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Studies on the Outdoor Cultivation of *Ulva lactuca* L.

T. A. DeBusk[1], M. Blakeslee and J. H. Ryther

Division of Applied Biology, Harbor Branch Foundation, RFD 1, Box 196A, Ft Pierce, Florida 33450, U.S.A.

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Abstract

Outdoor growth studies were conducted in central Florida with the chlorophyte *Ulva lactuca* L., a productive macroalga for which few cultivation data are available. Maximum yield of this species was found to occur in tanks receiving a rapid seawater exchange (12 tank volumes per day) with the seaweed kept in suspension by continuous aeration. Yields decreased, although not linearly, as either water flow or aeration times were reduced. Under both aerated and non-aerated conditions, maximum yields at weekly harvests were obtained at an initial standing crop of 0.8 kg wet wt m^{-2}. In a nitrogen uptake study, thallus N levels increased 3.6 and 2.8-fold when nitrogen-starved *U. lactuca* was held for 24 hours in media enriched with NH_4^+ and NO_3^-, respectively. Hence, pulse-fertilization, a nitrogen enrichment strategy useful in reducing epiphytization, can be employed as a culture technique for this alga. Productivity of *U. lactuca* in 700 l tanks over an eight-month period averaged 18.8 and 6.8 g dry wt m^{-2} day^{-1} under aerated and non-aerated conditions, respectively. Such high sustained yields suggest that the cultivation of this species as a feed or energy crop may be feasible.

Introduction

The chlorophyte *Ulva lactuca* L. is a marine macroalga which occurs along the eastern coast of the Americas from Newfoundland to the Caribbean (Taylor 1972). This species is capable of rapid vegetative growth, and in the presence of high nutrient levels, such as near sewage outfalls, the standing crop of this seaweed can assume nuisance proportions (Guist and Humm 1976). *U. lactuca* is considered an opportunist (Waite and Mitchell 1972), and thrives over a wide temperature and salinity range. Its wide ecological amplitude, rapid specific growth rate, and favorable nutritive content (Habig *et al.* 1984) make it a good candidate for cultivation as an animal feed (Boney 1965) or as a biomass source for anaerobic conversion to methane gas (Habig and Ryther 1983).

Previous studies have demonstrated that many *Ulva* species are difficult to maintain in a vegetative state in culture (Subbaramaiah 1970). Vegetative fragmentation, zoospore production by sporophyte plants and isogamete production by the morphologically identical gametophyte plants are all means by which *Ulva* disseminates propagules. These reproductive processes are triggered by a number of environmental factors such as nitrogen concentration and lunar periodicity (Mohsen *et al.* 1974, Rhyne 1973), and usually result in a disintegration of all or part of the thallus. Hence, large-scale cultivation data for *Ulva* spp. are lacking.

In 1981 and 1982, we obtained two clones of *U. lactuca* which are resistant to fragmentation. The present study was conducted to determine whether long-term outdoor tank cultivation of these *Ulva* clones is feasable, and to identify some of the operating conditions (e. g. tank aeration, water exchange) under which maximum yields in meso-scale tanks can be attained.

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[1] Present Address: Reedy Creek Utilities Co., Inc., Post Office Box 40, Lake Buena Vista, FL 32830, U.S.A.

Methods

Two *Ulva lactuca* L. clones, designated 007 and 010, were grown outdoors in 1.7 m^2 (700 l) concrete tanks at the Harbor Branch Foundation, Ft Pierce, Florida. The tanks were equipped to receive a constant flow of estuarine water (annual salinity range, 18 – 35‰), which exited the tank through a non-clogging, overflow drain. The seaweed was grown unattached, and in most experiments was kept suspended in the water column by means of vigorous aeration supplied through a perforated pipe placed along the bottom of each tank.

Nitrogen uptake experiments

Nitrogen-starved *U. lactuca* was obtained by culturing the seaweed (clone 007) in 700 l tanks for one month at a low seawater flow rate with no supplemental fertilization. This seaweed was then divided among four outdoor tanks (0.7 kg wet wt/tank), each containing 350 l of estuarine water (23 °C, 22‰ salinity; 4 μm N as NH_4^+, 6 μm NO_3^- + NO_2^-). Two tanks each received additional nitrogen (2000 μM) as NH_4Cl and $NaNO_3$, respectively. Supplemental phosphorus (150 μM $NaPO_4 \cdot 7H_2O$) was also added to each tank. Two of the tanks (one NO_3^-, one NH_4^+) were covered so that nutrient uptake could be measured in the dark. All tanks were continuously aerated to keep the seaweed mixed and in suspension. Nitrogen uptake by the seaweed was measured by changes in tissue N content. Duplicate algal samples were collected from each tank prior to incubation and after 3, 6, 12 and 24 hours of soaking in the nutrient media. The collected plant material was briefly rinsed in deionized water, dried (48 h at 70 °C), and ground to a fine powder. Nitrogen composition was determined on a Perkin Elmer 240A elemental analyzer.

Standing crop experiments

The relationship between *U. lactuca* productivity and plant standing crop was examined in both aerated and non-aerated tanks. Eight outdoor tanks were stocked with clone 010 at initial densities of 0.4, 0.8, 2.3 and 3.8 kg wet wt m^{-2}. Seawater was fed into the tanks at a flow rate of 10 volume exchanges per day. The seaweed was weighed and harvested back to initial stocking weights at weekly intervals for one month. Prior to restocking, the plants were soaked in enriched (2000 μM N, 150 μM P) seawater for 24 hours. At each weighing, plant samples were collected and oven dried (48 h at 70 °C) in order to determine dry weight to wet weight ratios.

Aeration experiments

In February, 1983, clone 010 was stocked at 1.0 kg wet wt m^{-2} into twelve outdoor tanks which received seawater at 10 volume exchanges per day. Duplicate tanks were aerated for 0, 1, 4, 12 and 24 hours daily. These aeration regimes were maintained with the use of electronically controlled solenoid valves on the culture tank air supply lines. The twelve hour aeration treatment consisted of one minute of aeration, followed by a one minute interval without aeration. This on: off aeration pattern was repeated continuously day and night. Aeration for the four hour treatment was on for one minute, off for five minutes, and that for the one hour treatment was on one minute: off 23 minutes. This experiment was conducted for one month, with seaweed in the tanks weighed weekly, soaked for 24 hours in a concentrated nutrient medium (2000 μM N and 150 μM P), and restocked to 1.0 kg m^{-2}. Between treatment differences in seaweed growth (as cumulative production for the one month period) were analyzed using a one-way ANOVA. Treatment means were then ranked using an a posteriori range test (Student – Newman – Keuls).

Water flow rate experiments

Clone 010 was stocked at a standing crop of 1.0 kg wet wt m^{-2} into eight continuously-aerated outdoor tanks. Duplicate tanks received either 1, 6, 12, or 24 seawater exchanges per day. The seaweed was weighed and fertilized weekly as described in the previous experiment. *U. lactuca* growth was measured under these conditions for three weeks during both October 1981 and April 1982. In April, pH and temperature of the tank waters were measured at 4 hour intervals during one 24 hour period. At the termination of the October experiment, plant samples were collected from each treatment for the determination of total N content. Between treatment differences in growth were examined for significance with a one-way ANOVA.

Annual production

During 1983, growth of *U. lactuca* (clones 010 and 007) was measured in outdoor tanks over an eight month period. The algae were cultured in both aerated and non-aerated tanks which received 10 volume exchanges per day of estuarine water. The seaweed in all tanks was stocked at a density of 1.0 kg m^{-2} and at intervals of one to two weeks was weighed, harvested back to the original stocking weight, and restocked. Fertilization was conducted as previously described. Plant samples were collected periodically for the determination of wet weight to dry weight ratios.

Results and Discussion

Nitrogen uptake experiments

Nitrogen starved *U. lactuca* (1.3 ± 0.1% N) assimilated inorganic nitrogen rapidly, particularly from the ammonium-enriched medium. Thallus N levels in the NH_4 exposed seaweed tripled within 24 hours, while that of the NO_3 exposed seaweed more than doubled (Fig. 1). Such a 'preference' for NH_4 has been demonstrated for many other macro- and microalgae (D'Elia and DeBoer 1978, Morris 1974).

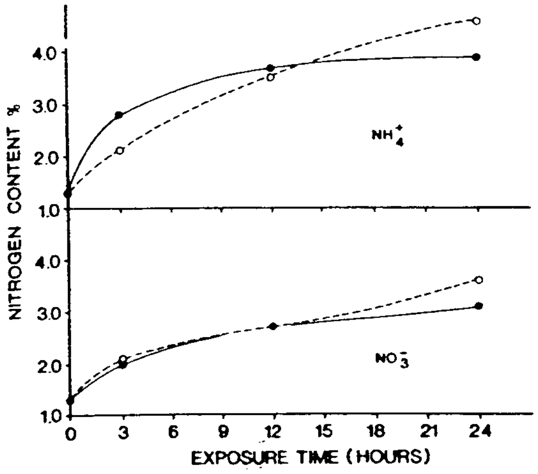

Fig. 1. Tissue Nitrogen levels of *U. lactuca* soaked for 24 h in seawater enriched with N as NH_4^+ (top) and N as NO_3^- (bottom). Closed circles indicate dark-held seaweed, open circles represent seaweed exposed to outdoor light. Initial (0 h) stocking time was 11.00 h. Standard deviation of the mean (n = 2) for each data point is 0.1% or less.

For both the NH_4^+ and N as NO_3^- treatments, 24 hour N uptake by light-exposed (normal day-night cycle) *U. lactuca* was greater than that by seaweed held in the dark. However, initial (3 and 12 h) N uptake by the NH_4 soaked alga was greater in the dark than in the light (Fig. 1). Habig *et al.* (1984) reported that N-starved *U. lactuca* thalli contain high levels of soluble carbohydrate. Syrett (1956 a, b) proposed that energy obtained from such carbohydrate reserves can be utilized to support rapid nitrogen uptake by dark-held algae.

The ability of N-starved *U. lactuca* to quickly absorb and store nitrogen indicates that a continuous supply in the medium is not required for algal growth, but that the alga's requirements can be met by periodic soaking in a concentrated nutrient medium. Benefits of pulse nutrient feeding in algal cultivation (e. g., epiphyte control) are discussed by Ryther *et al.* (1981).

Standing crop experiments

Maximum *U. lactuca* yields in both aerated and non-aerated tanks were obtained at an initial standing crop of 0.8 kg wet wt m^{-2}, with yields declining at both lower and higher stocking densities (Fig. 2). At 3.8 kg m^{-2}, the highest stocking biomass examined, aerated *U. lactuca* grew at one-half the rate of seaweed at the optimum stocking biomass. However, at 3.8 kg m^{-2} under non-aerated conditions, no net algal production was observed.

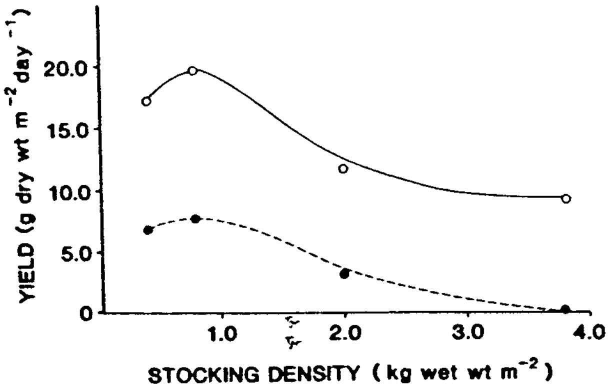

Fig. 2. Yields of *U. lactuca* at four initial stocking densities in both aerated (open circles) and non-aerated (closed circles) tanks.

Prior studies have shown that an optimum stocking density (or density range) exists for which maximum seaweed yields are attained (Neish and Knutson 1977, Lapointe and Ryther 1978). Macroalgae for which such density optima (in aerated culture tanks) have been reported include: *Hypnea musciformis*, 1.9 kg m^{-2}; *Iridea cordata* (Turner) Bory, 2.1 kg m^{-2}; *Chondrus crispus*, 3–4 kg m^{-2}; and, *Gracilaria tikvahiae*, 2–3 kg m^{-2} (Guist *et al.* 1982, Waaland 1976, Simpson *et al.* 1977, Lapointe and Ryther 1978). It is important to note that these density optima are 2–4 times higher than the standing crop optimum we found for *U. lactuca*, and the 0.8 kg wet wt m^{-2} optimum reported for *U. fasciata* (Lapointe and Tenore 1981). Under natural conditions, flat or sheet-like macroalgae such as *Ulva* spp. are considered among the most productive of seaweeds (Littler and Arnold 1982), at least when 'productivity' is reported as specific growth rate. However, because yield is a function of both standing crop and specific growth rate (Lapointe and Ryther 1978), the low standing crop optimum for *U. lactuca* requires that its specific growth rate be extremely high if its yields in cultivation are to approach those of macroalgae such as *Gracilaria tikvahiae* (Lapointe and Ryther 1978).

Aeration experiments

The amount of time for which aeration was supplied to the culture tanks had a pronounced effect on *U. lactuca* growth. Highest yields occurred in tanks receiving continuous (24 h) aeration, while lowest yields occurred in non-aerated tanks (Table I). However, the decline in algal yields with decreasing aeration time was not linear. Seaweed production in tanks with four hours of aeration was not significantly lower than that in tanks receiving aeration for 12 hours, whereas yields dropped sharply in tanks provided with only 1 hour of aeration per day (Table I).

Table I. Effect of aeration time on productivity of *U. lactuca* in 700 l outdoor tanks. Aeration regimes are explained in text.

Aeration time (h)	0	1	4	12	24
Productivity (g dw m^{-2} d^{-1})	4.7^a	7.6^b	11.3^c	12.1^c	16.0^d

Values followed by the same letter are not significantly different at the 0.05 level.

Although previous investigators have noted that culture tank mixing or agitation is required to obtain high seaweed yields (Neish and Knutson 1977), the exact means by which the aeration process stimulates growth is unknown. Blakeslee (1984) demonstrated that aeration supplies little atmospheric carbon to culture tank waters. Observations with the macroalgae *Ulva* sp. and *Gracilaria tikvahiae* (T. A. DeBusk, unpublished) suggest that the increased exposure of the algae to light and the physical removal of sediments and epiphytes from the thalli are the two main benefits of tank aeration.

Data from this study show that tank aeration can be reduced considerably without major sacrifices in *U. lactuca* yields. For example, by providing 4 hours of intermittant aeration daily (17% of the operating energy required for 24 h of aeration), *U. lactuca* yields are ca 72% of those attainable with continuous aeration. If improved light utilization by the algal culture is indeed the primary function of aeration, it is possible that *U. lactuca*'s aeration requirements could be further halved by the elimination of night time aeration, a strategy utilized in the cultivation of *Chondrus crispus* (Bidwell *et al.* 1985).

Water flow rate experiments

U. lactuca productivity was influenced by the flow rate of estuarine water through the culture tanks. In studies conducted both in October 1981 and April 1982, seaweed yields increased ($P < 0.01$) as the seawater turnover rate was raised from one to twelve exchanges per day (Fig. 3). Between 12 and 24 water exchanges per day, however, little further growth enhancement was observed. During April, water temperature differences between treatments were slight (< 2 °C) compared to the diel temperature fluctuations in each tank (ca 6 °C). In contrast, tank water pH was strongly influenced by water exchange, with widest pH fluctuations occurring in tanks with the lowest flow rate (Fig. 4).

During the October study, plant tissue nitrogen levels were found to vary only slightly with seawater flow rate. At the end of the final growth period, (one week after fertilization), the nitrogen content [x, (S. D.) n = 2] of *U. lactuca* cultured at 24, 12, 6 and 1 seawater exchanges averaged 3.2 (0.1), 3.3 (0.2), 3.0 (0.1), and 2.4 (0.4)%, respectively.

A rapid seawater exchange may stimulate algal yields by providing required macro- or micronutrients, moderating temperatures, or flushing out toxic metabolites. Waaland (1976) demonstrated that *Iridea* productivity in low flow culture tanks was equal to that

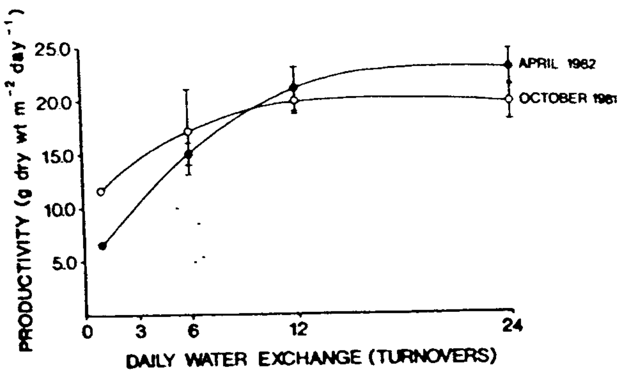

Fig. 3. Productivity of *U. lactuca* as a function of water exchange. Water flow was continuous, with rates adjusted to provide (daily) the number of tank volumes (700 l) indicated on the horizontal axis. Error bars denote one standard deviation (n = 2).

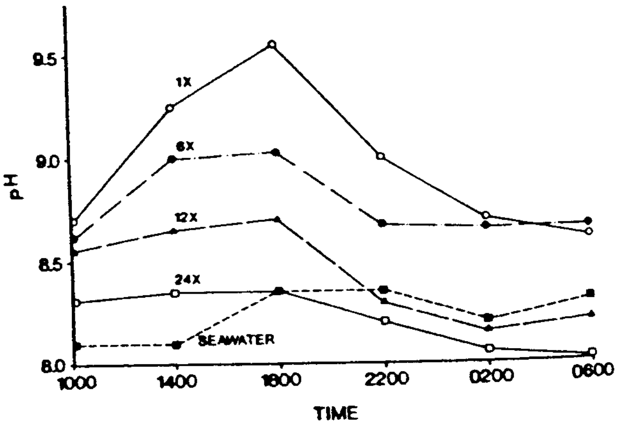

Fig. 4. Diel fluctuations in pH of seawater and *U. lactuca* culture tank waters at four different water exchange rates (1, 6, 12, and 24 exchanges).

in high flow tanks only when supplemental N was supplied. In the present study, the decrease in thallus N content in the tanks receiving the lowest water flow demonstrates the potential for a similar N limitation occurring for *U. lactuca* under low influent nitrogen loadings if the time between pulse-feedings is great enough.

Reduced inorganic carbon concentrations or high pH caused by photosynthetic carbon assimilation may also reduce yields of macroalgae in low flow culture tanks (DeBusk and Ryther 1984). The flow related elevations in culture tank pH coupled with the reduction in seaweed growth in the present study suggest high pH and/or reduced carbon availability was the factor limiting *U. lactuca* growth at low water exchanges. At locations where water pumping costs are high and a rapid water exchange is not required for nutrient supply or temperature regulation, it is likely that *U. lactuca*'s carbon/pH requirements can be met by the addition of CO_2 or carbonate salts, a strategy which has been successfully used in the cultivation of other macroalgae (Bidwell *et al.* 1985, DeBusk and Ryther 1984).

Annual production

During 1981, 1982, and early 1983, outdoor growth studies were conducted exclusively with the 010 *U. lactuca* clone. This clone grew well in the winter and spring, but fragmented and could not be maintained in culture during the summer months. In contrast, the 007 clone which was initially stocked outdoors during July 1983 grew well during the summer months, with only minor fragmentation occurring in the winter. Regardless of season, survival of these two clones under non-aerated conditions was poor. Without aeration, the seaweed was buoyed to the water's surface by photosynthetic oxygen bubble formation. Conditions of high temperature, dessication, and high light intensity caused death and decay of these plants, necessitating the replacement of the entire culture. In 1983, the non-aerated tanks had to be restocked approximately 5–6 times with healthy plant material from previously aerated cultures. The 8 month average growth rate for non-aerated *U. lactuca* was 6.8 g dry wt m^{-2} day^{-1} (Fig. 5).

The two *U. lactuca* clones grew relatively well, however, when continuous aeration was supplied: yields averaged 18.8 g dry wt m^2 day^1 for the eight months of 1983 in which growth was monitored. Highest productivity for clone 010 (29.9 g dry wt m^{-2} day^{-1}) was observed in May, while maximum yields for clone 007 (35.2 g dry wt m^{-2} day^{-1}) occurred in July (Fig. 5).

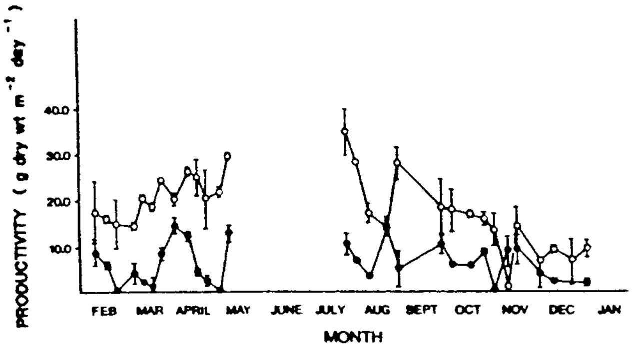

Fig. 5. Productivity of *U. lactuca* in aerated (open circles) and non-aerated (closed circles) 700 l tanks during 1983. Clone 010 was cultured from February – May, and clone 007 from July – January. Error bars denote one standard deviation (n = 2).

The seasonal growth pattern of clone 010 in the present study is similar to that of the *Ulva* populations in Florida which begin to spread in October and proliferate until April (Guist and Humm 1976). Clone 007, which thrived in our tanks during the summer months, may be more of a 'warm water' strain, since January was the only month in which thallus fragmentation was observed. However, because no attempt was made to manipulate temperature and light regimes in the outdoor tanks, the environmental parameters which triggered fragmentation of these clones could not be determined. Many *Ulva* species have been observed to release gametes on a 14–15 day cycle (Rhyne 1973). Periodic gamete release was not exhibited by either of the two *U. lactuca* clones cultured in the present study. Moreover, neither N-depletion nor N additions (of NH_4^+ or NO_3^-) to the tank waters stimulated algal fragmentation or gamete release. Because the infrequent fragmentation that we observed was not necessarily linked with reproductive activity, it is possible that these two *Ulva* clones are sterile. Further research on the fragmentation phenomenon in *U. lactuca* should be conducted so that an algal crop can be consistently maintained in culture.

Under conditions of high seawater exchange and continuous aeration, average 8 month *U. lactuca* yields were comparable to those reported for many other cultivated algae (Guist *et al.* 1982, Lapointe *et al.* 1976). Because maximum productivity of the two *U. lactuca* clones occurred in May and late July, it is probable that if continuous summer growth data were available (as would be possible with clone 007), mean yields over a 12 month period would be substantially higher than that of our eight month estimate.

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