Reference
Laboratory system : batch culture	San Francisco Bay (USA)	*Spirulina* - 20 °C - 35 ‰	28	–	Versichele and Sorgeloos (1980)
		Spirulina - 28 °C - 35 ‰	26	–	Versichele (1983)
		Spirulina - 20 °C - 90 ‰	76	–	
		Spirulina - 28 °C - 90 ‰	72	–	
		Spirulina - 20 °C - 180 ‰	38	–	
		Rice bran - 20 °C - 35 ‰	19	–	
		Rice bran - 28 °C - 35 ‰	22	–	
		Rice bran - 20 °C - 90 ‰	66	–	
		Rice bran - 28 °C - 90 ‰	78	–	
		Rice bran - 20 °C - 180 ‰	40	–	
		Spirulina - Baker's yeast - 25 °C - 90 ‰	83	–	
		Scenedesmus - 25 °C - 90 ‰	45	–	
		live *Dunaliella* - 25 °C - 90 ‰	85	–	
Laboratory system : closed flow-through culture (90 ‰ ; inert diets)	Lavalduc (France)	optimal feeding[1]	20.1	4.7	Lavens *et al.* (1986b)
		sub-optimal feeding[1]	14.5	2.8	
	San Francisco Bay (USA)	optimal feeding[1]	53.0	4.4	
		sub-optimal feeding[1]	42.4	5.4	
		optimal feeding regime[2]	63.7	7.8	
		sub-optimal feeding regime[2]	36.6	6.5	
Laboratory system : closed flow-through culture (inert diet)	Great Salt Lake (USA)	25 °C - 35 ‰	68.9	5.6	Lavens (unpubl.)

TABLE I. Continued

Type of production system	Strain origin	Experimental abiotic and biotic parameter(s)	Hatching percentage	Standard deviation	Reference
Laboratory system : open flow-through culture (live *Chaetoceros*)	Macau (Brazil)	25 to 28 °C - 35 ‰	61 (35 ‰) 84 (5 ‰)	8 –	Tobias (pers. commun.)
Laboratory system : batch culture (live *Dunaliella*)	Great Salt Lake (USA)	35 ‰ - optimal feeding	11.1	–	Tackaert *et al.* (1987)
		90 ‰ - optimal fedding	35.1	–	
	Chaplin Lake (Canada)	35 ‰ - optimal feeding	14.5	–	
		35 ‰ - suboptimal feeding	7.1	–	
		90 ‰ - optimal feeding	19.2	–	
	Tientsin (PR China)	35 ‰ - suboptimal feeding	30.1	–	
		35 ‰ - optimal feeding	51.8	–	
		90 ‰ - optimal feeding	60.2	–	
Outdoor culture ponds	San Francisco Bay (USA)	85 ‰	27.0	5.1	Naessens (pers. commun.)
		155 ‰	69.5	7.1	
Laboratory system : culture in vials (inert diet mixed with *Spirulina* powder)	Mono Lake (USA)	76 ‰	62	–	Dana and Lenz (1986)
		88 ‰	66	–	
		97 ‰	58	–	
		118 ‰	66	–	
		133 ‰	64	–	
		159 ‰	15	–	

[1] Feeding regimes were kept constant, and densities varied.

[2] Different feeding regimes : optimal and suboptimal refer to amounts of food respectively 10 and 15 % of biomass (wet weight).

TABLE II

Possible effects of exploitation factors on hatching quality of *Artemia* cysts

Exploitation step/technique		Possible effects
Harvesting	From pond-water surface	
	+ at regular intervals	Dormant (or quiescent) cysts ; their dehydration levels are determined by pond salinity
	+ at irregular intervals	Risks for exposure to dehydration/hydration cycles as a result of rainfall/salinity changes in the pond Risks for mixture with cysts temporarily accumulated on-shore
	From the shore	(Complete) dehydration Risks for rehydration (rainfall, air, humidity) and for rehydration/dehydration cycles Risks for exposure to high temperatures (> 40 °C) during sun drying Risks for exposure to UV-radiation
Storage before processing	In pond water	No effect when salinity does not change ; longterm effects not known
	In brine (saturated NaCl solution)	Dehydration to ± 20 % H_2O content
Cleaning	In brine (saturated NaCl solution)	Dehydration to ± 20 % H_2O content
	In tapwater	Rehydration (60 % H_2O content after 15 min at 20 °C)
	Eventual different cycles	Dehydration-rehydration cycles
Drying	Several techniques	Varying dehydration rates ; varying levels of final water content Possible exposure to high temperatures, possible exposure to UV radiation (for sun drying)
Stocking	Several techniques	Varying hatching quality when insufficiently dehydrated or stored in presence of oxygen

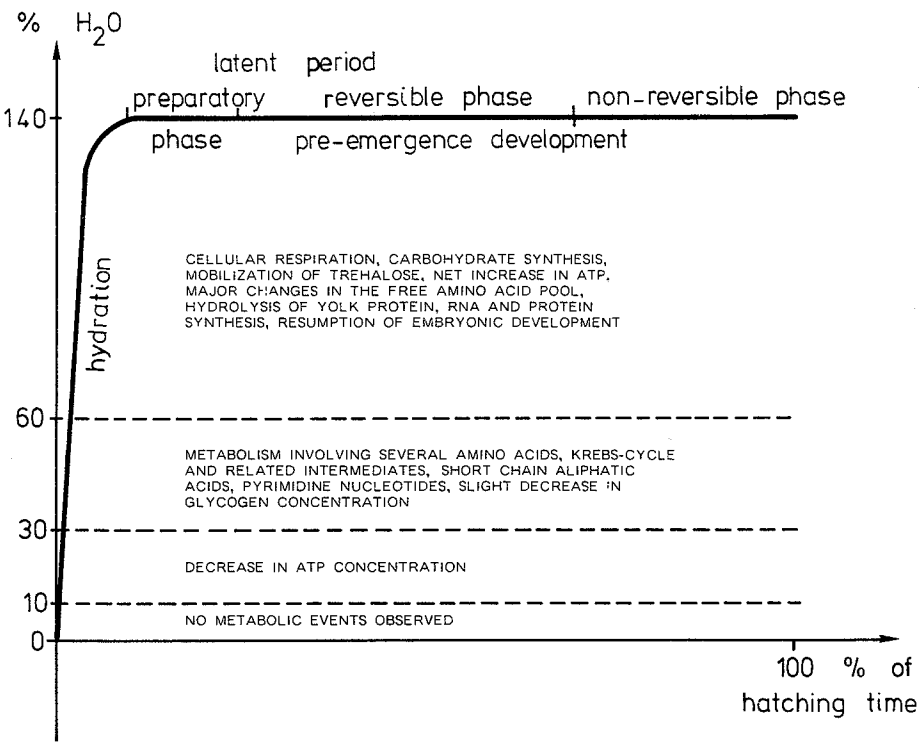

FIG. 3. Hydration dependence of cellular metabolism in *Artemia* cysts. (For supporting evidence see Morris, 1971 ; Clegg, 1974, 1976a-d, 1977, 1978ab ; Clegg and Cavagnaro, 1976 ; Clegg and Lovallo, 1977 ; Olson, 1979).

Cysts exposed for too long a period to water levels exceeding 65 % will have completed their pre-emergence embryonic development. Subsequent dehydration of these cysts will not result in an otherwise reversible interruption of the hatching metabolism (Morris, 1971 ; Olson, 1979) but in the killing of the differentiated embryos. Additional negative effects on hatchability are noted when hydrated cyst material is exposed to prolonged UV-irradiation or to temperatures above 40 °C (Nimura, 1968 ; Voronov, 1974).

Insufficient dehydration or rehydration resulting in water levels in the range 30-65 % H_2O will initiate metabolic activities which may critically reduce the energy contents down to levels which are insufficient to reach the state of emergence when incubated in seawater under optimal hatching conditions.

A depletion of the energy reserves is furthermore attained when the cysts undergo subsequent hydration/dehydration cycles. Long-term storage of such material results in the loss of the cumulative effect of hydration on development and hatching percentages decrease substantially (Morris, 1971 ; Vanhaecke and Sorgeloos, 1982).

Hatching quality in stored cysts is slowly decreasing when the cysts contain water levels from 10 to 35 % H_2O. Clegg and Cavagnaro (1976) detected indications of enzyme activity and a

serious drop in the ATP concentration. These processes may be retarded when the cysts are stored at freezing temperatures (Vanhaecke and Sorgeloos, 1982). The optimal intra-cystic water level (under 10 %) is not known. Iwasaki (1958) and Engel and Fluke (1962) reported a higher irradiation sensitivity for cysts that contained less than 1 % H_2O.

Sufficiently dehydrated cysts (less than 10 % H_2O) only keep their viability when stored under vacuum or in nitrogen (Dempster and Hanna, 1956 ; Clegg, 1962, 1967 ; Vanhaecke and Sorgeloos, 1982). The presence of oxygen results in a substantial depletion of the hatching percentage and rate. According to Crowe (1971) and Crowe and Clegg (1973) this is caused by the formation of highly detrimental free radicals, and not by a decrease of the energy content (Clegg, 1962).

Diapause deactivation conditions

Dehydration

In many cases the removal of intra-cystic water is an efficient way to terminate the state of dormancy. For *Artemia* cysts this can be achieved by drying the cysts at temperatures not exceeding 40 °C (Voronov, 1974 ; Sorgeloos *et al.*, 1976) or by suspending the cysts in a NaCl brine solution. Similar effects of dehydration have been reported for various cyst producing anostracans, notostracans, conchostracans, and cladocerans (Table III). However, depending on the technique applied, the hatching quality of the treated cysts may vary within the same (sub-)species. These differences seem to be highly correlated with either the level, the rate, or the period (moment of start and/or duration) of desiccation.

The relative humidity at which the drying cysts are kept seriously affects hatching (Hall, 1961 ; Hempel-Zawitkowska, 1971a ; Khalaf *et al.*, 1977 ; Scott and Grigarick, 1979). More pronounced desiccation may result in europlasticity, *i.e.* the resistance of *Triops* cysts against large variations in temperature becomes much higher (Carlisle, 1968 ; Hempel-Zawitkowska and Klekowski, 1968 ; Hempel-Zawitkowska, 1971b).

The influence of the length of the dehydration period on the hatchability of diapausing eggs of *Streptocephalus* (Moore, 1967 ; Bernice, 1972), *Chirocephalus* (Hall, 1953, 1961 ; Khalaf *et al.*, 1977), *Limnadia* (Bishop, 1968), and *Triops* (Takahashi, 1977) is very significant. Bernice (1972) illustrated the need for a drying period of more than 15 days to obtain 80 % hatching of *Streptocephalus* cysts. Too long desiccating periods, on the other hand, result in lower hatching percentages or hatching rates. Analogous findings for *Triops* are reported by Takahashi (1977).

Only minor hatching is recorded when drying is started immediately after deposition of the eggs of *Streptocephalus* or *Triops* (Prophet, 1963 ; Hempel-Zawitkowska, 1967, respectively). In the latter case the insertion of a rest period of at least 5 days before dehydration is started results in increased hatching levels up to 90 %.

Similar tendencies have been observed in *Artemia*, however, only scarce data are available on true diapausing cysts. Most information is indeed on cyst material that was already (partially) dehydrated : *e.g.* collected from highly saline waters or from the shore, or processed. Some of the effects noted in these cases are therefore not due to the dehydration techniques applied but to *e.g.* dehydration/hydration cycles (Vanhaecke and Sorgeloos, 1982) and will therefore not be discussed here.

TABLE III

Literature review on diapause terminating techniques for crustacean (non-*Artemia*) and rotifer cysts
(effect : + = positive, 0 = none, – = negative)

Diapause-inhibiting method		Species	Effect H %	Effect HR	Reference
	CRUSTACEA				
Desiccation	Anostraca	*Branchinecta lindahli*	+		Prophet (1963)
		Branchipus stagnalis	+		Mathias (1937)
		Eubranchipus serratus	+		Prophet (1963)
		vernalis	+		Castle (1938) ; Weaver (1943)
			–		Avery (1939)
		Chirocephalus diaphanus	0	–	Mathias (1926, 1929)
			+/–	–	Hall (1953)
			0	+	Hall (1959a, 1961) ; Khalaf *et al.* (1977)
			0	–	Nourisson (1964)
		Streptocephalus dichotomus	+		Bernice (1972)
		seali	+/–		Prophet (1963)
			0		Moore (1957)
			–		Moore (1967)
		texanus	+		Prophet (1963)
		Thamnocephalus platyurus	+		Prophet (1963)
	Notostraca	*Lepidurus apus*	+		Braswell (1967)
		Triops cancriformis	+	+	Hempel-Zawitkowska (1967, 1971b)
				–	Hempel-Zawitkowska and Klekowski (1968)
		granarius	+		Carlisle (1968), Takahashi (1977)
		longicaudatus	–		Scott and Grigarick (1979)
			+		Takahashi (1977)
	Conchostraca	*Eulimnadia antlei*	+		Belk (1972)
		Limnadia stanleyana	+		Bishop (1968)
		Caenestheriella gynecia	0	–	Mattox and Velardo (1950)

TABLE III. Continued

Diapause-inhibiting method		Species	Effect H %	Effect HR	Reference
	Cladocera	*Daphnia magna*	–		Van De Vel (1984)
			+		Doma (1979)
		pulex	–	–	Pancella and Stross (1963)
Dehydration/ hydration	Notostraca	*Triops cancriformis*	+		Fox (1949), Hempel-Zawitkowska (1967)
		longicaudatus	+		Takahashi (1977)
Low temperature	Anostraca	*Eubranchipus vernalis*	+		Weaver (1943)
		Chirocephalus stagnalis	+		Nourisson (1961)
	Notostraca	*Lepidurus apus*	+		Braswell (1967)
		Triops cancriformis	0/+		Hempel-Zawitkowska (1971b)
		granarius	0/+		Takahashi (1977)
		longicaudatus	0		Takahashi (1977)
	Copepoda (Calanoida)	*Pontella meadi*	+		Grice and Gibson (1977)
		Labidocera aestiva	+	+	Marcus (1979, 1980, 1984), Grice and Marcus (1981)
		Tortanus forcipatus	0		Kasahara and Uye (1979)
Light/UV	Notostraca	*Triops cancriformis*	0/+	+	Hempel-Zawitkowska (1970), Takahashi (1975, 1977)
		longicaudatus and *granarius*	+		Takahashi (1975, 1977)
	Conchostraca	*Eulimnadia antlei*	+		Belk (1972)
		Limnadia stanleyana	+		Bishop (1967)
	Cladocera	*Pleuroxus denticulatus*	+		Shan (1970)
	Copepoda (Calanoida)	*Labidocera aestiva*	+		Marcus (1982)
Osmotic shock	Anostraca	*Branchipus stagnalis*	+		Mathias and Bouat (1934)

TABLE III. Continued

Diapause-inhibiting method		Species	Effect H %	Effect HR	Reference
Decapsulation	Notostraca	*Triops granarius*	+		Takahashi (1977)
	Cladocera	*Daphnia magna*	+	+	Van de Vel (1984)
		pulex	+	+	Pancella and Stross (1963)
Organic chemicals	Anostraca	*Branchipus stagnalis*	+		Mathias and Bouat (1934)
Organic detritus	Anostraca	*Chirocephalus bundyi*	+		Broch (1965)
Anaerobiosis	Anostraca	*Chirocephalus bundyi*	+		Broch (1965)
	Notostraca	*Triops cancriformis*	+		Hempel-Zawitkowska (1971b)
Freshwater	Anostraca	*Chirocephalus diaphanus*	+		Hall (1959a)
Water depth	Anostraca	*Chirocephalus bundyi*	0		Broch (1965)
		diaphanus	−	−	Hall (1959a, 1959b)
		Streptocephalus dichotomus and *seali*	0		Moore (1967), Bernice (1972)
	ROTIFERA				
Desiccation	Monogononta	*Brachionus plicatilis*	+		Lubzens *et al.* (1980)
Hibernation		*Asplanchna* spp.	+	+	Nipkow (1961), Birky (1964)
		Brachionus plicatilis		+	Blanchot and Pourriot (1982b)
			−		Lubzens *et al.* (1980)
			+		Minkoff *et al.* (1983)
		rubens	+		Blanchot and Pourriot (1982a)
Light		*Brachionus plicatilis*	+		Minkoff *et al.* (1983), Hagiwara *et al.* (1985)
			−		Ito (1960)
		rubens	+		Blanchot and Pourriot (1982a)
Osmotic shock		*Brachionus plicatilis*	+		Hagiwara *et al.* (1985)

Experimental results that may help to interpret the possible roles of specific dehydration methods in the termination of diapause are summarized in Table IV. According to Versichele and Sorgeloos (1980) a minimum level of dehydration is required to obtain maximal hatchability, *i.e.* 80 % hatching at a water content below 20 %, *e.g.* when the cysts are suspended in saturated NaCl brine for at least 24 h (Clegg, 1978b). Further water removal to less than 10 % did not improve the hatching output (Godeluck, 1980 ; Versichele and Sorgeloos, 1980 ; Lavens *et al.*, 1986b ; Naessens, pers. commun.). On the other hand the inferior results recorded with the 48 h $CaCl_2$ treatment might be related to the extremely low water content achieved in these cysts, which seems to make them far more sensitive to radiation effects (Iwasaki, 1958 ; Engel and Fluke, 1962).

It furthermore appears from Table IV that the type of drying technique interferes with diapause inhibition, *e.g.* very significant increases in the hatching percentage are noted when San Francisco Bay cysts are oven dried at 40 °C instead of at 30 °C (Versichele and Sorgeloos, 1980) or when cysts from Lavalduc are dried in a fluidized bed dryer *versus* an oven (Godeluck, 1980). Apparently a faster water removal seems to improve hatchability. The small differences reported by Lavens (unpubl.) and Lavens *et al.* (1986b) can be interpreted in the same way. Cyst drying was, however, most probably an insufficient deactivation method and more specific diapause inhibitors were required.

Considering the criterion hatching rate, Versichele (1983) detected no correlation between the dehydration technique applied and the hatching rate of lab produced SFB cysts : both oven dried and brine-stored cysts yielded hatching curves analogous to those of the parental material.

Repeated dehydration/hydration cycles

Fox (1949), Hempel-Zawitkowska (1967), and Takahashi (1977) revealed that most of the dried cysts of *Triops*, which failed to hatch at their first incubation, did develop after one (or two) successive dehydration/hydration (D/H) cycles. Analogous observations were reported for *Artemia* by Barigozzi (1939), Morris (1971), and Metalli and Ballardin (1972). Up to 70 % hatching was recorded for these so-called 'delayed hatching cysts' which were distinguishable from the other cysts by structural differences : "cysts examined had the space between the shell and embryo filled in most regions with rosette-like particles assumed to be glycogen" (Morris, 1971). More recently, Browne (1980), Versichele (1983), Browne *et al.* (1984), and Lavens (unpubl.) studied the effect of up to 5 D/H cycles. They found high variations between the different sibling species or even within the same species (Table V). Generally speaking *Artemia parthenogenetica* and *A. franciscana* gave the best hatching output after 2 D/H cycles, while *A. persimilis* and *A. tunisiana* needed up to 5 D/H cycles. The fact that the hatching levels often did not reach 50 % indicates once more that this diapause treatment will only be effective in specific cases (see further). An overview of the effect of several dehydration/hydration cycles on the hatchability of *Artemia* cysts is given in Table V.

Several dehydrations may also have a negative effect on the hatching rate when the treated cysts are stored for a long time. Cysts exposed to 2 D/H cycles initially hatch faster – confirming the cumulative development theory of Morris (1971) – but after several weeks storage need 2 h extra incubation to reach hatching (Sorgeloos *et al.*, 1976 ; Vanhaecke and Sorgeloos, 1982). This delay may be due to the drying technique applied, *i.e.* the more slowly water is removed, the more the hatching rate is delayed after storage.

TABLE IV

Effect of various dehydration techniques on the hatching performance of *Artemia* cysts

Strain	Specific characteristics of the non-processed cyst material	Dehydration technique used	Hatching %	Reference
SFB	Lab produced cysts (90 ‰ S)	Saturated NaCl-brine for 1 h	6	Versichele and Sorgeloos (1980)
		Saturated NaCl-brine for 4 h	17	
		Saturated NaCl-brine for 6 h	27	
		Saturated NaCl-brine for 12 h	49	
		Saturated NaCl-brine for 24 h	76	
		Saturated NaCl-brine for 48 h	79	
		Oven drying at 30 °C for 24 h	20	
		Oven drying at 30 °C for 48 h	60	
		Oven drying at 40 °C for 48 h	81	
		Drying over $CaCl_2$ for 24 h	72	
		Drying over $CaCl_2$ for 48 h	23	
	Produced in extensive ponds (85 ‰ or 155 ‰*) in Kenya ; harvested from the water	Saturated NaCl-brine	12/56*	Naessens (pers. commun.)
		Oven drying at 35 °C for 6 h	15/59*	
GSL	Lab produced cysts (90 ‰) batch 210584	NaCl-brine	4	Lavens *et al.* (1986b)
		MgCl-brine	3	
		Oven drying at 35 °C for 24 h	11	
	batch 0483	$NaCl_2$-brine	46	Lavens (unpubl.)
		Oven drying at 35 °C for 24 h	69	
RAC	Lab produced cysts (90 ‰) batch 210584	NaCl-brine	35	Lavens (unpubl.)
		Oven drying at 35 °C for 24 h	40	
LVD	Harvested from the lake water (180 ‰)	NaCl-brine	37	Godeluck (1980)
		Oven drying at 35° C	41	
		Fluidized bed drying at 35 °C	90	
ML	Harvested from the lake water (90 ‰)	No treatment	17	Dana (1981)
		NaCl-brine	40	

TABLE V

Effect of several dehydration/hydration cycles on the hatchability of *Artemia* cysts produced under controlled conditions

Strain	Hatching % after 1 to 5 dehydration/hydration cycles (non-cumulative hatching data)					Cumulative hatch (%)	Reference
	1	2	3	4	5		
San Francisco Bay, USA	19	50	0	0	0	69	Browne (1980), Browne *et al.* (1984)
Puerto Rico, USA	6	9	8	0	0	23	
Hidalgo, Argentina	19	12	8	12	2	53	
Chott Ariana, Tunisia	2	5	2	12	2	23	
Larnaca, Cyprus	14	1	2	3	7	27	
Santa Pola, Spain	17	4	0	0	12	33	
Tuticorin, India (cited as Madras)	7	29	8	1	0	45	
Kutch, India	0	17	1	0	0	18	
Cadiz, Spain	1	12	1	0	12	26	
Izmir, Turkey	21	0	0	0	1	22	
Macau, Brazil	40	44	–	–	–	88	Versichele (1983)
Great Salt Lake, USA	2	3	0	–	–	5	Lavens *et al.*(1986b)
Lavalduc, France	30	20	–	–	–	50	Lavens (unpubl.)

Hibernation

The need for a cold preincubation treatment in other crustaceans and rotifers is documented in Table III. Weaver (1943) indicated that non-dried *Eubranchipus vernalis* cysts only hatched (33 %) after slow freezing and thawing. Another anostracan, *Chirocephalus stagnalis*, showed analogous effects when preincubated at 4 °C for daily cycles of 3 up to 18 h (Nourisson, 1961). Braswell (1967) noted the need for a hibernation period for the notostracan *Lepidurus apus*, that was, however, not essential for *Triops* (Hempel-Zawitkowska, 1971b ; Takahashi, 1977). Remarkable for the latter organism is that freezing can restore the negative effect of an inefficient drying. Marcus (1979, 1980) demonstrated for the copepod *Labidocera aestiva* that resting eggs which are chilled at 5 °C for a minimal period of 30 days, will hatch better and more synchronously. This seems also to be valid for *Pontella meadi* (Grice and Gibson, 1977). A far better synchronous hatching occurred also with cysts of the rotifer *Brachionus plicatilis* after hibernation for 3 months at 5 °C ; only 2 days instead of 3 months were required to obtain a 50 % hatching (Blanchot and Pourriot, 1982b).

Substantial evidence on the effect of a cold treatment as diapause inhibitor in brine shrimp cysts has been revealed by several authors. For Great Salt Lake (GSL) *Artemia* Rackowicz (1975) reported the need of a wintering period of at least 7 months, while Karmiol (1981) and Van der Haegen (1981) detected a hatching increase of 10 % when dehydrated cysts were frozen at -20 °C. Lavens *et al.* (1986b) used laboratory-produced GSL cysts to prove that the hibernation effect can be quantified : *i.e.* 8 weeks at 4 °C or -25 °C improved hatchability by 5 %, respectively 50 %, and a 70 % value was reached after 32 weeks storage at -25 °C. Two freezing periods interrupted by an acclimation period did not result in a cumulative deactivation : only 39 % hatching instead of 50 % was obtained after a 2×4 weeks preincubation at -25 °C. They also confirmed the earlier observation of Kinne (1977) that following hibernation, the cysts should be acclimated at room temperature for at least 1 week prior to incubation, otherwise poor hatching will prevail.

Experiments with cysts from Mono Lake (California, USA), harvested from 90 ‰ S lake water, revealed an analogous time-temperature dependent hibernation effect (Dana, 1981, 1982 ; Dana and Lenz, 1986 ; Thun and Starrett, 1987). As can be seen from Fig. 4 the preincubation period not only affected hatchability but also hatching rate : best results (> 80 % in less than 6 days) were obtained after storing the cysts at 4 °C for more than 100 days. The diapause inhibition was less effective at higher storage temperatures : only 12 % hatched when treated at 10 °C (Dana, 1981). Nonetheless Mason (1967) reported a good hatching after a longer preincubation period of 150 days at 15 °C.

In Lake Lavalduc (France) *Artemia* maximal hatchability (90 %) is ensured after 2 months storage of the dormant embryos at about -30 °C (Sorgeloos, 1979 ; Godeluck, 1980). Hibernation is also an efficient diapause inhibitor for brine shrimp cysts from the Soviet Union – salt lakes in Kazakstan and Siberia (own data, unpubl.) and the Shivash-saltworks along the Azov Sea (Voronov, 1973), and for *Artemia* from Chaplin Lake (Canada) (Sorgeloos, 1979 ; Van der Haegen, 1981 ; Sawchyn, 1985).

Peroxide or other chemicals

Mathias (1937) reported the positive effect (50 % hatching) of hydrogen peroxide on *Artemia* cysts that normally did not hatch. This was confirmed by Bogatova and Shmakova (1980) and Bogatova and Erofeeva (1985) for *Artemia* from the Soviet lakes near the Black Sea and the Altai.

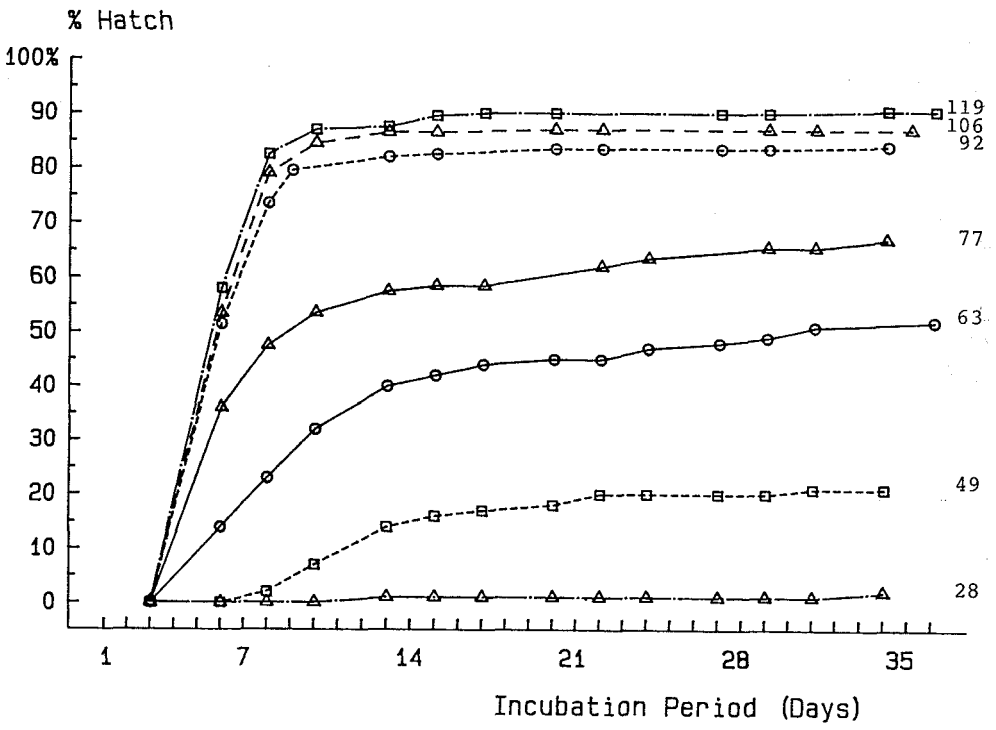

FIG. 4. Hatching rate and percentage of Mono Lake *Artemia* cysts incubated at 10 °C and 90 ‰ S ; this in function of various preincubation periods (28, 49, 63, 77, 92, 106, 119 days) at 4 °C (from Thun and Starrett, 1987).

Hatching yields increased from 5 to 90 % hatch and more by soaking the dried cysts for 15 min in a 3 % H_2O_2 solution prior to incubation, or when 30 up to 230 ppm H_2O_2 was added to the hatching medium. Small but significant variations were reported when different peroxide concentrations were used ; at higher peroxide doses the hatching rates also increased. Vu Do Quynh *et al.* (1987) studied Great Salt Lake *Artemia* cysts harvested from pond cultures in Vietnam ; the hatchability increased 10 to 20 % after treatment with a 1.5 %, respectively 2 % H_2O_2 solution in freshwater. Lavens *et al.* (1986b) reported an incubation-time effect : for Great Salt Lake cysts maximal larval outputs of 80 % were obtained after 30 min preincubation, whereas at longer periods hatching levels slightly decreased. For Lavalduc cysts the highest number of nauplii were hatched (52 %) after a 1 h pretreatment with 3 % H_2O_2 (Lavens, 1981).

More recent tests at our laboratory with Great Salt Lake cysts produced in inoculated ponds in Vietnam (Vung Tau, UN-Mekong Project, courtesy W. Tackaert) revealed an obvious dose-time effect (Table VI). High concentrations of H_2O_2 (5-10 %) have their optimal effect at short incubation periods (5, respectively 1 min) while lower doses need more exposure time (15-60 min) to reach comparable hatches. Diapause inhibition by peroxide treatment can be reversible, *i.e.* Bogatova and Shmakova (1980) detected a loss of the H_2O_2-effect when the cyst material was dried and stored ; we observed constant hatching levels after redrying and storage for 4 months under vacuum or in air, but storage in brine or exposure of the dry cysts to freezing temperatures seemed to affect hatchability (Lavens, unpubl.).

TABLE VI

Dose-time effect of H_2O_2 preincubation treatment
on the hatchability of *Artemia* cysts from Vung Tau (Vietnam).
Data are expressed as percentage of hatching results obtained at 2 %/15 min treatment (74 % hatch)
(Lavens, unpubl.)

Time (min.)	Doses (%)					
	0.5	1	2	3	5	10
1					46	105
2					94	
5			54	69	102	32
10	47		90	81	88	
15		46	100	76		
20			91	94	52	
30		91	95			
60	56	85		6	1	
120		15				
180	47					

The effect of the peroxide treatment on brine shrimp hatching is explained by Bogatova and Shmakova (1980) as a catalyzation, via the reactive oxygen atoms, of the oxidation-reduction process whereby trehalose is converted into glycerol and glycogen during embryogenesis. In this regard the observations of Van der Linden (pers. commun.) that the threshold for light sensitivity in hydrated *Artemia* cysts decreases in those cysts that have been preincubated in peroxide, may be of interest, especially since the illumination energy normally serves to catalyze oxidation-reduction processes in the cysts via captation by haempigments (Van der Linden *et al.*, 1986).

Other chemicals reported to be effective in increasing the hatchability of brine shrimp cysts are aceton, n-butanol, ethylether, xylene (Tazawa and Iwanami, 1974), and hypochlorite (Bruggeman *et al.*, 1980 ; Vanhaecke and Sorgeloos, 1983). The latter product is used for so-called cyst decapsulation, *i.e.* to dissolve the cyst envelope. It is not clear if decapsulation acts as a diapause inhibitor or if the positive effect is related to a beneficial role during the hatching metabolism. The breaking stage may be reached more easily if the thick shell barrier has been removed, or if the resistant outer cuticular membrane is affected. Evidence for the latter hypothesis might be found in *Daphnia* where hypochlorite treatment of the ephippia results in the release of the individual embryos from the protective envelope and in an increase of their hatching percentage and rate (Pancella and Stross, 1963 ; Van De Vel, 1984). On the contrary, preliminary experiments comparing the effect of peroxide and hypochlorite treatment on *Artemia* cysts support the first hypothesis, *i.e.* in both cases hatching percentages increased very significantly from about 5 to over 60 % hatch (Lavens, unpubl.).

Other diapause-terminating techniques

The following other diapause-terminating techniques exist :

- ionizing irradiation (Metalli and Ballardin, 1972) ;
- permanent magnetic fields (2 000 Oersted) for less than 24 h (Dolgopol'skaya *et al.*, 1969, 1970 ; Taneyeva and Dolgopol'skaya, 1973) ;

– illumination of the cysts in hydrated and aerobic conditions. Dana (1982) demonstrated that light is not needed when Mono Lake cysts were previously hibernated. Since Belk (1972) revealed that a light stimulus is not essential for *Eulimnadia antlei* cysts that have been properly dried, Vanhaecke *et al.* (1981) hypothesized that light could act as a diapause inhibitor rather than a trigger of the hatching metabolism. Since no further evidence has become available that proves that light can replace other diapause inhibitors (*e.g.* dehydration) we have transferred the detailed discussion on light effects to the section "hatching conditions".

Is diapause termination strain-specific ?

Dormancy plays a decisive role in the persistence of species under temporarily adverse conditions, and is a life-cycle strategy to synchronize population developments to the variations of their specific biotope. In this regard it is very likely that the process of diapause and its termination are adapted to the local environment. Such climatic adaptations might have contributed to strain-specific differences, *e.g.* inter-population differences in diapause deactivation sensitivity. Proofs of inter-population variations in cyst hatchability were provided by Browne *et al.* (1984), Lavens *et al.* (1986b), and Tackaert *et al.* (1987) who performed experiments with *Artemia* cysts from different geographical origin, produced under analogous conditions in the laboratory, and by Vu Do Quynh *et al.* (1987) with field tests in identical ponds but inoculated with different strains.

Two hypotheses may explain the inter-population differences : either the basic biochemical process involved in the induction of diapause is strain-specific, or different levels of diapause-deactivation sensitivity exist. The first hypothesis might have but limited value as it is most likely that the causal mechanism in establishing dormancy in the early embryo stages is universal within the genus *Artemia*, and maybe even within the phyllopods. Extra support for the hypothesis on differences in diapause-deactivation sensitivity are provided by the following observations. Firstly diapause deactivation is influenced by environmental conditions of the biotope, *e.g.* food quantity and salinity. This means that within the same population the possibility exists to adapt the diapause-deactivation sensitivity to a higher threshold value. Secondly, specific diapause terminating methods are not just on/off-reactions but have a quantitative effect : *e.g.* temperature-time dependence in hibernation and dose-time interaction in the peroxide treatment.

Strain-specificity in diapause termination is most probably explained by differences in the minimal threshold value for diapause deactivation. For example within the *A. franciscana* species San Francisco Bay *Artemia* cysts seem to have a low threshold value since a simple dehydration in brine for 24 h already results in a maximal hatching (> 90 %), whereas cysts from Great Salt Lake may have a high threshold value : dehydration is insufficient and more specific diapause deactivation techniques, *e.g.* hibernation and peroxide treatment, are needed to finally break dormancy. Specific levels of the intracellular pH – as suggested by Drinkwater and Crowe (1986) for Mono Lake cysts – may explain how differences in diapause-deactivation sensitivity may occur.

Differences between *Artemia* strains in diapause-deactivation sensitivity may furthermore be related to specific variations in habitat conditions and in this way may have an ecological significance. When considering the low temperature effect, it is striking that especially *Artemia* strains which occur in temperate regions need a more or less prolonged hibernation as diapause

terminator, *e.g.* Great Salt Lake, Lavalduc Lake, Mono Lake, Chaplin Lake, Azov Sea saltworks, and Kazakstan lakes. Their encysted offspring produced during summer will not develop when hatching conditions might become acceptable with the autumn rains because this might lead to the elimination of the future "seed-stock" which will be vital to generate a new population after the winter. The hibernation effect will ensure that in the spring all cysts are quiescent, resulting in a synchronous hatching and consequently a fast build-up of the new population. A similar adaptation has been reported by Marcus (1979, 1980) for the calanoid copepod *Labidocera aestiva.* Furthermore, this relation with the habitat explains why dehydration/hydration cycles do not affect *e.g.* Great Salt Lake cysts but do terminate dormancy in (sub-)tropical or coastal populations, *e.g.* Macau, Tuticorin, Hidalgo, San Francisco Bay, etc. (Table V).

A practical example of the ecological significance of specific dormancy terminating processes may be given by Vu Do Quynh *et al.* (1987) who suggested that the disappearance of the Macau population and the persistence of Great Salt Lake *Artemia* after the rainy season in inoculated salt ponds in Vietnam, was caused by differences in their cysts' diapause termination characteristics.

Differential adaptation of geographically separated populations has been documented in various other crustaceans, *e.g. Triops* (Table III). Until now, however, there is no evidence that differences in dormancy termination between various geographical brine shrimp strains are correlated with different phenotypes or genotypes ; almost all experiments mentioned in this review were dealing with cysts produced under controlled conditions only during one generation (laboratory systems) or a few generations (temporal inoculations in extensive systems).

Factors affecting cyst hatching in *Artemia*

Quiescent *Artemia* cysts resume their embryonic metabolism as soon as the environmental parameters become favorable, *e.g.* when sufficient water has been taken up (more than 65 % of the cyst dry weight). The carbohydrate metabolism is activated under aerobic conditions and trehalose is converted into glycogen (as an energy supply for respiration) and glycerol. The accumulation of the hygroscopic glycerol within the outer cuticular membrane leads to increased intra-cystic osmotic pressures eventually resulting in the breaking of the shell. The embryo now differentiates into an instar I nauplius within its hatching membrane which it can leave, head first, once a hatching enzyme is secreted supposedly in the head region of the nauplius. More details on the hatching metabolism can be found in the paper by Clegg and Conte (1980). As a consequence of differential, 'strain-specific' adaptations to their local environment, the interactions of the parameters may be slightly different for the various strains resulting in variable optimal ranges for hatching.

Salinity

The qualitative ionic composition of the incubation medium can interfere either with the embryo's toxicity tolerance, *e.g.* K^+, Ca^{2+} and Zn^{2+} (Sato, 1966b ; Bagshaw *et al.*, 1986 ; Rafiee *et al.*, 1986), the osmotic capacity of the emerging embryo (Conte *et al.*, 1977), or with specific activities, *e.g.* of the hatching enzyme (Sato, 1966ab, 1967). With regard to the latter hatching enzyme, its inhibition by Fe^{2+} or Cu^{2+} can be prevented by the addition in the hatching medium of $NaHCO_3$ or chelating agents. Ca^{2+} has an activating effect on the hatching enzyme provided

it is released from its heavy metals. When K^+ and/or Mg^{2+} are absent no effect on the rate of excystment has been observed (Sato, 1966a).

Quantitative effects of the incubation salinity on cyst hatching are related in the first place with the hydration-level that can be reached in the cysts. Above a threshold salinity insufficient quantities of water can be taken up to support the embryo's metabolism. This threshold value varies from strain to strain and is well documented in the literature, *i.e.* approximately 85-90 ‰ is the maximum salinity for most *Artemia* strains : GSL (D'Agostino, 1965 ; Von Hentig, 1971), SFB (Sorgeloos, 1975), Bonaire (Kristensen and Hulscher-Emeis, 1972), Lavalduc (Sorgeloos, 1979), Kiatuthlana Green and Red ponds (Cole and Whiteside, 1965). Lake Ontario and Mono Lake cysts hatch even at salinities higher than 95 ‰ (Ivanovskii *et al.*, 1981), respectively 125 or 190 ‰ (Dana, 1981 ; Thun and Starrett, 1987). On the contrary, a lower value was detected for Tuticorin (75 ‰) (Royan, 1975), Penley Lake (58 ‰) and Jesse Lake (32 ‰) (Collins, 1977).

In the second place the incubation salinity will interfere with the amount of glycerol that needs to be built up to reach the critical intra-cystic osmotic pressure. The fastest hatching rates will thus be noted at the lowest salinity levels since it will take less time to reach breaking. When considering high salinities, it is is very likely that cysts from a certain geographical origin contain insufficient quantities of carbohydrates to meet their varying hyperosmotic requirements (Vanhaecke, 1983). As a result optimal seawater salinity for cyst hatching varies from strain to strain in *Artemia* : *e.g.* 15-70 ‰ for GSL cysts (Von Hentig, 1971), 5-80 ‰ for SFB cysts (Sorgeloos, 1975), 40 ‰ or less for Mono Lake cysts (Dana, 1981), 5 ‰ for Buenos Aires cysts (Vanhaecke and Sorgeloos, 1983), 5-15 ‰ for Chaplin Lake cysts (Kurata, 1967 ; Smith, 1969 ; Provenzano and Goy, 1976 ; Vanhaecke and Sorgeloos, 1983). Differences found in salinity optima for this latter sulphate lake strain when incubated in sulphate or chlorine hatching media (Provenzano and Goy, 1976 ; Vanhaecke, 1983 ; Sawchyn, 1985) are attributed to the same phenomenon of osmotic pressure and not to qualitative differences in osmotic composition (Vanhaecke, 1983). The osmotic pressure of a 35 ‰ Na_2SO_4 water is far lower than that of a 35 ‰ NaCl medium (Weast, 1973), as a result of which Chaplin Lake cystst do hatch in the first medium with a normal salinity (35 ‰) and only after dilution of the second medium to 5-15 ‰.

Temperature

Hydrated *Artemia* embryos do not resist temperatures below the freezing point -10 °C (Hempel-Zawitkowska, 1971a) or above 40 °C (Voronov, 1974) (Fig. 5). Their metabolism is also arrested below 4 °C (Iwasaki, 1964 ; Iwasaki and Nakanishi, 1966), and above 33-37 °C (Sorgeloos, 1975 ; Vanhaecke, 1983). The latter reversible onset and arrest of the early metabolism seems to be controlled by the molecular sensor cytochrome oxidase which activation/deactivation pattern is analogously influenced at the same temperatures (Vallejo *et al.*, 1980). According to Von Hentig (1971) maximal hatching is ensured between 15 and 30 °C. Similar but more narrow ranges were confirmed : 20-30 °C by Jones (1972), 20-28 °C by Sorgeloos (1975), and 26-30 °C by Ivanovskii *et al.* (1981). Experiments carried out by Vanhaecke (1983) on cysts from 17 different strains revealed maximal hatchabilities in the temperature range 25-30 °C, except for Larnaca and Chaplin Lake cysts which yielded 65 % respectively 30 % less nauplii at 30 °C and for Tuticorin *Artemia* with a 30 % improvement at 30 °C *versus* 25 °C (confirming earlier findings of Royan, 1975).

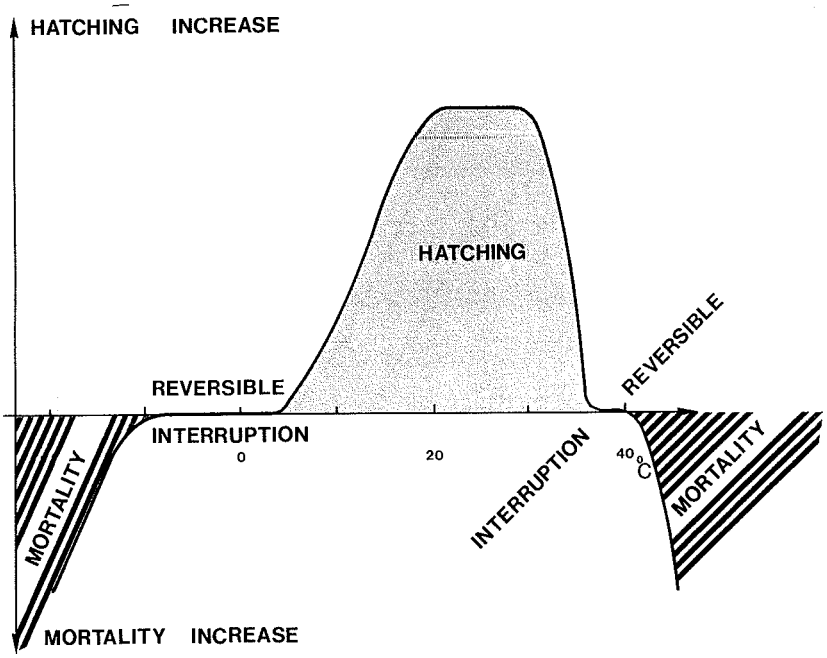

FIG. 5. General schematic diagram of the relationship between incubation temperature and hatching characteristics of *Artemia* cysts. Note that the Y-axis is not scaled with only relative hatching unit values used in this general model.

Vanhaecke (1983) furthermore showed that the hatching tolerance for 35 °C is specific for each sibling species, *i.e.* the proportional hatching decrease is limited for *Artemia franciscana* and *A. persimilis* whereas drastic decreases have been recorded for *A. parthenogenetica* and *A. tunisiana* (Fig. 6). No correlation could be detected between the temperature regime of the brine shrimp biotopes and their temperature tolerance for cyst hatching.

pH

Whereas the hatching rate is not affected by pH, a maximal hatching efficiency can be reached at alkaline pH's in the range 8-8.5 (Jennings and Whitaker, 1941 ; Nimura, 1968 ; Jones, 1972 ; Metalli and Ballardin, 1972). Sato (1967) correlated this finding with the optimal pH activity range for the hatching-enzyme which digests the inner cuticular membrane, eventually facilitating the release of the free-swimming nauplius. This may also explain why the addition of $NaHCO_3$, up to 2 g/l, to artificial or diluted seawaters, or to dense suspensions of cysts does result in improved hatching outputs. Under those circumstances increased buffer capacities are required to avoid a dropping of the pH of the hatching medium (Jones, 1972 ; Rogers and Johnston, 1977 ; Sorgeloos *et al.*, 1983 ; Lavens *et al.*, 1986a).

Oxygen concentration

Although it is well known that an essential requirement for the (aerobic) resumption of development is molecular oxygen at an adequate partial pressure, only few data are found in the

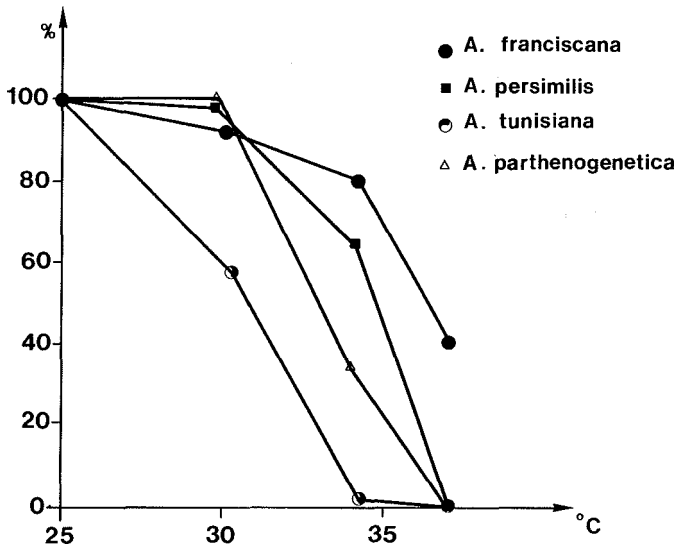

FIG. 6. Average decrease in hatching of *Artemia* cysts from different sibling species in relation to increasing incubation temperature (after Vanhaecke, 1983).

literature. Kurata (1967) described a linear effect on hatching percentage ; Nimura (1968) and Sorgeloos and Persoone (1975) detected an increasing hatching efficiency between the 0.6 ppm minimum O_2 level and 2 ppm O_2, and maximal larval hatching above this concentration. An analogous minimum oxygen concentration is required for the embryonic metabolism of the anostracan *Chirocephalus seali* (Moore, 1963, 1967) and the conchostracan *Limnadia stanleyana* (Bishop, 1967). To avoid oxygen gradients during hatching it is obvious that a good, homogenous mixing of the cysts in the incubation medium is required.

Cyst density

The cyst density may interfere with the other abiotic conditions which are essential for hatching, *i.e.* pH, O_2, illumination. Provided that the other prerequisites are fulfilled, the hatching process will not be affected by high cyst densities up to 17 g/l (Sorgeloos, 1975 ; Kurata, 1967).

Illumination

The effect of light on the hatching process was first described in 1973 by Sorgeloos. Compared to controls incubated in darkness, hatching increases up to 50 % were demonstrated for San Francisco Bay, Great Salt Lake, and Bulgarian *Artemia* cysts hatched in light (Sorgeloos, 1973). This photoquiescence phenomenon has been confirmed for the same as well as for other strains : San Francisco Bay (Meade, 1975 ; Spektorova and Syomik, 1979 ; Van der Haegen 1981), Tuticorin (Royan, 1976), Larnaca (Person-Le Ruyet and Salaun, 1977), Caspian and Crimean *Artemia* (Spektorova and Syomik, 1979). Fuchs (1976) and Van der Haegen (1981), however, detected no light sensitivity in cysts from San Francisco Bay and Lavalduc, respectively Great

Salt Lake. The fact that (part of) the encysted embryos do hatch in darkness was explained by Sorgeloos (1973) as a response to anteriorly captated and stored light stimuli.

According to Sorgeloos and Persoone (1975) brine shrimp cysts are susceptible to light triggering as soon as they have reached full hydration under aerobic conditions. They also found that a minimal dose of light energy is needed to trigger the onset of embryonic metabolism. Dose-response data from Van der Linden *et al.* (1985) confirm the relation between the light intensity/exposure time and the hatching effect. According to Vanhaecke *et al.* (1981), the critical light intensity threshold does vary from strain to strain, *e.g.* Chaplin Lake cysts require at least a continuous light intensity of more than 1 000 lux in order to hatch optimally. In other strains (Great Salt Lake, San Pablo Bay, Buenos Aires) only the hatching rate is affected by various illumination levels since progressively more accumulation time is needed to reach the triggering dose. These variations between strains may be attributed to differences in shell characteristics, *e.g.* envelope thickness (Vanhaecke *et al.*, 1981) or haematin pigment concentration (Gilchrist and Green, 1960), which can delay the light infiltration (Hempel-Zawitkowska, 1970 ; Van der Linden *et al.*, 1986). This is further supported by the fact that the light intensity threshold of decapsulated San Pablo Bay cysts has dropped from 100-500 lux, to 20-100 lux (Vanhaecke *et al.*, 1981). Analogous findings were reported for the cladoceran *Daphnia pulex* by Pancella and Stross (1963). Recently Van der Linden *et al.* (1985, 1986) identified the wavelength region of 400-600 nm to be the most effective one in triggering the onset of metabolism. The same authors furthermore reported that in total 21 600 $\mu E/m^2$ light energy is needed to ensure maximal hatching in San Francisco Bay cysts. It is assumed that a photoreceptor, which may be a haempigment, mediates in the light-induced hatching.

Conclusions and recommendations

Innumerable research efforts have been contributed to the elucidation of the biological, biochemical, ecological, and other aspects of *Artemia* cyst hatching. To a large extent this interest has been inspired by economic motives, *i.e.* hatching quality is of primary importance to aquaculturists and aquariologists. Further contributions were generated by its scientific relevance, *i.e.* its extensive use as a practical research object, and the human fascination for the 'state of suspended animation' as an excellent adaptation to survive extreme habitat conditions. However, this review shows that all these efforts have hitherto not resulted in a complete understanding of all processes involved. It is obvious that the major cause for this should be sought in the numerous factors which interact with the cyst's hatchability, some of which have often been disregarded. Consequently, experimental data have been misinterpreted sometimes resulting in confusion or contradictory statements in the literature.

Therefore we would like to make a strong plea for the use of standard cyst material, the antecedents of which are well known, to further study biochemical, biological or physiological effects related to the cryptobiotic state. In this regard we hope that the laboratory technique for controlled cyst production of Lavens and Sorgeloos (1984) can soon be scaled-up to produce sufficient amounts of these 'standard *Artemia* cysts'. Meanwhile, productions from more or less controllable extensive *Artemia* operations may be helpful. We also recommend more interdisciplinary cooperation to elucidate the mechanisms involved in diapause.

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