


Review on integrated production of the brine shrimp *Artemia* in solar salt ponds

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Abstract

The brine shrimp *Artemia* is a highly required, convenient and cost-effective live food used in fish and shellfish larviculture. Its cysts, originating from a limited number of inland salt lakes, are traded worldwide. Over the past decades, *Artemia* pond production in solar saltworks, integrated with salt production, has emerged as an alternative to cope with possible cyst shortages and high prices on the international market and as a tool to fulfill local cyst and *Artemia* biomass demands associated with local and regional aquaculture developments. This article reviews the principles of *Artemia* pond production and how it is practiced in terms of general management. It also describes the distinctive features that have evolved in various countries, due to geographical, environmental and socio-economic characteristics and the local aquaculture context. It highlights the lessons that may be learnt from previous successes and failures for new areas where the technique may be introduced. The principles of *Artemia* pond production are also discussed in the light of global and local *Artemia* biodiversity, as its protection is key to support the sustainability of the aquaculture industry. This review pioneers in bringing this information, much of which was restricted to grey literature until now, under the attention of the international scientific audience. Future research and development should focus on continued proper management to secure cyst production and to maximize cyst quantities. Moreover, using the information provided by the *Artemia* genome will allow as well to strive for improved cyst quality, by producing strains with specific market characteristics.

Key words: *Artemia*, biomass, cysts, integrated, pond production, salt.

Introduction

Aquaculture is thought to supplement and maybe eventually replace the over-exploited fisheries in fulfilling the need of the growing human population for high-quality animal proteins. Under this demanding scenario, the sustainable diversification of aquaculture practices emerges as a challenge ahead. The potential for diversification is enormous due to the diversity of existing aquatic species (Duarte *et al.* 2007), but the bottleneck of high larval mortalities threatens the realization of this potential.

One of the problems faced by the larval rearing of aquaculture organisms in captivity (larviculture) is the quantitative and qualitative supply of cysts of the brine shrimp *Artemia*, an anostracan branchiopod crustacean with natural populations spread worldwide in hypersaline inland lakes and coastal lagoons. Its freshly hatched nauplius larvae are an essential live food in the larviculture stage of marine crustaceans and fish. This is linked to its many advantages over alternative live diets in the market, among the most relevant being the availability and convenience of the dry cysts (diapausing

embryos) that can be kept in a viable state on-the-shelf (Lavens & Sorgeloos 2000; Conceição *et al.* 2010). *Artemia* cysts are the trade coin in the marine aquaculture business and are naturally harvested in a limited number of salt lakes, the main single producing location being Great Salt Lake (GSL) in Utah, USA, but with growing importance of salt lakes scattered over Russia, Kazakhstan and inland China. In a scenario of climate change and increasing harvesting pressure on these natural salt lakes, it is difficult to manage the dynamics of the *Artemia* population cycles and hence to predict mid- or long-term cyst harvests, even in GSL which is a well managed and monitored lake (Wurtsbaugh & Gliwicz 2001; Byron *et al.* 2011; Endebu *et al.* 2013; Eimanifar *et al.* 2015).

Consequently, new locations such as solar saltworks, suitable for *Artemia* biomass and cyst production in ponds, have been more or less successfully exploited for several decades as a way to overcome past, present and probable future cysts shortages. However, in spite of its relative success, peer-reviewed literature on *Artemia* pond production is scarce. A limited number of literature sources report on specific scientific experiments conducted in pond conditions, aiming to enhance *Artemia* production (De los Santos *et al.* 1980; Anh *et al.* 2010; Sui *et al.* 2013; Ronald *et al.* 2014; Van *et al.* 2014; Gao *et al.* 2017). A recent review has focused on the *Artemia* pond feeding protocols, as practiced in the Mekong Delta, Vietnam (Le *et al.* 2018). A lot of technical information on production protocols and technical developments in this sector is scattered in 'grey literature' such as manuals, reports and workshop proceedings. Much is only handed down through bilateral extension initiatives. Consequently, rather than reviewing the scarce scientific literature on *Artemia* pond production experimental work or compiling methodological details of existing production protocols, this paper reviews past and ongoing developments and trends in this field. We focus on a number of study cases in specific countries (China, Vietnam, Thailand) while sketching the broader framework within which these developments have taken place in the respective countries. For this purpose, we have reviewed non-English language literature and reports available in the target countries, and compiled information through our respective networks with local production companies, farmer associations, and stakeholder groups in general. In doing so, similarities and differences are evaluated in order to draw conclusions on how these examples may catalyze local or regional aquaculture and socio-economic developments. We thus hope that the information presented will help the emergence of new initiatives in areas in the South where aquaculture diversification is underway.

Rationale and general principles of *Artemia* pond production

The status of *Artemia* as an economic commodity began in the 1930s when some investigators adopted it as a convenient substitute for the natural plankton diet for fish larvae, thus realizing an early break-through in the culture of commercially important fish species (Sorgeloos 1980). With industrial fish and shrimp culture operations emerging from the early 1960s onwards, new marketing opportunities were created for *Artemia* cysts. However, by the mid 1970s increased demand, declining harvests from GSL, high import taxes in some countries and possibly artificial cyst shortages created by certain companies resulted in a substantial price rise for *Artemia* cysts (Bengtson *et al.* 1991). The dramatic impact of the cyst shortage on the expanding aquaculture industry invigorated research on the rationalization of the use of *Artemia* and the exploration of new cyst resources, not only in other inland salt lakes but also in coastal saltworks where seawater is concentrated by evaporation until crystallization (Dhont & Sorgeloos 2002).

More or less controlled production of *Artemia* is nowadays carried out in many coastal saltworks. Solar salt production is confined to (sub)tropical climates in arid zones, or to climates with a sufficiently long dry season. In areas with seasonal salt production there is often no natural occurrence of *Artemia*. Also in arid areas there is not always a natural population of *Artemia* (e.g. the area of Natal, northeast Brazil, see further). Under such conditions *Artemia* has been introduced deliberately, not only for the sake of cysts or biomass production (Zmora *et al.* 2002), but also because of the beneficial effect of *Artemia* presence on the salt production process itself (Jones *et al.* 1981; Davis 2000). The first attempts of inoculation and subsequent management of *Artemia* in this type of solar saltworks were performed in the 1970s in Brazil, soon followed by the Philippines, China, Thailand, Vietnam and a number of other countries (Vos *et al.* 1984; Lavens & Sorgeloos 2000). Whereas there were synergies in the development of production protocols (e.g. Vietnamese experts provided consultancy in countries such as India and Sri Lanka), alternative production techniques were developed in other countries (e.g. in Thailand). Finally, there are also countries (e.g. in Latin America, East Africa) where initial attempts did not lead to the establishment of a viable *Artemia* production sector so far.

Generally, introductions have been done with San Francisco Bay (SFB)-type *A. franciscana* Kellogg 1906, although occasionally GSL *A. franciscana* has been used as well, and more recently *Artemia* from Vietnam (Kuruppu & Ekaratne 1995; Sultana *et al.* 2011; Sui *et al.* 2012), which originates in its turn from SFB *Artemia*. Depending on the climatological conditions, these inoculations turned out to be

definitive when one or several inoculations lead to the permanent establishment of an adapted *Artemia* population. Local adaptation may also occur in seasonal production, as has been documented for Vietnamese saltworks where *A. franciscana* is inoculated again and again at the onset of each dry season (Clegg *et al.* 2000, 2001; Kappas *et al.* 2004). Both in permanent solar salt operations and in seasonal units, brine shrimp are mainly found in ponds at intermediate salinity levels since the organism has no defense mechanisms against predators (which are generally dwindling or absent above 80–100 g L⁻¹). At salinities exceeding 200–250 g L⁻¹ brine shrimp productivity tends to decrease due to osmoregulatory stress (Van Stappen 2002).

In addition to the numerous introduction initiatives, also non-intentional introduction is increasingly being reported, generally as a side-effect of intentional introduction and/or of aquaculture activities. As mentioned in the introduction, a well-documented example is the Bohai Bay area in coastal China, where the current *Artemia* populations are a mixture of species: first, various strains of autochthonous parthenogenetic *Artemia*, second, *Artemia sinica* Cai 1989, harvested in inland China and used in Bohai Bay coastal aquaculture facilities, from where it has spread into the environment. Finally, there is also *A. franciscana* in the Bohai Bay saltworks, deliberately inoculated and further dispersed through aquaculture effluents (Tackaert & Sorgeloos 1991a; Van Stappen *et al.* 2007). Depending on the abiotic conditions and the competition with local *Artemia* species or strains (if any), an allochthonous population may establish itself following its appearance in the new environment. *A. franciscana* has higher overall colonizing abilities than other species (Gajardo *et al.* 2002); the gradual dispersion of this species has become common in various parts of the world (Mediterranean area, India, Sri Lanka, South Africa, Australia, coastal China; see for example Amat *et al.* 2005; Vikas *et al.* 2012), where the newcomer has out-competed local populations.

Although the bulk of *Artemia* cyst product on the world market still originates from GSL and other inland salt lakes (mainly in China, Kazakhstan, Russian Federation), the production of cysts in solar saltworks may make up for a considerable share of local markets in terms of product value (Lavens & Sorgeloos 2000). Domestic production of *Artemia* cysts and/or biomass in solar saltworks may be vital for the development of the local aquaculture sector. In solar saltworks, active management to enhance productivity may be minimal, but the highest productivity is reached when intensive management procedures are applied (Baert *et al.* 1997; Anh *et al.* 2009, 2010), such as in the Mekong Delta, Vietnam, where *Artemia* pond production is firmly established in the local saltworks. The principle of this

integrated production is nowadays enjoying renewed attention in several countries in Asia and Africa as a tool to sustain locally emerging aquaculture developments. Issues such as the ability of the (often introduced) *Artemia* strain(s) to adapt to local ecological conditions and the socio-economic imperatives of the local aquaculture environment thus need to be considered. Expansion of *Artemia* pond production into new areas benefits from the increasingly scientific approach that has been followed in Vietnam in recent years, in contrast with the empirical approach of the past. So more understanding is available of how the *Artemia* population interacts with its abiotic and biotic environment. This facilitates prognosis on how the population will perform after introduction in a new environment.

It is not easy to identify in retrospect the factors that have been decisive for the success or failure, development or stagnation, of *Artemia* production in the countries where pond production is practiced or has been attempted. Generally, it turns out as a profitable economic activity integrated with salt production, provided salt producers are willing to adopt the technology and researchers support this endeavor with research and sustained dissemination activities. Other elements conducive for success are a flourishing and diversified local aquaculture sector in need of *Artemia*; a flexible and dynamic trading sector able to cope with the unavoidable fluctuations in quantity and quality of local *Artemia* production; and finally a legal framework facilitating all of the above.

Case studies

China

The Artemia market and history of Artemia production in China

China is the world's biggest salt producer, with the Bohai Bay coastal area in north-eastern China being one of the country's main production areas (27 million tons, corresponding with 43% of the national production in 2016; Zhu Guoliang, China Salt Corporation, pers. comm. 2018) with about 250 000 ha of salt evaporation ponds (data of 2009), mainly operated by large salt production companies. However, the production area has been decreasing over recent years as salt ponds have been turned into urban and industrial areas.

China is equally a leading country for aquaculture, with an annual aquaculture production of 49 million tons in 2016 (FAO 2018). The fast development of aquaculture has led over the past few years to a steadily increasing demand for *Artemia* cysts: from 600 tons of commercial dry product in 2000 to about 1500 tons in 2012, which corresponded with roughly 50% of the entire world demand. Recently, however, cyst consumption has decreased again,

with about 1000 tons consumed in 2016 (Fig. 1). The cysts are mainly used in the hatcheries of penaeid shrimp and of freshwater prawn *Macrobrachium rosenbergii*, as well as in larviculture of several marine fish species. Several hundreds of tons of cysts are harvested in various inland salt lakes in China and in coastal saltworks in the Bohai Bay area (Fig. 2a), but these are largely insufficient to meet the country's demands. Although statistical data are not centralized, it is estimated that an average 1300 tons of dry cysts are imported annually from Russia, Kazakhstan and USA; meanwhile about 400 tons of cysts are exported to Thailand, Korea and other countries (Zhang Bo, Tianjin University of Science and Technology; Guo Yan, Boaifeng Biological Products Co., pers. comm. 2018). In recent years, the number of cyst processors has decreased from about 100 to less than 30, with about 80% of total output produced by about a dozen enterprises with an annual production in the range 50–200 tons each.

In the late 1980s-early 1990s, several outdoor trials were conducted in China to enhance *Artemia* biomass production by inoculating imported *A. franciscana* (Tang & Lin 1993) or using local parthenogenetic strains. However, widely varying biomass yields in the experimental ponds (in the range 3600–7300 kg ha⁻¹ year⁻¹) were reported (Sun *et al.* 1991; Xu 1994; Zhao *et al.* 1995). Tackaert and Sorgeloos (1991a,b, 1993) described the main salt producing area, the Bohai Bay area, as highly productive (the result of extreme eutrophication). By that time the harvesting of cysts and biomass of the local parthenogenetic strains, used in shrimp hatcheries and grow-out facilities, had become a considerable industry employing several hundreds of people. Closer biological management of the salt ponds was proposed through the scientifically supported introduction of *A. franciscana*. The higher productivity of this species under local conditions, due to its fast growth and reproduction at relatively low temperatures and its high temperature and salinity resistance, was

demonstrated by Triantaphyllidis *et al.* (1995). The different fitness of the autochthonous and the introduced populations in the Bohai Bay conditions, coupled with the dispersal of non-native populations following introduction, thoroughly affected the biodiversity of the genus *Artemia* in this area. By 1997, Bohai Bay *Artemia* production approximated 600 tons for cysts and 20 000 tons for biomass, but allochthonous *A. franciscana* was prominently present in the area from the end of the century onwards (Zheng *et al.* 2004; Van Stappen *et al.* 2007). This was also illustrated by the fact that since the first introduction of *A. franciscana* in the Bohai Bay area in 1992, the average cyst diameter of locally produced cysts had decreased from 272 to 240 µm (Van Stappen *et al.* 2007), which is indicative of a growing share of small-type *A. franciscana* in local cyst production.

By the end of the previous century, due to the fast development of Chinese aquaculture and the shortage of *Artemia* cyst resources worldwide, extensive *Artemia* pond production enjoyed a lot of attention in the Bohai Bay area, particularly in Shandong Province. Large areas of salt land (about 33 000 ha) were used to construct ponds for *Artemia* pond production. The size of these ponds varied from several hundred m² to 100 ha, with cysts yields reaching 3–60 kg wet weight ha⁻¹ (Li & Xin 2003). In contrast to monsoon South East Asia, *Artemia* biomass and cysts were mostly produced in an extensive way: no organic manure or agriculture by-products were added to the ponds as supplemental feeds. However, this expansion was transient due to the dramatically decreasing cyst prices in the period 2000–2002 (Fig. 1).

Present status of *Artemia* production in China

In the period 2008–2012, about 180–200 tons of cysts out of an estimated total Chinese production of 900 tons originated from Bohai Bay saltworks (the remainder of the production being harvested in inland salt lakes). The cyst production season in the Bohai Bay generally starts in June

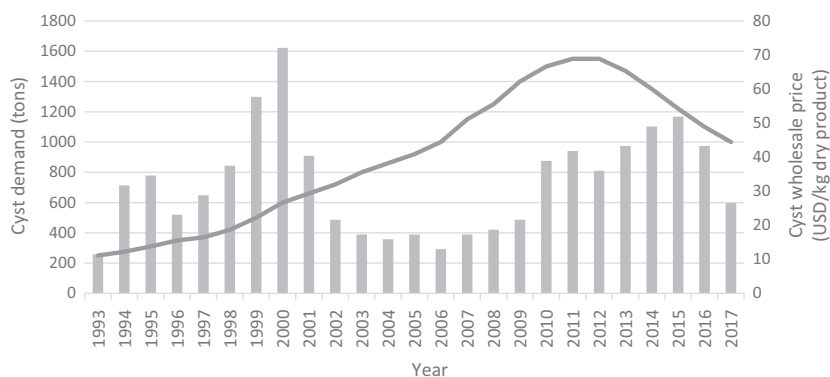


Figure 1 Fluctuations in price of commercial cyst product (bar graph) and of cyst demand (line graph) on the Chinese market in the period 1993–2017 (Source: Zhang Bo, Asian Regional Artemia Reference Center, Tianjin University of Science and Technology, China).

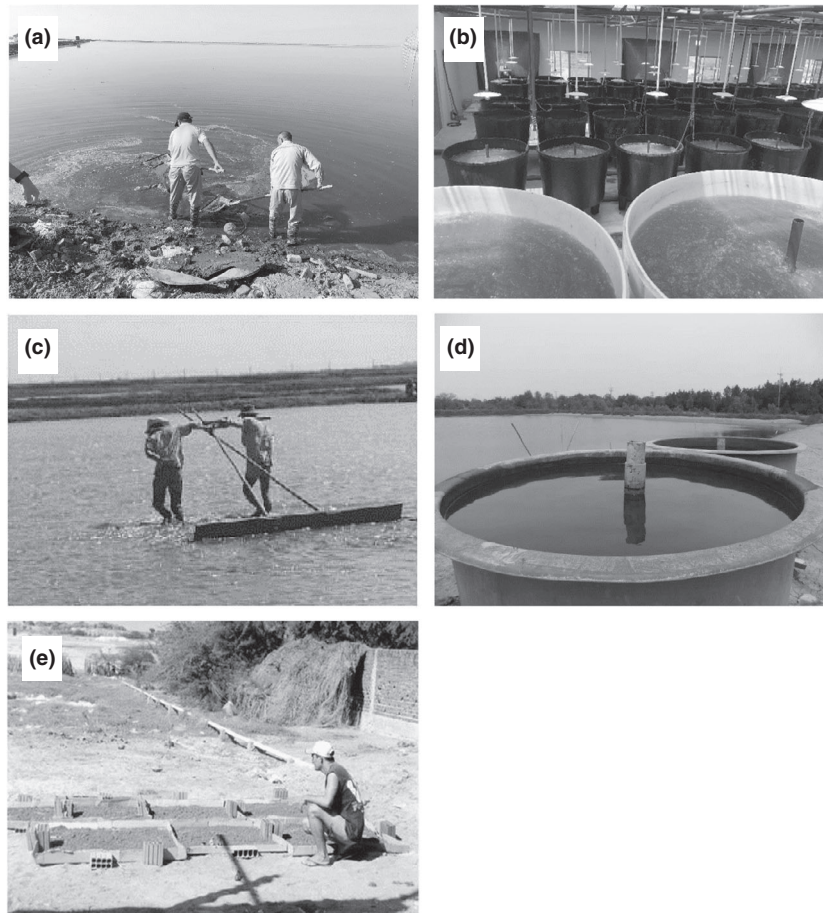


Figure 2 Management activities related to *Artemia* pond production: (a) harvesting of cysts from Bohai Bay saltworks, China; (b) nauplii hatching containers in company commercializing hatched nauplii, China; (c) raking of salt ponds, Mekong Delta, Vietnam; (d) ami-ami fermentation in fiber glass tanks, Thailand; (e) outdoors cyst drying, Brazil.

(about 1 month after the first spring emergence of *Artemia* nauplii) when the water temperature has increased to about 20°C. In the summer months July and August, water temperatures can peak up to 30–31°C in the high salinity ponds, that are usually shallow. The cyst production season ends toward the end of November when the water temperature drops to less than 10°C. The harvest is usually interrupted by heavy rains often occurring from July until mid August. Generally, the autumn harvest (September to end of November) accounts for 2/3 of the total harvest. Since 2010, interest in *Artemia* pond production has risen again, aiming to produce high-quality Bohai Bay *Artemia* cysts. This has led to the involvement of a number of saltwork managers in semi-intensive pond culture, including supplementation with chicken manure to stimulate algal blooms in the ponds. In 2012–2013, this type of semi-intensive *Artemia* pond production was conducted, with proper scientific support, over 60 ha of ponds in Shandong Chengkou Saltworks.

The price of *Artemia* cysts on the Chinese market fluctuates with cysts yields and with the demand of the market, both at international and domestic levels. Recently, the price of cysts from inland salt lakes has increased gradually from the lowest point of 13 USD kg⁻¹ in 2006 to 50 USD kg⁻¹ in 2015, after which prices have dropped again (Fig. 1). *Artemia* cysts from the Bohai Bay salt ponds on the other hand are known for higher hatchability and better nutritional value and are thus sold in China at a price roughly 150% higher than inland cysts, reaching 75–80 USD kg⁻¹ in 2016. Recently, so-called *Artemia* nauplii hatcheries (Fig. 2b) have been established in the vicinity of fish and shellfish hatcheries, supplying (shell)fish farmers with freshly hatched *Artemia* nauplii, as the specialized *Artemia* hatching enterprises are more professional and more efficient in cyst hatching procedures than the (shell)-fish hatcheries, especially the smaller ones. Also *Artemia* biomass is used, mostly as fresh feed for shrimp farms. Only a small percentage of biomass is frozen, to be used

during winter and early spring, when biomass is not available in the field. The price for fresh biomass was 0.5 USD kg⁻¹ wet weight in 2016.

In spite of occasional upsurges of initiatives, the Bohai Bay production has been stagnating or even declining in recent years due to the reduction in the overall size of the salt production area and to overharvesting. Yields have also decreased as a consequence of extensive discharge of effluents of desalination and bromine extraction plants into lower salinity evaporation ponds (usually at 50–60 g L⁻¹), which provide the intake water for *Artemia* production ponds. The bromine extraction effluents (often acidified to pH 2–3 and deprived of live plankton organisms) have a profoundly negative impact on the salt pond ecosystem. Although the buffering capacity of the soil allows recovery to weak alkalinity (pH 7–8) after three to four steps of the evaporation procedure, there has been a marked decline in harvested quantities and hatching quality of Bohai Bay *Artemia* cysts. The effect of acidity stress on *Artemia* populations has been studied by several Chinese authors; although no changes in morphology or microstructure were detected, cyst hatching, animal growth and survival were all impaired when pH decreased from 8.2 to 7.6 (Zheng *et al.* 2015), although tolerance to acidity stress increased as the animals grew to the adult stage (Sui *et al.* 2014). Moreover, as a function of culture time there was a clear effect of low pH on fatty acid content, with generally a reduced proportion of saturated fatty acids, but increased monounsaturated and polyunsaturated fatty acids at pH 7.6 (Gao *et al.* 2018).

As a result of the decrease of the salt production area and of the acidification of ponds due to discharge of bromine extraction effluents, the average annual production in the Bohai Bay area is presently less than 30% of the production in the 1990s. Unstable and unpredictable *Artemia* pond production thus remains a problem in China. Suitable techniques adapted to the local environment, such as fertilization regimes, still need to be developed. In contrast to Vietnam, little is known about the adaptation of the introduced *A. franciscana* populations to their new environment or how their performance can be enhanced, for example, by selective breeding. Progress in this field is slowed down by factors inherent to the local situation. A first factor is the large size of most of the salt production facilities, which are difficult to manage to enhance *Artemia* production. The organization of the salt production sector itself, which is generally under the ownership of large companies, may slow down developments but may also have the benefit of the scale (e.g. facilitating investment into processing equipment). The most important factor is probably the complexity and volatility of the Chinese *Artemia* market itself, with a variety of differently priced domestic and foreign *Artemia* cyst products being imported and locally harvested,

processed, locally consumed and/or exported. The resulting interplay of demand and offer so far has not provided sufficient incentive to intensify efforts for the local production of high-quality cysts in Chinese saltworks.

Vietnam

The Artemia market and history of Artemia production in Vietnam

In Vietnam, the controlled production of *Artemia* integrated with artisanal salt production is thoroughly documented. *Artemia* was inoculated in the country in the mid 1980s to produce cysts and biomass for larviculture and nursery stages of aquaculture species such as the giant freshwater prawn (*Macrobrachium rosenbergii* De Man 1879), whiteleg shrimp (*Litopenaeus vannamei* Boone 1931), tiger shrimp (*Penaeus monodon* Fabricius 1798) and marine fish. Since the first initiatives in 1983 in Cam Ranh Bay (Central Vietnam, Quynh & Lam 1987) and in 1986 in Vinh Chau salt fields (Mekong Delta, South Vietnam, Rothuis 1987) interest in the seasonal culture of *Artemia* in the Mekong Delta, especially in its Soc Trang and Bac Lieu provinces, has steadily expanded. *Artemia* farmers mainly aimed at cyst production. The know-how was gradually spread among artisanal salt farmers via extension from research institutes and universities, especially Can Tho University, toward local cooperatives. This integrated type of farming system, initially largely driven by empiricism, was increasingly successful and ended up in higher profits for salt farmers compared to their traditional low income from salt production alone. The low revenues from salt production and the fluctuations of salt prices over the years proved to be sufficiently stimulating for many salt farmers to turn entirely to *Artemia* production. Moreover, salt production in Vietnam has been negatively affected in recent years by unusual rains, low productivity, low prices and large stocks of low-quality salt with low market value. The salt production area has been fluctuating in recent years in the range 11 000–15 000 ha (Ministry of Agriculture and Rural Development, pers. comm. 2018). Whereas the country produced 1.5 million tons of crude salt in 2015, the production had dropped to 1.3 million tons in 2016, and further dropped in 2017 due to early onset of the rainy season.

Within nearly 20 years after the first inoculation, the originally inoculated San Francisco Bay (SFB)-type *A. franciscana* had evolved into the so-called Vinh Chau (VC) strain (after the Vinh Chau district in the Mekong Delta, the center of *Artemia* production) with characteristics different from the original feral strain, such as an increased temperature tolerance (Clegg *et al.* 2000, 2001; Kappas *et al.* 2004). The Mekong Delta is the only *Artemia* pond production area for which long-term production statistics are available and where production is systematically

monitored (Fig. 3). Over the years, cyst yields with the adapted strain have gradually improved, exceeding the amounts obtained originally with the introduced SFB strain (Hoa 2002). By 2001, the *Artemia* production area had increased up to over 1000 ha of salt fields, yielding almost 50 tons of raw cysts per dry season of 4 months (the yield of dry, processed cysts from the raw harvested product varies annually, but is typically 30–35%). However, periods of growth have alternated with periods of stagnation, as expansion of the production area was not always accompanied by appropriate planning and by proper instruction of the new farmers joining this type of activity. Also fluctuations at the demand side (with lower cyst demand leading to weakened interest of salt farmers) and occasional unusual weather conditions contributed to fluctuations in cyst yields (Nam *et al.* 2008). In the period 2002–2014, the culture area stabilized to 200–400 ha (Fig. 3), followed by a further expansion in more recent years. Since the early 2000s, the annual production has steadily grown from 15–20 to 50–60 tons of raw cysts. Yields have generally been fluctuating since the early 2000s in the range 50–70 kg raw cysts ha⁻¹ per ‘crop’. One crop corresponds with one production run until the productivity of the reproducing population goes down and the production is stopped (see further).

Prices of VC *Artemia* cysts have remained highly market-driven. Depending on offer and demand, both domestically and globally, VC cyst product found its way to the international market whereas in other years the entire production was utilized in Vietnamese aquaculture facilities, especially mud crab hatcheries. Cysts of the VC strain are considered of excellent quality for their small size, high hatching and nutritional qualities (especially levels of highly unsaturated fatty acids; Anh *et al.* 2009, 2010). Moreover, they are in high demand for use in mud crab hatcheries where the first larval stages of mud crab are fed with umbrellae of VC

Artemia, due to their smaller size, whereas imported cysts may be used for the older larval stages. VC cysts are therefore sold at prices which may be substantially higher than the price of imported cysts. Since 2013, the price of locally produced raw cysts has been in the range 45–60 USD kg⁻¹, and 150–200 USD kg⁻¹ for processed cysts. For local shrimp hatchery managers, these prices may be prohibitive and thus urge them to purchase other, cheaper non-Vietnamese cyst product. Presently, Vietnamese hatcheries require approximately 400–500 tons of dry *Artemia* cysts per year and consequently only a fraction of this need is covered by the production in the Mekong Delta.

Management of Artemia ponds in the Mekong Delta

The Mekong Delta is characterized by a tropical monsoon climate with high humidity and high temperatures year-round (annual average air temperature 26.1°C). In April–May, air temperatures by day frequently exceed 35°C. The dry season, generally from November/December to April/May, may have occasional rainfall. *Artemia* culture takes place in the dry season, or continues to a certain extent early in the wet season depending on the weather and the availability of water of sufficiently high salinity in the culture ponds. The system is sometimes described as static as there is no continuous flow of water from one pond to another. Within the series of consecutive ponds with increasing salinity (the ‘salt street’), brine shrimp are inoculated at high densities (>20 nauplii L⁻¹) in ponds as soon as the appropriate salinity of 80–90 g L⁻¹ has been reached by evaporation, which is generally in late January. Sometimes ponds are stocked already at lower salinity if the farmer wants to prolong (advance) the production season by starting as early as possible in the dry season.

As ponds are generally small (order of magnitude of up to few 1000 m²) they are intensively managed and production conditions are organized in a way targeting increasing

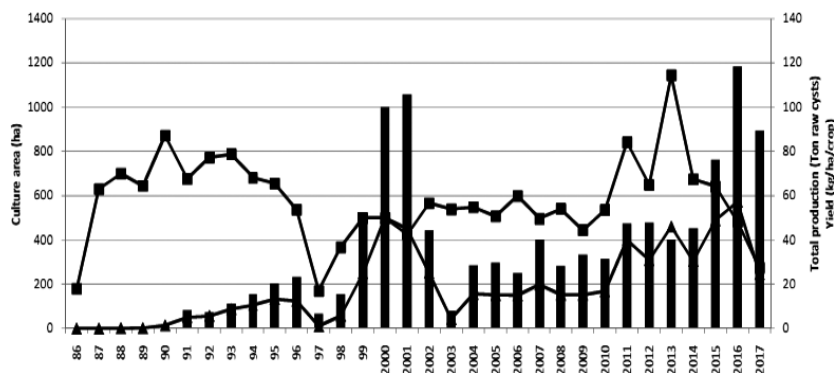


Figure 3 Evolution of culture area, total production and yield of *Artemia* cysts (raw product) in the Vinh Chau and Bac Lieu districts of the Mekong Delta, Vietnam (Source: College of Aquaculture & Fisheries, Can Tho University, Vietnam) since the first inoculation in 1986. ■ Culture area (ha), ▲ Total production (Ton raw), + Yield (kg/ha/crop).

yields and enhancing operational efficiency. For example, for better oxygen diffusion into the water column, ponds are oriented with the longest axis parallel with dominating winds. Wind action also helps to accumulate the floating cysts into one corner which facilitates harvesting. As salt ponds are shallow (generally 30–35 cm), excavating (part of) the pond bottom and/or heightening the dikes to increase the water depth are often done to enlarge the production volume and to reduce temperature stress for *Artemia*. Predators, such as fish (e.g. tilapia *Oreochromis mossambicus* Peters 1852), crab (e.g. fiddler crab *Uca* sp.), and food competitors (*Acartia* sp., *Microsetella* sp. and other copepod species; rotifers *Brachionus plicatilis* Müller 1786; ciliates *Fabrea salina* Henneguy 1889. . .) are kept out and/or removed from the *Artemia* ponds as much as possible and using a variety of tools, including screening of intake water, use of chemicals, and by keeping sufficiently high salinity (Hoa & Van Hong 2019). Generally, a 'kitchen' or 'fertilizer pond' (with a surface area about 30% of the total surface area of all *Artemia* culture ponds) at lower salinity (30–45 g L⁻¹) is fertilized to produce phytoplankton-rich green water as feed for *Artemia*. Chicken manure is used as organic fertilizer in combination with inorganic fertilizers (urea, diammonium phosphate) in order to continuously stimulate the development of microalgae in the fertilization pond; 'green water' from this pond is then drained daily into the *Artemia* culture ponds. Generally, the amount of green water supplied from the fertilizer pond to the *Artemia* ponds is limited to a volume corresponding with 1–2 cm water level increase per day, because salinity in the *Artemia* pond should be maintained in the range 80–100 g L⁻¹, while salinity in the fertilizer pond is much lower. The purpose of green water supply is not only to provide fresh algae as food for the *Artemia* ponds, but also to compensate for the amount of water lost daily by evaporation (Le *et al.* 2018) Green water may also first be mixed with high-salinity water to maintain sufficiently high-salinity levels in the *Artemia* ponds (Quynh & Lam 1987; Baert *et al.* 1997).

Several field studies have focused on the phytoplankton species composition in the fertilizer ponds, and on species resulting in good *Artemia* performance. Diatoms, cyanobacteria, chlorophytes and dinoflagellates all occur in green water and in *Artemia* ponds at different densities according to the ambient conditions, but especially diatoms such as *Chaetoceros* sp., *Rhizosolenia* sp., *Nitzschia* sp., *Pleurosigma* sp. and green algae such as *Tetraselmis* sp. and *Dunaliella* sp. are generally linked to good *Artemia* production (Van *et al.* 2012; Ut *et al.* 2013; Dao 2016; Hoa & Van Hong 2019). In the *Artemia* ponds, green water is supplemented with chicken manure and cheap agricultural by-products (such as micronized rice bran and soya pellets) which may be consumed directly by the brine shrimp

population or act as a substrate for proliferation of bacteria, which in their turn are consumed by *Artemia*. Frequent raking of the pond bottom is standard practice (Fig. 2c): it avoids the excessive growth of benthic macroalgae, re-suspends particles that have settled down and makes them accessible again for filtration uptake by *Artemia*. Abundance of macroalgae is affected by various factors such as pond depth, water temperature and salinity. Overall, especially green seaweeds of the genera *Enteromorpha*, *Cladophora* and *Chaetomorpha* may proliferate if salinity is not kept sufficiently high (Anh 2015).

In normal conditions, two to three weeks after inoculation *Artemia* starts reproducing, generally first by producing live nauplii (ovoviviparous reproduction) and later by producing cysts (oviparity). High production of cysts usually occurs in February and March as water temperature stays below 35°C. Toward the end of the dry season the productivity decreases by high temperature, food limitation and senescence of the population, which may collapse prematurely in extreme cases. To boost production, ponds are sometimes re-inoculated toward the second half of the dry season for a new production run ('crop' or 'cycle') but higher water levels are needed then in order to avoid excessive water temperatures.

Present developments in Artemia production in Vietnam

Present R&D efforts generally target at intensification of production by modifying the above described standard type of management. In contrast with the empirical approach of the past, these developments are generally sustained by scientific field experimentation. Modifications may include (i) deepening ponds (>50 cm water depth), which increases the culture volume and thus the population size; (ii) increasing stocking density (up to 100 nauplii L⁻¹), resulting in a more dense starting population; (iii) management of green water by an appropriate fertilization policy to stimulate more suitable algae (e.g. diatoms, green algae); (iv) optimizing feeding protocols by supplementation of green water with fermented agricultural waste products, formulated shrimp feed or artificial feed specifically formulated for *Artemia* production (Hoa *et al.* 2013; Le *et al.* 2018); (v) a variety of adaptations in daily management, such as providing aeration, optimized raking techniques; (vi) stimulation of bioflocs (either directly in the *Artemia* culture ponds or in ponds specifically maintained for biofloc production, similar to green water ponds), which provides additional food for the *Artemia* population (Le *et al.* 2018). Biofloc development and characteristics are affected by various parameters (De Schryver *et al.* 2008, 2012a,b). Especially in salinities exceeding that of seawater the effect of production conditions (oxygen levels, water turbulence, C source, C/N ratio. . .) on biofloc characteristics (size and texture, buoyancy, microbial composition, nutritional composition, other aspects such as

immunostimulatory effect. . .) largely remains to be investigated. In experimental conditions and using a combination of measures to promote intensified production, up to a doubling of the regular cyst yields has been achieved (Le *et al.* 2018).

Other recent initiatives in Vietnam aim at the geographical and temporal expansion of *Artemia* production. Other provinces than Soc Trang and Bac Lieu in the Mekong Delta, and other areas in Vietnam (e.g. Cam Ranh area in Central Vietnam) have a longer dry season and thus a potentially longer production season. A longer production period is also aimed at by development of protocols allowing for an earlier inoculation of the ponds at the onset of the dry season and/or for prolongation of the production through the beginning of the subsequent wet season. Other recent R&D lines have focused on the feasibility of field production of cysts selected for favorable market characteristics, such as small cyst size (Van *et al.* 2014) and on enhanced production of *Artemia* biomass as aquaculture food in live or frozen form, or processed into formulated feeds. A model was worked out to predict cyst yields based on biomass sampling (Baert *et al.* 2002) and guidelines on optimal biomass production have been developed (Anh *et al.* 2010): using specific biomass harvesting strategies, 25–30 kg wet weight biomass ha⁻¹ day⁻¹ can be produced (totaling 3–4 tons per crop of 3–4 months), in combination with 30–50 kg raw cysts ha⁻¹ per crop. To complement seasonal outdoor biomass production, recently also research into the feasibility of indoor *Artemia* biomass production has been done, in continuation of older studies (Dobbeleir *et al.* 1980; Lavens & Sorgeloos 1991) and aiming at the production of fresh biomass for pet fish or for fish and shrimp nurseries. Using circular tanks or race-ways with seawater, and culturing the ongrowing *Artemia* population with a mixture of green water, formulated feed and cheap agricultural products until adulthood, 2–5 kg wet biomass m⁻³ per batch run of 10 days could be achieved (Hoa & Van Hong 2019). Compared to pond production, biomass quantities harvested indoors are rather limited, but the technique allows for production year-round and for further intensification, for example by the use of bioflocs.

Economic assessment of Artemia production in the Mekong Delta

Optimization of *Artemia* culture techniques also includes the study of the socio-economic factors which may prove favorable or obstructive for further development of this economic activity. Presently, three cyst processors are active in the sector in the Mekong Delta, and they buy the raw product either directly from the farmer or through middlemen. Each has the capacity to process up to about 200 kg raw cysts per day, and all processors are operational for up to 4–5 months per year, which allows them to produce

8–10 tons of commercial product (the equivalent of 24–30 tons of raw cysts). The *Artemia* farmers themselves generally do not have the infrastructure to store the cysts in optimal conditions until the next production season and to hatch cysts into nauplii for inoculation. Moreover, they are inclined to capitalize a maximum of their harvest, in need of cash. So they generally buy the material to stock their ponds at the onset of the dry season back from the cyst processors, either as cysts or as hatched nauplii. A survey among *Artemia* farmers in the Mekong Delta has shown that factors such as shortage of available seawater, highly fluctuating farmgate cyst prices, lack of investment capital, and limited overall knowledge on correct production techniques are generally identified as obstacles for further expansion (Son 2010). *Artemia* yields in the region may thus further be increased by easier access to cheap credit and other capital support, by a better management of the water resources and the water in- and outflow system, and by more technical and training support from governmental agencies, universities and research institutes.

In spite of the above points of concern, 2011–2013 field surveys by Can Tho University (Quang 2012), in the Soc Trang and Bac Lieu provinces among 124 farmers demonstrated a general shift of salt farmers' activities from salt production toward *Artemia* production, with a total *Artemia* farming area per household in the range 1.1–1.9 ha and pond sizes in the range 0.24–0.31 ha. Nevertheless, surveying farmers involved in either *Artemia* monoculture or in *Artemia* combined with salt production showed that farmers had a higher rate of return (on average 2.4) when they produced *Artemia* together with salt, than when they focused on *Artemia* production only (rate of return 1.1; Quang 2012). It is difficult, however, to generalize these figures as salt and *Artemia* prices may fluctuate from year to year. The main production expenses (apart from labour, which accounts for about 25% of production costs, and land rental) are the *Artemia* cysts needed for stocking the ponds with nauplii at the onset of the dry season, ranging from about 600 to 850 g ha⁻¹ crop⁻¹, corresponding with about 17% of production costs. Other production costs are the purchase of chicken manure (0.5–2.2 tons ha⁻¹ per crop), inorganic fertilizers (10–250 kg ha⁻¹ per crop) and supplemental feeds such as rice bran (50–200 kg ha⁻¹ per crop). Fertilizers and supplemental feeds together account for about 24% of production costs. Also fuel costs for pumping account for a substantial part (13%) of production costs. With production costs in the order of about 1100 USD ha⁻¹ per crop and raw cyst prices off-farmgate increasing from 28 to 53 USD kg⁻¹ in 2011 and 2012, respectively, this resulted in a mean net profit per household of about 1200–2800 USD ha⁻¹ per crop for 2011 and 2012, respectively, with profit margins also depending on the type of management applied by the farmer. In 2013 and following years, the situation was similar to 2012

(Can Tho University, unpublished results). Finally, there is an increasing tendency of viewing the benefits of *Artemia* production as part of a broader framework of integrating production systems operated by coastal rural communities, for example rotation production systems with *Artemia* and salt in the dry season and shrimp or fish aquaculture in the wet season, but an economic assessment of these integrated systems still remains to be conducted.

As described above, *Artemia* pond production in Vietnam has developed over the past years into a dynamic sector, allowing salt producing rural communities to generate a substantial alternative income with short return and moderate investment. Although the *Artemia* production sector may not be of primary economic significance at the national Vietnamese level, it is an important factor for creation of jobs and wealth at the local level in some provinces of the Mekong Delta, with major socio-economic ramifications through the supply and processing business. It is estimated that over 500 families of salt farmers out of a total of 625 (or 80%) have improved their income with more than 5000 USD per household and per dry season through the production and sales of brine shrimp cysts, and recently also through sales of biomass harvested toward the end of the dry season through the first months of the rainy season (Hoa & Sorgeloos 2015). Moreover, it supplies high-quality cysts, primarily for the country's aquaculture sector, and this indirect role in contributing to socio-economic development through aquaculture should thus not be underestimated. Nevertheless, as in any type of primary production, the sector occasionally suffers from the dependence of unpredictable weather conditions; the *Artemia* farmers, who are generally in a position of limited financial resilience and/or land ownership, face unstable profit margins due to the fluctuations in demand and in prices of imported cysts on the Vietnamese aquaculture market. Especially the combination of abnormal weather conditions (shorter dry season; higher temperatures) and reluctance of farmers to adopt improved production techniques has resulted in decreased productivity since 2014, whereas total production still moderately increased until 2016 thanks to the further expansion of the culture area (Fig. 3). Sustained guidance needs to be provided by extension officers with proper scientific knowledge, in collaboration between local authorities, aquaculture decision makers and universities, and especially farmers' associations, as the farmers themselves have neither the schooling nor the financial and infrastructural means to engage into experimental initiatives aiming at enhancement of production protocols.

Thailand

History of Artemia production in Thailand

Following the successful demonstration of seasonal *Artemia* production in salt ponds in the Philippines (De los

Santos *et al.* 1980), Thailand became the first commercial producer of *Artemia* cysts in commercial salt farms. First attempts by the Department of Fisheries using the SFB strain were successful in 1979, with a production of 10 kg of dry *Artemia* cysts in a 0.24 ha earthen pond within 45 days (Vos & Tunsutapanit 1979). In 1980, *Artemia* farming for cyst production was launched by the Department of Fisheries in the saltfields of Chonburi, Chachoengsao and Samut Songkhram, which resulted in the production of about 600 kg of raw cysts from 5.7 ha earthen ponds. Since this initial period the nature of *Artemia* culture has changed as a function of aquaculture demands, causing a shift of *Artemia* production from cysts to biomass. By 1987, the yields of *Artemia* biomass had increased from initial values in the range 120–250 kg ha⁻¹ month⁻¹ up to 300–600 kg ha⁻¹ month⁻¹; for *Artemia* cysts the yield in that time was in the range 30–60 kg raw cysts ha⁻¹ month⁻¹.

Management of Artemia ponds in Thailand

Presently *Artemia* cysts are imported in Thailand and *Artemia* farming focuses mainly on biomass production. Now there are 24 *Artemia* production farms, covering 73 ha located in the central (Phetchaburi, Samut Songkhram) and eastern (Chachoengsao) parts of Thailand, and their number and culture area have remained stable over the past few years. *Artemia* production is practiced in former salt ponds or shrimp production ponds. In areas with limited precipitation, it may be practiced as a continuous culture throughout the dry and rainy seasons. Biomass culture is practiced according to the so-called 'traditional' (Samut Songkhram area) or 'developed' (Phetchaburi and Chachoengsao areas) system. The 'traditional' system uses rather shallow ponds (0.6–0.8 m), though the pond is generally deep enough to keep its salinity high enough for *Artemia*, also during the rainy season. Pond surface area is in the range 3000–4500 m². Pond management is rather limited; raking is not applied. The 'developed' system uses former shrimp ponds for *Artemia* biomass production, which are deeper (1–1.5 m). The ponds may have a bigger surface area, up to 1 ha, and management may be more intensive, for example, including raking from a boat. In principle, no water exchange is done during the culture period, but refilling (with highly saline water) or discharge (of low salinity water) may be needed during the rainy season. In some cases, pond aeration is applied. *Artemia* production generally starts at a salinity in the range 75–90 g L⁻¹; salinity may, however, go up to 150–180 g L⁻¹. If inoculation is needed (e.g. after the wet season in areas with substantial rainfall) *Artemia* biomass transported from other areas may be inoculated at 12–15 kg wet weight ha⁻¹.

In the past, pond fertilizers such as chicken or cow manure or urea have been used to induce primary production,

but nowadays both in the traditional and developed system the production in Thailand is entirely based on the use of the so-called 'ami-ami' as feed. Ami-ami is the waste product of the industrial production of monosodium glutamate (MSG), a flavor enhancer commonly used in Asia in the food industry. Most MSG is produced by bacterial fermentation in a process similar to the production of vinegar or yogurt: during fermentation, *Corynebacterium* bacteria, cultured with carbohydrates from, for example, sugar beets, sugar cane, tapioca or molasses, excrete amino acids into a culture broth from which MSG is isolated. The waste product is a dark colored viscous liquid, which is further fermented prior to application in the pond. This fermentation may be done over various weeks or months in recipients or tanks of various sizes (several hundreds up to thousands of liter; Fig. 2d), in which the fermented product may occasionally be stirred to resuspend settled particles. This fermented product is applied in the ponds at various rates (up to about 100 L ha⁻¹ day⁻¹, but generally lower), depending on the state of the product, the primary production in the pond, the overall pond turbidity, and the *Artemia* densities in the pond. The same stock tank may be used over a prolonged period (e.g. several weeks): as part of the stock is used in the *Artemia* ponds, new water may be added to the remaining stock, where the bacterial fermentation further continues.

Artemia biomass may be harvested manually with harvesting nets, by light attraction, or semi-automatic. Yields are nowadays in the range 1500–3000 kg wet weight ha⁻¹ month⁻¹. If transported alive, the biomass is acclimated at 40–50 g L⁻¹ salinity before packing. Given the small size of the sector, proper economic assessment of *Artemia* production has not been done. With a production cost estimated at about 0.65 USD kg⁻¹, fresh biomass is sold at about 1 USD kg⁻¹, and frozen biomass at 1.25 USD kg⁻¹. The annual production of *Artemia* biomass was estimated to be 500–600 tons in 2015 with a market value (for fresh and frozen product combined) of 615 000–737 000 USD. Of this amount, most is used for the domestic market, either live, chilled or frozen, in hatcheries and nurseries of penaeid shrimp and high-value fish (such as Asian sea bass and grouper), for shrimp broodstock (as a substitute for blood worm *Glycera* sp. and sandworm *Arenicola* sp.) and for ornamental fish (replacing e.g. cladocerans or the sludge worm *Tubifex tubifex*). Until a few years ago, a quarter of the total production was exported as frozen *Artemia* biomass to Europe and several Asian countries. Nowadays only small quantities are exported to Belgium and India.

As the *Artemia* production procedure in Thailand looks technologically relatively simple, it may be an attractive alternative for more elaborate production methods requiring the maintenance of an algae-rich fertilizer pond,

supplemental feeds to sustain dense *Artemia* populations etc., such as common in Vietnam. Yet the culture success with ami-ami in Thailand highly depends on the skills and experience of the farmer: his 'gut feeling' is crucial in keeping the ami-ami stock tank in optimal conditions, in respecting the 'expiry date' by which the stock should be consumed and fresh product should be purchased, and especially in determining the quantities of product daily supplied to the ponds. Based on their intuition or experience, farmers may thus give preference to a specific intake product, supplied by a specific company according to a specific MSG production process. Procedures may vary depending on the farmer's personal insights and the characteristics of his farm. In absence of scientific study of the biochemical and biological processes ongoing in the ami-ami production tanks and of the nutrient dynamics in the *Artemia* ponds, extension of the technique chiefly relies on the 'trial and error' principle.

Artemia pond production in other geographical areas

In addition to the countries discussed above, *Artemia* pond culture has been initiated with more or less success in numerous other areas in the world. By no means this review aims to present a complete geographical overview. For example, there has been to some extent *Artemia* pond production on the Indian subcontinent, and presently there are emerging initiatives in Cambodia, Laos and Myanmar. However, this geographical overview wants to conclude by highlighting areas where the technique was once initiated, but so far did not firmly consolidate itself, for example in sub-Saharan Africa and South America.

In sub-Saharan Africa, aquaculture is an industry in full development, with mortality of fish fry at a very young age being one of the key challenges. Aquaculture in sub-Saharan Africa is characterized by the production of freshwater fish species such as various tilapia species and African catfish *Clarias gariepinus*. Although the use of *Artemia* as live food in the larval stages of these species is not always a must, larval performance often benefits from a restricted period of feeding *Artemia* nauplii or decapsulated cysts, as proven for example for catfish (Onura *et al.* 2018), and feeding *Artemia* may help in diversifying inland aquaculture by solving nutritional issues in larviculture of species under study, such as Nile perch *Lates niloticus*. Moreover, in countries such as Kenya and Tanzania, where development of aquaculture of marine fish and shrimp species (for which live food for larval stages is generally a necessity) is underway, securing a proper supply of *Artemia* cysts and biomass is of utmost importance. Local and affordable production of *Artemia* could thus be an important breakthrough: now the cyst prices are high due to importation costs and suboptimal distribution

channels, and often the scarce and expensive product is partially wasted due to ignorance on appropriate storage and application procedures.

Artemia occurrence has been reported in coastal salinas and inland saline lakes throughout Africa (Kaiser *et al.* 2006), but except for some isolated attempts *Artemia* is harvested in none of these. The natural or man-induced occurrence of *Artemia* in man-operated saltworks has been poorly described, except for some documented cases in Kenya. In this country, the first inoculation attempts with *A. franciscana* in one of the coastal saltworks around Malindi along the coast of the Indian Ocean were conducted in the mid 1980s. A recent salt work survey confirmed that the original SFB population has established itself along the belt of adjacent saltworks (Ogello *et al.* 2014a,b) and even into Tanzania (Glen Bieber, pers. comm. 2017). The saltworks in Kenya and Tanzania are generally large and industrially exploited by international companies, with salt ponds one to several hectares in size. These companies are generally aware of the benefits of the presence of *Artemia* in the salt production process and thus occasionally the ponds are restocked with nauplii hatched from imported cysts or from cysts harvested locally, for example, in conditions of insufficient *Artemia* occurrence to graze away phytoplankton blooms. Management as such, intended to maximize production and aiming at commercialization, has so far been non-existing. However, urged by the need to develop aquaculture to ensure food security, there is a revived interest in several African countries such as Kenya, Tanzania, Uganda and Mozambique for local *Artemia* production in salt works or salt lakes. Feasibility studies have been conducted or are ongoing through north-south or north-south-south (with Vietnam) collaboration with local research partners (Sserwadda *et al.* 2018). However, the quantities of cysts and biomass produced, if any, are still small and/or inadequately processed and stored, and hardly find their way to the local market. Production so far has not gone beyond the experimental or pilot phase, due to a combination of factors: lack of knowledge on proper production technology, adapted to local ecological conditions; insufficient dissemination of production technology to potential producers (artisanal salt farmers and industrial salt works); lack of interest in *Artemia* production due to high salt prices; lack of policy framework stimulating this type of economic activity; legal issues on the use of land and water resources. Sometimes interest of potential target groups is dwindling when progress can only be acquired stepwise, especially as this type of primary production requires a close monitoring and frequent adjustment of production conditions (e.g. fertilization scheme; water circulation), particularly when practiced in a new environment by non-experienced farmers. Nevertheless, modest success in cyst production has recently been booked by

artisanal salt farmers in coastal Kenya (Morine Mukami, Kenya Marine & Fisheries Research Institute, pers. comm. 2018) and encouraging small-scale initiatives like this may act as a nucleus for further dissemination of the technique in the region.

In South America, *Artemia* pond production is practiced mainly in Brazil and to a certain extent in Ecuador, alongside the expansion of marine fish and crustacean aquaculture as a way to enhance its cost-effectiveness. In other countries there have been only scattered attempts of *Artemia* production, for example, in the estuary of Virrilá, in Piura, Peru (Salgado 2001; Cisneros & Vinatea 2009). However, in spite of the limited initiatives for man-managed *Artemia* pond production, South America should be regarded as a reservoir of natural *A. franciscana* populations which may be valorized for aquaculture development (Gajardo *et al.* 1998, 2006), in addition to the GSL strain which is omnipresent on the aquaculture market, and to the resources from other pond production sites where generally the SFB type has been introduced outside its natural distribution area.

In Brazil, *Artemia* production is limited to the northeastern states of Rio Grande do Norte and Ceara. This region, located in a semi-arid climate zone, is the largest salt-producing area of the country (approximately 7 million tons year⁻¹, which represents 95–97% of the national production. The dry season lasts for 7–8 months (June to January) and large pond systems (up to 4000 ha) are operated in this area with a high degree of mechanization, producing 200 000–1 000 000 tons of salt per year, and employing directly and indirectly 15 000–50 000 people. In these enterprises, *Artemia* is a by-product used to guarantee the salt quality. Also smaller artisanal saltworks exist, ranging just 2–50 ha in size with a yearly production of 200–20 000 tons, but salt quality is generally considered as low due to the largely artisanal salt extraction procedures.

Populations of *Artemia* have been described for different states of Brazil, but it is only in the state of Rio Grande do Norte that *Artemia* is exploited to some extent since the inoculation of 250 g of SFB cysts in 1976 in the salt ponds in Macau. Only few months after this first inoculation, the first ton of cysts was harvested. Since then, brine shrimp has established itself in saltworks stretching over about 250 km of coastline (Camara 2001). In spite of this wide semi-natural distribution, there have been very few initiatives (e.g. launched by the Brazilian Mariculture Linkage Program, BMLP) in valorizing and enhancing this natural resource and there has been limited dissemination of good management techniques. As a result, the annual production of *Artemia* (about 3–4 tons of cysts, and an estimated 25–30 tons of biomass) has been stagnating for years. The production is highly monopolized and is insufficient to supply the domestic market of small fish and shrimp hatcheries

and small ornamental fish breeders. Sometimes a rudimentary restocking is done by inoculating *Artemia* hatched from local cysts or by transplanting biomass harvested in higher salinity ponds into lower salinity ponds. Man-managed intentional production of *Artemia*, however, is non-existent and there has been virtually no investment into appropriate production management and processing. A lot of the local product commercialized is actually the result of illegal harvesting, in which the product is exposed to sub-optimal processing (e.g. sun drying; Fig. 2e), storage and transport conditions (De Medeiros Rocha *et al.* 2011).

In spite of the interesting intrinsic characteristics, such as small-sized nauplii and relatively high HUFA levels, prices of this low-quality product are thus low (e.g. 50–70 USD kg⁻¹). Over recent years, the production has decreased due to lower demand from the aquaculture industry, because of its low quality. Moreover, ovoviviparity has become the predominant mode of reproduction in the local strain. This shift in reproductive behavior has been linked to the relatively stable abiotic conditions in the vast salt ponds, which do not stimulate cyst production (Camara & Tackaert 1994; Camara 2001). Yet about 50 000 ha of salt pans are considered as financially not profitable due to their small size and mode of operation, and thus have potential to be turned into family size *Artemia* farms. However, to remediate the present under-exploitation and deficient production logistics, a governmental strategy to monitor the present brine shrimp populations, to assess and enhance the production potential and to disseminate knowhow on production and application technology is of utmost importance (De Medeiros Rocha *et al.* 2012).

Conclusions, emerging issues, and opportunities related to *Artemia* pond production

Implementation of proper management to maximize cyst quantities

Artemia pond production can be seen as a form of extractive aquaculture, in which low-cost products are turned into high-quality products that are of value for aquaculture expansion, such as cysts and protein-rich biomass. The case studies highlighted above are examples of integrated *Artemia* biomass and salt production in specific socio-economic contexts, the target being cyst and/or biomass harvest, primarily for local use in farming of aquaculture species of local interest. Such endeavors have contributed to the expansion of industrial activities as in China, the world's leading salt producer and second world source of *Artemia* cysts (both inland and coastal), but have also proven successful in leveling-up welfare of rural communities, especially in the case of Vietnam. In many countries in Asia (e.g. Bangladesh, Myanmar, India) seasonal salt production

is becoming a marginal artisanal activity, although the livelihood of millions of households depends on this traditional practice. Integrating this activity with *Artemia* production in the dry season and fish/crustaceans in the rainy season might open up better socio-economic perspectives for these often impoverished communities.

A common problem throughout various scenarios of *Artemia* pond production is the difficulty to keep production at a certain level of profitability. Fluctuations in yields are common, with cycles of quantitative expansion followed by declines, making production difficult to predict even for the next production season. Partially this is inherent to this type of primary production where yields depend, among others, on natural factors such as the duration of the dry season, ambient temperatures, occasional rainfall, fluctuations in the abundance of algae species and so forth. In future, climate change and continued eutrophication of coastal waters may bring about new challenges; in recent years, periods with very high water temperatures or unusual patterns of phytoplankton occurrence (e.g. higher abundance of cyanobacteria such as *Phormidium* sp., *Anabaena* sp. and *Oscillatoria* sp.) have become more common in the Mekong Delta, negatively affecting *Artemia* yields (Van *et al.* 2012; Ut *et al.* 2013).

Fluctuations in yield may, however, also have managerial origins: not all farmers have the same level of experience or an open attitude toward innovation. If off-farm cyst prices are low (and especially when at the same time salt prices are high), farmers may be less inclined to maintain the constant managerial discipline needed to maximize production. Disinterest may also follow discouragement because of initial yields being below expectation, for example when simply copying production protocols proving successful elsewhere into a new local environment. Procedures for optimal management, leading to maximal yields, are highly entwined with fluctuations in abiotic and biotic field conditions, and the alertness and knowledge of the farmer to properly respond to them. Moreover, and on top of all the above, farmers may temporarily face low or variable profit margins as a consequence of annual price fluctuations on the international and local *Artemia* cyst market, which may provoke a reluctant attitude toward expansion or intensification of this activity.

Keeping *Artemia* production above a certain level is a challenge when promoting this sort of extractive aquaculture in countries in need of *Artemia* cysts and biomass to boost their local aquaculture production, as for example in many countries in Africa but also in countries with a well established aquaculture sector (such as in Latin America) where programs for diversification of the sector are underway. Such diversification programs need to overcome the many difficulties inherent to the production of species in captivity that has poorly been studied in terms of the

nutritional requirements of the larval stages. Maintaining proper *Artemia* yields over various successive production seasons requires understanding of what should be 'proper management' given the ecological characteristics of the ponds and the dynamics of the *Artemia* population over the production cycles, allowing to predict future yields. Even in a well-documented situation like the Mekong Delta, these aspects of brine shrimp production have hardly been studied so far. This requires monitoring the genotype composition of the *Artemia* population over time associated to key phenotypic traits, together with monitoring the critical stressors or selective forces acting on the gene pool over the production cycle.

Generally, the large scale of industrial salt operations does not facilitate proper management and control: it is difficult to maintain optimal *Artemia* production conditions (e.g. in terms of phytoplankton densities) in ponds with surface areas of several hectares. As explained above, salt work managers working in large facilities often have sufficient expertise and scientific schooling and are convinced of the beneficial role of *Artemia* in the salt production system. Yet they may show reluctance in actively implementing measures, aiming at improved *Artemia* production, if these measures are perceived to affect the salt yields targeted by the business plan. Although small-scale production units thus allow for more intensive and more controlled production and easier application of experimental protocols, large production facilities may contribute to securing local or regional cyst supply by a more extensive type of *Artemia* production, where findings, observed at small experimental scale may be validated.

Optimal use of the Artemia gene pool to improve cyst quality

The developments seen so far have in common that *Artemia* pond production has been initiated or accompanied with inoculation of non-indigenous *Artemia* species, with *A. franciscana* being the species of choice (with cysts often from San Francisco Bay, USA). Although this has hardly been studied, one may assume that culture success has been determined among others by the degree to which this strain has adapted to the local conditions. Nevertheless, starting with the same inoculation material, each of these introduction initiatives has evolved with distinctive features due to the local or regional geographical, environmental and socio-economic characteristics, and also due to the dynamic and temporal evolution of the *Artemia* population over a succession of production cycles.

Furthermore, the deliberate introduction of non-native *A. franciscana*, together with the dispersal of this species from hatchery effluents into the local environment, has put pressure on the overall natural biodiversity of *Artemia* which is based on six regional endemic sexual species with a long history of geographic isolation, and numerous

parthenogenetic types (Gajardo *et al.* 2002). Translocating commercially demanded *Artemia* species or locally adapted populations which are themselves the result of such translocation, brings about additional risks such as compromising the stability of local brine shrimp species, if these are present, that may have evolved relatively isolated in the region. The Bohai Bay saltworks in China with their extensive cyst production are a well-documented case in this regard. The introduction of the invasive American species *A. franciscana*, the most frequently used species in aquaculture, has led to the near-disappearance of the rich diversity of parthenogenetic types naturally evolved in the Bohai Bay area. Moreover, bottlenecks associated to introductions may reduce the genetic diversity of the introduced species and its ability to respond to changing local environmental circumstances.

Consequently, the authors of this article also wish to alert on the need to protect the local natural *Artemia* resources and their hypersaline habitats that are under threat in some places (Wurtsbaugh *et al.* 2017), and which are key to support sustainability of the aquaculture industry. The biodiversity impact of *Artemia* introduction needs to be evaluated and minimized in order to comply with international agreements to protect biodiversity. In any case the need to conserve local *Artemia* biodiversity should be of paramount importance when designing future inoculation activities, as *Artemia* resources stand as a natural patrimony supporting the sustainable expansion of aquaculture. On the positive side such introductions can also be seen as a model system and opportunity to study adaptation, selection and interspecies competition processes. Moreover, the information provided by the annotated *Artemia* genome (De Vos *et al.* 2019) will allow studying the processes associated with natural adaptation and with man-managed production in a targeted and much more efficient way than has been possible in the past, by identifying and monitoring the genes related to local adaptation, and by studying how their expression is regulated upon translocation. Locally adapted strains (such as the Vinh Chau strain, but also *A. franciscana* now prevailing in Bohai Bay area) should be protected in their turn, and be considered as valuable material for initiatives elsewhere. Additionally, potential research and development, making use of the *Artemia* genome, should focus on selection programs targeting at *Artemia* with higher cyst production, wider temperature tolerance, and/or with specific market characteristics in terms of cyst (nauplius) size and nutritional profile. Finally, numerous recent laboratory studies have studied to what extent and how *Artemia* interacts with its surrounding microflora, and how these processes can be manipulated to enhance protection to larval fish and shrimp, fed *Artemia* nauplii, against biotic and abiotic stressors (Sung *et al.* 2008; Defoirdt *et al.* 2011; De

Schryver *et al.* 2012a,2012b). Also the possible role of *Artemia* cysts as vector of aquaculture pathogens such as *Vibrio* sp. in hatcheries has been studied for a long time (López-Torres & Lizárraga-Partida 2001; Vadstein *et al.* 2018). Consequently, studying how *Artemia* pond conditions can be manipulated, so that *Artemia* cysts are produced, hatching into nauplii that are not only nutritionally beneficial, but that also convey maximal protection and stress resistance for fish and shrimp larvae, is an entirely novel field of research. All the above research fields require a close collaboration between various experts, active in either *Artemia* field production, hatchery use or genomics, but experience in the past has shown that progress in *Artemia* pond production has been realized where a thorough science-based approach has been followed.

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