The Brine Shrimp Artemia. *1980. Vol. 1. Morphology, Genetics, Radiobiology, Toxicology*
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Differentiation in *Artemia* strains from Spain[1]

F. Amat Domenech

Planta Piloto de Acuicultura
Torre de la Sal, Castellon de la Plana, Spain

Abstract

A total of 36 different *Artemia* strains, found in the Iberian peninsula, have been characterized with regard to their mode of reproduction and ploidy.

A detailed biometrical analysis of 17 spanish and 4 foreign strains (7 bisexual and 14 parthenogenetic) has been performed on animals harvested from wild populations or cultured under laboratory conditions. Most of the distinctive morphological characteristics found, appear to be single consequences of allometric phenomena, and are also related to a different degree of sexuality, ploidy, and size of nucleus in different strains.

Data are given on the biometrics of the telopodite filtering-apparatus, larval growth rate, and adult fertility under different environmental conditions, as a first step in the determination of the relative value of different *Artemia* strains for aquaculture purposes.

The data presented suggest the existence of a specific North-American strain which has to be considered as a different species as well as various Spanish and Mediterranean autochtonous strains.

Introduction

The brine shrimp *Artemia* has become a species that is cultured worldwide as a source of food in aquaculture projects. As a result, the strictly scientific problem of the existence of a variety of strains, which was a study object of genetics, evolution, biogeography, and ecology, is now suscitating more and more interest from the practical point of view. Different strains indeed possess different characteristics and are as a consequence better or less suited for particular uses. The best known and most used strain, namely the one from San Francisco Bay, California, USA, can now be replaced by specific strains available locally in various countries. In this regard Spain has undoubtedly a large potential because a variety of *Artemia* strains have been discovered at different sites. In the near future this will hopefully permit to select the appropriate strain among the geographical races of Spanish brine shrimp in function of particular nutritional or other prerequisites for specific purposes and uses.

[1] This paper is a synthesis of the Ph. D. thesis "Diferenciacion y distribucion de las poblaciones de *Artemia* (Crustaceo Branquiopoda) de Espanã" by Amat Domenech (1979).

Distribution of *Artemia* in the Iberian peninsula and neighboring islands

The geographical distribution of 36 different strains of *Artemia* occurring naturally in Spain is given in Fig. 1. Initial strain-differentiation is based on morphological comparison of the Spanish strains with foreign ones previously studied by Artom (1922, 1925, 1926, 1929), Stella (1933), Barigozzi (1934, 1941, 1957, 1974), Barigozzi and Tosi (1959), Clark and Bowen (1976), Goldschmidt (1952) and kindly made available to us by several scientists from different countries. The strains which were found are generally diploid bisexual, though several diploid and tetraploid parthenogenetic races and several intermediate (also parthenogenetic) forms have been encountered. It is interesting to note that at some locations mixed populations of bisexual and parthenogenetic *Artemia* are co-existing.

FIG. 1. Geographical distribution of *Artemia* strains in the Iberian peninsula and neighboring islands.

Morphological analysis

Juvenile and adult *Artemia* from 21 different locations (17 spanish, 4 foreign, 7 bisexual, 14 parthenogenetic, Table I) were narcotized in a chloroform-saturated water solution and measured under a dissection microscope. The following measurements were performed (Fig. 2): total length, length of abdomen, maximal width of brood pouch, width of 3rd abdominal segment, length of furca, number of setae on each branch of the furca, width of head, length of 1st antenna, maximal diameter and distance between the compound eyes, total length of and number of filtering setae on the telopodite of the 6th thoracopod. Measurements were performed on animals harvested from nature (Spanish strains) and on animals cultured from wild cysts (Spanish and foreign strains) under standard laboratory conditions (*Tetraselmis* food, 25 °C, and seawater of 30-32 ‰ salinity).

TABLE I

List of geographical strains studied

Strain		Origin
Bisexual (diploid)		
San Francisco Bay, California, USA		Metaframe, San Francisco California, USA
San Félix San Fernando		San Fernando, Cádiz, Spain
Salinera Española Bras de Port		Santa Pola, Alicante, Spain
San Pedro del Pinatar		San Pedro del Pinatar, Murcia, Spain
Ibiza		La Canal de San José, Ibiza, Spain
Parthenogenetic		
Gerri de la Sal	(diploid)	Gerri de la Sal, Lérida, Spain
Calpe	(diploid)	Calpe, Alicante, Spain
Bras de Port	(diploid)	Santa Pola, Alicante, Spain
Bonmati	(diploid)	Santa Pola, Alicante, Spain
Cabo de Gata	(diploid)	Cabo de Gata, Almeria, Spain
Ayamonte	(diploid)	Ayamonte, Huelva, Spain
Isla Cristina	(diploid)	Isla Cristina, Huelva, Spain
San Fernando	(diploid)	San Fernando, Cádiz, Spain
Janubio	(diploid)	Yaiza, Lanzarote, Spain
Sanlucar	(diploid and tetraploid)	Sanlucar de Barrameda, Cádiz, Spain
San Antonio	(tetraploid)	River Ebro delta, Tarragona, Spain
Alcochete	(tetraploid)	Alcochete, south of Lisbon, Portugal
Larache	(diploid and tetraploid)	Larache, atlantic coast, Morocco
Comacchio	(tetraploid)	Comacchio, Emilia Romaña, Italy

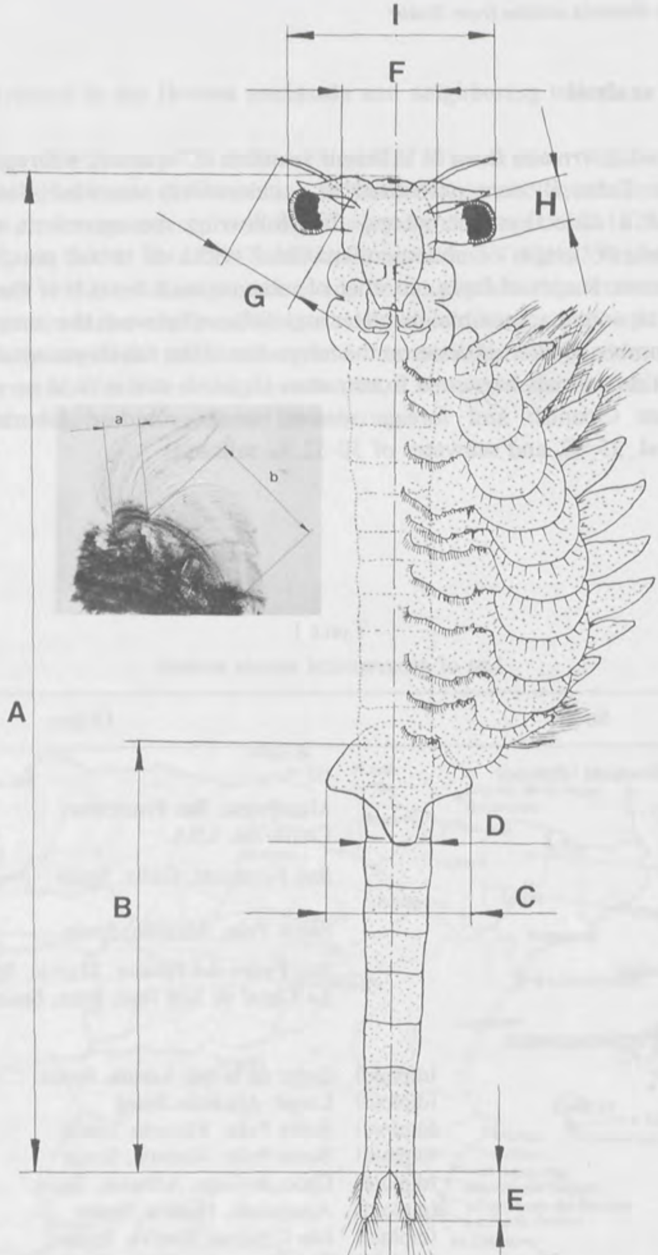

FIG. 2. Schematic drawing of adult *Artemia* and microscopic detail of the telopodite of the 6th thoracopod with indications of the various size measurements. A : total length ; B : abdomen length ; C : maximal width of brood pouch ; D : width of 3rd abdominal segment ; E : furca length ; F : width of head ; G : length of 1st antenna ; H : maximal diameter of complex eye ; I : distance between complex eyes ; a + b = total telopodite length of 6th thoracopod.

Biometrics of animals cultured under standard laboratory conditions

1. From Fig. 3 and 4 it appears that there is a positive correlation between abdomen length and total individual length. This correlation is more obvious in females than in males, especially in the bisexual individuals of Spanish origin.
2. The maximal width of the brood pouch (ovisac) and of the 3rd abdominal segment are both proportional to the total individual length (Fig. 5 and 6). It was furthermore noted that the shape of the ovisac can be used to distinguish Spanish strains from San Francisco Bay *Artemia* (Fig. 7).
3. The length of the furca as well as the number of setae on each furcal branch increase with the size of the animals (Fig. 8 and 9). The morphology of the furca is different in the strains studied (Fig. 10).
4. As shown in Fig. 11 the width of the head increases in all *Artemia* strains, proportionally with the total individual length. In San Francisco Bay *Artemia* the head is elliptical, while it is smaller and of a more irregular shape in the Spanish strains. San Francisco Bay and Spanish bisexual strains can be differentiated through the male claspers : *i.e.* their relative size is larger in San Francisco Bay *Artemia*, the shape of the frontal knob is subspherical in San Francisco Bay, but subconical in Spanish *Artemia* (Fig. 12).
5. In all *Artemia* strains studied a positive correlation was noted between the length of the animals and the length of their 1st antenna, the diameter of and the distance between the compound eyes (Fig. 13, 14 and 15). Antennulae in San Francisco Bay *Artemia* are shorter than in the bisexual strains from Spain. The compound eyes appear to be larger in males than in females, especially in San Francisco Bay *Artemia*.

Biometrics of animals harvested from their natural habitat or cultured in the laboratory

1. Increasing salinities induce a reduction in size of the length of the furca (Fig. 16) and the number of setae on the furca (Fig. 17).
2. The eye diameter and the width of the ovisac do not change much in function of the salinity of the medium (Fig. 18, 19 and 20). However, it appears that in bisexual *Artemia* (Fig. 19) sexual maturity is reached at a smaller width of the brood sac than in parthenogenetic animals (Fig. 20).
3. The relative length of the abdomen, on the contrary, increases with the salinity of the medium (Fig. 21).
4. Whereas in San Francisco Bay *Artemia* the ratio of the total number of filter setae to the telopodite length is constant, it appears that in all Spanish strains studied, there is a positive correlation between the number of setae and the telopodite length (Fig. 22). It is interesting to note that animals harvested from high salinity salt ponds have smaller telopodites and reduced numbers of setae as compared to controls cultured in the laboratory under standard conditions (*cf.* stripped areas in Fig. 22). This could be an adaptation to the decreased size of food particles in media of high salinity (Erhardt *et al.*, 1971).

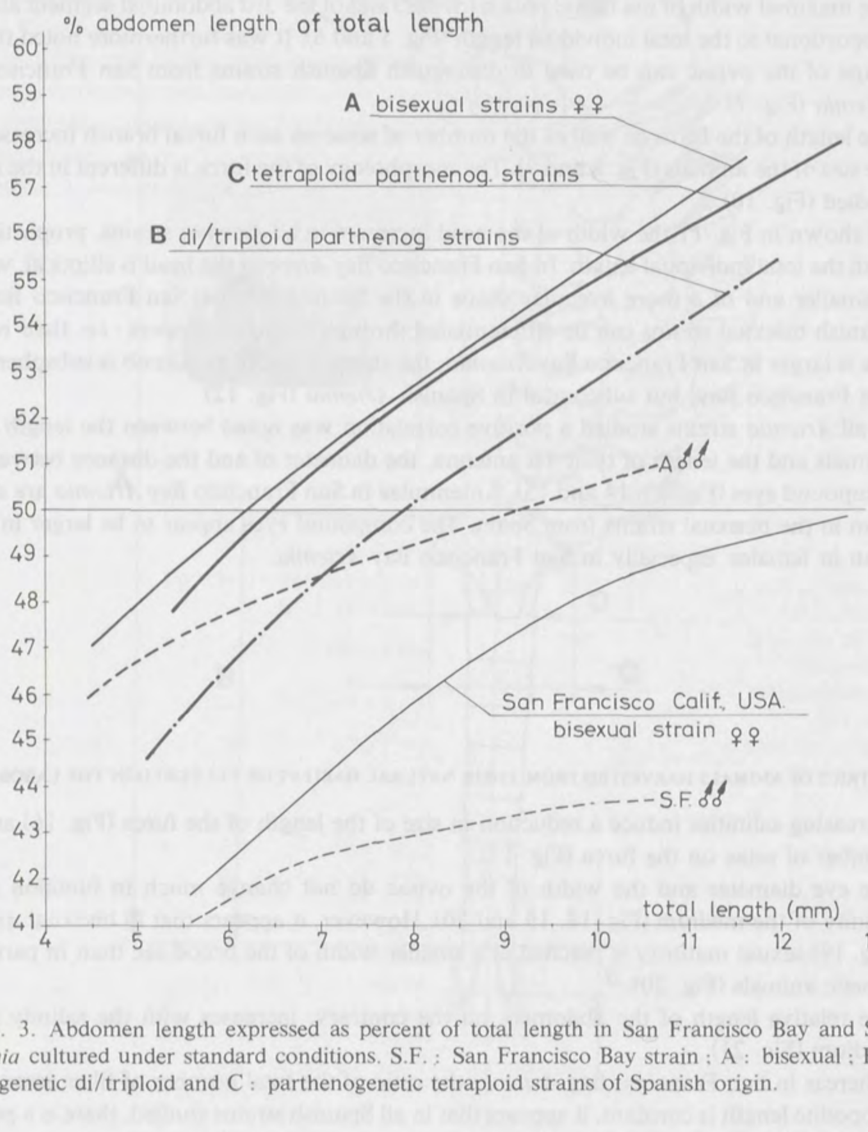

FIG. 3. Abdomen length expressed as percent of total length in San Francisco Bay and Spanish *Artemia* cultured under standard conditions. S.F. : San Francisco Bay strain ; A : bisexual ; B : parthenogenetic di/triploid and C : parthenogenetic tetraploid strains of Spanish origin.

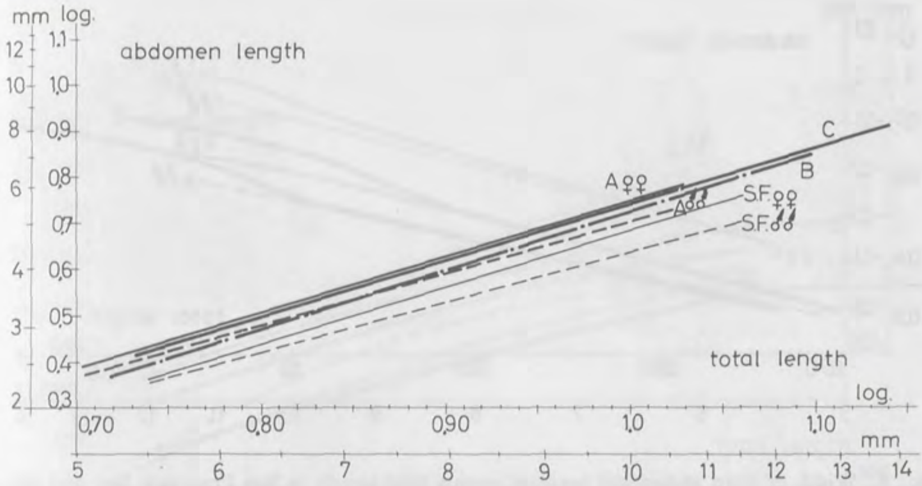

FIG. 4. Abdomen length *versus* total length in San Francisco Bay and Spanish *Artemia* cultured under standard conditions (legend to letters A, B, and C see Fig. 3).

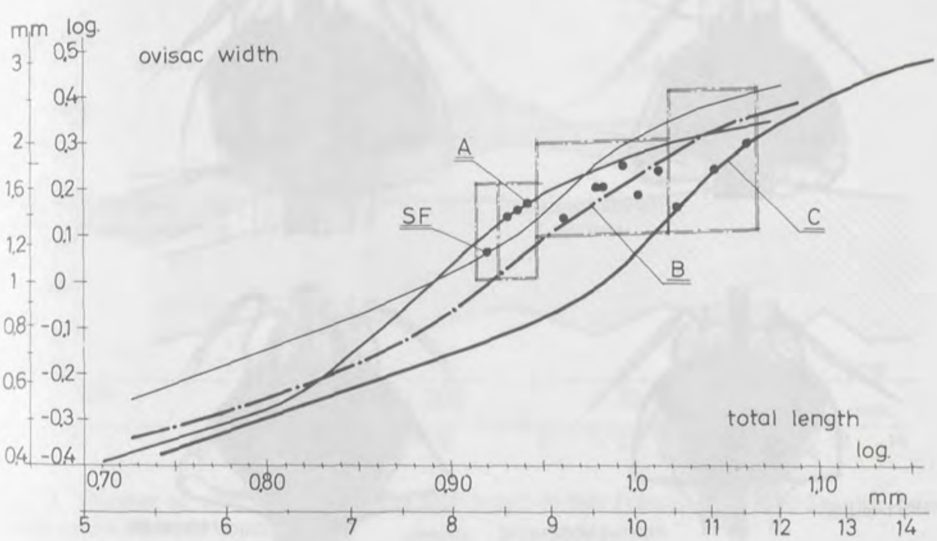

FIG. 5. Width of ovisac *versus* total length in San Francisco Bay and Spanish *Artemia* cultured under standard conditions (full circles represent a population of which 50% of the females carry their first offspring; legend to letters A, B, and C see Fig. 3).

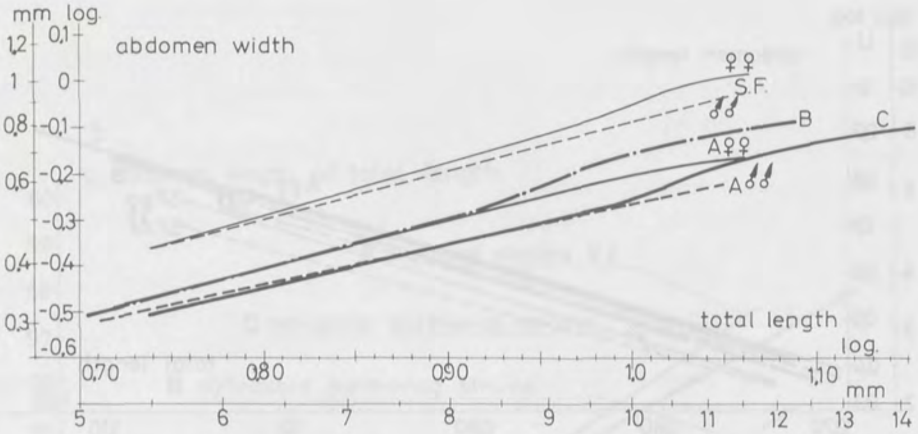

Fig. 6. Width of third abdominal segment versus total length in San Francisco Bay and Spanish *Artemia* cultured under standard conditions.

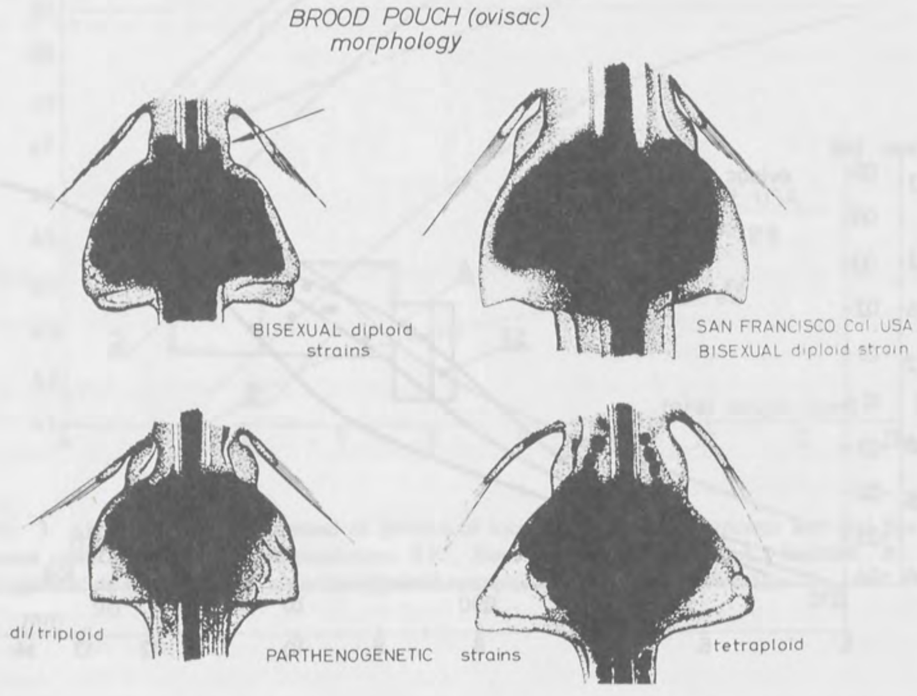

Fig. 7. Morphology of ovisac in San Francisco Bay and Spanish (bisexual and parthenogenetic) strains.

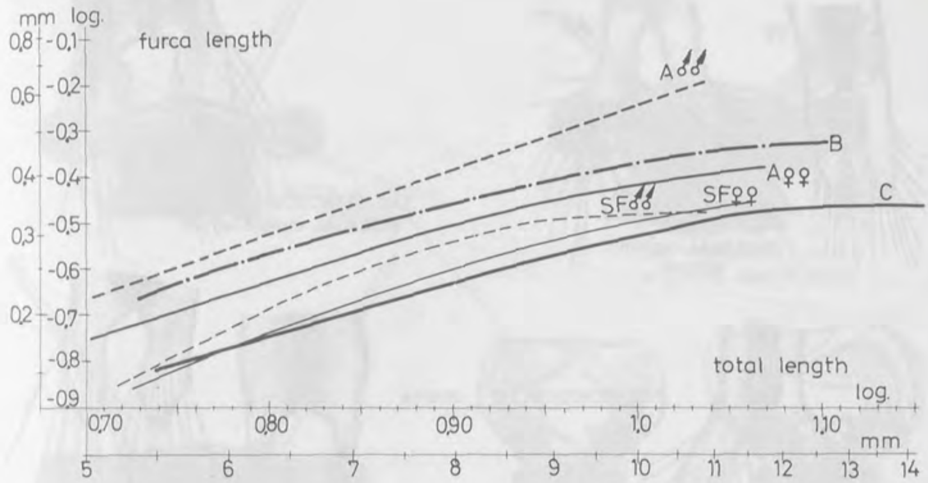

Fig. 8. Furca length *versus* total length in San Francisco Bay and Spanish *Artemia* cultured under standard conditions.

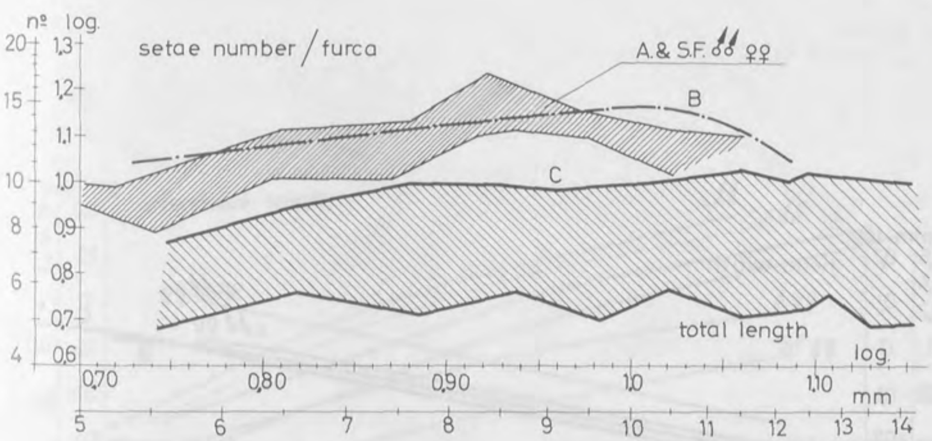

Fig. 9. Number of setae per furca *versus* total length in San Francisco Bay and Spanish *Artemia* cultured under standard conditions.

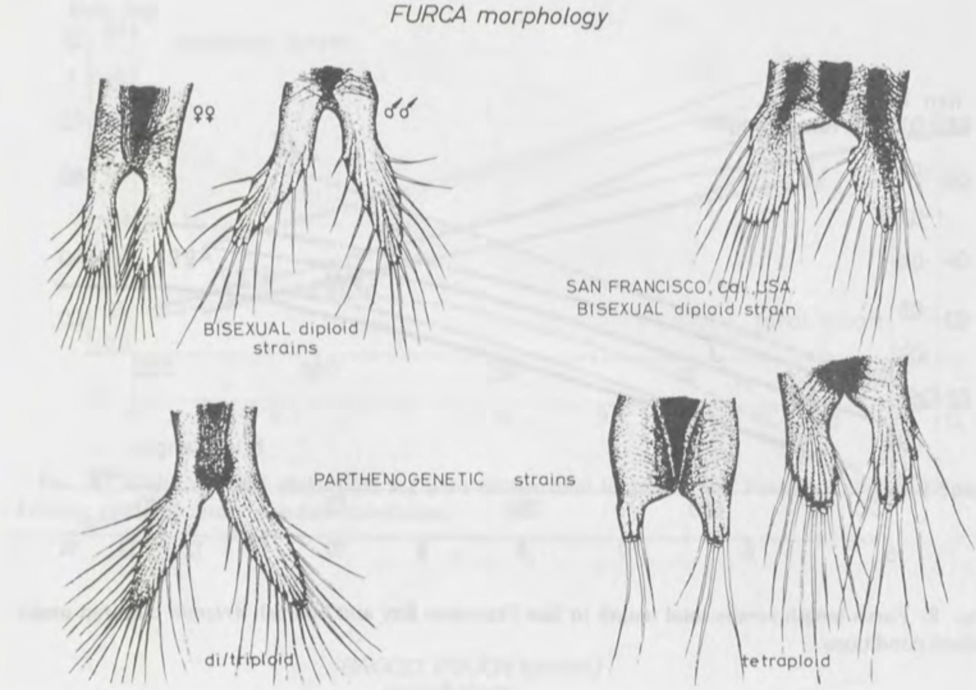

FIG. 10. Morphology of the furca in San Francisco Bay and Spanish (bisexual and parthenogenetic) strains.

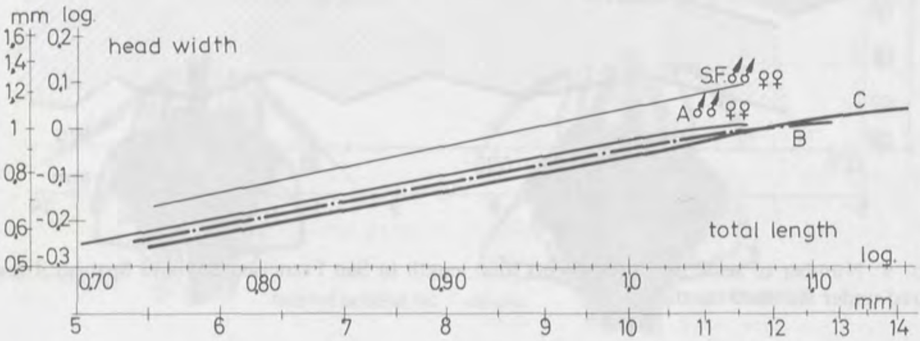

FIG. 11. Width of head *versus* total length in San Francisco Bay and Spanish *Artemia* cultured under standard conditions.

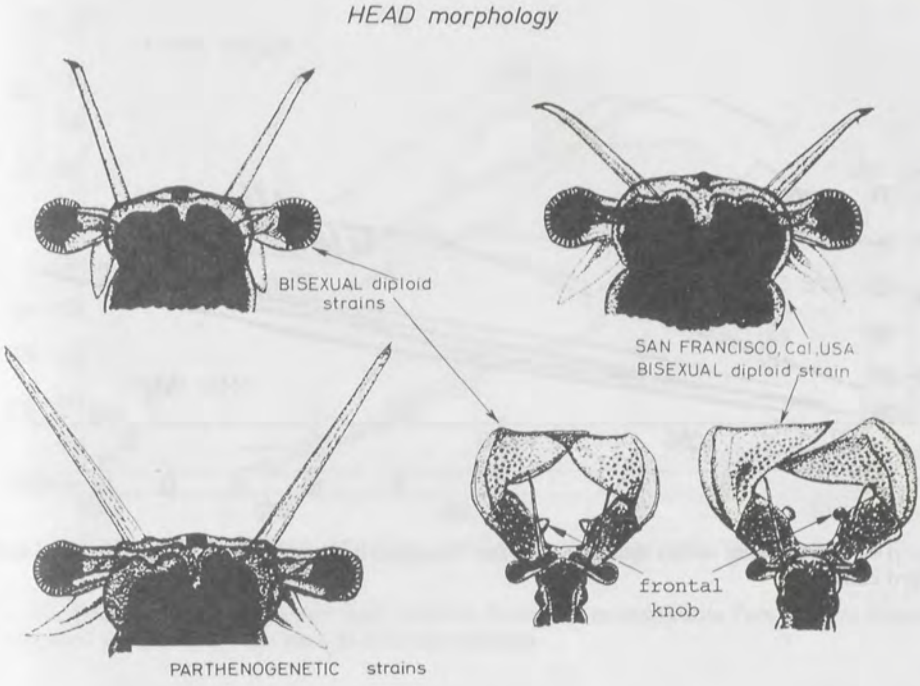

FIG. 12. Morphology of the head in San Francisco Bay and Spanish (bisexual and parthenogenetic) strains.

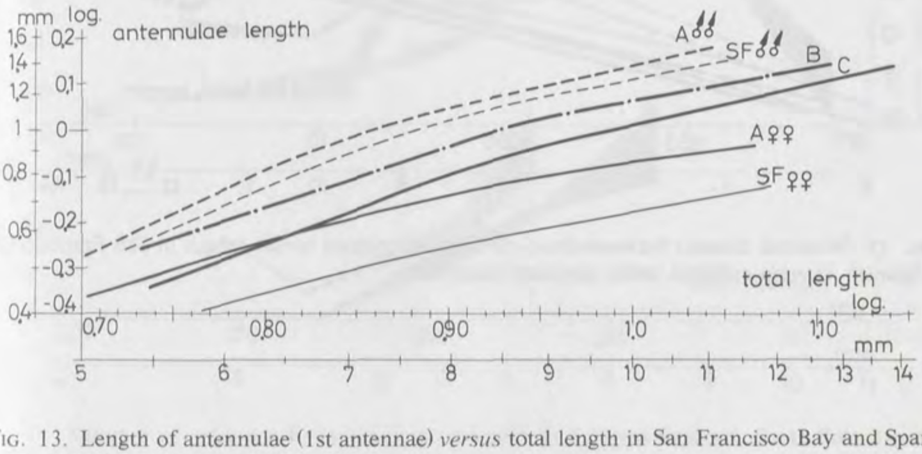

FIG. 13. Length of antennulae (1st antennae) *versus* total length in San Francisco Bay and Spanish *Artemia* cultured under standard conditions.

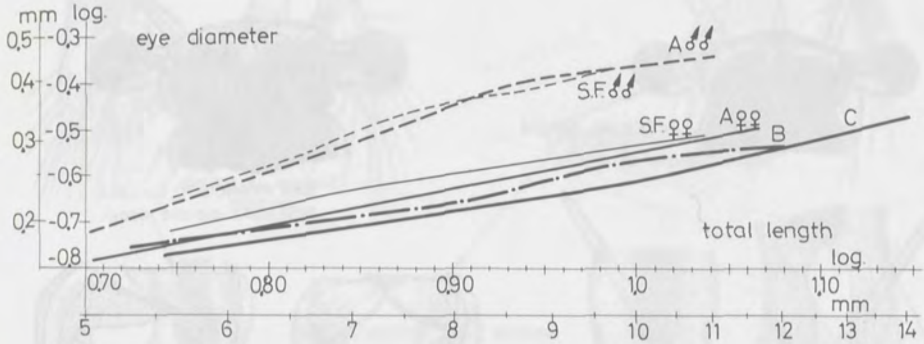

FIG. 14. Eye diameter *versus* total length in San Francisco Bay and Spanish *Artemia* cultured under standard conditions.

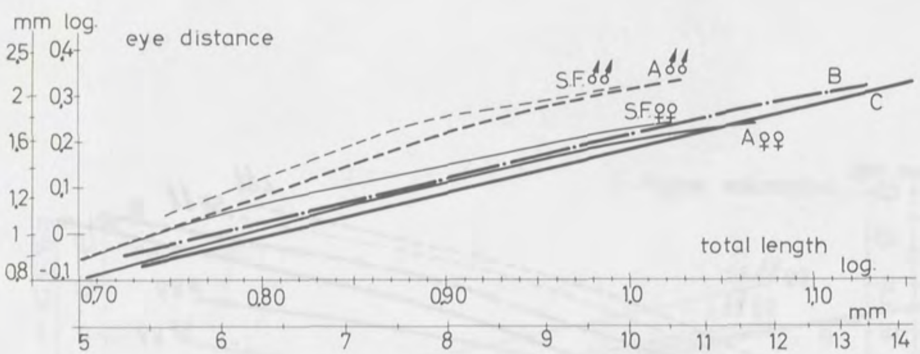

FIG. 15. Maximal distance between the eye's external contour *versus* length in San Francisco Bay and Spanish *Artemia* cultured under standard conditions.

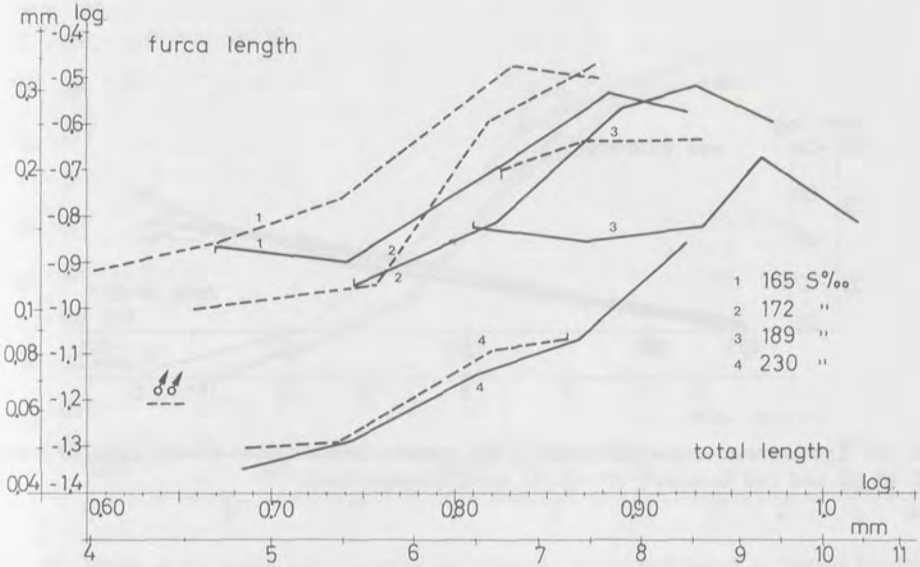

FIG. 16. Length of the furca *versus* total length in Salinera Espanola-Santa Pola *Artemia* (bisexual diploid strain) sampled from salt pans at different salinities.

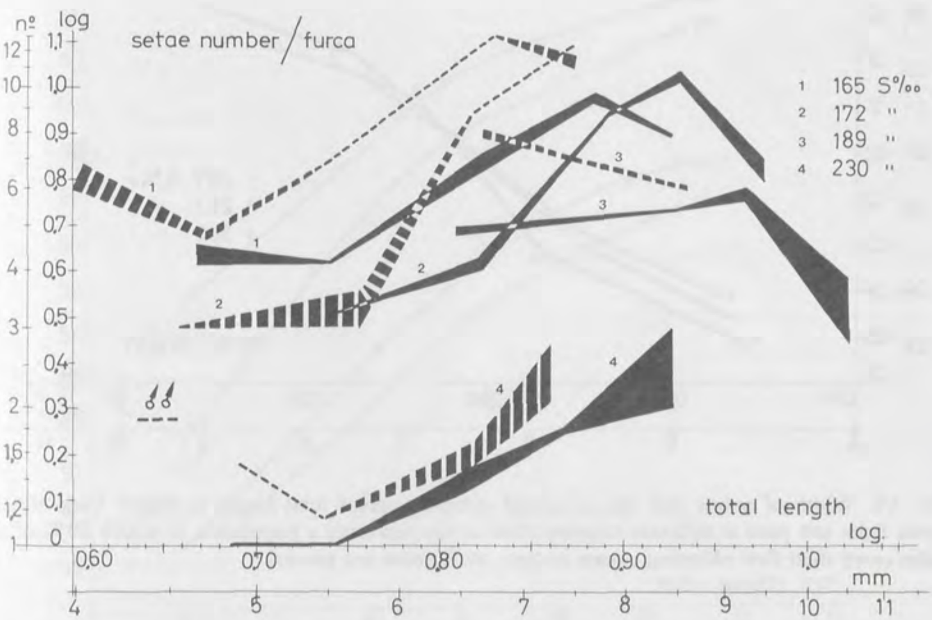

FIG. 17. Number of setae per furca *versus* total length in Salinera Espanola-Santa Pola *Artemia* (bisexual diploid strain) sampled from salt pans at different salinities.

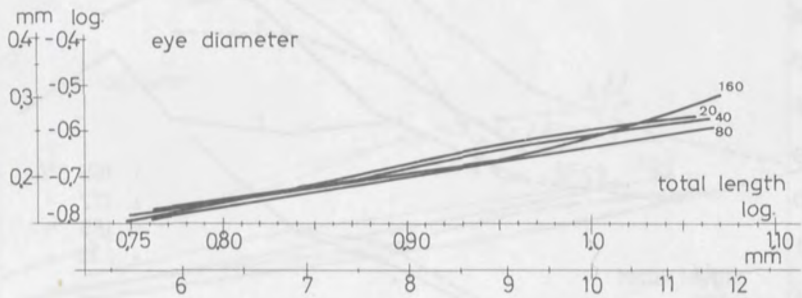

FIG. 18. Eye diameter *versus* total length in San Antonio-Delta del Ebro-*Artemia* cultured in media of 20, 40, 80, and 160 ‰ salinity (t° = 25 °C, dried *Spirulina* food).

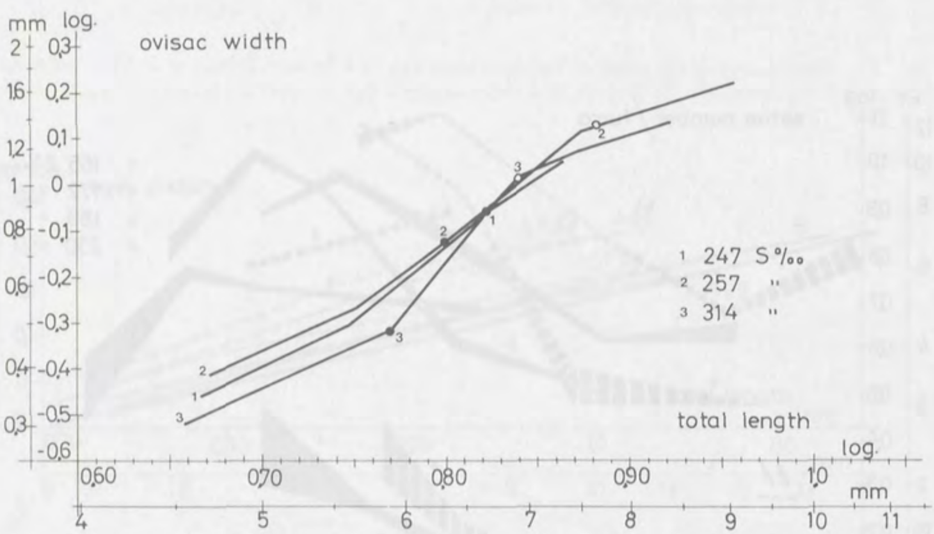

FIG. 19. Width of ovisac and rate of sexual maturity versus total length in Salero Viejo-Villena *Artemia* from salt pans at different salinities (full circles represent a population of which 50 % of the females carry their first offspring ; open circles : all females are gravid).

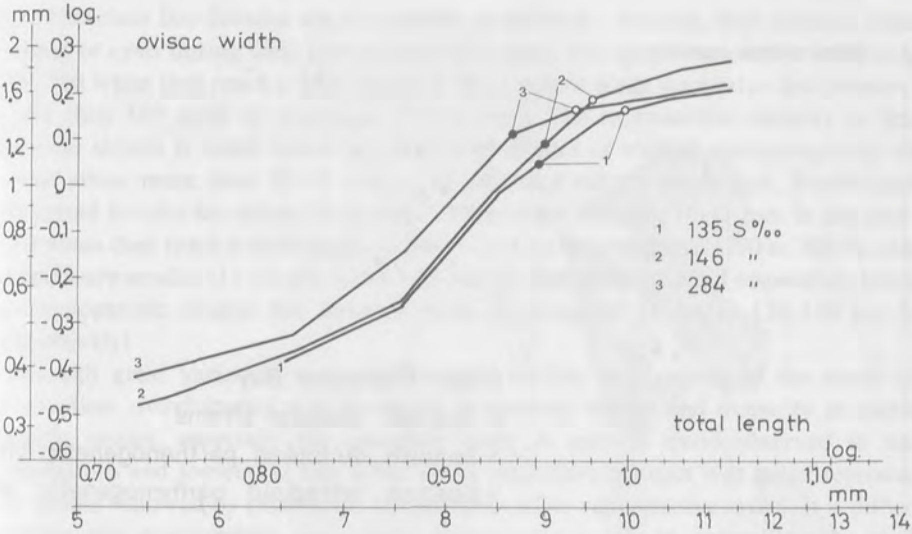

FIG. 20. Width of the ovisac and rate of sexual maturity *versus* total length in Salinas Maritimas-Calpe *Artemia* sampled from salt pans at different salinities (full circles represent a population of which 50% of the females carry their first offspring ; open circles : all females are gravid).

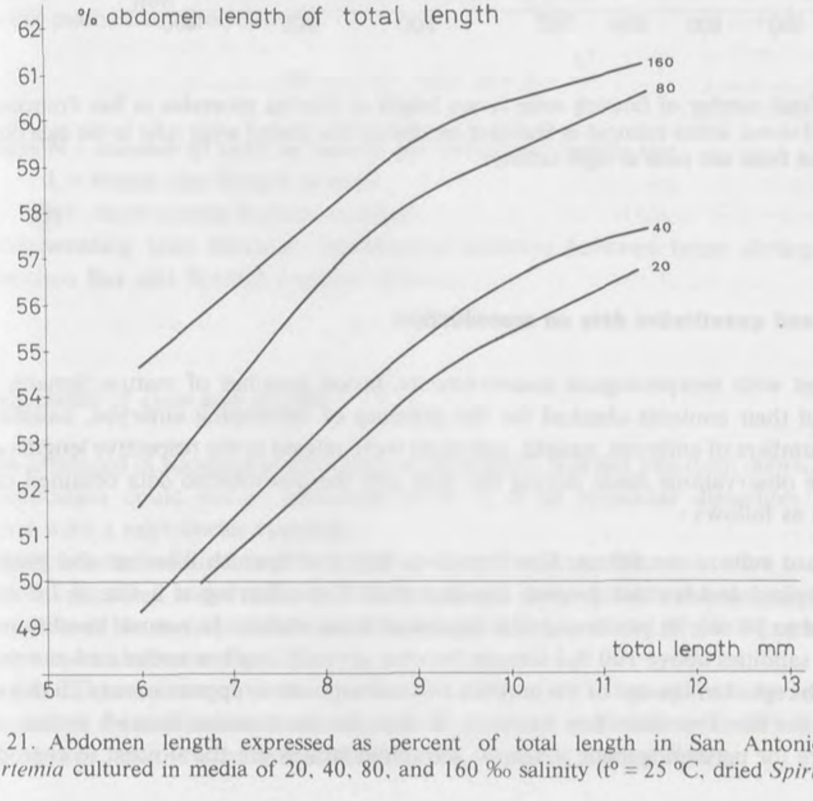

FIG. 21. Abdomen length expressed as percent of total length in San Antonio-Delta del Ebro-*Artemia* cultured in media of 20, 40, 80, and 160 ‰ salinity (t° = 25 °C, dried *Spirulina* food).

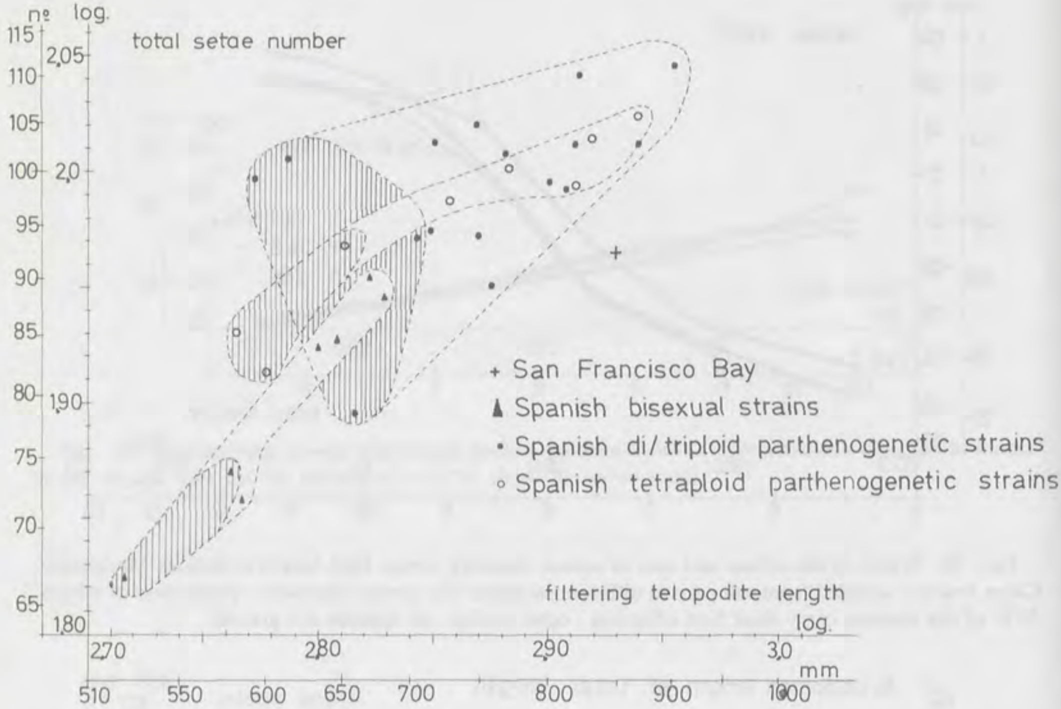

FIG. 22. Total number of filtering setae *versus* length of filtering telopodite in San Francisco Bay and Spanish *Artemia* adults cultured in Standard conditions (the shaded areas refer to the data obtained for individuals from salt pans at high salinity).

Qualitative and quantitative data on reproduction

Concurrent with morphological measurements, brood pouches of mature females were dissected and their contents checked for the presence of developing embryos, nauplii, and cysts. The numbers of embryos, nauplii, and cysts were related to the respective lengths of the females. The observations made during the tests and the quantitative data obtained can be summarized as follows :

1. In standard culture conditions. San Francisco Bay and Spanish bisexual and parthenogenetic diploid and triploid *Artemia* produce their first offspring at a size of 7-8 mm as compared to 10 mm in parthenogentic tetraploid brine shrimp. In natural conditions (salt ponds at salinities above 100 ‰) females become sexually mature earlier and at a smaller size. With regard to the age of the animals this corresponds to approximately 22 days after hatching for San Francisco Bay *Artemia*, 28 days for the bisexual Spanish strains, and a little more for parthenogenetic *Artemia* ; tetraploid strains are the slowest to reproduce.

2. San Francisco Bay females are remarkably proliferous : whereas they produce about 50 nauplii or cysts during their first reproductive cycle, this figure increases to an average of 300-350 when they reach a total length of 14-15 mm (it is not unusual to find females with more than 500 cysts or nauplii in their ovisac). The reproductive capacity in Spanish *Artemia* strains is much lower : bisexual and diploid or triploid parthenogenetic strains never attain more than 12-13 mm under standard culture conditions. Parthenogenetic tetraploid females lay about 30 nauplii or cysts when they are 10-11 mm in size and 150-160 when they reach a total length of 16-17 mm. In natural brines (100 to 300 ‰ salinity) animals are smaller (11-12 mm and 13-16 mm in diploid and triploid respectivey tetraploid parthenogenetic strains) but produce more offspring (45-50 up to 120-130 per brood respectively).
3. Although great variation was noted among strains with regard to the mode of reproduction, ovoviviparity was dominant in bisexual strains and oviparity in parthenogenetic strains, especially the tetraploid ones. A general trend observed in natural populations and sometimes also noted in the laboratory cultures was initial reproduction by nauplii followed by production of cysts after a few reproductive cycles. It is difficult to explain this phenomenon since many factors play a role in controlling the mode of reproduction, *e.g.* salinity of the medium, food quality, environmental conditions, intrinsic factors related to the genetical constitution of the strains studied, *etc.*
4. The number of cysts or nauplii per brood can be allometrically related to the female's size and to the haploid chromosome number (Margalef, 1953). This relation can be expressed by the mathematical equations :

$$N = a \times L^3 \quad \text{and} \quad N = b \times \frac{L^3}{n}$$

where N = number of cysts or nauplii per brood and female size
L = female size (length in mm)
n = chromosome haploid number.

5. Cross-breeding tests revealed reproductive isolation between brine shrimp from San Francisco Bay and Spanish bisexual *Artemia*.

Characteristics of cysts and nauplii

Cysts produced in standard culture tests were isolated, washed free from debris, and before any dessication could occur, measured under a Wild binocular dissection microscope equipped with a micrometer eyepiece.

Nauplii collected within 2 hr after hatching or removal from the ovisac were narcotized in a chloroform-saturated water solution and their length determined under the microscope.

The results of the measurements are summarized in Tables II and III. Histograms of the size of the cysts are shown in Fig. 23.

The size differences are obvious : San Francisco Bay cysts and nauplii are the smallest of all strains studied. In the Spanish strains bisexual *Artemia* are smaller than parthenogenetic brine shrimp ; in the latter group the tetraploid strains score the highest figures.

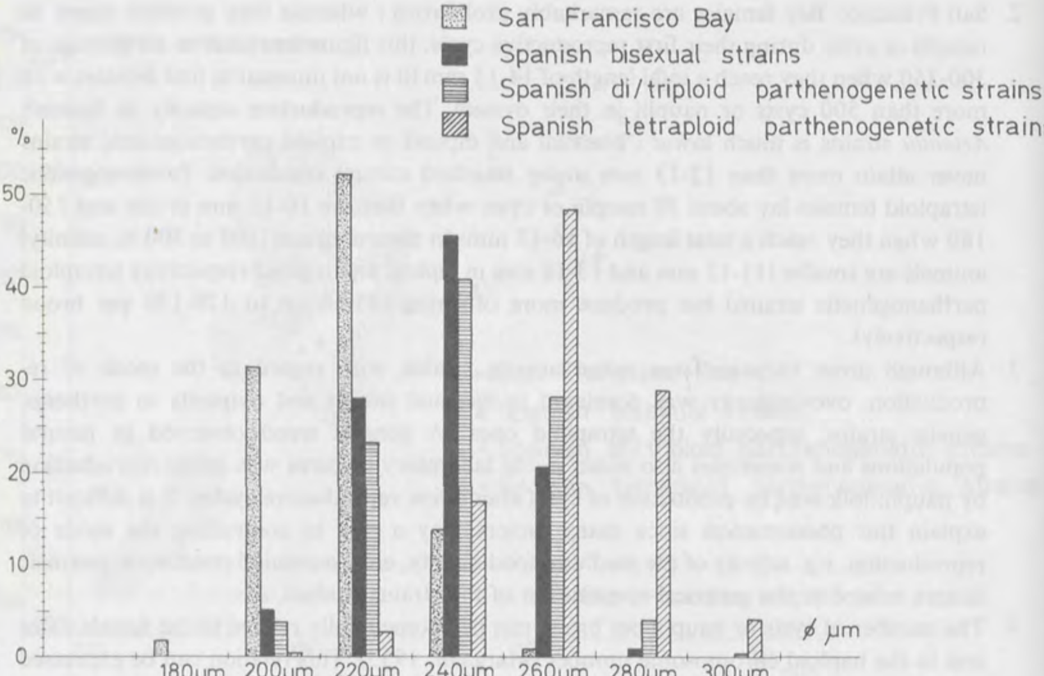

FIG. 23. Size frequency distribution of cysts harvested from standard culture tests of San Francisco Bay and Spanish *Artemia* strains.

It is interesting to note that ovoviviparous nauplii are larger than nauplii hatched from cysts ; this can perhaps be explained by the more energy-expensive mode of reproduction in oviparity as compared to ovoviviparity (Clegg, 1964, 1974 ; Morris, 1971).

TABLE II

Mean diameter and cyst volume of the San Francisco Bay strain and Spanish strains (harvested from standard cultures at 30-32 ‰ salinity)

Strains	Mean diameter (µm)	Mean volume (10^6 µm^3)	Number of cysts measured
San Francisco Bay	216	5.27	1 080
Spanish			
Bisexual diploid	236	6.88	4 625
Parthenogenetic diploid and triploid	240	7.23	7 498
Parthenogenetic tetraploid	262	9.41	4 558

TABLE III

Average length of ovi- and ovoviviparous nauplii from the San Francisco Bay strain and from Spanish strains

Strains	Oviparous nauplii (μm)	Number measured	Ovoviviparous nauplii (μm)	Number measured
San Francisco Bay	412.27	417	421.57	114
Spanish				
Bisexual diploid	445.95	366	474.57	328
Parthenogentic diploid or triploid	447.72	1 075	476.48	1 455
Parthenogentic tetraploid	469.70	431	501.19	134

Cytogenetical characteristics

To obtain more information to classify the strains studied into bisexual diploid, parthenogenetic diploid or triploid and parthenogenetic tetraploid, either chromosome counting or nuclei surface area measurements are necessary. Since the classic squash and orcein staining method proved to be laborious and unreliable, a method was worked out to measure the surface area of nuclei in epithelium cells of the digestive tract extending from the 3rd to the 5th abdominal segment. Dissected abdomens were fixed in Bouin's solution, dehydrated, inbedded in parafin, cut in sections of 7 μm, stained with Weighert's and Mallory's hematoxylin, and finally mounted in Canada balsam. Elliptically outlined sections of nuclei were measured under a Wild microscope, with a calibrated eyepiece, at a magnification of 1 500 X. 200 measurements were performed for each strain or group of strains.

The following strains were studied :

- San Francisco Bay, California, USA ;
- bisexual strains from San Felix, Spain and San Fernando, Spain ;
- parthenogenetic strains from the Spanish salinas Maritimas-Calpe, Cabo de Gata, and Ayamonte ;
- parthenogenetic strains from San Antonio-Delta del Ebro, Spain and Comacchio, Italy.

The numerical data obtained are summarized in Table IV. The bisexual diploid San Francisco Bay strain has the smallest nuclei of all strains studied ; bisexual *Artemia* from Spain have a slightly larger nucleus and a wide variation in nucleus size is found among the parthenogenetic groups. The data allow to make a distinction between diploid bisexual and parthenogenetic strains (16 to 21 μm^2) and the tetraploid parthenogenetic group of strains from San Antonio-Delta del Ebro, Alcochete, Larache, and Comacchio. In fact the ploidy nature of the Comacchio strain was already known from the work by Artom (1922), Stella (1933) and Barigozzi (1957).

The small differences in nuclei surface areas are considerably increased when the data are converted into nuclei volumes :

TABLE IV

Nuclei area of epithelium cells of the digestive tract of bisexual and parthenogenetic *Artemia* groups

Strains	Mean nuclei surface area ± s.d. (μm^2)
San Francisco Bay	16.41 ± 2.52
Bisexual strains from San Felix and San Fernando	17.96 ± 3.26
Parthenogenetic strains from Salinas Maritima-Calpe	19.05 ± 3.27
Cabo de Gata	19.89 ± 2.61
Ayamonte	21.67 ± 3.85
Parthenogenetic strains from San Antonio-Delta del Ebro and Comacchio	37.77 ± 6.77

- the Ayamonte strain has a nucleus volume which is 1.5 times larger than that calculated for the Spanish bisexual strains ; this suggests triploidy in the Ayamonte strain (to be confirmed by chromosomal counts) ;
- the San Antonio strain has a nucleus volume 3 times that of the Ayamonte strain, suggesting the existence of a higher ploidy.

Such wide variation in ploidy is very common in parthenogenetic animals and plants (Margalef, 1974).

Conclusion

All the data gathered in this study with *Artemia* strains from Nearctic and Palearctic regions allow to distinguish at least two different species :

- *Artemia salina* (Linnaeus, 1758) Leach 1819, bisexual diploid, distributed all around the Palearctic region (North Africa, Spain, Mediterranean area in general)
- *Artemia gracilis* (Verrill, 1869), bisexual diploid, distributed in the Nearctic region, mainly the United States of America, the Caribbean Sea islands and the northern part of South America.

The parthenogenetic strains in the Palearctic region probably have their origin in the bisexual diploid strain as a result of chromosome mutations (Stefani, 1964).

It is difficult to say when or how these mutations occurred ; they have resulted, however, in a genetical barrier between bisexual and parthenogenetic forms that precluded their further crossing.

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