CHAPTER – VIII

REPLACEMENT OF LIVING MICROALGAE DIET WITH SPRAY-DRIED ALGAE

Microalgae are used as live feed for all stages of molluscs, for the early larval stages of crustaceans and for prey species such as rotifers, copepods etc. which are used in turn as food for late larval and juvenile crustaceans and fishes. But, the live algae culture system pose limitations like unreliability, need for space, high cost of production, maintenance, energy, nutrients, crop failures or change in algal culture's nutritional quality (due to change in culture conditions) and presence of contaminating organisms.

In hatcheries, the algal requirement is variable depending on the availability of spawner, quantity and stage of the larvae in the tanks. Algal biomass production which is expensive can represent 30-50% of hatchery operation cost (Jeffrey and Garland, 1987) is often produced in excess. Any type of processing and preservation of the microalgae (provided the nutritional value is unaltered, cost effective and pathogen–free) would help to stockpile the same until needed. Such an off-the-shelf diet may supplement or replace live algae reducing the dependability on the fresh live algae and help in streamlining the production process as well as in reducing costs.

Various approaches have been tried to preserve microalgae concentrates, like drying, chilling, cryopreservation etc. Such processed microalgae have been tried as feed for molluscan larvae and juveniles (Laing and Verdugo, 1991; Namaguchi and Nell, 1991;

Langdon and Onal, 1999; Enright *et al.*, 2002), for rotifers (Gatesoupe and Robin, 1981; Ben-Amotz and Rosenthal, 1981; Lubzens *et al.*, 1995), for *Artemia* (Takano, 1967; Barclay and Zeller, 1996) and as feed component for fish (Stanley and Jones, 1976; Matty and Smith, 1978 ; Sandbank and Hepher, 1978).

With penaeid larviculture, various artificial diets have been tried to overcome or reduce the reliance on live algae (Jones *et al.*, 1987; Tackaert *et al.*, 1989). But, usage of microalgae was found inevitable as 100% replacement resulted in reduced growth (Galgani and Aquacop, 1988; Kurmaly *et al.*, 1989b). The reasons attributed included the presence of gut enzyme stimulants associated with microalgae (Amjad *et al.*, 1993; Jones *et al.*, 1993, 1997; Le Vay *et al.*, 1993; Rodriguez *et al.*, 1994; Kumlu and Jones, 1995) and also possible probiotic action (Alabi *et al.*, 1999a). Moreover, comparing with artificial diets, as algae are the natural food for filter feeding early larval stage of shrimp, a preparation of algae preserved by freezing or drying is expected to perform better.

Attempts to utilise various forms of preserved algae for shrimp larvae started decades back. Frozen algae (Mock and Murphy 1970; Brown, 1972), frozen centrifuged concentrates (Mock *et al.*, 1980) and chilled algae concentrates (D'Souza *et al.*, 2000, 2002) have been attempted as algal replacement diets. Another form of preparation that has been tried is the dried algae. Millamena and Aujero (1978) tried sun-dried algae and got better survival with *Chaetoceros calcitrans* followed by *Tetraselmis chuii* and *Isochrysis* sp. as sole food sources. Biedenbach *et al.* (1990) fed spray-dried *T. suecica* to *Litopenaeus vannamei* larvae as a partial replacement (66%) for fresh algae and recorded growth, survival and development similar to larvae fed 100% fresh algae. Millamena *et al.* (1990) had more success feeding sun-dried *T. chuii* and *C. calcitrans* to *Penaeus monodon* larvae as complete replacement for fresh algae.

In this investigation, two experiments were carried out separately to evaluate the usage of spray-dried *Isochrysis sp.* (T-iso), a commonly used microalgae, as a substitution diet for live algal mixture at various levels for the larviculture of *F. indicus*. The larval criteria used for assessment included the larval survival till postlarvae 1 (PL_1), percentage of larvae metamorphosed to postlarvae and the development index. The live algae used included a diatom (*Chaetoceros muelleri*) and two flagellates viz. *Isochrysis* sp. (clone T-iso) and *Tetraselmis suecica*. In experiment 1, the live algal mixture used included *C. muelleri* and *T. suecica*, in the second one *C. muelleri* was replaced by *Isochrysis* sp.

MATERIALS AND METHODS

Spray–dried algae

Spray-dried *Isochrysis* used in these experiments was sourced from the Conwy Fisheries laboratory of CEFAS, Ministry of Agriculture, Fisheries and Food, United Kingdom. Hydration was done by adding the spray-dried powder to filtered seawater and blending for about 20 seconds in a domestic blender. The dissolving of 1 g powder in one litre seawater yielded an algal cell concentration of 26.7 x 10^6 cells/ml.

Live algae

Stock cultures of live algae (100 ml) were brought from the CSIRO collection of living microalgae (CSIRO Marine Research, Tasmania, Australia) and maintained in the algae culture facility of the hatchery. The strains of *Chaetoceros muelleri* Lemmermann (CS-176), *Tetraselmis suecica* (CS-187) and *Isochrysis* sp. (Tahitian strain, clone T.Iso, CS-177) were cultured in 2 L Erlenmeyer flasks with 1.5 L f_2 medium (Guillard and Ryther, 1962) with 12:12 h light:dark cycles, illuminated with white fluorescent lights giving 70-80 µ mol photon/m^2/s and maintained at 23±1 °C.

The algae used for the experiments were supplied from the 40 l polyethylene bag cultures maintained in semi-continuous mode. The bags containing 25 l of f/2 media, prepared with 30 ppt seawater (filtered down to 0.5 micron), bags were inoculated with algae from 2 l stock cultures. The cultures were allowed to grow initially for 2-3 days with diatoms and 4-5 days with flagellates and were illuminated from one side using fluorescent lamps. The nutrient for the diatoms included sodium metasilicate ($Na_2SiO_3.5H_2O$). The harvest started after the initial growth period and was carried out on alternate days, by removing 50% of culture volume for flagellates and 75% for diatoms and replenishing with fresh media to maintain the culture at the log phase. Each bag culture was maintained for 2 to 3 weeks based on the algal condition. Cell counts (5 replicate counts per species) were done using improved Neubauer haemocytometer. The culture density obtained in the bags were 10-13 x 10^6 cells per ml for *Chaetoceros*, 8-10 x 10^6 cells / ml for *Isochrysis* and 0.8-1 x 10^6 cells / ml for *Tetraselmis*.

Larviculture Experiments

In both the experiments (1 and 2), nauplii (from single spawning) were acclimated as well as allowed to stay in aerated treatment solutions containing algal density and combinations similar to the one that would be used in the experiment. Upon metamorphosis to zoea 1 (Z_1), they were counted and stocked in 1 ton tanks holding 70% water volume, with salinity 30 ppt. The stocking density was 100 larvae/l. Water exchange regime involved filling up to reach 100% volume next day, 30% for Z_3 and 50% for mysis (M) stages.

The various feeding regimes followed in the experiments are shown in table 29. The algal density in larviculture flasks was checked for every 4 hours. All treatments were in triplicates.

Experiment was stopped when 100% the larvae in any of the replicates transformed to postlarvae. Supplementation of *Artemia* in control as well as in substitution treatments was to allow evaluation of spray-dried product in feeding regimes including live diets. No artificial diets were used in these experiments.

Development index was calculated using the formula given by Villegas and Kanazawa (1979). Development index = A/total number of larvae staged, where A=Σ (stage value x number of larvae at that stage). The stage values assigned were 1, 2, 3, 4, 5, 6 and 7 for Z1, Z_2, Z_3, M_1, M_2, M_3 and PL respectively. Therefore, higher the proportion of advanced stage larvae in the treatment, the higher the development index. From each replicate 75 larvae were sampled randomly for the assessment of larval stage. Staging of larvae was according to Silas *et al.* (1978). Survival was assessed by five random samples from different parts of the tanks with 500 ml beaker.

Dry weight estimation

The algae from 100ml volume, filtered and retained on the pre-weighed glass-fibre were used. The salt on the filtrate was removed by rinsing the filter with 2M of ammonium formate. The filter was dried in an oven at 105° C and reweighed.

Biochemical analysis

The total protein, carbohydrate and lipid content of the dried as well as live algae used in the experiments were estimated in triplicate using standard methods. For analysis, live algal cells were harvested by filtering through pre-combusted (450° C; 16 h), glass-fiber filters (Whatman GF/F; 25 mm). When required the filters were placed in polypropylene cryogenic vials and immediately frozen by immersion in liquid nitrogen.

For protein estimation, the Coomassie-blue dye method of Bradford (1976) was carried out using the Bio-Rad Quick start protein assay kit (Bio-rad Labs, Hercules, CA, USA), with bovine serum albumin (BSA) as standard.

Total carbohydrate was determined by colorimetric method using phenol-sulphuric acid method (Dubois *et al.*, 1956). The algal sample preparation (acid hydrolysis) for analysis was done according to Brown *et al.* (1993).

Lipids were extracted from algae immediately after filtration. Extraction was done by homogenization in chloroform-methanol-water mixture (1:2:0.8 v/v/v) using a modified method of Bligh and Dyer (1959) as mentioned by (Mansour *et al.*, 1999).

Data analysis

The significance between survival values, metamorphosis of postlarvae and biochemical composition between the feeds were estimated using single factor Anova. The mean comparison was carried out using Tukey's multiple comparison test. Data were expressed as mean with standard error.

RESULTS

The biochemical composition of all three diet combinations used in the experiment are given in table 29. Compared to live algae mix, the spray-dried powder was distinct in having the highest carbohydrate content ($P<0.001$). But, the spray-dried algae had the lowest content of protein and lipid. Though the protein content of spray-dried algae was significantly lower ($P<0.05$) than live *Isochrysis-Tetraselmis* mix (IT), it was lower than

Chaetoceros-Tetraselmis mix (CT). While, the CT mix had the highest lipid content ($P<0.01$), the highest protein content was with IT mix.

Table.29. Biochemical composition (% dry weight) of spray-dried *Isochrysis*, live *Chaetoceros muelleri* plus live *Tetraselmis suecica* mix and live *Isochrysis* sp. and live *Tetraselmis* mix.

Sample	Protein	Lipid	Carbohydrate
Spray-dried. *Isochrysis*	29.92 ± 1.04^{a}	9.63 ± 0.26^{a}	42.21 ± 0.40^{a}
Live *Chaetoceros* – *Tetraselmis* mix	32.83 ± 0.56^{a}	15.71 ± 0.36^{b}	18.18 ± 0.51^{b}
Live *Isochrysis* – *Tetraselmis* mix	38.21 ± 0.69^{b}	11.34 ± 0.43^{c}	16.60 ± 0.29^{b}

Means (± S.E.) in the same column with different superscripts are significantly different ($P<0.05$).

EXPERIMENT 1

The results for the experiment 1 are presented in table 30 and fig. 23.

Survival of the prawn larvae

Analysing the results separately for non-*Artemia* treatments, the larval survival of 33S and 66S treatments did not differ significantly, from control (C) treatment. Interestingly, the survival was slightly higher for the 33S treatment than C, though not significant ($P>0.05$). The 100% dry algae substitution (100S) resulted in the lowest survival of all treatments and was significantly ($P<0.05$) lower than C and 33S treatments.

As seen in table 30, the inclusion of *Artemia* in the feeding regime improved survival when compared to their counterparts, but did not result in any significant improvement.

Table 30. Experiment 1.The survival (from zoea 1 to postlarvae 1) and percentage of zoea that metamorphosed to postlarvae in tanks fed different combinations of spray-dried algae and live algae.

Feeding regime from Z_I to PL_I	Survival (%)	Metamorphosis to PL_I (%)
Control (C)	75.7 ± 1.96^{a}	90.7 ± 1.66^{a}
33% substi. (33S)	77.7 ± 1.91^{a}	91.7 ± 1.66^{a}
66% substi. (66S)	72.3 ± 1.44^{ab}	73.3 ± 2.88^{b}
100% substi. (100S)	49.7 ± 2.77^{b}	4.7 ± 0.98^{c}
Control + *Artemia* (C+A)	87.3 ± 3.14^{a}	98.3 ± 0.98^{a}
33% subst.+Art. (33S+A)	82.0 ± 2.36^{ab}	96.3 ± 1.91^{a}
66% subst.+Art. (66S+A)	77.0 ± 2.38^{ab}	89.7 ± 1.66^{ab}
100% subst.+Art. (100S+A)	63.0 ± 4.65^{b}	31.3 ± 3.35^{d}

Means (± S.E.) in the same column with different superscripts are significantly different ($P<0.05$)

Among *Artemia*-fed (+A) treatments, the best survival was obtained with C+A treatment and survival dropped proportionately with increasing dried algae substitution. Even with +A treatments, 100S+A gave significantly poor survival compared to its respective control, C+A. Considering all the eight treatments, best two survivals were from +A treatments and third one from the non-*Artemia* treatment (33S). Noticeably, the 100S+A treatment, even with *Artemia*, gave poor survival that it was not significantly different ($P>0.05$) from 66S and 100S. Moreover, the survival percentage of 100S+A was lower than 66S. The treatments in the order of decreasing survival are as below:

(C+A) > (33S+A) > 33S > (66S+A) > C > 66S > (100S+A) > 100S

Metamorphosis to postlarvae

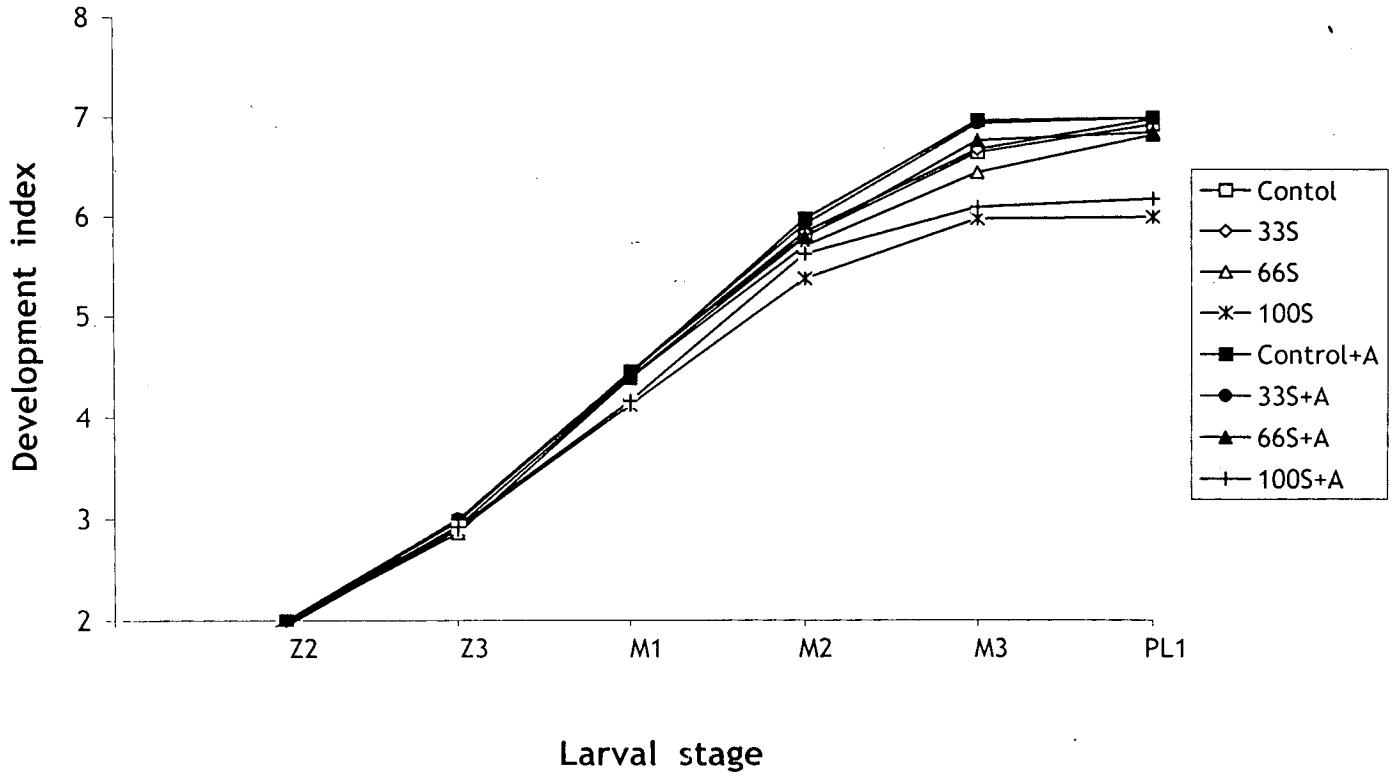
Results from both –A and +A treatments showed that the impact of substitution was more on metamorphosis than survival, especially with substitution at 66% or above. This is evident by the fact that the percentage metamorphosis obtained with 66S was significantly lower than C, while this is not the case with survival. The impact of 66% substitution seems to differ with the presence of *Artemia*. Though 66S+A gave values slightly less than C+A, it was drastically low in the case of 66S versus C. At the end of the experiment, the lowest number of postlarvae were found with 100S substitution treatment, where two of the replicates did not have even a single larvae metamorphosed to postlarval stage. Addition of *Artemia* to the same, resulted in all replicates having postlarval stages. The control treatment containing *Artemia* (C+A) significantly improved the metamorphic rate, compared to control with 100% live algae (C), confirming the importance of *Artemia* feed in larval rearing. Noticeably, with or without *Artemia*, there was a significant drop in value with 100% substitution. The treatments in decreasing performance order are as presented below.

(C+A) > (33S+A) > 33S > C > (66S+A) > 66S > (100S+A) > 100S

Development of the prawn larvae

The development index (DI) was calculated for every stage (Fig. 23) On the fourth day, with all treatments the zoea started moulting to mysis. The lowest DI value at all stages was obtained with 100% substitution treatment, followed by 100S+A. All the other treatments showed nearly similar value. At the end of experiment, the best DI values were obtained with C+A treatment and 33S+A treatments, while C and 33S treatments gave lesser values indicating the role of *Artemia* in boosting metamorphosis.

Fig. 23. Experiment 1. Development index of larval stages fed different combinations of spray-dried microalgae and live algae



The lowest DI values were obtained with 100% substitution treatment, and the addition of *Artemia* to the same treatment slightly promoted the development of larvae.

Some of the treatments (66% substitution, 100% substitution and 100% substitution with *Artemia*) had some larvae remaining at mysis 2 stage even at the end of the experiment. The different treatments, with DI value in decreasing order are as below.

(C+A) > (33S+A) > 33S > C > (66S+A) > 66S > (100S+A) > 100S

Survival rate, % metamorphosis to PL and development rates combined

Comparison of diets, confirmed that all three parameters exhibited similar trend. This is to say that the treatment which gave the best survival gave the best percentage metamorphosis to PL as well as highest DI value, and others followed almost the same trend. Best results were obtained with control plus *Artemia* treatment (C+A), which was closely followed by 33% substitution with *Artemia* (33S+A).

Data showed that with or without *Artemia*, 66% substitution treatments gave survival slightly less (NS, $P>0.05$) than their controls, but, percentage metamorphosis to postlarvae and DI were significantly lower for the 66S treatment. With all three parameters, 66S+A was not significantly different from C+A ($P>0.05$). Without *Artemia,* the 33% substitution gave better results when compared to control. The lowest value for all the three parameters were reported with 100% substitution treatment.

EXPERIMENT 2

The results from experiment 2 are presented in table 31 and fig. 24.

Survival of the prawn larvae

In general, the survival obtained with the treatments in this experiment was lesser than experiment 1. The effect of substitution on survival was also less here, even with +A treatments. For example, in contrast to experiment 1 results, here the 100S treatment survival was not significantly lower than 33S treatment. Again, control gave closely better survival than 33% substitution. Lowest survival reported here with 100S treatment (47.3%) was closer to survival value (49.7%) obtained for the same treatment with experiment 1. Both 66S and 66S+A did not differ statistically ($P>0.05$) from their respective controls. The survival in treatments can be summarized as below in decreasing order.

(C+A) > (33S+A) > (66S+A) > C > 33S > (100S+A) > 66S > 100S

Metamorphosis to postlarvae

As with experiment 1, C+A treatment had more number of postlarvae at the end of the experiment and was closely followed by 33S+A. Contrary to experiment 1 results, here the third best percentage metamorphosis was by 66S+A. Moreover, unlike experiment 1, 66S value for percentage metamorphosis did not differ significantly from C and 33S. The lowest value 47.3% (with 100S) in this experiment were almost closer to that of experiment 1 (49.7%). Even at the end of the experiment, 100% substitution treatment had two replicates without even single postlarva. The treatments with metamorphosis value in descending order are as below.

(C+A) > (33S+A) > (66S+A) > C > 33S > 66S > (100S+A) > 100S

Table 31. Experiment 2.The survival (from zoea 1 to postlarvae 1) and percentage of zoea that metamorphosed to postlarvae in tanks fed different combinations of spray-dried algae and live algae.

Feeding regime from Z_1 to PL_1	Survival (%)	Metamorphosis to PL_1 (%)
Control (C)	73.3 ± 3.85^{a}	86.3 ± 1.44^{ab}
33% substi. (33S)	70.7 ± 3.79^{ab}	84.7 ± 1.96^{ab}
66% substi. (66S)	65.3 ± 1.66^{ab}	74.0 ± 3.09^{a}
100% substi. (100S)	47.3 ± 4.07^{b}	8.3 ± 1.66^{c}
Control + *Artemia* (C+A)	81.0 ± 2.16^{a}	97.0 ± 1.42^{b}
33% subst.+*Art.*(33S+A)	78.3 ± 3.35^{a}	91.7 ± 2.42^{ab}
66% subst.+*Art.*(66S+A)	76.3 ± 1.52^{a}	87.0 ± 2.06^{ab}
100% subst.+*Art.*(100S+A)	68.3 ± 1.44^{ab}	36.7 ± 3.21^{d}

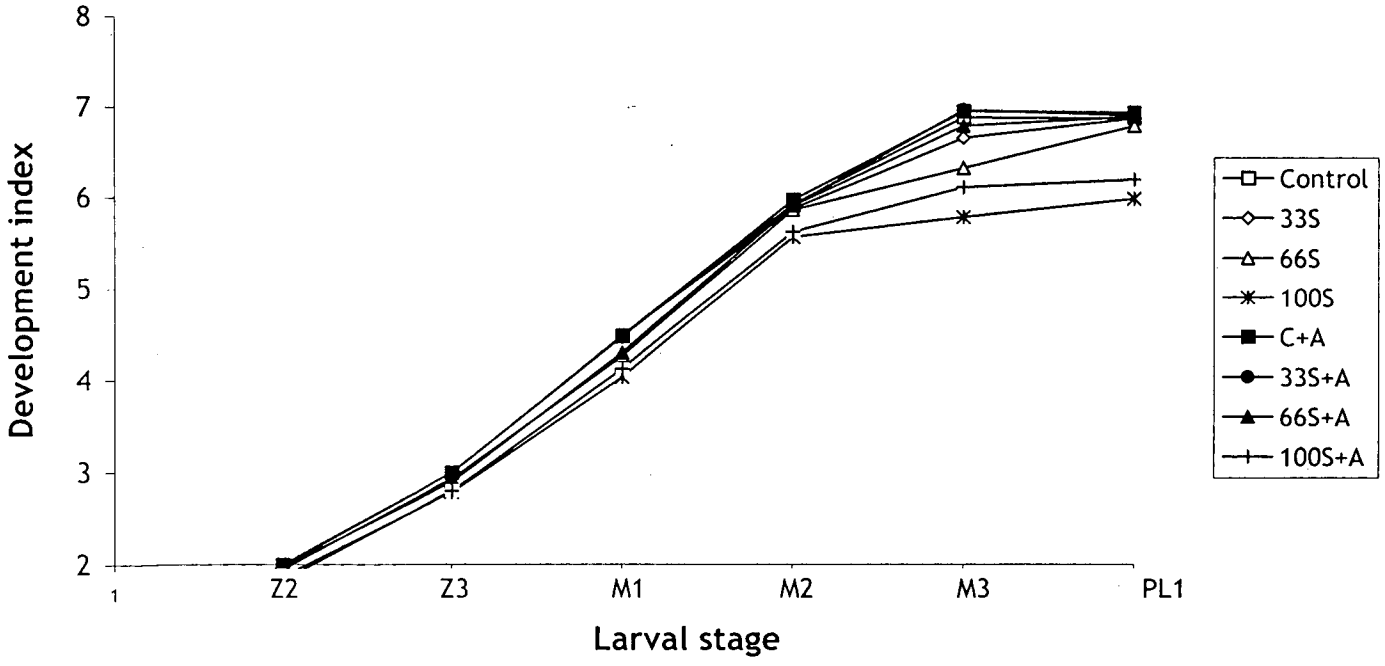
Means (± S.E.) in the same column with different superscripts are significantly different ($P<0.05$)

Development of the prawn larvae

As in the case of experiment 1, C+A gave the highest DI value at all larval stages, closely followed by 33S+A and control (Fig. 24). Among the treatments without *Artemia,* control recorded the maximum DI value at all larval stages, and 100% substitution the lowest. In the case of +A treatments also, 100% substitution gave the lowest value. It is important to note that compared to experiment 1, though the temperature conditions were similar, experiment 2 took an extra 5.5 hours for the completion of experiment. The results can be summarized as below in descending order of DI value.

(C+A) > (33S+A) > C > (66S+A) > 33S > 66S > (100S+A) > 100S

Fig. 24. Experiment 2. Development index of larval stages fed different combinations of spray-dried microalgae and live algae



Survival rate, % metamorphosis to PL and development rates combined

Results for all the parameters combined showed that the C+A gave the best results. Contrary to the results obtained in experiment 1, here the 66S+A gave better results, especially with survival and DI, when compared to respective C+A value. Among treatments without *Artemia,* control gave best results, followed by 33S with acceptable reduction in all three values. When fed with *Artemia,* even with 66% substitution (66S+A) only a minor reduction was noticed in all parameter values when compared to 33S+A and C+A.

DISCUSSION

A plethora of literature is available on the efforts to replace live algae with processed algae, especially with molluscs and the major ones are the report by Coutteau and Sorgeloos (1992) and review by Jones *et al.* (1993). Coutteau and Sorgeloos (1992) discussed in detail the various feeds attempted (microencapsulated diets, yeast based products, spray-dried algae etc.) to replace live algae with reference to molluscs. Jones *et al.* (1993) broadened the review and incorporated a detailed picture on the algal replacement efforts made with crustacean larviculture, especially shrimp. However, most of the research with spray-dried algae have been with molluscs (Laing *et al.*, 1990; Laing and Verdugo, 1991) and literature available is scant with reference to shrimp.

Results from the two experiments, plainly indicate that even with inclusion of *Artemia* in the larval feeding regimes, 100% replacement of live microalgae with spray-dried algae was not technically feasible. 66% substitution using spray-dried algae with *Artemia* (66S+A) inclusion gave results comparable to control (C+A) without much compromise on survival, percentage metamorphosis and development rate. Substitution

above this level affected the results. In 66% substitution treatments without *Artemia* (i.e.66S), the results were variable. In experiment 1, 66S resulted in significant reduction of percentage metamorphosis and in experiment 2 also, 66S resulted in more than 10% reduction in metamorphosis. Though the percentage value was not significantly different from control (C), the 10% reduction compared to the previous level of substitution (33S) suggests slightly lesser level of substitution.

The results reiterated the requirement for inclusion of live algae with spray-dried algae in larval feeding regime, at least to a minimum percentage. Similar reports on the inevitable inclusion of live algae with processed algae to attain better results have been previously reported. Using spray-dried *Tetraselmis suecica,* Biedenbach *et al.* (1990) reported that survival of *L. vannamei* larvae fed on 100% spray-dried algae was significantly lower than that of larvae fed on 33% substitution. He also noted that 100% live algal replacement resulted in significantly lower larval metamorphic rate than other treatments.

With *Tapes philippinarum* and *Crassostrea gigas* juveniles, Laing and Verdugo (1991) reported that 20% inclusion of live *Chaetoceros calcitrans,* significantly improved the dry weight based daily growth rate, compared to juveniles fed 100% spray-dried *Tetraselmis suecica.* Similarly, Laing and Millican (1992) obtained significantly lower growth rates in Manila clam *Tapes phillipinarum,* fed 100% spray-dried algae (*T. suecica*) compared to animals fed 90% dried algae and 10% live algae (*Skeletonema costatum*) mix. Enright *et al.* (2002) tried freeze-dried algae to replace live algae for the juvenile American oyster *Crassostrea virginica.* The authors noted that on day 23, oysters fed on 50% live : 50% freeze-dried algae showed a significantly higher growth rate than those fed with 100% freeze-dried algae.

In this study, the results from both experiments were nearly the same and exhibited similar trend. When the spray-dried algae was new addition to the feeding mix (as with CT mix combination in experiment 1), the substitution to some extent (up to 33%) seemed to improve the results especially in the absence of *Artemia.* This could be due to the fact that spray-dried algae contributed some nutrients to the feeding mix that were needed in limited quantities. Similarly, the lesser impact noticed with substitution in experiment 2 could be due to the fact that the same nutrients supplied by live algae (*Isochrysis*) were also provided by the spray-dried algae (*Isochrysis* sp.).

The inability of processed algae to replace live algae totally has been widely reported and results vary in the percentage replacement possible. As mentioned before, only limited literature is available with the usage of spray-dried algae for shrimp larvae and so the possible extent of live algae replacement. The only report on the total replacement of live algae by dried algae for shrimp larvae was by Millamena *et al.* (1990). Evaluating sun-dried *Chaetoceros* sp. for *P. monodon* larvae, the authors obtained better survival results with sun dried algae than the live algae. However, no report was made on the growth rate. Biedenbach *et al.* (1990) indicated that 66% replacement of live algae (*Chaetoceros gracilis* and *Tetraselmis chuii*) for *L. vannamei* larvae with spray-dried *T. suecica* did not significantly affect growth and survival.

Using spray-dried *Schizochytrium* (commercial product named Algamac), for partial replacement of live algae, Boeing (2004) reported that 50% replacement of live algae (*Chaetoceros* sp) for *L. vannamei* larvae with Algamac (@4mg/l/d) did not affect the percentage survival, metamorphic rates and total length measurements. D'Souza *et al.* (2002) tried a number of flocculated microalgae for *P. monodon* larvae. The researchers noted that fresh *C. muelleri* promoted the highest survival, greatest weight gain and

fastest development to mysis 1. Larvae fed this diet were twice as weighty as those fed with other diets. Concentrated *C. muelleri* or *Thalassiosira pseudonana* promoted similar survival rates to that of larvae fed fresh *C. muelleri* although development rates were slower. The results from the present experiment also corroborate with the above results in the point that live algal replacement resulted in the slower development of larvae.

Doroudi *et al.* (2002) conducted live algae replacement trials with larvae of the black-lip pearl oyster, *Pinctada margaritifera.* The authors tried to replace living algae (an equal mixture of Tahitian *Isochrysis* aff. *galbana* and *Pavlova salina*) either partially or completely with heterotrophically grown spray-dried algae (*Tetraselmis suecica*). The workers found that only 25% substitution of live micro-algae with spray-dried algae was possible without affecting growth of *P. margaritifera* larvae. According to Ponis *et al.* (2003) substitution of 50% (trial 1) or 80% (trial 2) of fresh *C. calcitrans* with the preserved *Pavlova lutheri* concentrates did not adversely affect growth rate or survival of *Crassostrea gigas* larvae. Algal concentrates fed to the larvae and spat of Sydney rock oyster, *Saccostrea commercialis* (Heasman *et al.*, 2000) and Pacific oyster, *Crassostrea gigas* (Brown and Robert, 2002) were effective as partial diets (e.g. up to 80%) with growth rates similar to, or marginally inferior to, complete live diets.

Comparatively lower values obtained with experiment 2, for all the parameters, may be explained by the fact that phytoflagellates are less efficient as food source for penaeid shrimp larvae, compared to diatoms (Wilkenfield *et al.*, 1984). The inclusion of *Artemia* is very much recommended as the same seems to improve the results, especially the growth (high percentage metamorphosis and DI). It is vital to note that the microalgae was the sole zoea food used in these experiments and *Artemia* feeding (only for +A

treatments) was only from mysis 1 stage. As it is well known that poorly-fed zoea results in unhealthy mysis (Jones *et al.,* 1997), the results from *Artemia*-fed treatments would also be influenced by the type and quality of microalgae fed to zoea.

The effect of live algae inclusion on improving results could be due to a number of reasons. Jones *et al.* (1989) with *P. monodon,* found that addition of only 10 algal cells/µl to the culture water containing microcapsules during the zoeal stages was sufficient to promote growth similar to live feed. It was proposed that live algae supplied, stimulated the production of enzyme trypsin. Trypsin activity is currently being used as an index for the evaluation of artificial diets (Pedroza-Islas *et al.*, 2004). Moreover, the addition of the live algae to the culture medium may provide a beneficial bacterial population which assists in the control of rearing water microbial population (Alabi *et al.*, 1999a). Control of bacterial populations by microalgae has been previously reported (Kogure *et al.*, 1979; Kellam and Walker, 1989; Austin and Day, 1990).

The failure of spray-dried algae to match live algae could be due to various reasons. The biochemical composition analysis clearly indicated the poor nutritional quality of spray-dried algae when compared to algae mix. The higher carbohydrate content of spray-dried algae reported here has been reported previously (Laing *et al.*, 1990). Meanwhile, the compromise on lipid needs to be noticed. Importance of lipids in larval development of crustaceans has been well documented and they are the major cellular reserve utilized to meet the energy demand of the growing larvae (Ward *et al.*, 1979; Teshima and Kanazawa, 1982; Kanazawa, 1990).

Though not analysed, the fatty acid content of the fed microalgae preparation is also vital and it is reasonable to believe that lower lipid will reduce the supply of fatty acids too. Relevant to this, Volkman *et al.* (1989) reported the low abundance of 20:5 n3 (eicosapentaenoic acid) and high abundance of 22:6 n3 (docosahexaenoic acid) fatty acids in *Isochrysis* sp. (Clone T. Iso). Content of both these fatty acids in zoea diet had major impact on the survival and growth in later stages of *L. stylirostris* (Leger *et al.*, 1985). Similarly, high moulting rate and best weight gain were obtained with *M. japonicus* larvae fed diets supplemented with oils rich in n-3 fatty acids (Guary *et al.*, 1976). It has also been pointed out that resistance to stress is related to the presence of adequate levels of DHA and EPA in crustacean larvae (Dhert *et al.*, 1992). This is in agreement with findings of Montano and Navarro (1996) that, wild post larvae contain higher levels of both DHA and EPA, and are in turn more resistant to handling stress, resulting in higher survival when introduced to the grow-out ponds. Numerous other studies also insist on the value of HUFA in larval nutrition (Jones *et al.*, 1979; Kurmaly *et al.*, 1989b; Sorgeloos and Leger, 1992).

The assessment of problems with spray-dried algae, well expose the possible quality improvement. Algal cells can be damaged or leak their contents during centrifugation step. The spray-drying process has resulted in a powder with much lesser lipid content than live algae. Similar loss of total fat by drying process was also reported by Esquivel *et al.* (1993). High temperature could have caused loss of other nutrients too.

Other problems with spray-dried algae are formation of cell aggregates (Lin, 1985) and loss of viability (Grima *et al.*, 1994). The combination of oxidative losses and leaching of ascorbic acid (and possibly other nutrients) may explain the lower nutritional value of dried algal preparations in mariculture (Laing *et al.*, 1990).

Non-living feeds like spray-dried algae needs mechanical means to keep them suspended. The aeration level applied in the experiments (optimum for the larvae), resulted in some settling of spray-dried feed, proportionate to the quantity used in treatments. This could also be a reason for the reduced growth rate.

To conclude, the experiments vividly show that 100% substitution of live algae is not possible with the currently available quality of spray-dried algae. The maximum substitution level without much compromise on vital parameters was 66% with the inclusion of *Artemia* in the feeding regime. Levels of substitution may be increased by improving the quality of the product, inclusion of additional dry algae species or dry feeds, trying higher cell density of dry algae for the larvae. Further studies are also needed to find out leaching of nutrients, if any, during hydration.

Practical dried feed would provide feed of consistent quality and quantity at any time, with less contamination problem; it would reduce operational costs and eliminate investments related to algae production facilities. The same would also help in establishing a standard control diet for comparison of nutritional research among laboratories. As, microalgae are used in hatchery rearing of other animals like oysters, mussels and fishes, development of dried feed would have a much bigger role in the aquaculture industry.