Consiglio Nazionale delle Ricerche

PROGRAMMA SHORT – TERM MOBILITY – ANNO 2008

Scientific report on the results of the research activity carried out by

dr. Michele Cognetti

at the

Laboratory of Aquaculture & Artemia Reference Center, Faculty of Bioscience Engineering - Department of Animal Production - Ghent University, Rozier 44, B-9000 Gent, Belgium.

REPORT TITLE

Taxonomy and strain studies on cysts of the brine shrimp Artemia from Margherita di Savoia solar Saltworks (Apulia, southern Italy).

Promotor: *Dr. Trotta Pasquale*

User: *Dr. Cognetti Michele*

2008

This report was prepared during the course of a three week's stay at the Laboratory of Aquaculture & Artemia Reference Center (Faculty of Bioscience Engineering - Department of Animal Production - Ghent University, Rozier 44, B-9000 Gent, Belgium). The stay was funded, through a mobility grant, by the Italian National Council of Research (CNR) as a result of positive assessment delivered by the Group of Experts of the Council, under the Short Term Mobility Program 2008.

For bibliographic purposes this document should be cited as follows:

M. Cognetti (2008). Taxonomy and strain studies on cysts of the brine shrimp *Artemia* from Margherita di Savoia solar saltworks (Apulia, southern Italy). Report of the Short Term Mobility Program 2008 (En).

Acknowledgments

I would like to thank the Italian National Research Council (CNR) for the positive assessment gives to the program submitted under the Short Term Mobility Program 2008.

Special thanks to Dr. Pasquale Trotta for having supported and promoted the submission of the application and Prof. Dr. Patrick Sorgeloos for hosting and providing me with all the facilities at Ghent University.

I want express my deep gratitude to Dr. Gilbert Van Stappen, for his timely guidance in planning my experiments and for helping me during my first visit to ARC.

Thanks to Ms. Lucia Longari for having helped and supported me in the submission of the application and thanks to scientific staff, PhD staff and Technical & Administrative staff of the ARC for their help and for their patience in front of my bad English.

Contents

INTRODUCTION

This report describes the results of a 3 weeks study-stay at the Artemia Reference Center (Ghent University, Belgium) in the frame of the Short Term Mobility Program funded by the Italian National Council of Research (CNR). During the period from the 28 of February to the 22 of March 2008, some activities were performed in order to run taxonomy and strain studies on cysts of the brine shrimp *Artemia* from Margherita di Savoia solar Saltworks (Apulia, southern Italy). The studies conducted at the laboratories of the Artemia Reference Center have proved of great importance in order to deepen the knowledge of Artemia of Italy, especially for the population from Margherita di Savoia solar saltworks (the biggest European solar saltworks).

CHAPTER 1

THE BRINE SHRIMP ARTEMIA

1.1. Taxonomy and biogeography of *Artemia*

The systematic classification of the genus is:

Phylum: Arthropoda
Subphylum: Crustacea
Class: Branchiopoda
Order: Anostraca
Family: Artemidae
Genus: Artemia

Artemia is a genus of anostracan crustaceans, commonly known as “brine shrimps”, that inhabitant extreme (hypersaline) habitat, but at the same time able to tolerate physiologically large changes in salinity, ionic composition, temperature and oxygen tension of water.

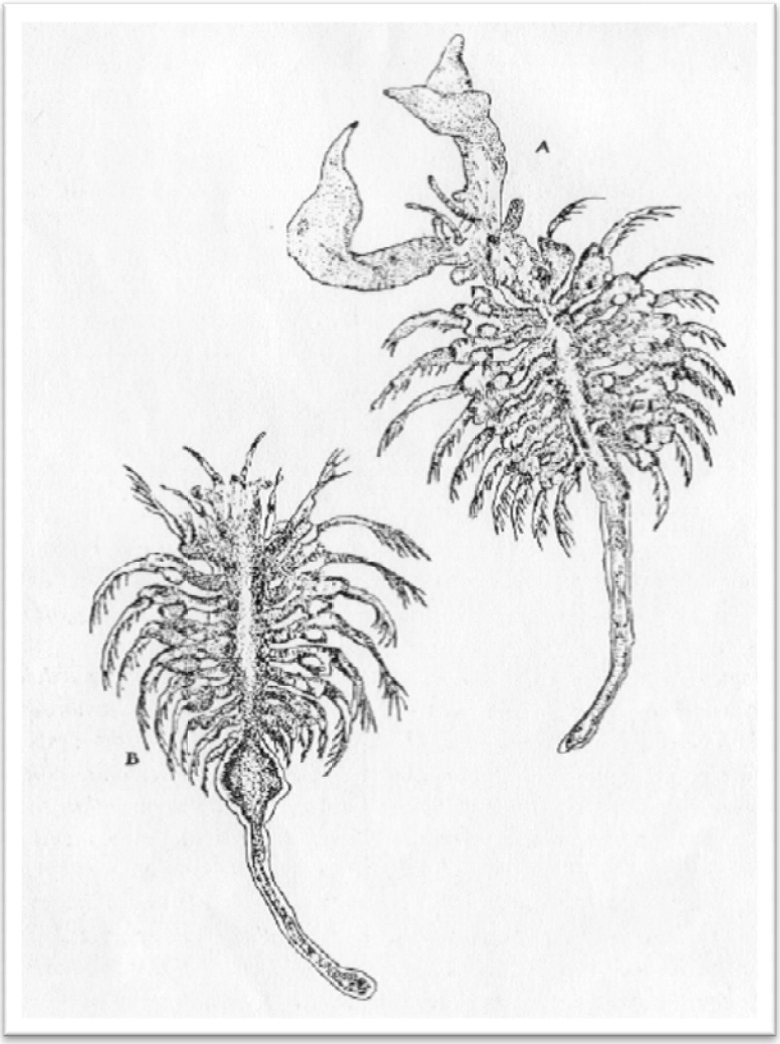


Fig. 1: Schlosser's drawing of a male (A) and a female (B) brine shrimp *Artemia* (From Kuenen and Baas-Becking, 1938).

The genus *Artemia* is a complex of sibling species and superspecies, defined largely, though not completely, by the criterion of reproductive isolation. The earliest scientific reference to brine shrimp is in 1756, when Schlösser reported their existence in the saltpans of Lymington, England. Later on, the profusion of names was abandoned and all brine shrimp was referred to as *Artemia salina* Linnaeus 1758 (Sorgeloos, 1980). Some authors continue this practice today, but must be noted that the scientific community had decided (supported by modern genetical evidence) to utilize the binomen *Artemia salina* only for European bisexual populations. However there are a considerable amount of publications (especially in past decades) in which there is a misuse of the name because early taxonomists assigned species names to populations with different morphologies, collected at different temperatures and salinities. Generally, different names are assigned to reproductively isolated populations or clusters of populations:

A*. salina* Linnaeus 1758*:* Lymington, England (now extinct), Mediterranean area;

A*. tunisiana* Bowen and Sterling 1978 (synonym of *A. salina*);

A*. parthenogenetica* Barigozzi 1974, Bowen and Sterling 1978: Europe, Africa, Asia, Australia;

A*. urmiana* Gunther 1990: Iran;

A*. sinica* Yaneng 1989: Central and Eastern Asia;

A*. persimilis* Piccinelli and Prosdocimi 1968: Argentina;

A*. franciscana* superspecies: Americas, Carribean and Pacific islands, including populations reproductively isolated in nature like *A.(franciscana) franciscana* Kellogg 1906 and *A.(franciscana) monica* Verrill 1869 (Mono Lake, California);

Artemia *sp.* Pilla and Beardmore 1994: Kazakhstan.

Artemia is a tipical primitive arthropod with a segmented body divided into three tagma called head, thorax, and abdomen (Fig. 2). The total length is usually about 8 – 10 mm for the adult male and 10 – 12 mm for the female. The head and its appendages are specialized, as they were in the ancestral crustacean. The thoracic appendages are similar to each other and are unspecialized phyllopods. The abdomen lacks appendages. The entire body is covered with a thin, flexibile exoskeleton of chitin to which muscles are attached internally.

1.2. Head

The head at the anterior end is composed of six segments which represents the most specialized region of the body (Fig. 2, 3). It bears a pair of stalked, lateral, compound eyes and a single, median, unstalked naupliar eye at the anterior end (Fig. 2, 3). The eyes, although stalked, are not considered to be segmental appendages.

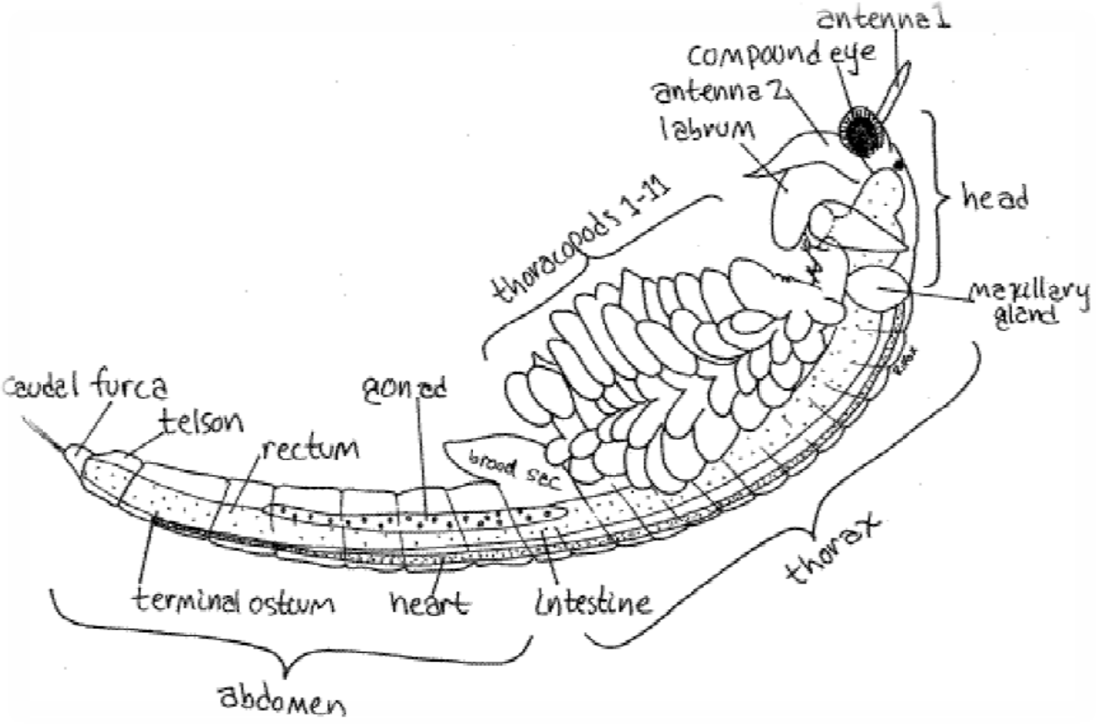


Fig. 2: A female brine shrimp *Artemia* (thoracopod setae omitted for clarity).

The small chemosensory first antennae or antennulae are the appendages of the first head segment (Fig. 2, 3, 4, 6). They are uniramous, and unjointed. The second antennae (Fig. 3, 4, 6) are larger and are sexually dimorphic. Those of adult males, termed claspers are very large and modified to form a clasping organ to hold the female during copulation (Fig. 1A). They are composed of two articles. Female second antennae are smaller, about the length of the first antennae but much thicker, and are composed of a single article (Fig. 1B). The labrum (or upper lip) is a large, median, ventral fold of body wall arising just posterior to the bases of the second antennae (Fig. 2, 3, 4). It is not paired and is not a segmental appendage. It extends posteriorly and covers the ventral surface of the head, including the mouth. The two, oval, bulging mandibles lie on either side of the head and are the appendages of the third head segment (Fig. 2, 3, 4). The mandibles curve medially and touch each other on the midline where the ventral borders bear teeth. The mouth is on the ventral midline between the two mandibles (Fig. 3, 4). It may be necessary to move the labrum aside to see the ventral ends of the mandibles and the mouth. The first and second maxillae are small and difficult to see. The first maxilla is larger than the second and bears a bundle of anteriorly directed setae on its medial edge (Fig. 3, 4). The first maxillae are immediately posterior to the mandibles on the ventral surface of the head and are used to transfer food from the thoracic appendages to the mouth. The tiny, conical second maxillae are vestigial and bear a few setae and the nephridiopores (Fig. 3, 4). The adult excretory organs are the two maxillary glands, or coxal glands, in the segment of the second maxillae where they form conspicuous bulges on its dorsolateral surfaces (Fig. 4). These are typical crustacean saccate nephridia. Their coiled ducts may be visible within the bulges. The maxillary glands open via the nephridiopores on the second maxillae.

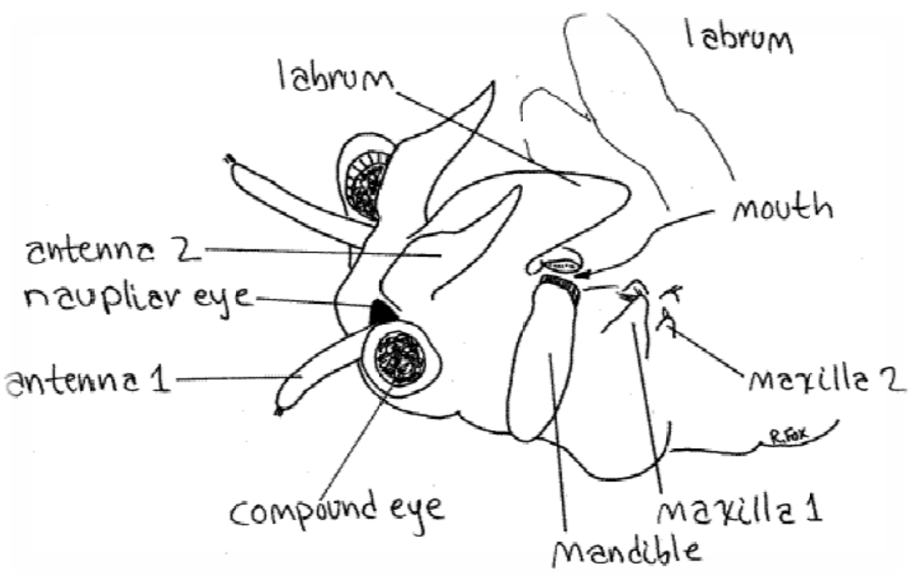


Fig. 3: Oblique view of the right side of the head of a female brine shrimp *Artemia*.

1.3. Thorax

The remainder of the body is the segmented trunk consisting of an anterior, limb-bearingthorax and limbless, posterior abdomen. The thorax consists of 11 independent segments. No carapace is present and, since none of the thoracic segments is fused with either the head or with each other, there is no cephalothorax. Each thoracic segment bears a ventral pair of leaflike thoracopods (= thoracic appendages) known as phyllopods (Fig. 2, 5). The 11 pairs of phyllopods are similar to each other and exhibit no regional specialization, differing only in size. The phyllopods are turgor appendages in which the exoskeleton is thin and flexible and blood pressure is required to keep the appendages stiff. The phyllopods are used for swimming, feeding, and respiration. The appendages are functionally uniramous although they have parts that are thought to be homologous to the two rami of a biramous appendage. Each appendage is flat and leaflike (phyll = leaf) and thus resembles the phyllopodous portion of the ancestral biramous mixopod. There is no stenopodous (cylindrical) portion.

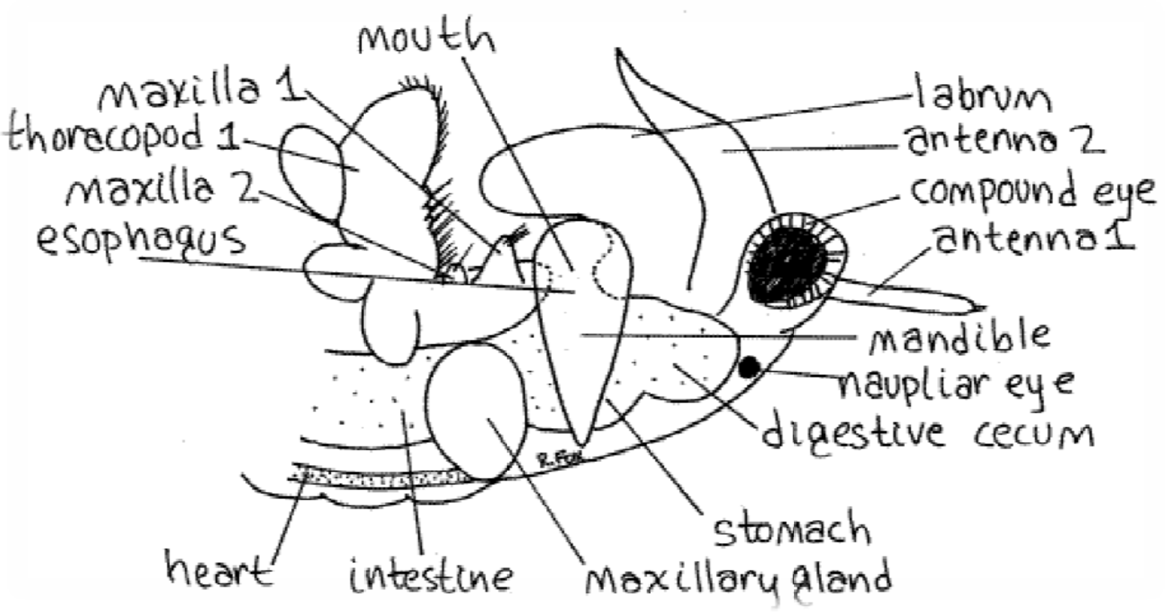


Fig. 4: Lateral view of the left side of the head and anterior thorax of a female brine shrimp *Artemia*.

Several processes extend from the lateral and medial borders of the protopod. Any process from the lateral border of a crustacean limb is an exite and any process from the medial border is an endite. Five or six endites, some of which are very small, extend from the medial margin of the protopod (Fig. 5). The proximal and distal endites are the largest, while three much smaller, middle endites lie between the proximal and distal endites. The densely setose proximal endite is often referred to as the gnathobase. Its setae form a setal comb, or filter, of finely spaced setae used to filter food particles from the water. The large distal endite may be homologous to the endopod of the ancestral biramous appendage. The proximal and middle exites do not bear setae. The middle exite is the epipod, which was once thought to be a gill although it now appears to be involved in osmoregulation. The distal exite may be homologous to the exopod of the biramous appendage. It is the only process attached to the protopod by an articulation. It bears long plumose natatory setae used for swimming. Plumose setae are feather-like to increase their effectiveness as oars.

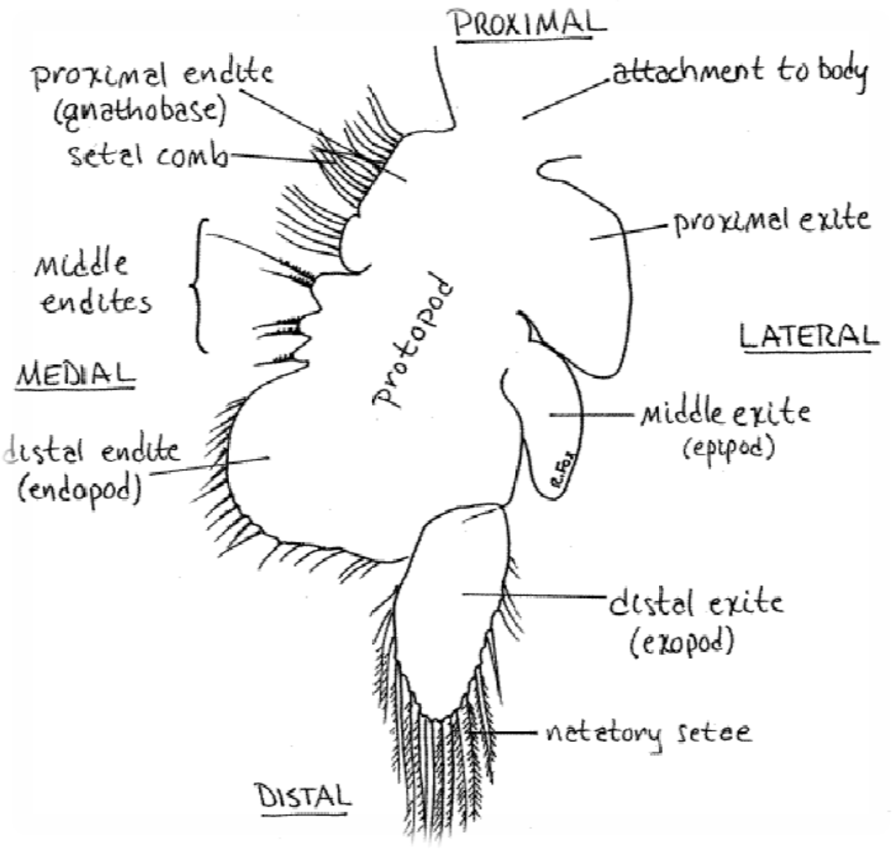


Fig. 5: Phyllopod of brine shrimp *Artemia* viwed from its anterior surface.

1.4. Abdomen

The abdomen lies behind the torax and consists of eight annular segments. The two segments posterior to the thorax are the genital segments and bear the external genitalia. Females have a conical pouch called the brood sac (= ovisac) which may contain eggs (Fig. 2, 6). Males bear a pair of tubular, retractile penes which can be extended to four times their resting length. The posterior end of the body is the telson. There is a caudal furca with two short rami on the end of the telson. None of the abdominal segments bears appendages. The anus is located on the telson at the base of the caudal furca (Fig. 6).

1.5. Digestive system

Most anostracans are suspension feeders although a few are carnivores that consume other species of anostracans. A longitudinal, midventral food groove lies between the gnathobases of the phyllopods (Fig. 2). The mouth faces the anterior end of the groove. Swimming movements of the phyllopods draw a water current into the groove. The water is then forced laterally through the setal comb on the gnathobases and food particles are prevented from leaving the groove. The food is moved anteriorly in the groove by the setae of the gnathobases. At the anterior end it is entangled in mucus from the labrum and transferred to the mouth by the setae of the first maxillae. The gut is a simple tube extending the length of the animal. The mouth is located on the ventral midline of the head between the opposing surfaces of the mandibles (Fig. 3, 4). The short vertical esophagus extends dorsally from the mouth to open into the stomach above the mouth (Fig. 2, 4). The mouth and esophagus make up the foregut and arise during ontogeny from the stomodeum, an invagination of surface ectoderm. The stomach is an expanded region of the gut in the middle of the head (Fig. 4). Two spherical digestive ceca bulge from the anterolateral walls of the stomach. The intestine is a long tube extending posteriorly from the stomach through the thorax and most of the abdomen (Fig. 2, 6). The stomach, ceca, and intestine make up the midgut and are endodermal derivatives. The midgut is the site of enzyme secretion, digestion (hydrolysis), and absorption. It is surrounded by the hemocoel and bathed in blood so that uptake of materials occurs across its thin walls. The intestine joins the short rectum, or hindgut, in segment 4 of the abdomen (Fig. 2, 6). The hindgut, like the foregut, is ectodermal and is lined by a chitinous exoskeleton. It is responsible for formation of fecal pellets and opens to the exterior via the anus between the caudal furcae. The anus is equipped with a sphincter. The rectum develops from an ectodermal invagination, the proctodeum. Early in development the rectum is not yet continuous with the midgut being separated from it by a partition.

1.6. Circulatory system

The anostracan hemal system is a good example of the primitive arthropod condition. The heart is a long, median, dorsal tube extending the length of the trunk (Fig. 2). The heart is surrounded by a special compartment of the hemocoel, the pericardial sinus. It is separated from the rest of the hemocoel, which lies ventral to it, by a perforated, horizontal septum through which blood flows on its way back to the heart. Contractions of the heart force blood out its open anterior end of the aorta into the body hemocoel. Blood flows through the hemocoel and over the tissues while making its way posteriorly. It is aided in its flow by movements of the appendages and their muscles. Blood flows from the body hemocoel into the pericardial sinus through the perforations in the horizontal partition and then passes posteriorly in the sinus and enters the ostia of the heart.

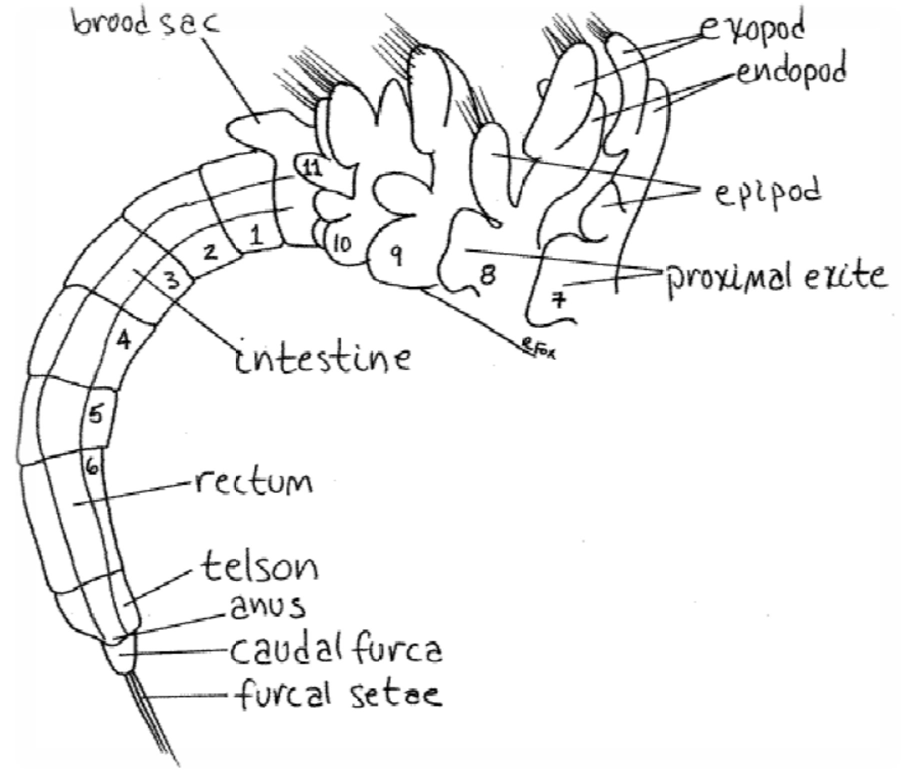


Fig. 6: Left side of the posterior thorax and the abdomen of an immature female of brine shrimp *Artemia*.

1.7. Respiratory and excretory / osmoregulatory system

Most gas exchange is accomplished across the permeable surfaces of the phyllopods. The two maxillary glands in the segment of the second maxilla (Fig. 2, 3) are usually referred to as excretory organs but, in fact, their role is largely osmoregulatory and they have little to do with the excretion of metabolic wastes. Nitrogen is lost as ammonia across the phyllopod surfaces. Each maxillary gland consists of an enclosed end sac, derived from a coelomic space, from which a long excretory duct leads to the nephridiopore located on the tiny second maxilla. The gland is surrounded by hemocoel and bathed with blood. The epithelium of the end sac is equipped with podocytes and forms an ultrafiltrate of the blood into the lumen of the end sac. The ultrafiltrate is modified as it passes down the duct to the exterior. *Artemia* is an efficient osmoregulator and is strongly euryhaline, being tolerant of an impressively wide range of salinities. *Artemia* can keep its blood hyposmotic to environments more saline than about 10 parts per thousand. This is something most marine invertebrates cannot do, at least not to the same extent. *Artemia* drinks brine and actively secretes salts from the maxillary glands, epipods, and gut. The maxillary glands can produce urine four times as salty as the blood. Maintenance of a hyposmotic blood is facilitated by the impermeability of most of the integument. The exoskeleton, with the exception of the epipods, is impermeable to salts. The epipods are major sites of active salt secretion. *Artemia* belongs to a predominantly freshwater taxon and presumably evolved from freshwater, not marine, ancestors.

1.8. Sensory system

The nervous system consists of a dorsal brain, paired circumenteric connectives, and double, ventral nerve cord with segmental ganglia. The brain is a mass of translucent tissue surrounding the naupliar eye in the dorsal, anterior part of the head. The sensory system includes the median naupliar eye which appears in the earliest larval instar and persists throughout life. It consists of three black pigment cups. Two cups face laterally and one points ventrally. Two stalked, lateral compound eyes, each composed of numerous black ommatidia are also present.

1.9. Reproductive system and reproduction

The female reproductive system consists of ovaries and oviducts leading into a single, median ovisac wherein several clusters of shell glands open. Adult female *Artemia* ovulate approximately every 140 hours depending on rearing conditions (also, strain specific) and whether development occours oviparously or ovoviviparously. The male reproductive system, consisting of paired testes, *vasa deferentia*, accessory glands and a double penis.

The gonads of both sexes are paired tubes located dorsolaterally in the posterior thorax and anterior abdomen (Fig. 1, 6). In the bisexual strains, during mating the male approaches the dorsal side of the female and holds her with his enlarged second antennae. The male twists his body around the female, inserts the penes into the brood pouch, and deposits sperm. The partners remain coupled for several hours during which copulation may occur every few minutes. In the phartenogenetic (from the Greek παρθένος parthenos, "virgin", + γένεσις genesis, "creation") strains (diploid or poliploid) there is an asexual form of reproduction where growth and development of embryos occurs without fertilization by males. The offspring produced by parthenogenesis almost always are female. As *Artemia* is an inhabitant of biotopes characterized by unstable environmental conditions, its survival during periods of extreme conditions (i.e. desiccation, extreme temperatures, high salinities, low oxygen levels) is ensured by the production of dormant embryos. *Artemia* females can indeed easily switch from live nauplii production (ovoviviparity) to cysts formation (oviparity) as a fast response to fluctuating circumstances. Although the basic mechanisms involved in this switch are not yet fully understood, sudden fluctuations seem to trigger oviparity. The triggering mechanism for the induction of the state of diapause is however not yet known. Both in

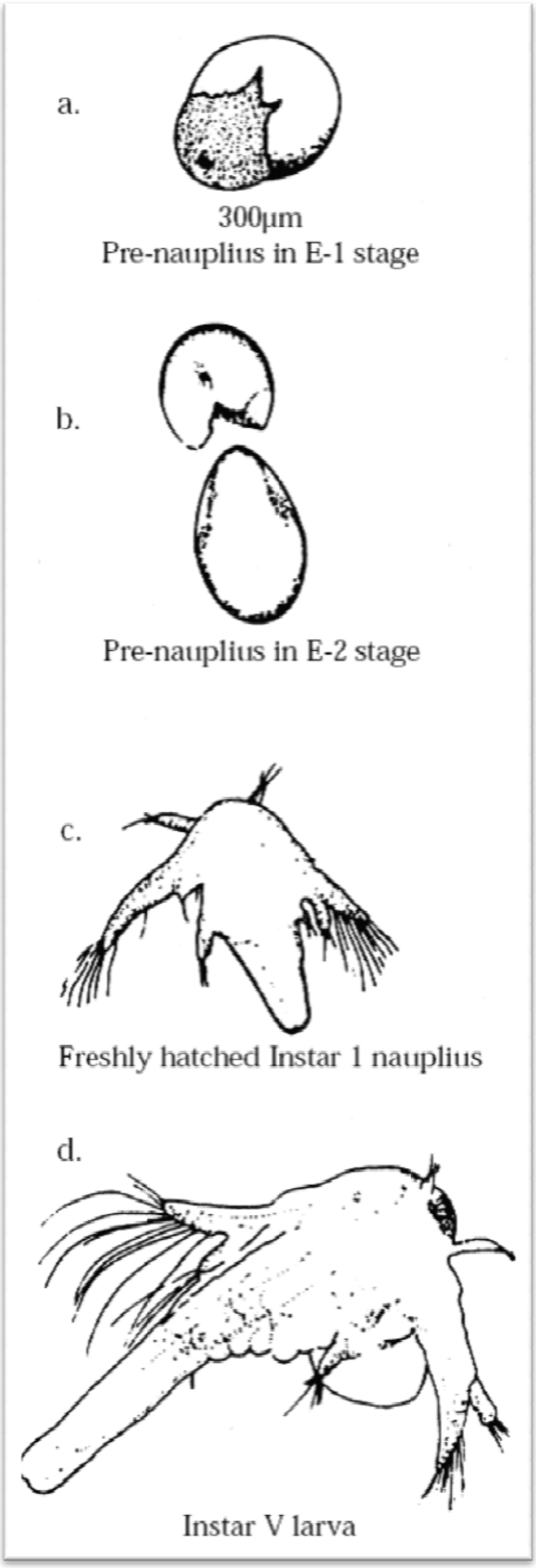
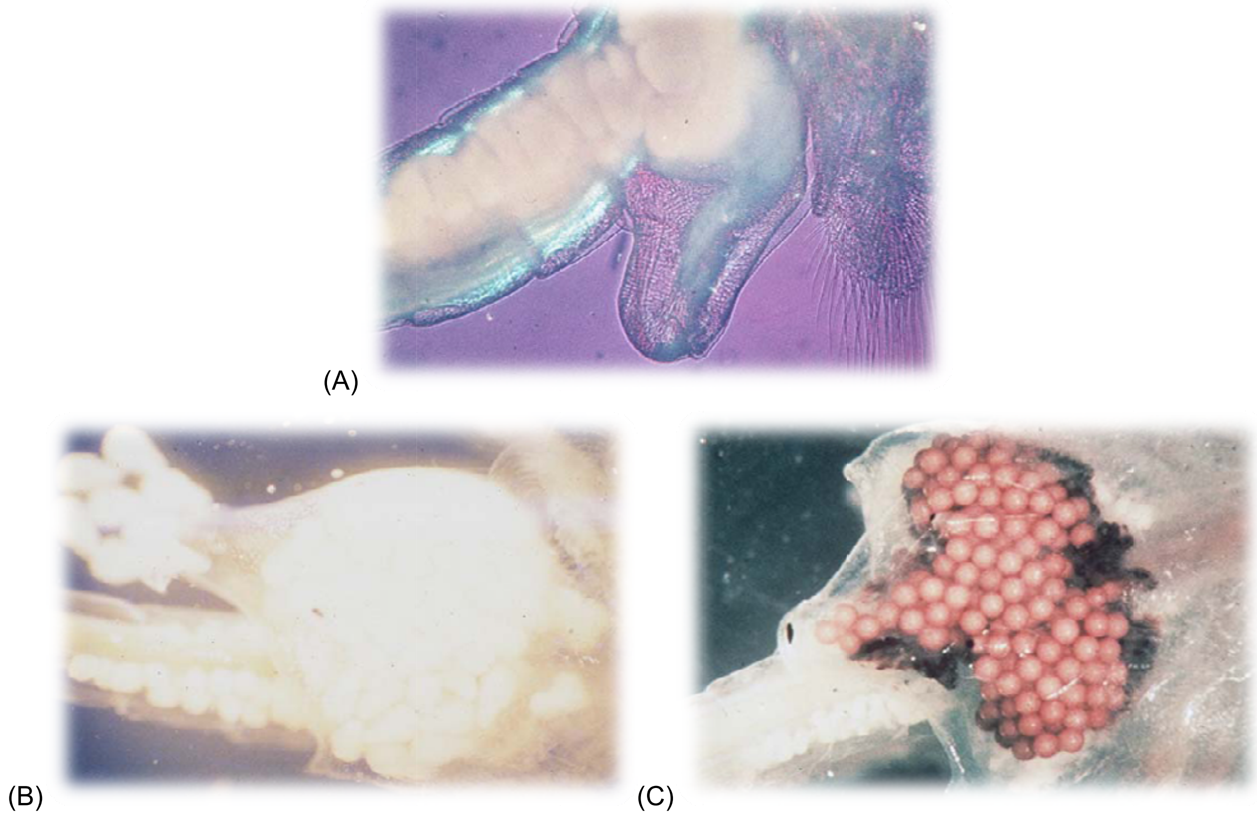


Fig. 7: Development of brine shrimp *Artemia* cyst to Instar V larva.

bisexual and in parthenogenetic strains eggs develop in two tubular ovaries in the abdomen (Fig. 8A). Once ripe they become spherical and migrate via two oviducts into the unpaired uterus. Fertilized eggs normally develop into free-swimming nauplii (= ovoviviparous reproduction) (Fig. 8B.) which are released by the mother. In extreme conditions the embryos only develop up to the gastrula stage. At this moment they get surrounded by a thick shell (secreted by the brown shell glands located in the uterus), enter a state of metabolic standstill or dormancy (diapause) and are then released by the female (=oviparous reproduction) (Fig. 8C). *Artemia* embryos released as cysts in the medium are in diapause and will not resume their development, even under favorable conditions, until they undergo some diapause deactivating environmental process. Both oviparity and ovoviviparity are found in all *Artemia* strains, and females can switch in-between two reproduction cycles from one mode of reproduction to the other (Fig. 9). Upon the interruption of diapause, cysts enter the stage of quiescence, meaning that metabolic activity can be resumed at the moment they are brought in favorable hatching conditions, eventually resulting in hatching (Fig. 7).



(A)

(B)

(C)

Fig. 8: Abdomen and uterus in fertile female *Artemia*: ripe eggs in ovary and oviduct (A), uterus of ovoviviparous *Artemia* filled with nauplii (B), uterus of oviparous *Artemia* filled with cysts (C) (From Sorgelos et al.).

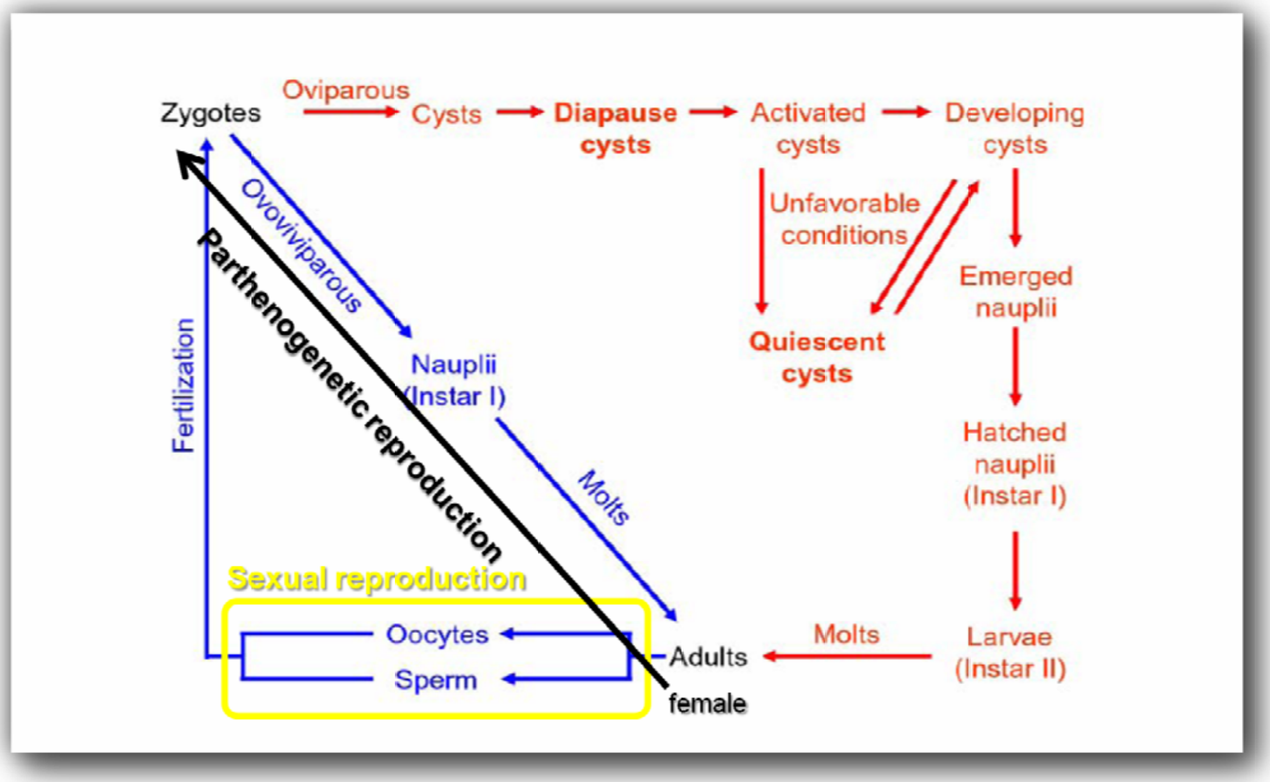
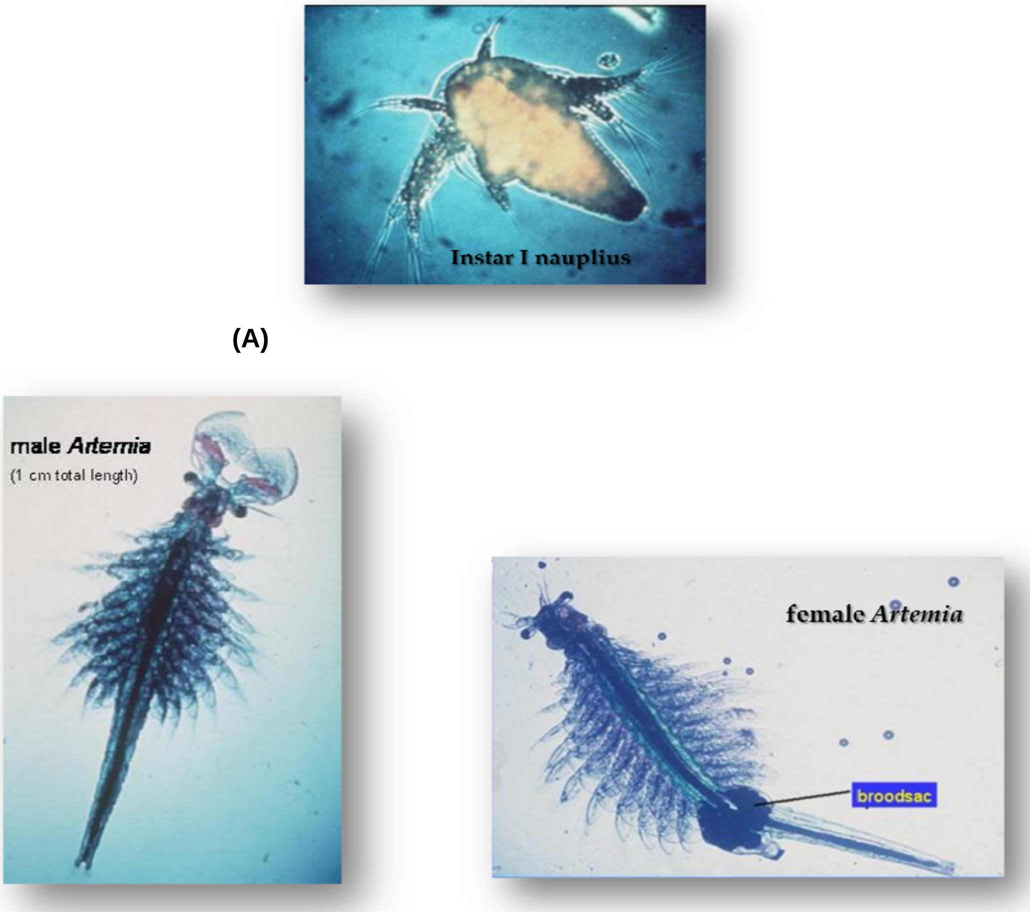


Fig. 9: ***Artemia*** **life cycle (From T. H. Mac Rae, modified).**

At this moment the biconcave-shaped cysts hydrate, become spherical, and within the shell the embryo resumes its interrupted metabolism. After about 20 h the outer membrane of the cyst bursts (= "breaking") and the embryo appears, surrounded by the hatching membrane. While the embryo hangs underneath the empty shell (= "umbrella" stage) the development of the nauplius is completed and within a short period of time the hatching membrane is ruptured (= "hatching") and the free-swimming nauplius is born (Fig. 10A). The first larval stage (instar I; 400 to 500 µm in length) has a brownish-orange colour, a red nauplius eye in the head region and three pairs of appendages: *i.e*. the first antennae (sensorial function), the second antennae (locomotory + filter-feeding function) and the mandibles (food uptake function). The ventral side is covered by a large labrum (food uptake: transfer of particles from the filtering setae into the mouth). The instar I larva does not take up food as its digestive system is not functional yet; it thrives completely on its yolk reserves. After about 8 h the animal molts into the 2nd larval stage (instar II). Small food particles (*e.g*. algal cells, bacteria, detritus) ranging in size from 1 to 50 µm are filtered out by the second antennae and ingested into the functional digestive tract. The larva grows and differentiates through about 15 molts. Paired lobular appendages are appearing in the trunk region and differentiate into thoracopods. On both sides of the nauplius lateral complex eyes are developing. From the 10th instar stage on, important morphological as well as functional changes are taking place: *i.e*. the antennae have lost their locomotory function and undergo sexual differentiation. In males (Fig. 10B) they develop into hooked graspers, while the female antennae degenerate into sensorial appendages (Fig. 4, 10C). The thoracopods are now differentiated into three functional parts, namely the telopodites and endopodites (locomotory and filter-feeding), and the membranous exopodites (gills). Under optimal conditions brine shrimp can live for several months, grow from nauplius to adult in only 8 days' time and reproduce at a rate of up to 300 nauplii or cysts every 4 days.



(A)

(B)

(C)

Fig. 10: Instar I nauplius (A), male (B) and female (C) of Artemia (From Sorgelos et al.).

CHAPTER 2

MARGHERITA DI SAVOIA SOLAR SALTWORKS

2.1. The Study site

Margherita di Savoia is the largest solar saltworks in Italy and one of the most extensive in the Mediterranean region. Located along the Adriatic coast (41°22′53″ N, 16°05′52″ E) in the province of Foggia (Apulia region, southeastern Italy), with a total area of about 4,00 ha and producing a total of about 5,500,000 quintals of salt per year, Margherita di Savoia is the main salt producing saltwork in Europe (Fig. 11A, 11B). The saltworks run lengthways, parallel to the Adriatic coast, for a distance of approximately 20 km, with a maximum width of 4 km and cover a surface area of approximately 4,000 hectares, with 3,781 subject to protection.

Due to its importance as a habitat for avifauna (waterfowl, wading and migratory birds), it was declared a natural reserve in 1977 (managed by the National Forestry Service) and was included in the list of preserved wetlands of international importance (Ramsar Convention). It is a zone of special protection in terms of the Birds directive 79/409/CEE and a site of community importance (Habitat directive 92/43/CEE).

The climate can be considered semi-arid, with mild winters and hot-dry summers. The average monthly air temperature ranges between 8.8 °C in February and 26.9 °C in July. In the period from 1988 to 2000, the mean annual precipitation ranged from 300 to 464 mm. In the same period, the average evaporation varied between 94 mm in January and 342 mm in July. Approximately 70% of the total yearly evaporation occurs from May to November (Meteo Service Archive of ex Monopolio di Stato, presently E.T.I.).

2.2. Story and origin of the Margherita di Savoia saltworks

The Margherita di Savoia saltworks originated from the ancient Salpi lagoon, a basin lying in the north-eastern part of the Apulia region.

During the last glaciation, the sea level was 120 m lower than present, and the coastline was about 70 km offshore of the present coast (Caldara *et al*., 1994). The Daunian rivers incised and widened their valleys.

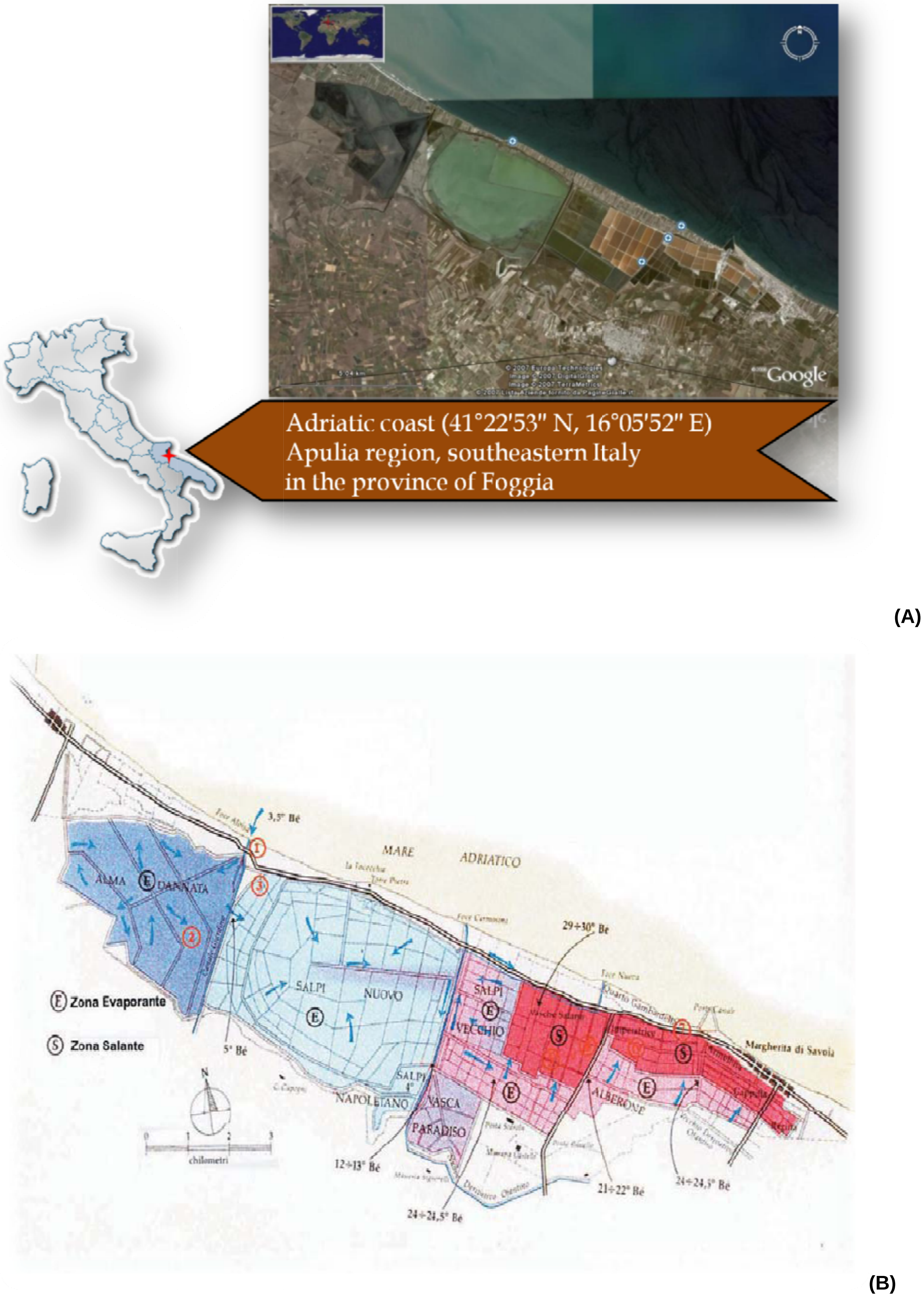


Fig. 11: Margherita di Savoia solar saltworks: location and coordinate (A) and solar salt production process (B).

At the beginning of the Neolithic sea level was at about 10 to 15 m compared to the present and a narrow coastal ridge developed which became larger as the lagoon became isolated. During this period the climate warmed. The change in climate prompted massive colonization by a population from the eastern Adriatic Sea coast. The new inhabitants settled the wide plain of this region and, in particular, the inner lagoon shores and the areas close to the rivers flowing into the lagoon itself. The inner lagoon shoreline was indented due to the shape of the mouths of the rivers flowing into it. These streams delivered significant amounts of water during the whole of the year. During this phase the largest extent of human settlement was reached, most of which were close to the inner shore of the lagoon, where the inhabitants of the villages either lived by fishing or farming, depending on the season (Jones, 1987).

The last period of the Middle Neolithic was characterized by a new phase of depopulation of the land. This period was characterized by intense aridity and the whole coastal area fell under semi-desert conditions (Boenzi *et al*., 2001). As a result, the lagoon, no longer communicating with the sea, turned to a sabkha. Rivers and streams flowing into the lagoon with wide outlets went through deep transformations. The presence of large areas totally lacking in vegetation favoured accelerated erosion along slopes and thick alluvial and colluvial sediments deposited in the valleys. The sediments were re-distributed at the outlet of rivers and ephemeral streams allowing the formation of large, flat alluvial cones. As a result of this sedimentation the bottom of the ancient lagoon was raised and the whole basin got smaller.

The areas close to the lagoon were again inhabited from the Middle Bronze Age.

During the Iron Age the areas surrounding the lagoon reached their maximum density of population. Reliable accounts of the characteristics of the lagoon at this time come both from the archeological record and from the works of many authors such as Lycophron (Alexandra, 1129), Strabo (Geographia, VI, 5, 283), Vitruvius (De Architectura I, 4, 12), Titus Livius (Ab Urbe Condita Libri, XXXIX, 2), Appianus (De Bello Annibalico, XLV, Bellum Civile, I, 52), Lucanus (Pharsalia, V, 377), Plinius (Naturalis Historia, III, 103) and other minor chroniclers.

Apart from the geological and archeological studies, the most ancient information about the lagoon comes from the Roman period, and relate in particular to the presence of the harbour of Salapia (Di Biase, 1985; Caldara and Pennetta, 1990). This town was founded in the IX century B.C. by populations from the Dalmatian coast. Salapia represented an important port of shipment for the foodstuffs from the south of Italy, competing with the port of Sypontum (Tinè Bertocchi, 1975).

Livius wrote that around the III century the lagoon area started getting silted up due to an increasingly of turbid flow from the rivers. Areas all around Salapia became malarial and the town began a long period of decay. Nevertheless Vitruvius reported that, as the harbour was located in an important place for the trades of Rome, the inhabitants of Salapia founded a new town upon a low hill 12 m in height, whose present name is Monte Salpi. In spite of many efforts, communication between the lagoon and the sea could not be established and the lagoon continued to infill and evolve into a swamp. Cicero (De Lege Agraria, II, 27, 71) opposed the proposal for Roman settlements being located "in the pestilential swamp of Salapia". Some years later Seneca (Epistulae Morales, I, 34) gave a similar opinion, describing the land as an "insalubrious, desert area". Livius reported that, due to the malaria that occurred between 195 and 185 B.C., the inhabitants of Sypontum were compelled to move to another site, a few kilometers south of the present Manfredonia. Livius himself held that the fill of the lagoon was the effect of a sudden and strong increase in the turbid flow of the inflowing rivers. Sediment discharge from the Carapelle stream in particular divided the lagoon into two basins: the so-called Salpi lake in the southern area and the Salso lake in the northern area.

There is no satisfactory documentation regarding the environmental conditions of the damp area during the Middle Ages. Reports regarding this area can be inferred from ancient geographic maps, by old ledgers of notaries and excisemen in charge of the collection of taxes and by monastic chronicles. Analysis of these reports suggests that the lagoon changed its configuration many times, and ultimately became a swamp in which malaria was endemic. The author who first hypothesized geological and climatic causes for the changes in the lagoon area was Aloe (in Pascale, 1912); in fact he postulated that up to the XI century the entire Salpi area (the new name for Salapia) was affected by a continuous slow subsidence as a result, the buildings of Salpi were partially submerged.

About at the beginning of the XI century the coastal areas close to Salpi were covered by a wood whose extension was so large that it became one of the most important in the whole empire of Frederick II of Swabia. Furthermore, historical studies (Masson, 1978) show that in that period the area was depopulated, uncultivated and covered by thick vegetation, whereas the areas surroundings the lagoon had a high density of population.

During the XII century, the first circumstantial reports regarding the physical environment of the land can be found in Geographica, by Guidone (1119) where the thriving activity at the Salpi salt pans was documented. Besides the chronicles by the monks and the noblemen, the first cartographic maps and documents started to be published in this period. The area was represented for the first time by the Arabian voyager Edrisi (Libro del Re Ruggero, 1139 - 1154). Other maps representing the region can be found in the Cronaca by Jordanus (1134 - 1339 in manoscr. Vat. Lat. n. 1960) and in the so called Carta d'Italia del Trecento (Archivio di Stato, Firenze).

Reliable reports describe this part of the plain as a damp area date back the first half of the XIII century. Around the year 1230, when the place takes the name of Casale di Sancta Maria de' Salinis, Emperor Frederick II of Swabia allowed the ships of the Venetian Republic to be admitted freely to the lagoonal harbour of Salpi (Caldara and Pennetta, 1992). This means that the lagoon was again navigable. Furthermore, in the year 1255, the Montevergine Monastery received from the Cardinal Ottaviano a "residence" in Salpi and the annuity of "two lakes" (the former, smaller, fresh-watered and placed in the internal area, the latter, closer to the sea, with salt-water) divided by a narrow ridge. The town was again destroyed in the 1255 due to another devastating earthquake which caused irreparable damage.

Between the XV and the XVII century long dry spells occurred, which caused serious famines in the whole southern Italy. Maybe due to the drought itself, the entire coastal area turned to a marsh: it was in this period that the town of Salpi was abandoned due to malaria (Alberti, 1551). At the beginning of the 1500 it was reported that the old name Casale di Sancta Maria de' Salinis was changed in Regie Saline di Barletta (Bulifon, 1690; Parrino, 1770) and during the XVI century the area was a desolate landscape, subject to famines after long periods of drought. A great number of swamps started to dry up. During the following years a deep change in climatic conditions occurred. The increase inflows of the Candelaro river and the Cervaro stream caused water levels in the swamps all around Siponto to rise. The historical maps show the presence of three little basins in the southern area of Manfredonia: the areas on the left of the Candelaro river were occupied by the saltworks, which represented one of the few sources of income in the region; the saltworks continued to be productive up to the first years of the XVIII century; on the right of the Candelaro river the ancient maps show the presence of two lakes: the Bersentino lake (or Versentino lake), and the Salso lake. A detailed representation of the damp area near Salpi can be seen in a geographic map by Magini (in: Angelini and Carlone, 1986). The map shows the swamp, a little pond in the internal area, some little Salt pans close to the mouth of the Ofanto river, the Salso lake, divided by the Salpi lake by the Carapelle stream, the Salt pans of Siponto, and a swamp fed by the Cervaro river, whose mouth does not reach the Candelaro river (Fig. 12).

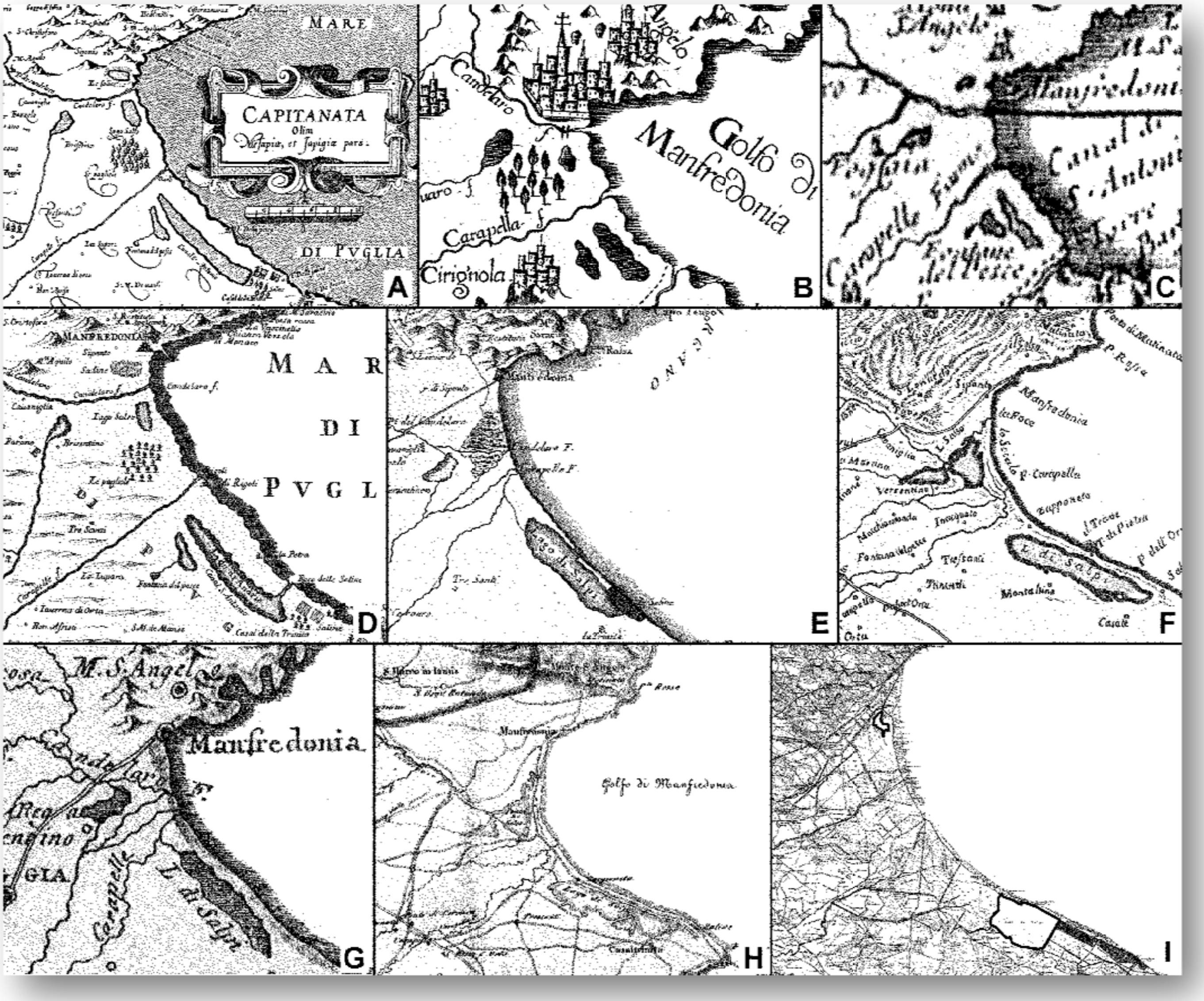


Fig. 12: Salpi lake as represented in cartography between the XVII and XX Century; A) Magini, 1620; B) from the Capucinorum Atlas, 1649; C) Coronelli, 1688; D) Pietrasanta and De Rossi, 1714; E) Zatta, 1779 - 1785; F) Rosati, 1787; G) Anonymous, 1789; H) Marzolla, 1836; I) from the Italian Istituto Geografico Militare historical cartography 1909. A, B, D, F and H from ANGELINI and CARLONE, 1986; C, E from FONSECA, 1984; G from a map called Parte Occidentale del Regno di Napoli, kept at "De Gemmis" library in Bari (From M. Caldara, L. Pennetta and O. Simone).

In the middle of the XVIII century the king Charles III of Bourbon, ascended to the throne in Naples, asked the famous architect Luigi Vanvitelli to redesign that area for a more modern and functional process of extraction and production of salt. The architect went for the first time to the saltworks in the 1754 with the intent to understand the mechanisms for salt production and the problems of the area. He noticed that the main issues were the insufficient flow of water especially in the summer months and the continuous floods of the Carapelle, Carapellotto and Ofanto rivers. Luigi Vanvitelli accque the confluence of the Ofanto river flooding towards the Caputo ditch with the aim to separate permanently the saltworks from the Salpi lake and suggested to reopen the existing mouths and maintain them always open in order to allow the normal replacement of water in the lake (Fig. 13).

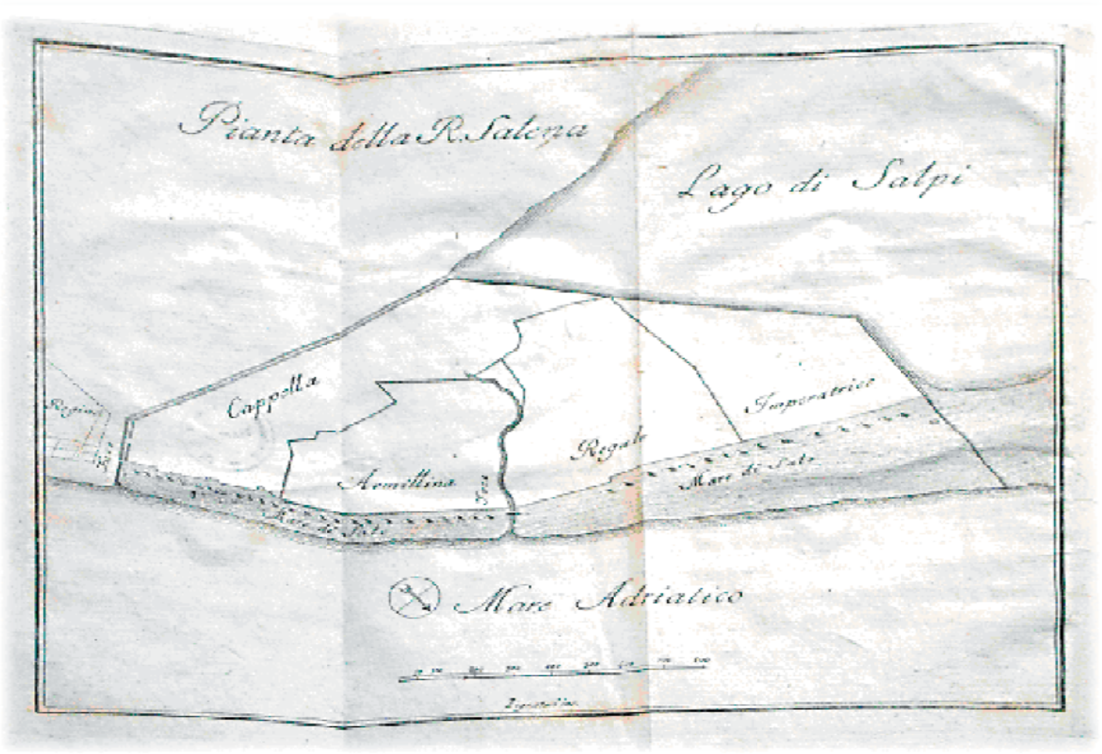


Fig. 13: Map of the Regia Salina di Barletta (Source Vincenzo Pecorari "Memoria sulla Regia Salina di Barletta", Naples 1784.

After the Vanvitelli's studies on the mouths, he was charged in 1758 to design the extension of the saltworks. He also modified the shape, the size and the depth of the ponds and the ideation and the realization of this project accompanied the architect up almost to his death (Fig. 14).

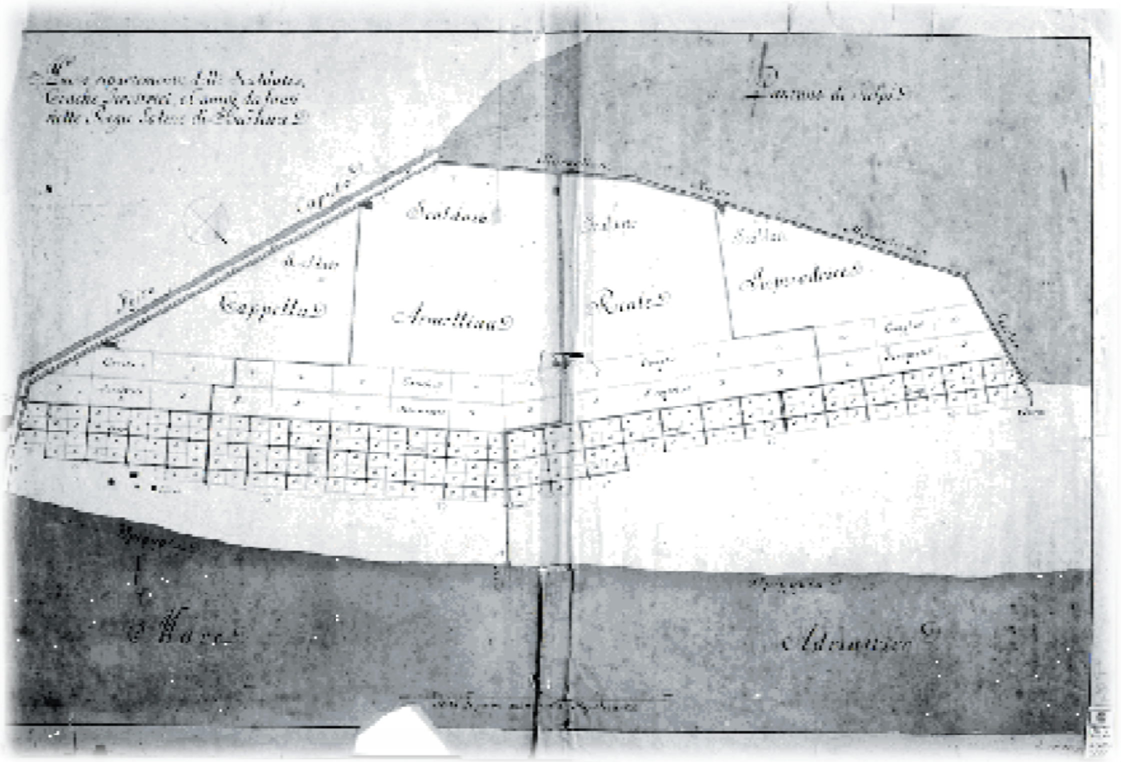


Fig. 14: Planimetry of the Regia Salina di Barletta; Luigi Vanvitelli 1757-1761. (Source Soprintendenza per i Beni A. A. A. S. Caserta, Palazzo Reale – depositi, inv. 16331/1).

In the XIX century the area continued to be affected by malaria, in spite of the attempts at reclamation of lands, among which was the impressive project of Afan De Rivera of 1808, which was never completed (Afan De Rivera, 1845).

After the Unity of Italy the Apulia region landscape was still changing. Maps and documents of the XX century show that the coastal swamps existed up to the 1930s. During these years projects for the reclamation of lands were provided by the Serpieri-Iandolo Law and, afterwards, by the "1938 Project for agrarian transformation" (Piano di Trasformazione Agraria) and by the "1948 Project for Land Transformation" (Piano di Trasformazione Fondiaria). After 1933 these lands came under the administration of the "Consorzio per la Bonifica della Capitanata", which realized the plan to reclaim the marsh, by filling the coastal lakes: the Salso and the Salpi lakes (Colacicco, 1955; Ciccone and Como, 1984). Following major reclamation work in the 1936 and in the 1950, the rest of the Salpi lake (the areas so called “Salpi Nuovo” first and “Alma Dannata” after), was annexed to the saltworks, increasing considerably their surface area. Now the Margherita di Savoia saltworks have the same conformation and the same extension that the saltworks had reached in the ages ’50s (Fig. 11B, 15).

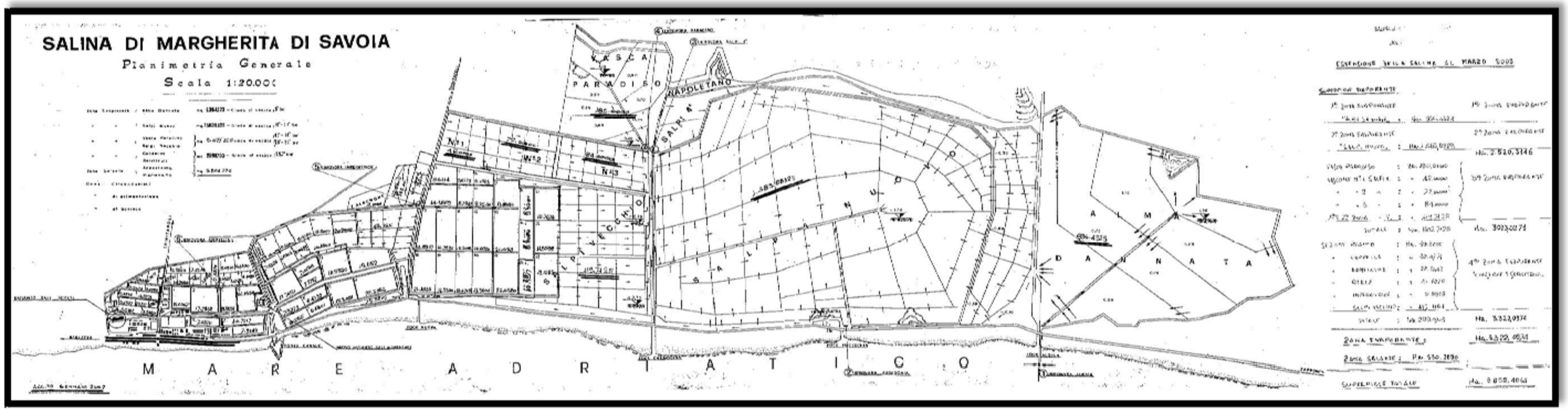


Fig. 15: 1:20.000 scale geneal plan of Margherita di Savoia saltworks updated to 2003 (with the permission of Atisale S.p.A.)

CHAPTER 3

ARTEMIA ECOLOGY AND STUDIES

3.1. *Artemia*, saline lakes, coastal lagoons and solar saltworks

Artemia populations and cysts of this small crustacean are widely distributed all over the world in a variety of biotopes (Fig. 16). Biomass and cysts are available year-round in large quantities along the shorelines of hypersaline lakes, coastal lagoons and solar saltworks scattered over the five continents (Persoone and Sorgeloos, 1980).

The brine shrimp *Artemia* has been known, with different names (brineworm, salztierchen, verme de sale sòfèreg, bahar el dud, fezzanwurm, ecc.), from centuries by different ethnic groups who attributed to the presence of this small crustacean in brine pools, the capacity to ameliorate the salt production.

Salt has, always been very precious to man, not only because of its dietary value, but also as an indispensable tool for preserving food. In ancient times salt was a means of payment. This explains the etymology of the word salary, used in so many languages. All over the world local populations have developed methods for solar salt production, i.e. the extraction of salt from seawater. Management techniques were developed to maximise evaporation, to allow sequential precipitation of carbonates and sulphates so as to eventually collect the sodium chloride. What man didn't realize, until very recent times, was the fact that these very salty waters, often considered lifeless, were the habitat of a remarkable invertebrate organism: the brine shrimp *Artemia*.

Artemia populations are now found in about 600 natural salt lakes and man-made saltworks scattered throughout the tropical, subtropical and temperate climatic zones, along coastlines as well as inland. This list still remains provisional as more extensive survey work should lead to the discovery of many more *Artemia* biotopes in different parts of the world (Fig. 16 and Tab. 1).

The distribution of *Artemia* is discontinuous and not all highly saline biotopes are populated with *Artemia*. Although brine shrimp thrive very well in natural seawater, they cannot migrate from one saline biotope to another via the seas, as they depend on their physiological adaptations to high salinity to avoid predation and competition with other filter feeders. As *Artemia* is incapable of active dispersion, wind and waterfowl (especially flamingos) are the most important natural dispersion vectors; the floating cysts adhere to feet and feathers of birds, and when ingested they remain intact for at least a couple of days in the digestive tract of birds. Consequently the absence of migrating birds is probably the reason why certain areas that are suitable for *Artemia* (*e.g.* salinas along the northeast coast of Brazil) are not naturally inhabited by brine shrimp.

Thalassohaline waters are concentrated seawaters with NaCl as major salt. They make up most, if not all, of the coastal *Artemia* habitats where brines are formed by evaporation of seawater in salt pans. Other thalassohaline habitats are located inland, such as the Great Salt Lake in Utah, USA. Athalassohaline *Artemia* biotopes are located inland and have an ionic composition that differs greatly from that of natural seawater: there are sulphate waters (*e.g.* Chaplin Lake, Saskatchewan, Canada), carbonate waters (*e.g.* Mono Lake, California, USA), and potassium-rich waters (*e.g.* several lakes in Nebraska, USA).

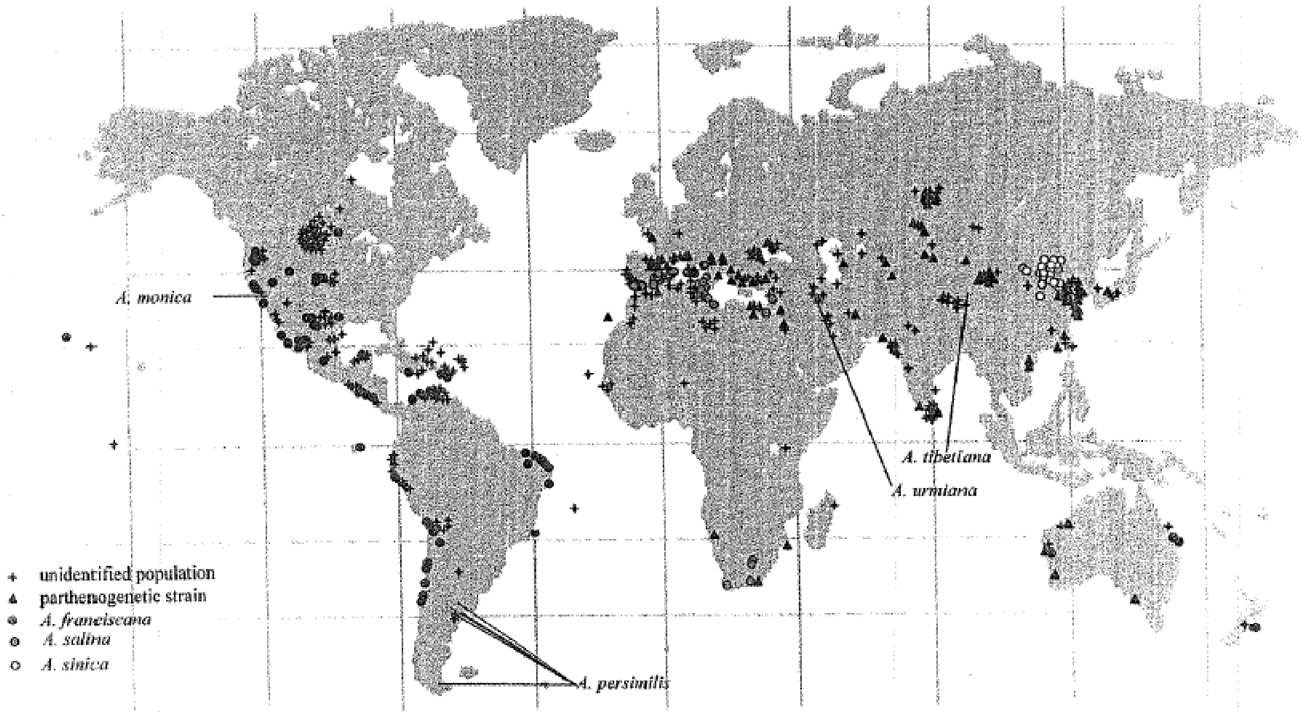
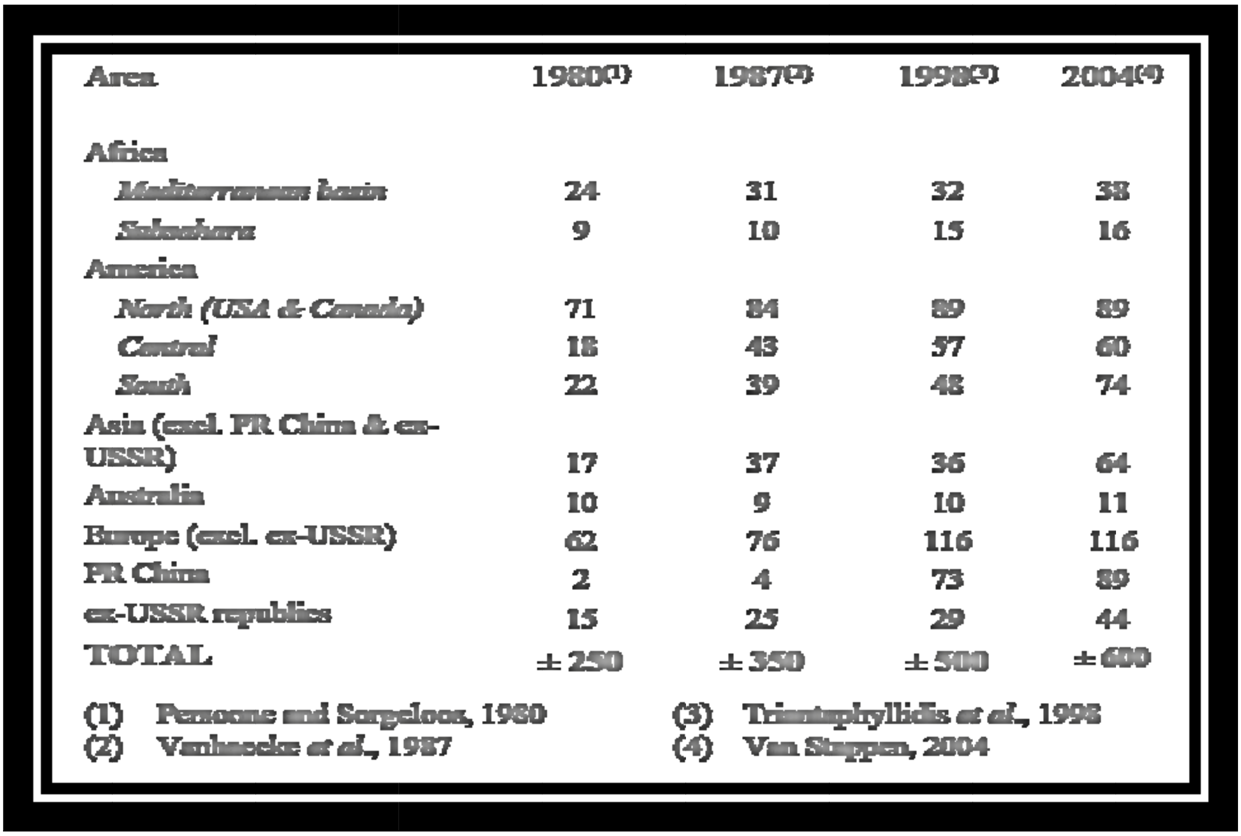


Fig. 16: World distribution of *Artemia* species (From Artemia: basic and applied biology).

Artemia is a non-selective filter feeder of organic detritus, microscopic algae as well as bacteria. The *Artemia* biotopes typically show a very simple trophical structure and low species diversity; the absence of predators and food competitors allows brine shrimp to develop into monocultures. As high salinity is the common feature determining the presence of *Artemia*, the impact of other parameters (temperature, primary food production, *etc.*) may at most affect the abundance of the population and eventually cause a temporary absence of the species.

The brine shrimp *Artemia is a* very efficient osmoregulatory animal and have the capacity to synthesize very efficient respiratory pigments to cope with the low O_2 levels at high salinities, furthermore his ability to produce dormant cysts when environmental conditions endanger the survival of the species. As a result of extreme physiological stress and water toxicity *Artemia* dies off at salinities close to NaCl saturation, *i.e*. 250 g/l and higher. Different geographical strains have adapted to widely fluctuating conditions with regard to temperature (6-35°C), salinity and ionic composition of the biotope.



Area	1980(1)	1987(2)	1998(3)	2004(4)
Africa				
Mediterranean basin	24	31	32	38
Subsahara	9	10	15	16
America				
North (USA & Canada)	71	84	89	89
Central	18	43	57	60
South	22	39	48	74
Asia (excl. PR China & ex-USSR)	17	37	36	64
Australia	10	9	10	11
Europe (excl. ex-USSR)	62	76	116	116
PR China	2	4	73	89
ex-USSR republics	15	25	29	44
TOTAL	± 250	± 350	± 500	± 600

(1) Persoone and Sorgeloos, 1980
(2) Vanhaecke *et al*., 1987
(3) Triantaphyllidis *et al*., 1998
(4) Van Stappen, 2004

Tab. 1: Distribution of known natural *Artemia* sites (From G. Van Stappen).

3.2. *Artemia* studies and utilization

Although *Artemia* has been known to man for centuries, its use as a food for the culture of larval organisms apparently began only in the 1930s, when several investigators found that it made an excellent food for newly-hatched fish larvae. Its high food value for aquaculture and aquarium pet organisms was soon appreciated and in less than two decades commercial harvests reached over 1000 mt of cysts and over 10000 mt of biomass annually. Worldwide sales in 1992 of *Artemia* cysts and biomass are estimated at over US$50 million (Sorgeloos, 1995).

During the 1940s, most commercially available brine shrimp cysts represented collections from natural saline lakes and coastal saltworks. With the growing interest for tropical hobby fish in the late 1940s, commercial value was attached to brine shrimp, thereby establishing a new industry.

Early pioneers exploited in 1951 the cyst production of *Artemia* at the Great Salt Lake in Utah, USA. First harvests of the lake yielded 16 tons of finished product. During the mid-1950s, commercial attention for brine shrimp was turned to controlled sources for production in the San Francisco Bay region. Here it was found that brine shrimp and their cysts could be produced as a by-product of solar saltworks. Since salt production entails management of the evaporation process, yearly cyst and biomass productions could be roughly predicted.

In the 1960s, commercial provisions originated from these few sources in North America and seemed to be unlimited.

However, with the expansion of aquaculture production in the 1970s, the demand for *Artemia* cysts soon exceeded the offer and prices rose exponentially, turning *Artemia* into a bottleneck for the expansion of the hatchery aquaculture of marine fishes and crustaceans. In particular, many developing countries could hardly afford to import the very expensive cysts. At the Kyoto FAO Technical Conference on Aquaculture in 1976 it was claimed that the cyst shortage was an artificial and temporary problem. During the following years research efforts were made to prove the possibility of local production of *Artemia* in developing countries.

At present, *Artemia* is being produced and exploited on the five continents. Despite this, a large part of the cyst market is still supplied by harvests from one location, the Great Salt Lake. Already in the late 1970s it appeared that the nutritional value of *Artemia*, especially for marine organisms, was not constant but varied among strains and within batches of each strain, causing unreliable outputs in marine larviculture. Through multidisciplinary studies in the 1980s both the causes for the nutritional variability in *Artemia* and the methods to improve poor-quality *Artemia* were identified. Genotypic and phenotypic variation (*i.e.* cyst size, cyst hatching characteristics, caloric content and fatty acid composition of the nauplii) determine if a particular cyst product is suitable for hatchery use of specific fish or shrimp species. By bio-encapsulating specific amounts of particulate or emulsified products rich in highly unsaturated fatty acids in the brine shrimp metanauplii, the nutritional quality of the *Artemia* can be further tailored to suit the predators' requirements. Application of this method of bioencapsulation, also called *Artemia* enrichment or boosting, has had a major impact on improved larviculture outputs. The same bio-encapsulation method is now being developed for oral delivery of vitamins, chemotherapeutics and vaccines.

Furthermore, a better knowledge of the biology of *Artemia* was at the origin of the development of other *Artemia* products such as disinfected and decapsulated cysts, various biomass preparates, which presently have application in hatchery, nursery and broodstock rearing

Among the live diets used in the larviculture of fish and shellfish, nauplii of the brine shrimp *Artemia* constitute now the most widely used food item. Indeed, the unique property of the small branchiopod crustacean *Artemia* to form dormant embryos, socalled 'cysts', may account to a great extent to the designation of a convenient, suitable, or excellent larval food source that it has been credited with.

During the last years, annually, over 2000 metric tons of dry *Artemia* cysts are marketed worldwide for on-site hatching into 0.4 mm nauplii. At present, *Artemia* is being produced and exploited on the five continents. Despite this, a large part of the cyst market is still supplied by harvests from one location, the Great Salt Lake. This situation makes the market still extremely vulnerable to climatological phenomenon.

CHAPTER 4

STUDIES ON *ARTEMIA* CYSTS FROM MARGHERITA DI SAVOIA SOLAR SALTWORKS (APULIA, SOUTHERN ITALY).

4.1. Introduction

The studies on the brine shrimp *Artemia* population existing in the Margherita di Savoia solar saltworks, in the southern of Italy, started at the end of 1970s, during the years in which research efforts were made to prove the possibility of local production of *Artemia* for aquaculture use. During these years the first Italian scientists who studied *Artemia* in Margherita di Savoia site were Claudio Barigozzi and Pasquale Trotta. In the few studies conduct in the Margherita di Savoia site was assessed that the autochthonous population that inhabitant this Italian saltworks was the parthenogenetic (diploid and tetraploid) one.

From the early 1980s however, field studies in the Mediterranean environments established the occurrence, in some site, of the allochthonous and invasive *A. franciscana* populations. The American invasive species *A. franciscana* was first documented in Portugal, in Morocco, in several sites in Spain and also in France (Amat et al., 1987, 2005; Thièry and Robert, 1992; Green et al., 2005). In the frame of this phenomenon, the determination of spatial and temporal invasion and colonization patterns and the evaluation of possible impacts to endemic species are imperative to analize.

A recent study (Mura et al., 2006) on cysts collected at Margherita di Savoia site in the 2004, revealed the presence in the saltworks of a bisexual invasive species (1,6%), although autochthonous strains are still predominant (~98.4%). Over the last ten years, intense research on *Artemia* biodiversity throughout the world has provided ample data on patterns on intra- and interspecific differentiation. As result, for species and/or strain characterization is suggested a multi-trait approach and an interdisciplinary and collaborative research.

4.2. Strain studies carried out at the *Artemia* Reference Center

In the Laboratory of Aquaculture & Artemia Reference Center at Ghent University, has been carried out analysis for the characterization of *Artemia* populations living and reproducing in Margherita di Savoia solar saltworks, in order to determine the presence, the origin and the status of the invasive bisexual species. This characterization has been carried out in the frame of a collaborative research and using a multi-trait approach. On cysts of Artemia collected during the 2007, from the Margherita di Savoia solar saltworks were performed the following characterizations:

Biometry of cysts and nauplii

- Cysts biometrics (cysts decapsulated diameter, cysts non decapsulated diameter, chorion thickness);
- Size (length) of Artemia instar I nauplii;

Hatching quality

- Hatching percentage (number of naplii per 100 full cysts);
- Hatching efficiency (number of nauplii per gram cysts material);

Reproductive characteristics

FAME (Fatty Acid Methyl Esters) analyses.

4.2.1. *Artemia* cysts sampling, sample preservation and diapause termination

Cysts floating at the water surface and accumulate along the windward side of the ponds in the Salpi Nuovo area of the saltworks, has been collected simply towing a net whit a mesh size in the range 100-120 µm. Sampled cysts have been on-site cleaned by washing with pond water over screens with different mesh widths in order to remove debris larger and smaller. Freshly sampled cysts, preserved at least for some days in pond water, as “raw cysts”, is used as a temporary storage method between harvesting and further brine processing. The processing of cysts including the size and density separation with brine, the wash and density separation in freshwater, have been performed according to the to the guidelines given in Baert et al. (1996). For long-term storage (several months), the hydration level of the cysts has be reduced by dehydration in saturated brine (> 300 g/l), and after some week by drying. The drying procedure in order to obtain the termination of the state of diapause has been achieved, according to guidelines given in Baert et al. (1996), by layer drying in oven of the cysts at temperatures not exceeding 35-40°C, in order to maintain maximum viability of cysts.

4.2.2. *Biometry of cysts and nauplii*

Prior to analyse, cysts have been washed and cleaned and all salt or debris has been washed out before proceeding with the sample hydration. The cysts diameter is determined on fully hydrated cysts, when the shape of the embryos approximates a sphere. Generally the dimensions of the cysts vary considerably from strain to strain and this parameter is used, together with the other parameters, to distinguish mixed cysts samples. According to the technique of Bruggeman et al. (1980) hydrated non-decapsulated cysts, hydrated decapsulated cysts, the length of instar-I nauplii and the chorion thickness were measured. The incubation of cysts for collection of instar I nauplii and the cysts decapsulation were performed according to Sorgeloos et al. (1986). The cysts diameter and the length of the nauplii were measured using light microscope equipped with eyepiece micrometer calibrated with a stage graticule. A random sample of 350 booth decapsulated and non-decapsulate cysts was measured. The average value of the diameter observed for non-decapsulated cysts is 236.5 ± 57.5 μm (mean value ± standard deviation), for decapsulated cysts is 210.4 ± 58.2 μm and the chorion thickness observed is 13.1 μm. Those data and in particular the double peak in the size frequency distribution of decapsulated cysts (Fig. 17B) and the high value of the chorion thickness, seem to confirm the presence of a mixed population in the Margherita di Savoia saltworks.

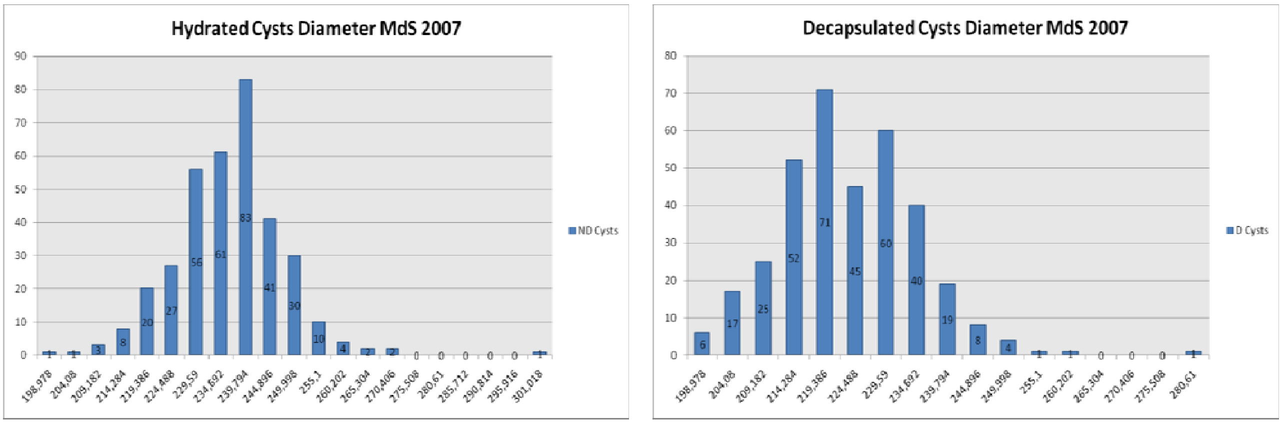
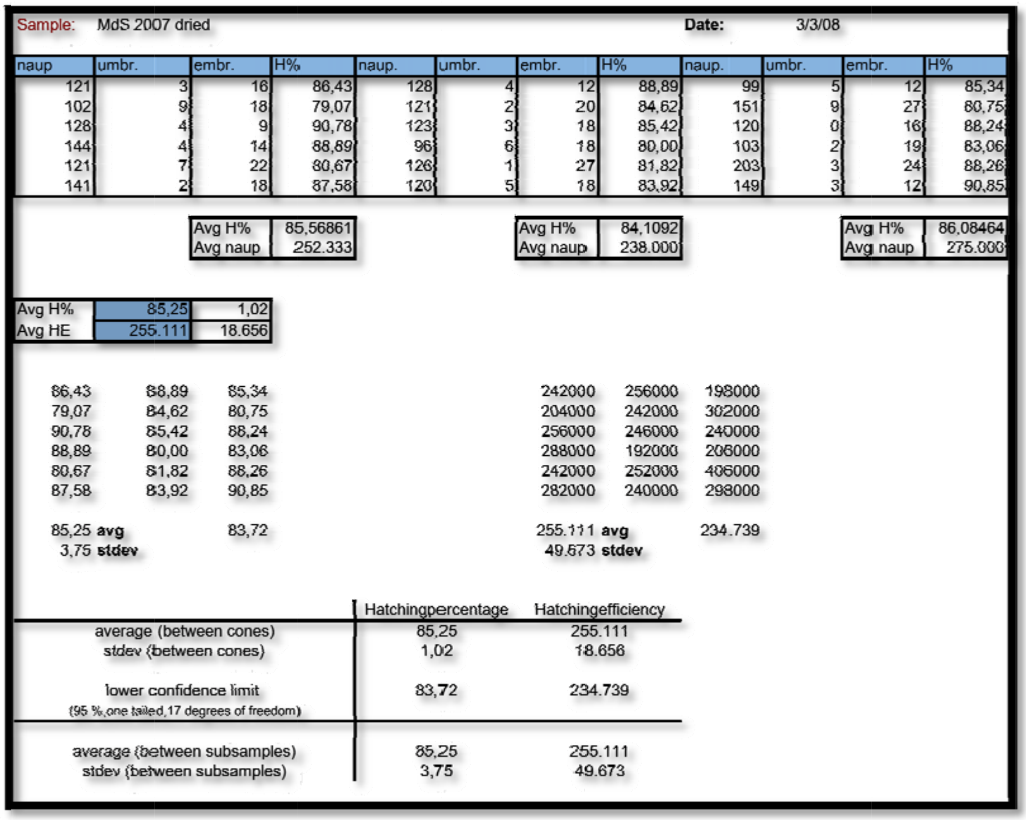


Fig. 17: Cysts *size frequency distribution of decapsulated (A) and non-decapsulated (B) cysts*.

Also the average value of the length of the instar I nauplii, 465.6 ± 267.2 μm, appears to confirm the mixed population. The cysts diameter of Margherita di Savoia saltworks populations resembles those of the New World bisexual populations, significantly smaller than the parthenogenetic one, autochthonous of this saltworks.

4.2.3. *Hatching quality*

Cysts has been incubated in natural seawater (35 - 37 g.l $^{-1}$) at a temperature of 25 – 28 ºC at 1000 lux, according to standardized methods (Vanhaecke and Sorgeloos, 1980). Hatching analyses were carried out according to Vanhaecke & Sorgeloos (1983) The analyses performed are the hatching efficiency (HE), the number of nauplii which can be produced out of 1 gram dry cyst product when incubated under standard hatching conditions, and the hatching percentage (H%), number of nauplii which can be produced from 100 full cysts containing an embryo. Table 2 show the high hatching efficiency (HE) and hatching percentage (H%), respectively 255,111 ± 49,673 and 85.25 ± 3.75.



Sample: MdS 2007 dried Date: 3/3/08

naup	umbr.	embr.	H%	naup.	umbr.	embr.	H%	naup.	umbr.	embr.	H%
121	3	16	86,43	128	4	12	88,89	99	5	12	85,34
102	9	18	79,07	121	2	20	84,62	151	9	27	80,75
128	4	9	90,78	123	3	18	85,42	120	0	16	88,24
144	4	14	88,89	96	6	18	80,00	103	2	19	83,06
121	7	22	80,67	126	1	27	81,82	203	3	24	88,26
141	2	18	87,58	120	5	18	83,92	149	3	12	90,85

Avg H%	85,56861	Avg H%	84,1092	Avg H%	86,08464
Avg naup	252.333	Avg naup	238.000	Avg naup	275.000

Avg H%	85,25	1,02
Avg HE	255.111	18.656

86,43	88,89	85,34	242000	256000	198000
79,07	84,62	80,75	204000	242000	302000
90,78	85,42	88,24	256000	246000	240000
88,89	80,00	83,06	288000	192000	206000
80,67	81,82	88,26	242000	252000	406000
87,58	83,92	90,85	282000	240000	298000
85,25 **avg**		83,72	255.111 **avg**		234.739
3,75 **stdev**			49.673 **stdev**		

	Hatchingpercentage	Hatchingefficiency
average (between cones)	85,25	255.111
stdev (between cones)	1,02	18.656
lower confidence limit (95 %,one tailed,17 degrees of freedom)	83,72	234.739
average (between subsamples)	85,25	255.111
stdev (between subsamples)	3,75	49.673

Tab. 2: Hatching characteristics of cysts from collected in the Margherita di Savoia saltworks in 2007.

The results of the hatching characteristics from Margherita di Savoia cysts collected in 2007 and their comparison with the date from the existing bibliography (Vanhaecke and Sorgeloos, 1983) reveal the high quality of the cysts and their compatibily with the San Francisco Bay bisexual population.

4.2.4. *Reproductive characteristics*

Experiments carried out in the laboratory on the reproductive characteristics has been considered for a range of objectives, from comparative studies on the reproductive performance and life span of different strains to the taxonomic verification of species, including inferences (in combination with other traits) on the degree of divergence among populations, within or between species (Gajardo et al. 1999). The methodology used considers the observation of single animals in single vials (Fig. 18). For this, freshly hatched nauplii has been cultured under standard hatching conditions and then transferred in a common aquarium. Prior to sexual maturity randomly-taken single animals are transferred into separate vials containing seawater (35 - 37 g/l) and different reproductive parameters are evaluated for a period of time. Vials (60ml Falcon tube) has been fed daily with microalgae and maintained under standardized temperature and light conditions. In the sample tested, the sex ratio (female/male) observed during the culture period was 0,87.

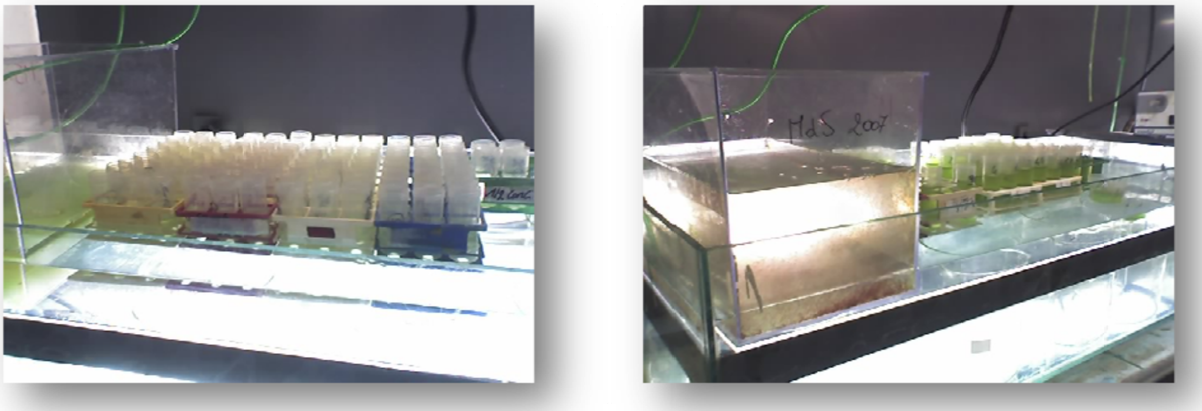


Fig. 18: Experiment on the reproductive characteristics carried out at the Artemia Reference Center.

Survival levels and percentage of animals attaining adulthood were recorded every three days. Adult virgin females in each individual 50ml Falcon tubes, were observed in order to determine the sexual or asexual reproductive mode. The observation carried out show that the survival after 15 days was about 70% and that only the 1% of the females cultured in absence of males produced free-swimming nauplii (parthenogenetic reproduction).

4.2.5. F.A.M.E. (Fatty Acid Methyl Esters)

The analyses of lipids extracted from freshly hatched nauplii (Tab. 3), following the procedure of gas chromatography, have been compared with the data present in literature. The comparison of our data with the data in literature on cysts collected in different years in Margherita di Savoia saltworks and on cysts collected in other biotopes (San Francisco Bay and Great Salt Lake, in particular) reveals great similarities. This similarity makes it impossible to discriminate on the presence or absence of an invasive population in the Margherita di Savoia saltworks.

4.3. Discussion

Over the last few years, multidisciplinary approaches in the strain studies of the genus *Artemia* have given answers to a wide range of questions related to the extent and nature of *Artemia* biodiversity. Assessments of species status correlation between the data obtained from the analyses carried out at the Artemia Reference Center confirm the presence of a bisexual population in the Margherita di Savoia saltworks. Previous work on the population dynamics in Margherita di Savoia saltworks on cysts collected before the year 2004 (Mamone et al., 1998; Nascetti et al., 2003; Amat et al., 2005), did not record any presence of an invasive population. The first data on the presence of a bisexual invasive species in the saltworks are from a recent study (Mura et al., 2006) on cysts collected at Margherita di Savoia site in the 2004. The data obtained in the study carried out at the Artemia Reference Center during the short term mobility program 2008, revealed that the invasive population is now predominant (~99%) on the autochthonous strains, while in the Mura’s study was assessed that the autochthonous strains are still predominant (~98.4%). All this data show the worrying speed of this invasion phenomenon, but at the same time the limit of few and fragmented studies on *Artemia* living in the biggest solar saltwork of Europe.

MdS 2007 - Instar I					
Code	Peak	Area%		mg/g DW	
		average	St.Dev.	average	St.Dev
* 1 R	14:0	1,0	0,1	1,2	0,1
* 2	14:1(n-5)	0,7	0,0	0,9	0,1
3	15:0	0,6	0,0	0,7	0,0
4	15:1(n-5)	0,5	0,0	0,6	0,0
* 5 R	16:0	11,9	0,1	14,0	1,0
* 6	16:1(n-7)	10,0	0,0	11,8	0,7
* 7	17:0	1,1	0,0	1,3	0,1
* 8	17:1(n-7)	2,2	0,0	2,6	0,1
* 9 R	18:0	5,6	0,1	6,7	0,5
* 10	18:1(n-9)	17,0	0,0	20,1	1,2
* 11	18:1(n-7)	12,2	0,1	14,4	0,7
* 12	18:2(n-6)-t	0,1	0,0	0,2	0,0
* 13 R	18:2(n-6)-c	7,2	0,1	8,5	0,6
* 14	19:0				
* 15	18:3(n-6)	0,2	0,1	0,3	0,1
16	19:1(n-9)	0,4	0,0	0,5	0,0
* 17	18:3(n-3)	3,2	0,0	3,7	0,2
18	18:4(n-3)	0,5	0,0	0,6	0,0
* 19	20:0	0,2	0,0	0,3	0,0
* 20	20:1(n-9)	0,6	0,0	0,7	0,0
21	20:1(n-7)	0,2	0,0	0,2	0,0
* 22 R	I.S.				
* 23	21:0	0,0		0,0	
* 24	20:3(n-6)	0,2	0,0	0,2	0,0
* 25	20:4(n-6)	2,5	0,0	3,0	0,2
* 26	20:3(n-3)	0,1		0,1	
27	20:4(n-3)	0,3	0,0	0,4	0,0
* 28	22:0	0,7	0,0	0,8	0,0
29 R	20:5(n-3)	11,7	0,0	13,8	0,8
* 30	22:1(n-9)				
31	22:1(n-7)				
* 32	23:0				
33	21:5(n-3)	0,1		0,1	
* 34	23:1(n-9)	0,1		0,2	
* 35	22:4(n-6)	0,2	0,0	0,3	0,0
* 36	22:3(n-3)				
37	22:5(n-6)				
38	22:4(n-3)				
* 39	24:0	0,0		0,0	
40	22:5(n-3)	0,1	0,0	0,2	0,0
* 41	24:1(n-9)	0,1		0,1	
* 42 R	22:6(n-3)	0,0	0,0	0,1	0,0
Sum (n-3) >or= 20:3(n-3)		**12,3**	**0,1**	**14,5**	**0,7**
Sum (n-6) >or= 18:2(n-6)-t		**10,5**	**0,1**	**12,4**	**0,7**

Further analytical information

		average	St.Dev.	average	St.Dev
g wet	**g dry**	0,2511	0,0057	0,0170	0,0004
% DW	**XF 0.5/DW**	6,755	0,0000	29,485	0,6684
Total mg FAME/g DW		118,0	7,0		
Total lipid % on DW					

Tab. 3: Composition of fatty acid methyl esters (F.A.M.E.) of artemia nauplii hatched from cysts collected in the Margherita di Savoia slatworks in 2007.

CONCLUSIONS

Artemia biodiversity is a complex and multi-faceted issue. The need for a combined use of techniques, including the tests carried out in the present study and other tests as, cytogenetics, allozyme studies, nuclear and mitochondrial DNA analysis, ecc., to approach the genus' taxonomy, speciation and phylogeny, has been amply documented and adequately sustained by other authors (Gajardo et al., 2002, 2006; Bossier et al., 2004). This invasion at the studied Italian saltwork may have taken place most recently, or might be that the invasion dynamics in this saltwork is the result of a temporal or spatial nature. The cyst samples analyzed represent a small fraction of the total cyst stock present in the environment, and its species composition may significantly deviate from the biomass occurring at a particular time in a particular area.

The controversy about the species status of the *Artemia* population(s) from Lake Urmia, Iran, where conclusions of successive studies were based on single laboratory samples or on sets of field data that were too limited in space and/or time (Clark and Bowen, 1976; Badaracco et al., 1987; Barigozzi et al., 1987; Azari Takami, 1989; Ahmadi et al., 1990; Browne et al., 1991; Pador, 1995; Van Stappen et al., 2001), is exemplary for how valuable but rather fragmentary research efforts may fail to see the broader picture. It illustrates how the interpretation of laboratory results needs to be matched with a broad set of field data – if possible covering several production seasons - and vice versa. Even so, it may require a considerable time and multiple efforts but this is necessary for well understanding a solar saltwork that indisputably have a certain degree of ecological value (as advocated by the 1st International Conference on the Ecological Importance of Solar Salt works, 2006, Santorini; Greece), especially if is operative from centuries, and have entered into an ecological equilibrium with the surrounding ecosystems.

References

Afan De Rivera C. (1845). *Del bonificamento del Lago di Salpi, coordinato a quello della pianura della Capitanata.* Stamperia e Cartiere del Fibreno, Napoli: 638 pp.

Ahmadi M. R. M., Leibovitz H., Simpson K. (1990). *Characterization of Uromiah Lake Artemia (Artemia uromiana) by electrofocusing of isozyme patterns.* Comparative Biochemistry and Physiology, 95: 115-118.

Alberti L. (1551). *Descrizione di tutta Italia.* Venezia: 203 pp.

Amat, F., Hontoria F., Ruiz O., Green A. J., Sánchez M. I., Figuerola J., Hortas F. (2005). *The American brine shrimp as an exotic invasive species in the western Mediterranean.* Biol. Invasions, 7: 37-47.

Angelini G., Carlone G. (1986). *Atlante storico della Puglia, -1- La Provincia di Foggia. Capone ed., Manduria: 69 pp.*

Azari Takami G. (1989). *Two strains of Artemia in Urmia Lake (Iran).* Artemia Newsletter, 13: 5.

Baert P, Bosteels T, Sorgeloos P (1996). *Artemia: Pond production. In: Manual on the production and use of live food for aquaculture.* Lavens, P., Sorgeloos, P. (Eds). FAO Fisheries Technical Paper No. 361: 295 pp.

Barigozzi C., Varotto V., Baratelli L., Giarrizzo R. (1987). *The Artemia of Urmia Lake (Iran): mode of reproduction and chromosome numbers. In: Atti della Accademia Nazionale dei Lincei.* Rendiconti Classe di Scienze fisiche, matematiche e naturali, 81(1): 87-90.

Boenzi F., Caldara M., Pennetta L. (2001). *History of the Salpi lagoon-sabkha (Manfredonia Gulf, Italy).* Il Quaternario, 14 (2).

Bossier P., Xiaomei W., Catania F., Dooms F., Van Stappen G., Naessens E., Sorgeloos P. (2004). *An RFLP database for authentication of commercial cyst samples of the brine shrimp Artemia spp. (International Study on Artemia LXX).* Aquaculture, 231: 93-112.

Browne R. A., Li M., Wanigasekara G., Simonek S., Brownlee D., Eiband E., Cowan J. (1991). *Ecological, physiological and genetic divergence of sexual and asexual (diploid and polyploid) brine shrimp (Artemia).* Advances in Ecology, 1: 41-52.

Bruggerman E., Sorgeloos P., Vanhaecke P. (1980). *Improvements in the decapsulation technique of Artemia cysts. In: The brine shrimp Artemia, Vol. 3. Ecology, culturing, use in aquaculture.* Persoone, G., Sorgeloos, P., Roels, O., Jaspers, E. (Eds). Universa Press, Wetteren, Belgium: 261-269.

Bulifon A. (1690). *Cronicamerone, ovvero Annali e Giornali historici delle cose notabili accadute nella città e nel Regno di Napoli fino all'anno 1690.* Napoli.

Caldara M, Pennetta L, Simone O (2002). *Holocene Evolution of the Salpi Lagoon (Puglia, Italy).* Journal of Coastal Research: 124-133.

Caldara M., Pennetta L. (1990). *Evoluzione dell'ambiente olocenico nel basso Tavoliere di Puglia.* Bonifica, 6 (4): 47-66.

Caldara M., Pennetta L. (1992). *Interpretazione paleoclimatica di dati preistorici e storici relativi all'entroterra del Golfo di Manfredonia.* Memorie della Società Geologica Italiana, 42: 197-207.

Caldara M., Pennetta L., Zito G., (1994). *L'area umida alla foce del Candelaro: osservazioni paleoambientali e climatiche.* II Workshop on Parchi naturali ed aree protette del Progetto Strategico Clima Ambiente e Territorio nel Mezzogiorno, S. Maria di Salina: 211-234.

Ciccone S., Como R. (1984). *Cinquant'anni di bonifica nel Tavoliere.* Bastogi ed., Foggia: 664 pp.

Clark L. S., Bowen S. T. (1976). *The genetics of Artemia salina. VII. Reproductive isolation.* The Journal of Heredity, 67: 385-388.

Colacicco G. (1955). *La Bonifica del Tavoliere. Consorzio di Bonifica della Capitanata.* Foggia: 66 pp.

Di Biase P. (1985). *Puglia medievale e insediamenti scomparsi. La vicenda di Salpi.* Schena ed., Fasano: 293 pp.

Gajardo G. M., Sorgeloos P., Beardmore J. A. (2006). *Inland hypersaline lakes and the brine shrimp Artemia as simple models for biodiversity analysis at the population level.* Saline Systems, 2: 14.

Gajardo G., Abatzopoulos T. J., Kappas I., Beardmore J. A. (2002). *Chapter V. Evolution and speciation. In: Artemia: basic and applied biology.* Abatzopoulos, T. J., Beardmore, J. A., Clegg, J. S., Sorgeloos, P. (Eds). Kluwer Academic Publishers, Dordrecht, the Netherlands: 225-250.

Gajardo G., Mercado C., Beardmore J.A., Sorgeloos P. (1999). *International Study on Artemia. LX. Allozyme data suggest that a new Artemia population in southern Chile (50° 29’ S; 73° 45’ W) is A. persimilis.* Hydrobiologia 405: 117-123.

Green A., Sánchez J., M. I., Amat F., Figuerola J., Hontoria F., Ruiz O., Hortas F. (2005). *Dispersal of invasive and native brine shrimps Artemia (Anostraca) via waterbirds.* Limnol. Oceanogr. 50: 737-742.

Jones G. D. B. (1987). *Apulia Vol. 1: Neolithic settlement in the Tavoliere.* Reports of the Research Committee of the Society of Antiquaries of London, XLIV: 237 pp.

Kuenen D J, Baas-Becking L G M (1938). *Historical notes on Artemia salina (L).* Zool. Med., 20: 222-230.

Lavens P, Sorgeloos P. (1996). *Manual on the production and use of live food for aquaculture.* FAO Fisheries Technical Paper. No. 361. Rome: 295 pp.

Linneaus C (1758). *Systema naturae.* Ed. X., Hafniae. 634 pp.

Mamone R., Ceccarelli R., D’ambria R., Maccaroni A., Rampacci M., Tancioni L. (1998). *Studio della popolazione di Artemia sp. nella più grande salina europea (Margherita di Savoia-Italia meridionale).* Biol. Mar. Medit. 5: 665-670.

Masson G. (1978). *Frederick II of Hohenstaufen.* Martin Secker and Warburg Ltd., London: 457 pp.

Mura, G., Kappas, I., Baxevanis, A.D., Moscatello, S., D'Amico, Q., Lopez, G.M., Hontoria, F., Amat, F., Abatzopoulos, T.J. (2006). *Morphological and molecular data reveal the presence of the invasive Artemia franciscana in Margherita di Savoia salterns (Italy).* International Review of Hydrobiology, 91(6): 539-554.

Nascetti G., Bondanelli P., Aldinucci A., Cimmaruta R. (2003). *Genetic structure of bisexual and parthenogenetic populations of Artemia from Italian brackish-hypersaline waters.* Oceanol. Acta, 26: 93-100.

Pador E. (1995). *Characterisation of the Artemia urmiana Gunther 1900 from Lake Urmia, Iran.* MSc. Thesis Vrije Universiteit Brussel and Laboratory of Aquaculture-*Artemia* Reference Center, University of Ghent, Belgium.

Parrino D. A. (1770). *Teatro eroico e politico de' governi de' Vicerè del Regno di Napoli, dal tempo del Re Ferdinando il Cattolico fino al presente.* Ricciardo ed., Napoli.

Pascale L. (1912). *L'antica e la nuova Siponto.* Tip. Marietti Torino: 217 pp.

Persoone G., Sorgeloos P. (1980). *General aspects of the ecology and biogeography of Artemia. In: The brine shrimp Artemia, Vol. 3. Ecology, culturing, use in aquaculture.* Persoone, G., Sorgeloos, P., Roels, O., Jaspers, E. (Eds). Universa Press, Wetteren, Belgium: 3-24.

Proceedings of the 1st International Conference on the Ecological Importance of Solar Saltworks (2006). Lekkas T. D., Korovessis N. A. (Eds). Santorini Island, Greece.

Sorgeloos P. (1980a). *Life history of the brine shrimp Artemia. In: The brine shrimp Artemia. Vol. 1. Morphology, genetics, radiobiology, toxicology.* Persoone G, Sorgeloos P, Roels O, Jaspers E (Eds). Universa Press, Wetteren, Belgium: 19-23.

Sorgeloos P., Dehasque M., Dhert P., Lavens P. (1995). *Revew of some aspects of marine fish larvicolture.* ICES Mar. Sci. Symp., 201: 138–142.
Sorgeloos P., Lavens P., Legér P., Tackaert W., Versichele D. (1986). *Manual for the culture and use of brine shrimp Artemia in aquaculture.* State University of Ghent, Belgium.

Thiéry A., Robert F. (1992). *Bisexual populations of the brine shrimp Artemia in Sète-Villeroy and Villeneuve saltworks (Languedoc, France).* Int. J. Salt Lake Res., 1: 47-63.

Tiné Bertocchi F. (1975). *Formazione della civiltà dauna dal X al VI secolo a.C. Civiltà preistoriche e protostoriche della Daunia.* Atti del Colloquio Internazionale di Preistoria e Protostoria della Daunia, 271-285.

Van Stappen G., Fayazi G., Sorgeloos P. (2001). *International Study on Artemia LXIII. Field study of the Artemia urmiana (Günther, 1890) population in Lake Urmiah, Iran.* Hydrobiologia, 466: 133-143.

Vanhaecke P., Sorgeloos P. (1980). *International Study on Artemia. XIV. Growth and survival of Artemia larvae of different geographical origin in a standard culture test.* Marine Ecology Progress Series 3: 303-307.

Vanhaecke P., Sorgeloos P. (1983). *International Study on Artemia. XIX. Hatching data for ten commercial sources of brine shrimp cysts and re-evaluation of the "hatching efficiency" concept.* Aqaculture (30).