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Formalin and salinity stress induced cyst induction in *Artemia parthenogenetica*

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Abstract

The effect of different concentrations (0.01 to 3.0%) of formalin on cyst induction in Artemia parthenogenetica was studied as a function of rearing salinity (20 to 200ppt). The survival of A. parthenogenetica was found to be influenced by both the concentration of formalin and also exposure duration. At the higher exposure duration of 6h, mortality coincided with cyst induction. Two factor ANOVA test inferred that in all the tested concentration of formalin, the influence of exposure duration was statistically more significant than the independent influence of salinity. At the tested stress duration, the number of cysts released showed positive relation with concentration of formalin. During 1h stress, the cyst induction ranged from 1.52 ± 0.02 to 7.32 ± 0.024 number. The maximum cyst induction by A. parthenogenetica was also found to depend on salinity and formalin concentration. At 2 and 3% formalin, maximum number of cyst was released during 1h at 20 and 40 ppt and at 20 and 80 ppt, respectively. Two Factor ANOVA test inferred that the influence of salinity and exposure duration was statistically more significant at low concentration (0.01% and 0.05 %), when compared to that of higher concentrations..

Keywords: *Artemia parthenogenetica*; formalin; salinity; cyst induction

Introduction

The brine shrimp *Artemia* (crustacea-Anostraca) is found in high saline environments especially in coastal salt works. Natural *Artemia* population is found in about 360 sites in 55 countries on the five continents of the world [1]. *Artemia* both in larval stage and in adult form (*Artemia* biomass) constitute not only the best, but also the most versatile of all live feeds in aquaculture. It is fed to larval fish and shrimp as well as to adult in grow out ponds [2].

The presence of brine shrimp, *Artemia* in sufficient number is essential in saltpans not only to control the algal blooms [3], but also to provide the essential requirements and suitable substrate in the form of *Artemia* metabolites and decaying *Artemia* for the development of halo bacterium in the crystallizer pond [4]. High concentration of the red colour halo bacteria ensure an increase in heat absorption which in turn increases the salt quantity as well as the sedimentation of the dissolved organic (viscosity) level, resulted in the formation of large salt crystals and thereby improving salt quality [5]. *Artemia* can be easily preyed on by predators such as insects, larval fish, crustaceans and other carnivorous species. The only effective defense against predation is the adaptation to environment of high salinity, which eliminates most of the predators [6].

Artemia are non selective filter feeders [7], and feed on particulate matter of biological origin as well as on the living organisms of the appropriate size range (microscopic algae and bacteria). It reproduces either by oviparity or ovoviviparity based on environmental conditions prevailed in the culture system. Ovoviviparous reproduction (nauplii as offspring) occurs mostly at optimum salinity levels; whereas, cysts (oviparous reproduction) are produced at stressed salinities.

Hatchery research and added interest in aquaculture of marine fish and shrimp have resulted in increased demands of live feed like *Artemia* with high quality in large biomass and cysts. The utilization of *Artemia* biomass was 1,000 tones in the late seventies but increased to 3,500 tones in 1987 and the demand was increased to 20,000 tones during the year 2000 [8]. The same way a market survey estimated that the *Artemia* cyst consumption by aquaculture was about 1,000 tones in 1992 [9]. Hence it becomes inevitable to carry out intense research on the production of cysts and thereby biomass of the widely used and demanded live feed.

The cysts not only survive in the adverse environmental conditions, but also facilitate the wide distribution of *Artemia* population through wind action and birds especially the flamingo that migrate and carry it over long distances [6, 10]. One of the major advantages of using *Artemia* as live feed in aquaculture is availability of *Artemia* in the form of dry cysts which can be stored for long time and hatched easily whenever required [11]. This eliminates the biological, technological and survival problems of stock requirements and culturing of *Artemia* [12].

Research carried out in California (USA) revealed a positive correlation between the presence of iron in the medium, increased hemoglobin synthesis and cyst production [13]. Factors such as salinity [14], temperature [15], formalin [16] and herbal products [17] induced cyst production in *Artemia*.

Previous studies on *Artemia* clearly revealed that the cysts induction could be made within short duration of one hour by using formalin as the chemical stimulatory agent as compared to the longer duration required in natural condition. But the average size of the induced cyst is different from that of the cyst from natural population [16]. In natural population, the release of nauplii and cyst depends on the environmental as well as the genetic factors. The intensity of stress by the environmental factors can be identified by the production of nauplii and cyst.

In the current study, the interacting effect of formalin and salinity on survival, nauplii and cyst production of *Artemia* was assessed to find out their respective optimum level for maximizing the intended parameters.

Materials and methods

Collection of Artemia and hatching of Artemia cysts

The brine shrimp, *Artemia parthenogenetica* is a locally available Indian strain with high reproductive potential. For the present study cysts of *A. parthenogenetica* collected from Thamaraikulam saltworks extension II about 16 km south of Nagercoil were rinsed with tap water and incubated in 1 liter transparent cylinder at a concentration of 1.5 g/l of seawater (35 ppt). Hatching temperature was maintained at 28 ± 1^0C. The pH was adjusted to 8.0 throughout hatching and the container was supplied with strong aeration. The light (2000 lux) was provided by a fluorescent lamp placed near the hatching cylinders. The photoperiod was maintained at 16: 8D/L. After 24 h of incubation, nauplii were observed and transferred into a 50l capacity fiber glass tank and were reared to adult stage. For rearing of *Artemia,* the culture

medium was prepared by dissolving commercially available solar salt in seawater. The salinity of culture medium was maintained at 80 ppt and it was measured by using Salinity Refractometer. During early morning and evening hours, rice bran suspension was given as feed to the culture stock. Rice bran has been reported to be a cheap and suitable feed source for intensive *Artemia* culture [18].

Formalin Stress

Experimental design

Stock cultures of *A. parthenogenetica* were kept in tanks maintained at $27^0C \pm 1^0C$ and were fed with rice bran. The salinity of the culture medium was maintained at 80 ppt. The concentration of formalin solution selected for this experiment was 40%. The test animals were exposed to different salinities of 20, 40, 80, 120, 160 and 200 ppt and were fed with commercially available rice bran. The healthy adult *A. parthenogenetica* were isolated for the induction experiment. To assess the formalin effect, the same age group of adult *A. parthenogenetica* was exposed to different concentrations of formalin 0.01, 0.05, 0.1, 0.5, 1.0, 2.0 and 3.0% at six different salinities, i.e. 20, 40, 80, 120, 160, 200 ppt. The salinities were prepared by means of using sea water and / or by adding fresh water and NaCl salt. The number of surviving animals and produced cysts was counted and recorded at different intervals of 1, 3, 6 and 24 h. The whole investigation was carried out at 27 ± 1^0C.

Statistical Analysis

The results obtained in the current study were subjected to the ANOVA test. The partitioning of the total variance into variance due to the different experimental condition (e.g. salinity, temperature etc.) was carried out by following the procedure described by Zar [19].

Results

Survival

A. parthenogenetica cultured at 80 ppt, exposed to different concentrations of formalin at different salinities, released cysts; but the survival was 88% during the first hour at low concentrations (0.01, 0.05, 0.1 and 0.5) and 60% at high concentrations (1, 2 and 3%). After the 6^{th} hour, the cyst induction was followed by mortality. The percentage of survival was reduced to 0% at the highest concentration during 24h (Fig.1 to 7) in all the tested salinities.

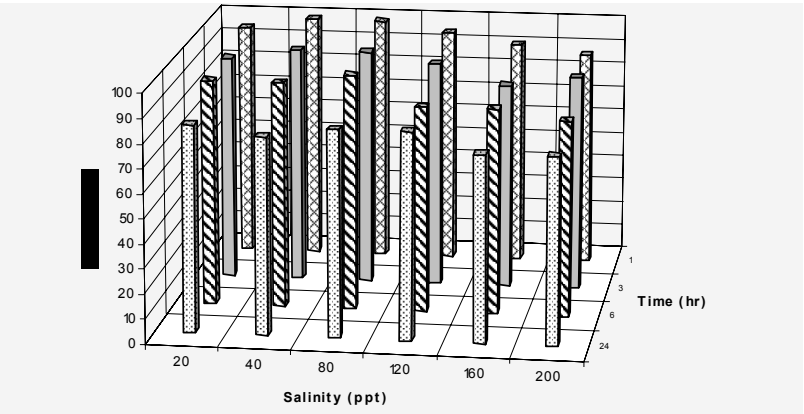

Figure 1. Survival of *A. parthenogenetica* at 0.01% formalin concentration

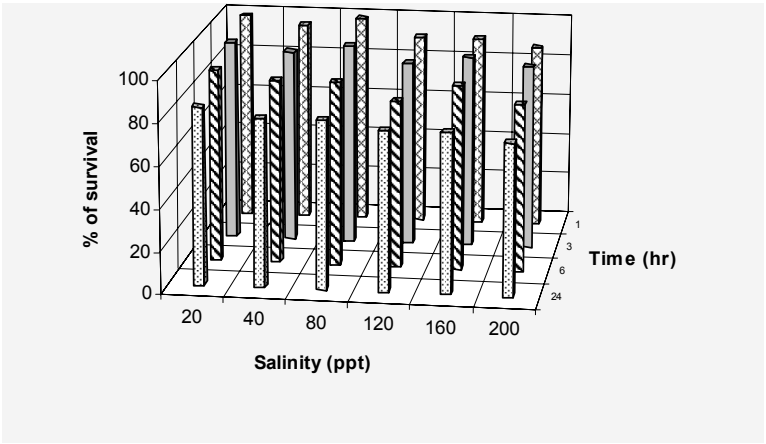

Figure 2. Survival of *A. parthenogenetica* at 0.05% formalin concentration

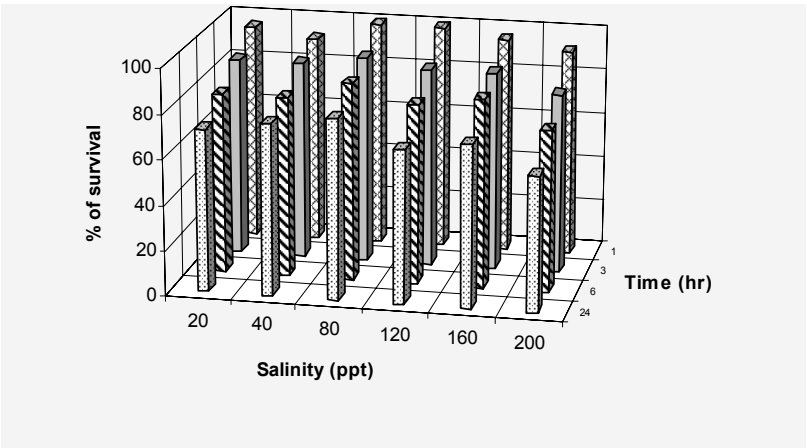

Figure 3. Survival of *A. parthenogenetica* at 0.1% formalin concentration

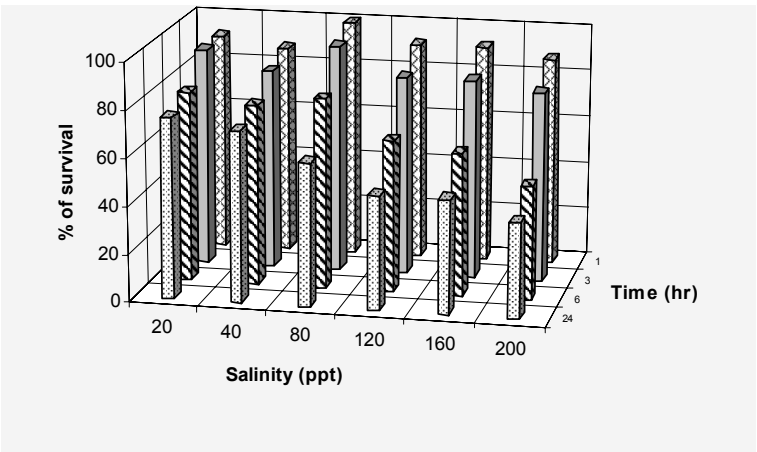

Figure 4. Survival of *A. parthenogenetica* at 0.5% formalin concentration

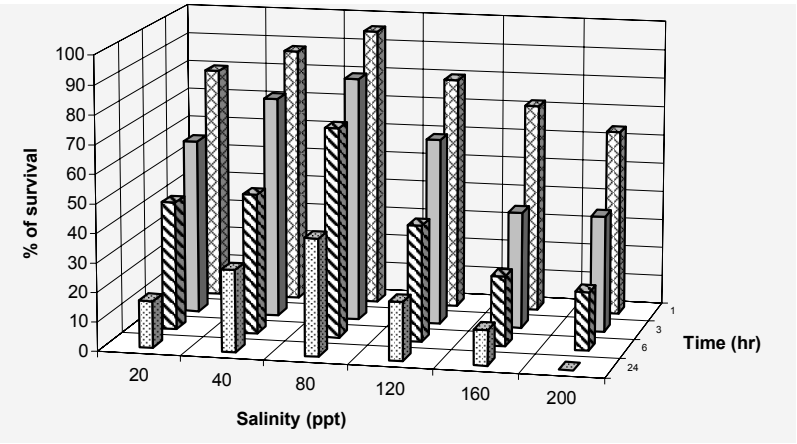

Figure 5. Survival of *A. parthenogenetica* at 1% formalin concentration

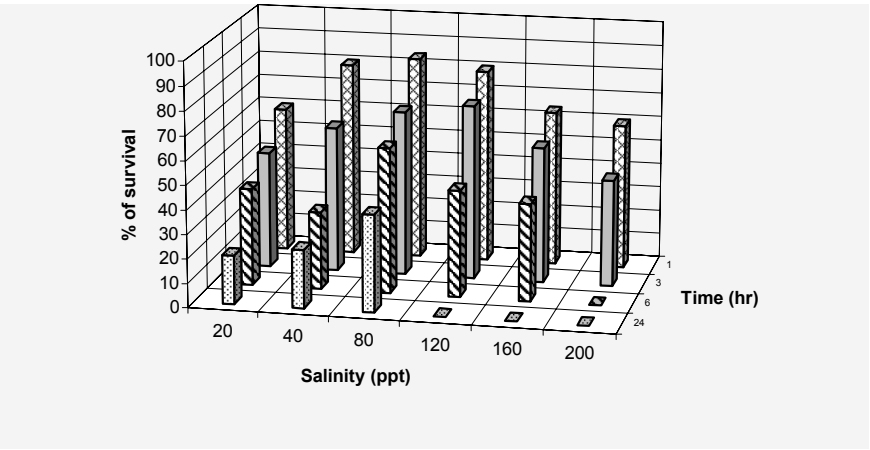

Figure 6. Survival of *A. parthenogenetica* at 2% formalin concentration

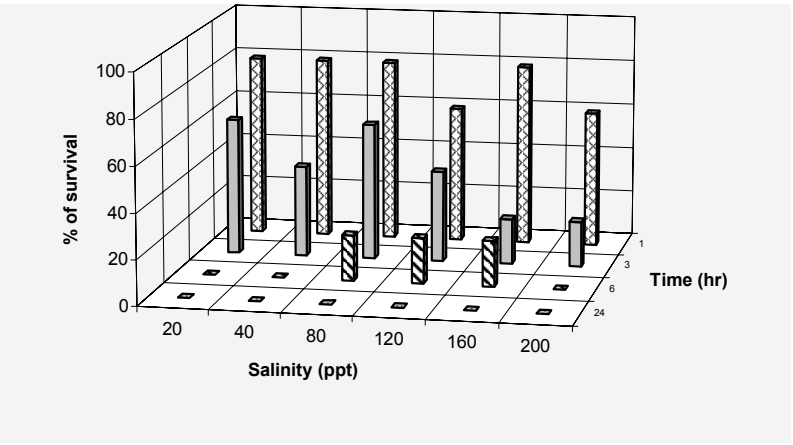

Figure 7. Survival of *A. parthenogenetica* at 3% formalin concentration

Two-way analysis of variance revealed that irrespective of the different formalin concentrations, *A. parthenogenetica* exposed to different salinities died with advancing time and the magnitude of the effect was high at 3% formalin concentration.

Nauplii production

It is interesting to note that the nauplii production was nil at the tested concentrations of formalin for all tested salinities.

Cyst induction

Fig. 8 to 14 illustrate the results obtained for cyst induction in *A. parthenogenetica* adult exposed to different concentrations of formalin, i.e. 0.01, 0.05, 0.1, 0.5, 1.0, 2.0 and 3.0 % during an experimental period of 24 h. At one hour, the number of cyst released were 1.52 ± 0.02, 2.20 ± 0.245, 4.08 ± 0.024, 3.20 ± 0.20, 3.88 ± 0.040, 7.32 ± 0.024, 1.40 ± 0.548, respectively at 0.01, 0.05, 0.1, 0.5, 1.0, 2.0 and 3% concentration at 80 ppt. The maximum number of cysts was released at 3% formalin concentration during the first hour of the experiment at low salinities (20 and 40 ppt). At 2% concentration, the maximum number of cysts was released during the 6th hour of the experiment at 20 and 80 ppt salinities.

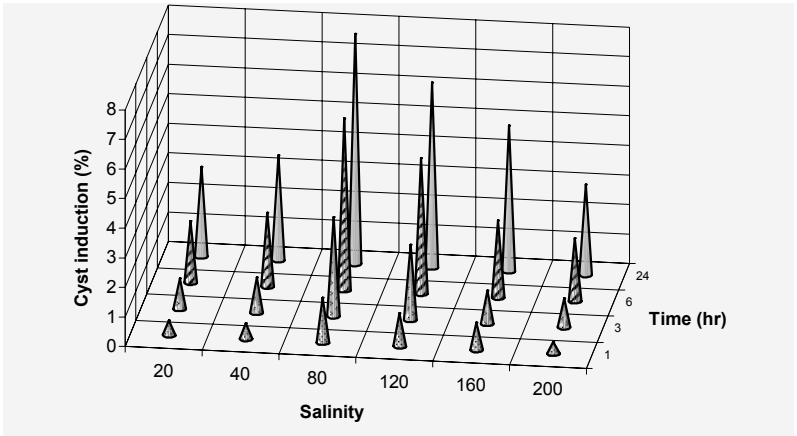

Figure 8. Cyst induction of *A. parthenogenetica* at 0.01% formalin concentration

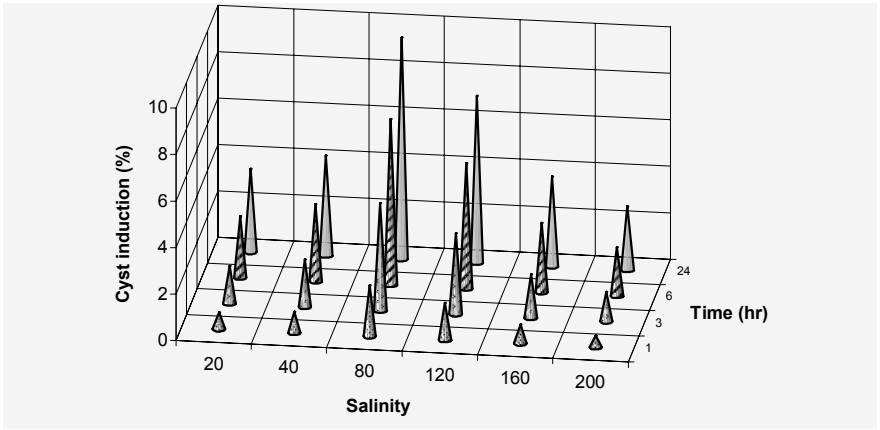

Figure 9. Cyst induction of *A. parthenogenetica* at 0.05% formalin concentration

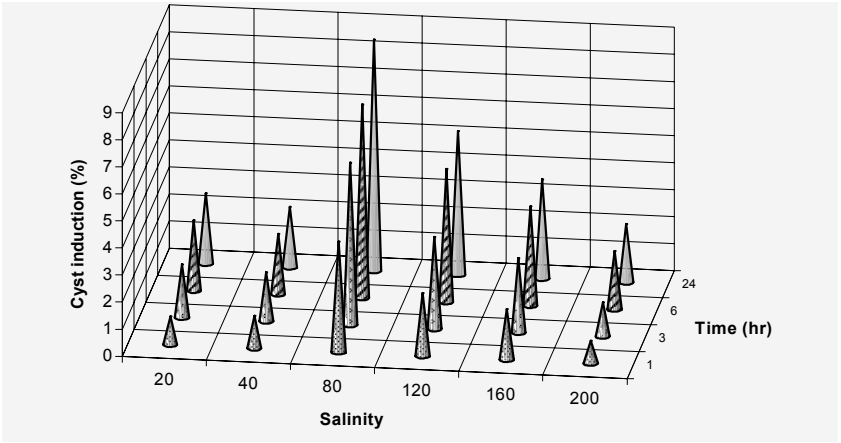

Figure 10. Cyst induction of *A. parthenogenetica* at 0.1% formalin concentration

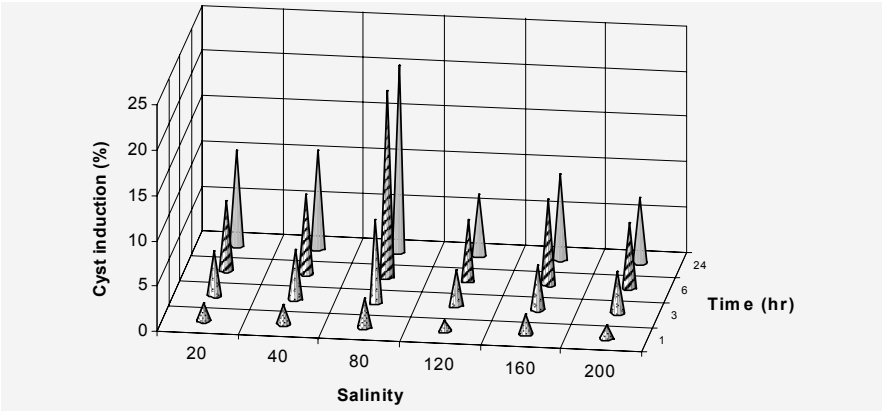

Figure 11. Cyst induction of *A. parthenogenetica* at 0.5% formalin concentration

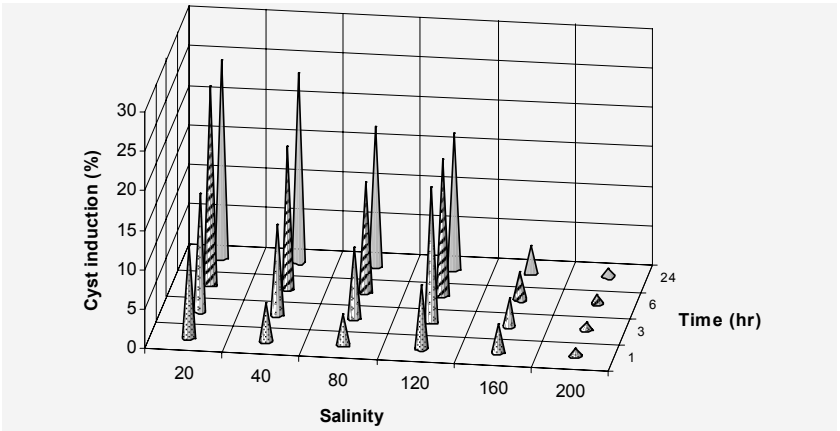

Figure 12. Cyst induction of *A. parthenogenetica* at 1% formalin concentration

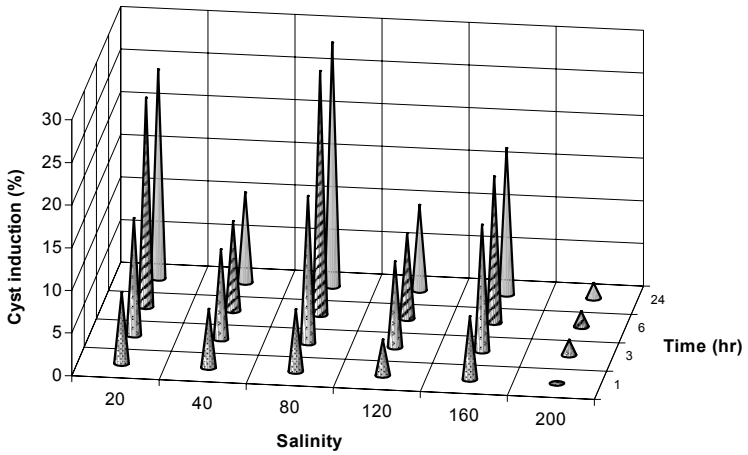

Figure 13. Cyst induction of *A. parthenogenetica* at 2% formalin concentration

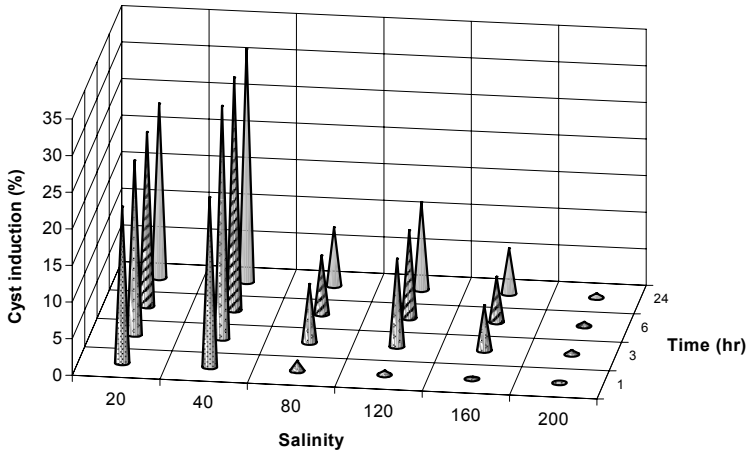

Figure 14. Cyst induction of *A. parthenogenetica* at 3% formalin concentration

The ANOVA test revealed that the effect of salinity and exposure duration exhibited more influence at low concentrations of formalin than at high concentration. It is also revealed that at the highest concentration (3%), the influence of both salinity and experimental duration was statistically non significant ($p > 0.05$).

Discussion

The brine shrimp *Artemia* are distributed in diversified environments where they encounter high salinity, extreme temperature, high doses of ultraviolet radiations and very low oxygen tensions [20, 21]. These challenging ecological settings imply that *Artemia* could be considered as a useful model organism for the stress response at all levels of biological organization [22].

Ovoviviparity and oviparity are the two modes of reproduction in the brine shrimp *A. parthenogenetica. Artemia* resorts to ovoviviparity under favorable environmental conditions and oviparity when these factors become adverse. The culture trials with *Artemia* have been done extensively by several workers in different aspects such as food, temperature,

salinity, density etc. The quality and quantity of food are also responsible for cyst production [23]. In addition to this, the content of iron in water [13] and chlorophyll [24] are also found to play a significant role in cyst production. As early as 1915, Abonyi [14] found that salinity is one of the factors inducing cyst production in *Artemia*.

The aim of this study is to develop a standard routine method of inducing *Artemia* populations to shift from ovoviviparity to oviparity i.e., formation of cysts. This may become a very practical tool for the techniques involving mass culture of *Artemia* biomass through such induced cyst. In the present study, extreme salinity affects the pattern of reproduction. Working on *A. parthenogenetica*, Balasundaram and Kumaragure [25] reported a positive linear increase in cyst production with rearing salinity. In this study, the induction of cyst production was decreasing with the increased salinity 20 > 40 > 160 > 120 > 80.ppt

The importance of salinity fluctuation leading to extreme dilution and its effect on the survival of *Artemia* in the saltpan has been reported by Bhargava *et al.* [26]. Wide fluctuations in salinity either due to solar radiation or dilution due to sudden rains play a major role in inducing the mode of reproduction in *Artemia*. These kinds of abrupt salinity fluctuations adversely affect the reproducing adult. Kuruppu and Ekaratne reported that changes occurred in salinities and water depth influenced the *Artemia* population size [27]. In the present study, the salinity ranging from 20 to 200 ppt (lower and higher salinities) supported the cyst production. *A parthenogenetica* were unable to tolerate higher salinities and survived only for a short span of time.

Cole and Brown [28] stated that high concentration of certain compounds like CO_3, bicarbonate and potassium were lethal to *Artemia*. In the present study, formalin, the well known effective germicide used in aquaculture was selected because it does not form residues in the body of the animals that are exposed to it. The results indicated that, the number of cysts released after exposed to 3% formalin concentrations at 20 and 40 ppt medium was higher in the first hour. The reason probably may be due to the sudden change in culture medium and high concentration of formalin. The cyst gain per hour was naturally lower because of the reducing formalin solution concentration by evaporation from the saline medium. The study by John [16] in *A. parthenogenetica* (Thamaraikulam salt works extension II) also revealed that *Artemia* produced cysts within 1 h when the formalin was applied**.** Lavens and Sorgeloos [29] have reported that in laboratory condition oviparity occurs in media with low dissolved oxygen concentration or in the presence of chelated iron (Ferric EDTA).

According to Dutrieu [24] only low oxygen levels induce haemoglobin synthesis (facilitating respiration). *Artemia* females used their haemoglobin as a basic element for cyst shell formation. The positive optimum dissolved oxygen concentration in 20 ppt medium acts as an inherent physiological barrier in the *Artemia* females (in spite of formalin solution treatments) preventing cyst release to a greater extent. The cyst release is more retarded in 0.1 and 0.5 concentrations. This may be due to the very low concentration of formalin solution. At 2 and 3% formalin solution, the cyst release was higher.

Working on the *A. parthenogenetica*, Lavens and Sorgeloos [29] have induced 60 g cyst/m^3/day in a population of 10000 nos/*l* by applying inert gas nitrogen. In *A. franciscana* and *A. persimillis* cysts were produced by applying sudden salinity stress (from 40 to 10 ppt). The cyst production induced by applying the chemical stimulant formalin in *A. parthenogenetica* accelerated not only the size, but also the hatching characteristics [16].

During recent years, commercial scale use of *Artemia* biomass harvested from local salt works or produced in manured salt works [30] is gaining more and more interest especially in fish weaning and shrimp nursing. Dobblier *et al.* [31] have reported the use of

non-soluble waste products from agricultural crops or from the food processing industries such as rice bran, corn bran, soybean pellet, lactoserum, and sugarcane molasses as for high density culture of *Artemia*.

Dutrieu [24] and Berthelemy–Okazaki and Hedgecock [32] reported that hypoxia might trigger cyst production. But the studies by Vanden *et al* [33], Heip *et al*. [34] and Lavens and Sorgeloos [29] revealed that the specific hemoglobin alone induced cyst production in *Artemia*. Several environmental factors such as photoperiod, temperature and animal density affected the reproductive mode in *Artemia.*

The study of cyst induction is important because the cysts have two important functions, i.e. they secure the survival of the population during unfavorable conditions and they are the effective dispersal agents [10].

Conclusion

It is evident from the current study that the stimulatory agents such as formalin (chemical) and salinity (environmental factor) were induced oviparity in bisexual brine shrimp *A. parthenogenetica*. The interacting effect of these tested parameters yielded noteworthy information's on survival and cysts induction of studied *Artemia* population. In 2 or 3% formalin concentration and at 20 and 40 ppt or 20 and 80 ppt salinities *A. parthenogenetica* released maximum number of cysts.

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