

International study on *Artemia*. LVI. Characterization of two *Artemia* populations from Namibia and Madagascar: cytogenetics, biometry, hatching characteristics and fatty acid profiles

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Abstract

Two parthenogenetic *Artemia* populations from southern Africa, one from Swakopmund saltworks (Namibia) and another from Ankiembe saltworks (Madagascar) have been studied. The population from Namibia is mainly diploid ($2n=42$) with few tetraploid individuals ($4n=84$), while the one from Madagascar was found to be triploid ($3n=63$). No chromocenters have been observed in either of the two populations. The Namibian population has smaller cysts and nauplii compared to those of the Madagascar population. Discriminant analysis revealed significant differences in the biometry of the adults from the two populations. The two populations exhibited very good hatching characteristics. The study of fatty acid methyl esters revealed that the Namibian population belongs to the 'fresh water' type of *Artemia* showing low levels of eicosapentaenoic acid, whereas the population from Madagascar displayed exceptionally high levels of eicosapentaenoic acid, belonging to the 'marine water' type.

Introduction

The brine shrimp *Artemia* is widely distributed on every continent except Antarctica (Browne & MacDonald, 1982) and is of considerable economic importance in fish and shellfish larviculture (Bengtson et al., 1991). Although the distribution of *Artemia* is well studied in many countries in the Americas, Europe and Australia, very few reports exist on the occurrence of *Artemia* in Africa. Moreover, little work has been done to characterize the known populations that exist in Africa.

Mariculture of finfish and Crustacea uses freshly-hatched nauplii of brine shrimp as part of the live food chain. Annual *Artemia* cyst consumption by aquaculture hatcheries increased dramatically from 60 metric tones in 1980 (Bengtson et al., 1991) to about 2000

metric tones by 1994 (Triantaphyllidis et al., 1994). Increased demands for fish fry and shrimp postlarvae as well as the expected extension of the list of new commercially cultured species (e.g. mahi-mahi, grouper, halibut) will increase *Artemia* cyst demand in the coming years (Bengtson et al., 1991). Nowadays, there is a serious shortage of *Artemia* cysts resulting from poor harvests last winter in the Great Salt Lake (Utah, USA), the single source responsible for about 95% of cyst production in the market (Sorgeloos, 1995). This shortage of cysts reinforces earlier studies that urged attention to focus on exploitation and development of alternative or complementary sources of cysts (Sorgeloos, 1979; Bengtson et al., 1991), in order to avoid a serious bottleneck in many aquaculture developments.

We have recently studied two native south African *Artemia* populations, one from Namibia and one from Madagascar. The first written report about the presence of *Artemia* in Namibia dates back to 1986, when a sample of cysts from Vineta, Swakopmund, was sent to the *Artemia* Reference Center (ARC) for analysis and reported to be a parthenogenetic population (Sorgeloos, 1986). Less information is available for *Artemia* in Madagascar. Vanhaecke et al. (1987) reported the existence of an *Artemia* population in Salins de Diego Suarez, Madagascar, based on a personal communication.

The present report provides the first findings on the chromosome numbers of two *Artemia* populations from Namibia and Madagascar, the biometric characteristics of cysts, instar-I nauplii and adults, and compares these with other, previously studied, populations. The hatching characteristics of cysts, such as hatching rate, hatching percentage and hatching efficiency and the fatty acid profiles of instar-I nauplii are also studied.

Materials and methods

The cysts from Namibia (SWA population) have been provided by the Salt Company and collected from the saltworks of Swakopmund (ARC cyst bank number No: 1186). The cysts from Madagascar (ANK population) were collected in June 1992 from the Ankiembe saltworks, situated 5 km south of the city of Toliara (ARC No: 1314). Upon their arrival in ARC, the cysts were stored at -10°C in plastic bags under vacuum.

Decapsulation of cysts was performed according to Sorgeloos et al. (1986). Decapsulated and non-decapsulated cysts were hydrated in a 10 ppt artificial Dietrich & Kalle (D & K) medium (Kalle, 1971) which was prepared following the modifications of Vanhaecke et al. (1984) and filtered through a $0.45\ \mu\text{m}$ cartridge filter (Sartobran[®]-PH capsule from Sartorius). The cysts measured under a microscope equipped with an eyepiece containing a graticule. The graticule calibrated against a standard and the measurements had an accuracy of $1\ \mu\text{m}$. Instar-I nauplii were fixed in 1% lugol solution at 35 ppt D & K medium and measured under a microscope to the nearest μm .

For the biometry of adults, cysts were incubated in 35 ppt D & K medium. The hatched nauplii were transferred to one litre cylindrical glass tubes containing $0.45\ \mu\text{m}$ filtered D & K medium of 50 ppt salinity and initial density of 2 nauplii per ml. The density was reduced after day 8 to one animal per 4 ml. The temper-

Table 1. Mean diameter of cysts and decapsulated cysts and length of oviparous instar-I nauplii from Swakopmund (Namibia) and Ankiembe (Madagascar).

Population		Sample size	Mean (in μm)	Standard deviation
Namibia	Cysts	501	246.7	11.0
	Decapsulated cysts	500	233.1	9.8
	Instar-I nauplii	300	449.6	20.0
Madagascar	Cysts	500	258.9	11.6
	Decapsulated cysts	501	246.2	11.7
	Instar-I nauplii	500	491.4	26.6

ature was maintained at $25 \pm 1^{\circ}\text{C}$, and mild aeration was applied from the bottom of the tubes, which were covered with perforated Petri dishes to minimize evaporation. For each population three replicates were set up. The animals were fed on a mixed diet of the alga *Dunaliella tertiolecta* Butcher and the yeast-based formulated feed LANSY PZ (INVE Aquaculture SA, Belgium), following the feeding schedule of Triantaphyllidis et al. (1995). Survival was monitored at each water renewal i.e. on day 8, 11 and every 3 to 4 days thereafter until the animals matured and started to reproduce. As soon as the animals started releasing their offspring they were examined for the following morphometric parameters: total length, abdominal length, length from the third abdominal segment to the end of the abdomen, length of the eighth abdominal segment, width of the ovisac, width of the head, width of the third abdominal segment, length of furca, number of setae on each branch of the furca, length of the first antenna, maximum distance between the compound eyes and maximum diameter of the compound eye. The animals were anaesthetized in chloroform-saturated seawater (Gilchrist, 1960) and measured under a dissection microscope equipped with a camera lucida and using a digitizer. The populations were compared by means of analysis of variance (Anova) and the multivariate procedure of discriminant analysis following Hontoria & Amat (1992a, b) and Triantaphyllidis et al. (1995).

Hatching efficiency, hatching percentage and hatching rate were analysed following Sorgeloos et al. (1978), Bruggeman et al. (1980) and Vanhaecke and Sorgeloos (1982).

Chromosome number and chromocenters were studied using instar-I nauplii, following the method of Abatzopoulos et al. (1986). *Artemia* nauplii contain cells with various ploidy levels. Since a diploid nau-

plius has some polyploid cells (e.g. nurse cells; Criel, 1991), examination of a sole mitosis could give false results. For this reason we examined several mitoses per nauplius, excluding from the results observations of only one mitosis per preparation. All preparations were studied under a Leitz Laborlux-D optical microscope equipped with a purpose-built Wild MPSS1 photomicrographic camera connected to a WildMPS45 camera control box and photographed with an Agfapan APX25 professional film.

Fatty acid methyl esters (FAME) were prepared through direct acid-catalysed transesterification following a modified method of Lepage & Roy (1984). An internal standard, 20:2(n-6), was added before the reaction. FAME were extracted with hexane. After evaporation of the solvent, FAME were prepared for injection by redissolving them in iso-octane (2 mg ml⁻¹). Quantitative determination was performed by a Chrompack CP9001 gas chromatograph equipped with an autosampler. Injections (of 0.2 µl each) were performed into a polar 50 m capillary column BPX70 (SGE Australia), of 0.32 mm diameter and a layer thickness of 0.25 µm, connected to a 2.5 m methyl-deactivated precolumn. The carrier gas was hydrogen at a pressure of 100 Kpa and the detection mode FID. The oven temperature was programmed to rise from the initial 85 °C to 150 °C, at a rate of 20 °C min⁻¹, from 150 °C to 152 °C at 0.1 °C min⁻¹, from 152 to 174 °C at 0.7 °C min⁻¹, from 174 to 180 °C at 10 °C min⁻¹ and to remain at 180 °C for 2 minutes. Identification was based on standard reference mixtures (Nu-Chek-Prep, Inc., USA). Integration and calculations were performed on a 486 computer, using the 'Maestro' software (Chrompack).

Data analyses followed Sokal and Rohlf (1981), using the statistical packages Statistica (release 4.3) and SPSS (release 6.0) in their Windows' versions.

Results and discussion

The two populations studied inferred to be parthenogenetic since no males were found in the cultures. Cysts of both populations were also incubated at 36.5 °C for 48 hours, in order to check for possible contamination with *A. franciscana* cysts (Triantaphyllidis et al., 1994). However, no nauplii emerged, suggesting that both samples were free from *A. franciscana* material.

Cytogenetics

The Namibian population was inferred to be diploid (2n=42) after examination of 66 nauplii, although some rare tetraploid nauplii (4n=84) were observed (6 out of 66). The population from Madagascar was inferred to be triploid (3n=63) after examining 78 nauplii. In both populations no distinct chromocenters were observed in the nuclei, such as those exhibited in different populations of *A. franciscana* and *A. persimilis* (Barigozzi & Baratelli Zambruni, 1982). Figure 1 shows a diploid (plate a) and a tetraploid nucleus (plate b) from Namibia, while Figure 2 depicts a triploid nucleus from Madagascar.

Biometry of cysts and instar-I nauplii

The results of the biometry of cysts and instar-I nauplii are presented in Table 1. Figure 3 shows the size frequency distribution of the decapsulated and non-decapsulated cysts. The cysts from Namibia, measured both with chorion and decapsulated, were significantly smaller compared to the cysts from Madagascar (Anova, $P < 0.05$). The cyst diameter data fit to the normal distribution as the Chi squared as well as the non-parametric Kolmogorov-Smirnov tests revealed (Figure 3). Values for skewness (a measure of the asymmetry of the distribution) and kurtosis (a measure of the extent to which a distribution is 'tail heavy' compared to a normal distribution) were not significantly different from zero, suggesting that the distributions indeed fit to the normal distribution.

The two populations exhibit notable differences in their cyst diameter and length of instar-I nauplii. These differences can be attributed to the different ploidy levels that characterize each population, which affect the size of the cells and thus the diameter of cysts as well as the size of instar-I nauplii.

The population from Namibia has a mean cyst diameter of 246 µm, and together with the Spanish diploid populations (Amat, 1980), they show the smallest values that have ever been recorded for parthenogenetic *Artemia*. The size of the cysts from Namibia is comparable to that of the Great Salt Lake *A. franciscana* cysts (Vanhaecke & Sorgeloos, 1980) which are one of the main sources of brine shrimp cysts for use in aquaculture (Triantaphyllidis et al., 1994). This feature makes the population from Namibia very attractive for commercial use. Conversely, the population from Madagascar has larger cysts compared to those of the Namibian population and other bisexual populations.

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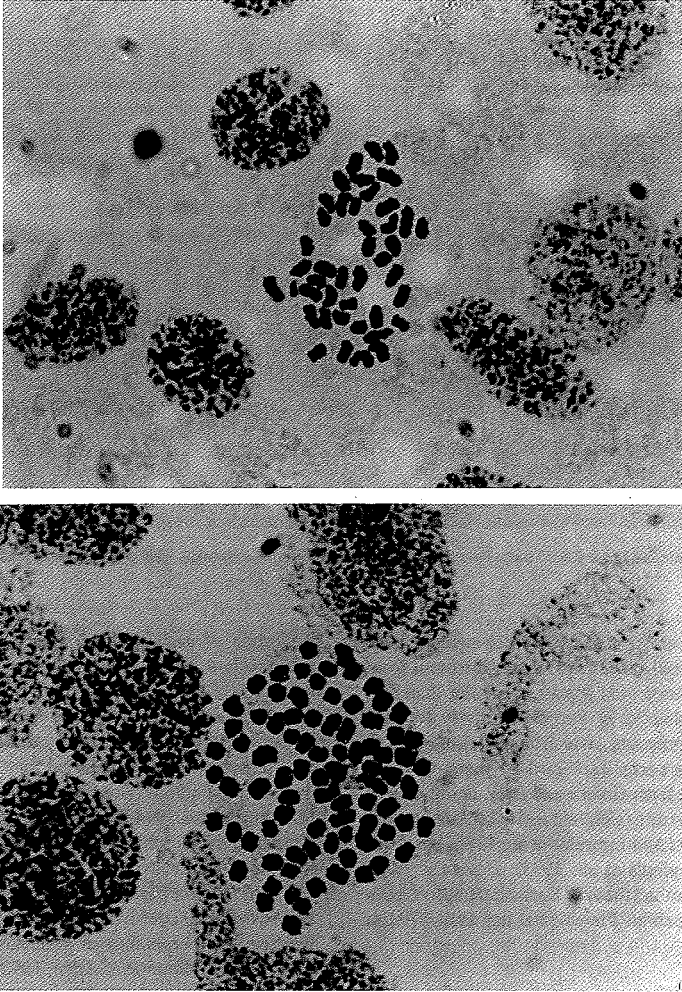


Figure 1. Acetic orcein-fast green stained metaphases from Namibia (Swakopmund saltworks). This population exhibited mainly diploid (plate a) and a few tetraploid (plate b) nuclei. No distinct chromocenters were observed.

However, these are quite small for parthenogenetic and polyploid *Artemia*, since Vanhaecke & Sorgeloos (1980) reported parthenogenetic cyst diameters of up to 284.9 μm for the Margherita di Savoia population (Italy) and 283 μm for a population from Tuticorin (India). The triploid population from Madagascar has a cyst diameter more closely resembling that of Spanish and Greek polyploid (mainly tetraploid) populations

(Amat, 1980; Abatzopoulos et al., 1989; Triantaphyllidis et al., 1993).

Instar-I nauplii from Namibia were significantly smaller than the ones from Madagascar (Anova, $P < 0.05$). Chi squared and Kolmogorov-Smirnov tests showed that both populations fitted to the normal distribution (see Figure 4). The triploid ANK population exhibited larger nauplii than the mainly diploid SWA population. The length of instar-I nauplii from SWA is

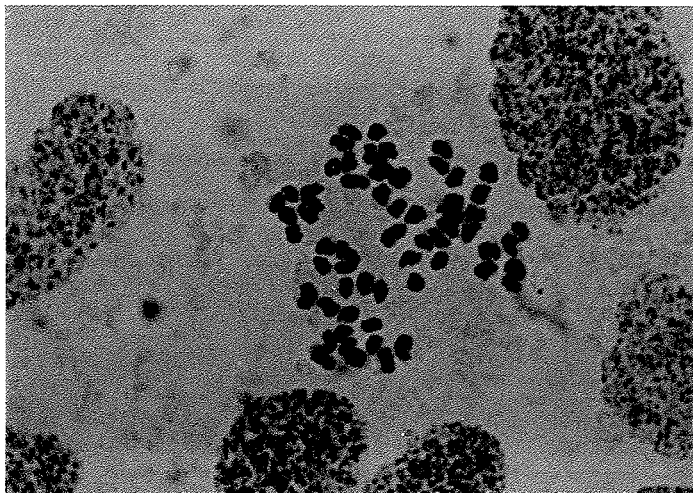


Figure 2. Acetic orcein-fast green stained metaphase from Madagascar (Ankiembe saltworks). This population was found to be consisted of triploid individuals.

similar to those of Spanish diploid populations studied by Amat (1980). The mean length of nauplii from the ANK population ($491.4 \mu\text{m}$) is smaller than that reported by Vanhaecke & Sorgeloos (1980) for other polyploid populations, which ranged from 509 to $521 \mu\text{m}$. This is partly explained by many of these populations, e.g. from Greece and Italy, being mainly tetraploid or a mixture of diploid and tetraploid individuals respectively (Abatzopoulos et al., 1986; Baratelli, 1987; Triantaphyllidis et al., 1993). However, ANK nauplii are larger than those of Spanish tetraploid populations, which exhibit a mean total length of $470 \mu\text{m}$ (Amat, 1980).

Biometry of adults

The mean values of the various morphological parameters measured are shown in Table 2. Analysis of variance revealed statistically significant differences between the two populations for most of the characteristics studied, except for the width of the head and the length of the antennae. The characters that contributed most to the discrimination between the two populations (and thus resulting in the highest F ratios) were the length of the telson, the length from the third abdominal segment to the end of the abdomen and the abdominal length. Discriminant analysis using

the origin of each population as a separation criterion resulted in one canonical discriminant function and 100% of grouped cases correctly classified to their own population. The use of the multivariate procedure of discriminant analysis has produced useful results in discriminating both bisexual and parthenogenetic *Artemia* populations (Hontoria & Amat, 1992a, b; Pilla & Beardmore, 1994; Triantaphyllidis et al., 1995). In this study, the morphometrical discrimination between the two populations proved to be very straightforward. The difference in ploidy levels between the two populations certainly contributed to this, as ploidy is known to affect the morphology of parthenogenetic *Artemia* (Amat, 1980; Hontoria & Amat, 1992a).

Hatching characteristics

The cysts from both sources displayed a very good hatching quality (Table 3). Contrary to what is observed in several other populations, incubation at a lower salinity of 5 ppt did not improve the hatching percentage nor the hatching efficiency ($P > 0.05$) (Vanhaecke & Sorgeloos, 1983). The study of the hatching rate curves revealed that the SWA population has better hatching synchrony (6.5 hours) compared to the ANK population (11 hours).

Table 2. Mean values and standard deviations of morphometric and meristic characters of adult animals from Swakopmund, Namibia and Ankiembe, Madagascar (n = number of animals analysed). A: total length, B: abdominal length, C: length from the third abdominal segment to the end of the abdomen, D: length of the eighth abdominal segment, E: width of third abdominal segment, F: length of furca, G: width of head, H: length of first antenna, I: distance between eyes, J: diameter of complex eye, K: number of setae on the left branch of the furca, L: number of setae on the right branch of the furca, M: width of ovisac. F is the ratio between the groups mean square and the within groups mean square and is an indication of the extent of the differences between the two means. Wilks' lambda (or U statistic) has values between 0 and 1. A lambda value of 1 occurs when all observed group means are equal (Norusis, 1993).

Morphometrical parameter	Namibia ($n=32$)	Madagascar ($n=31$)	F ratio	Wilk's lambda	F probability
A	9.68±0.51	11.53±0.55	183.45	0.2495	***
B	4.63±0.35	6.33±0.42	295.95	0.1709	***
C	3.41±0.25	5.00±0.35	436.81	0.1225	***
D	0.90±0.09	1.38±0.14	273.42	0.1824	***
E	0.77±0.05	0.66±0.04	103.71	0.3703	***
F	0.49±0.04	0.27±0.03	548.49	0.1001	***
G	0.92±0.04	0.93±0.04	1.69	0.973	n.s.
H	1.20±0.05	1.20±0.08	0.04	0.9994	n.s.
I	1.63±0.06	1.80±0.08	86.52	0.4135	***
J	0.27±0.02	0.29±0.02	4.74	0.9277	*
K	8.84±1.17	7.90±1.25	9.22	0.8687	**
L	9.31±1.53	7.52±1.34	23.81	0.7192	***
M	2.15±0.11	1.98±0.18	18.83	0.7641	***

*** = $P < 0.001$

** = $P < 0.01$

* = $P < 0.05$

n.s. = not significant

Table 3. Hatching characteristics of *Artemia* cysts from Swakopmund (Namibia) and Toliara (Madagascar).

Hatching characteristics	Namibia	Madagascar
<i>Hatching percentage</i>		
35 ppt	87.75 (±2.26)	85.22 (±3.35)
5 ppt	90.76 (±1.85)	83.49 (±2.80)
<i>Hatching efficiency</i>		
35 ppt	226,667 (±29,814.25)	216,000 (±15,379.99)
5 ppt	234,442 (±30,836.94)	194,133 (±33,587.30)
<i>Hatching rate characteristics*</i>		
T0	18	17
T10	19.5	18
T90	26	29
Ts	6.5	11

* Values refer to the time period (in hours) from the beginning of incubation until the appearance of the first nauplii (T0), or the incubation time til the appearance of 10% (T10) and 90% (T90) of the hatching efficiency. Ts = T90 - T10 and is a measure of the hatching synchrony.

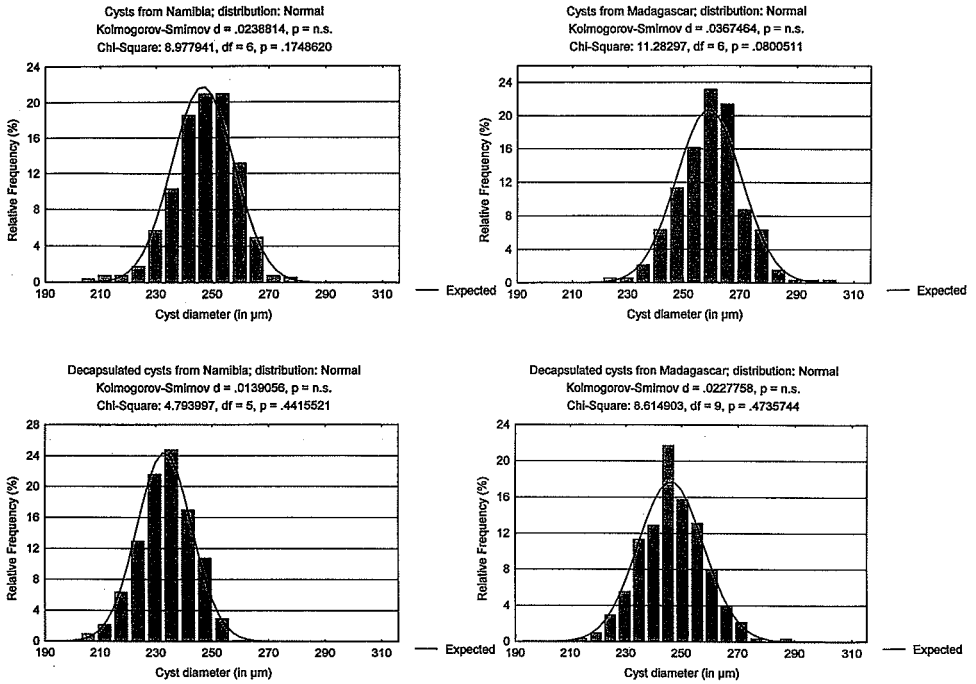


Figure 3. Size frequency distribution of the diameter of non-decapsulated (top) and decapsulated cysts (bottom) from Swakopmund (Namibia) and Ankiembe (Madagascar).

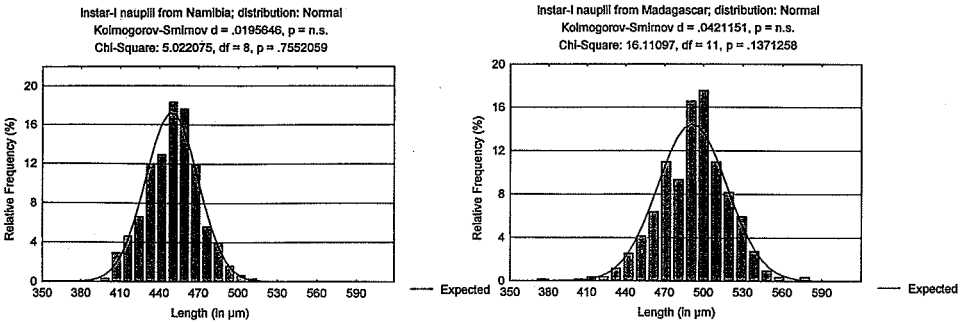


Figure 4. Size frequency distribution of instar-I naupliar length from Swakopmund, Namibia (a) and Ankiembe, Madagascar (b).

FAME analyses

The FAME analysis of freshly-hatched instar-I nauplii revealed significant differences between the two populations. The levels of 18:3(n-3) (linolenic acid) and 18:4(n-3) were much higher in the SWA population

than in the ANK population. Conversely, the levels of 20:5(n-3) (eicosapentaenoic acid) were very low in the SWA population and exceptionally high in the ANK population. The total highly unsaturated fatty acids (HUFA) were highest in the ANK population.

Table 4. Fatty acid methyl esters (FAME) of instar-I *Artemia* nauplii hatched from cysts collected in Swakopmund (Namibia) and Ankiembe (Madagascar). The results are means from two analyses. The asterisks denote statistically significant differences between the two populations (t-test, $P=0.05$).

FAME	Namibia		Madagascar	
	Area percent	mg g ⁻¹ dry weight	Area percent	mg g ⁻¹ dry weight
14:0*	1.15	1.45	2.35	3.3
14:1(n-5)	1.3	1.8	1.15	1.65
15:0*	0.2	0.3	0.75	1.05
15:1(n-5)	0.55	0.7	0.45	0.6
16:0	13.4	18.15	13.15	18.55
16:1(n-7)*	5.4	7.25	14.35	20.2
17:0	0.5	0.65	0.8	1.15
17:1(n-7)*	1.15	1.55	1.8	2.55
18:0	4.55	6.15	5.2	7.35
18:1(n-9)	16.9	22.9	15	21.15
18:1(n-7)*	9.75	13.15	12.8	18.15
18:2(n-6)-c	7.45	10.05	5.25	7.4
18:3(n-6)	0.55	0.75	0.4	0.55
18:3(n-3)*	26.55	35.85	4.45	6.25
18:4(n-3)*	3.85	5.2	0.6	0.9
19:1(n-9)	0.4	0.55	nd	nd
20:1(n-9)*	0.55	0.75	0.2	0.2
20:4(n-6)	1.05	1.35	1.15	1.6
20:3(n-3)	0.1	0.1	nd	nd
20:4(n-3)	0.1	0.1	0.1	0.2
20:5(n-3)*	1	1.35	17.3	24.45
Sn-3:≥20:3(n-3)*	1.25	1.7	17.45	24.65
Total FAME*		135.2		141.4

nd: not detected

The results of the FAME analyses are summarized in Table 4.

The study of the fatty acid methyl esters of the two populations revealed that the SWA population, due to the high content of 18:3(n-3) and the low levels of 20:5(n-3), is suitable for use as food source for freshwater organisms, according to the classification proposed by Watanabe et al. (1978). Conversely, the ANK population exhibited high levels of 20:5(n-3) which, to our knowledge, are the highest ever reported in the literature and can be classified as belonging to the 'marine' type *Artemia*.

Why has the ANK population such a high 20:5(n-3) content? The fatty acid composition of *Artemia* is considered to be more environmentally than genetically determined (Bengtson et al., 1991). Millamena et al. (1988) and Lavens et al. (1989) demonstrated that the fatty acid profiles of *Artemia* adults and the cysts they produce strongly reflect the fat-

ty acid profile of their diet. This finding confirmed later on by Navarro and Amat (1992) and Navarro et al. (1993). Therefore, one should primarily seek the reasons of high HUFAs content in the habitat conditions. In Madagascar, production of cysts takes place in relatively low salinity ponds, i.e. from 80–100 ppt. Under these conditions, it is possible that microalgal species such as diatoms, some Haptophyceae (Prymnesiophytes) and most Cryptophytes – which contain significant amounts of eicosapentaenoic acid and docosahexaenoic acid (Volkman et al., 1989) – are present and enhance the nutritional value of *Artemia*. Moreover, it has been reported that the total lipid content of the diatom *Navicula* sp. increased when the salinity of the medium increased from 30 to 100 ppt, but declined at 146 ppt (Al-Hasan et al., 1990), while the total fatty acid content of the marine alga *Porphyridium cruentum* (Rhodophyceae) increased in salinities from 26 to 88 ppt (Lee et al., 1989). In addition, these studies

showed that the levels of poly-unsaturated fatty acids either remained constant or increased slightly as the salinity went up. In salinities above 120 ppt halophyte green microalgae, such as *Dunaliella* sp., (which are known to be low in HUFA content) prevail (Volkman, 1989). Further studies of the microalgal composition of the Madagascar saltworks are needed to isolate the species that are responsible for the high eicosapentaenoic acid levels in *Artemia*.

We think that characterizing new *Artemia* populations must be a multidisciplinary approach. It is essential to apply various techniques although these methods can be seemingly disparate. It is important, for example, to know if a population is a 'mixture' of individuals with different ploidy level. Focusing, especially, on mixed populations, such as the one from Namibia, we must be very careful to use the term population characterization if the whole study is based upon one sample or batch; one should take into consideration the difficulty to obtain samples from distant areas. Therefore, it could be better to refer to it as 'batch characterization'.

Conclusions

The two *Artemia* populations from Namibia and Madagascar showed marked differences in their biometric and morphological characteristics. The key parameter that affects these characteristics is probably the ploidy level. Chromosome numbers, clearly affected the size as well as the 'appearance' of the adults, and enabled discrimination of the two populations with a very high degree of certainty. The biometric characteristics of the SWA population are of great importance for its 'commercial' exploitation: the size of cysts and nauplii are among the smallest known for parthenogenetic populations. The very small size of cysts and nauplii of the SWA population, together with the exceptionally high level in 20:5(n-3) of the ANK population makes these *Artemia* sources of particular interest for commercial development. Small-scale production in man-made saltworks is technically feasible and successful in several countries in Southeast Asia, especially in Vietnam (Vu Do Quynh, 1987). As Bengtson et al. (1991) mentioned 'contribution to the world cyst supplies might not be significant, however, management strategies could provide interesting opportunities for local commercial developments with restricted import opportunities, and where local availability of *Artemia* cysts is the first requirement in consideration of a viable hatchery industry'.

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The population dynamics of the parasitic copepode *Lernaeocera lusci* (Bassett-Smith, 1896) on its definitive host

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ABSTRACT: The mesoparasitic copepod *Lernaeocera lusci* (Bassett-Smith, 1896) was recovered from first-year bib (*Trisopterus luscus* L.) in the Voordelta (Southern Bight of the North Sea) from May until December 1989. Analysis of the seasonal abundance and of the population structure showed that transmission of infective stages to bib mainly occurred from June to September. From September to December the overall prevalence fluctuated around 70%. Maximum parasite population size ($47/10^4\text{m}^2$) and the highest total egg number were recorded in September and October, respectively. It was found that total parasite mortality was significantly influenced by mortality of hosts carrying parasites. Natural mortality probably contributed a small percentage to total parasite mortality. Calculation of the temporal mean-variance regression equation revealed that the parasites were aggregated within the definitive host population.

INTRODUCTION

Some of the most conspicuous parasite species of the North Sea belong to the genus *Lernaeocera* (Pennellidae, Crustacea). Several authors have studied the population dynamics of *Lernaeocera branchialis*, a pathogenic parasite which infects economically important gadoid species such as whiting (*Merlangius merlangus*) and cod (*Gadus morhua*) (e.g. Whitfield et al., 1988; Pilcher et al., 1989). In contrast, comparatively little information is available on a related species, *Lernaeocera lusci*, which infects the bib (*Trisopterus luscus*). Some authors even doubted the validity of the latter parasite species (Heegaard, 1947; Bastide-Guillaume et al., 1985). Recently, Tirard (1991) studied both species by enzyme electrophoresis and decisively confirmed both *L. branchialis* and *L. lusci* as valid species. She also proposed a morphological distinguishing characteristic: antennary processi are absent in *L. branchialis* but are present in *L. lusci*.

Aspects of the biology of *L. lusci* have been studied by Evans et al. (1983), Eiras (1986) and Tirard (1991). The life cycle of this species was studied in detail by Slinn (1971), who found that sole *Solea solea* (L.) is the typical intermediate host in the North Sea. Several authors reported on the (broad) host specificity towards the definitive host