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FACTORS INFLUENCING ARTEMIA POPULATIONS IN ANTILLEAN SALINES

by

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In order to get an insight into the ecology of *Artemia salina* on Curaçao and Bonaire a dozen of natural salt flats and salt pans were surveyed, more or less regularly, in the period of 1960 to 1964. Some of the results have already been summarized (KRISTENSEN, 1971), but details were not given.

One of the most fascinating aspects is the ability of *Artemia salina* to adjust to wide ranges of environmental conditions. As *Artemia* is represented by different forms (or "races") in water of varying salinities it seemed worth while to study these forms in relation to their environment.

In addition to a field study on *Artemia* some experimental data have been collected in order to corroborate the findings derived from field observations. The two principal aspects in the investigation seek to answer the following questions:

Are the morphological characteristics of the *Artemia salina* "forms" inherited, or are they brought about by the environmental factors?

Which factors have a role in other variations of *Artemia salina*?

What causes fluctuation in *Artemia* populations?

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HABITAT

In the Antilles, *Artemia* is only found in supersaline water from 45‰ upwards. The lower limit of salinity is much higher than the lowest limit recorded for *Artemia* in Europe or in the U.S.A., where salinities of about 35‰ are mentioned. Brines are formed in land-locked bays or lagoons, especially in those of shallow depth. From direct observations we know that, in inland bays, the evaporation of seawater is 4–7 mm a day depending on sun radiation, wind strength, relative humidity, temperature, etc. In shallow lagoons extensive areas may turn into salt flats during the dry season; these areas are the so called "salinjas." In former times some of them were excavated and enclosed by low dikes. These salt pans were sources for salt production. Most of them are no longer in use. The salinity in the salinjas and pans may vary considerably over a period of weeks or even days because of evaporation, flooding by seawater, or by run-off of rainwater from neighbouring hills.

In the dry season the temperature in the saltpans is rather constant: in winter it varies from 26° (at night) to 30°C (in the afternoon) and, in summer, the respective values are 28 to 32°C. After rains however, an unusual rise may occur; temperatures up to 61°C have been measured. This takes place when a layer of fresh water covers a concentrated brine; because of the differences in specific gravity the brine cannot rise to the surface and, therefore, will not be cooled. Very high temperatures occur where the bottom is black and a layer of brine of only a few cm is covered by fresh-water.

A survey of the fauna is given below. Concerning the animals some data on the salinity range of the species were collected. WAGENAAR HUMMELINCK and ROOTH sampled algae in the same area, prior to our study, and KOSTER (1963) published a paper on the algae found by them and by us. Besides algae, bacteria are present, especially the dark red species which conspicuously redden the water and salt of highly concentrated brines. Dinoflagellates, both coloured and colourless, are abundant in most brines. However, we found no *Dunaliella viridis* or *D. salina*, although this species has been recorded from near-by Puerto Rico (WALKER, 1962) and appears to be world-wide in its distribution.

FAUNA

		Salinity in ‰
Aves	*Phoenicopterus ruber*, feeding in water of	36–250
Pisces	*Rivulus marmoratus*, found in water of	0– 70
	Cyprinodon dearborni	0–130
	Poecilia sphenops	0–130
Insecta	Corixidae	0– 95
	aquatic Coleoptera	0– 95
	Ephydra gracilis larvae	36–300
	Odonata larvae	0– 64
Gastropoda	*Batillaria minima*	34– 60
	Cerithidea costata	34– 60
	Cerithium variabile	18– 47
	Neritina virginia	18– 42
	trochophora of Gastropoda	32– 90
Rotatoria		0–162
Crustacea	Harpacticida	32–113
	Cyclopidae	0– 82
	Ostracoda	0– 90
	Artemia salina	45–360
Protozoa	*Vorticella* spec.	68– 92
	Volvocales	72–159

Besides *Phoenicopterus* other bird species were observed more or less regularly, viz *Pelecanus occidentalus, Himantopus himantopus*, and various species belonging to Charadriidae, Scolopacidae, Ardeidae and Laridae.

Already in 1930 special attention has been paid to the organisms of the marine salines of the Netherlands Antilles. As a result of HUMMELINCK's collecting work RAMMNER (1936) published a paper on *Artemia salina*, whilst other authors gave descriptions of Cyanophyceae (FRÉMY, 1941), Polychaeta (AUGENER, 1936), Ostracoda (KLIE, 1939), Amphipoda (STEPHENSEN, 1933), Oribatidae (WILLMANN, 1936) and Corixidae (NIESER, 1969), occasionally occurring together with brine shrimps.

INFLUENCE OF THE ENVIRONMENT

1. THE "FORMS" *arietina* AND *milhauseni*

About a century ago, many *Artemia* species were identified and described; subsequently they were all found to be one and the same species, with possibly one exception (vide KUENEN, 1939). As some morphological and physiological differences do exist between European and North American *Artemia* and hybridization has not been accomplished, it is quite possible that more than one species do exist. As we made no attempts at crossing Antillean with other *Artemia* we have no information on this question.

When observing *Artemia* from various salinities the morphological differences are quite obvious. Moreover, these variations are clearly exhibited in populations from various localities throughout the world. The Antillean ones have been thoroughly studied by RAMMNER (1936), who had at his disposal 23 samples from 11 localities collected by WAGENAAR HUMMELINCK. RAMMNER found two forms known as forma *arietina* and forma *milhauseni*. These forms differ in the length of the abdomen, the shape of the abdominal plates, and the number of the setae (vide Fig. 9). Forma *arietina* was found in concentrated brines of 200‰ as well as at lower concentrations, and f. *milhauseni* was found only in high concentrations, together with some specimens of the other form. In the light of our further observations we are now able to present more detailed and supplementary data on the *Artemia* – salinity relationship. On Curaçao and on Bonaire f. *arietina* is predominant in brine of 45–95‰ but many exceptions are found: it may predominate in stronger

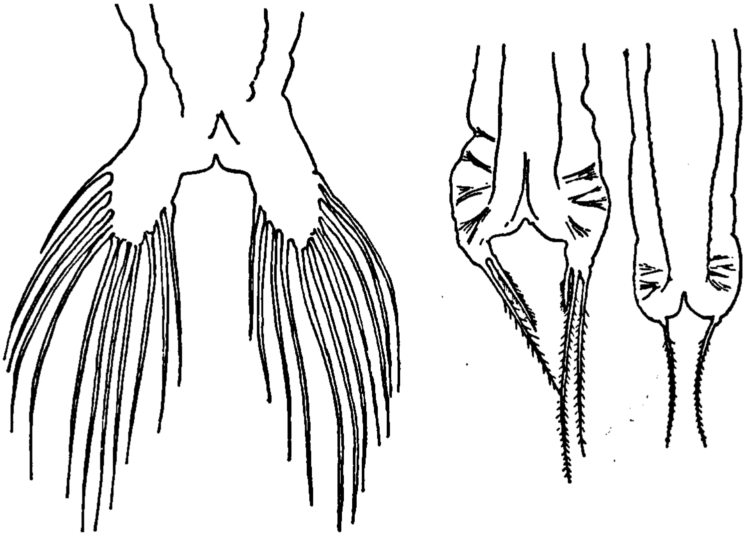

Fig. 9. Postabdomen of *Artemia salina* from Bonaire (scale 1 : 65). – To the left: forma *arietina*; to the right, both figures: forma *milhauseni*. [After RAMMNER, 1936, figures 1 and 3].

brines also. Forma *milhauseni* is found in concentrated brine and only rarely in brines under 120‰. Very often, a population consists of a mixture of both forms together with a number of intermediate forms so that a complete gradient of characteristics may be present. This is illustrated by a random sample from Curaçao (Cas Abau, 9.VII.1963), where the salinity was 157‰. This sample of adult individuals was divided into groups on the basis of the increasing number of setae (Table 1).

TABLE 1

Sample of 300 adult *Artemia* from Cas Abau salinja, grouped according to the number of setae on each postabdominal plate.

	f. *milhauseni*				f. *arietina*		
number of setae	1–2	3–4	5–6	7–8	9–10	11–12	13–14
% represented	16%	17%	20%	11%	13%	11%	12%

From these figures it is clear that the separation into two forms is an arbitrary one. Furthermore, mating between the two forms is not less frequent than mating of individuals of the same form. Definite proof of the validity of the characteristics might be shown by raising f. *arietina* at various salinities. A "pure" *arietina*-population was obtained from a Curaçao salt pan (San Nicolas) with a salinity of 59‰. When cultivated in the laboratory at 59‰ most of the offspring, but not all, belonged to the parental form *arietina*; however, when the salinity level was raised to 211‰ (gradually, over a two week period, by natural evaporation) the resulting nauplii all developed into f. *milhauseni* (Table 2).

TABLE 2

Setae counts of offspring from forma *arietina* (originating from 59‰ sal.) in water of relatively low and high salinity. (Both samples 300 specimens)

	number of setae in the offspring			
	1–2 setae (f. *milhauseni*)	3–4 setae	5–6 setae	7–14 setae (f. *arietina*)
at sal. 59‰	4%	3%	7%	86%
at sal. 211‰	100%	0%	0%	0%

While it is clear that the number of setae is influenced by the salinity the mechanism responsible for the characteristics of the "forms" is unknown. It is remarkable that the same experiment has been done repeatedly in Europe (ABONYI, 1915), with about the same result. On the other hand, it has been impossible to obtain the change of form characteristics in North American *Artemia* (KUENEN, 1939).

2. GROWTH AND BODY SIZE

Very remarkable is the fact that growth is always very uniform within each population: all individuals will reach the same size, regardless of the fact that the populations may be a mixture of *arietina* and *milhauseni* forms.

Nauplii and adult *Artemia* are particle feeders. They are constantly in motion – day and night – capturing their microplankton prey. Microscopic analysis of the content of the digestive tracts reveals the presence of coloured and uncoloured dinoflagellates, clumps of chlorococcales, unicellular algae and detritus. Undigested algae may be present in any part of the tract. Detritus may be in evidence, even though the plankton contains no detritus. Although a suspension feeder, *Artemia* is found browsing on submerged stones, clumps of blue-green algae, etc., and on detritus too.

Under conditions that favour rapid growth *Artemia* will mature in 8 days and will reach the maximum length of 11 mm in 10 days. When growth is stunted maturation takes 14–16 days and the final length of 5.5 mm is not reached before 20 or more days after hatching

Both from observations in nature and from experiments it is known that size is influenced by salinity (MARTIN & WILBUR, 1921). Growth rates are stated to be less at very low and at very high concentrations of the brine. In the Antilles, as a rule, the *Artemia* from the lowest concentrations (45‰ to about 190‰) have a length of 10–11 mm, and in higher concentration their maximum length is less, sometimes not more than 5.5 mm. In our cultures, growth rate and final size was maximal between 32‰ and 200‰ salinity. Below 32‰ and above 200‰ growth was inhibited, however, exceptions have been observed, in nature as well as in our experiments. Occasionally, even under controlled conditions an unexpected growth rate occurred in extreme salinities. On the other hand, growth can be stunted at any concentration of the brine. One factor is very important in this respect: the turbidity of the water. In natural waters no *Artemia* will be found when the water is very turbid, whether the turbidity is caused by algae, by organic matter or by inorganic salt. When small cages of nylon gauze with eggs, nauplii and adults were put into such water the population either died within a couple of days, or the growth rate was impaired when compared with growth in other water.

Turbid water used in the laboratory brought about the same deleterious effect – either mortality or greatly lessened growth rate. When the particles causing the turbidity are large their effect on the growth rate can be readily explained; the appendages of *Artemia* become loaded with particles. In clay suspension *Artemia* do not show normal growth, although the particles are extremely small and do not cling to the appendages. In some cases, the digestive tracts were found empty or nearly empty although food was available as in all experiments. Examples of particle feeders that starve when non-edible particulate matter adulterates their food are well documented, e.g. LOOSANOFF & TOMMERS, 1948, and KRISTENSEN, 1957, reported a similar phenomenon in oysters and cockles – no food was taken in, hence starvation. In some *Artemia*, however, the tract was packed with black matter. The origin and significance of these deposits are unknown.

3. OVIPARITY AND VIVIPARITY

We made the following observations. The ovary may contain up to 50 ripe eggs. About one day after copulation the eggs are released into the water (oviparous) or are passed to the egg sac (viviparous). In case of viviparity the number of eggs is always small, rarely surpassing 15. If the ovary contains more eggs than the egg sac can accommodate the surplus eggs are released as in oviparous females. In these instances, however, the number of immediately released eggs is always small, 1–20, and never as many as 50 as in oviparous females.

Females in a saline media of more than 85‰ are for the most part viviparous. In brines of concentrations less than that most, if not all females are oviparous. Only when brines of high density have been recently diluted still viviparity occurs.

Artemia from temperate areas differ from the Antilles *Artemia* in their reaction to salinity: *Artemia* from the north exhibit oviparity in high and viviparity in low salinities (ABONYI, 1915). In a cold climate, this might be advantageous to the species as most eggs are produced at the end of the summer when the salinity is highest and the temperatures have fallen. The majority of the eggs will not hatch, thus securing the survival of the species over the winter. In a dry tropical climate, however, the reverse seems to be advantageous; in this area survival depends on ability to withstand drought. When the brine becomes more concentrated the eggs no longer can hatch, nevertheless eggs still are being shed in small quantities, as has been mentioned. Such eggs will not hatch before the water has been diluted by rainwater to a concentration of less than 85‰ (Table 3). In the Antilles, hatching occurs over a wide salinity range, from complete freshwater to concentrations of 60–85‰.

TABLE 3

A. The amount of shed eggs as a percentage of the total spawn (shed eggs + live born nauplii) of an *Artemia* population in relation to salinity ("production of eggs"). B. The percentages of eggs that hatch after having been shed at various salinities ("hatching of eggs").

salinity in ‰	0	12	36	51	60	72	85	98	138	254
A. production of eggs	—	100	100	100	100	83	49	1–4	2	0–4
B. hatching of eggs	91	85	89	90	88	53	6	0	0	0

The disadvantage of a large salinity range for the hatching of eggs is understandable: if a salinja has been covered by water of low salinity for a prolonged period all *Artemia* eggs will have hatched but the larvae run into difficulties being subject to great attrition from competition and predation. Thus, it may require months for the brine to concentrate and a re-population of the salinja to take place. The advantage of the large salinity range for the hatching of eggs is that the *Artemia* can reoccupy its area immediately after rainfall, and, because of the strong evaporation, the rather short time left for development can be used to its full extent.

The difference between oviparity and viviparity is not caused by inherited factors: all females raised at low salinities become oviparous, but their young become viviparous when raised at high salinities (vide Table 4).

TABLE 4

Artemia eggs put into seawater of 10‰ salinity.

	number of ♀♀	oviparous	viviparous
after 10 days	250	250	0

Newly hatched nauplii from oviparous ♀♀ put into 150‰ salinity.

	number of ♀♀	oviparous	viviparous
after 10 days	250	15	235

We were not able to convert oviparous females into viviparous ones by moving them to more concentrated brines. On the other hand, viviparous females moved to lower concentrations will produce more eggs "for immediate release."

It is possible that microscopic investigations will reveal that this matter is more complicated as in some Mediterranean salt lakes: STEFANI (1963, 1964, 1967) reports on the differences between "amphigonic" and "parthenogenetic" strains or races - not only ecological differences, but also in sex ratio, fertility, cytological egg development, chromosomes, and so on.

4. COLOUR

The colour variability has often been discussed, but until now no explanation has been found for the diversity of colours. Food seems to play an important role, but there are no feeding experiments that confirm this view (vide KUENEN, 1939).

In the Antilles, the adult *Artemia* show a coloration varying from transparent or milkish white to yellow, orange, red, or blue-green. In less saline brines the *Artemia* are often pale, but there are exceptions. In concentrated brines the colour is often dark red, but here too, exceptions are many. Under laboratory conditions *Artemia* always turned pale even when they were kept in water from whence they were taken. Nauplii always are bright orange in colour.

According to GILCHRIST (1954, 1960) the red colour is probably due to synthesis of haemoglobin under oxygen deficient conditions. However, the brines in the Antillean salines are nearly always saturated with oxygen. Of course, the amount of oxygen in brines is much less than in freshwater, but the tension is not lowered by the high concentrations, and that is the only factor of physiological importance as long as no crowding of organisms occurs. Animals that, under laboratory conditions, have lost their red colour will thrive and propagate even in the most concentrated brines, so the red colour is no condition sine qua non. It is possible, however, that red *Artemia* are better adapted to lower oxygen levels, but this has not been tested by us so far.

Usually the *ovaries* of *Artemia* have a brownish colour, but in some populations some or all females possess bright blue-green ovaries. No correlation of colour and salinity can be shown. The blue-green colour is inherited: all offspring show the same colour.

5. SALINITY PREFERENCE AND SALINITY TOLERANCE

In our rearing experiments we have found that the eggs will hatch in distilled water as well as in water of 60‰. From 60 to 85‰ the hatching time is prolonged and part of the eggs do not hatch. Above 85‰, no hatching occurs. In salinities from zero to 5‰ nauplii soon die, but from 5‰ and up, they will develop to adulthood. In nature, however, *Artemia* are found only at concentrations of at least 45‰. This discrepancy can be caused either by mortality in the low salinities, or by a preferential migration, when possible, by the *Artemia* from the low salinity environment. The preference of *Artemia* for certain salinities might also explain the fact that, in salines with a salinity gradient, the *Artemia* sometimes accumulate where salinity is extreme: in brines of low concentration they accumulate in the briniest parts, in salines of high salinity they are found crowding in the less saline parts. It would appear, then, that *Artemia* can choose – high or low salinity. In order to test for this salinity preference an experiment was designed, using adults as well as nauplii.

For testing, the method described by BAGGERMAN (1958) was adopted. Her technique had to be modified conform to the needs of *Artemia*. A trough was divided into 10 compartments by glass walls which did not reach to the top of the trough. The compartments were filled with water of various salinities (23–300‰) and, then, on top a layer of water of the lowest salinity was added so that *Artemia* could move from one compartment to the other. Equal numbers of animals were put in each compartment. The experiments were performed in a dark room to rule out phototaxis. During the next days, the numbers present in each compartment were counted. Additional experiments were done in small aquaria with only two compartments (for detailed techniques vide KRISTENSEN & FELTKAMP, 1970). The large trough offered the advantage of permitting a large gradient series to be used, but also had limitations because the rapid movement of the shrimps made counting difficult. The aquaria with 2 compartments permitted ease in counting, but the salinity differences between both compartments were rather large, which involved occasional problems.

In the trough, a preliminary series of 8 experiments each with 100–150 *Artemia*, gave insignificant results concerning the preference of the brine shrimps: in four experiments no preference could be detected, and in the others there appeared to be a preference for salinities in the 50 to 150$^0/_{00}$ range. Wheter this apparent preference was real could not be proved because of the high mortality which occurred, especially in the extreme salinity compartments.

In a second series one experiment was performed with 1020 brine shrimps from a saltpan of rather low salinity (71‰), and a second experiment with 1085 shrimps from a brine of 210‰. Both groups of shrimps were adapted to 150‰ for some days before being put into the trough. The results (Table 5) show that:

1. There is no significant preference for any salinity.
2. There is a significant difference in mortality between the two groups of brine shrimps: the shrimps from the low salinity show a relatively high mortality in very briny water, and the shrimps from the high salinity salt pan partly succumbed in brackish water.

The results in the series where adults and nauplii had to choose between two different salinities have been tabulated in Table 6. In the area of low salinities the *adult Artemia* show a marked

TABLE 5

Salinity preference of *Artemia* adults in a trough with a salinity gradient.
A. *Artemia* adapted to low salinity.
B. *Artemia* adapted to high salinity.

Adult *Artemia*	initial number	salinity compartments 24‰ → 290‰ no. 1	2	3	4	5	6	7	8	9	10
A. from salinity 71‰	1020 specimens										
mortality % (total mortality 31.9%)		0.5	0.7	0.4	1.0	0.7	1.1	2.0	5.3	8.7	11.5
distribution %		12	9	11	14	8	10	9	8	9	10
B. from salinity 210‰	1085 specimens										
mortality % (total mortality 30.7%)		8.5	10.1	4.6	1.1	1.3	0.2	0.2	1.0	2.1	1.6
distribution %		13	9	12	10	9	11	6	8	15	6

negative preference for the lowest salinities (below 20‰), and a positive preference for salinities above 60‰. Very high salinities (170‰) were avoided.

The preference of the nauplii is more obscure. In the trough experiments (not tabulated) the nauplii moved constantly towards the most concentrated brine where they expired.

The high mortality rate could not be explained. When the nauplii had to choose between two salinities they appeared to prefer the lower concentrations. From Table 6 it can be deducted that the nauplii should be expected to show a strong preference for fresh water; however, this is a false interpretation, as the very low concentrations simply trapped the nauplii.

If they entered into low salinity they appeared to have difficulty in leaving. At least the "preference" for salinities of 5 and 0‰ is actually no preference at all. Whether the preference between 10 and 25‰ is a voluntary or a compulsory one is difficult to say, but we are inclined to think that a real preference was involved as the nauplii were quite able to return to 35‰. This reversal occurred almost immediately.

The "preference" shown by the nauplii for extremely high salinities (170 and 270‰) is again the effect of being trapped.

TABLE 6

Salinity preference of *Artemia* adults and nauplii.
Choice between two salinities only.

choice between	ADULTS		NAUPLII	
	numbers	ratio	numbers	ratio
0–35‰	30–58	0.5	83–5	16.6
5–35‰	16–67	0.2	63–12	5.3
10–35‰	23–75	0.3	68–20	3.4
15–35‰	34–64	0.5	72–18	4.0
20–35‰	34–83	0.4	84–12	7.0
25–35‰	48–58	0.8	68–33	2.6
30–35‰	46–52	0.9	54–43	1.3
35–35‰	58–49	1.2	52–40	1.3
40–35‰	57–51	1.1	67–53	1.3
50–35‰	42–45	0.9	53–56	0.9
60–35‰	83–38	2.2	75–63	1.2
100–35‰	65–31	2.1	44–52	0.8
170–35‰	38–63	0.6	85–31	2.7
270–35‰	30–72	0.4	89–23	3.9

The results of the preference experiments of *Artemia* do not explain their total absence in water of salinities below 45‰. In order to secure some insight into this preference behaviour another experiment was undertaken. Nauplii were raised from eggs in glass 1.5 l aquaria which contained various saline concentrations. Yeast was added as food. The larvae hatched in all salinities between 0 and 60‰ as shown above (Table 3). The nauplii did not survive in completely fresh water (Table 7), and even at 5‰ salinity mortality was considerable. From 12‰ and higher, on the other hand, there was almost no mortality, and the larvae matured in one or two weeks.

TABLE 7

Influence of low salinity upon mortality of *Artemia*.

salinity in ‰	0	5	12	20	36
hatching of eggs in %	91	82	85	90	89
hatchlings reaching adulthood in %	0	12	96	93	91

These results offered no explanation for the question as to why in nature, Artemias are not found in water of salinity lower than 45‰. In order to further inquire into this subject *Artemia* were placed in cages which were immersed in natural waters of various salinities.

The cages measuring 25 × 25 × 25 cm were made of plankton gauze with meshes of 45–55 μ. Some eggs, nauplii and adults were put into the submerged cages. During two weeks the cages were inspected every 3 or 4 days. Some cages were damaged by various causes, but twelve cages yielded pertinent information, among which three cages with water of relatively low salinity: one cage in a pond in Rio Canario (salinity 11‰), one in the sea (36‰) and one in the salt pan of Cas Abau where the salinity was 40‰.

Although no exact figures on the mortality rate are available it was observed that in all cages the population had increased either by young ones hatched from the eggs which had been introduced, or by propagation of the adults – or by both. This clearly shows that no abiotic factors can be responsible for the absence of *Artemia* in water with a salinity below 45‰.

These experiments were repeated with cages clothed with gauze with larger meshes (about 100 μ). Although the adult *Artemia* could not escape they vanished from the cages within 2 days, presumably because of small predators of less than 100 μ.

6. LIGHT PREFERENCE

Artemia always swims upside down. As has been shown by SEIFERT (1932) they are light-orientated: if the (artificial) light is changed *Artemia* will respond by changing its swimming position. Under natural light conditions European *Artemia* appear to be indifferent in this respect (KUENEN, 1939). In the laboratory the Antillean *Artemia* behave like the European shrimps, although the latter appear to be more negatively phototactic. In the Antillian *salines*, *Artemia* are seldom found at the surface, rather at least 10 cm below the surface. At sunset, however, the upper 10 cm become inhabited. No experiments have been done to investigate this phenomenon.

7. TEMPERATURE PREFERENCE AND TEMPERATURE TOLERANCE

In the Antilles, the temperature is very constant. Under normal conditions the extreme water temperatures throughout the year are 26°C and 32°C, and no temperature differences are found between surface and bottom water as long as no salinity differences occur. During the night the water temperature drops for about two degrees. The preference of *Artemia* for rather high temperatures can be observed early in the morning when the greatest concentration of the shrimps occupies the region which registers the first rise (approximately 1°C) in temperature. As has been indicated previously, after a rainfall the water may become layered which brings about a sudden rise in temperature near the bottom. *Artemia* has never been found in water of a temperature exceeding 35°C, but if the water is layered they will be found quite near to the warm water layer.

In order to test the temperature preference of adults and nauplii an aquarium in a (dark) room was filled with temperature layered water of 71‰ salinity. A layer of 20°C was placed at the bottom. Water of 25°C was gently added, followed by a layer of 30°, of 35° and of 40°C. Although there was quite some intermixing of the layers, a rather regular gradient from 22° at the bottom to 37° at the surface was obtained. About one hundred adults and one hundred nauplii were released on the bottom. After half an hour a census was made. The figures given in Table 8 are the average of three experiments. No *Artemia* were found in water from 34°C upwards. A clear preference was shown for the water between 29 and 34°C. The animals found at the bottom (at 22–25°C) were crawling along the walls and bottom and were probably led by some tigmotaxis.

TABLE 8

Distribution of 100 *Artemia* adults and 100 nauplii in a water columm with a temperature gradient of 22°C at the bottom to 37°C near the surface.

temperature	distribution of adults	distribution of nauplii
37–34°	0%	0%
34–32°	47%	40%
32–29°	33%	44%
29–25°	8%	1%
25–22°	12%	15%
	100%	100%

The preference of nauplii and adults for 32–34°C is remarkable as that temperature is quite near to the lethal limit. If the temperature rises above 36°C all *Artemia* are killed as we experienced more than once in our experiments.

8. AGGREGATION IN NON-STAGNANT WATER

Artemia nauplii, like most other plankton animals do not show any rheotaxis. Adults similarly do not show rheotaxis to any marked degree. This suggests that brine shrimps are readily carried by horizontal currents, caused by wind action or by differences in the water level. However, as soon as the water movement has a vertical component, the shrimps may oppose the current, but for other reasons, such as their predilection for a certain light intensity. Thus, it appears that water movements play a subordinate role in the formation of *Artemia* aggregations.

9. COMPETITION

Food competitors seem to be very rare. On some occasions, however, it has been observed that in salines where rotatorians were present, no *Artemia* were found (ROOTH, 1965, p. 69–70). At this time we can report on a simple experiment dealing with this subject (see Table 9).

Unidentified rotatorians from Salinja Tam (Bonaire), salinity 71‰, were placed together with some nauplii and adult *Artemia*, and a number of green flagellates was added. In one aquarium, each day a new portion of flagellates were added; both *Artemia* and rotatorians flourished. In the second aquarium, the flagellates were presented only on the first day, but not afterwards. After four days, the digestive tract of the nauplii and adult *Artemia* appeared to be empty. The rotatorians on the other hand appeared to have ingulfed food; their stomachs contained green material. After one week, all *Artemia* were dead.

Rotatoria seem to be more effective in their method of collecting food than *Artemia*. Unfortunately, the experiment could not be repeated because of a lack of rotatorians from supersaline water.

TABLE 9

Food competition between *Artemia* and Rotatoria in aquaria of 6 l.

A.		daily fed with flagellates	
first day	± 2000 rotatoria	107 *Artemia* ad.	± 250 *Artemia* juv.
after 4 days	± 1500 —	± 200 — —	± 800 — —
after 7 days	± 1200 —	± 500 — —	± 2000 — —
B.		no feeding after first day	
first day	± 2000 rotatoria	121 *Artemia* ad.	± 200 *Artemia* juv.
after 4 days	± 1400 —	± 175 — —	± 500 — —
after 7 days	± 1100 —	0 — —	0 — —

In most places where *Artemia* is found no other plankton feeder is present. The only food competition will be among the *Artemia* themselves. We have no evidence whether this intraspecific food competition may be of significance, nor do we have evidence concerning other forms of competition, as for example, oxygen or space.

10. PREDATORS

All small fish are fond of *Artemia.* The slow brine shrimps will never elude or even attempt to escape from predators. Hence, in waters containing fish no *Artemia* will be found. This fact has been repeatedly mentioned in literature (HEDGPETH, 1957). In the Antilles *Artemia* is confined to areas where the salinity is too high for fish like *Poecilia sphenops* and *Cyprinodon dearborni,* which abound in most supersaline waters of a salinity level to 80‰ and sometimes in a higher level of 130‰. A third fish, *Rivulus marmoratus,* is also an avid *Artemia* feeder, although of negligeable importance as the species is rare in brine of more than 70‰. Moreover, it does not occur anywhere in large numbers like the two other cyprinodont species (KRISTENSEN, 1970).

It is noteworthy that neither *Poecilia* nor *Cyprinodon* are specialized *Artemia* feeders: they are both omnivorous species. After heavy rainfall has lowered the salinity throughout the entire salinja these Cyprinodonts may enter the area and wipe out the *Artemia* in no time. They can devour this dietary source unpunished as they will switch over to algal food instead. Thus *Artemia* has no chance to survive in areas containing fish.

Omnivorous fish may actually be more hazardous to the *Artemia* than when they would have preyed upon *Artemia* only. Otherwise, they would starve to death without the *Artemia* as in the case of the carnivorous species *Rivulus marmoratus* (KRISTENSEN, 1970). As it is, *Poecilia* and *Cyprinodon* become algae feeders, so that the *Artemia* will never be free of fish predation.

One bird in the salines is an *Artemia* feeder; this is the flamingo. However, according to ROOTH (1965), *Artemia* is not consumed in quantity, so this predation may be of little importance. The same may hold for Odonata larvae and aquatic Hemiptera and Coleoptera.

At rather low salinities, one small predator has proven to be extremely successful in exterminating an *Artemia* population; this is a cyclopoid copepod. It is found in brackish and in seawater as well as in supersaline water of less than 82‰, but is never abundant above 45‰. Cyclopoids ferociously attack *Artemia*. Some five or six cyclopoids surround one *Artemia* and with powerful strokes on the body and appendages of the brine shrimp actually dismember their victim. Nauplii are similarly destroyed. There is no explanation for this behaviour as cyclopoids do not feed on larger prey. Possibly the fragmented *Artemia* yields edible material. In the experiments of raising *Artemia* at low salinities we had to use the finest gauze (45–55μ) as copepods would enter when larger meshes were used, and bring about the extermination of the *Artemia* population.

FLUCTUATIONS IN ARTEMIA POPULATIONS

1. After a rainless period the shallow parts of salinjas and salt pans become dry by evaporation and cause a firm crust of salt to cover the bottom. Of course, no live *Artemia* are found here, but viable eggs are present everywhere in these areas: a sample of salt or bottom material will yield a multitude of nauplii if water is added; this phenomenon has been observed by us many times when the samples were taken to the laboratory.

When such dried up areas again are flooded, the repopulation will start immediately and after one week a second generation may start. If this flooding is caused by heavy rain falls so that a freshwater layer of 10 cm or more covers the salt flats, large numbers of eggs will hatch. After one week the females are mature and will produce eggs. Some eggs hatch immediately, others float ashore and many hatch subsequently after another rainfall. The first *Artemia* generations always consist of the *arietina* form and are oviparous. When the salinity of the brine increases to 150‰ or more the next generation of females will be viviparous and possess abdomens which tend to have the *milhauseni* form. This is the normal development when a dry period has existed for some time. It can be duplicated completely in the laboratory. However, several exceptions have been observed, which are listed below.

2. If a dry salt flat is covered by only a few cm of rainwater the salinity may increase to 20–40‰ during the very first day. The *Artemia* eggs present on the flat will hatch immediately, and numerous nauplii are seen by the next day. However, they mostly die within a few days, probably because of the relatively high amount of potassium in newly formed brine.

The phenomenon was investigated in detail only twice: Feb. 23, 1963: water layer of 3 cm on salt crust in pans of Fuik, Curaçao. May 17, 1964: 2–4 cm of water on salt in the Salinja of Slagbaai, Bonaire. When the brines were analysed the salinities proved not to be very high (85 and 131‰), but the ratio of the cations was abnormal; potassium was present in a quantity amounting to almost 0.2 of sodium. In this ratio potassium is poisonous to *Artemia* (JACOBI & BAAS BECKING 1933). When nauplii were put into this brine they succumbed in half a day.

3. If the surface of a brine pool, containing no *Artemia* is covered by rainwater, shrimp eggs present will hatch and populate the pool. If, however, the two fluid layers – rainwater and brine – are not disturbed by the wind or currents and no convection occurs, the bottom brine region does not cool, and radiant heat from the sun may bring about a considerable rise in the bottom temperature, especially if the floor of the pool is black mud. Because many organisms cannot avoid the heated bottom zone, they will succumb. As a result, oxygen deficiency and eventual mass mortality occur. The only organisms that may survive under such conditions are certain anacrobic bacteria and the larvae of the brine fly, *Ephydra gracilis*.

These phenomena have been observed several times, but only on three occasions exact data have been obtained (Table 10).

TABLE 10

Salinity and temperature near the bottom and near the surface by absence of turbulence.

date	name of saltpan	depth	salinity bottom	salinity surface	temperature bottom	temperature surface
23.IX.1960	Cas Abau	50 cm	223‰	111‰	51°C	32°C
30. I.1961	Cas Abau	50 cm	245‰	92‰	55°C	32°C
25.IV.1964	Fuik	40 cm	240‰	85‰	61°C	31°C

On these occasions *Artemia* was found in the *upper* layers, but the population succumbed after about one week, probably as the result of oxygen depletion and/or hydrogen sulphide poisoning (a strong H_2S smell was noticed in all cases).

4. If the flooding of a saline makes it possible for *Poecilia, Cyprinodon* or copepods to enter they will exterminate the young *Artemia* population within a week or so. If the wind or other causes bring eggs from the surrounding areas, newly hatched nauplii will be found constantly, but they too fail to escape from the predators. When, by evaporation, the concentration of the brine will have passed 100‰ most of the predators mentioned will be killed, but the brine is then too concentrated for the hatching of *Artemia* eggs. It may take months before *Artemia* will return although all conditions would seem to be favourable. However, a light shower is enough to lower the salinity in some isolated shallow corner, and some eggs will hatch which will yield a new population of *Artemia.* Pertinent data were collected after the torrential rains at the end of August, 1961, when many salt flats were covered with a considerable amount of fresh water (Table 11).

TABLE 11

The influence of disappearance of predators on the *Artemia* population.

date, 1961	salt pan	salinity	predator	*Artemia*
Sept. 5	Cas Abau, 5 pans	43– 54‰	*Poecilia*	nauplii only
Oct. 12	ibidem	56–111‰	*Poecilia*	very few nauplii
Dec. 10	ibidem	124–155‰	none	none
Dec. 23	ibidem	112–154‰	none	a few nauplii
Sept. 11	Fuik, 4 pans	15– 64‰	Cyclopoids	nauplii only
Oct. 13	ibidem	34– 85‰	Cyclopoids	none
Dec. 15	ibidem	124–155‰	none	none
Dec. 30	ibidem	95–158‰	none	a small population
Sept. 18	Pekelmeer, 3 pools	28– 45‰	*Cyprinodon*	nauplii only
Dec. 17	ibidem	140–205‰	none	none

On December 10, 15 and 17 resp. water from the three locations was taken into the laboratory and stocked with *Artemia*. They thrived, and after the brine had been diluted to 52–64‰, nauplii hatched from eggs that apparently were present in the brine. This illustrates that the absence of *Artemia* in general does not imply that the conditions have to be adverse – it may be caused by changes in the environment which took place months previously, their long-range effect having not yet disappeared.

The same picture is seen when the pans are not flooded by rains, but by seawater. In April 1962, an extraordinarily high tide caused flooding of large parts of Pekelmeer, and its average salinity dropped to 64‰. *Cyprinodon* invaded the area and exterminated the *Artemia* population in about one month.

In August, the salinity had increased to 160–210‰. *Cyprinodon* was then restricted to the border area where, because of seepage, the salinity is always below 90‰. This condition existed until October when after some showers the salinity of Pekelmeer decreased in shallow places so that *Artemia* eggs could hatch – the start of a repopulation.

In May, 1964, a very high tide in Curaçao caused flooding of old saltpans in Rifwater. After the flooding, *Cyprinodon*, *Poecilia*, cyclopoids, and a few *Artemia* nauplii were found, at a salinity of 42‰. On June 11, the salinity had increased to 180‰ and no predators or *Artemia* were present. At the end of June some minor showers caused a decrease of the salinity to 164‰, and in shallow places to 56‰; no predators could enter, and *Artemia* came back. The absence of predators in June had presumably been caused by the salinity increase, and the absence of *Artemia* because they had been consumed. Furthermore, the salinity before the end of June had been too high to permit any eggs to hatch, although, as we ascertained in the laboratory the water did not harm nauplii and adult *Artemia*.

5. When brines become supersaturated by evaporation the *Artemia* population is not affected; the shrimps thrive even at 300‰ and higher concentrations. However, when gypsum (calcium sulphate) starts to precipitate *Artemia* die within a few days. The

remains well-preserved by the salt can then be found on the bottom. After precipitation has more or less abated the brine of less salinity is not repopulated unless the salinity has decreased considerably by rainfall or flooding from the sea.

This has been observed frequently. This phenomenon can be reproduced under laboratory conditions. Under laboratory conditions, but not observed in nature, supersaturated brine becomes cloudy as the result of rapid formation of gypsum and other crystals. In the laboratory *Artemia* will not tolerate the presence of crystals. Their appendages become loaded with salt crystals, so it is quite possible that the cause of death represents the same phenomenon as has been found in turbid water.

The death of *Artemia* as the result of precipitation of gypsum is remarkable as the presence of brine shrimps is supposed by the "briners" to be an obligate condition for cleaning a brine with a gypsum suspension (in "pickle ponds"). Therefore, they call the shrimps "clearer-worms". After precipitation of gypsum the clear brine is transported to the "salterns" where no *Artemia* are to be found (BAAS BECKING, 1932), which conforms to our observations in nature and in our experiments: if a brine clouded by gypsum crystallization is filtered until the water is clear, it is still poisonous to *Artemia*, probably because the equilibrium between calcium and magnesium has been changed too much (JACOBI & BAAS BECKING, 1933). The viability of the eggs, on the other hand, is not affected at all, even after a prolonged stay in this brine. Subsequently, the brine turns red because of red bacteria which are typical for brines without calcium ions. After dilution with seawater the equilibrium of ions will be partially restored and the brine becomes suitable again for hatching and maturing of *Artemia*, thus the possibilities for a reestablishment are present.

Although we are well aware that the description of population fluctuations of *Artemia* presented here is far from complete, it may illustrate the susceptibility of *Artemia* to adverse biotic and abiotic factors and also the remarkable ability of *Artemia* to reestablish itself after extermination by physical and biotic factors.

The several aspects in the life history of *Artemia* in this paper do not, except perhaps in a general way, apply to the European *Artemia*. The European environment is so vastly different from the tropics that different adaptation to the ecological requirements are to be expected in so variable a species like *Artemia*. The differences mentioned are so great that it would seem quite probably that inherited characteristics are responsible.

SUMMARY

In the Netherlands Antilles two extreme "forms" of *Artemia salina*, f. *arietina* and f. *milhauseni*, show all intermediate gradations. They hybridize constantly. When forma *arietina* is moved from lower concentrations into highly concentrated brine it will produce offspring that exhibit characteristics of the *milhauseni* form. The forms, therefore, do not represent groups of any systematical importance.

Growth rate and body size show great variation. Often, greatest growth rate and largest size are found at salinities between 45 and 200‰. Below and above that concentration growth is usually stunted, and mortality is high.

Artemia is not capable of getting rid of adhering silt. Moreover, silt seems to prevent the uptake of food.

Artemia is oviparous at low salinity concentrations, but from 85‰ upwards they become viviparous although viviparous females also produce some eggs.

Differences in body colour are induced by the environment, but the colour of the ovary appears to be inherited.

Adult *Artemia* prefer salinities from 50 to 150‰, but this preference is variable. Nauplii show a marked preference for freshwater and for more concentrated brines. This preference, however, appears to be only apparent, as the nauplii may have become trapped by the exigency of the environment.

Artemia has a preference for light intensities that are somewhat less than that of a sunny day.

Artemia prefers a temperature of 29 to 34°C.

Interspecific food competition seems to be rare. There is an indication that rotatorians may be competitors. Intraspecific competition may occur, but no proof has been obtained.

Poecilia sphenops and *Cyprinodon dearborni* prey upon *Artemia*; the fact that they are not specialized *Artemia* feeders is considered to be a disadvantage to *Artemia*. The salinity barrier for these two predators lies between 80 and 130‰, leaving the higher concentrations as a refuge to *Artemia*. However, *Artemia* will never attempt to escape from its predators. At lower salinities cyclopoids have been observed attacking *Artemia*.

The absence of *Artemia* in water of low salinity can be attributed to predation. Omnivorous predators are more effective in this respect than carnivorous predators.

A survey is given of the population fluctuations and of the factors involved.

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