# Developing feed for the culture of brine shrimp *Artemia franciscana* using marine algae as major dietary source

Received for publication, June 1, 2007
Accepted, July 5, 2007

**S. M. JOSEPHINE PUNITHA, M. MICHAEL BABU, G. IMMANUEL***
*Marine Biotechnology Laboratory, Centre for Marine Science and Technology*
*M. S. University, Rajakkamangalam - 629 502, Kanyakumari District,*
*Tamilnadu, India*
*Phone: + 91 4652 253078*
*Email: gimmas@gmail.com (or) g_immas@yahoo.com*

## Abstract

*The present study was undertaken to develop a suitable diet to culture Artemia franciscana using three different groups of algal species i.e., red algae (Acanthophora spicifera, Gelediella acerosa and Gracilaria edulis), brown algae (Turbinaria ornate, T. conoides and Sargassum wightii) and green algae (Ulva spiralis, Enteromorpha intestinalis and Halimeda tuna). The individual algal powders were supplemented with rice bran extract in different combinations (0, 25:75, 50:50, 75:25 and 100%) as dietary source. The prepared diets were fed to A. franciscana nauplii (instar I stage), reared in managed culture condition and observed the survival, growth and reproductive performance for 30 days. The survival and growth were higher in 100% rice bran fed group than algal powder supplemented diets except that of 50:50% combinations of rice bran and green algae U. spiralis. Similar trend was also observed in the reproductive characteristics of A. franciscana. The 50:50% ricebran and U. spiralis fed group exhibited good performance among the reproductive characters such as reduction in maturation period, gestation period, increased number of broods, increased fecundity rate etc.*

Keywords: *Artemia*; Red algae; Brown algae; Green algae

## Introduction

In the intensive culture of live feed *Artemia*, agricultural by-products such as corn flour and soybean [1], groundnut husk, groundnut oil cake [2], wheat flour [3] and rice bran [4, 5, 6] have been used as inert cheap feed. Even though rice bran has been used as a universal cheap feed for the intensive culture of *Artemia*, the growth attained by *Artemia* on this feed is not satisfactory. Hence, other plant products such as grass powder [7] and different species of dried seaweeds such as *Chlorella* sp. [8], *Enteromorpha* sp. [6, 9], *Ulva* sp. [6] etc. have been tested. The aim of this work was to use the seaweed as a cheapest diet to culture *Artemia*.

Seaweeds are commercially important marine living and renewable resources all over the world. Red algae (Rhodophyceae), brown algae (Phaeophyceae) and green algae (Chlorophyceae) are the major groups of seaweeds and they occur in inter-tidal and sub-tidal regions of the sea. The nutrient content of seaweeds is quite enormous and they contain more than 60 trace elements, minerals, protein, iodine, bromine, vitamins and several bioactive substances. The presence of minerals, trace elements, protein and vitamins account for their nutritional value.

Presently, in certain regions of many countries, animals still regularly feed upon fresh seaweed or a processed seaweed food. Formerly the use of seaweed as food was restricted to coastal areas near the source of supply. Modern practice of seaweed processing involves drying and grinding the seaweeds into a meal. It is evident that during the First World War, there was considerable exaggeration of the nutritional value of seaweed meals, though claims have been made that their use increased the fertility and birth rate of animals. Presence of fucoxanthin in seaweed meals improves the yolk colour of eggs and their iodine content [10]. The reality behind the use of seaweed meals as food is that one cannot expect a quick return after adding seaweed meal to animal rations, but continued use is essential. Experiments have shown that the effectiveness of the meal varies with the algal species, the locality, time of harvest, method of preparation and storage time.

In the present study, an attempt was made to use the seaweeds as one of the feed combinations along with the rice bran brine shrimp *Artemia*. For this purpose, seaweeds of nine species belonging to red algae (*Acanthophora spicifera, Gelidella acerosa,* and *Gracilaria edulis*), brown algae (*Turbinaria ornata, T. conoides,* and *Sargassum wightii*) and green algae (*Ulva spiralis, Enteromorpha intestinalis,* and *Halimeda tuna*) were selected to find out the suitability of seaweed (s) to be incorporated along with the universally used inert feed rice bran, so that a suitable combinations of seaweed and rice bran which ensure high survival, growth and fecundity in the brine shrimp *Artemia* can be formulated for the culture of *Artemia franciscana*.

## Materials and Methods

### *Preparation of culture medium*

Seawater was brought to the laboratory and filtered through a fine filter (pore size 20µm) to remove planktonic organisms and suspended particles. The salinity of seawater was maintained at 60 ppt by adding adequate quantity of solar salt (sodium chloride). The salinity was measured by using a salinity refractometer (New S-100, Tanaka Sanjiro, Japan; sensitivity ±1ppt). The temperature of the experimental medium was maintained at 27 ± $1^0$C. The temperature was monitored using a thermometer (Hermes, India; sensitivity ± $1^0$C).

### *Experimental diets*

To prepare the experimental diets, three species each of red algae (*A. spicifera, G. acerosa* and *G. edulis*), brown algae (*T. ornata, T. conoides, S. wightii*) and green algae (*U. spiralis, E. intestinalis, H. tuna*) were used. These seaweeds were collected from seaweed culture centre, Central Marine Fisheries Research Institute (CMFRI), Mandapam, Tamilnadu, India.

***Preparation of feeds***

The selected seaweeds were shade dried and ground well separately and sieved through a mesh (50 µm size). The powdered products were again dried and stored in separate screw capped polythene containers and used for this experiment. The rice bran purchased from the market was sieved to 50 µm size before using as feed.

Five different feed combinations were formulated by mixing rice bran and the individual seaweed powder in the ratios of 100 : 0 (A- Control; Rice bran alone), 75 : 25 (B), 50 : 50 (C), 25 : 75 (D) with combination and 0 : 100 (E ; Seaweed alone). The appropriate feed suspension was prepared individually by mixing 20 g of the feed (s) in one liter of seawater

***Artemia cysts hatching procedure***

Cysts of the brine shrimp *Artemia franciscana* (Great Salt Lake strain) were purchased from San Francisco Bay Brand (San Francisco, California) and allowed to hatch under optimum hatching conditions (35‰ salinity; 27 ± $1^0$C temperature and 1000 lux light) in the laboratory. After complete hatching (24 h), the nauplii were separated, reared in 50‰ saline water medium.

***Experimental setup***

The experiment was carried out in transparent flat bottomed culture vessels (plastic bowls) of 300 ml capacity. The culture vessels were stocked with 25 randomly selected *A. franciscana* nauplii of 12 to 24 h old (II instar stage). These nauplii were carefully isolated from the stock and transferred into each culture vessel containing 150 ml of the culture medium (seawater at 60 ppt). The animals were fed with 1 ml respective feed twice a day (09:00 and 17:00 h). 90% of the culture medium was changed daily from the culture vessel in the morning before feeding. The animals were also observed daily for their maturity.

To study the offspring production, the matured animals were removed from the culture vessels and were restocked individually in separate culture vessels containing 100 ml of the same culture medium. The same type of feed was offered at the rate of 1 ml twice a day and 90% of the culture medium was changed daily. Simultaneously five replicates were maintained for each combination.

***Determination of growth and reproductive traits***

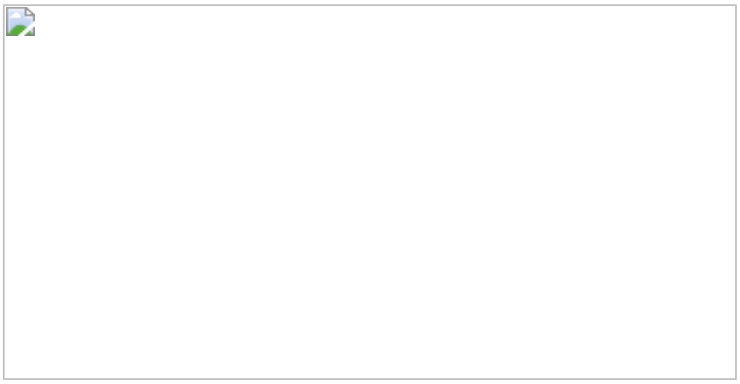
Survival, growth and reproductive characteristics of the *Artemia* were recorded to evaluate the efficiency of the experimental diets. Survival was recorded once in five days by direct counting while changing the water medium. Growth was determined by weighing the *Artemia* and individual wet weight was recorded in every five day using an electronic balance of Shimadzu make (0.0001g accuracy). Before taking weight, the *Artemia* were placed on a blotting paper to absorb the external water.

The ovary development of *Artemia* was observed daily. The length of maturation period was scored from the day of hatching until the development of ovary. The gestation period was timed from the appearance of ovary and the first brood parturition day. After the development of the brood pouch and the brood, careful observation was made daily for the release of nauplii. All the nauplii released from each dietary source and replicate were removed by pipette and counted. Cysts (if any) released were removed, by filter paper and stored for subsequent counts using a dissection microscope. The data obtained were subjected for relevant statistical analysis described by Zar [11].

## Results

***Survival of Artemia***

*A. franciscana* fed with rice bran and brown algae namely *T. ornata, T. conoides* and *S. wightii* at 60 ppt salinity displayed different levels of survival. In the first group, *T. ornata* supplemented diet, only 100% ricebran fed *Artemia* exhibited maximum (52%) survival, but in the other two groups such as *T. conoides* and *S. wightii,* the maximum survival was noticed in 25 and 75% combination at the end of the culture period (Fig. 1).



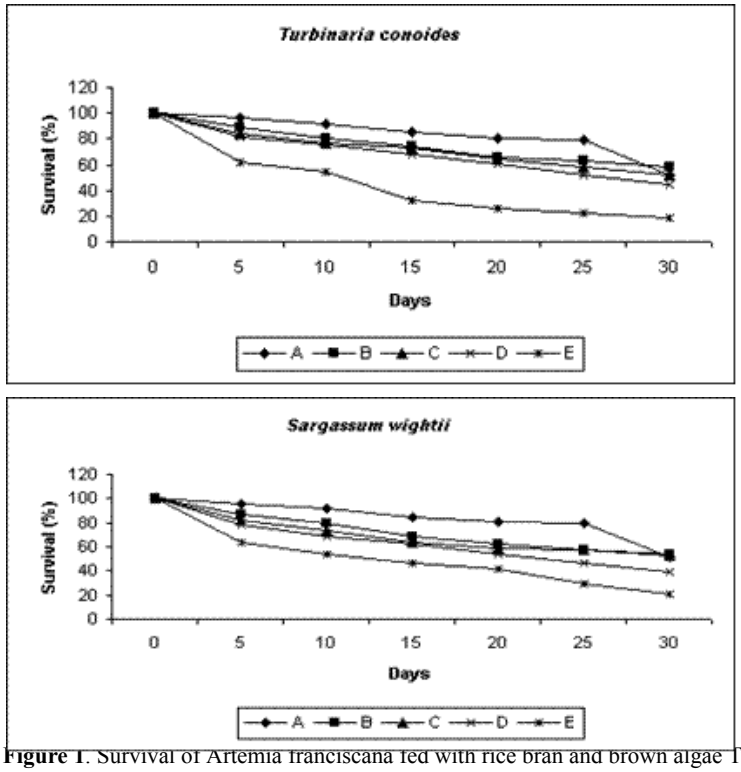
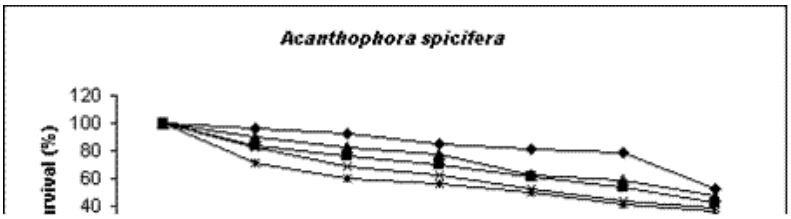



**Figure 1**. Survival of Artemia franciscana fed with rice bran and brown algae Turbinaria ornata, Turbinaria conoides and Sargassum wightii (Each value is a mean of five replicates)

The survival of *A. franciscana* fed with rice bran and red algae such as *A. spicifera, G. acerosa, G. edulis* in different combination is given in Fig 2. The survival of *A. franciscana* was higher in rice bran alone fed *A. franciscana*. Among the three red algae tested, *G. edulis* fed animals exhibited better survival than other seaweeds.



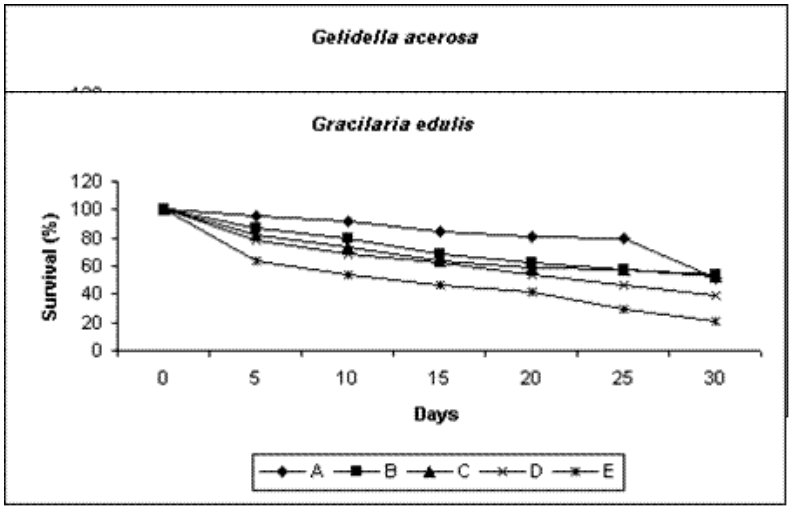



**Figure 2**. Survival of Artemia franciscana fed with rice bran and red algae Acanthophora spicifera, Gelidella acerosa and Gracillaria edulis (Each value is a mean of five replicates)

The survival of *A. franciscana* fed with rice bran and green algae *U. spiralis, E. intestinalis* and *H. tuna* in different combinations are given in Fig.3. Among the three green algae tested, *U. spiralis*, resulted maximum survival (62%) of *Artemia* at the combination of 50: 50 ratio.

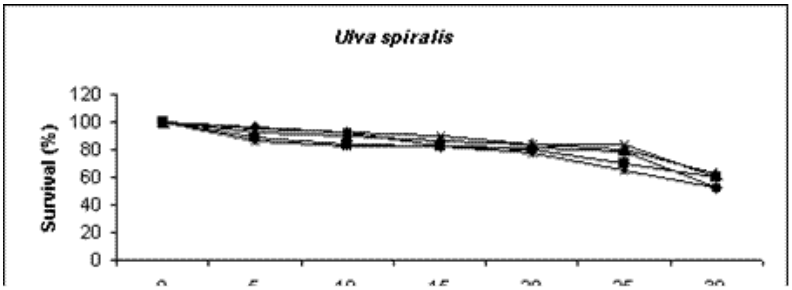
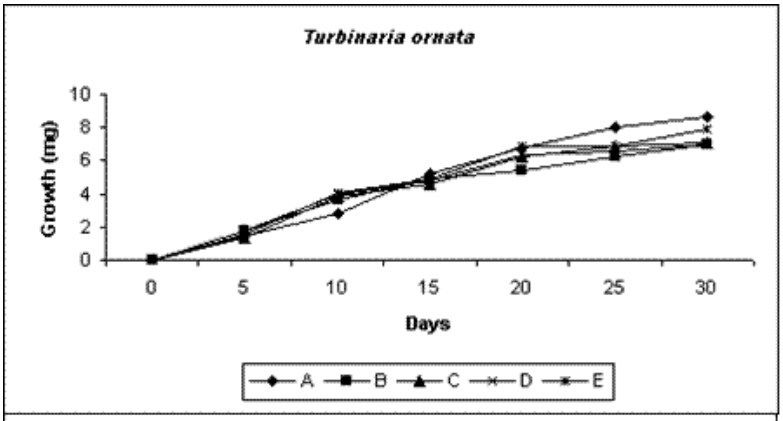

Ulva spiralis
Survival (%)
120
100
80
60
40
20
0

**Figure 3**. Survival of Artemia franciscana fed with rice bran and green algae Ulva spiralis, Enteromorpha intestinalis and Halimeda tuna (Each value is a mean of five replicates)

***Growth of Artemia***

The growth pattern of *A. franciscana* fed with the three brown algae is given in Fig.4. The rice bran fed *A. franciscana* exhibited highest growth (8.6 mg), whereas the respective brown algae mixed with rice bran at 50:50 combination supported good growth between 7.1 and 7.3 mg. Among the tested three red algae, *G. edulis* supported better growth performance (Fig.5).


Turbinaria ornata
Growth (mg)
10
8
6
4
2
0
0
5
10
15
20
25
30
Days
A
B
C
D
E

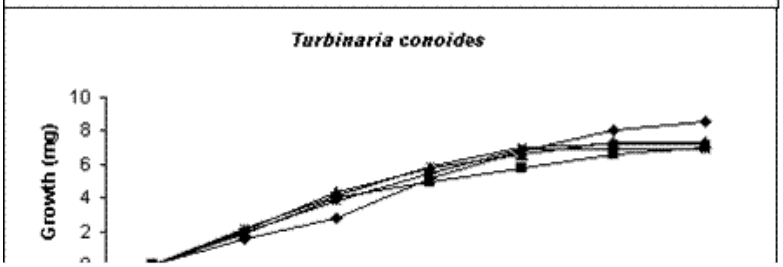
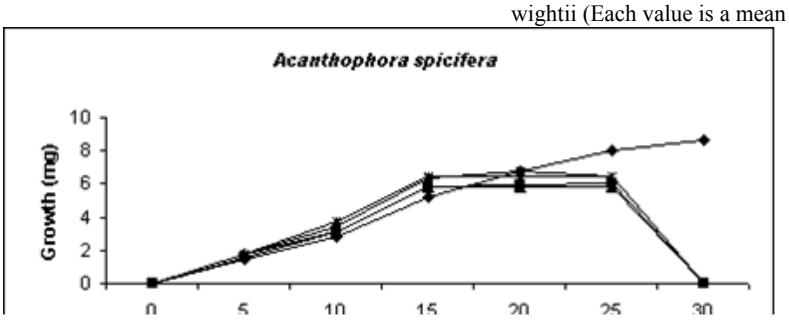

Turbinaria conoides
Growth (mg)
10
8
6
4
2

**Figure 4**. Growth of Artemia franciscana fed with rice bran and brown algae Turbinaria ornata, Turbinaria conoides and Sargassum wightii (Each value is a mean of five replicates)



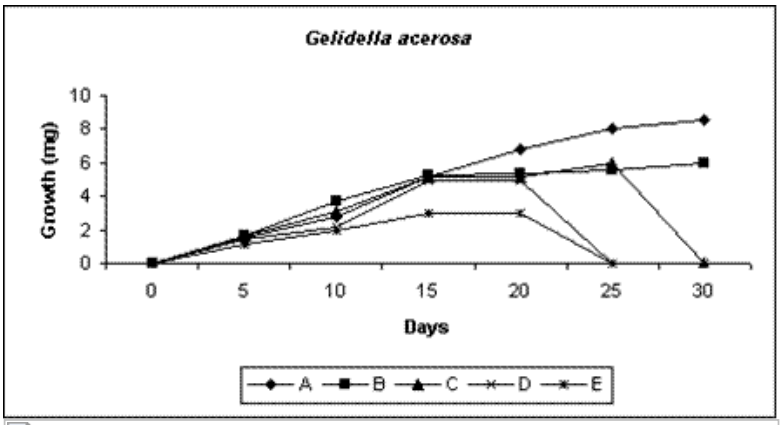




**Figure 5**. Growth of Artemia franciscana fed with rice bran and red algae Acanthophora spicifera, Gelidella acerosa and Gracillaria edulis (Each value is a mean of five replicates)

Fig 6. shows the growth performance of *A. franciscana* fed with rice bran and green algae. From this figure, it is clear that *U. spiralis* in a combination with equal percentage of rice bran (50 : 50) supported higher growth (9.7 mg) when compared to other treatments.

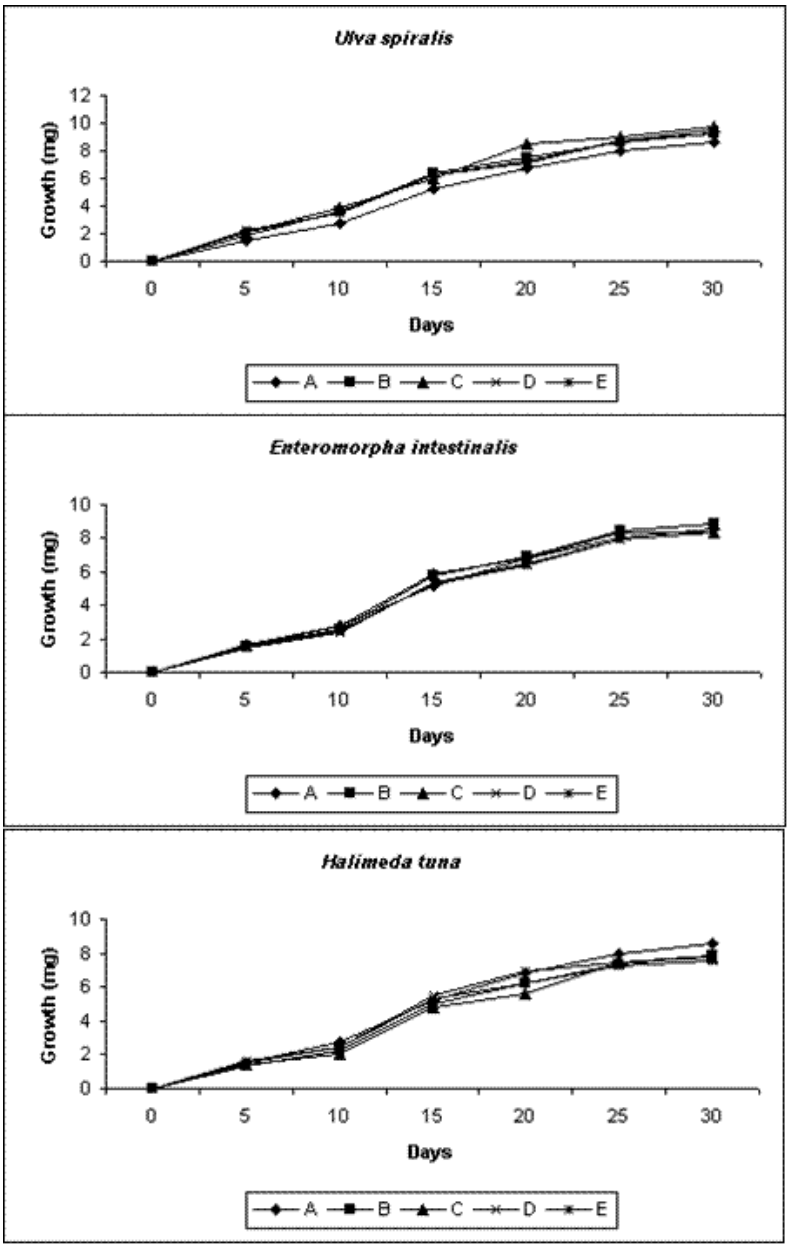


**Figure 6**. Growth of Artemia franciscana fed with rice bran and green algae Ulva spiralis, Enteromorpha intestinalis and Halimeda tuna (Each value is a mean of five replicates)

### *Reproduction of Artemia*

*A. franciscana* fed with rice bran and brown algae in different combinations exhibited different reproductive potentials. Irrespective of different combinations, the brown algae did not significantly ($P > 0.05$) improve reproduction of *A. franciscana* (Table 1). The poor reproductive performance indicated that the red algae are not supporting the reproductive characteristics (Table 2).

**Table 1**. Reproductive characteristics of *Artemia fransicana* fed with rice-bran (Rb) and brown algae (T.o. - *Turbinaria ornata ;* T.c. - *Turbinaria conoides ;* S.w. - *Sargassum wightii*) in different combinations

| ***Turbinaria ornata*** | | | **Total life span (days)** | **Maturation (days)** | **Gestation (Days)** | **No. of Broods** | **No. of nauplii / brood** | **Inter brood (Days)** | **No. of Nauplii / *Artemia*** |
|---|---|---|---|---|---|---|---|---|---|
| **Rb** | | **T.o.** | | | | | | | |
| 100 | : | 0 | 56 | 18 | 6.0 | 3 | 32 | 5.0 | 96 |
| 75 | : | 25 | 43 | 17 | 6.5 | 2 | 23 | 5.5 | 48 |
| 50 | : | 50 | 46 | 19 | 6.5 | 2 | 28 | 5.5 | 73 |
| 25 | : | 75 | 52 | 19 | 6.0 | 2 | 24 | 5.0 | 62 |
| 0 | : | 100 | 43 | 18 | 6.0 | 2 | 21 | 5.0 | 43 |
| ***Turbinaria conoides*** | | | **Total life span (days)** | **Maturation (days)** | **Gestation (Days)** | **No. of Broods** | **No. of nauplii / brood** | **Inter brood (Days)** | **No. of Nauplii / *Artemia*** |

| Rb | | T.c. | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 100 | : | 0 | 56 | 18 | 6.0 | 3 | 32 | 5.0 | 96 |
| 75 | : | 25 | 51 | 19 | 6.0 | 1 | 18 | - | 18 |
| 50 | : | 50 | 44 | 19 | 6.0 | 1 | 18 | - | 18 |
| 25 | : | 75 | 44 | 20 | - | - | - | - | - |
| 0 | : | 100 | 32 | 20 | - | - | - | - | - |

| ***Sargassum wightii*** | | | **Total life span (days)** | **Maturation (days)** | **Gestation (Days)** | **No. of Broods** | **No. of nauplii / brood** | **Inter brood (Days)** | **No. of Nauplii / *Artemia*** |
|---|---|---|---|---|---|---|---|---|---|
| **Rb** | | **S.w.** | | | | | | | |
| 100 | : | 0 | 56 | 18 | 6.0 | 3 | 32 | 5.0 | 96 |
| 75 | : | 25 | 43 | 19 | 6.5 | 2 | 22 | 5.5 | 44 |
| 50 | : | 50 | 52 | 19 | 6.0 | 3 | 26 | 5.5 | 78 |
| 25 | : | 75 | 41 | 20 | 6.0 | 2 | 16 | 5.5 | 32 |
| 0 | : | 100 | 33 | 20 | 6.0 | 1 | 20 | - | 20 |

**Table 2**. Reproductive characteristics of *Artemia franciscana* fed with rice bran (Rb) and red algae (G.a. - *Gelidella acerosa ;* A.s. - *Acanthophora spicifera ;* G.e. - *Gracilaria edulis*) in different combinations

| ***Acanthophora spicifera*** | | | **Total life span (days)** | **Maturation (days)** | **Gestation (Days)** | **No. of Broods** | **No. of nauplii / brood** | **Inter brood (Days)** | **No. of Nauplii / *Artemia*** |
|---|---|---|---|---|---|---|---|---|---|
| Rb | : | A.s. | | | | | | | |
| 100 | : | 0 | 56 | 18 | 6 | 3 | 32 | 5.0 | 96 |
| 75 | : | 25 | 28 | 23 | - | - | - | - | - |
| 50 | : | 50 | 26 | 22 | - | - | - | - | - |
| 25 | : | 75 | 26 | - | - | - | - | - | - |
| 0 | : | 100 | 26 | - | - | - | - | - | - |

| ***Gelidiella acerosa*** | | | **Total life span (days)** | **Maturation (days)** | **Gestation (Days)** | **No. of Broods** | **No. of nauplii / brood** | **Inter brood (Days)** | **No. of Nauplii / *Artemia*** |
|---|---|---|---|---|---|---|---|---|---|
| Rb | : | G.a. | | | | | | | |
| 100 | : | 0 | 56 | 18 | 4 | 3 | 32 | 5.0 | 96 |
| 75 | : | 25 | 32 | 21 | 6 | 1 | 16 | - | 16 |
| 50 | : | 50 | 28 | 22 | 7 | 1 | 21 | - | 21 |
| 25 | : | 75 | 23 | - | - | - | - | - | - |
| 0 | : | 100 | 21 | - | - | - | - | - | - |

| ***Gracilaria edulis*** | | | **Total life span (days)** | **Maturation (days)** | **Gestation (Days)** | **No. of Broods** | **No. of nauplii / brood** | **Inter brood (Days)** | **No. of Nauplii / *Artemia*** |
|---|---|---|---|---|---|---|---|---|---|
| Rb | : | G.e. | | | | | | | |
| 100 | : | 0 | 56 | 18 | 6 | 3 | 32 | 5.0 | 96 |
| 75 | : | 25 | 43 | 22 | 7 | 1 | 18 | - | 18 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 50 : 50 | 46 | 21 | 7 | 1 | 23 | - | 23 |
| 25 : 75 | 43 | 22 | - | - | - | - | - |
| 0 : 100 | 39 | 32 | - | - | - | - | - |

The results obtained with rice bran and green algae in different combinations are given in Table 3. *U. spiralis* and rice bran in 50:50 ratio reduced the maturation time and gestation period from 18 – 14 and 4 – 5 days respectively. The number of broods increased from three to four. The number of nauplii increased from 32 to 38 and prolonged final fecundity from 96 to 152 numbers during the life span of 63 days. Among the three seaweeds, *U. spiralis* supported highest survival, growth and the life span as well as high fecundity.

**Table 3**. Reproductive characteristics of *Artemia franciscana* fed with rice bran (Rb) and green algae (E.i. - *Enteromorpha intestinalis*; U.s. - *Ulva spiralis* ; H.t. - *Halimeda tuna*) in different combinations

| ***Ulva spiralis*** | **Total life span (days)** | **Maturation (days)** | **Gestation (Days)** | **No. of Broods** | **No. of nauplii / brood** | **Interbrood (Days)** | **No. of Nauplii / *Artemia*** |
|---|---|---|---|---|---|---|---|
| **Rb : U.s.** | | | | | | | |
| 100 : 0 | 52 | 18 | 5.0 | 3 | 32 | 5.0 | 96 |
| 75 : 25 | 58 | 17 | 5.0 | 3 | 32 | 5.0 | 96 |
| 50 : 50 | 63 | 14 | 4.0 | 4 | 38 | 5.0 | 152 |
| 25 : 75 | 60 | 16 | 4.0 | 3 | 33 | 5.0 | 99 |
| 0 : 100 | 58 | 16 | 4.0 | 3 | 34 | 5.0 | 102 |

| ***Enteromorpha intestinalis*** | **Total life span (days)** | **Maturation (days)** | **Gestation (Days)** | **No. of Broods** | **No. of nauplii / brood** | **Interbrood (Days)** | **No. of Nauplii / *Artemia*** |
|---|---|---|---|---|---|---|---|
| **Rb : E.I.** | | | | | | | |
| 100 : 0 | 52 | 18 | 6.0 | 3 | 32 | 5.0 | 96 |
| 75 : 25 | 56 | 18 | 6.0 | 3 | 32 | 5.0 | 96 |
| 50 : 50 | 58 | 19 | 6.0 | 3 | 34 | 5.5 | 102 |
| 25 : 75 | 58 | 18 | 6.0 | 3 | 33 | 5.0 | 99 |
| 0 : 100 | 58 | 17 | 6.0 | 3 | 32 | 5.0 | 96 |

| ***Halimeda tuna*** | **Total life span (days)** | **Maturation (days)** | **Gestation (Days)** | **No. of Broods** | **No. of nauplii / brood** | **Interbrood (Days)** | **No. of Nauplii / *Artemia*** |
|---|---|---|---|---|---|---|---|
| **Rb : H.t.** | | | | | | | |
| 100 : 0 | 52 | 18 | 6.0 | 3 | 32 | 5.0 | 96 |
| 75 : 25 | 54 | 18 | 6.0 | 3 | 33 | 5.0 | 99 |
| 50 : 50 | 56 | 18 | 6.0 | 3 | 32 | 5.5 | 96 |
| 25 : 75 | 58 | 19 | 6.0 | 3 | 32 | 5.0 | 96 |
| 0 : 100 | 56 | 18 | 6.0 | 3 | 32 | 5.5 | 96 |

## Discussion

A wide range of diet including unicellular algae (live, frozen or dried), yeast, bacteria, and microcapsules have been used for the culture of *Artemia* [12]. Sorgeloos [13] used micronized rice bran, spray dried spirulina, dried yeast and dried algae as dietary source for culture of *Artemia.* While Roels et al. [14] used monospecific algae culture in combined culture medium as food source for *Artemia*. Apart from these, vitamins and trace minerals were also used with rice bran for the culture of *Artemia* [15].

Roels et al. (14) used 3 types of feed such as dried *Spirulina*, rice bran and baker yeast as food in the culture of *Artemia*. Douillet [16] used dried *Spirulina*, yeast, defatted rice bran and soyabean powder for the culture of *Artemia*. Jumalon et al. [17] used clam meat suspension, squid powder, rice bran, squeezed ground nut oil cake and wheat flour extract. Rice extract has been used by Basil et al. [2] and Yashiro [3]. Ribeiro - Vieiva [18] used micronized rice bran, dried *Ulva* and dried *Spirulina* powder for the culture of *Artemia*.

In the present study, nine species of seaweeds have been tested as part of the ingredients along with rice bran for the preparation of diet. Most of the seaweeds belonging to the brown and red algae did not support the survival as compared with rice bran.

Among the tested three species of red algae, *A. spicifera* and *G. acerosa* did not support the survival, growth and reproduction of *Artemia*. When the animals were fed with *A. spicifera,* high mortality was recorded as the concentration of seaweed increased. Complete mortality was observed in all combinations within 30 days. The seaweeds also did not support the growth of animals. The *Artemia* fed with *A. spicifera* in different combinations (75: 25; 25: 75 and 0: 100) supported a maximum growth of 5.5 mg in 25 days. This seaweed did not support the reproduction of animals. Maturation was found in animals fed with 75: 25 combination of feed and the ovary that was formed became brownish in colour in two days and then the animals died. The other combinations did not support any maturation. As the concentration of the seaweed increased the animals were found inactive.

The reason for the maximum survival in green algae may be due to the presence of maximum protein content. Boussiba and Richmond [19] studied that the protein in the red algae and the phycobiliproteins, has antioxidant property helps to increase the survival of the *Artemia*. The phenolic content in the algae also play a major role in influencing the survival of *Artemia*. But naturally the phenolic level of green algae is low.

In the present study, the red seaweed *G. acerosa* did not support the survival, growth and reproduction of animals as the concentration of the seaweed increased. The red seaweed *G. edulis* supported a maximum survival of 49 and 48% in 30 days in the combinations of 50: 50 and 25: 75 respectively when compared to other seaweeds, *G. edulis* which supported a maximum growth of 7.5 mg in 25: 75 combination. This seaweed supported a release of one set of nauplii in the combinations of 75: 25, 50: 50 and 25: 75. When *Artemia* were fed with rice bran alone, they exhibited 56% survival, attained a maximum growth of 7.6 mg and released 3 broods.

Compared to the red algae, the brown algae supported more survival and the life span had been increased to the maximum of 52 days when they were fed with in a combination of 25% of rice bran and 75% of the red algae (*T. ornata*), 50% rice bran and 50% of *S. wightii*. Maturation was delayed by one or two days in those fed with the brown algae and there was no change in the gestation period as well as the interbrood duration. The number of brood and the number of nauplii per brood were reduced considerably in those fed with brown algae.

The green algae, especially the *U. spiralis* had significantly ($P < 0.05$) increased the life span (63 days), reduced the maturation period to 14 days and the gestation period to 4 days in a combination of 50% seaweed and 50% rice bran. *U. spiralis* increased the number of broods as well as the fecundity and finally the nauplii production was increased from 96 to 150. This shows that *U. spiralis* could support survival, growth and reproduction in *A. franciscana* in the combination of 50: 50 (50% rice bran and 50% seaweed).

Seaweeds present a number of challenges to chemist and biochemists. Few types of seaweed are available in laboratory culture; hence chemist must usually begin with collected samples whose growth conditions and metabolic history are poorly known. These samples are likely to host an abundance of epiphytic and endophytic organisms including other algae, bacteria and fungi. The copious cell wall materials complicate extraction of hydrophilic constituents and together with other factors may hamper biosynthetic studies involving isotrophically labeled intermediates.

## Conclusion

From this study, it is understood that brine shrimp *A. franciscana* can be cultured by feeding rice bran and green algae *U. spiralis* in equal combination to ensure high survival, growth rate and high reproductive potential.

## References

1. LAVENS, P., DE MEULEMEESTER, A., SORGELOOS, P.: Evaluation of mono and mixed diets as food for intensive *Artemia* culture. In: *Artemia* Research and its Applications. Vol. 3. Ecology, Culturing, Use in aquaculture. Sorgeloos, P., D.A. Bengtson, W. Decleir and E. Jaspers (Eds.). Universa Press, Wetteren, Belgium, 309 – 318 (1987).
2. BASIL, J.A., SELVARANI, D., JEBAKUMAR, S.R.D., ISREAL, P., MATHURAM, G.: Open-air culture systems of *Artemia* (Tuticorin strain) using chosen agricultural byproducts. Abstract. *Artemia* News Letter, 12: 77 (1989).

3. YASHIRO, R.: 1985.: Effects of salinity on reproduction cycle of brine shrimp (*Artemia salina L.*). Proceedings of the 3rd Seminar on Coastal Aquaculture Bangkok, 1985, 268 – 278 (1985).
4. BOSSUYT, E., SORGELOOS, P.: Technological aspects of the batch culturing of *Artemia* in high densities. In: The brine shrimp *Artemia*. Vol.3. Ecology, Culturing, Use in aquacultue. Persoone G., Sorgeloos, P., Roels, O. and Jaspers, E. (Eds.). Universa Press, Wetteren, Belgium, 133 – 152 (1980).
5. PLATON, R.R, ZAHRADNIK, J.W.: 1987. Scale up studies on the culture of brine shrimp *Artemia* fed with rice bran. In: *Artemia* Research and its Applications. Vol. 3. Ecology, Culturing, Use in aquaculture. Sorgeloos, P., D.A. Bengtson, W. Decleir and E. Jaspers (Eds.). Universa Press, Wetteren, Belgium, 347 – 348 (1987).
6. VIJAYARAGAVAN, S., KRISHNAKUMARI, L., ROYAN, J.P.: Evaluation of different feeds for optimal growth and survival of parthenogenetica brine shrimp, *Artemia.* Indian Journal of Marine Science, **16(4)** : 253 – 255 (1987)
7. MONY, C.S.: Studies on the use of some ayurvedic products for improving the reproductive performance in parthenogenetic *Artemia* from Thamaraikulam. South India, Ph.D. Thesis, M. S. University, Tirunelveli, India, 232 P (1998).
8. CASTELO-BRANCO, D., VIELA, M.A.: Larval growth of a Portuguese *Artemia* strain fed on dried microalgae produced in JNJP. Workshop on Nutrition in Aquaculture. Libson, 29th January 1985, 12: 29-38 (1987).
9. BASIL, J. A., PANDIAN, T.J.: Culturing of *Artemia* (Tuticorin strain) in organic and Argicultural wastes at different salinities. Hydrobiologia, **212**: 11 – 17 (1991).
10. JENSEN, A.: Carotenoids of Norwegian brown seaweeds and of seaweed meals. Nov. Inst. Taug. Tare Forsle. Rep., **31**: 1 – 138 (1966).
11. Zar, J. E.: Biostatistical analysus, Prentice-Hall, New Jersy, USA, 620 pp (1974).
12. SORGELOOS, P.: The influence of algal preparation on its nutritional efficiency for *Artemia salina* larvae. Thalassia Jugosl, **10**: 313 – 320 (1974).
13. SORGELOOS, P.: The use of the brine shrimp *Artemia* in aquaculture. In: The brine shrimp *Artemia* Vol. 3, Ecology, Culturing, Use in aquaculture. Sorgeloos, P., D.A. Bengtson, W. Decleir and E. Jaspers (Eds.). Universa Press, Wetteren, Belgium, 25 – 46 (1980).
14. ROELS, O. A., SHARFSTEIN, B. A.: TOBIAS, W.J.: Cultivation of the brine shrimp *Artemia*. Final Report for the work supported by NOAA, Sea grant Project No. NA – 79 – AA – D – 00039, 256 pp (1979).
15. DE LOS SANTONS, C., SORGELOOS, P., LAVINA, E., BERNARDINO, A.: Successful inoculation of cysts in manmade salterns in the Phillippines. In: The brine shrimp *Artemia,* Vol. 3. Ecology, Culturing, Use in aquacultue. Persoone G., Sorgeloos, P., Roels, O. and Jaspers, E. (Eds.). Universa Press, Wetteren, Belgium, 556 P (1980).
16. DOUILLET, P.: Effect of bacteria on the nutrition of the brine shrimp *Artemia* fed on dried diets. Paper presented in Aquaculture Eurpoe 87 and Proceedings of the II International Symposium on the brine shrimp *Artemia*, Sep.1 – 5, Antwerpen (1985).
17. JUMALON, N. A., ESTENOR, D.G., OGBURN, D.M.: Commercial production of *Artemia* in the Philippines. 231 – 238. In: *Artemia* research and its applications. Vol. 3. Sorgeloss, P., D. A. Bengston, W. Decleris, E. Jaspers (Eds.), Universa Press, Wetteren, Belgium, 556 P (1987).
18. RIBEIRO-VIEIVA, M. N.: Culture performance of *Artemia* from Vievo (Portugal) fed with wheat bran and seaweed. Proceedings of the II International Symposium on the brine shrimp *Artemia*, Antwerp, Belgium, Sep. 1 – 5 (1985).
19. BOUSILBA, S., RICHMOND, A.E.: C-Phycocyanin as a storage protein in the blue-green alga *Spirullina platensis*. Archfur Microbiologia., **125**: 143 – 147 (1980).