(Manuscript received on 1 December 1988)

# INTERNATIONAL STUDY ON ARTEMIA (\*). XLVII. THE EFFECT OF TEMPERATUGRE ON CYST HATCHING LARVAL SURVIVAL AND BIOMASS PRODUCTION FOR DIFFERENT GEOGRAPHICAL STRAINS OF BRINE SHRIMP ARTEMIA SPP.

by

PAUL VANHAECKE (\*\*) and PATRICK SORGELOOS

Artemia Reference Center, Faculty of Agricultural Sciences
State University of Ghent
Rozier 44, B-9000 Ghent (Belgium)

### SUMMARY

The effect of temperature on different geographical strains of *Artemia* has been studied for the following criteria: the hatchability of the cysts, the resistance of the larvae to high temperature (34° C) and the biomass production of larvae cultured under standard conditions. Experimental temperatures ranged from 25° to 37° C for the hatching criteria and from 20° to 32.5° C for the biomass production.

Both the hatching percentage and growth performance are affected by temperature. However, the relative and quantitative impact of temperature varies widely among *Artemia* strains. This is also the case for the temperature resistance of the larvae.

The impact of increasing temperatures on cyst hatching as well as the temperature resistance of the larvae is related to the genetic classification of *Artemia* in different sibling species, *i.e. Artemia franciscana* strains are most resistant whereas *Artemia tunisiana* and *Artemia persimilis* strains are very sensitive to high temperatures. Intraspecific differences in tolerance may be due to genetic differentiation, or in the case of survival tolerance to genetic adaptation.

On the basis of these results guidelines are provided for the utilisation of *Artemia* strains in aquaculture, e.g. optimal hatching temperature, strain selection for inoculation purposes and for biomass production purposes, temperature selection for culturing.

### INTRODUCTION

Temperature is known to be an important abiotic parameter affecting and conditioning the life processes in aquatic organisms (Kinne, 1970). In *Artemia*, temperature has been reported to interact with cyst hatchability and hatching rate (Von Hentig, 1971; Sorgeloos, 1975; Royan, 1975), molting rate and developmental rate of nauplii (Hentschel, 1968; Sorgeloos *et al.*, 1976), growth rate (Reeve, 1963; Von Hentig, 1971) and reproductive capacity (Von Hentig, 1971; Iwasaki, 1976).

(\*\*) Ecolas, Noorderlaan 147, B-2030 Antwerpen, Belgium.

<sup>(\*)</sup> International interdisciplinary study on *Artemia* strains coordinated by the Artemia Reference Center, State University of Ghent, Belgium.

Various geographical strains of Artemia have been found to inhabit widely isolated biotopes with specific temperature conditions (Persoone and Sorgeloos, 1980; Vanhaecke et al., 1987). Substantial genetic differences have been recorded between Artemia strains resulting in their classification into different sibling species (Abreu-Grobois, 1987). Moreover, several publications have revealed biometrical, biochemical and physiological differences, as well as variations in temperature resistance and optimal temperature for survival between Artemia strains (Sorgeloos et al., 1976; Claus et al., 1977; Vanhaecke et al., 1984; Léger et al., 1986).

The purpose of the present study was to detect and quantify the effect of temperature on three criteria which are important when using Artemia in aquaculture, i.e. the hatching of the cysts, the survival of the nauplii, and the biomass production of cultured larvae. This study not only aims to better characterize the different Artemia strains and to relate the strain characteristics to the genetic classification of Artemia, but especially to provide practical guidelines on strain selection and temperature selection for the use of Artemia in aquaculture.

## MATERIALS AND METHODS

Details on the exact origin and the major characteristics of the Artemia strains used in this study are provided in Table 1. All cyst samples were first cleaned according to the procedures described in Sorgeloos et al. (1978). Unless specified otherwise cysts were always incubated under the following hatching conditions: 25°C, natural seawater of 35 ppt salinity, 1000 lux illumination (SorgeLoos, 1980). The hatchability was studied at four test temperatures (25, 30, 34 and 37°C), using water baths kept at  $+0.2^{\circ}$ C of the test-temperature. For each sample, cysts were equally distributed (density 2-2.5 g/l) over two cylindroconical tubes each containing 80 ml seawater. After 1 h 20 ml seawater was added to each tube. 48 hours after incubation five subsamples were taken from each tube using an automatic micropipet, adjusted to such a volume as to collect about 100 to 150 individuals per subsample. The subsamples were transferred to petridishes and 1 ml water was added. After fixation with lugol-solution, nauplii were counted under a dissection microscope. In order to make an accurate count of the number of unhatched embryos, the remaining cysts were decapsulated by the addition to the petridishes of one drop of NaOH and three drops of NaOCl; within a few minutes all cyst shells are dissolved and the non-emerged embryos can easily be counted. For each experimental temperature the mean hatching percentage was calculated from the duplicate series of five subsamples.

The survival of the Artemia larvae was studied at a temperature of 34°C and at two salinities (35 and 120 ppt), this in order to detect eventual tolerance differences as a function of salinity. The tests were carried out according to the procedure described in Vanhaecke et al. (1984). During the nine day experimental period larvae were fed with Dunaliella tertiolecta Butch cells according to the feeding schedule in Table 2 (25-32.5°C values). For each experiment the Great Salt Lake strain (1977) was used as an inner standard for intercalibration purposes.

The biomass production experiments were carried out in cylindroconical tubes as described in Vanhaecke et al. (1984), but with 150 nauplii in 300 ml seawater (35 ppt), Five test temperatures were studied in four duplicates: 20, 22.5, 25, 27.5 and 30°C. For those strains which showed a good temperature tolerance an additional temperature (32.5°C) was tested. During the nine day experimental period the larvae were fed once a day with a suspension of *Dunaliella tertiolecta* Butch cells. Since

TABLE 1

List of Arternia strains and batches studied

Source of cysts	Year of harvest (batch number)	$\begin{aligned} & \text{Mode of} \\ & \text{reproduction (1)} \\ & B = \text{bisexual} \\ & P = \text{parthenogenetic} \end{aligned}$	Sibling species (2)	Ploidy number (3)
San Francisco Bay (CA., U.S.A.) Macau (Brazil) Macau (Brazil) Macau (Brazil) Macau (Brazil) Barotac Nuevo (Philippines) Mu ndra (India) Bangpakong (Thailand) Great Salt Lake (UT., U.S.A.) Great Salt Lake (UT., U.S.A.) Great Salt Lake (Canada) Lake Tahoka (TX., U.S.A.) Manaure (Colombia) Galera Zamba (Colombia) Bahia Salinas (Puerto Rico) Bahia Getuu) Virrila (Peru) Bonaire (Netherlands Antilles)	1976 (288-2596) 1978 (871172) 1979 (971051) 1978 1980 1979 1977 1977 1977 1977 1977 1980 1980	***************************************	A. franciscana	**************************************

(1) From Barigozzi and Tosi (1959), Clark and Bowen (1976), Bowen et al. (1978), Abreu-Grobois and Beardmore (1980, 1982).
(2) From Bowen et al. (1978, 1980), Barigozzi (1980), Abreu-Grobois and Beardmore (1980, 1982).
(3) From Barigozzi (1974), Abreu-Grobois and Beardmore (1982).

TABLE 1 (continued)

List of Artemia strains and batches studied

Source of cysts	Year of harvest (batch number)	Mode of reproduction (1) B = bisexual P = parthenogenetic	Sibling species (2)	Floidy number (3)
December (Percedon)	1081	щ	A framonomona	į,
Tacoa (recaca)	1001	n p	A face of contract	1 4
For Araya (Venezuela)	1010	<b>a</b> 6	A. J'uncescuira	,
Buenos Aires (Argentina)	1977	R	A. persimilis	2n + 1
Larnaca (Cyprus)	1980	В	A. tunisiana	žr.
Barbanera (Spain)	1978	В	A. tunisiana	2n
Shark Bay (Australia)	1977 (113)	ď	A. parthenogenetica	2r
Shark Bay (Australia)	1979 (199)	Ъ	A. partehnogenetica	3n
Salin du Giraud (France)	1979	ď	A. parthenogenetica	2n
Tientsin (P.R. China)	1978	Q.	A. parthenogenetica	2n + 4n
Lavaldue (France)	1979	Ы	A. parthenogenetica	2n + 4n
Margherita di Savoia (Italy)	1977	<u>a</u>	A. parthenogenetica	2n + 4n
Eilat (Israel)	***************************************	д	A. parthenogenetica	2n + 1
Tuticorin (India)	1978	<del>d</del>	A. parthenogenetica	3n
Izmir (Turkey)	1977	Ъ	A. parthenogenetica	3n + 5n

	TABLE 2
at	Feeding schedule (in 10 <sup>6</sup> Dunaliella cells per day) the different test temperatures (after Vanhaecke, 1983)

	80° C	22.5° C	25-32.5° C
Day 1	18.0	20.7	22.5
Day 2, 3, 4	36.0	41.4	45.0
Day 5, 6	54.0	63.0	67.5
Day 7	72.0	82.8	90.0
Day 8	90.0	106.2	112.5
Day 9	126.0	147.6	157.5
Total numbers of cells	522.0	607.5	652.5
Total dry weight (µg)	45075	52458	56343

the food requirements of *Artemia* may alter as a function of temperature (Hernandorena, 1976), the optimal feeding schedule under the prevailing experimental conditions was determined for two strains in a series of preliminary experiments (Vanhaecke, 1983). The optimal feeding schedules for the different temperatures tested are given in Table 2.

At the end of the experiment the larvae were filtered off, rinsed thoroughly with distilled water, counted and dried for 24 h at 60°C.

The following parameters were analysed:

- % survival (S),
- total biomass in  $\mu g$  dry weight DW  $(W_t)$ ,
- total biomass production in  $\mu g$  DW (W<sub>t</sub> W<sub>o</sub>),
- food conversion =  $F/W_t$   $W_o$ ,
- specific growth rate  $k = \ln (W_t/W_o)/T$ ,

with: W<sub>o</sub> = biomass (μg DW) of 150 instar I nauplii (from Vanhaecke and Sorgeloos, 1980a; Vanhaecke, 1983),

 $F = \mu g DW$  of food provided during the experimental period,

T = duration of the experiment (in days).

In order to be able to calculate the food conversion on a dry weight basis, and since no literature data could be found, a method was developed to determine the dry weight of Dunalliella cells, i.e.  $86.35 \,\mu\text{g}/10^6$  cells (Vanhaecke, 1983). For each experiment the San Francisco Bay strain (batch nr 288-2596) was used as an inner standard at 25°C for intercalibration purposes and for the quantitative comparison of the production data. For all experiments, results were statistically analysed by means of a one way analysis of variance, Model I (Sokal and Rohlf, 1969), followed by Duncan's test. Survival data were normalised through an arcsin  $\sqrt{\frac{9}{2}}$  transformation prior to statistical treatment.

### RESULTS

The statistical data on the hatching results (Table 3) reveal that the incubation temperature significantly affects cyst hatchability in all Artemia strains studied. The hatching percentage is always maximal at 25 up to 30°C. For most strains no significant difference can be noted within this range. Several strains however perform better at 25 than at 30°C. Whereas for most strains the drop in hatching percentage at 25 vs 30°C is moderate, a significant decrease is observed for the cysts from Larnaca. The strain from Tuticorin yields even the highest hatchability

TABLE 3

The effect of the incubation temperature on the hatching percentage of Artemia cysts from different geographical origin

Geographical strain (year of harvest, or batch)	25° C	30° C	34° C	37° C
San Francisco Bay	71.58 (*)	71.3a	67.7b	41.5°
Macau (871172)	84.5a	77.9ab	72.2b	43.5 <sup>c</sup>
Macau (971051)	81.0a	76.6a	70.8 <sup>b</sup>	48.3c
Barotac Nuevo	77.8a	69.3b	67.4 <sup>b</sup>	40.5 <sup>c</sup>
Great Salt Lake	44.2ab	48.0a	41.7b	15.0c
Great Salt Lake	70.5a	63.9b	37.9 <sup>c</sup>	5.5d
Chaplin Lake	19.0a	13.9b	10.9 <sup>b</sup>	3.7c
Manaure	25.6a	25.3a	25.3a	5.3b
Bahia Salinas	74.5a	70.3a	63.7 <sup>b</sup>	$20.4^{\circ}$
Bahia de Ceuta	85.6a	76.9b	73.0 <sup>b</sup>	50.4 <sup>e</sup>
Buenos Aires	62.7a	61.8a	41.0 <sup>b</sup>	$0.0^{c}$
Larnaca	87.0a	38.5 <sup>b</sup>	0.0c	0.0c
Barbanera	23.0a	18.5 <sup>b</sup>	0.7c	0.0c
Shark Bay (114)	88.4 <sup>a</sup>	86.3a	75.0 <sup>b</sup>	2.7 <sup>c</sup>
Shark Bay (1980)	85.8ª	85.0a	32.3b	$0.0^{c}$
Tientsin	73.9 <sup>a</sup>	75.1 <sup>a</sup>	47.4 <sup>b</sup>	$0.0^{c}$
Lavalduc	81.4 <sup>a</sup>	80.0a	$20.4^{\rm b}$	$0.0^{\circ}$
Margherita di Savoia	68.0a	67.6a	27.8 <sup>b</sup>	0.0c
Eilat	73.8ª	62.7b	13.8c	0.0d
Tuticorin	58.6b	73.8a	28.2c	0.0d

<sup>(\*)</sup> For each strain the means with the same superscript (a, b, c, d) are not significantly different at the P < 0.05 level.

at 30°C. With temperature increasing to 34°C hatching percentage of all strains studied further drops significantly, exception made for the Manaure strain. The hatchability drop continues with temperature increasing up to 37°C. However, at the highest temperatures tested the relative change in hatchability clearly differs from one strain to another.

Whereas Larnaca and Barbanera cysts practically do not hatch anymore at 34°C, several strains (*i.e.* San Francisco Bay, Macau, Barotac Nuevo, Great Salt Lake, Chaplin Lake, Manaure, Bahia Salinas and Bahia de Cueta) still yield a substantial hatching output at 37°C.

The results for survival at 34°C for the different Artemia strains are summarized in Table 4. The sequence of the strains corresponds with increasing mean survivals at the two salinities tested. It is clear that substantial differences in survival exist at both salinities. No larvae of the strains from Larnaca, Barbanera, Buenos Aires and Shark Bay survived at 34°C neither at 35 nor 120 ppt. Other strains such as Chilea, Port Araya, Bahia de Cueta, Great Salt Lake, Galera Zamba and Manaure

TABLE 4

Mean survival (in %) of different geographical strains of brine shrimp at 34° C and two salinities

Geographical strain (year of harvest)	35 ppt	120 ppt
Larnaca	0	0
Barbanera	0	0
Buenos Aires	0	0
Shark Bay (1979)	0	0
Salin de Giraud	0	3
Margherita di Savoia	9	16
Chaplin Lake	0	31
Virrila	22	19
Izmir	20	25
Pacoa	22	31
Lavaldue	23	30
Tientsin	28	38
Eilat	34	48
Tuticorin	33	56
Lake Tahoka	50	39
Bahia Salinas	43	46
Bonaire	45	50
Chilca	52	49
Port Araya	55	56
Bahia de Ceuta	68	46
Great Salt Lake (1979)	53	62
Galera Zamba	62	57
Great Salt Lake (1977)	60	62
Manaure	69	62

withstood quite well the elevated test temperature with a survival of about 50 % or more. For some strains, in particular Chaplin Lake, Eilat, Tuticorin and Bahia de Cueta temperature tolerance is clearly conditioned by salinity.

The temperature tolerance in *Artemia* strains which originate from the same parential material (San Francisco Bay) but produced in biotopes with higher average water temperatures (Vos *et al.*, 1984) seems to be significantly higher (at least at 120 ppt) as compared to the parental material (see Table 5). The results in Tables 4 and 5 furthermore reveal no significant difference in temperature tolerance between cyst batches from the same strain.

TABLE 5

Mean larval survival (in %) at 34° C and two salinities for Artemia strains and batches originating from the same parental material

Geographical strain (batch number)	35 ppt	120 ppt
San Francisco Bay (288-2596)	12al	17a
Mundra	13a	27b
Macau (871172)	18a	30р
Macau (973060)	13a	28b
Barotac Nuevo	33p	26 <sup>b</sup>
Thailand	38p	26 <sup>b</sup>

(1) Results with the same superscript are not significantly different at the P < 0.05 level.

The biomass results (Table 6) have been expressed as a percentage of the biomass produced by the reference San Francisco Bay strain (batch nr 288-2596) at 25°C. The average total biomass produced after nine days by this strain over the different experiments was  $15.861 \,\mu g$  (s =  $1104 \,\mu g$ ). This represents an individual dry weight production of 111 µg, corresponding with an average survival of 95 %. No statistical analysis data were provided for the biomass production data since the results are similar to those obtained for specific growth rates. From the statistical analysis it is clear that temperature significantly affects production performance for all strains studied. In all cases a temperature below 20°C significantly reduces growth rate. This is also valid for temperatures above 30°C, exception made however for the Manaure strain. Furthermore the impact of temperature seems to vary from one strain to another. Not only the optimal temperature for production but also the optimal temperature range differs between strains, e.g. at 30°C production levels of Barbanera and Larnaca strains have practically dropped to zero whereas Great Salt Lake and Galera Zamba strains yield their maximal biomass production at this temperature.

TABLE 6 The effect of temperature on different production parameters for various geographical strains of Artemia

Geographical strain	Temperature (°C)						
	20	22.5	25	27.5	- 30	32.5	
San Francisco Bay		-					
Survival (%)	97	97	94	91	66	(3)	
Biomass production (%) <sup>1</sup>	75	101	100	94	88		
Specific growth rate (1)	0.431d(2)	$0.464^{a}$	0.463ab	$0.456^{\rm b}$	0.448c	_	
Food conversion (1)	3.89b	$3.35^{a}$	$3.64^{\rm b}$	$3.87^{b}$	4.15 <sup>c</sup>		
Macau						•	
Survical (%)	97	94	98	96	60		
Biomass production (%)	77	100	99	94	87		
Specific growth rate	$0.428^{\circ}$	0.456a	$0.455^{a}$	$0.450^{a}$	0.441b	—	
Food conversion	$3.77^{\rm b}$	$3.39^{a}$	3.65 <sup>ab</sup>	$3.86^{\rm b}$	4.16 <sup>c</sup>	<u> </u>	
Great Salt Lake							
Survival (%)	77	85	89	89	87	88	
Biomass production (%)	69	104	122	128	135	78	
Specific growth rate	$0.392^{\mathrm{e}}$	$0.437^{c}$	$0.454^{b}$	$0.460^{ab}$	$0.465^{a}$	0.406d	
Food conversion	$3.79^{c}$	2.90 <sup>b</sup>	2.65 <sup>ab</sup>	2.52a	$2.40^{a}$	4.14 <sup>d</sup>	
Chaplin Lake			:				
Survival (%)	72	75	77	65	50		
Biomass production (%)	78	102	108	106	90	<u> </u>	
Specific growth rate	$0.422^{c}$	0.452ab	$0.459^{a}$	$0.456^{a}$	0.437bc	_	
Food conversion	3.42a	$3.00^{a}$	3.03ª	3.11a	3.72b		
Manaure							
Survival (%)	94	94	91	93	86	77	
Biomass production (%)	52	102	112	110	104	105	
Specific growth rate	$0.379^{c}$	$0.451^{b}$	$0.462^{a}$	$0.460^{ab}$	$0.454^{ab}$	0.455al	
Food conversion	5.82b	3.43a	3.36 <sup>a</sup>	3.40a	3.62a	3.56a	
Galera Zamba							
Survival (%)	. 94	96	98	96	96	92	
Biomass production (%)	74	107	110	106	113	80	
Specific growth rate	$0.390^{\rm b}$	$0.431^{a}$	0.433a	$0.427^{a}$	$0.437^{a}$	$0.399^{b}$	
Food conversion	4.05 <sup>b</sup>	3.26a	3.42a	$3.55^{a}$	$3.31^{a}$	$4.68^{\circ}$	

<sup>(1)</sup> For more details cf. Materials and Methods. (2) Means with the same superscript are not significantly different at the P < 0.05level.

<sup>(3)</sup> Not analyzed.

TABLE 6 (continued)

~	Temperature (°C)						
Geographical strain	20	22.5	25	27.5	30	32.5	
Buenos Aires				-			
Survical (%)	96	90	94	91	80		
Biomass production (%)	93	113	115	98	96		
Specific growth rate Food Conversion	$0.462^{\rm b} \ 2.78^{\rm a}$	$0.483^{a}$ $2.66^{a}$	0.485a 2.81a	$0.468^{ m b} \ 3.29^{ m b}$	$0.465^{\rm b} \ 3.38^{\rm b}$	_	
Larnaca							
Survival (%)	98	94	89	62	3	<del>-</del>	
Biomass production (%)	61	94	95	83	0		
Specific growth rate Food conversion	$0.384^{\rm b} \\ 4.29^{\rm b}$	0.430a 3.23a	0.432a 3.43a	0.417 <sup>c</sup> 3.93 <sup>b</sup>	0q		
rood conversion	4.29	5.25	3.43	5.95~	_	-	
Barbanera							
Survival (%)	89	89	94	93	28	<u> </u>	
Biomass production (%)	70	103	101	97	8		
Specific growth rate	$0.379^{b}$	0.421a	$0.419^{a}$	$0.416^{a}$	0.163c		
Food conversion	$4.62^{\rm b}$	3.66a	4.02ab	4.13a	50.22	<del></del>	
Shark Bay							
Survival (%)	97	96	97	92	68	_	
Biomass production (%)	48	67	84	75	66		
Specific growth rate	$0.332^{c}$	$0.369^{b}$	0.393a	0.381ab	0.367b	_	
Food conversion	6.49 <sup>c</sup>	5.35ab	4.57 <sup>a</sup>	5.08a	5.81bc	-	
Tuticorin							
Survival (%)	93	92	93	92	96	90	
Biomass production (%)	57	89	104	94	89	78	
Specific growth rate	0.331d	$0.380^{\rm b}$	0.396a	$0.386^{\rm b}$	$0.379^{b}$	$0.365^{\circ}$	
Food conversion	5.06e	$3.74^{\mathrm{b}}$	$3.45^{a}$	3.81bc	4.06 <sup>c</sup>	4.62d	
Tientsin			,				
Survival (%)	95	94	91	93	84	54	
Biomass production (%)	41	61	80	92	85	16	
Specific growth rate	$0.299^{c}$	$0.343^{\rm b}$	0.371a	$0.387^{a}$	$0.378^{a}$	0.208d	
Food conversion	$7.22^{\mathrm{e}}$	$5.42^{b}$	4.46ab	3.84 <sup>a</sup>	4.22ab	22.04 <sup>d</sup>	
Margherita di Savoia							
Survival %	96	93	92	95	91	_	
Biomass production %	59	68	90	94	84	_	
Specific growth rate	0.318d	0.333c	0.363ab	0.368a	$0.356^{\rm b}$		
Food conversion	5.48 <sup>c</sup>	5.56 <sup>c</sup>	4.47ab	$4.26^{a}$	4.77b		

### DISCUSSION

It appears from the data obtained in this study, that the temperature has an important impact on the hatchability of *Artemia* cysts and their biomass production, two characteristics which are important parameters in the determination of the quantitative value of an *Artemia* product. Moreover the effect of temperature on hatching and growth performance varies as a function of the geographical origin of the brine shrimp cysts.

Apparently the observations made for one or two specific strains by Von Hentig (1971), Jones (1972) and Sorgeloos (1975) that hatching is constant within the temperature range of 15-30, 20-30, respectively 20-28°C may not be generalized for all Artemia strains. Indeed, several strains yield significantly better hatching results at 25 than at 30°C whereas the Tuticorin cysts hatch best at 30°C. The latter observation confirms the results obtained by ROYAN (1975) with this strain. Although in an exceptional case improved hatching has been reported at temperatures below 25°C (e.g. Dana, 1981 for the Mono Lake strain) such low temperatures are not of practical use in aquaculture (too slow hatching rates, Sorgeloos, 1980) and were therefor not taken into account for the present study.

The claim of Dutrieu (1960), Teramoto and Kinoshita (1961) and Walne (1967) that a temperature of 28°C is optimal for the culture and growth of *Artemia* may not be generalized in analogy with the earlier remarks made for the hatching parameter, *i.e.* the optimal temperature for production indeed depends on the *Artemia* strain used,

A comparison of the hatching performances for the different strains at different temperatures with their genetic classification into sibling species (see Table 1) seems to reveal some interesting correlations between temperature tolerance and genetic differentiation (Fig. 1): the bisexual Artemia tunisiana strains do not seem to be tolerant to high temperatures as far as their hatchability is concerned. Hatchability is already neglegable at 34°C, Although to a lesser extent, this is also the case for the Artemia parthenogenetica and Artemia persimilis strains. For these strains hatching is completely inhibited at 37°C, exception made for the Shark Bay cysts from 1977. According to ABREU-GROBOIS and BEARDMORE (1980) this sample contains a small percentage of bisexual Artemia franciscana forms, which might explain the hatching of some cysts at 37°C. Artemia franciscana strains indeed seem to be rather tolerant to high temperatures. Even at 37°C still substantial hatching percentages could be recorded for all strains tested from this sibling species. However, a considerable variation in temperature tolerance with respect to the hatching also seems to exist among Artemia strains from the same sibling species (ABREU-Grobois and Beardmore, 1982). Based on the relative constancy of the impact of temperature on the hatching performances in cysts harvested from subtropical (endemic strains) and tropical localities (inoculated with San Francisco Bay franciscana material, Vos et al., 1984) genetic adaptation to the specific niche does not seem to be of prime importance in determining temperature tolerance at hatching. Anyhow, the criterium temperature tolerance of cyst hatching appears to be a helpful tool to carry out a quick screening and preliminary classification of Artemia strains.

Difference in naupliar survival at a high temperature seems to be related to the genetic classification of *Artemia* as well. Furthermore, the sequence of the sibling species in function of temperature tolerance seems to be analogous with

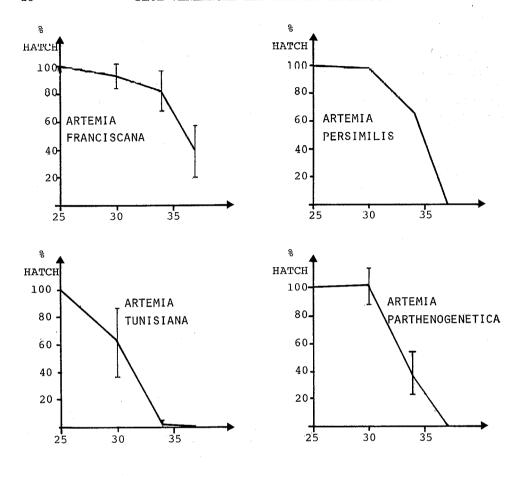


Fig. 2. — Mean impact of temperature on the hatchability of cysts from different sibling species of *Artemia* (vertical bar = standard deviation).

TEMPERATURE (°C)

the findings for cyst hatching. Artemia tunisiana and Artemia persimilis strains show the lowest tolerance towards high temperatures. Artemia franciscana strains tolerate best high temperatures (mean survival of all Artemia strains tested is  $42\pm17$ % at 35 ppt and  $40\pm18$ % at 120 ppt), whereas Artemia parthenogenetica strains take an intermediate position (mean survival of all strains tested is  $18\pm14$ % at 35 ppt and  $26\pm20$ % at 120 ppt). However, substantial variance occurs among different strains belonging to the latter two sibling species. The variance between Artemia parthenogenetica strains may be attributed to different degrees of ploidy, i.e. diploid strains (Salins de Giraud and Shark Bay) are clearly less resistant than the other parthenogenetic strains which are mixed diploid and tetraploid, triploid or pentaploid. According to the observations of Artom (1931), Charman (1968) and Metalli and Ballardin (1972) polyploidy seems to provoke a higher genetic variability, as such better buffering the population against extreme conditions.

Differences among strains may furthermore be due to genetic differentiation and/or genetic adaption within the same sibling species, e.g. increased tolerance in the harvested products from tropical waters (in Macau, Barotac Nuevo, Thailand and Mundra) that were inoculated with subtropical San Francisco Bay Artemia franciscana indicates that a heritable adaptation has occurred.

Differences among Arlemia strains in biomass production at different temperatures match well with their survival tolerance pattern for temperature (Vanhaecke et al., 1984). The production figures obtained at 25°C correspond very well with the growth results obtained for different strains in the standard culture test of Vanhaecke and Sorgeloos (1980b).

In conclusion, results reported in this study provide useful information as well as a practical guideline for the utilisation of *Artemia* strains in aquaculture, with regard to e.g.

- the selection of the optimal hatching temperature for each strain;
- the selection of the optimal culturing temperature for each strain;
- the selection of specific strains for inoculation purposes under different climatological conditions;
- the selection of specific strains for production purposes at specific temperature conditions.

Exception made for the Tuticorin strain, maximal hatching is always attained at 25°C. When a faster hatching rate is of interest, incubation temperatures may be increased up to 30°C for most strains.

For each strain studied, the most appropriate temperature range for production purposes is schematically shown in Fig. 2. In this temperature range maximal growth rate and minimal food conversion was obtained (no significant differences among temperatures). From this graph it is clear that in moderate temperature conditions intensive cultures of most Artemia strains do not have to be heated beyond 22.5-25°C, which can result in important energy savings. On the other hand, since most sites suitable for Artemia inoculation purposes are located in the tropical and subtropical belt, where water temperatures of 30°C or more are very common (De los Santos et al., 1980; Vos et al., 1984), the success of inoculation trials is greatly dependent on the selection of Artemia strains with high temperature resistance and good production performance at high temperatures. In this regard the Artemia strains from Galera Zamba, Great Salt Lake and Manaure should be selected. On the basis of their temperature resistance the Artemia franciscana strains from Bahia de Ceuta, Bonaire, Port Araya, Chilca, Bahia Salinas and Tuticorin may also be taken into consideration.

Taken into account the quantitative production data of the *Artemia* strains at different temperatures, the following strain selection guideline can be provided for culturing purposes at different temperatures:

20°C : Buenos Aires, Chaplin Lake, Macau, San Francisco Bay,

22.5°C: Buenos Aires, Galera Zamba, Great Salt Lake, Chaplin Lake,

25°C : Great Salt Lake, Buenos Aires, Manaure, Galera Zamba, Chaplin Lake,

27.5°C : Great Salt Lake, Galera Zamba, Manaure, Chaplin Lake,

30°C : Great Salt Lake, Galera Zamba, Manaure,

 $32.5{\rm ^{o}C}$ : Manaure, Galera Zamba, Great Salt Lake, Tuticorin.

# TEMPERATURE (°C)

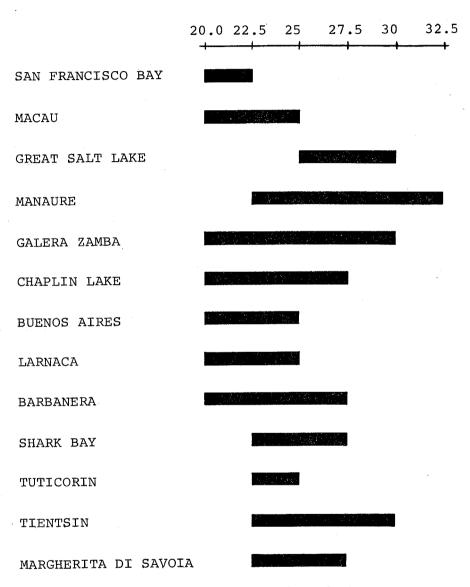


Fig. 2. — The optimal temperature range for production purposes of different *Artemia* strains.

### ACKNOWLEDGEMENTS

This study was supported by the Belgian Administration for Development Cooperation and by the Belgian National Science Foundation (grant FKFO 2001282) of which P. Sorgeloos is a senior scientist.

### REFERENCES

- ABREU-GROBOIS, F. (1987) A review of the genetics of Artemia. In: Artemia research and its applications. (P. Sorgeloos, D. A. Bengtson, W. Decleir, E. Jaspers, eds.), Universa Press, Wetteren, Vol. I, 61-100.
- ABREU-GROBOIS, F. A. and J. A. BEARDMORE (1980) International Study on Artemia. II. Genetic characterization of Artemia populations An electrophoretic approach. In: The brine shrimp Artemia. (G. Persoone, P. Sorgeloos, O. Roels, E. Jaspers, eds.), Universa Press, Wetteren, Vol. I, 133-146.
- ABREU-GROBOIS, F. A. and J. A. BEARDMORE (1982) Genetic differentiation and specification in the brine shrimp *Artemia*. In: *Mechanism of Speciation*. *Progress in Clinical and Biological Research*. (C. Barigozzi, ed.), Alan R. Liss, Inc., New York, 245-376.
- Artom, C. (1931) L'origine e l'evoluzione della partenogenesi attraverso i differenti biotipi di una specie collettiva (*Artemia salina* L.) con speciale referimento al biotipo partenogenetico di Sète. *Memorie R. Accad. Ital.*, 2 (1), 1-57.
- Barigozzi, C. (1974) *Artemia*: A survey of its significance in genetic problems. In: *Evolutionary Biology* (T. Dobzhansky, M. K. Hecht, W. C. Steere, eds.), Plenum Press, New York, Vol. VII, 221-252.
- Barigozzi, C. (1980) Genus *Artemia*: Problems of systematics. In: *The brine shrimp* Artemia. (G. Persoone, P. Sorgeloos, O. Roels, E. Jaspers, eds.), Universa Press, Wetteren, Vol. I, 147-153.
- Bargozzi, C. and M. Tosi (1959) New data on tetraploidy of amphigonic Artemia salina L. and on triploids resulting from crosses between tetraploids and diploids. Atti Ass. genet. Ital., Ric. Sci. Suppl., 29, 129-132.
- Bowen, S. T., J. P. Durkin, G. Sterling and L. S. Clark (1978) *Artemia* hemoglobins: genetic variation in parthenogenetic and zygogenetic populations. *Biol. Bull.*, **155**, 273-287.
- Bowen, S. T., M. L. Davis, S. R. Fenster and G. A. Lindwall (1980) Sibling species of *Artemia*. In: *The brine shrimp* Artemia. (G. Persoone, P. Sorgeloos, O. Roels, E. Jaspers, eds.), Universa Press, Wetteren, Vol. I, 155-167.
- Chapman, J. (1968) The relative adaptive values of parthenogenesis and zygogenesis. Thesis, San Francisco State University, U.S.A., 76 pp.
- CLARK, L. S. and S. T. BOWEN (1976) The genetics of Artemia salina. VII. Reproductive isolation. J. Hered., 67 (6), 285-388.
- CLAUS, C., F. BENIJTS and P. SORGELOOS (1977) Comparative study of different geographical strains of the brine shrimp Artemia salina. In: Fundamental and applied research on the brine shrimp, Artemia salina (L.) in Belgium. (E. JASPERS and G. PERSOONE, eds.), EMS Special publication No. 2, Inst. Mar. Sc. Res., Bredene, 91-105.
- Dana, G. L. (1981) Comparative population ecology of the brine shrimp *Artemia*. *Thesis*, San Francisco State University, U.S.A., 125 pp.
- De Los Santos, C., P. Sorgeloos, E. Lavina and A. Bernardino (1980) Successful inoculation of *Artemia* and production of cysts in man-made salterns in the Philippines. In: *The brine shrimp* Artemia. (G. Persoone, P. Sorgeloos, O. Roels, E. Jaspers, eds.), Universa Press, Wetteren, Vol. III, 159-163.
- Durtieu, J. (1960) Observations biochimiques et physiologiques sur le développement d'Artenia salina Leach. Arch. Zool. exp. gén., 99, 1-134.

- Hentschel, E. (1968) Die postembryonalen Entwicklungsstadien von Artemia salina Leach bei verschiedenen Temperaturen (Anostraca, Crustacea). Zool. Anz., 180, 372-384.
- HERNANDORENA, A. (1976) Effects of temperature on the nutritional requirements of Artemia salina. Biol. Bull., 151 (2), 314-321.
- ТWASAKI, T. (1976) Reproductive patterns of Artemia with special regard to food and temperature. Zool. Mag., Tokyo, 85 (3), 229-236.
- Jones, A. J. (1972) An inexpensive apparatus for the large scale hatching of Artemia salina L. J. Cons. int. Expl. Mer., 34 (3), 351-356.
- KINNE, O. (1970) Chapter 3. Temperature. 3.3 Animals. 3.31 Invertebrates. In: *Marine Ecology*. Wiley Interscience, New York, Vol. I, 407-514.
- Leger, Ph., D. A. Bengtson, K. L. Simpson and P. Sorgeloos (1986) The use and nutritional value of *Artemia* as a food source. *Oceanogr. Mar. Biol. Ann. Rev.*, 24, 521-623.
- METALLI, P. and E. Ballardin (1972) Radiobiology of Artemia: radiation effects and ploidy. Current Topics in Radiation Research Quarterly, 7 (2), 181-240.
- Persoone, G. and P. Sorgeloos (1980) General aspects of the ecology and biogeography of *Artemia*. In: *The brine shrimp* Artemia. (G. Persoone, P. Sorgeloos, O. Roels, E. Jaspers, eds.), Universa Press, Wetteren, Vol. III, 3-24.
- REEVE, M. R. (1963) Growth efficiency in *Artemia* under laboratory conditions. *Biol. Bull.*, **125** (1), 133-145.
- ROYAN, J. P. (1975) Influence of salinity and temperature on the hatching of the brine shrimp, *Artemia salina*. *Mahasagar*, 8 (3, 4), 183-185.
- Sokal, R. R. and F. J. Rohlf (1969) *Biometry*, W. A. Freeman Co., San Francisco, 776 pp.
- Sorgelos, P. (1975) De invloed van abiotische en biotische faktoren op de levenscyclus van het pekelkreeftje, *Artemia salina* L. *Thesis*, State University of Ghent, 235 pp.
- Sorgeloos, P. (1980) The use of the brine shrimp Artemia in aquaculture. In: The brine shrimp Artemia. (G. Persoone, P. Sorgeloos, O. Roels, E. Jaspers, eds.), Universa Press, Wetteren, Vol. III, 25-46.
- Sorgelos, P., M. Baeza-Mesa, F. Benijts and G. Persoone (1976) Current research on the culturing of the brine shrimp *Artemia salina* L. at the State University of Ghent, Belgium. In: *Proc.* 10th European Symposium on Marine Biology (G. Persoone, E. Jaspers, eds.), Universa Press, Wetteren, Vol. I, 473-495.
- Sorgeloos, P., G. Persoone, M. Baeza-Mesa, E. Bossuyt and E. Bruggeman (1978)

   The use of *Artemia* cysts in aquaculture: the concept of «hatching efficiency» and description of a new method for cyst processing. In: *Proc. 9th Ann. Meeting WMS*. (J. W. Avault, jr., ed.), Louisiana State University, Baton Rouge, 715-721.
- TERAMOTO, K. and S. KINOSHITA (1961) Some informations on the culture of Artemia (in Japanese, English abstract). Bull. Jap. Soc. scient. Fish., 27 (8), 801-804.
- Vanhaecke, P. (1983) Vergelijkende studie van diverse geografische rassen van het pekelkreeftje, *Artemia*, ter verbetering van zijn gebruik in de aquakultuur. *Thesis*, State University of Ghent, Belgium, 420 pp.
- VANHAECKE, P., S. E. SIDDALL and P. SORGELOOS (1984) International Study on *Artemia*. XXXII. Combined effects of temperature and salinity on the survival of *Artemia* of various geographical origin. *J. exp. mar. Biol. Ecol.*, 80 (3), 259-275.
- Vanhaecke, P. and P. Sorgeloos (1980a) International Study on *Artemia*. IV. The biometrics of *Artemia* strains from different geographical origin. In: *The brine shrimp* Artemia (G. Persoone, P. Sorgeloos, O. Roels, E. Jaspers, eds.), Universa Press, Wetteren, 393-405.
- Vanhaecke, P. and P. Sorgeloos (1980b) International Study on *Artemia*. XIV. Growth and survival of *Artemia* larvae of different geographical origin in a standard culture test. *Mar. Ecol. Progress Ser.*, 3 (4), 303-307.

- Vanhaecke, P., W. Tackaert and P. Sorgeloos (1987) The biogeography of Artemia: an updated review. In: Artemia research and its applications (P. Sorgeloos, D. A. Bengtson, W. Decleir, E. Jaspers, eds.), Universa Press, Wetteren, Vol. I, 129-155.
- Von Hentig, R. (1971) Einfluss von Salzgehalt und Temperatur auf Entwicklung, Wachstum, Fortpflanzung und Energiebilanz von Artemia salina. Mar. Biol., 9, 145-182.
- Vos, J., Ph. Leger, P. Vanhaecke and P. Sorgeloos (1984) Quality evaluation of brine shrimp *Artemia* cysts produced in Asian salt ponds. *Hydrobiologia*, **108** (1), 17-23.