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OYSTER GROWTH AND NUTRIENT NITROGEN COST IN BIVALVE MOLLUSCAN MARICULTURE

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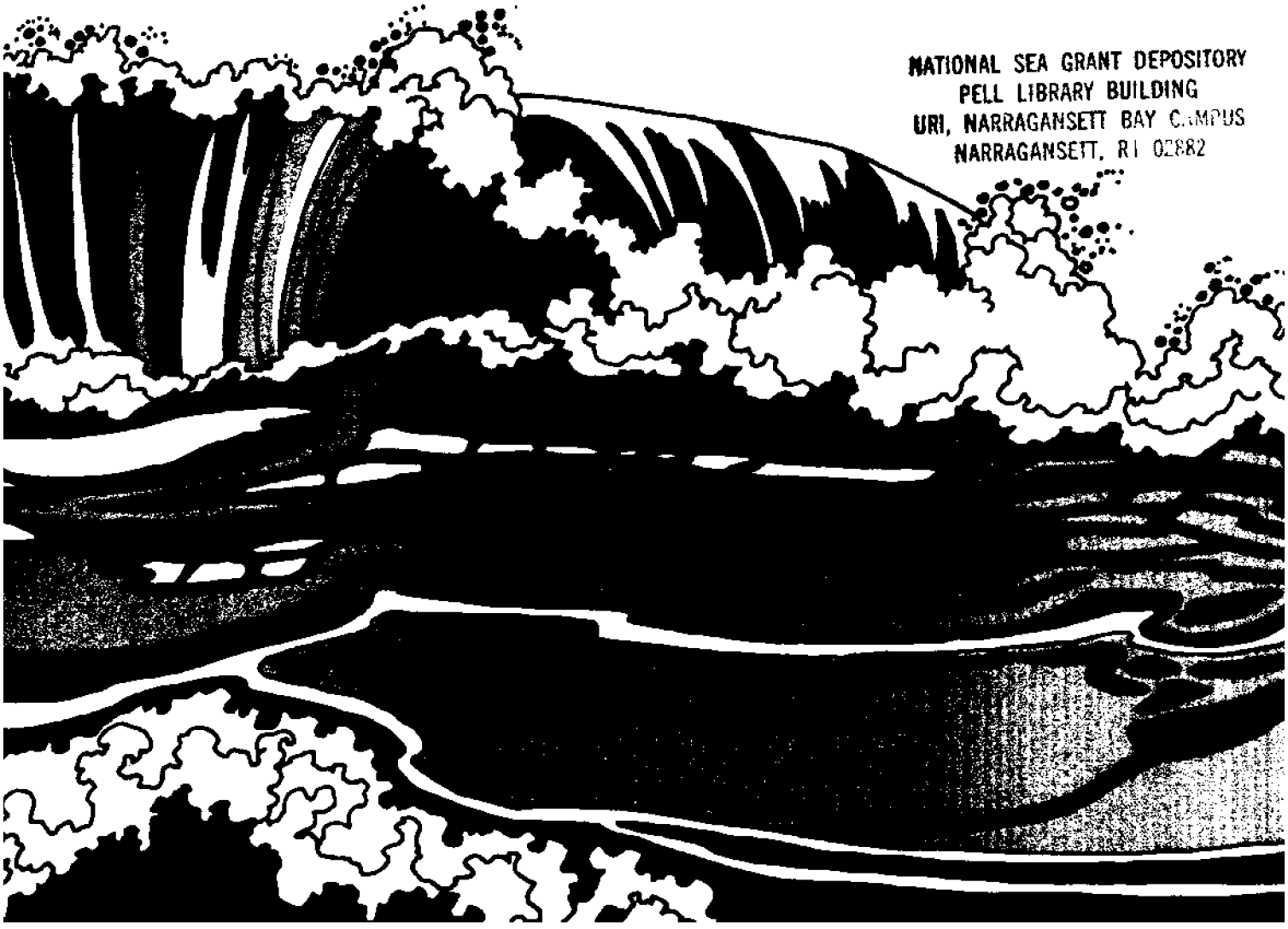

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ABSTRACT

The University of Delaware Project is working toward development of commercial, closed-cycle, controlled environment shellfish mariculture. As the result of several years' effort, the technical feasibility of raising bivalve molluscs from egg to market size in a recirculating system on a diet of cultured algae has been demonstrated.

System optimization and cost reduction efforts are leading to an economically feasible system. Progress toward this goal has been made in several areas, including improved oyster growth rate, reduction in make-up nutrient cost, and incorporation of a foam fractionation device for water quality maintenance and waste removal.

INTRODUCTION

The Delaware Mariculture Project, supported by the Office of Sea Grant and the State of Delaware, has as its objective the rapid development of commercial, closed-cycle, controlled environment shellfish mariculture. The project, now in its fifth year, is making orderly progress toward this goal.

The effort involves development of an efficient, reliable, and economical process and hardware system for raising selected bivalve molluscs from egg to marketable size. To be economically feasible, such a system must provide and maintain the physical, chemical, and biological conditions suitable for rapid growth of such commercially desirable bivalve molluscs as Crassostrea virginica (Gmelin), the Eastern oyster, and Mercenaria mercenaria (Linné), the hard clam. More specifically, the closed-cycle controlled environment process and hardware system must provide and distribute life support and growth factors, collect and remove undesirable liquid and solid wastes and organisms, and treat the material removed for disposal and recycling.

This paper reviews improvements in the growth rate of oysters, a reduction in the cost of adding nitrogen, and an improved method of removing undesirable liquid and solid wastes.

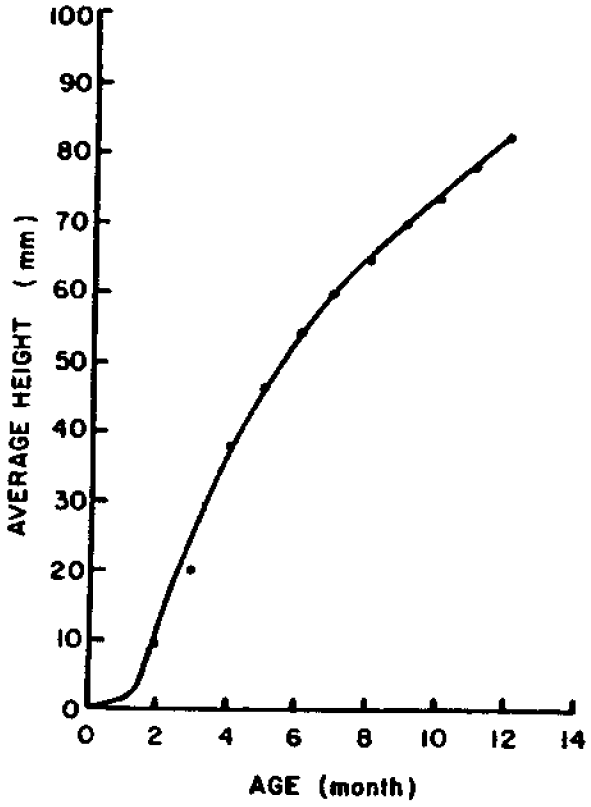

Fig. 1 Expected growth by oyster plant Cyanamid (1968)

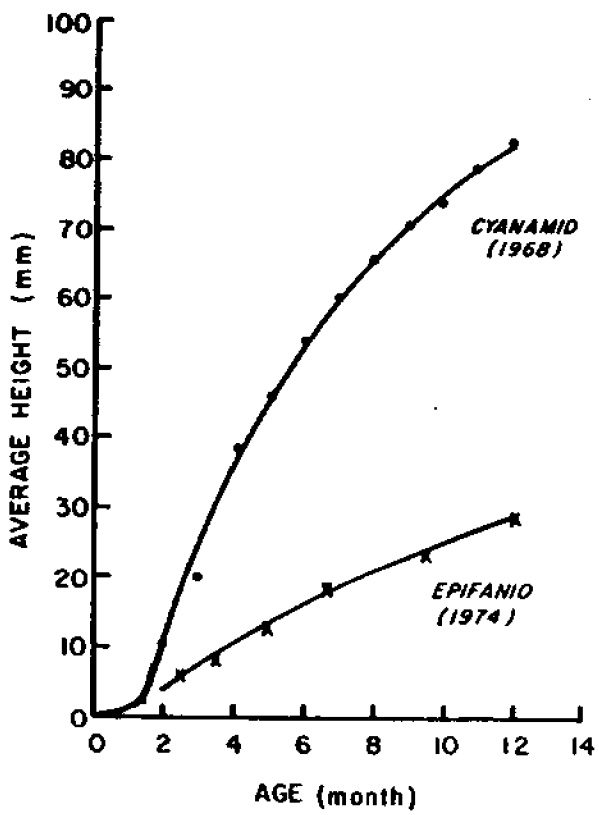

Fig. 2 Comparison of oyster growth reported by Epifanio (1974) with oyster growth expected by Cyanamid (1968)

IMPROVED OYSTER GROWTH RATE

American Cyanamid (1968) selected the fastest growth rate data for the oyster (Crassostrea virginica) reported from hatchery and raft experiments by Mattheissen and Toner (1966), eliminated zero growth periods (hibernation), and constructed a growth curve representing what might be expected in a maricultural factory situation (Figure 1). Epifanio et al. (1974) reared oysters in a largely recirculating system, monitoring the growth of oysters fed on seven different mixed algal diets. The highest growth rate was achieved on a four-part diet of Phaeodactylum tricornutum, Isochrysis galbana, Carteria chuii, and Croomonas salina. These 46-week data, when extrapolated, indicate a post-set to market-size period of 110 weeks. This period was shorter than the 156-to-260-week period estimated for wild oysters to reach market size in Delaware Bay (Maurer and Aprill, 1973). A comparison of oyster growth reported by Epifanio and expected growth for an oyster factory by Cyanamid is shown in Figure 2. It was essential for us to resolve the obvious discrepancy in rate of growth. Doubling the time required for an oyster to reach market size would be highly uneconomical and would adversely affect the future of molluscan mariculture.

Accordingly, during a three-month period (June through August, 1975), 300 oysters, suspended in trays, were grown in the Broadkill River as part of a comparison of oyster growth in natural sites. Average weight (total weight including shell) of these oysters almost doubled (39.6 to 77.0 grams) and average height increased from 40 mm to 58 mm in the 12-week period. A comparison between oyster growth in the Broadkill and the growth rate expected by Cyanamid is shown in Figure 3. The latter appears reasonable for local Delaware oysters in a good growing environment.

Concurrently with these field experiments, we were achieving accelerated oyster growth in the laboratory with oysters fed cultured algae. Utilizing the setting and spat-growing hardware developed by Dupuy (1972), and the feeding, care, and maintenance techniques devised by Greenhaugh, we have maintained accelerated growth rates of oysters for 5½ months from time of

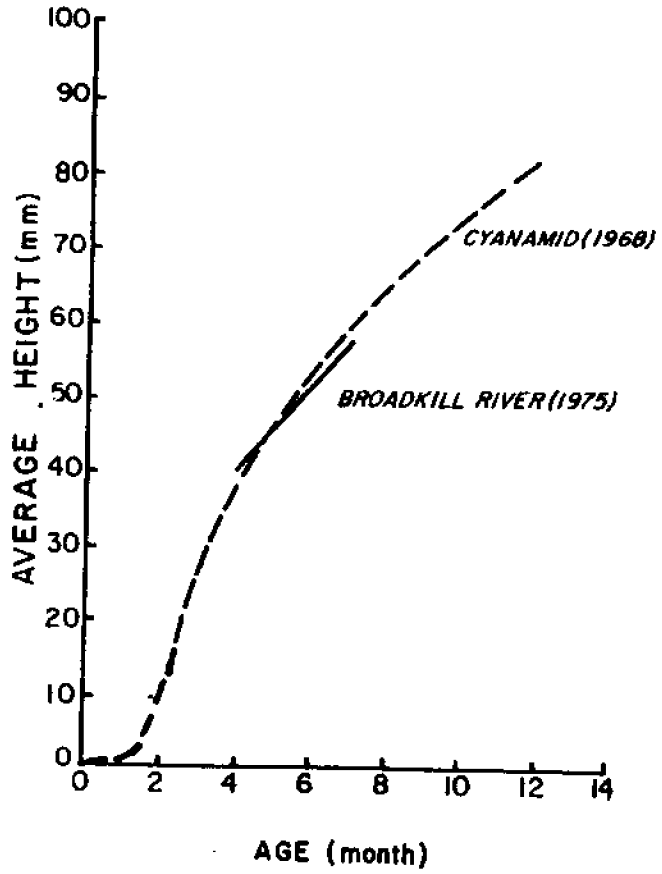

Fig. 3 Comparison of oyster growth in Broadkill River reported by Pruder in 1975 with oyster growth growth expected by Cyanamid (1968)

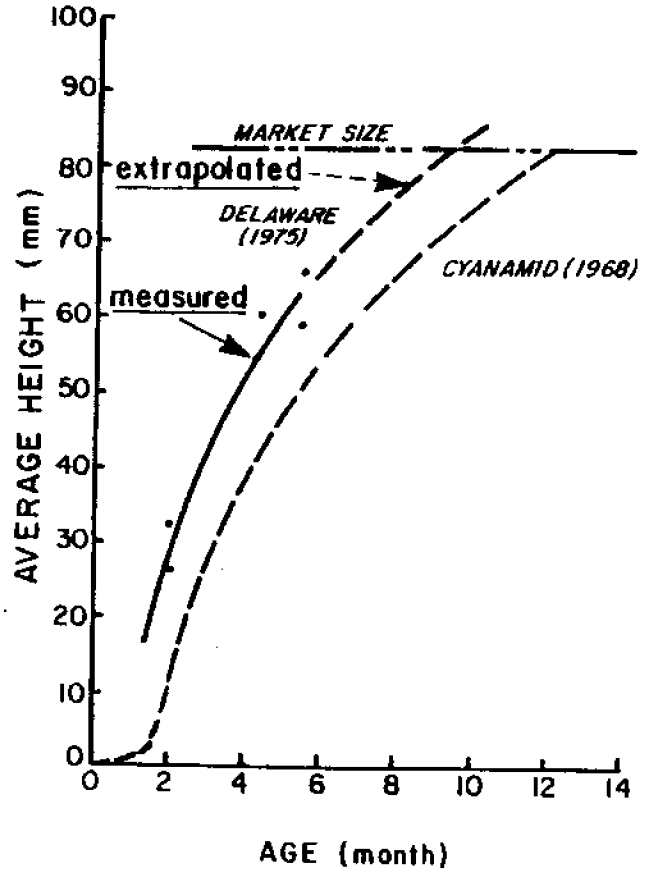

Fig. 4 Comparison of oyster growth in Delaware Mariculture Laboratory in 1975 with oyster growth expected by Cyanamid (1968)

fertilization of the ova. Figure 4 compares this growth rate with that expected by Cyanamid. These oysters are being cultured between 26° and 28° C on a mixed diet of approximately equal number of cells of Thalassiosira pseudonana (3H) and Isochrysis galbana with a complete change of water every other day. Feeding rate by cell count (Coulter Counter) averages 10 x 10^8 cells/gram/day for oysters in the 0.5 to 1.0 gram range. This success on a combined diatom and naked flagellate diet appears to support the recent work of Toner (1975) on juvenile bay scallops, Argopecten irradians irradians Lamarck.

Considerable gains would be possible if a single algal species could support the accelerated growth of oysters, particularly in a controlled environment system. The potential gains of returning viable undigested cells to the algal culture and growing algae and oysters at the same temperature are considerable in a unialgal system. Our most recent experiments were carried out with oysters setting on Mylar sheets. All oysters on each sheet were weighed to measure growth rate. There were approximately 2000 oysters per sheet, and each sheet was suspended vertically, one each in two 400-liter containers. One group of oysters received a combined diet of T. pseudonana and I. galbana and the other group received only T. pseudonana.

Eight weeks after setting, both groups exceeded Cyanamid's expectation of weight gain and the height exceeded 25 mm, which is above the Cyanamid expected height referenced earlier in this report. Thalassiosira pseudonana thus appears to be an excellent food by itself. Difficulty of matching oyster mass prevented us from ruling out the possible superiority of the two-part diet. Epifanio (1975a) reported equal growth rates for oysters being fed solely T. pseudonana and oysters being fed the best four-part diet discussed earlier. The excellent growth rate and wide temperature and salinity range of T. pseudonana make it a prime candidate as food for oysters being grown in a controlled environment maricultural system. Whether a second alga is required to achieve maximum growth rates, has yet to be determined.

The quantity of algae required to feed oysters depends upon the desired rate of oyster growth, the number and size of oysters being grown, and the type and condition of the algae. There is considerable variation in the

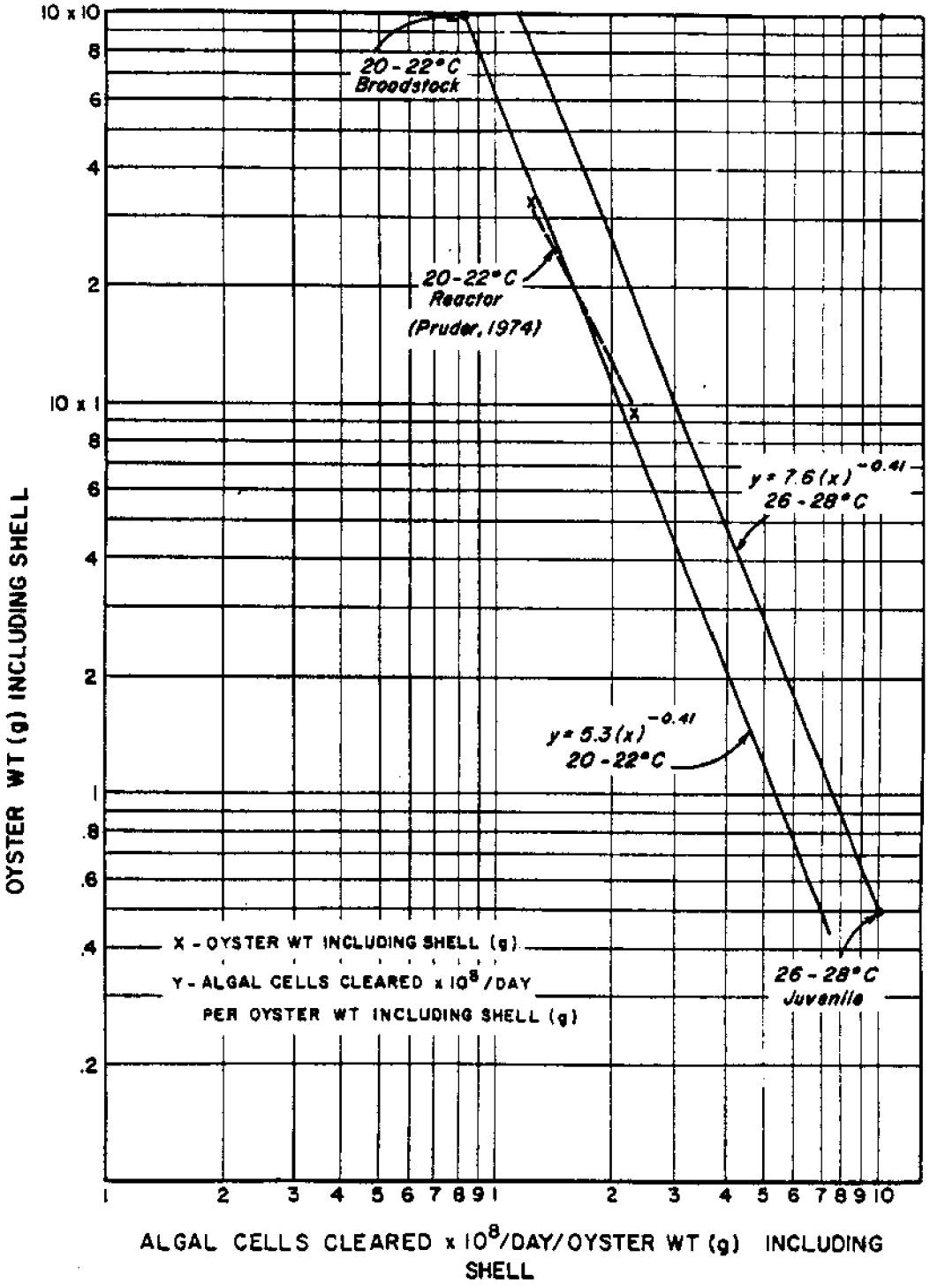

Fig. 5 Relationship between number of algal cells cleared and oyster weight at two temperatures

reported literature on the amounts of food consumed by oysters (Epifanio et al. 1975b). In our laboratory we have achieved rapid growth of oysters and the successful conditioning of broodstock, using Thalassiosira pseudonana as food. Data gathered here, coupled with previous results on oyster clearing rates obtained in a tubular reactor (Pruder 1974), allow us to assess the specific algal needs of oysters with reasonable confidence.

The tubular reactor data were reported as an expression relating the number of cells cleared per day as a function of individual oyster biomass (dry weight). These data were obtained in a flowing system with algal concentrations ranging from 3×10^4 to 1×10^5 cells (Phaeodactylum tricornutum). Based on a dry weight to total weight percentage of 2.7% for oysters in good condition (unpublished), the reactor data may be expressed in terms of total oyster weight including shell, as follows: $Y = 7.65 \times^{0.53}$

Y = algal cells cleared x 10^8/g total oyster weight including shell/day

X = g total oyster weight including shell

This relationship is applicable for oysters in the size range from 10 to 33 grams weight including shell at 20° - 22° C in the tubular reactor.

Our best estimate of the number of cells required to support the vigorous growth of small oysters (0.5 g total wt.) at 26° - 28° C is 10×10^8 cells/g oyster weight including shell/day. The number of algal cells required to support the conditioning of broodstock at 20° - 22° C is approximately 0.85×10^8 cells/g oyster weight including shell/day for 100 g size oysters. Data from feeding the juveniles (0.5 g) at 26° - 28° C, adults (100 g) at 20° - 22° C, and the 10-33 g oysters at 20° - 22° C from the tubular reactor are combined to yield summary expressions (Figure 5):

$Y = 7.6\ (x)^{-0.41}$ @ 26° - 28° C

$Y = 5.3\ (x)^{-0.41}$ @ 20° - 22° C

Y = algal cells cleared 10^8/g oyster weight including shell/day

X = g oyster weight including shell

For analytical purposes the $Y = 7.6\ (x)^{-0.41}$ expression for feeding rate should be used when projecting nine months to reach market size and $Y = 5.3\ (x)^{-0.41}$ expression for feeding rate used when projecting twelve months to reach market size.

REDUCTION IN MAKE-UP NUTRIENT COST

The addition of nutrients, trace metals, and vitamins to seawater to enhance the growth of desired algae is common laboratory practice. The "$f/_2$" enrichment, described by Guillard (1974), is widely used for this purpose in many parts of the world and success has been achieved in culturing even the most fastidious pelagic algae. The "$f/_2$" basic enrichment was used by Delaware investigators in culturing Carteria chuii, Croomonas salina, Isochrysis galbana, Monochrysis lutheri, Thalassiosira pseudonana, and Phaeodactylum tricornutum to support growth experiments on juvenile oysters Crassostrea virginica, and clams Mercenaria mercenaria.

The results of our studies, discussed in the previous section, indicate that the single algal species Thalassiosira pseudonana appears to support the rapid growth of oysters and affirm the central role to be played by T. pseudonana in maricultural development.

System optimization and cost reduction include the judicious addition of raw materials, particularly those representing out-of-pocket costs. Guillard (1975) encouraged us to consider the "$f/_2$" enrichment only as a starting point and to pursue the identification and satisfaction of the specific needs of Thalassiosira pseudonana. Clearly, the culture of T. pseudonana, as an integral part of a controlled environment molluscan mariculture system, differs from usual laboratory practice. The type and quantity of material (nutrients, etc.) added to the system must replace those utilized or discarded by the system to avoid unsatisfactory build-ups and/or deplations.

Using "$f/_2$" and standard techniques in culturing Thalassiosira pseudonana, we achieved cell doubling each six to eight hours when initial cell densities were below 1×10^5 cells/ml. Maximum cell concentration achieved in batch cultures ranged from 2.4×10^6 cells/ml to 3.0×10^6 cells/ml based upon Coulter Counter measurements. It was found that only 20 percent of the nitrogen was utilized by the algae.

Piecing together information from Guillard (1973), Tenore (1973), Goldman (1974), and Parsons (1961) we approximated the partial composition of Thalassiosira pseudonana.

dry weight	1.54×10^{-11} grams/cell
dry weight (ash free)	1.08×10^{-11} grams/cell
Carbon	0.54×10^{-11} grams/cell
Nitrogen	0.10×10^{-11} grams/cell
Silicon	0.13×10^{-11} grams/cell

At a cell density of 2.4×10^{9} cell/L the weight of nitrogen is 2.4 mg/L. The "$f/_2$" enrichment in comparison provides 12.35 mg/L nitrogen. It must be recognized that similar imbalances probably exist in phosphates, trace metals, and vitamins, and efforts are under way to identify and correct them.

It is important to consider the cost of alternate sources of raw materials. Nitrogen is an especially important consideration. The use of nitrogen occurring in human waste (Ryther 1975) and nutrients upwelled from the deep (Roels 1969), is being explored for maricultural purposes. At Delaware we use commercially available chemicals for nitrogen. The economic significance of dependence upon commercial sources is considered. How much nitrogen is required? -- in what form? -- and at what cost? -- needs to be answered. Reasonable estimates are sufficient to project the economic impact of nitrogen acquisition.

It is instructive to estimate the total number of Thalassiosira pseudonana cells required to grow an oyster from egg to market size. The total weight of individual oysters as a function of time was estimated over a 12-month period required to reach market size (Cyanamid, 1968). Using these data and combining them with the size-consumption relationship for 20° - 22° C adduced in the previous section, we make an estimate of the total number of cells consumed (see Table 1). The cumulative amount of algal cells cleared by an individual oyster growing from egg to market size in 12 months (20° - 22° C) is plotted in Figure 6.

One oyster in growing to market size will consume approximately 1.28×10^{12} cells of Thalassiosira pseudonana in 12 months at 20° - 22° C. For

TABLE 1

Age in Months (2)	End Period Wt. (3)	Period Aver. Wt.	Period	Cells/day (1) oyster	Total Cells clear/period	Cells cleared & accumulative
2	0.5 g	0.1 g	60 days	1.4×10^8	8×10^9	8×10^9
3	2.7	1.6	30 days	7.0×10^8	21×10^9	29×10^9
4	12.0	7.35	30 days	1.72×10^9	52×10^9	81×10^9
5	19	15.5	30 days	2.7×10^9	81×10^9	162×10^9
6	27.5	23.2	30 days	3.4×10^9	102×10^9	264×10^9
7	38	32.5	30 days	4.1×10^9	123×10^9	387×10^9
8	48	43.0	30 days	4.8×10^9	144×10^9	531×10^9
9	56	52.0	30 days	5.5×10^9	165×10^9	696×10^9
10	66	61.0	30 days	6.0×10^9	180×10^9	876×10^9
11	76	71.0	30 days	6.6×10^9	196×10^9	1072×10^9
12	85	80.5	30 days	7.1×10^9	213×10^9	1285×10^9

(1) using $Y = 5.3 (x)^{-0.41}$

(2) from egg to market size for individual oyster

(3) Cyanamid (1968)

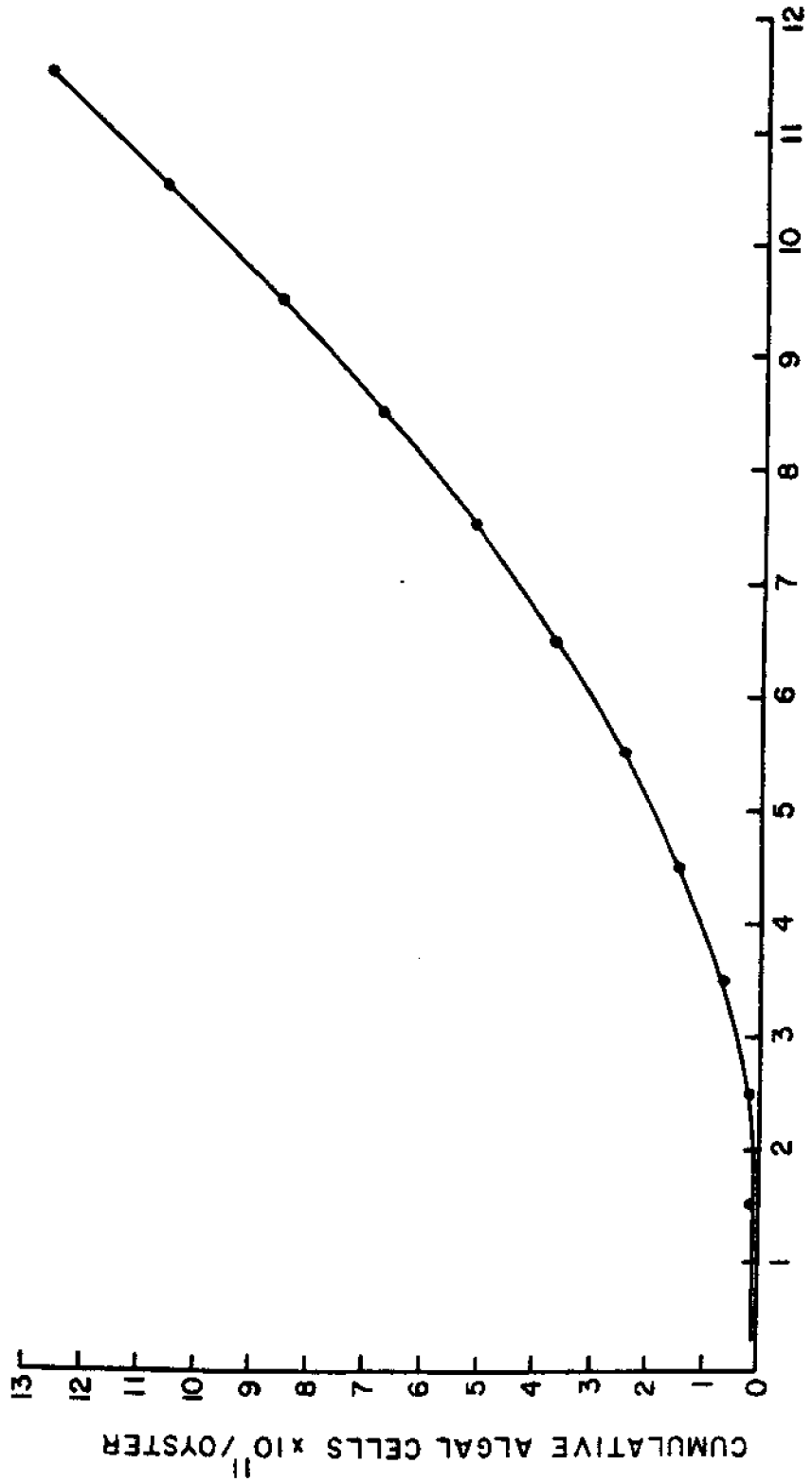

Fig. 6 Cumulative algal cells cleared by oyster growing from egg to market size in 12 months (20 - 22 °C)

cost comparison purposes, a bushel of 85 g oysters is assumed to contain about 300 individuals. Production of one bushel of oysters will require 3.8×10^{14} cells of T. pseudonana.

The particulate nitrogen supplied as algae per bushel of oyster is

$$3.8 \times 10^{14} \frac{\text{cells}}{\text{bushel}} \times .10 \times 10^{-11} \frac{\text{gram N}}{\text{cell}} = 3.8 \times 10^{2} = \frac{380 \text{ g N}}{\text{bushel}}$$

The cost to purchase inorganic nitrogen to supply this need depends upon the particular form selected.

Material	Cost/100#*	Cost Elemental N	Cost Elemental N/bushel
$NaNO_3$	\$22.00/100#	\$3.00/\$Kg	\$1.14/bushel
NH_4Cl	\$12.75/100#	\$1.08/\$Kg	\$ .41/bushel
NH_4NO_3	\$ 9.20/100#	\$0.60/\$Kg	\$ .23/bushel

The potential to recycle and further decrease the cost is made evident by the small percentage of N that is utilized by the oyster. An 85 gram oyster will yield 2.7 g of dry tissue weight of which 10% (0.27 g) is nitrogen. The shell contains ½ percent organics (0.35 grams), of which .03 g is nitrogen.

The intake is 380 g N per bushel and what is utilized is 90 g N per bushel yielding a conversion efficiency of 24.0 percent. This may suggest diet alteration. However, it is difficult to envision, with recycling, the nitrogen cost exceeding 10¢ per bushel. Urea is currently being evaluated for its combined CO_2 and N contribution.

Experience has shown that Thalassiosira pseudonana can utilize either NH_4 or NO_3 and can switch from one to the other. Thus, the general idea of the level of nitrogen oxidation in the nutrients supply is opened to economically meaningful exploration.

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*From Southern State Cooperative, Nassau, Delaware

APPLICATION OF FOAM FRACTIONATION

The primary purpose for including this section in this report is to recall some information potentially valuable for mariculture. We built and incorporated a foam fractionation device into our controlled environment system. The design of the device called a "protein skimmer" by National Fisheries Center personnel, was presented in a Department of Interior publication (Hagen, 1970) which is no longer in print. The device is promising and many requests for design information have been received in our laboratory. The design drawings are included in Figures 7 and 8, taken directly from the publication referenced above.

The device is under active development in our laboratory.

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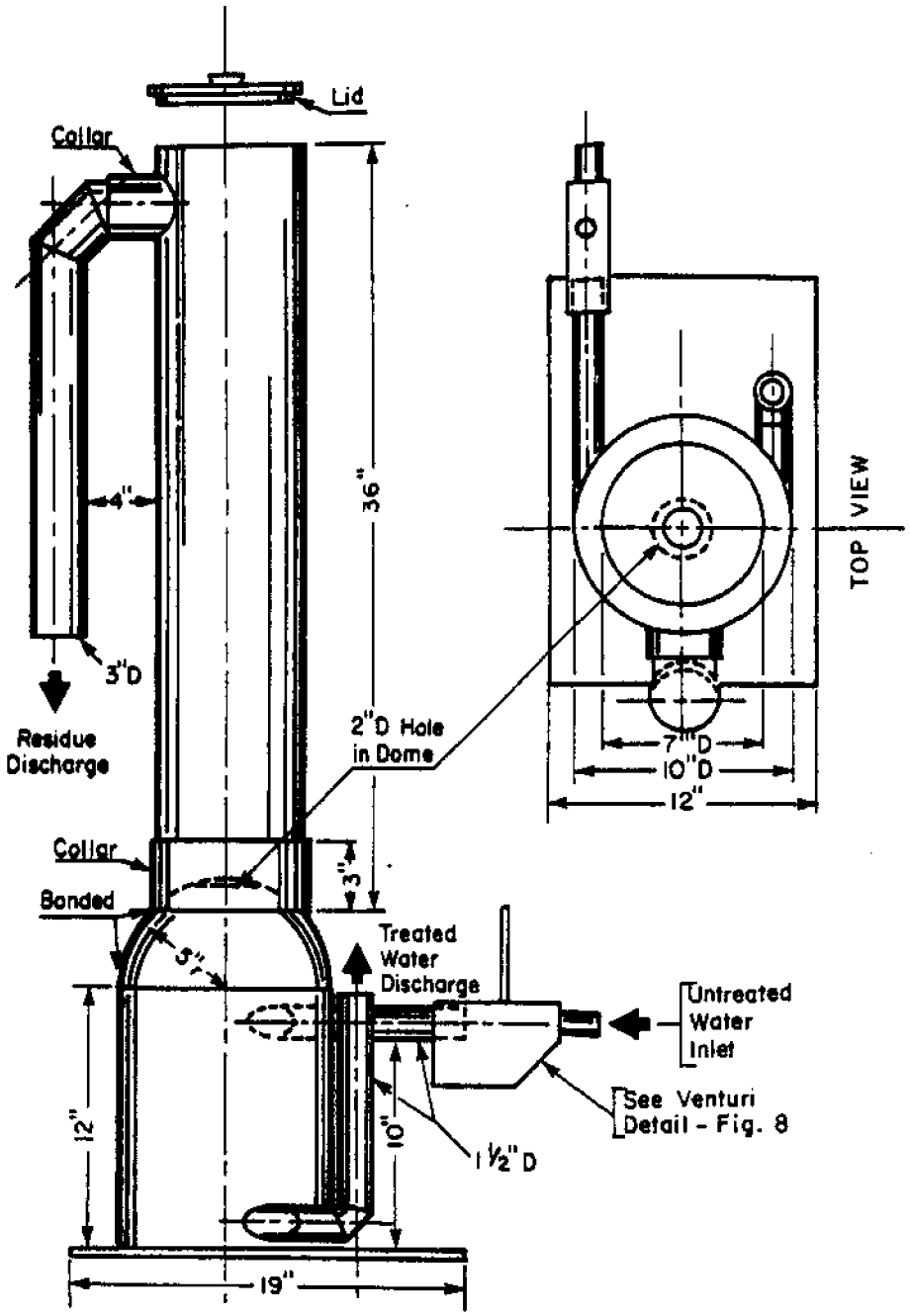

Fig. 7 Design of the protein skimmer (Hagen, 1970)

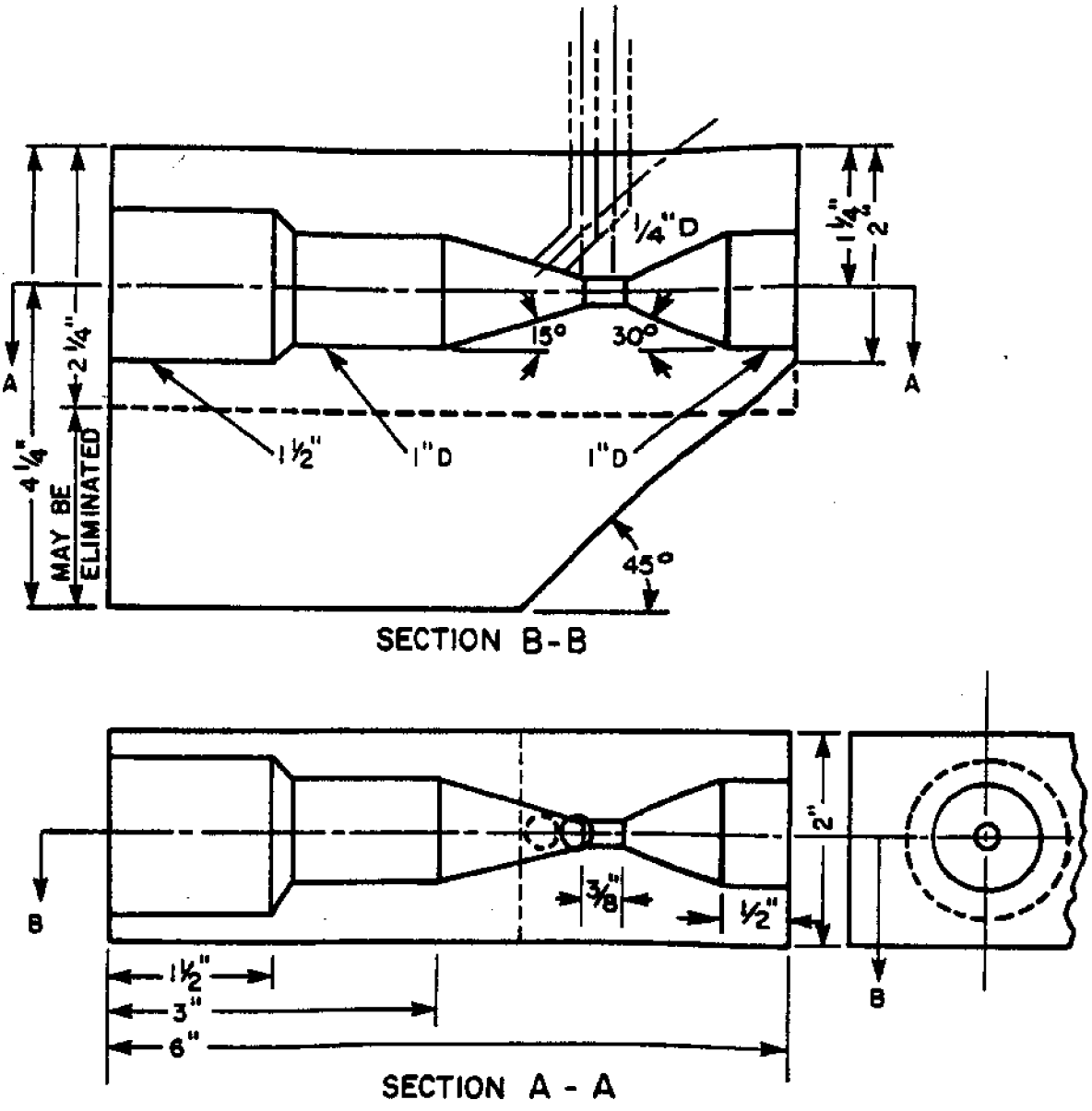

Fig. 8 Detail of the Venturi protein skimmer (Hagen, 1970)

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