

## Cost effective medium for the laboratory culture of live feed micro algae

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### ABSTRACT

Extracts of the green seaweed *Ulva lactuca* promoted the growth and the multiplication of three species of micro algae, *Tetraselmis gracilis*, *Isochrysis galbana* and *Chaetoceros calcitrans* 250-325% more than those cultures supplemented with vitamins (B<sub>1</sub> and B<sub>12</sub>). When these microalgae were cultured in seawater supplemented with varying levels of extracts of garden soil and *Ulva lactuca*, *Isochrysis galbana* and *Tetraselmis gracilis* registered 16% and 58% increase in growth respectively and 19% decrease in growth by *Chaetoceros*. The results are discussed in the light of preparations of a low cost effective and ready to use recipe for the mass culture of these live feed organisms.

### Introduction

With the rapid increase in aquaculture production, there is an ever-growing interest in live feed culture. Live feeds constitute the inevitable input in hatchery operation of any aquaculture system. Being the primary link in the food chain, phytoplankton (micro algae) among live feeds plays a very important role. Thus the culture and maintenance of these feed organisms becomes equally important. Gopinathan(1982) has described the batch culture method for the mass culture of phytoplankton for shellfish hatcheries. Although batch culture is relatively easy to carry out, its efficiency is very poor and the cultures are prone to crash. Considering the advantages of continuous and semi continuous culture systems over the traditional

batch culture systems, a number of workers (Persoone and Sorgelos, 1975; Boussiba *et. al.*, 1988; Janes and Al Khars, 1990; Feberga *et. al.*, 1996; Lambade and Mohamed, 2001) have reported on several designs for the continuous production of micro algae in high densities.

Like culture methods, culture media also play a major decisive role in live feed culture. There are a number of conventional media, such as, Walne's, Scheiber's, Miquel's etc., being used for the culture and maintenance of micro algae in the laboratory as well as in hatchery. These media contain inorganic recipes and procurement of the ingredient chemicals is tedious and often expensive. It is imperative that to make the hatchery production of shellfish and finfish profitable, the essential operational inputs are

to be minimized for all stages of hatchery programmes including feed development by adopting low cost productions. Although Kumaraswamy Achari and Kaumudi Menon (1993) have reported a simple medium to isolate and culture species of *Chromulina*, *Pavlova* and *Chlorella*, the new medium contains higher concentrations of phosphate and vitamins. An attempt has been made in the present study to formulate a new medium for the culture of micro algae, which is derived from organic ingredients, which are cost effective and are easily available. The study aims at the formulation of a low cost culture medium, which can be easily prepared and at the same time can offer the desired growth rate.

### Materials and Methods

The medium comprised of extracts of the green seaweed *Ulva lactuca* and garden soil.

#### Preparation of *Ulva* extract

100g wet weight of *Ulva lactuca* was cut into small pieces and boiled in about 500 ml distilled water for about 20 minutes with constant stirring. It was then squeezed and filtered through a 5 $\mu$  mesh. The final extract thus obtained was made up to 500 ml in a standard flask. This was then autoclaved, cooled and refrigerated for further use.

#### Preparation of garden soil extract

One kg of garden soil was sieved to remove stones and such other materials. This was then boiled in one litre distilled water for about 30 minutes to get the extract. It was filtered, autoclaved, cooled and then kept under refrigeration for further use.

### Experimental set up

Two experiments were conducted with three species of micro algae namely *Tetraselmis gracilis*, *Isochrysis galbana* and *Chaetoceros calcitrans*, which are the most commonly used live feeds.

Experiment I consisted of culturing these species of micro algae in Walne's medium (Walne, 1974) with *Ulva* extract. The extract was added at levels of 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 to the culture flasks containing the inocula and Walne's medium (devoid of vitamins).

Experiment II consisted of growing these species with *Ulva* extract and gardensoil extract (0.5-5.0ml). In both the experiments, Walne's medium enriched with vitamins served as the control. The particulars of experimental set up was given in Table 1.

Both the experiments were carried out for 8 days duration. Counts/ml were recorded on zero days and then on every alternate day to determine the growth and multiplication of the culture, using a haemocytometer with improved Neubauer ruling. Measurement of growth, rate of multiplication/day was calculated as per Herrero *et. al.* (1991). The growth and net yield were analysed using ANOVA for test of significance (Snedecore and Cochran, 1967) and the analysis was done using SPSS/PC software.

### Results:

The Experiment I showed that 1-2 ml of *Ulva lactuca* extract supplemented to Walne's medium increased the net growth of 315% in *Isochrysis galbana* ( $P < 0.05$ ), 323% in

Table 1. Details of experimental setup

	Inoculum	Seawater	<i>Ulva</i> extract	Soil extract	Walne's medium
Experiment I	10ml	85-90ml	0.5-5.0ml	Nil	2 ml
Experiment II	10ml	80-90ml	2.0ml	0.5-5.0%	Nil
Control	10ml	90ml	Nil	Nil	0.2 ml

Table 2. Effect of extract of *Ulva lactuca* on the growth of *Isochrysis galbana* ( $n \times 10^4$ ) cultured in Waime's medium

Treatment (ml)	One	Three	Five	Seven	Net growth (%)
0.5	28.50	51.70	92.60	66.75	234
1.0	27.50	62.50	80.00	96.20	350
2.0	16.75	19.25	57.50	128.50	767
3.0	19.50	21.50	86.50	88.00	451
4.0	18.33	n.d	83.50	88.70	484
5.0	17.60	n.d	76.00	82.00	470
Control	18.70	21.00	88.50	84.50	452

n. d. - not detected

Table 3. Effect of extract of *Ulva lactuca* on the growth of *Chaetoceros calcitrans* ( $n \times 10^4$ ) cultured in Waime's medium

Treatment (ml)	One	Three	Five	Seven	Net growth (%)
0.5	33	23.75	42.25	103.0	312
1.0	30	64.75	136.5	166.5	555
2.0	30	26.50	60.25	73.30	245
3.0	30	17.25	65.75	59.75	171
4.0	32	87.75	48.25	61.0	190
5.0	31	43.75	51.75	53.75	173
Control	25	34.25	47.75	58.0	232

Table 4. Effect of extract of *Ulva lactuca* on the growth of *Tetraselmis gracilis* ( $n \times 10^4$ ) cultured in Waime's medium

Treatment (ml)	One	Three	Five	Seven	Net growth (%)
0.5	16.0	27.0	36.0	42.0	263
1.0	19.0	29.0	34.0	84.0	442
2.0	11.0	18.0	25.0	66.0	600
3.0	11.0	23.0	22.0	45.0	409
4.0	16.0	n.d	27.0	40.0	250
5.0	15.0	22.0	31.0	40.0	267
Control	12.0	22.0	32.0	41.0	342

n. d. - not detected

Table 5. *Effect of extract of Ulva lactuca (2%) and garden soil (0.5 - 5.0 %) on the growth of Isochrysis galbana ( $n \times 10^4$ ) Cultured in Walne's medium*

Treatment (ml)	One	Three	Days Five	Seven	Net growth (%)
0.5	13.25	24.5	44.5	45.75	345
1.0	11.75	34.00	42.25	53.00	451
2.0	14.75	29.70	21.00	63.00	427
3.0	15.50	26.00	39.00	51.03	329
4.0	15.82	35.25	45.50	57.00	360
5.0	19.50	41.75	38.25	58.50	300
Control	19.25	23.70	88.50	83.75	435

Table 6. *Effect of extract of Ulva lactuca (2%) and garden soil (0.5 - 5.0%) on the growth of Chaetoceros calcitrans ( $n \times 10^4$ ) Cultured in Walne's medium*

Treatment (ml)	One	Three	Days Five	Seven	Net growth (%)
0.5	19.00	32.00	32.75	39.25	207
1.0	15.25	26.25	33.00	40.00	262
2.0	16.25	20.25	28.75	35.00	215
3.0	18.00	31.25	25.50	38.25	213
4.0	19.50	22.50	37.75	48.75	247
5.0	17.00	37.00	35.00	35.50	206
Control	20.00	33.50	45.75	56.25	281

Table 7. *Effect of extract of Ulva lactuca (2%) and garden soil (0.5 - 5.0 %) on the growth of Tetraselmis gracilis ( $n \times 10^4$ ) Cultured in Walne's medium*

Treatment (ml)	One	Three	Days Five	Seven	Net growth (%)
0.5	15.00	25.70	23.83	36.00	240
1.0	10.00	17.14	27.00	31.00	310
2.0	12.00	25.00	32.00	33.00	358
3.0	14.00	37.43	44.00	42.00	300
4.0	18.00	40.33	45.00	46.00	256
5.0	17.00	41.00	46.00	45.00	264
Control	14.00	24.00	33.00	42.00	300

*Chaetoceros calcitrans* ( $P < 0.05$ ) and 258% in *Tetraselmis gracilis* ( $P < 0.10$ ) higher than their respective controls (Tables 2, 3 and 4; Fig. 1). Levels higher than 2ml extract of *Ulva lactuca* did not enhance the growth and multiplication of micro algal cells proportionately.

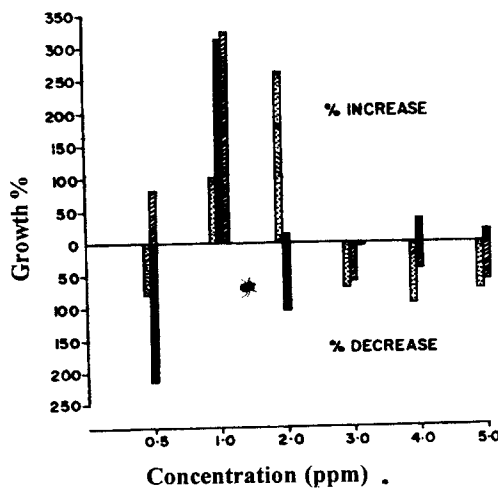


Fig. 1. Effect of extract of *Ulva lactuca* on the growth of live feed algae cultured on Walne's medium

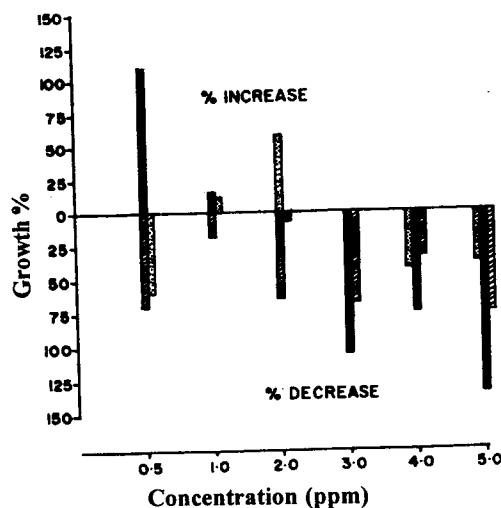


Fig. 2. Effect of extract of *Ulva lactuca* and garden soil on the growth rate of live feed algae cultured in sterile seawater

Culture of these micro algal cells carried out in the Experiment II in the seawater supplemented with extracts of garden soil and *Ulva lactuca*, without any inorganic salts and vitamins resulted in considerable increase (Tables 5-7; Fig. 2) in cell numbers than their controls (Walne's medium). However, the net increase in cell numbers on the 7th day in 2% *Ulva lactuca* and 4% soil extracts showed 16% increase in *Isochrysis galbana* and a combination of 2% *Ulva* extract and 4% soil extract showed a net increase of 58% in *Tetraselmis gracilis* ( $P < 0.05$ ), whereas cultures of *Chaetoceros calcitrans* showed 19% decrease ( $P < 0.05$ ) than the control (Table 5-7; Fig. 2).

**Discussion**

*Ulva lactuca* is one of the most commonly available green seaweed along the seacoast and hence there is practically no difficulty in collecting them, as they inhabit the intertidal regions of the coastal areas. Chemical composition of *Ulva lactuca* is known from Chennubhotla *et. al.* (1991) and that of garden soil extract from Thompson and Troch (1979). Experiments on growth efficiency may offer some valuable clues in regard to the ecological success of an organism (Kinne, 1960). The present study indicates that extracts of the seaweed and the soil are rich in nutrients, which are cost effective and easy to obtain.

Addition of extracts of *Ulva lactuca* to Walne's medium (without vitamins) for the culture of *Isochrysis*, *Chaetoceros* and *Tetraselmis* enhanced the growth and multiplication of cells considerably (Tables 2-4). However, soil extract in the place of inorganic salts did support the growth, more or less comparable to the control although, not proved superior to the control (Tables 5-7) indicating the possibilities of culturing micro algae in seawater with these extracts only. It is observed that higher concentrations of *Ulva* extracts beyond 2% and 4% of soil extract are not favouring the growth and multiplication of microalgal cells but enables to achieve their exponential phase quicker than the other treatments (Tables 5-7)

The sterile extracts of *U. lactuca* and garden soil can be stored for about six months in the form of a ready to use ampoule mixed in appropriate proportions or as separately, that can form a medium when heated with known quantity of filtered seawater. This simple medium stands in sharp contrast to the widely used commercial media in terms of economic viability as the cost of production for this new medium would be just 1/10<sup>th</sup> required for the preparation of the commercial medium.

The performance of this simple medium can perhaps be improved by mixing the extracts of many other seaweeds belonging to Rhodophyceae and Phaeophyceae, also at various proportions. The major advantage of this new medium for culturing marine micro algae (live feeds), besides cost effectiveness, is that this medium reduces the risk of accumulation of inorganic salts in the micro algal cells and thereby their transport into the feeding organisms as well as into the culture environment.

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