NUTRITIONAL REQUIREMENTS FOR GAMETOGENESIS IN *LAMINARIA SACCHARINA* (L.) LAMOUROUX

by

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ABSTRACT

Several aspects in the development of gametophytes of Laminaria saccharina (L.) Lamouroux were examined in relation to selected nutrients. Growth, morphology, gametogenesis and metabolites were studied in different concentrations of nitrate, phosphate, iodide, iodate and chloride/iodide ratios in axenic culture, using a synthetic seawater medium under optimal light and temperature conditions.

Nitrate and phosphate were required for the various stages of gametophyte development and gametogenesis. Under nitrate and phosphate concentrations optimal for growth and gametogenesis maximum quantities of DNA, RNA, protein, carbohydrate, and chlorophylls a, c and low quantities of lipids were produced. Further, nutrient concentrations which produced high percentage fertility corresponded with high ratios of RNA/DNA and protein/RNA.

Iodide and iodate were not essential for antheridial and oogonial production, but a small addition of iodine had a stimulatory effect. At low concentrations, iodide was more effective than iodate. At high concentrations iodate had a less inhibitory effect than iodide. Inhibition occurred at

iodine concentrations 60,000 times greater than that in natural seawater. This inhibition diminished with increasing chloride concentration.

Antheridial production occurred under a wider range of nutrient concentrations than oogonial production. Further, percent fertility was greater for the male gametophyte. This indicated that the female gametophyte was the limiting agent in sexual fusion.

The phenology of *in situ* gametogenesis was monitored every 10 days for three years in Burrard Inlet, B.C., Canada. The surface water temperature, salinity, pH, nitrate, nitrite, phosphate, and total inorganic iodine were monitored weekly for the three years. The waters overlying natural *L. saccharina* populations were characterized as having considerable diurnal and seasonal variation in all the monitored properties.

In situ gametophytes, which developed from meiospores labelled with Calcofluor White, produced gametes throughout the year. Antheridial production was earlier than oogonial production. Gametogenesis took longer during the late autumn-early winter than the rest of the year. On the basis of the nutritional studies and the seasonal distribution of nutrients

the longer period required for gametogensis during the late autumn-early winter was not due to nutrient deficiency but most likely temperature and light.

Gametogenesis did not limit production of new macroscopic sporophyte generations; rather the limiting action was the response of microscopic sporophytes to their environment.

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CHAPTER I

GENERAL INTRODUCTION

Efforts to increase seaweed production has led to an interest in the mariculture of Laminariales. Definition of optimal and inhibitory growth conditions of the gametophyte - the sexual stage - must be known if successful mariculture is to be achieved by other than trial-and-error methods.

Numberous studies have been made on the response of laminarialean gametophytes to culture conditions. These include light intensity (Saito, 1956b; Segi and Kida, 1957, 1958; Hasegawa, 1962; Kain, 1964; Yabu, 1964), light quality (Harries, 1932; Saito, 1956a; Anderson and North, 1966), temperature (Saito, 1956a, b; Kain, 1964; Yabu, 1964; Druehl, 1967), inorganic nutrients (Harries, 1932; Yabu, 1964) and orange juice (Carter, 1935).

In none of the above mentioned studies have defined media or axenic cultures been employed. Microorganism contaminates may compete for or alter the nutrients and produce inhibitory or stimulative substances thus changing the culture environment. The use of natural seawater or fortified seawater precludes knowledge of the organic and inorganic substances

in the culture system. Thus, the significance of nutrient studies is greatly enhanced when axenic culture in defined media are used.

In studies preparatory to the investigation, a means of obtaining axenic cultures for the Laminariales was developed (Druehl and Hsiao, 1969). A defined synthetic seawater medium, suitable for the culture of Laminariales, was obtained by modification of ASP 2 (Provasoli et al, 1957).

The present investigation explored, for the first time, the effects of selected inorganic nutrients on gametophyte development and gametogenesis in defined media under axenic conditions.

Parallel to this investigation a study of natural variation of selected environmental parameters and the gametophyte response to these in situ conditions was conducted.

This thesis investigates four problems:

1. How do various nitrate and phosphate concentrations affect morphology, gametogenesis and metabolite production of gametophytes (Chapter II)?

2. How do various forms and concentrations of iodine affect morphology, gametogenesis and metabolite production of gametophytes? And are these effects altered by different

chlorinities (Chapter III)?

3. What is the natural variation in seawater temperature, salinity, pH, nitrate, nitrite, phosphate and iodine concentrations in an environment occupied by L. saccharina (Chapter IV)?

4. When during the year are gametophytes fertile in their natural environment (Chapter V)?

Chapter VI is a brief summary in which I discuss the interrelated aspects of the above mentioned problems.

CHAPTER II

CORRELATION OF NITRATE AND PHOSPHATE CONCENTRATIONS WITH GAMETOGENESIS AND SELECTED METABOLITES OF L. SACCHARINA

INTRODUCTION

All biological growth processes require nitrogen for the synthesis of cellular proteins, amino acids, purines, pyrimidines, and nucleic acids, since nitrogen is an important constituent in enzymes and many of their substrates. Phosphorus participates in an essential way in a number of cell functions, including photosynthesis, glycolysis, oxidative phosphorylation, protein synthesis and nucleic acid synthesis (Katchman, 1961).

It is well documented that nitrogen and phosphorus concentrations correlate with phytoplankton growth (Harvey, 1940; Jeener, 1953; Ketchum et al, 1958). In nitrogen deficient cultures, chlorophyll concentrations decrease (Ketchum et al, 1958; Fogg, 1959; Newmark and Fujimoto, 1959; Richardson et al, 1969) and carotenoid content is reduced (Ketchum et al, 1958; Fogg, 1959). Cell division and photosynthesis (Maslov, 1969) and protein synthesis (Fogg, 1956; Klyachko-Gurvich and Zhukova, 1966) are inhibited. Several studies have shown

that carboydrates and fats are accumulated in nitrogen deficinet cultures (Maslov, 1968; Richardson et al, 1969; Trukhin, 1968; Zhukova et al, 1969). Phosphorus deficiency in cultures causes cessation of cell division (Thomas and Dobson, 1968; Maslov, 1969) and breakdown of protein synthesis (Bezlyudnyi and Belenkevick, 1970). There is also a decline in chlorophyll concentration, a decrease of chloroplast numbers, suppressed photosynthesis and accumulation of a large amount of fat (Epstein and Allaway, 1967; Kuenzler and Ketchum, 1962; Maslov, 1969; Round, 1965).

Ribonucleic acid (RNA) is a necessary precursor to protein synthesis. It has been used as a growth indicator in microorganisms (Maaløe and Kjeldgaard, 1966; Leick, 1968), marine zooplankton (Sutcliffe, 1965) and invertebrates (Sutcliffe, 1970). Davidson and Leslie (1950) used deoxyribonucleic acid (DNA) as a measure of cell multiplication, and as a standard of reference in the expression of biochemical changes in cell growth and differentiation. The ratios RNA/DNA and RNA/protein are linear functions of the growth rate in bacterial cells (Neidhardt and Magasanik, 1960; Neidhardt, 1964; Leick, 1968), a colorless alga (Jeneer, 1953), and fish (Bulow, 1970).

Concentrations of DNA, RNA and protein in cultured microorganisms are quantitatively affected by the chemical composition of the culture media (Schaechter et al, 1958; Neidhardt and Magasanik, 1960; Kjeldagaard, 1961; Wacker, 1962; Rosset et al, 1966; Pogo et al, 1966; Epstein and Allaway, 1967; Carell et al, 1970). Nitrogen deficiency causes a decrease of RNA in Chlorella (Newmark and Fujimoto, 1959) and marked decrease in the RNA and DNA of Monodus subterranius cells (Fogg, 1959). Phosphorus deficiency decreases the growth rate and RNA concentrations in Polytomella coeca (Jeener, 1952), reduces the amount of DNA and RNA in Euglena gracilis (Epstein and Allaway, 1967). Deficiency of phosphorus also inhibits the rates of DNA, RNA and protein synthesis in E. gracilis (Parenti et al, 1971).

Zhukova et al (1969) showed that when Chlorella cells were grown in nitrogen deficient medium, lipids accumulated accompanied by intensified division and tiny cells were formed at the initial stage of nitrogen deficiency. When biosynthesis was directed toward carbohydrate formation, large cells were formed.

In all these studies bacterial cells and unicellular algae were used. The purpose of the present study was to

determine the effect of various concentrations of nitrogen and phosphorus on DNA, RNA, protein, carbohydrate, lipid and pigment concentrations in the gametophytes of L. saccharina. In addition meiospore germination, gametophyte development, morphology and gametogenesis were determined under various concentrations of nitrate and phosphate.

MATERIALS AND METHODS

Axenic meiospore suspension

Mature sori of L. saccharina were collected from Lumberman's Arch, Burrard Inlet (49°18'10" N, 123°07'32" W) at the 0.1 foot tide level. Axenic meiospore suspension was obtained according to Druehl and Hsiao's technique (1969). A sample of the axenic meiospores was counted using a hemacytometer (American Optical Co.). The meiospore density was then adjusted by dilution of ASP 2M basal medium to give a uniform suspension of 1.2×10^5 meiospores/ml. Volumetric 1-ml inoculations were taken from the thoroughly stirred spore suspension to assure a uniform spore inoculum of 1.2×10^5 meiospores in 15 ml test media.

Basal medium

The basal medium based on ASP 2M (Druehl and Hsiao, 1969)

was prepared without nitrate, phosphate and iodide. Test media were then made up with different concentrations of the test nutrient.

Culture apparatus and conditions

Each culture tube (180 x 15 mm) containing two glass slides (76 x 13 mm) and capped with white plastic caps were used as culture vessels. The glass slides provide a substrate for meiospore attachment and were used for monitoring meiospore germination and gametophyte development.

The cultures were incubated in Psycrotherm incubators (New Brunswick Scientific Co.) under optimal conditions for gametogenesis: 860 lx, 18 h photoperiod and 10 C (Hsiao and Druehl, 1971).

Every 10 days sterility tests were done as described by Druehl and Hsiao (1969), and the media changed. All cultures were shaken continuously starting three days after meiospore inoculation.

Evaluation of meiospore germination and fertility

Three days after inoculation duplicate cultures were fixed by adding 0.5 ml of IKI solution (Hsiao and Druehl, 1971). More than 300 meiospores on each slide were scored as having germinated or not on the basis of germ tube production.

Every 10 days one set of cultures was preserved and later observed for gametophyte development. Six weeks after inoculation, all plants of duplicate cultures were fixed by adding 1 ml of concentrated formalin. Later, the number of cells, oogonia and antheridia per gametophyte and percentage of fertile gametophytes to the total number of gametophytes were determined for more than 100 male and female plants.

Determination of metabolites

L. saccharina gametophytes were harvested from 6-week old cultures for the various tests as follows: 2 sets of 5 duplicates for DNA, RNA and protein; duplicates for carbohydrates; 2 sets of duplicates for pigments and 2 sets of quadruplicates for lipids. A large number of replicates were required to make up a significant quantity of gametophytes for analysis of the various metabolites.

The gametophytes were scraped from the glass slides with a rubber spatula and centrifuged. After centrifugation the medium was removed with a micropipette attached to a vacuum pump. The algae were washed once with ASP 2M basal medium and prepared for assay of DNA, RNA and protein based on the method of Smillie and Krotkov (1960). Each sample was extracted twice successively with 10 ml of cold methanol containing 0.05 M formic acid, cold 5% trichloracetic acid,

cold 95% ethanol, boiling 95% ethanol, boiling ethanol-ether mixture (2:1) and boiling ether. After each treatment, the samples were centrifuged for 10 min. The dried residue from the ether washing was extracted with 5 ml of 5% perchloric acid at 90 C for 15 min. After centrifugation the supernatant was assayed for DNA by diphenylamine test and for RNA by the orcinol method (Schneider, 1957); the precipitate was resuspended in 1 ml of 1 N NaOH for 60 min at room temperature, and then assayed for protein by the method of Lowry et al (1951). Pigments, total lipids and total carbohydrates were determined by the methods of Strickland and Parsons (1968).

RESULTS

The nitrate and phosphate experiments on L. saccharina gametophytes were carried out in October, 1969 and February, 1970. There was no significant difference between the results on the two occasions, and therefore the data have been averaged.

The effect of nitrate and phosphate on meiospore germination

Percent germination of L. saccharina meiospores increased with increasing phosphate concentrations up to 15 μg-at/l in

all nitrate concentrations tested (Table 1). All phosphate concentrations tested with nitrate concentrations 5 and 80 μg-at/l promoted germination.

The effect of nitrate and phosphate on gametophyte morphology, development and gametogenesis

Considerable variation in gametophyte morphology was observed between various nitrogen and phosphorus treatments (Fig. 1 A-K).

In nitrogen- and phosphorus-free medium, the meiospores developed into gametophytes with a few cells. By the end of the 6-week experiment, these plants had produced neither antheridia nor oogonia (Fig. 1 A). In the phosphorus-free medium, male gametophytes produced antheridia only with nitrate at 80 μg-at/l or higher but they produced antheridia in a nitrogen-free medium with phosphate at 20 μg-at/l or higher (Table 2). The female gametophytes did not produce oogonia in these conditions, neither in a phosphorus-free medium with nitrate up to 160 μg-at/l, nor in a nitrogen-free medium with phosphate above 25 μg-at/l. In high concentrations of nitrate and phosphate, the meiospores developed into gametophytes

Table 1. Effect of nitrate and phosphate on percentage meiospore germination of Laminaria saccharina (-- = no test).

NO_3-N µg-at/l	PO_4-P µg-at/l										
	0	1	3	5	7	10	15	20	25	28.7	57.4
0	80	80	81	81	82	82	82	83	85	--	--
5	80	81	82	82	82	83	84	--	--	--	--
10	80	81	82	82	83	84	86	--	--	89	97
20	81	82	82	84	83	85	87	88	88	--	--
30	82	82	83	84	84	85	87	--	--	--	--
40	82	84	83	84	85	86	89	88	89	90	94
80	82	--	--	--	--	86	90	92	91	--	--
160	84	85	--	--	--	89	91	93	92	91	88
588.3	--	87	--	--	--	--	97	--	--	90	84
1176.6	--	87	--	--	--	--	96	--	--	91	82

Figure 1 A-K. Examples of morphological variability in Laminaria saccharina gametophytes grown in ASP 2M basal medium plus 50 μg I^--I/l as KI and with different concentrations of nitrate and phosphate observed at the end of six weeks culture.

A. Nitrogen- and phosphorus-free medium

B. 40 μg-at NO_3-N/l and 1 μg-at PO_4-P/l

C. 160 μg-at NO_3-N/l and 1 μg-at PO_4-P/l

D. 588.3 μg-at NO_3-N/l and 1 μg-at PO_4-P/l

E. 1176.6 μg-at NO_3-N/l and 1 μg-at PO_4-P/l

F. 10 μg-at NO_3-N/l and 28.7 μg-at PO_4-P/l

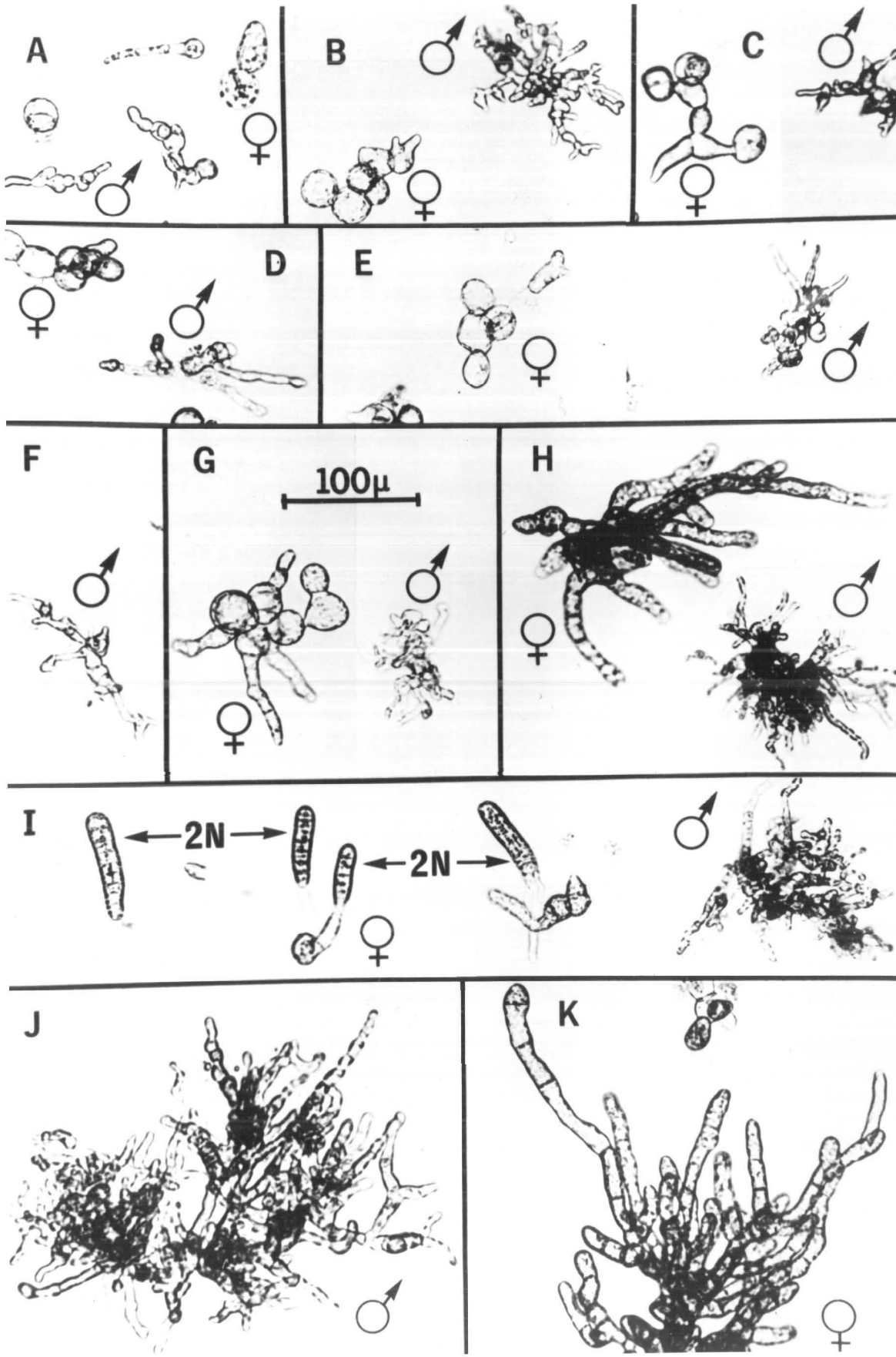
G. 40 μg-at NO_3-N/l and 10 μg-at PO_4-P/l

H. 160 μg-at NO_3-N/l and 10 μg-at PO_4-P/l

I. 588.3 μg-at NO_3-N/l and 15 μg-at PO_4-P/l

J. 1176.6 μg-at NO_3-N/l and 15 μg-at PO_4-P/l

K. 1176.6 μg-at NO_3-N/l and 28.7 μg-at PO_4-P/l


A
B
C
D
E
F
G
100μ
H
I
2N
2N
J
K

with more cells (Fig. 2 and 3) and profuse branching (Fig. 1 J, K). The lowest combined nitrogen-phosphorus concentration which produced oogonia was 5 μg-at NO_3-N/l and 10 μg-at PO_4-P/l. Antheridia however were produced with 5 μg-at NO_3-N/l and 5 μg-at PO_4-P/l (Table 2).

The percentage fertility of male gametophytes was greater than female in all test media (Fig. 4 and 5). Generally, a greater percentage fertility for both male and female gametophytes was obtained as concentrations of nitrate and phosphate were increased. But in media with concentrations above 588.3 μg-at NO_3-N/l and 15 μg-at PO_4-P/l, the male gametophytes were slightly inhibited in antheridial production and the female gametophyte greatly inhibited in oogonial production.

The ratio of female to male gametophytes (both fertile and sterile) in the various concentrations of nitrate and phosphate ranged from 0.91 to 1.11 (Table 2). The average ratio was 0.98 for all concentrations used.

The effects of nitrate and phosphate on selected metabolites

The following data are based on the amount of metabolite produced per culture, i.e. by 1.2×10^5 meiospores after 6 weeks culturing.

Figure 2. Number of cells per male gametophyte of Laminaria saccharina in different nitrate and phosphate concentrations. Vertical bars denote 99% confidence limits.

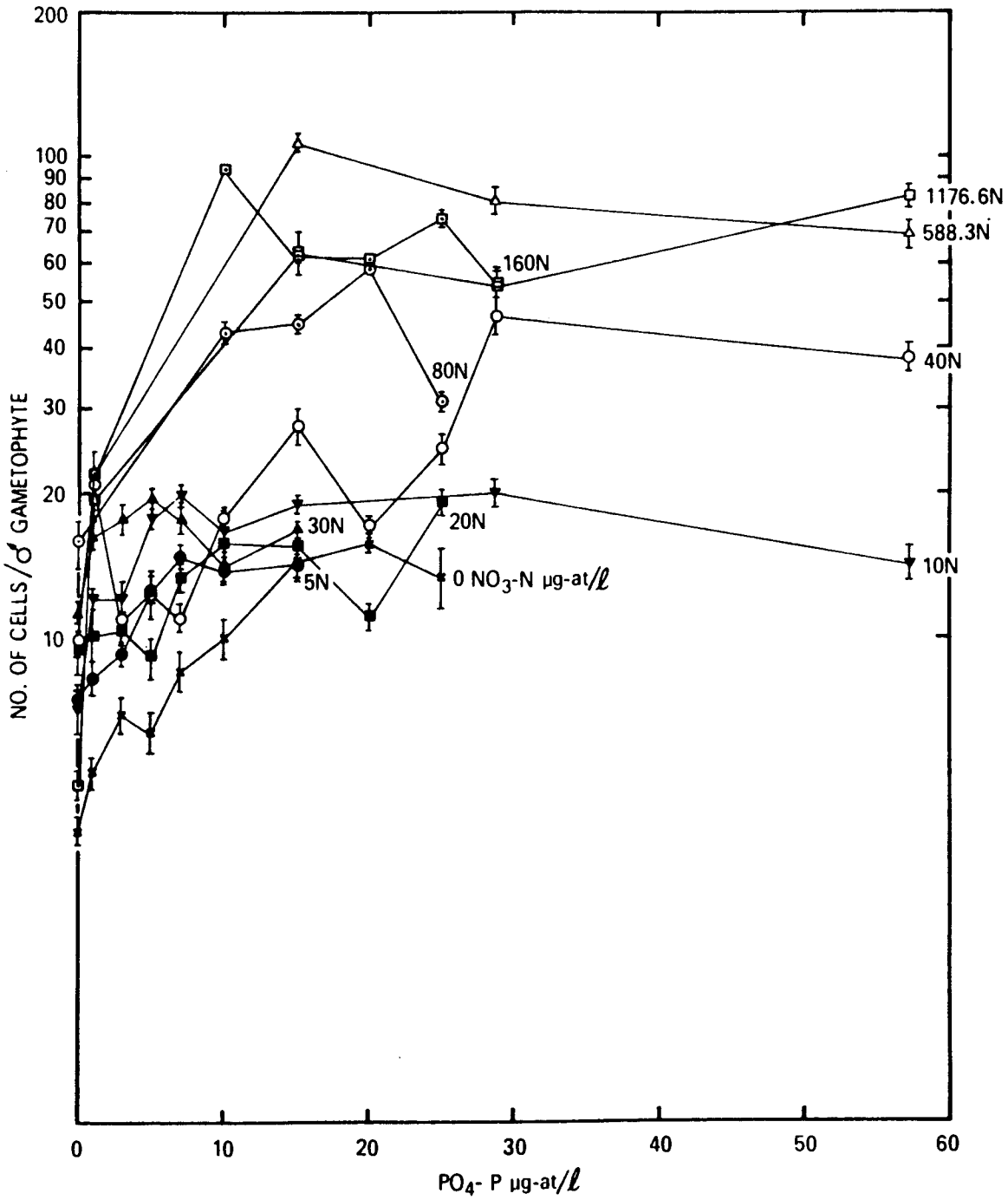

200
100
90
80
70
60
50
40
30
20
10
NO. OF CELLS/♂ GAMETOPHYTE
0
10
20
30
40
50
60
PO4- P µg-at/ℓ
1176.6N
588.3N
160N
80N
40N
30N
20N
10N
5N
0 NO3-N µg-at/ℓ

Figure 3. Number of cells per female gametophyte of Laminaria saccharina in different nitrate and phosphate concentrations. Vertical bars denote 99% confidence limits.

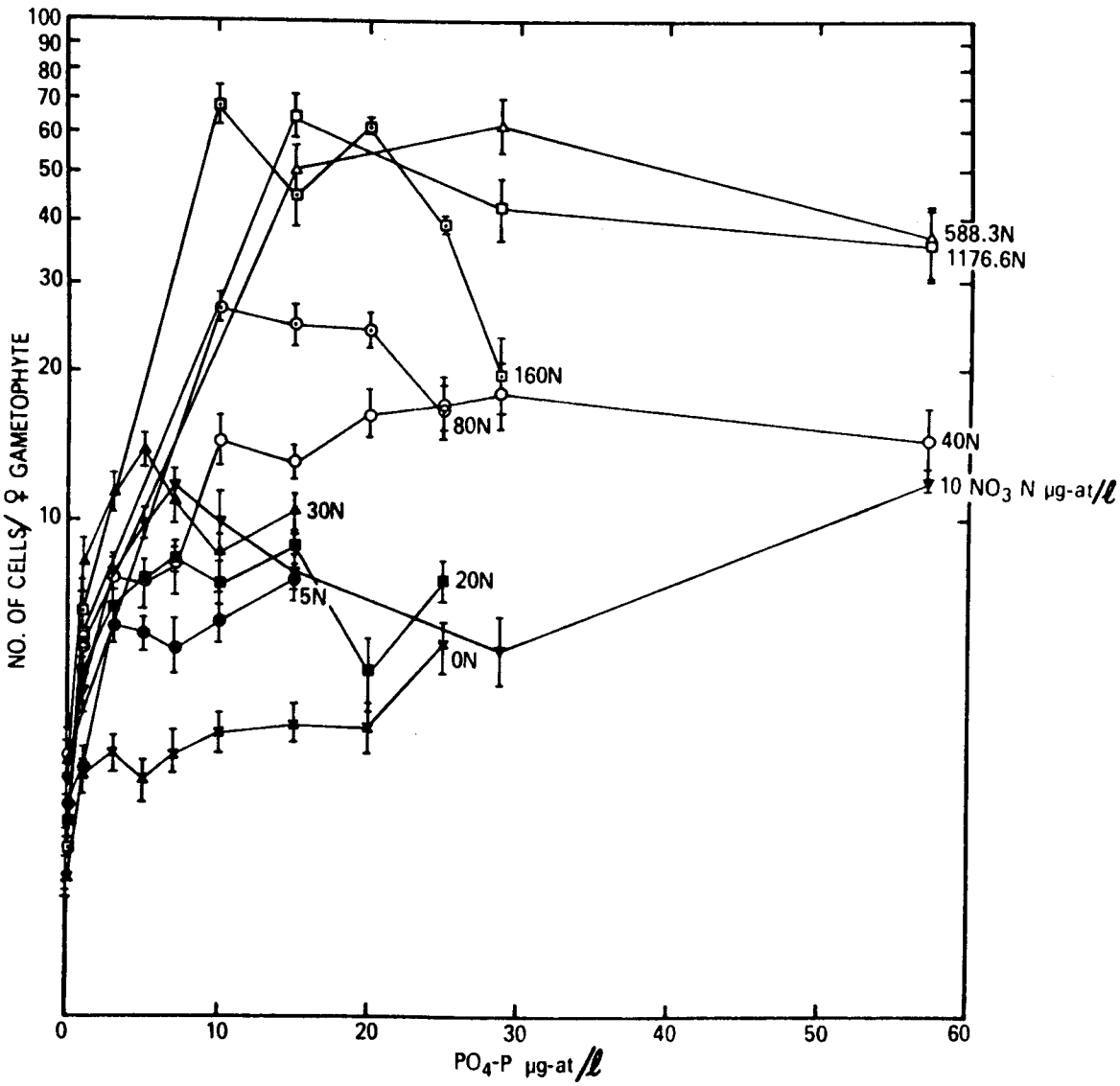

NO. OF CELLS/ ♀ GAMETOPHYTE
100
90
80
70
60
50
40
30
20
10
0
10
20
30
40
50
60
PO4-P µg-at/ℓ
588.3N
1176.6N
160N
80N
40N
10 NO3 N µg-at/ℓ
30N
20N
5N
0N

Table 2. The ratio of female to male gametophytes (fertile and sterile) and the percentage distribution of oogonia and antheridia produced per gametophyte for Laminaria saccharina in all nitrate and phosphate concentrations.

Nitrate and phosphate concentrations µg-at/l	Total ♀/♂	No. of oogonia/Gametophyte							No. of antheridia/Gametophyte							
		0	1	2	3	4	5	6	0	1	2	3	4	5	6	7-11
0 N 0 P	1.01	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0
0 N 1 P	0.98	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0
0 N 3 P	0.98	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0
0 N 5 P	1.04	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0
0 N 7 P	0.97	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0
0 N 10 P	1.02	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0
0 N 15 P	0.98	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0
0 N 20 P	1.04	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0
0 N 25 P	0.97	100	0	0	0	0	0	0	91	9	0	0	0	0	0	0
5 N 0 P	0.95	100	0	0	0	0	0	0	89	11	0	0	0	0	0	0
5 N 1 P	0.96	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0
5 N 3 P	1.03	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0
5 N 5 P	1.07	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0
5 N 7 P	0.98	100	0	0	0	0	0	0	80	18	2	0	0	0	0	0
5 N 10 P	1.11	98	2	0	0	0	0	0	72	15	8	5	0	0	0	0
5 N 15 P	1.04	98	2	0	0	0	0	0	68	17	11	4	0	0	0	0
10 N 0 P	0.97	100	0	0	0	0	0	0	61	8	16	10	4	1	0	0
10 N 1 P	0.95	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0
10 N 3 P	0.95	100	0	0	0	0	0	0	86	9	2	2	1	0	0	0
10 N 5 P	1.07	99	1	0	0	0	0	0	85	13	2	0	0	0	0	0
10 N 7 P	1.09	99	1	0	0	0	0	0	72	23	4	1	0	0	0	0
10 N 10 P	0.91	98	2	0	0	0	0	0	59	22	13	6	0	0	0	0
10 N 15 P	1.01	98	2	0	0	0	0	0	52	22	10	10	4	2	0	0
10 N 28.7P	1.02	97	2	1	0	0	0	0	44	11	20	16	4	3	1	1
10 N 57.4P	1.06	95	5	0	0	0	0	0	44	8	18	21	2	4	1	2
									20	16	16	28	20	0	0	0

Table 2 (cont'd)

Nitrate and phosphate concentrations µg-at/l	Total ♀/♂	No. of oogonia/Gametophyte							No. of antheridia/Gametophyte							
		0	1	2	3	4	5	6	0	1	2	3	4	5	6	7-11
20 N 0 P	0.96	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0
20 N 1 P	0.98	100	0	0	0	0	0	0	79	19	2	0	0	0	0	0
20 N 3 P	0.97	98	2	0	0	0	0	0	68	30	2	0	0	0	0	0
20 N 5 P	1.03	98	2	0	0	0	0	0	50	34	8	8	0	0	0	0
20 N 7 P	1.05	98	2	0	0	0	0	0	45	30	16	8	1	0	0	0
20 N 10 P	1.03	97	3	0	0	0	0	0	41	23	21	14	1	0	0	0
20 N 15 P	0.98	97	3	0	0	0	0	0	37	19	19	14	8	2	1	0
20 N 20 P	0.96	96	3	1	0	0	0	0	35	18	17	14	13	2	1	0
20 N 25 P	1.03	96	2	2	0	0	0	0	33	4	23	12	14	9	3	2
30 N 0 P	0.98	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0
30 N 1 P	1.05	98	2	0	0	0	0	0	62	26	6	4	2	0	0	0
30 N 3 P	0.95	97	2	1	0	0	0	0	49	18	19	8	5	1	0	0
30 N 5 P	0.98	94	4	2	0	0	0	0	40	21	15	14	8	2	0	0
30 N 7 P	0.97	94	6	0	0	0	0	0	35	17	15	15	9	5	3	1
30 N 10 P	1.02	94	5	1	0	0	0	0	30	17	18	21	10	3	1	0
30 N 15 P	0.96	94	4	2	0	0	0	0	25	10	9	15	5	12	12	12
40 N 0 P	0.98	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0
40 N 1 P	1.03	97	3	0	0	0	0	0	4	13	21	23	20	12	7	0
40 N 3 P	0.97	95	5	0	0	0	0	0	5	8	29	31	16	8	3	0
40 N 5 P	1.08	94	5	1	0	0	0	0	4	7	3	16	24	17	16	13
40 N 7 P	0.98	94	6	0	0	0	0	0	4	6	7	8	5	1	20	49
40 N 10 P	1.06	93	6	1	0	0	0	0	5	7	2	7	7	21	16	35
40 N 15 P	0.99	92	6	1	1	0	0	0	3	4	4	7	13	18	13	38
40 N 20 P	0.95	90	7	2	1	0	0	0	2	3	7	10	17	18	7	36
40 N 25 P	1.02	91	5	1	2	1	0	0	2	2	5	2	7	16	13	53
40 N 28.7P	0.96	75	18	6	1	0	0	0	3	2	6	11	8	15	13	42
40 N 57.4P	1.04	54	21	7	2	2	4	0	2	0	17	12	24	10	17	18

Table 2 (cont'd)

Nitrate and phosphate concentrations µg-at/l	Total ♀/♂	No. of oogonia/Gametophyte							No. of antheridia/Gametophyte							
		0	1	2	3	4	5	6	0	1	2	3	4	5	6	7-11
80 N 0 P	0.95	100	0	0	0	0	0	0	96	4	0	0	0	0	0	0
80 N 10 P	0.97	76	22	2	0	0	0	0	4	42	23	21	10	0	0	0
80 N 15 P	1.06	73	21	4	2	0	0	0	4	42	29	14	7	3	1	0
80 N 20 P	0.99	69	25	6	0	0	0	0	4	38	32	16	7	2	1	0
80 N 25 P	0.96	65	27	8	0	0	0	0	3	29	20	23	16	9	0	0
160 N 0 P	1.02	100	0	0	0	0	0	0	94	6	0	0	0	0	0	0
160 N 1 P	1.04	87	10	3	0	0	0	0	4	26	34	28	2	4	2	0
160 N 10 P	0.98	70	21	6	2	1	0	0	4	39	19	26	7	5	0	0
160 N 15 P	0.96	58	32	7	2	1	0	0	2	27	38	22	6	4	1	0
160 N 20 P	1.04	58	31	9	2	0	0	0	3	28	44	9	9	4	3	0
160 N 25 P	1.02	58	30	10	2	0	0	0	3	13	16	26	21	16	5	0
160 N 28.7P	1.06	57	30	9	3	1	0	0	4	4	15	33	19	19	4	2
160 N 57.4P	1.02	55	22	11	5	4	2	1	2	5	5	28	19	28	9	4
588.3N 1 P	0.98	85	13	2	0	0	0	0	3	22	27	19	19	8	2	0
588.3N 15 P	1.03	13	27	33	20	4	3	0	0	2	5	2	21	19	10	41
588.3N 28.7P	1.03	33	38	17	8	2	1	1	0	0	3	17	17	35	14	14
588.3N 57.4P	1.05	57	22	19	1	1	0	0	1	4	6	19	10	21	14	25
1176.6N 1 P	0.97	86	14	0	0	0	0	0	4	17	25	23	19	8	4	0
1176.6N 15 P	0.95	17	31	27	17	7	1	0	0	2	0	5	8	15	13	57
1176.6N 28.7P	1.02	35	34	15	11	4	1	0	2	2	5	20	18	29	16	8
1176.6N 57.4P	0.98	56	22	17	2	2	1	0	3	8	4	20	12	20	12	21

Figure 4. Effect of nitrate and phosphate concentrations on percentage fertility of Laminaria saccharina male gametophytes.

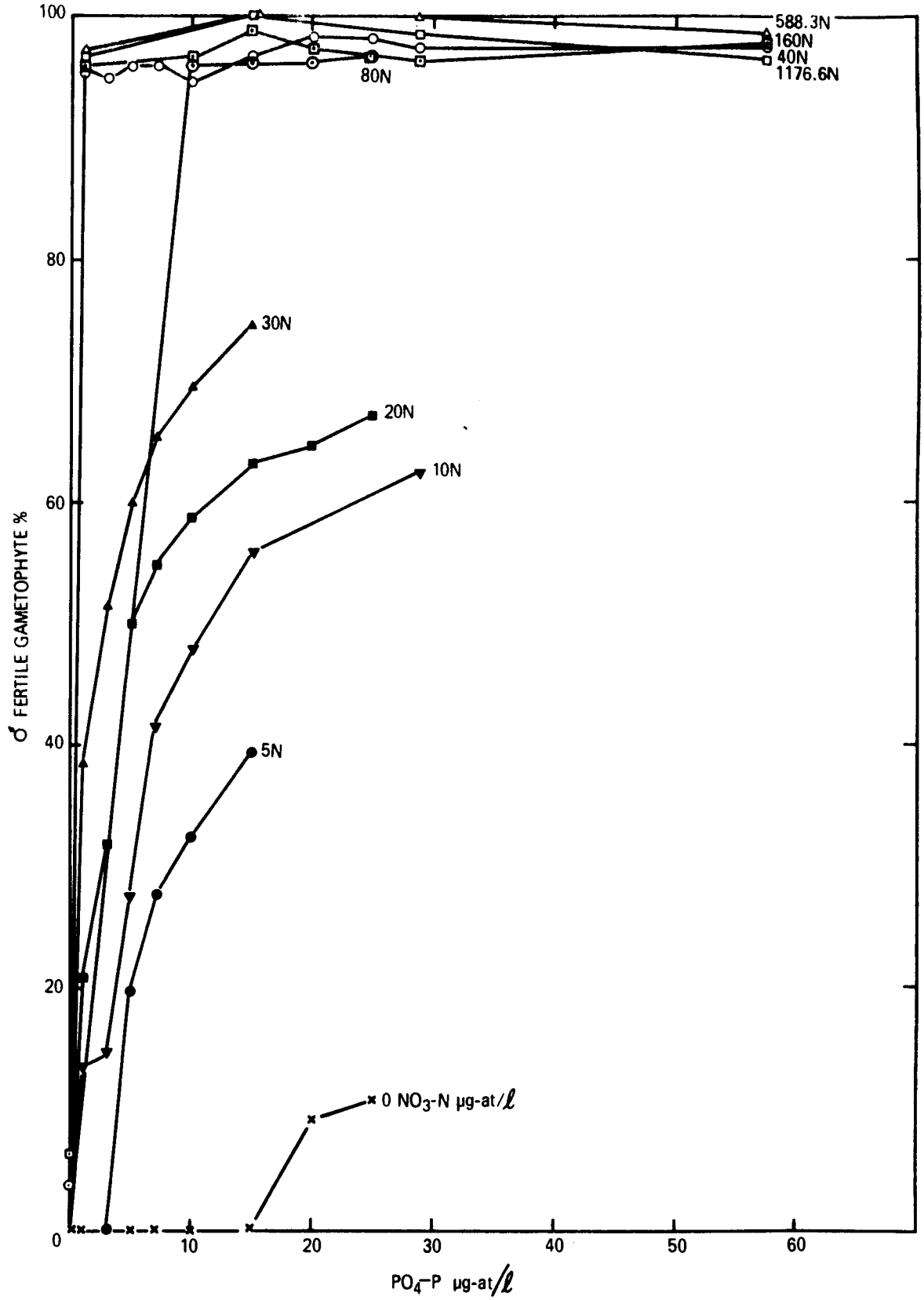
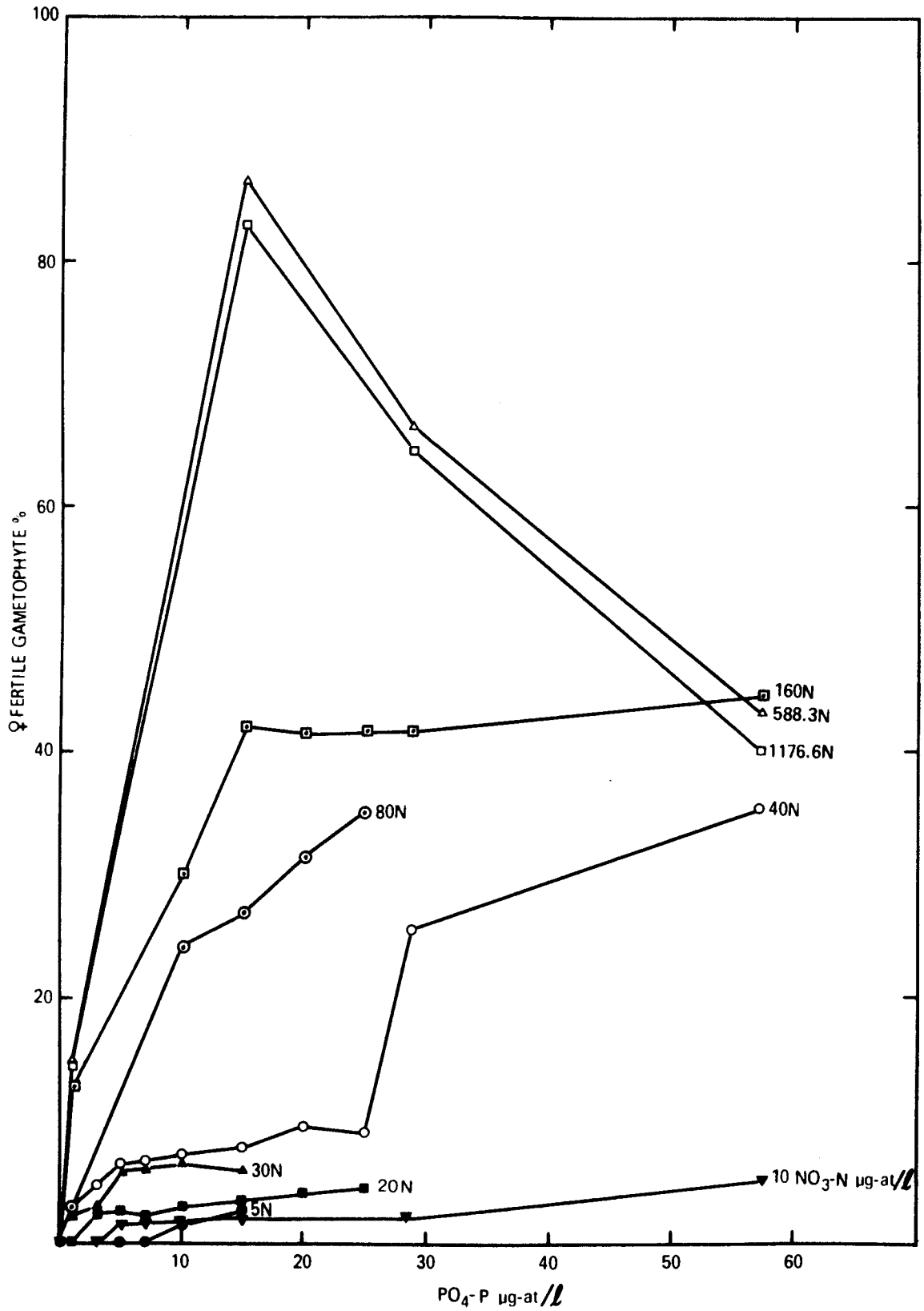

100
80
60
40
20
0
10
20
30
40
50
60
♂ FERTILE GAMETOPHYTE %
PO4-P µg-at/ℓ
588.3N
160N
40N
1176.6N
80N
30N
20N
10N
5N
0 NO3-N µg-at/ℓ

Figure 5. Effect of nitrate and phosphate concentrations on percentage fertility of Laminaria saccharina female gametophytes.


100
80
60
40
20
♀FERTILE GAMETOPHYTE %
160N
588.3N
1176.6N
80N
40N
30N
20N
5N
10 NO3-N µg-at/ℓ
10
20
30
40
50
60
PO4-P µg-at/ℓ

The effects of varying concentrations of nitrate and phosphate on concentrations of DNA, RNA, protein and carbohydrate were very similar (Table 3, 4, 5, and 6). The optimal nitrate-phosphate concentration for production of these metabolites was 588.3 μg-at NO_3-N/l and 15 μg-at PO_4-P/l. At the maximum concentrations of nitrate and phosphate the concentration of all four metabolites decreased.

RNA/DNA ratios increased with increasing phosphate concentrations up to 15 μg-at/l in all nitrate concentrations (Fig. 6). The greatest RNA/DNA ratio occurred in the culture of 588.3 μg-at NO_3-N/l with 15 μg-at PO_4-P/l. At phosphate concentrations higher than 15 μg-at/l, the ratios steadily increased with increasing nitrate up to 40 μg-at/l; then decreased with higher nitrate concentrations except the cultures of 160 μg-at/l.

Protein/RNA ratios were low at concentrations of nitrate below 80 μg-at/l in all phosphate concentrations (Fig. 7). They increased with greater concentrations of nitrate and phosphate. The greatest ratio was obtained at 160 μg-at NO_3-N/l and 10 μg-at PO_4-P/l; it slightly decreased with phosphate higher than 10 μg-at/l. The protein/RNA ratios in the cultures of 588.3 μg-at NO_3-N/l increased with increasing phosphate up

Table 3. Effect of nitrate and phosphate concentrations of DNA concentration (μg/culture) of Laminaria saccharina gametophytes (-- = no test).

NO_3-N μg-at/l	PO_4-P μg-at/l										
	0	1	3	5	7	10	15	20	25	28.7	57.4
0	0.81	0.81	0.82	0.81	0.82	0.82	0.83	0.85	0.89	--	--
5	0.85	0.86	0.88	0.87	0.89	0.88	0.89	--	--	--	--
10	0.92	0.94	0.98	1.00	1.02	1.02	1.04	--	--	1.09	1.21
20	0.90	0.95	1.00	1.05	1.05	1.10	1.12	1.21	1.29	--	--
30	0.95	1.00	1.00	1.08	1.10	1.10	1.25	--	--	--	--
40	1.00	1.14	1.03	0.98	1.15	1.12	1.13	1.22	1.25	1.28	1.33
80	1.03	--	--	--	--	1.29	1.32	1.41	1.48	--	--
160	1.08	1.29	--	--	--	1.32	1.42	1.47	1.50	1.55	1.59
588.3	--	1.32	--	--	--	--	1.64	--	--	1.58	1.53
1176.6	--	1.13	--	--	--	--	1.45	--	--	1.49	1.42

Table 4. Effect of nitrate and phosphate concentrations on RNA concentrations (μg/culture) of Laminaria saccharina gametophytes (-- = no test).

NO_3-N μg-at/l	PO_4-P μg-at/l										
	0	1	3	5	7	10	15	20	25	28.7	57.4
0	0.51	0.51	0.53	0.58	0.58	0.60	0.61	0.69	0.91	--	--
5	0.61	0.68	0.69	0.69	0.72	0.75	0.78	--	--	--	--
10	0.71	0.88	0.88	0.89	0.93	0.99	1.05	--	--	1.89	2.43
20	0.80	0.89	0.89	0.96	1.04	1.08	1.17	1.39	1.51	--	--
30	0.85	0.96	1.02	1.07	1.08	1.21	1.39	--	--	--	--
40	0.88	1.22	1.40	1.49	1.54	1.68	1.91	2.88	3.25	3.68	3.87
80	0.95	--	--	--	--	4.15	4.99	4.89	4.95	--	--
160	1.05	2.43	--	--	--	4.92	5.68	6.08	6.48	6.73	7.09
588.3	--	2.88	--	--	--	--	11.65	--	--	10.36	8.82
1176.6	--	2.28	--	--	--	--	9.57	--	--	9.13	8.07

Table 5. Effect of nitrate and phosphate concentrations on protein concentrations (μg/culture) of *Laminaria saccharina* gametophytes (-- = no test).

NO_3-N μg-at/l	PO_4-P μg-at/l										
	0	1	3	5	7	10	15	20	25	28.7	57.4
0	4	8	10	11	11	13	13	14	14	--	--
5	15	15	16	19	21	22	23	--	--	--	--
10	19	22	22	24	24	26	26	--	--	33	34
20	24	27	29	30	31	32	32	34	34	--	--
30	27	34	38	40	40	41	48	--	--	--	--
40	30	40	41	48	50	52	54	60	78	84	98
80	32	--	--	--	--	127	136	169	160	--	--
160	82	87	--	--	--	866	959	970	936	916	898
588.3	--	92	--	--	--	--	1520	--	--	1390	1352
1176.6	--	100	--	--	--	--	1352	--	--	1382	962

Table 6. Effect of nitrate and phosphate concentrations on carbohydrate concentrations (μg/culture) of Laminaria saccharina gametophytes (-- = no test).

NO_3-N μg-at/l	PO_4-P μg-at/l										
	0	1	3	5	7	10	15	20	25	28.7	57.4
0	444	465	483	502	507	523	594	688	713	--	--
5	841	855	877	900	925	975	1112	--	--	--	--
10	865	910	954	1005	1028	1056	1193	--	--	2174	1935
20	896	972	1126	1250	1252	1421	1648	2060	2065	--	--
30	1300	1325	1558	1616	1753	1822	2104	--	--	--	--
40	1692	1892	1897	1925	2028	2267	2489	2459	2515	2667	2528
80	1738	--	--	--	--	2427	3387	3093	2881	--	--
160	1952	2138	--	--	--	2805	3637	3202	3073	3154	2728
588.3	--	2721	--	--	--	--	4709	--	--	3375	2397
1176.6	--	2942	--	--	--	--	3787	--	--	3284	2123

Figure 6. RNA/DNA ratios of Laminaria saccharina gametophytes in different nitrate and phosphate concentrations.

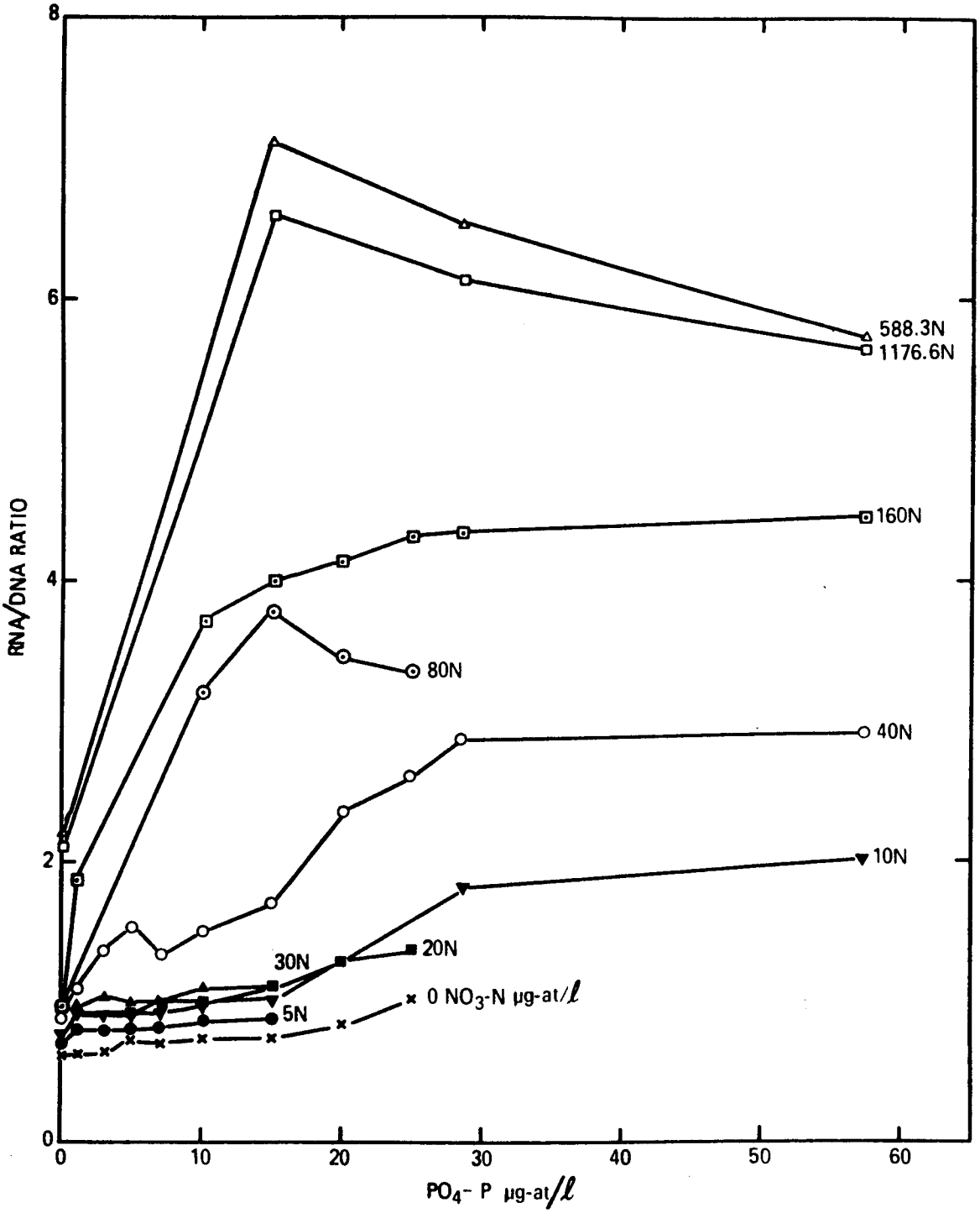
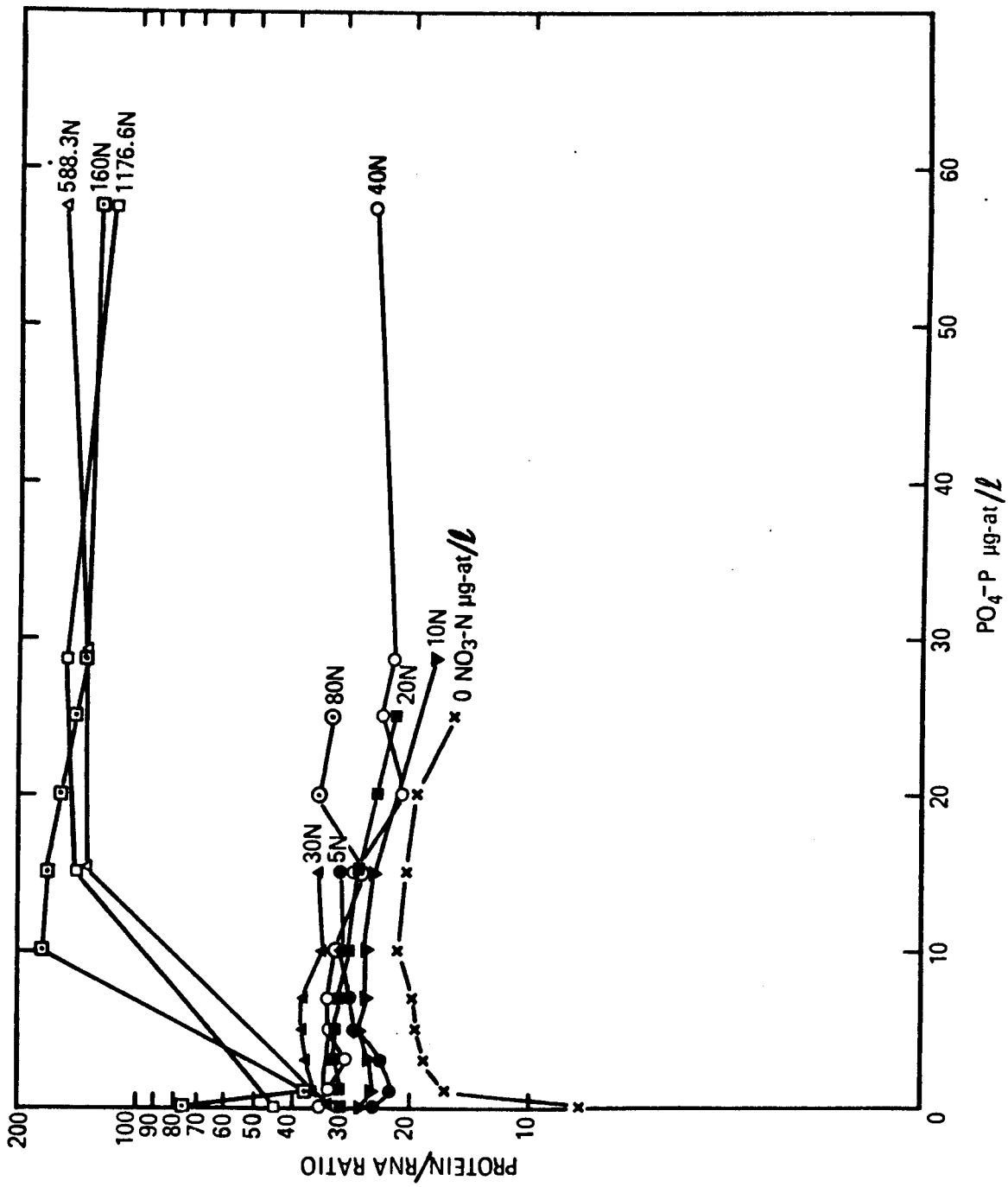

RNA/DNA RATIO
0
2
4
6
8
0
10
20
30
40
50
60
PO4- P µg-at/ℓ
588.3N
1176.6N
160N
80N
40N
10N
20N
30N
5N
0 NO3-N µg-at/ℓ

Figure 7. Protein/RNA ratios of Laminaria saccharina gametophytes in different nitrate and phosphate concentrations.


588.3N
160N
1176.6N
40N
80N
20N
10N
0 NO3-N µg-at/l
30N
5N
PROTEIN/RNA RATIO
PO4-P µg-at/l
200
100
90
80
70
60
50
40
30
20
10
0
10
20
30
40
50
60

to 15 μg-at/l and then remained constant. Ratios in the cultures of 1176.6 μg-at NO_3-N/l were the highest at 28.7 μg-at PO_4-P/l, and somewhat decreased at 57.4 μg-at PO_4-P/l.

Total lipids decreased with increasing phosphate concentrations in the media (Table 7). The greatest amount of lipids was synthesized in low concentrations of nitrate and phosphate. In high nitrate and phosphate concentrations the lipid concentration decreased. The highest concentration of lipids was found in 30 μg-at NO_3-N/l in a phosphorus-free medium.

Both chlorophylls a and c were at maximum concentration in the medium containing 588.3 μg-at NO_3-N/l and 15 μg-at PO_4-P/l (Table 8 and 9). At nitrate concentrations from 0 to 80 μg-at/l chlorophyll c concentrations were greater than chlorophyll a at all phosphate concentrations. From 160 μg-at NO_3-N/l the concentrations of these two pigments were quite similar.

Total carotenoids of the gametophytes increased with increasing phosphate concentrations up to 20 μg-at/l in all nitrate concentrations tested, but decreased when phosphate was higher than 20 μg-at/l (Table 10). Nitrate concentrations above 80 μg-at/l produced a significantly greater concentration of carotenoids up to 28.7 μg-at PO_4-P/l.

Table 7. Effect of nitrate and phosphate concentrations on lipid concentrations (μg/culture) of Laminaria saccharina gametophytes (-- = no test).

NO_3-N μg-at/l	PO_4-P μg-at/l										
	0	1	3	5	7	10	15	20	25	28.7	57.4
0	11.8	7.8	7.6	4.7	4.6	3.6	3.1	3.2	3.0	--	--
5	13.5	10.2	6.7	6.5	6.1	5.3	3.4	--	--	--	--
10	13.8	11.3	8.4	6.8	6.5	6.1	5.1	--	--	5.3	5.0
20	14.6	12.5	9.5	8.7	6.7	6.3	5.3	5.5	5.4	--	--
30	15.5	13.9	10.8	9.5	8.4	6.9	6.3	--	--	--	--
40	14.8	13.1	11.0	8.8	7.4	6.4	5.5	5.3	5.2	5.1	4.9
80	8.5	--	--	--	--	6.2	5.4	5.2	5.1	--	--
160	7.3	6.5	--	--	--	5.0	5.2	5.0	4.9	4.9	4.2
588.3	--	5.6	--	--	--	--	4.7	--	--	4.2	3.1
1176.6	--	4.5	--	--	--	--	3.7	--	--	2.8	0.6

Table 8. Effect of nitrate and phosphate concentrations on chlorophyll a concentrations (μg/culture) of Laminaria saccharina gametophytes (-- = no test).

NO_3-N μg-at/l	PO_4-P μg-at/l										
	0	1	3	5	7	10	15	20	25	28.7	57.4
0	3	11	18	29	29	35	33	39	42	--	--
5	4	18	25	29	36	40	43	--	--	--	--
10	18	29	37	40	42	42	44	--	--	50	51
20	25	29	38	41	43	45	47	50	51	--	--
30	29	35	41	43	46	45	48	--	--	--	--
40	36	41	43	47	51	55	60	74	79	84	86
80	48	--	--	--	--	236	284	298	360	--	--
160	51	263	--	--	--	708	915	985	918	946	820
588.3	--	431	--	--	--	--	1214	--	--	1119	907
1176.6	--	525	--	--	--	--	1101	--	--	981	854

Table 9. Effect of nitrate and phosphate concentrations on chlorphyll c concentrations (μg/culture) of Laminaria saccharina gametophytes (-- = no test).

NO_3-N μg-at/l	PO_4-P μg-at/l										
	0	1	3	5	7	10	15	20	25	28.7	57.4
0	3	18	27	36	38	54	57	68	82	--	--
5	6	52	65	72	80	88	90	--	--	--	--
10	50	68	109	124	117	107	129	--	--	93	90
20	53	68	102	88	65	69	68	80	77	--	--
30	44	60	71	84	102	116	141	--	--	--	--
40	36	51	62	81	99	150	195	280	295	256	260
80	69	--	--	--	--	326	375	447	581	--	--
160	72	288	--	--	--	640	770	991	920	886	616
588.3	--	568	--	--	--	--	1163	--	--	1050	702
1176.6	--	576	--	--	--	--	1051	--	--	980	666

Table 10. Effect of nitrate and phosphate concentrations on total carotenoids concentrations (μg/culture) of Laminaria saccharina gametophytes (-- = no test).

NO_3-N μg-at/l	PO_4-P μg-at/l										
	0	1	3	5	7	10	15	20	25	28.7	57.4
0	11	20	26	38	42	44	55	54	55	--	--
5	13	22	32	45	50	56	67	--	--	---	--
10	29	38	46	61	66	76	86	--	--	92	94
20	41	49	64	72	80	92	97	118	111	--	--
30	42	51	66	77	83	93	98	--	--	--	--
40	52	55	70	82	89	97	123	168	162	136	110
80	56	--	--	--	--	318	438	478	528	--	--
160	59	156	--	--	--	888	1057	1248	1092	1057	398
588.3	--	372	--	--	--	--	1210	--	--	1151	503
1176.6	--	389	--	--	--	--	1036	--	--	960	468

DISCUSSION

The experimental data show that meiospore germination was stimulated with increasing phosphate concentrations under optimal concentrations of nitrate, but inappropriate combinations of nitrate and phosphate concentrations produced an adverse effect. These findings may be explained if meiospore germination requires phosphate for energy source and as an initiator of the germination sequence, and nitrate as a trigger for the utilization of phosphate. Srinivasan and Halvorson (1961) demonstrated that ^{32}P was taken up into nucleotides during germination of bacterial spores. Nishi (1961) reported that intracellular utilization of reserve phosphates, such as polyphosphate and phospholipid, in germination spores of Aspergillus niger, was largely affected by the composition of the germination medium, especially at a deficiency of nitrogen.

The optimal concentrations of nitrate and phosphate in ASP 2M for gametogenesis in L. saccharina were 588.3 μg-at NO_3-N/l and 15 μg-atPO_4-P/l. These nutrient concentrations in culture were approximately 32 times higher than nitrate and 7 times higher than phosphate than those in natural seawater where the plants were collected (Chapter IV). It is not

known if all nutrients are utilized by the algae or bound in the chelating agent, EDTA in the culture, to form complexing compounds which are relatively unavailable to the algae. Wiessner (1962) has shown that additional quantities of the nutrient elements particularly trace metals must be added to the culture medium with EDTA as a chelator, to compensate for the unavailability of bound nutrients.

The optimal concentrations of nitrate and phosphate in ASP 2M for growing L. saccharina meiospores into gametophytes were 588.3 μg-at NO_3-N/l and 15 μg-at PO_4-P/l. In this condition, the meiospores had highest germination, actively divided, and the resultant gametophyte had the highest percentage fertility. Consequently, these gametophytes had the greatest number of cells per plant, and had the greatest biomass as determined by metabolite concentrations. Nitrogen and phosphorus are required for the growth, development and reproduction of algae (Chu, 1943; Provasoli, 1958) and participate directly or indirectly in most of the reactions associated with photosynthesis. The former is a constituent of the various algal pigments, essential in protein synthesis and also of all the active protein and peptide molecules that serve as enzymes in photosynthesis. When cultures were grown

either in nitrogen- and/or phosphorus-free media, or in the media containing suboptimal combinations of nitrate and phosphate concentrations, cell division and development were inhibited. It might be expected that photosynthesis would be reduced, resulting in less production of carbohydrates and proteins.

The amount of nitrogen available to L. saccharina would determine the amount of lipid accumulation, since nitrogen deficiency limits growth, and causes photosynthetic activity to be directed to lipid synthesis. This has been demonstrated in several freshwater algae by Fogg and Collyer (1964). They also concluded that the accumulation of lipids regarded as characteristic of certain algae, perhaps may depend more on environmental conditions under which such species habitually grew than on genetically determined peculiarities of metabolism.

Many physiological processes are limited by phosphate concentrations, since the availability of inorganic phosphate and ADP determine the amount of ATP synthesis. It may be inferred that the amount of carbohydrate and protein produced in L. saccharina increases with increasing phosphate concentrations, but lipid synthesis is in the reverse direction.

The amount of DNA per nucleus is fairly constant for a given species of animal cells (Mirsky and Ris, 1951). Davidson et al (1949), and Hull and Kirk (1950) reported that the nucleic acid content of cultured cells derived from freshly explanted tissues varied with the nutritional state of the culture. A fast and better growing culture, with 588.3 μg-at NO_3-N/l and 15 μg-at PO_4-P/l, consisted of gametophytes that, on average, had a larger number of cells per plant, denser contents, and contained more DNA, RNA and protein than the cells in poor, slow growing cultures, particularly those which were nitrogen-and/or phosphorus-free media. Higher concentrations of metabolites, excepting lipid, reflect the greater biomass of cultures grown under optimal conditions, but rapidly growing cultures contain not only more nuclei and protoplasm but a protoplasm richer in RNA. Hotchkiss (1955) showed that DNA molecules are metabolically relatively stable in most tissues and are produced in rather strict relation to growth. One may conclude that the total amount of DNA present is an important measure of cell number and reflects the occurrence of growth.

As concentrations of nitrate and phosphate were increased, up to the optimum the concentration of both RNA and DNA in-

creased, however the ratio of RNA/DNA also increased, indicating that RNA increased to a greater extent and was therefore more susceptible to change in nitrate and phosphate concentrations. A similar effect was seen at suboptimal concentration of nitrate and phosphate 10 μg-at NO_3-N/l and 15 μg-at PO_4-P/l where the quantities of both RNA and DNA decreased, but the decrease in RNA was greater than DNA producing a low RNA/DNA ratio. Smellie (1955) showed that DNA might be a comparatively inert component, whereas RNA was actively engaged in the metabolic processes of the cell. The amount of RNA may vary considerably in different stages of life cycle and growth in different nutrients (Smellie, 1955; Epstein and Allaway, 1967; Carell et al, 1970; Hobson and Pariser, 1971; Parentia et al, 1971). The protein/RNA ratios were affected by the low concentrations of nitrate. They were high when the cultures were sufficiently supplied with nitrate and phosphate and the plants had better growth and greater fertility. The higher ratio of RNA/DNA in L. saccharina gametophytes were consistent with the higher percentage fertility of both male and female gametophytes. It seems reasonable to infer that the high RNA/DNA is correlated with conditions optimal for gametogenesis in L. saccharina.

The ratios of RNA/DNA and protein/RNA are considered the more accurate indices of metabolic activity than DNA, RNA and protein alone since the ratios are not affected by differences in biomass of L. saccharina gametophytes developed in the various nitrogen and phosphorous treatments.

Since the concentrations of metabolites introduced, with the meiospores are not known, I was not able to determine the effects of different NO_3 and PO_4 concentrations on metabolite synthesis, only the standing crop of metabolites after six week culture.

CHAPTER III

RESPONSE OF L. SACCHARINA GAMETOPHYTES TO DIFFERENT IODINE CONCENTRATIONS, AND CHLORIDE AND IODIDE RATIOS

INTRODUCTION

Iodine is highly concentrated by some members of the Phaeophyta and Rhodophyta, particularly by the Laminariales (Fritsch, 1945; Black, 1948; Grimm, 1952). Concentrations can reach 30,000 times that in seawater, and can constitute 1% of the dry weight of Laminaria digitata (Black, 1948; Vinogradov, 1953; Levring et al, 1969). The greatest accumulation of iodine is in the meristematic tissue at the base of the Laminaria blade (Fritsch, 1945). It can be accumulated as inorganic iodide, iodoamino-acids and iodoproteins (Scott, 1954; Tong and Chaikoff, 1955; Roche et al, 1963; Levring et at, 1969). The mechanisms of iodide uptake and accumulation have been demonstrated in L. flexicaulis, L. saccharina (Roch and Yagi, 1952) and L. digitata (Shaw, 1959). Shaw (1960, 1962) found that iodide uptake by L. digitata was accompanied by a vigorous burst of respiration.

Iodine was necessary for the growth of Asparagopsis (von Stosch, 1964), Polysiphonia urceolata (Fries, 1966), the Conchocelis phase of Porphyra tenera (Iwasaki, 1957), Ecto-

carpus fasciculatus (Pedersen, 1969) and Petalonia fascia (Hsiao, 1969).

Vinogradov (1953) showed that more saline seas and arctic zones favored the accumulation of iodine in Phaeophyceae. He also showed that Phaeophyceae collected near the shore and at relatively shallow depths contained less iodine than those taken from the open sea.

A direct relationship between chloride and iodine was found by Lewis and Powers (1941) who found that chloride had an antagonistic effect on iodine toxicity in corn.

Iodine had a stimulatory effect on seed germination of corn (Powers, 1939), on pollen germination (Portyanko and Kudrya, 1966; Kostina et al, 1969) and amino acid synthesis in higher plants (Portyanko et al, 1969). Sokoloff et al (1963) reported that iodine (thyroxine) intensified protein synthesis by activating amino acids to attach to soluble RNA. Powers (1939) noted that iodine seemed to promote development of chlorophyll in plants.

Iodovolatisation usually occurs when Laminaria Andersonii is exposed to the air during low tide (Blinks, 1951). The released iodine may influence algal development, growth and reproduction.

The following experiments were carried out to learn the

effects of different iodine concentrations, as well as the interrelated effects of chloride and iodide on meiospore germination, gametophyte development and gametogenesis of L. saccharina in a defined medium under axenic culture conditions.

MATERIALS AND METHODS

About 1.2×10^5 axenic meiospores were inoculated into 15 ml of test media, prepared with 588.3 μg-at NO_3-N/l and 15 μg-at PO_4-P/l, which produced maximum growth and development of L. saccharina gametophytes, as described in the preceding Chapter. To these media were added, 0, 20, 40, 60, 80, 10^2, 10^3, 10^4, 10^5 and 10^6 μg iodine/l in the forms of NaI, KI, $NaIO_3$ and KIO_3 these concentrations include the range measured over 3 years in Burrard Inlet (2.9-29.2 μg/l); and the value of iodine in open ocean waters determined at 60 μg/l by Barkley and Thompson (1960). The higher concentrations were used as representative of localized conditions of iodovolatisation at low tide.

The effects of chloride on iodide toxicity in L. saccharina gametophytes were studied by placing the meiospores in ASP 2M basal medium containing 588.3 μg-at NO_3-N/l and 15 μg-at PO_4-P/l, optimal for growth and development, to which 10^6 μg iodine/l were added. This concentration inhibited gametophyte. Sodium chloride was then added in quantities of 9, 13.5, 18, 27 and 36

g/l. The ratios of chloride to iodide were 5.84, 8.56, 11.29, 19.75 and 22.21 in the above media, and respective salinities were 13.3, 16.7, 23.8, 30.0, and 38.9%.

The culture conditions, evaluation of meiospore germination and fertility, determination of metabolites and analysis of results were made in the same way as described in Chapter II. Meiospore germination was counted 3 and 10 days after inoculation in experiments using 10^6 μg I^--I/l and in experiments using chloride and iodide together, as there was no germination in the first 3 days.

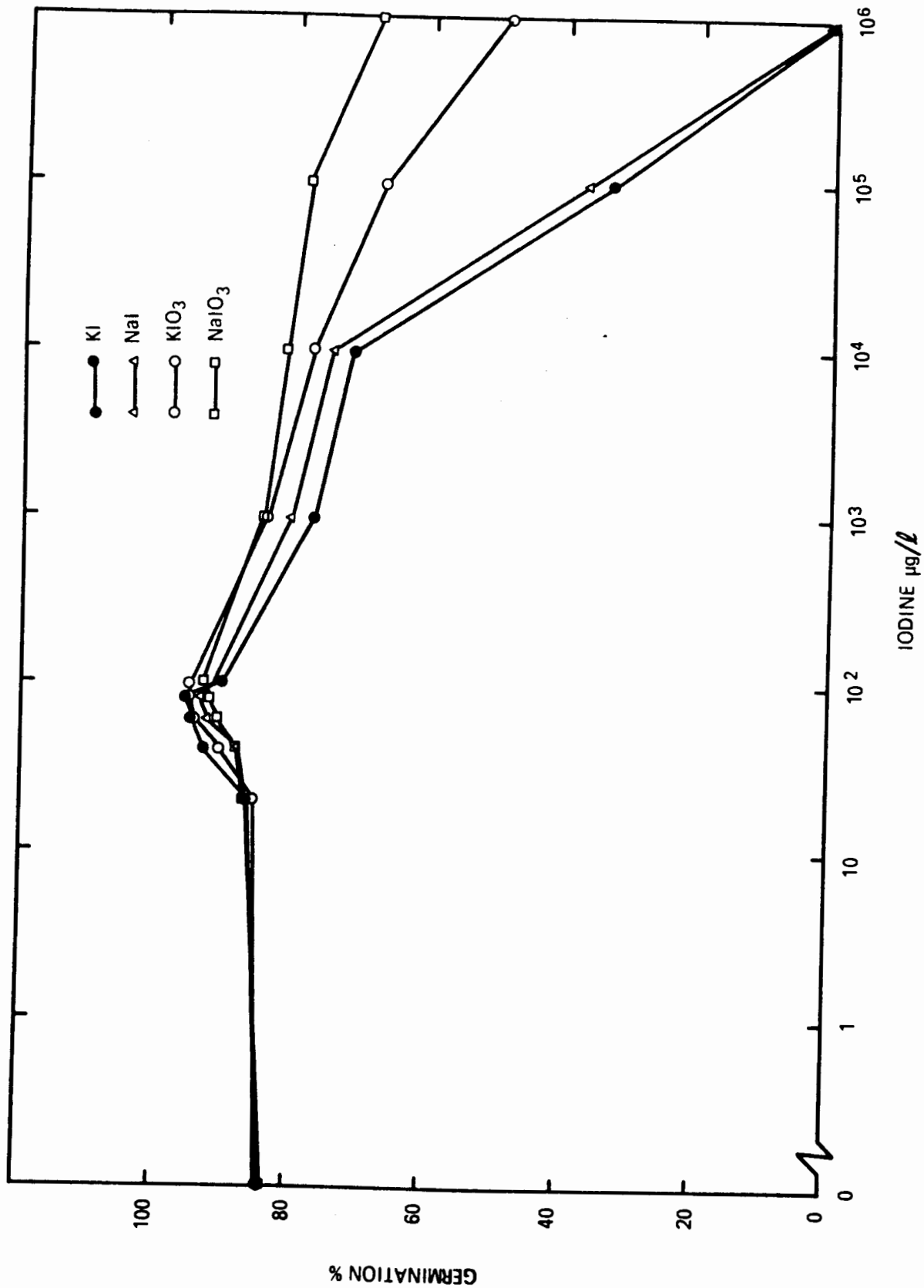
RESULTS

These experiments were performed on two occasion, October, 1970 and May, 1971. As there was no significant difference between the results, the averages of the data are presented below.

The effect of iodine concentrations on meiospore germination

Meiospore germination of L. saccharina increased with increasing iodine as iodide up to 80 μg/l and increasing iodine as iodate up to 100 μg/l (Fig. 8). Germination decreased with higher concentrations of iodine in both these forms. Inhibition began at iodine concentrations higher than 10^3 μg/l, and was greater with iodide. No meiospores had germinated 3 days

Figure 8. Effect of iodine concentrations on meiospore germination of *Laminaria saccharina* 3 days after inoculation.


KI
NaI
KIO3
NaIO3
GERMINATION %
0
20
40
60
80
100
0
1
10
10 2
10 3
10 4
10 5
10 6
IODINE µg/ℓ

after inoculation in the cultures containing 10^6 µg I^--I/l and by the tenth day there was only 13.1% germination in KI and 14.5% in NaI cultures.

Generally, in concentrations lower than 80 µg/l iodine as potassium salts gave a greater percent germination than sodium salts. At concentrations above 100 µg/l, iodate had a higher percent germination than iodide.

The effect of iodine concentrations on gametophyte development, morphology and gametogenesis

The meiospores of L. saccharina developed into normal gametophytes in both iodine-free medium and the medium with the highest iodine concentration. There were no significant morphological variations in all test media (Fig. 9 A-I). Male gametophytes produced antheridia about 21-30 days after inoculation in all iodine concentrations tested, up to 10^6 µg I^--I/l, where antheridial production took 31-40 days. The time required for oogonial production was 21-30 days when the female gametophytes were grown in iodine concentrations from 0 to 10^4 µg/l, 31-40 days in 10^5 µg IO_3^--I/l and 41-42 days in 10^6 µg I^--I/l.

The percentage fertility of male gametophytes was greater than female gametophytes in all tested media (Fig. 10).

Figure 9 A-I. Examples of morphological variability observed in Laminaria saccharina gametophytes grown in ASP 2M basal medium plus 588.3 μg-at NO_3-N/l and 15 μg-at PO_4-P/l, and with different forms and concentrations of iodine.

A. Iodine-free medium

B. 80 μg IO_3^--I/l as $NaIO_3$

C. 10^6 μg I^--I/l as KI

D. 60 μg I^--I/l as KI

E. 60 μg IO_3^--I/l as KIO_3

F. 20 μg I^--I/l as KI

G. 10^3 μg IO_3^--I/l as $NaIO_3$

H. 10^6 μg IO_3^--I/l as KIO_3

I. 10^6 μg I^--I/l as NaI

Figures A, C, F, G are magnified to the scale shown in Fig. G. Figures B, D, E, H, I are magnified to the scale shown in Fig. B.

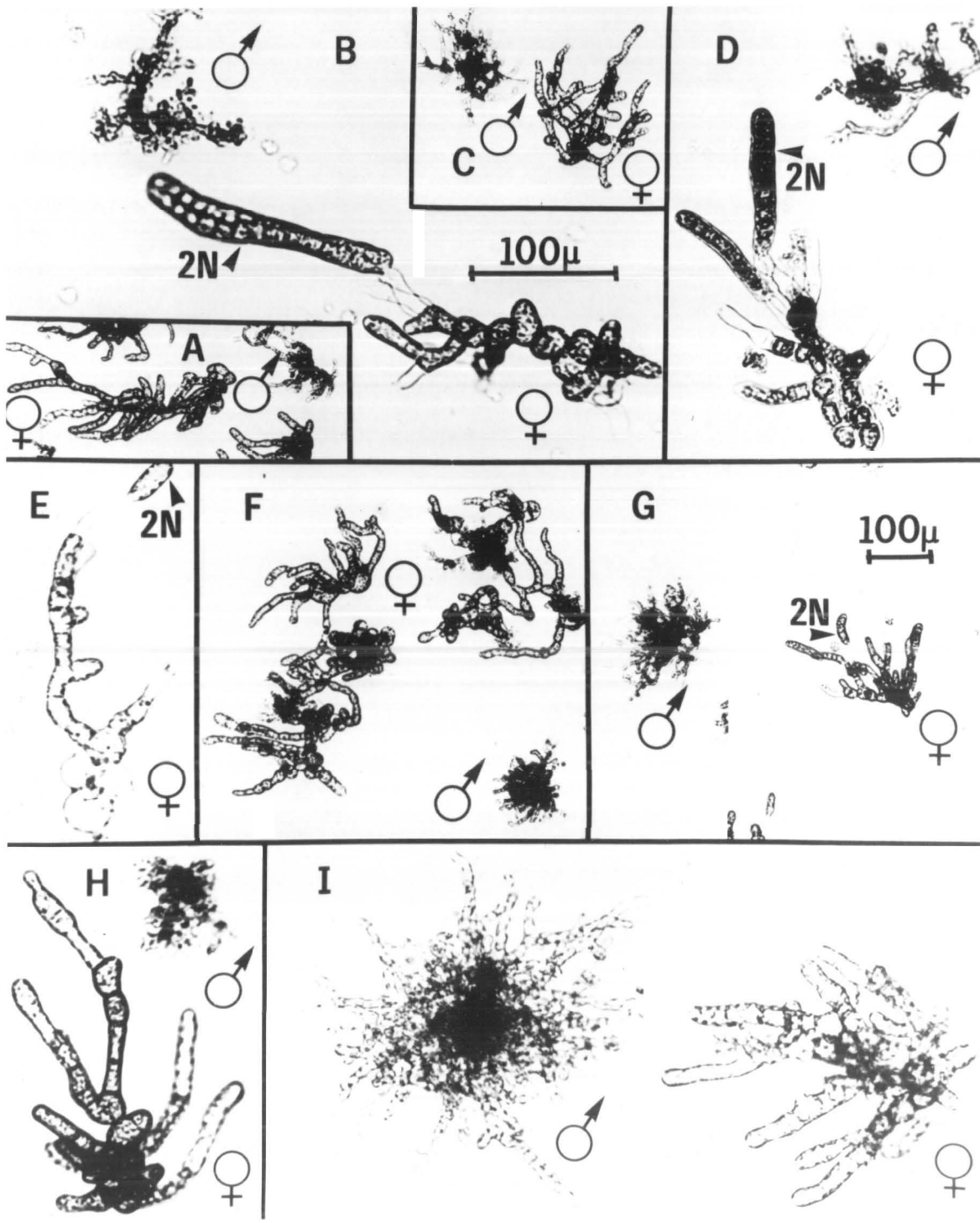
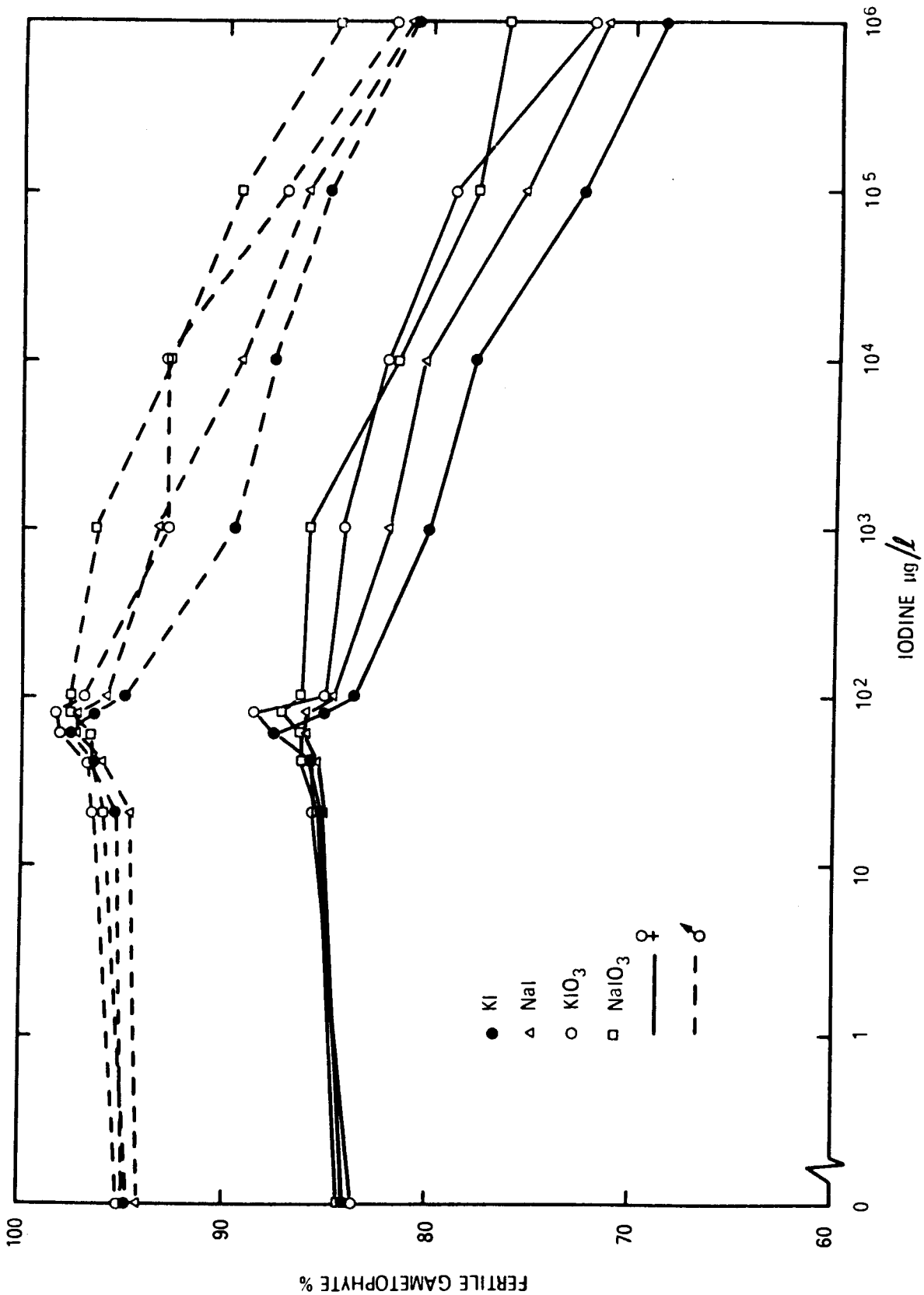

A
B
C
D
E
F
G
H
I
2N
2N
2N
100μ
100μ

Figure 10. Effect of iodine concentrations on fertility of Laminaria saccharina gametophytes as a percentage of total gametophytes.


FERTILE GAMETOPHYTE %
100
90
80
70
60
0
1
10
10^2
10^3
10^4
10^5
10^6
IODINE µg/l
KI
NaI
KIO_3
$NaIO_3$
♀
♂

The greatest fertility of both male and female gametophytes was found in KIO_3 at 80 μg IO_3^--I/l, and the lowest fertility in KI at 10^6 μg I^--I/l.

The ratio of female to male gametophytes (both fertile and sterile) in different iodine concentrations varied from 0.97 to 1.03 (Table 11), with an average ratio of 1.00 for all conditions.

The effect of iodine concentrations on selected metabolites

The effects of iodine were quite similar on the concentrations of DNA, RNA and protein (Fig. 11-13). There was an increase in concentrations of all 3 metabolites, up to the maximum, which occurred at about 80 μg iodine/l in all four iodine forms. At higher iodine concentrations, the quantities of DNA, RNA and protein decreased, the minimum quantities occurring at the highest concentration of iodide, 10^6 μg/l (Fig. 11-13). Although there was a marked decrease in DNA and RNA at iodine concentrations above 100 μg/l, the RNA/DNA ratio remained quite constant for both iodide and iodate up to the highest concentration, where the ratio decreased appreciably in the media containing iodide (Fig. 14).

Protein/RNA ratios increased with increasing iodine concentrations up to approximately 80 μg/l (Fig. 15), and

Table 11. The ratio of female to male gametophytes (fertile and sterile) and the percentage distribution of oogonia and antheridia produced per gametophyte for Laminaria saccharina in different iodine concentrations.

Iodine Concentration µg/l	Total ♀/♂	No. of oogonia/Gametophyte									No. of antheridia/Gametophyte								
		0	1	2	3	4	5	6	7	8-9	0	1	2	3	4	5	6	7	8-11
As KI																			
0	1.02	16	32	26	22	3	1	0	0	0	5	16	22	8	11	9	8	15	6
20	0.98	15	23	31	25	5	1	0	0	0	5	17	22	16	13	9	8	8	2
40	1.01	14	27	33	22	2	2	0	0	0	3	6	5	8	21	16	15	10	16
60	0.99	13	29	30	23	4	1	0	0	0	2	7	6	12	22	16	12	11	12
80	1.02	15	30	26	28	1	0	0	0	0	4	9	17	14	13	16	11	9	7
10^2	0.98	16	21	25	32	5	1	0	0	0	5	4	12	0	8	15	18	16	22
10^3	1.03	20	36	28	16	0	0	0	0	0	10	16	18	3	10	12	13	8	10
10^4	1.01	22	38	26	13	1	0	0	0	0	12	17	16	10	17	10	9	6	3
10^5	1.01	28	38	25	8	1	0	0	0	0	15	19	17	22	10	8	7	2	0
10^6	0.98	32	44	18	6	0	0	0	0	0	19	33	15	16	9	7	1	0	0
As NaI																			
0	0.98	16	29	24	25	4	2	0	0	0	6	22	16	11	8	0	13	14	10
20	1.02	15	24	28	22	7	4	0	0	0	5	10	17	8	9	8	14	14	15
40	0.97	15	24	20	29	8	4	0	0	0	4	11	8	13	11	10	13	16	14
60	1.01	14	14	25	17	16	7	3	3	1	3	8	11	0	10	17	17	14	20
80	0.99	14	33	18	15	11	6	2	1	0	3	18	10	4	8	15	16	11	15
10^2	1.02	15	34	18	19	8	5	1	0	0	4	26	10	7	7	12	14	8	12
10^3	0.98	18	34	20	16	6	4	1	1	0	6	29	14	7	8	9	12	6	9
10^4	0.99	20	33	25	16	5	1	0	0	0	11	31	13	13	7	8	10	5	2
10^5	1.02	25	42	24	9	0	0	0	0	0	14	17	22	25	8	7	5	2	0
10^6	0.99	29	42	17	8	4	0	0	0	0	19	30	17	19	12	3	0	0	0

Table 11 (cont'd)

Iodine Concentration µg/l	Total ♀/♂	No. of oogonia/Gametophyte									No. of antheridia/Gametophyte								
		0	1	2	3	4	5	6	7	8-9	0	1	2	3	4	5	6	7	8-11
As KIO_3																			
0	1.02	16	30	25	23	5	1	0	0	0	5	23	14	10	10	8	9	15	6
20	0.99	14	12	32	30	9	3	0	0	0	3	17	13	15	8	9	12	15	8
40	1.03	14	13	22	31	14	4	2	0	0	3	8	16	2	14	13	15	16	13
60	1.03	12	12	22	27	16	6	4	1	0	2	0	3	15	14	16	15	16	19
80	0.97	10	7	23	28	16	7	5	2	2	2	2	8	2	13	16	18	13	26
10^2	1.01	15	18	23	29	8	5	1	1	0	3	14	14	8	10	10	9	11	21
10^3	1.00	16	25	19	27	8	4	1	0	0	7	14	9	16	14	10	10	5	15
10^4	1.02	18	35	24	16	5	2	0	0	0	7	16	16	14	16	10	9	4	8
10^5	0.99	21	32	33	11	2	1	0	0	0	13	9	20	16	13	11	9	6	3
10^6	1.00	28	36	24	10	2	0	0	0	0	18	20	22	14	12	10	3	1	0
As $NaIO_3$																			
0	0.99	16	23	30	26	4	1	0	0	0	5	21	19	14	9	11	6	10	5
20	1.02	15	16	30	28	7	2	2	0	0	4	9	17	16	15	10	11	12	6
40	0.96	14	11	27	26	13	5	3	1	0	4	7	0	14	17	14	16	15	13
60	1.02	14	11	24	20	14	8	5	3	1	3	3	11	4	12	14	19	15	19
80	0.98	13	9	10	24	17	13	7	5	2	2	4	7	4	10	13	16	17	27
10^2	1.02	14	16	21	16	15	10	5	2	1	3	8	10	7	9	16	15	16	16
10^3	0.98	14	15	12	20	23	9	5	2	0	4	16	15	9	9	17	16	6	8
10^4	1.02	18	33	22	19	6	2	0	0	0	7	16	16	13	9	11	10	7	11
10^5	0.99	22	30	29	14	3	2	0	0	0	11	12	17	19	15	10	6	2	8
10^6	1.02	24	33	28	11	4	0	0	0	0	15	14	16	17	15	12	8	2	1

Figure 11. Effect of iodine concentrations on DNA concentrations of *Laminaria saccharina* gametophytes.

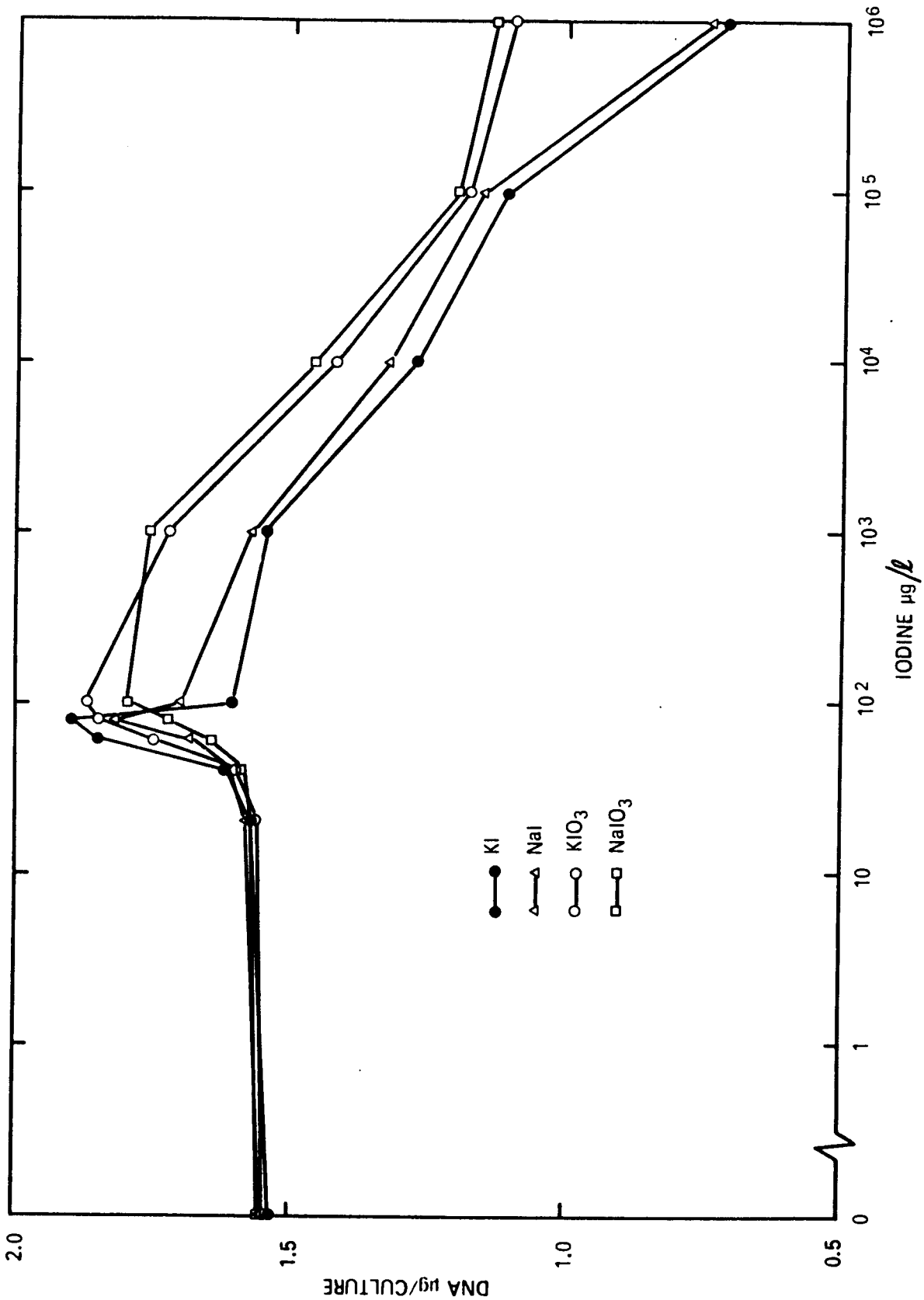

DNA µg/CULTURE
2.0
1.5
1.0
0.5
0
1
10
10^2
10^3
10^4
10^5
10^6
IODINE µg/ℓ
KI
NaI
KIO_3
$NaIO_3$

Figure 12. Effect of iodine concentrations on RNA concentrations of Laminaria saccharina gametophytes.

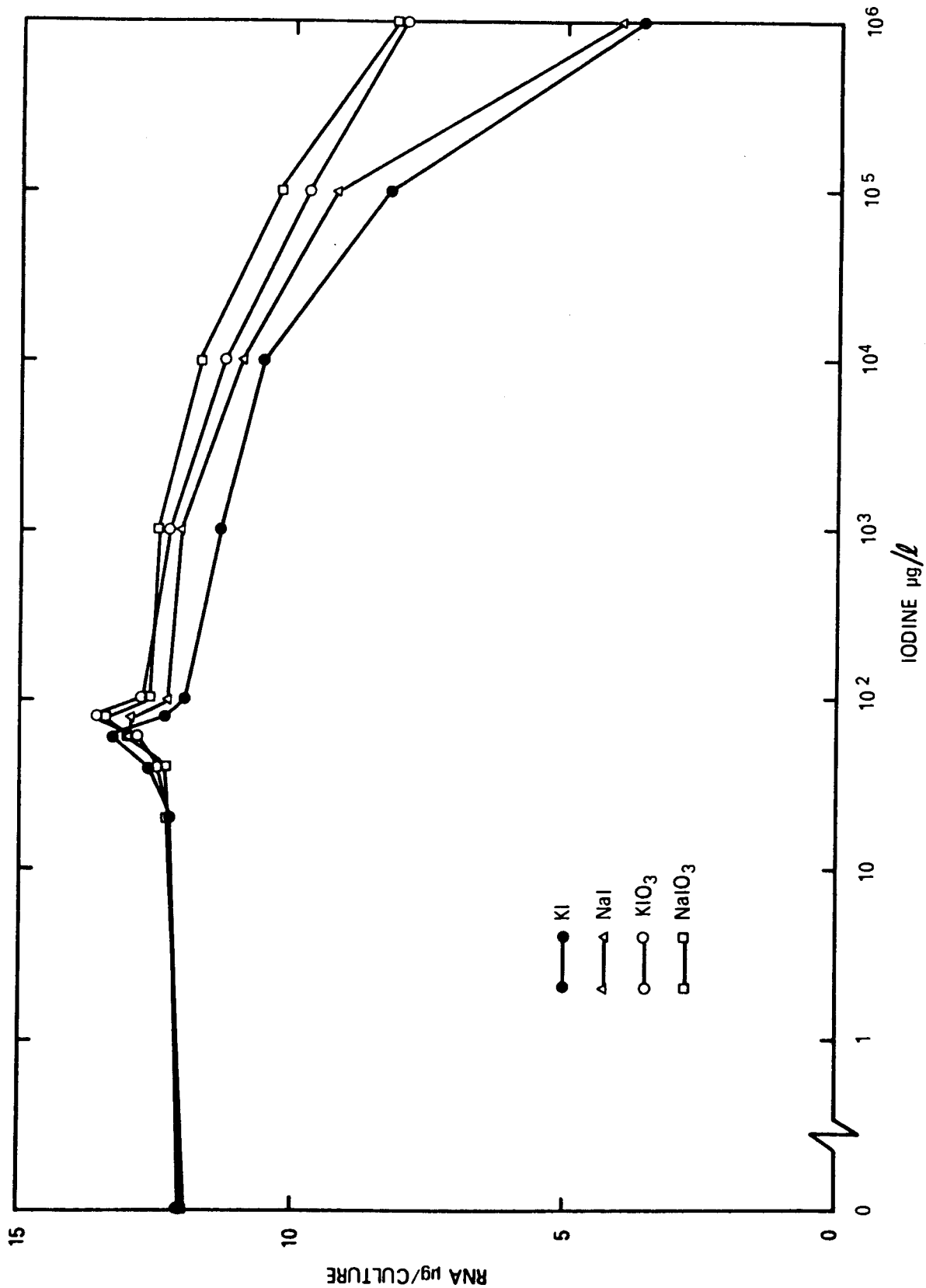

RNA µg/CULTURE
15
10
5
0
0
1
10
10^2
10^3
10^4
10^5
10^6
IODINE µg/ℓ
KI
NaI
KIO_3
$NaIO_3$

Figure 13. Effect of iodine concentrations on protein concentrations of Laminaria saccharina gametophytes.

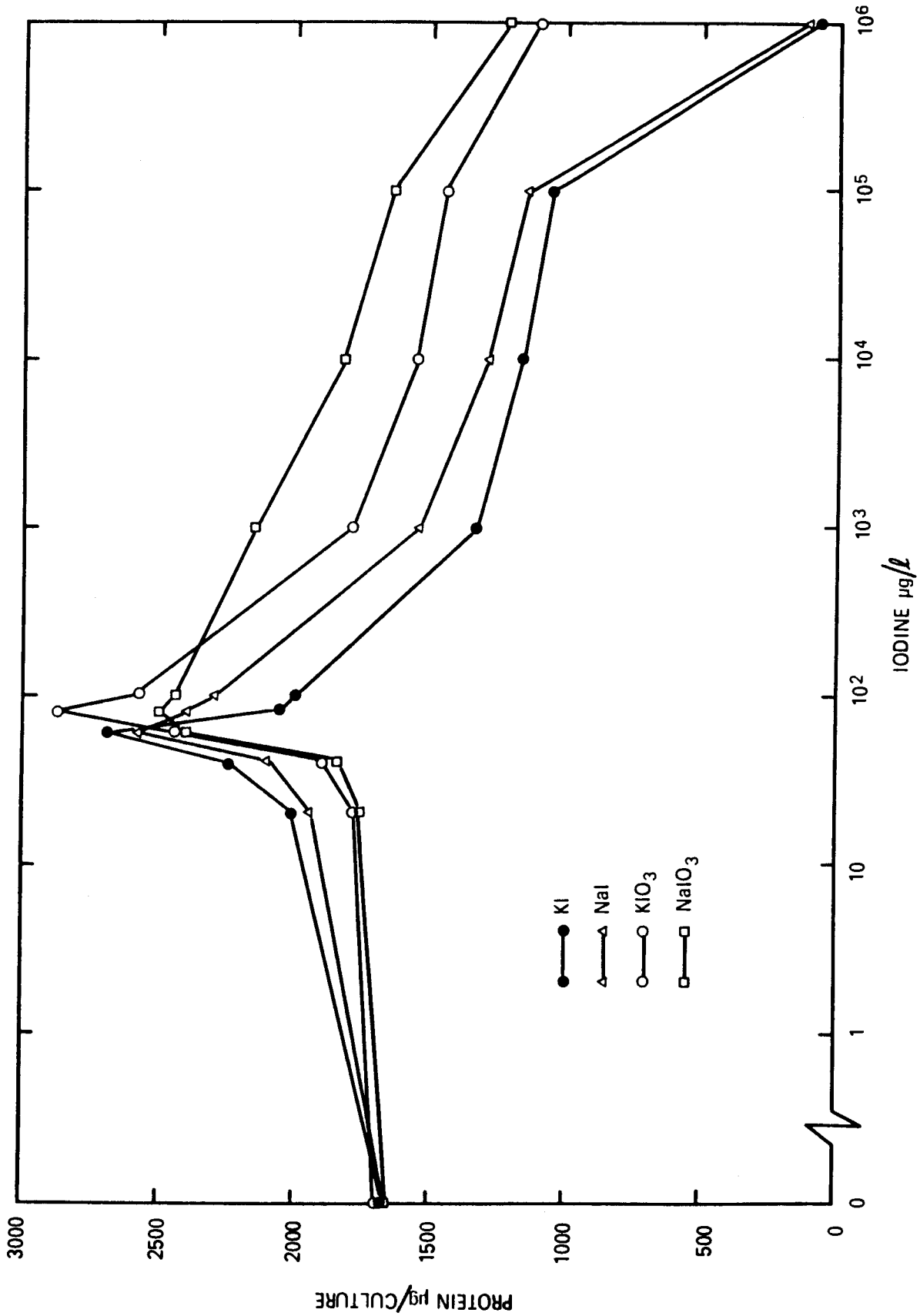

PROTEIN µg/CULTURE
3000
2500
2000
1500
1000
500
0
0
1
10
10^2
10^3
10^4
10^5
10^6
IODINE µg/ℓ
KI
NaI
KIO_3
$NaIO_3$

Figure 14. Effect of iodine concentrations on RNA/DNA ratios in *Laminaria saccharina* gametophytes.

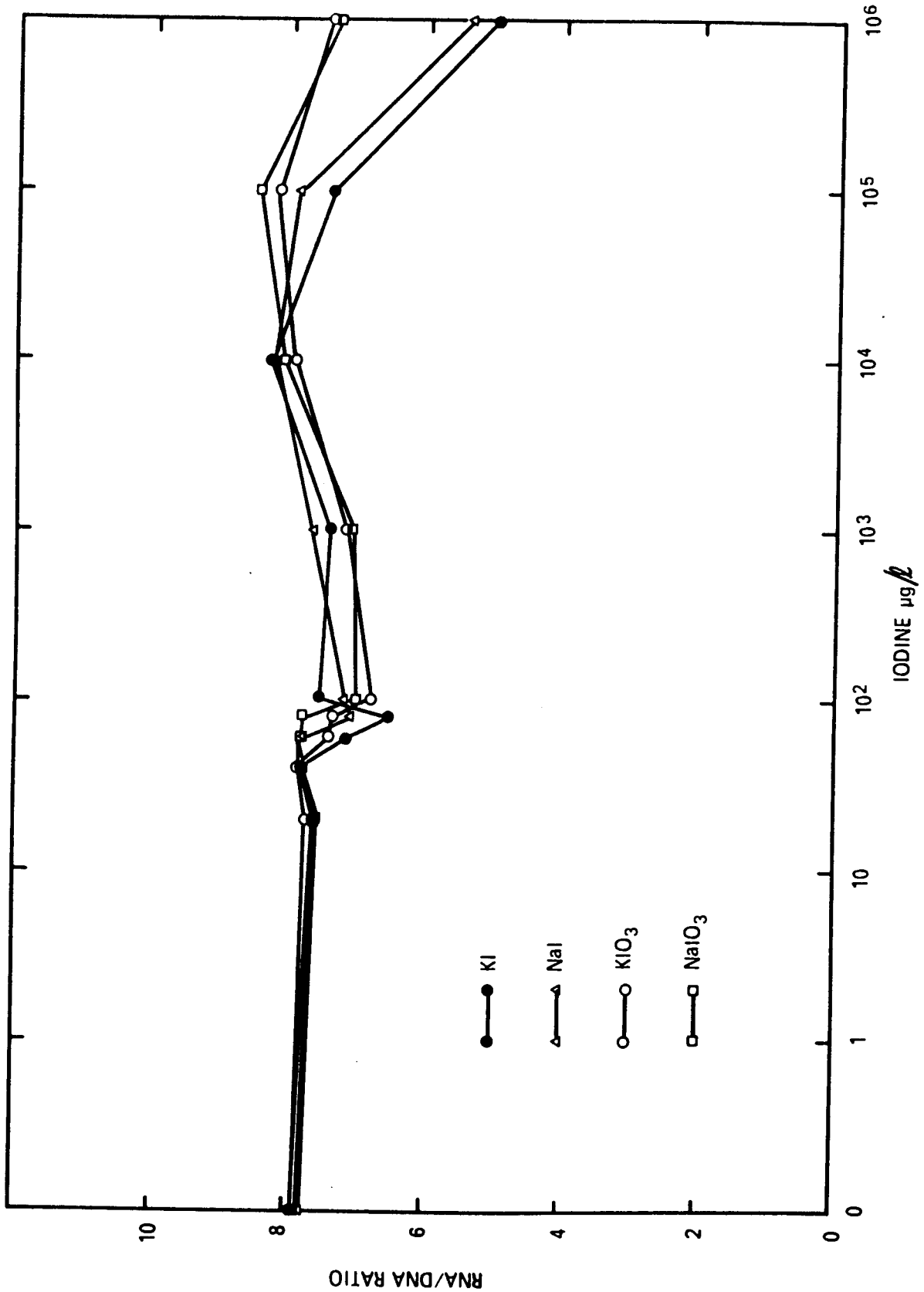
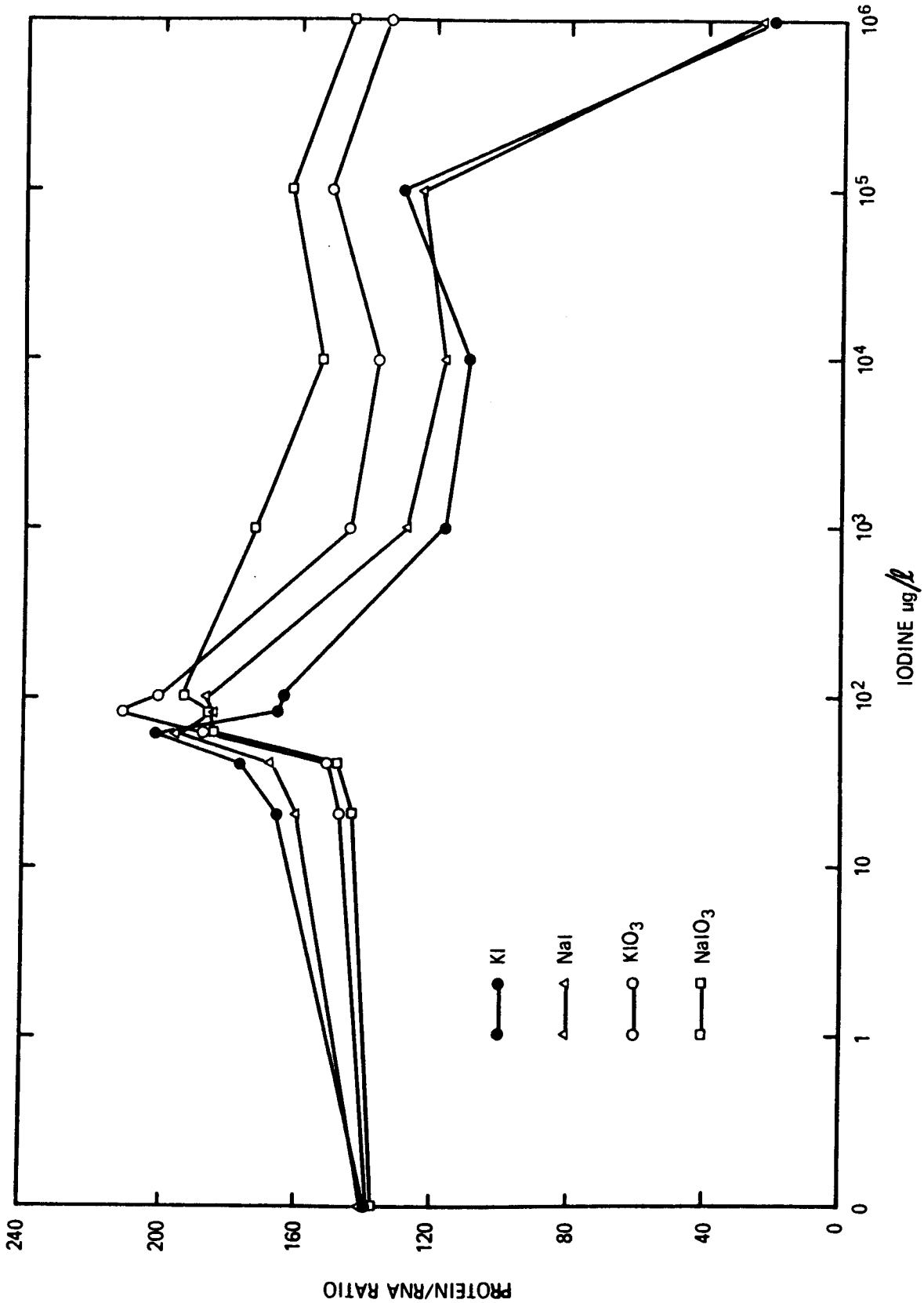

RNA/DNA RATIO
0
2
4
6
8
10
IODINE µg/ℓ
0
1
10
10²
10³
10⁴
10⁵
10⁶
KI
NaI
KIO3
NaIO3

Figure 15. Effect of iodine concentrations on protein/RNA ratios in Laminaria saccharina gametophytes.


PROTEIN/RNA RATIO
240
200
160
120
80
40
0
0
1
10
10^2
10^3
10^4
10^5
10^6
IODINE ug/l
KI
NaI
KIO_3
$NaIO_3$

decreased with higher iodine concentrations, with the minimum ratio in the highest concentration of iodide.

The carbohydrate content of gametophytes increased with increasing iodine concentrations up to 80 μg/l, and decreased with higher iodine concentrations (Fig. 16). Carbohydrate concentrations diminished in the media containing iodide from 10^3 μg/l and iodate from 10^4 μg/l. The iodides showed a greater inhibitory effect than iodate.

The amount of chlorophyll a, chlorophyll c and carotenoids reached a maximum at iodine concentration between 60 and 80 μg/l (Fig. 17-19). All pigment quantities decreased in higher concentrations of iodine, to a minimum at 10^6 μg I^--I/l.

The effects of chloride and iodide on meiospore germination, gametophyte development, morphology, gametogenesis and selected metabolites

In media with 10^6 μg I^--I/l as KI and different amounts of NaCl, meiospores of L. saccharina germinated between 4 and 10 days after inoculation, but the percent germination was very low, ranging from 8.1 to 13.1% (Fig. 20). Germination increased with increasing ratios of chloride and iodide up to 11.29 and a salinity of 23.8‰, and decreased with greater Cl^-/I^- ratios and higher salinities.

Figure 16. Effect of iodine concentrations on carbohydrate concentrations of Laminaria saccharina gametophytes.

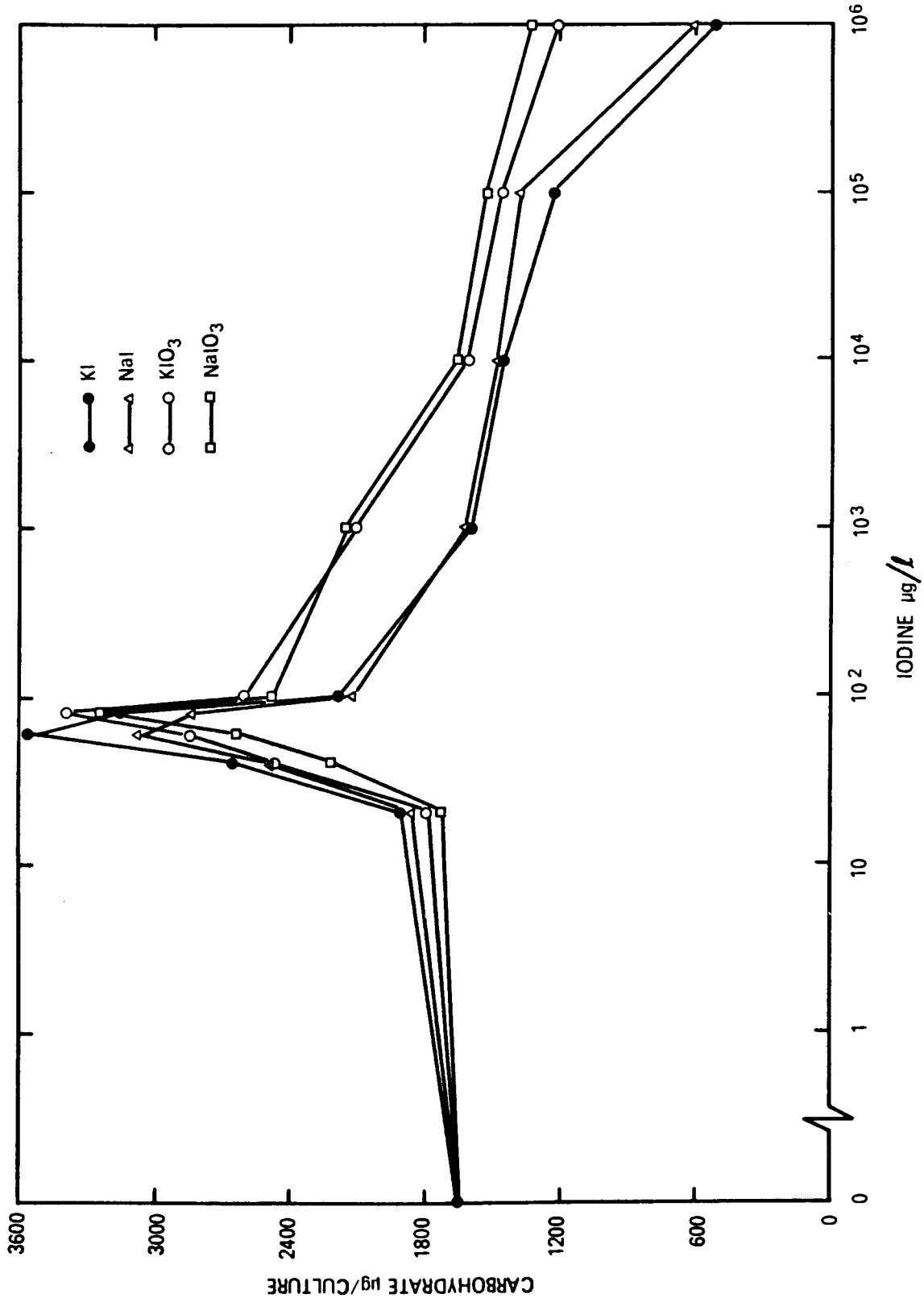

KI
NaI
KIO3
NaIO3
CARBOHYDRATE µg/CULTURE
3600
3000
2400
1800
1200
600
0
0
1
10
10 2
10 3
10 4
10 5
10 6
IODINE µg/l

Figure 17. Effect of iodine concentrations on chlorophyll a concentrations of Laminaria saccharina gametophytes.

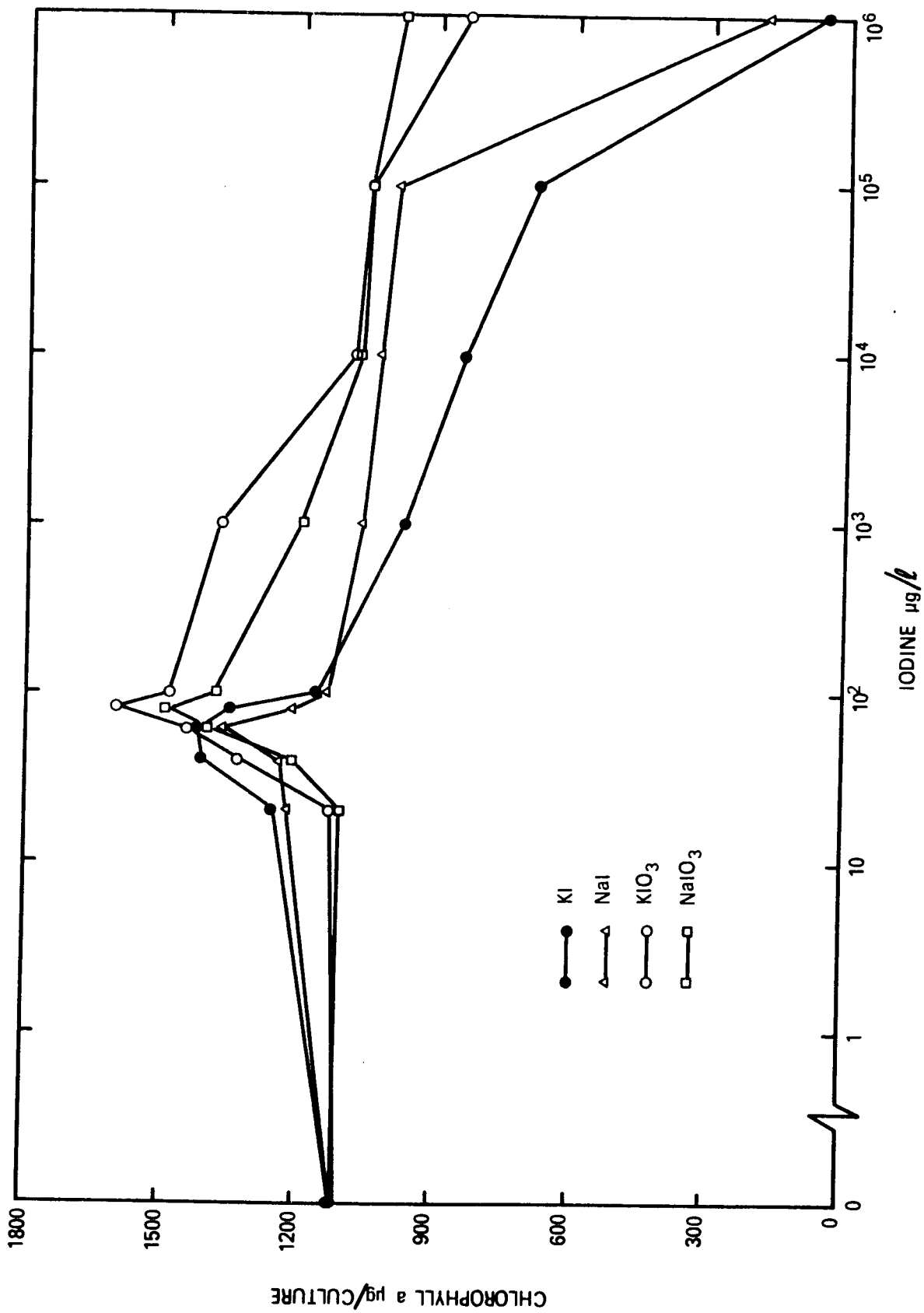

CHLOROPHYLL a µg/CULTURE
1800
1500
1200
900
600
300
0
0
1
10
10^2
10^3
10^4
10^5
10^6
IODINE µg/ℓ
KI
NaI
KIO_3
$NaIO_3$

Figure 18. Effect of iodine concentrations on chlorophyll c concentrations of Laminaria saccharina gametophytes.

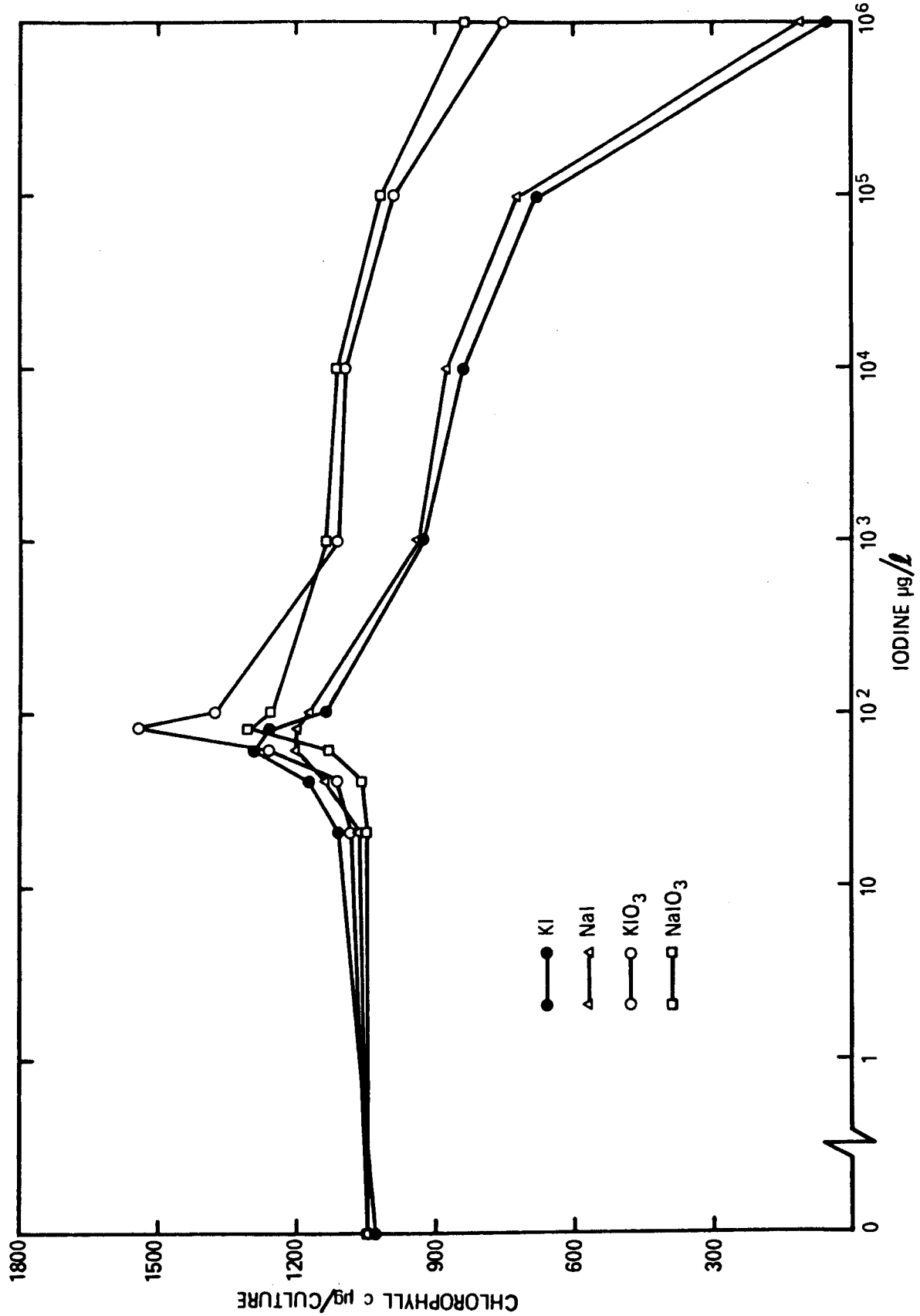

CHLOROPHYLL c µg/CULTURE
1800
1500
1200
900
600
300
0
1
10
10^2
10^3
10^4
10^5
10^6
IODINE µg/ℓ
KI
NaI
KIO_3
$NaIO_3$

Figure 19. Effect of iodine concentrations on total carotenoid concentrations of Laminaria saccharina gametophytes.

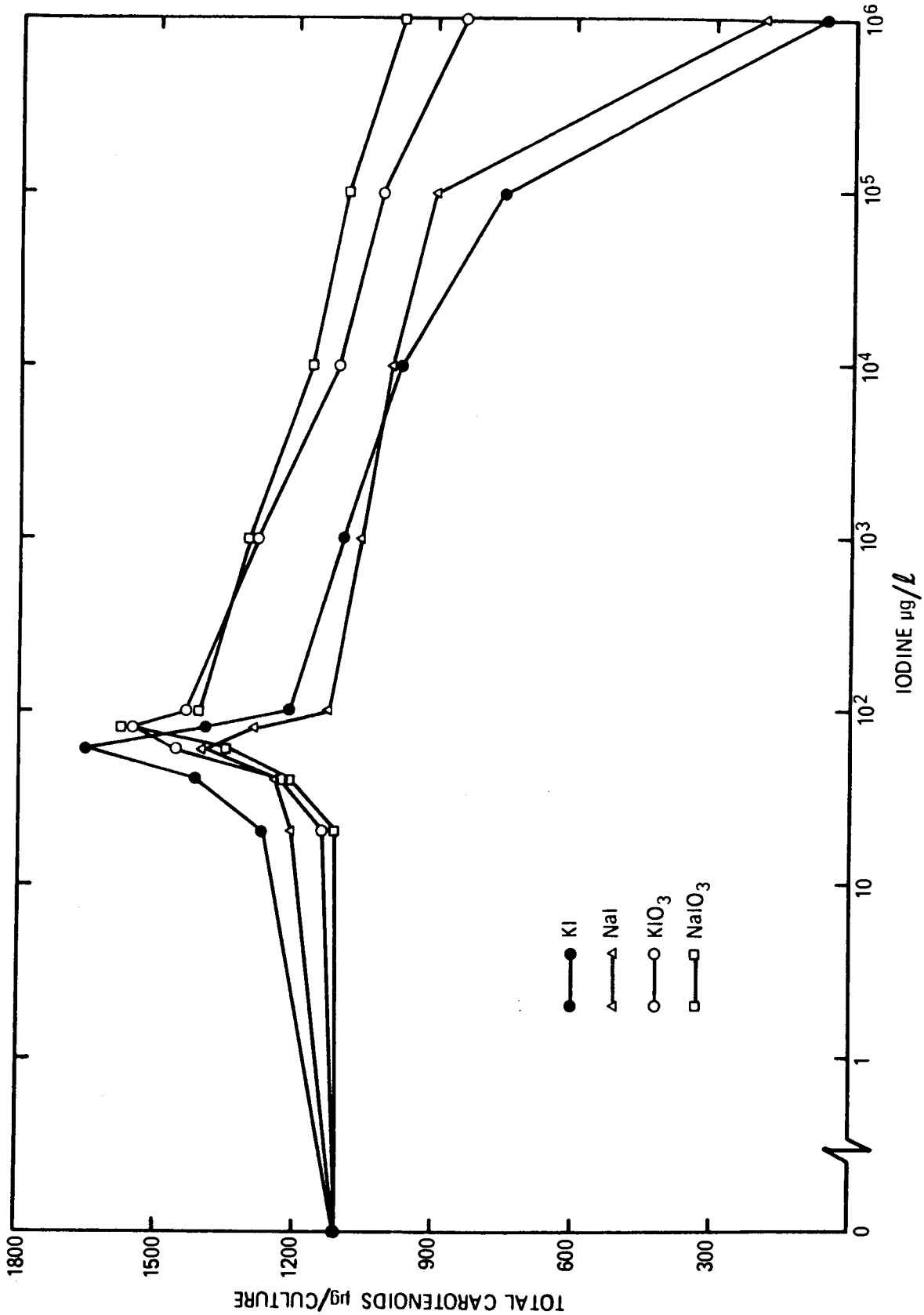
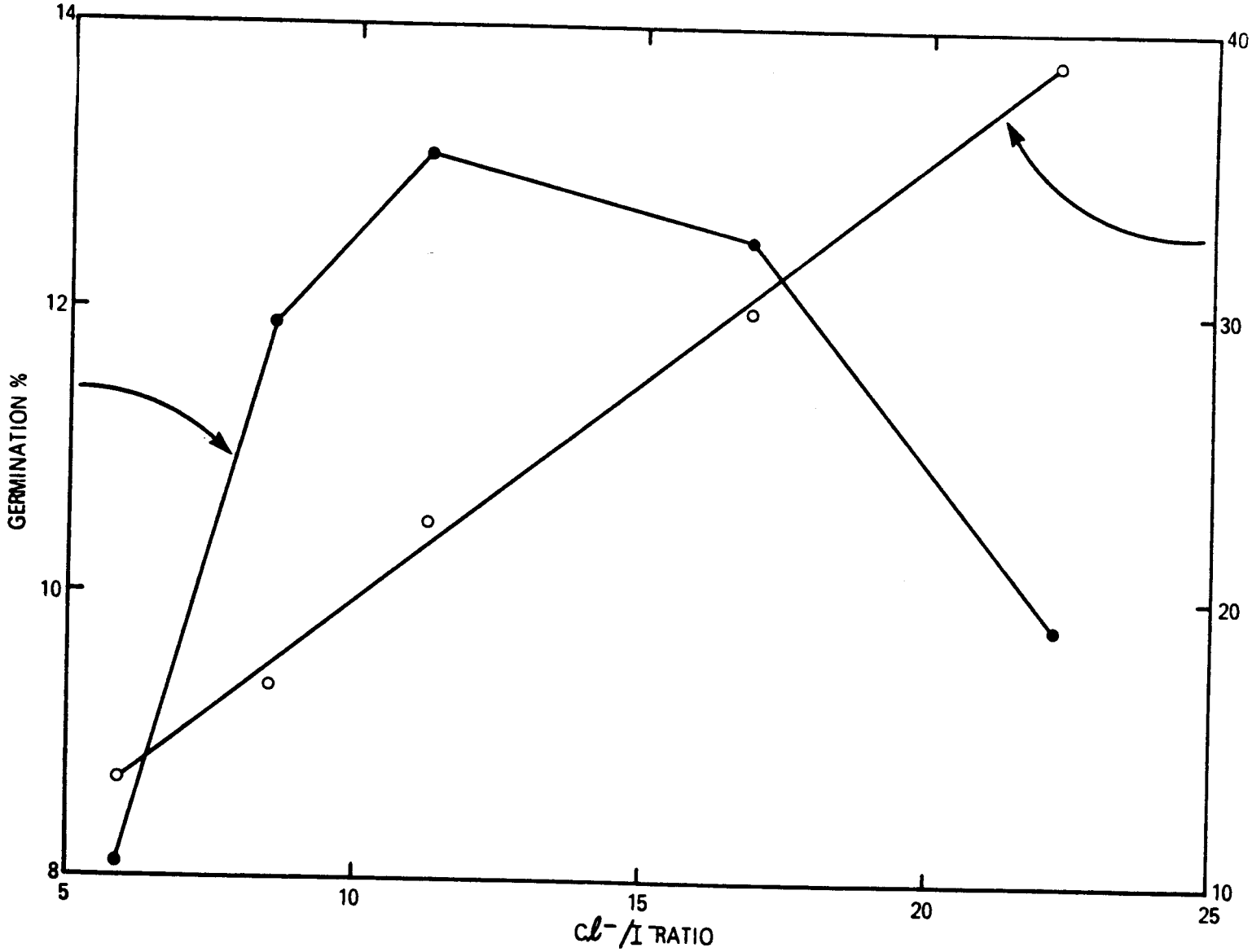

TOTAL CAROTENOIDS µg/CULTURE
1800
1500
1200
900
600
300
0
1
10
10^2
10^3
10^4
10^5
10^6
IODINE µg/ℓ
KI
NaI
KIO_3
$NaIO_3$

Figure 20. Effect of Cl^-/I^- ratios on meiospore germination of Laminaria saccharina.


14
12
10
8
GERMINATION %
40
30
20
10
SALINITY ‰
5
10
15
20
25
Cl⁻/I⁻ RATIO

Meiospores developed into gametophytes in all Cl^-/I^- ratios tested. Gametophyte morphology varied with Cl^-/I^- ratios (Fig. 21 A-E). The filaments were more compacted in the lowest Cl^-/I^- ratio, and more profusely branched with greater Cl^-/I^- ratios up to 11.29, but with higher ratios the gametophytes became less branched. Generally, the male gametophytes were more branched than the female gametophytes.

At five ratios of Cl^-/I^- between 8.56 and 22.21, male gametophytes produced antheridia 31 to 40 days after inoculation, and female gametophytes produced oogonia 41-42 days after inoculation. No oogonia were formed in the lowest Cl^-/I^- ratio by the end of the 6-week experiment.

The percentage fertility of male gametophytes was greater than female gametophytes in all Cl^-/I^- ratios tested (Fig. 22). The greatest fertility of both male and female gametophytes was found in Cl^-/I^- ratio = 11.29. The percentage fertility decreased with higher or lower Cl^-/I^- ratios.

In the studies on interrelated effects of chloride and iodide the ratio of female to male gametophytes (both fertile and sterile) varied from 0.98 to 1.02 (Table 12) and the average was 0.99

The Cl^-/I^- ratio of 11.29 produced the greatest amounts

Figure 21 A-E. Examples of morphological variability observed in Laminaria saccharina grown in different Cl^-/I^- ratios.

A. Cl^-/I^- ratio of 5.84

B. Cl^-/I^- ratio of 8.56

C. Cl^-/I^- ratio of 11.29

D. Cl^-/I^- ratio of 19.75

E. Cl^-/I^- ratio of 22.21

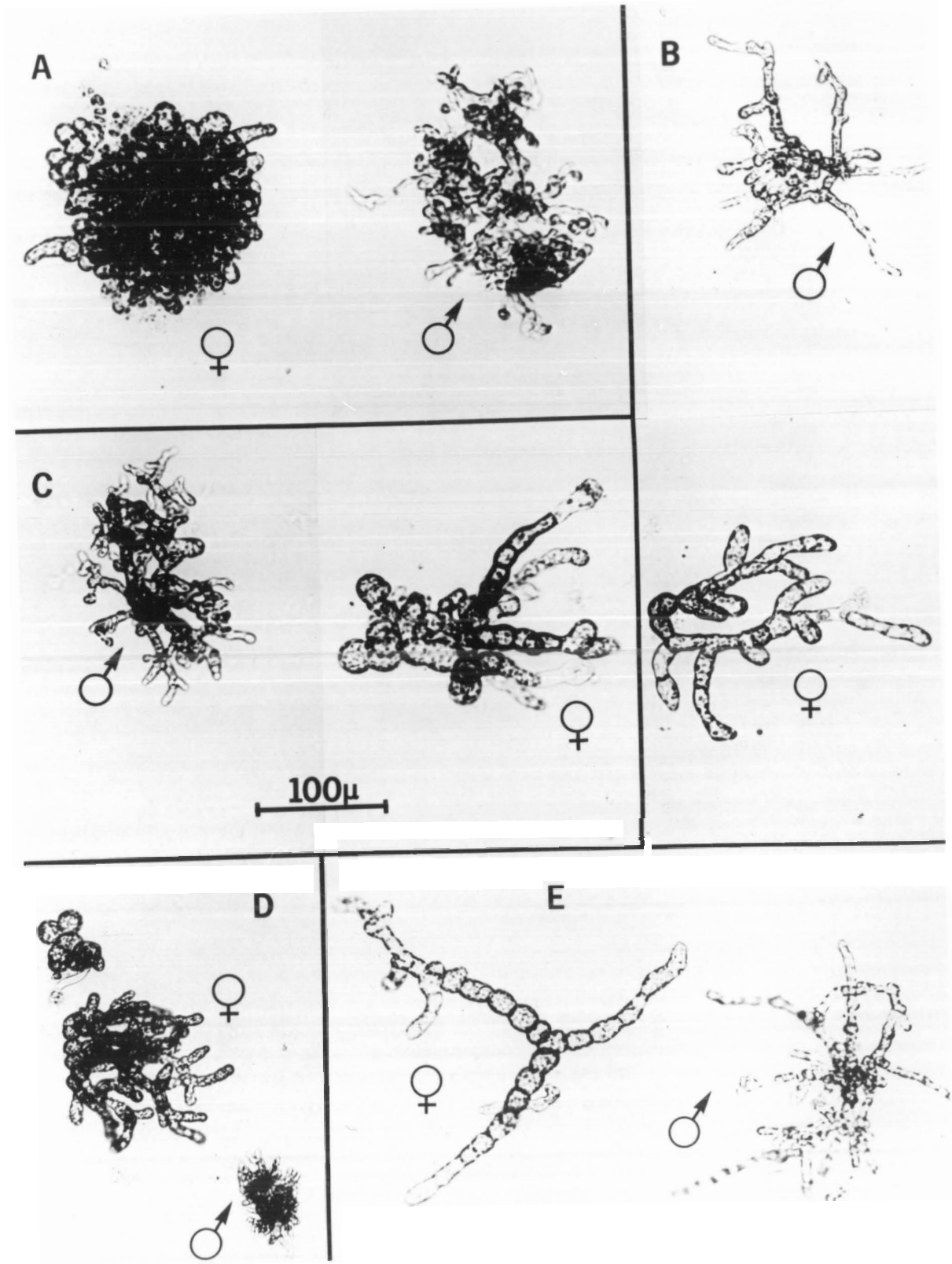

A
B
C
100µ
D
E

Figure 22. Effect of Cl^-/I^- ratios on fertility of Laminaria saccharina gametophytes as a percentage of total gametophytes.

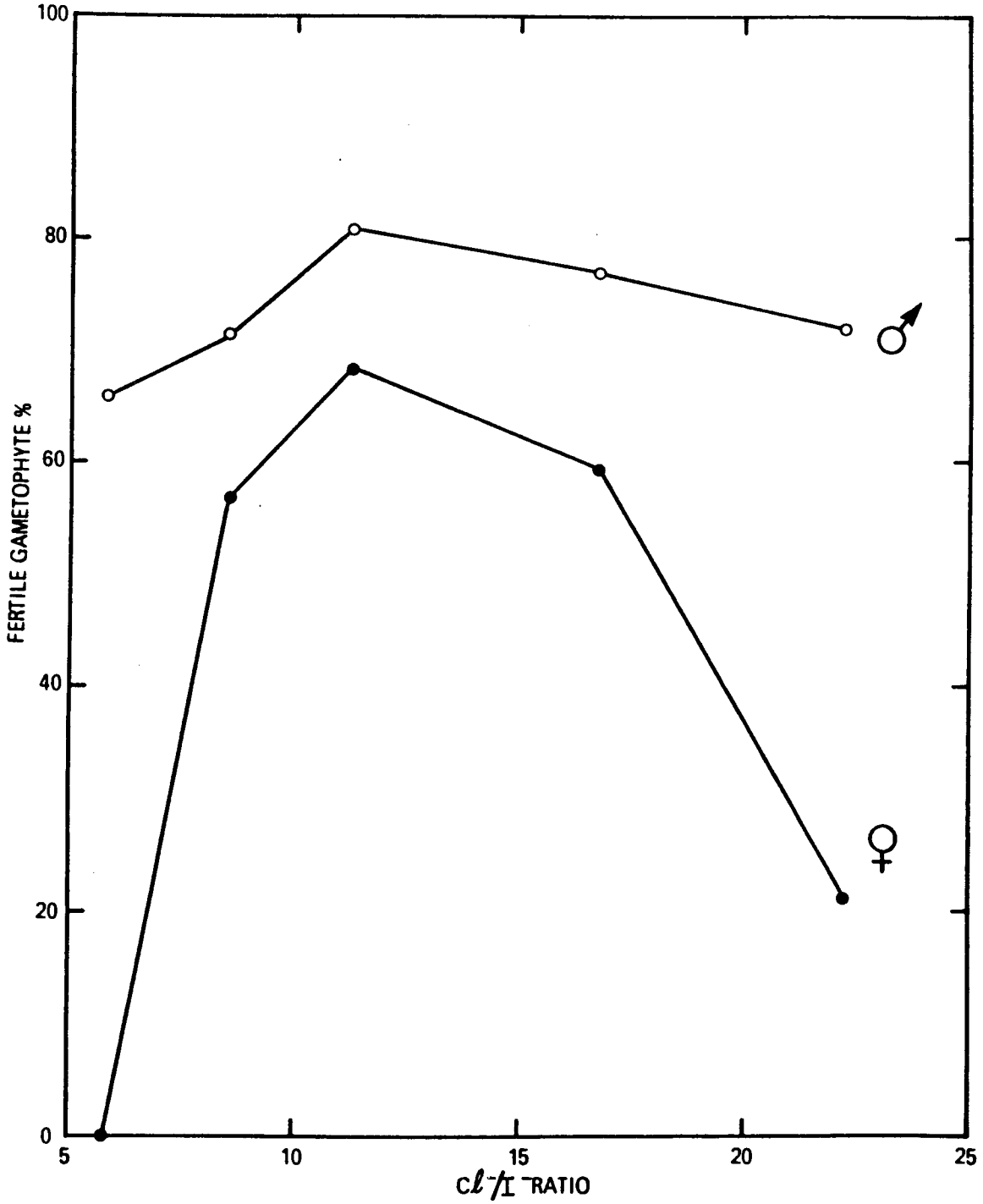

100
80
60
40
20
0
FERTILE GAMETOPHYTE %
5
10
15
20
25
Cl⁻/I⁻ RATIO
♂
♀

Table 12. The ratio of female to male gametophytes (fertile and sterile) and the percentage distribution of oogonia and antheridia produced per gametophyte for Laminaria saccharina in different Cl^-/I^- ratios.

Cl^-/I^- ratio	Total ♀/♂	No. of oogonia/Gametophyte				No. of antheridia/Gametophyte						
		0	1	2	3	0	1	2	3	4	5	6
5.84	1.02	100	0	0	0	34	45	13	4	3	1	0
8.56	0.99	43	37	15	5	28	40	13	9	8	2	0
11.29	0.98	32	43	18	7	19	33	15	16	9	7	1
16.75	0.99	40	39	15	6	23	45	9	12	6	4	1
22.21	0.98	79	20	1	0	28	36	16	10	6	3	1

of DNA, RNA, protein, carbohydrate, chlorophylls a, c and carotenoids in L. saccharina gametophytes (Fig. 23-25). With lower or higher Cl^-/I^- ratios, the concentrations of metabolites were greatly diminished. The greatest RNA/DNA (Fig. 26) and protein/RNA ratios (Fig. 27) were found in Cl^-/I^- ratios of 11.29. These ratios decreased with lower and higher Cl^-/I^- ratios.

DISCUSSION

Iodine stimulated meiospore germination in L. saccharina when iodides were less than 80 μg I^--I/l and iodates were less than 100 μg IO_3-I^-I/l in the media. All forms inhibited germination at higher concentrations. Portyando and Kudrya (1966) found iodine a stimulator of pollen germination in higher plants. They suggested that iodine either might intensify the activity of the oxidative enzymes or be used as a catalyst in the generation of chain reactions when the pollen germinated. Kelly (1953) reported that radioactive iodine uptake by Ascophyllum was related to respiration, and uptake was stimulated by the addition of glucose and sucrose to the medium, which he suggested function as respiratory

Figure 23. Effect of Cl^-/I^- ratios on DNA and RNA concentrations of Laminaria saccharina gametophytes.

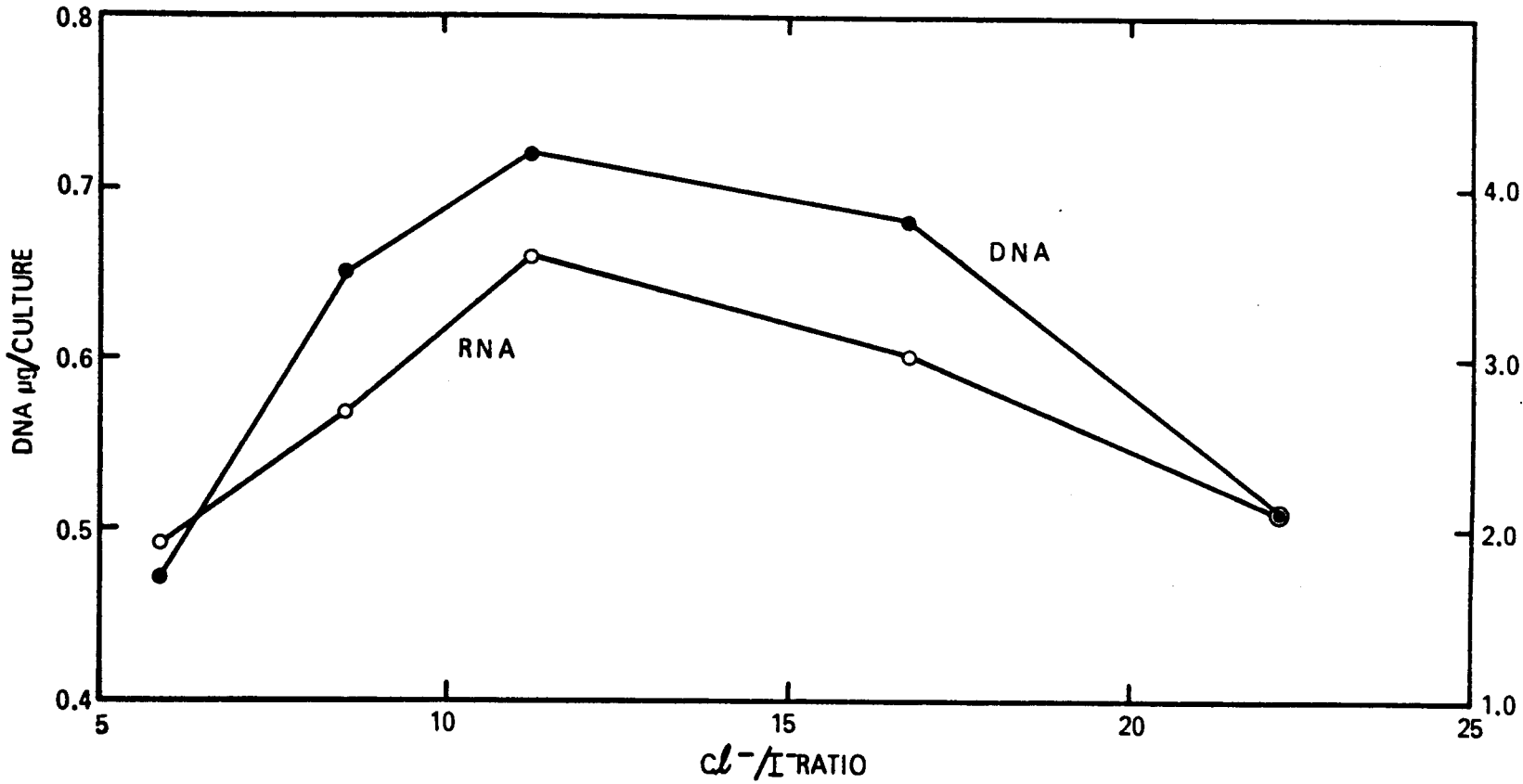

0.8
0.7
0.6
0.5
0.4
DNA µg/CULTURE
4.0
3.0
2.0
1.0
RNA µg/CULTURE
DNA
RNA
5
10
15
20
25
Cl⁻/I⁻RATIO

Figure 24. Effect of Cl^-/I^- ratios on protein and carbohydrate concentrations of Laminaria saccharina gametophytes.

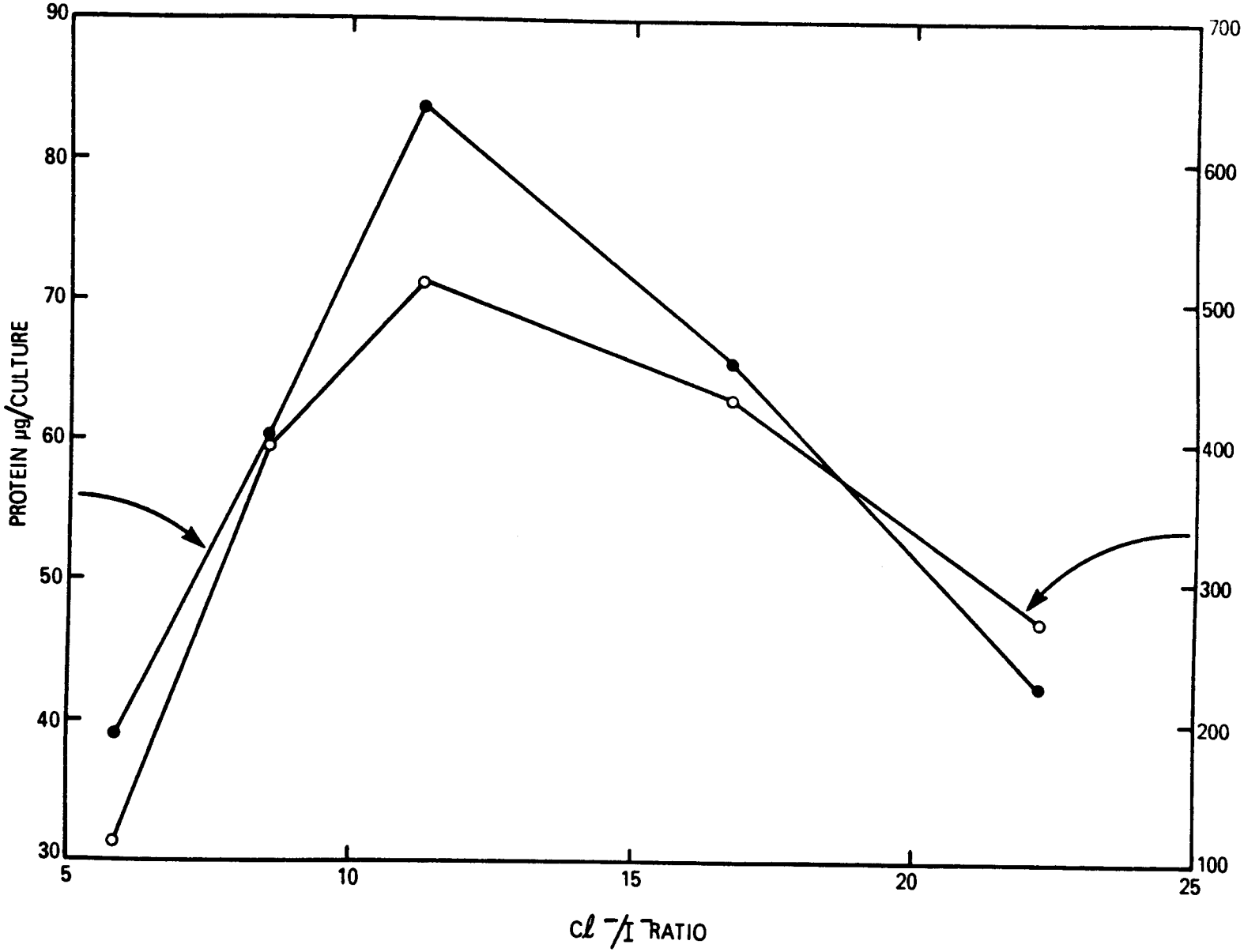

90
80
70
60
50
40
30
PROTEIN µg/CULTURE
700
600
500
400
300
200
100
CARBOHYDRATE µg/CULTURE
5
10
15
20
25
Cl⁻/I⁻ RATIO

Figure 25. Effect of Cl^-/I^- ratios on chlorphylls a, c and total carotenoid concentrations of Laminaria saccharina gametophytes.

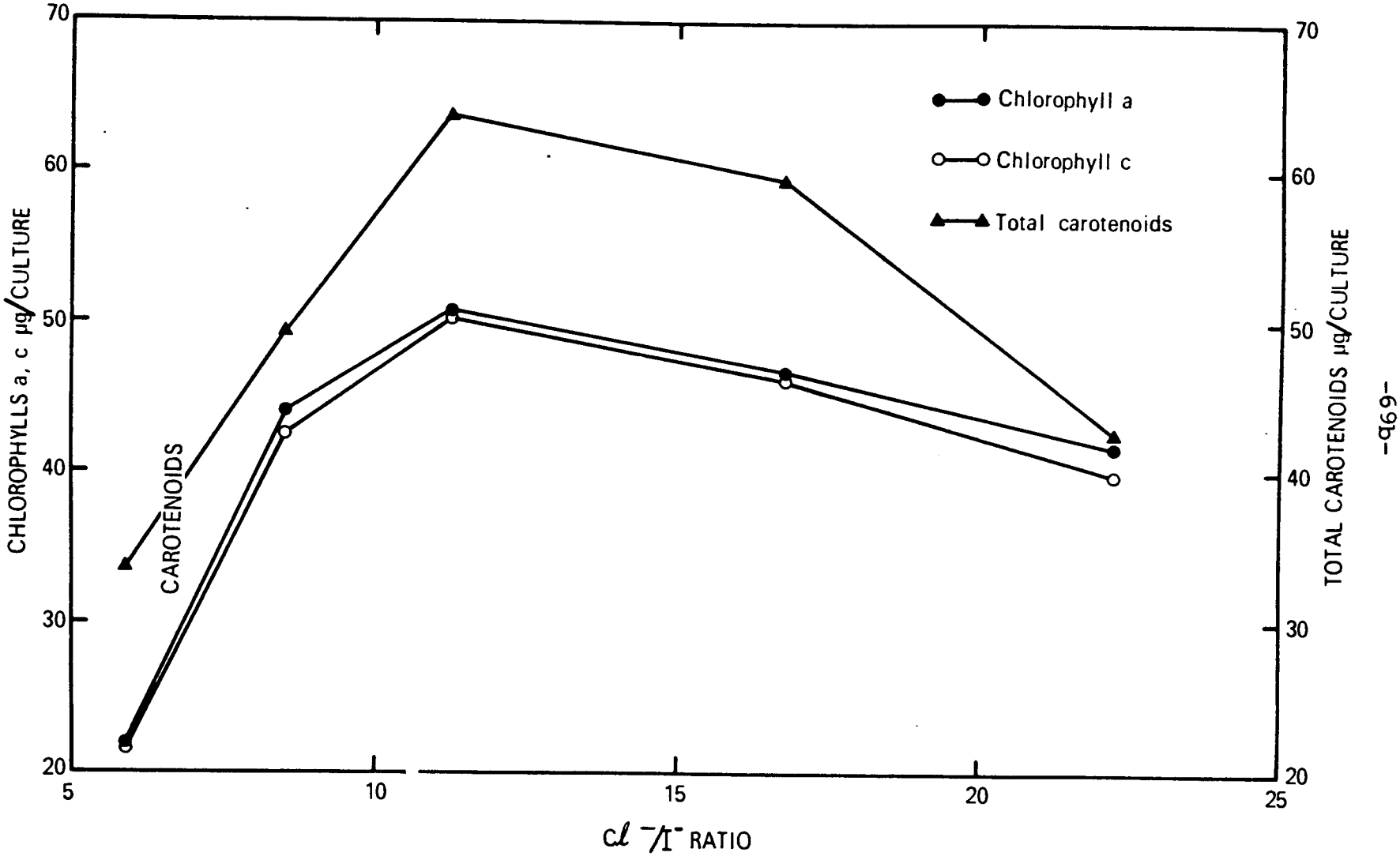

Chlorophyll a
Chlorophyll c
Total carotenoids
CAROTENOIDS
CHLOROPHYLLS a, c µg/CULTURE
TOTAL CAROTENOIDS µg/CULTURE
Cl⁻/I⁻ RATIO
70
60
50
40
30
20
5
10
15
20
25

Figure 26. Effect of Cl^-/I^- ratios on RNA/DNA ratios in *Laminaria saccharina* gametophytes.

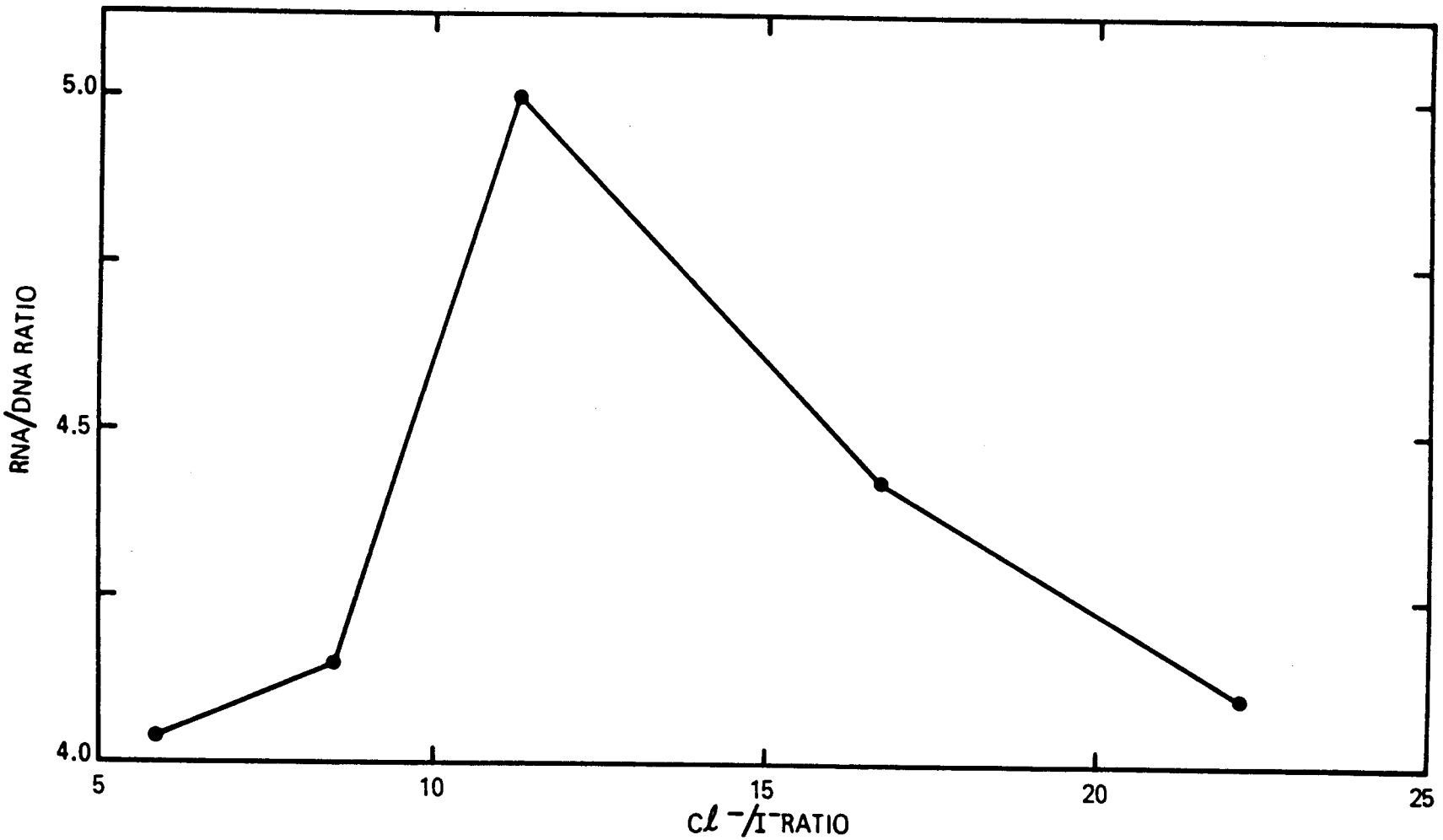
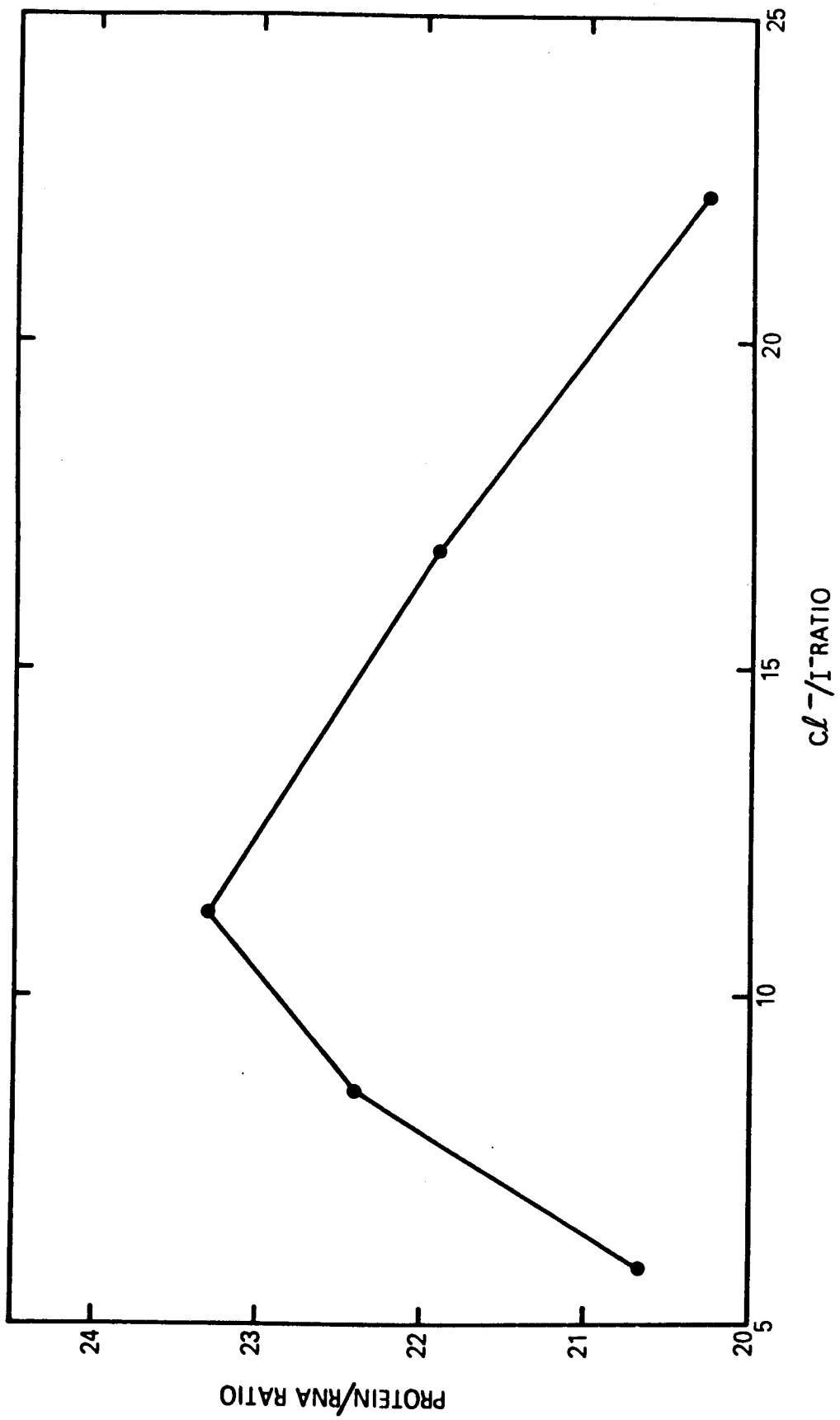

5.0
4.5
4.0
RNA/DNA RATIO
5
10
15
20
25
Cl⁻/I⁻RATIO

Figure 27. Effect of Cl^-/I^- ratios on protein/RNA in Laminaria saccharina gametophytes.


PROTEIN/RNA RATIO
20
21
22
23
24
5
10
15
20
25
Cl⁻/I⁻ RATIO

intermediates. Shaw (1960) showed that iodide uptake by L. digitata blades was accompanied by approximately a fivefold increase in the rate of oxygen consumption. In my study meiospore germination was inhibited by high iodine concentrations. This may be due to a stimulation of carbohydrate utilization by iodine, so that energy necessary for germination was not available.

After the meiospores had germinated, gametophytes grew in all the iodine concentrations tested without significant morphological variation. They produced antheridia and oogonia even in the iodine-free medium and in the medium with the highest iodine concentration. This indicates that iodine in the medium is not essential for growth and gametogenesis of L. saccharina. However, small additions of iodine stimulated growth, development and fertility. Iodine concentrations ranging from 80 to 10^2 μg I^--I/l and from 10^2 to 10^3 μg IO_3^--I/l were less effective. Concentrations greater than 10^3 μg I^--I/l or 10^4 μg IO_3^--I/l had an inhibitory effect.

Von Stoch (1964) showed that Asparagopsis grew twice as well in 10 μ mol KI/l than 2 μ mol KI/l, and abnormal growth occurred in iodine-free medium. Fries (1966), using both inorganic and organic iodine, demonstrated that there was a

linear correlation between iodine concentration and growth of Polysiphonia urceolata at least for concentrations from 1-8 μ mol/l. However, she found that growth of Nemalion multifidum and Goniotrichum elegans did not increase with increasing concentrations. The Conchocelis phase of Porphyra grew best with 10 μg iodine/l as KI; higher concentrations inhibited growth (Iwasaki, 1967). Pedersen (1969) reported that iodine was an absolute requirement for growth of Ectocarpus fasciculatus, but a concentration of 64 μ mol KI/l was inhibitory, while Lithosiphon pusillus grew best at this iodine concentration. Hsiao (1969) demonstrated that different stages of the life history of Petalonia fascia required different iodide concentrations for their development: protonemata and plethysmothalli survived in iodide-free medium, and for Ralifsia-like thalli and blades the minimal iodide concentrations required were 50.76×10^2 μg I^--I/l as KI and 50.76×10^1 μg I^--I/l as KI respectively. Harries (1932) found that a small amount of iodide, 50.8 μg I^--I/l as KI added to natural seawater gave optimal development of L. cloustoni gametophytes. Increased concentrations did not produce a corresponding effect on growth and reproduction, and iodide concentrations higher than 50.8×10^3 μg I^--I/l were inhibitory.

My results agree with Harries', despite the difference in the culture medium.

Sokoloff et al (1963) showed that 6.5 x 10^{-5}M thyroxine (3, 5, 3', 5'-tetraiodothyronine) intensified protein synthesis by promoting the attachment of amino acids to RNA. Portyanko et al (1969) demonstrated that 0.001% KI stimulated amino acid synthesis in corn and barley. These two studies indicate that iodine is related to RNA and protein synthesis. At high concentration iodide had a marked effect on the protein/RNA ratio, the protein being appreciably reduced. The direct effect of iodine on RNA and protein synthesis can not be deduced from this study. Further studies of the effects of iodine concentration on respiration and the attendant production of metabolites might give a clearer picture of the direct effect of iodine on the cell metabolism of L. saccharina gametophytes.

Powers (1939) showed that iodine seemed to stimulate development of chlorophyll in higher plants. In my study of L. saccharina gametophytes it was found that concentrations of chlorophylls a and c did increase up to the optimal iodine concentration. The acutal mechanism involved is unknown for

marine algae.

Generally, optimal concentrations of iodide for meiospore germination, fertility and metabolite concentrations were lower than iodate. At high concentrations, iodide had a much greater inhibitory effect.

Iodine was taken up as iodide by Phaeophyta (Roche and Yagi, 1952; Shaw, 1959, 1960), but not directly as iodate (Baily and Kelly, 1955). Iodate must first be reduced by the plants before it can be absorbed (Borst Pauwels, 1961). According to Ekdahl (1948), iodide has a marked weakening effect on the cell membranes of root hairs of young wheat plants. This might make the cell membrane more permeable to iodide than to iodate which did not have such an effect. Borst Pauwels (1962) showed that the rate of iodine uptake by oats as iodide was more than double that taken up as iodate. Umaly and Poel (1971) explained such differential rates of iodine uptake by plants as due to the large potential difference between the oxidation state of +5 in iodate and -1 in iodide. The lower forms of iodine appear to be taken up more easily than the heavier and higher forms. From this I suggest that the optimum iodine concentration for meiospore germination and growth of L. saccharina gametophytes as well as

syntheses of metabolites was surpassed sooner with iodide than with the equivalent concentration of iodate.

No meiospores germinated 3 days after inoculation in the medium containing 10^6 µg I^--I/l, an iodine concentration 60,000 times greater than that in natural seawater. Meiospore germination and gametophyte growth, as well as DNA, RNA, protein, RNA/DNA, protein/RNA, carbohydrate and pigment content in L. saccharina gametophytes increased as the Cl^-/I^- ratios increased up to 11.29 at a salinity of 23.8‰. They decreased with higher or lower chloride concentrations. Male gametophytes produced antheridia in all Cl^-/I^- ratios, whereas female gametophytes produced oogonia in the range from 8.56 to 22.21 and at a salinity of 16.7-38.9‰. Druehl (1967) grew L. saccharina gametophytes in natural seawater with a broad range of salinities from 17 to 32‰ at 10 C, and they all became fertile. Lewis and Powers (1941) found that iodine uptake and accumulation by a corn plant was not affected by the chloride concentrations in the culture solutions, but that chlorine uptake and accumulation was increased markedly with increasing iodide concentrations. They concluded that the presence of adequate chloride seemed to reduce the toxic action of iodides. Black (1948) found

that L. digitata taken from open sea contained more iodine than in that from the loch. Vinogradov (1953) attributed the greater iodine accumulation by Phaeophyceae from the open sea to the greater iodine concentrations of these waters. I suggest that chloride rather than salinity counteracts inhibition of meiospore germination, gametogenesis and metabolite production caused by high iodide concentrations in L. saccharina. Therefore, marine algae can grow in a plentiful supply of chloride and at the same time permit the accumulation of iodine without toxic effects.

CHAPTER IV

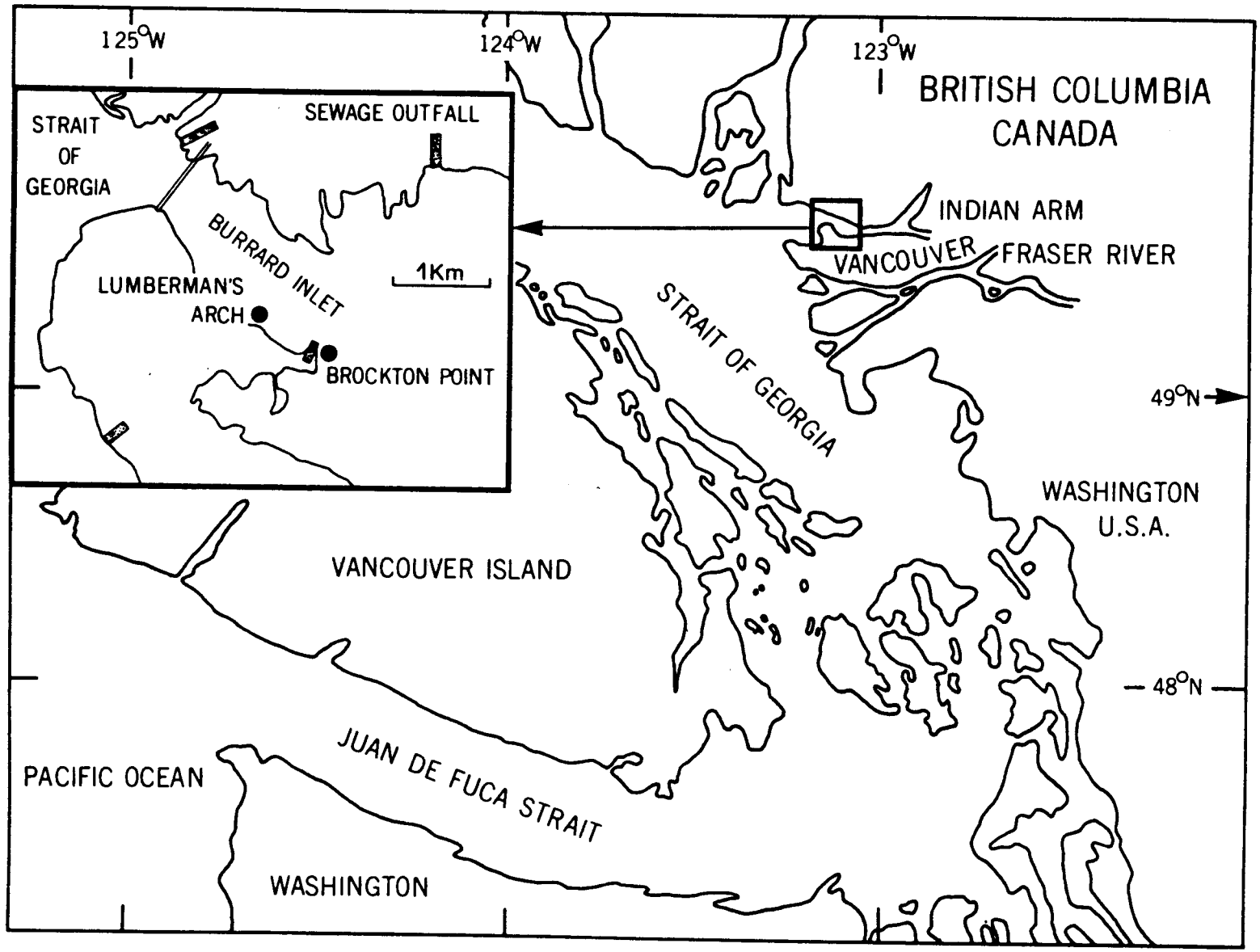
ENVIRONMENTAL CHARACTERISTICS OF STUDY SITES

INTRODUCTION

Laminaria saccharina is widely distributed in the northeast Pacific from Adak Island, Alaska to Coos Bay, Oregon, with an isolated population at Santa Catalina Island, California (Druehl, 1969). Laboratory and field experiments demonstrated that *L. saccharina* had a wide range of tolerance to temperature and salinity, and its distribution along southern British Columbia coasts was probably controlled by water motion (Druehl, 1967).

The purpose of the present study was to describe general features, as well as seasonal and diurnal variations of temperature, salinity, pH, nitrate, nitrite, phosphate, and iodine at Lumberman's Arch (49°18'10" N, 123°07'32" W), Burrard Inlet, B.C., Canada (Fig. 28), from where the plants were collected for the preceding culture experiments. This information was also correlated with *in situ* phenological studies of *L. saccharina* gametophytes at Lumberman's Arch (Chapter V). Similar data were obtained at Brockton Point (49°18'03" N, 123°06'56" W) to learn how the sewage outfall

Figure 28. The location of Burrard Inlet and Lumberman's Arch and Brockton Point (insert).


125°W
124°W
123°W
BRITISH COLUMBIA
CANADA
STRAIT
OF
GEORGIA
SEWAGE OUTFALL
BURRARD INLET
1Km
LUMBERMAN'S
ARCH
BROCKTON POINT
INDIAN ARM
VANCOUVER
FRASER RIVER
STRAIT OF GEORGIA
49°N
WASHINGTON
U.S.A.
VANCOUVER ISLAND
48°N
PACIFIC OCEAN
JUAN DE FUCA STRAIT
WASHINGTON

might affect the environmental parameters under consideration and the growth of L. saccharina.

Burrard Inlet is oriented in an east-west direction and connects with the Strait of Georgia at its mouth. Indian Arm Inlet opens into Burrard Inlet near its head and is one source of freshwater. Freshwater from the Fraser River flushes in at the west end of Burrard Inlet. There are several sewage outfalls along the shores of Burrard Inlet (Fig. 28). One of them is located at Brockton Point.

MATERIALS AND METHODS

Surface water samples were collected weekly from June 30, 1968 to June 26, 1971 on the ebbing tide, within half an hour of low tide. Variation in monitored parameters as a function of the diurnal tides was determined over 3 years at the time of the greatest tidal amplitude for the middle month of the 3-month periods, related to periods of runoff as described by Gilmartin (1962) i.e. winter minimum runoff (January-March), spring maximum runoff (April-June), summer minimum runoff (July-September) and autumn maximum runoff (October-December). The four periods listed above will be

referred to as winter, spring, summer and autumn respectively.

A plastic bucket and plastic beaker were used to collect water samples. Beakers of water were scooped from the water/beach interface at short intervals along approximately 20 m of shore. The final sample was a composite of usually more than 20 subsamples. Separate thermometers were used to measure air and seawater temperatures. The pH was measured with a battery powered Fisher Accumet pH meter model 210, and salinity was determined in the field with a Goldberg refractometer (American Optical Co.). Water samples for analysis of nitrate, nitrite, phosphate, and iodine were immediately filtered through glass wool, tightly plugged into the neck of a plastic funnel. The filtered samples were placed in 8-oz polyethylene screw-cap bottles, and quick-frozen in an ice-chest with dry ice. The frozen samples were then stored at -25 C until analysed.

Colorimetric chemical analyses were performed with a Hitachi Perkin-Elmer model 139 UV-Vis spectrophotometer using the following methods: nitrate (Wood et al, 1967); nitrite (Bendschneider and Robinson, 1952); phosphate (Strickland and Parsons, 1968) and total inorganic iodine (Dubravčić, 1955). The results are mean values of at least duplicate

samples.

RESULTS

The information obtained at Lumberman's Arch and Brockton Point were, in all but a few cases, identical. The sewage outfall at latter site did not appear to appreciably affect the parameters measured. The data for Lumberman's Arch are discussed and the data from Lumberman's Arch and Brockton Point are presented in illustrations (Fig. 29-39).

General Features

Grained rocks form a major portion of the intertidal area at Lumberman's Arch. Clayed silts and shells are found between the rocks. Laminaria saccharina grows on rocks and shells. The other kelps found are Alaria tenuifolia Setchell, Costaria costata (Turner) Saunders and Nereocystis luetkeana (Mert.) Postels and Ruprecht.

In Burrard Inlet the tides are the mixed semi-diurnal type with a maximum range of 15.9 ft (Canadian Hydrographic Service 1968-1971). There are two high and two low tides every 24 hours, each with a duration of about 6 hours (Fig. 29-32). The spring low tides occurred around mid-day during

Figure 29. Average diurnal variations of pH, salinity, seawater temperature, nitrate, nitrite, phosphate, and total inorganic iodine during one complete tidal cycle for the winters of 1969-71.

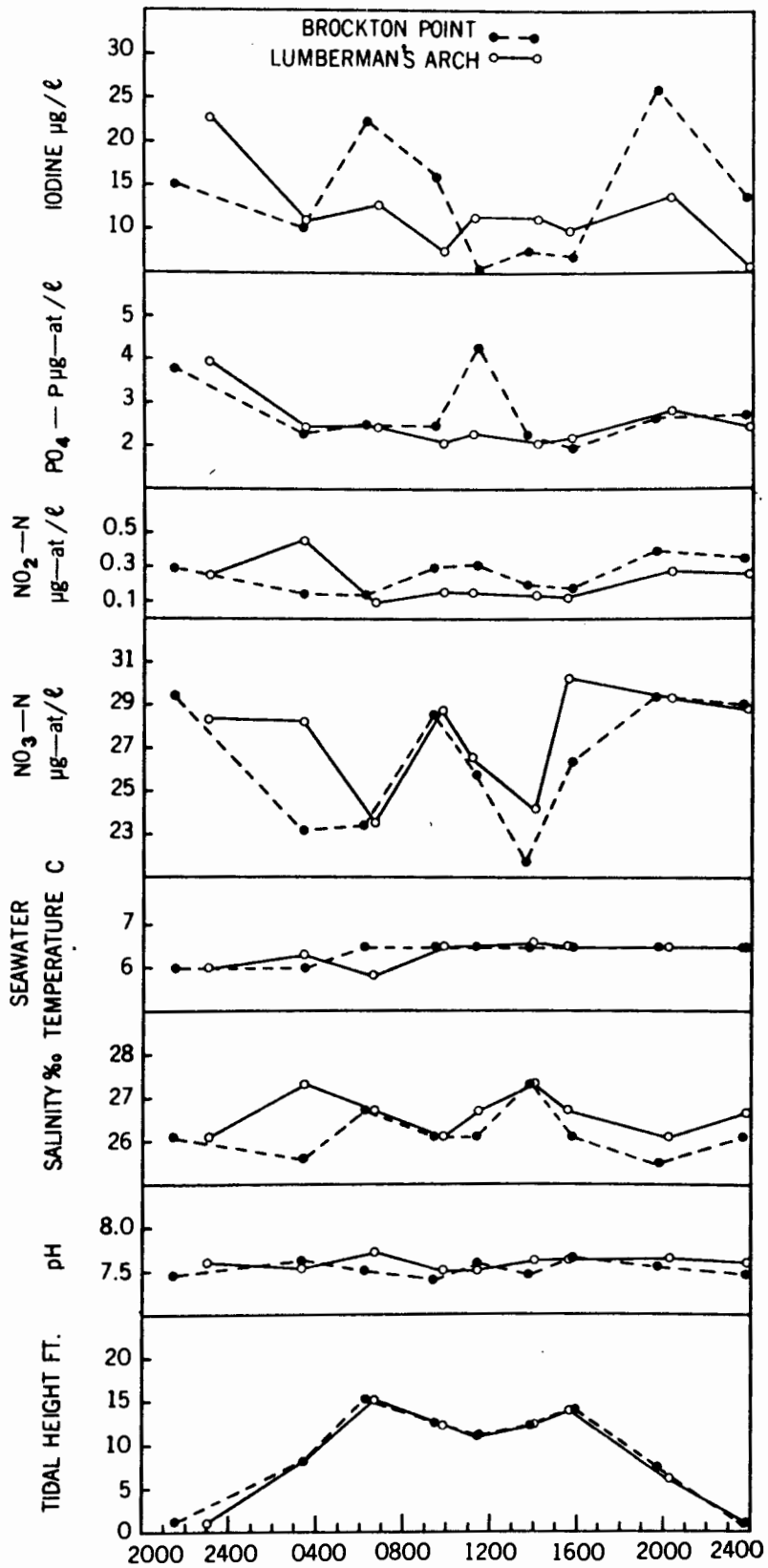

BROCKTON POINT
LUMBERMAN'S ARCH
IODINE µg/ℓ
30
25
20
15
10
PO4 — P µg—at/ℓ
5
4
3
2
NO2—N µg—at/ℓ
0.5
0.3
0.1
NO3—N µg—at/ℓ
31
29
27
25
23
SEAWATER TEMPERATURE C
7
6
SALINITY ‰
28
27
26
pH
8.0
7.5
TIDAL HEIGHT FT.
20
15
10
5
0
2000 2400 0400 0800 1200 1600 2000 2400
HOUR OF DAY (P.S.T.)

Figure 30. Average diurnal variations of pH, salinity, seawater temperature, nitrate, nitrite, phosphate, and total inorganic iodine during one complete tidal cycle for the springs of 1969-71.

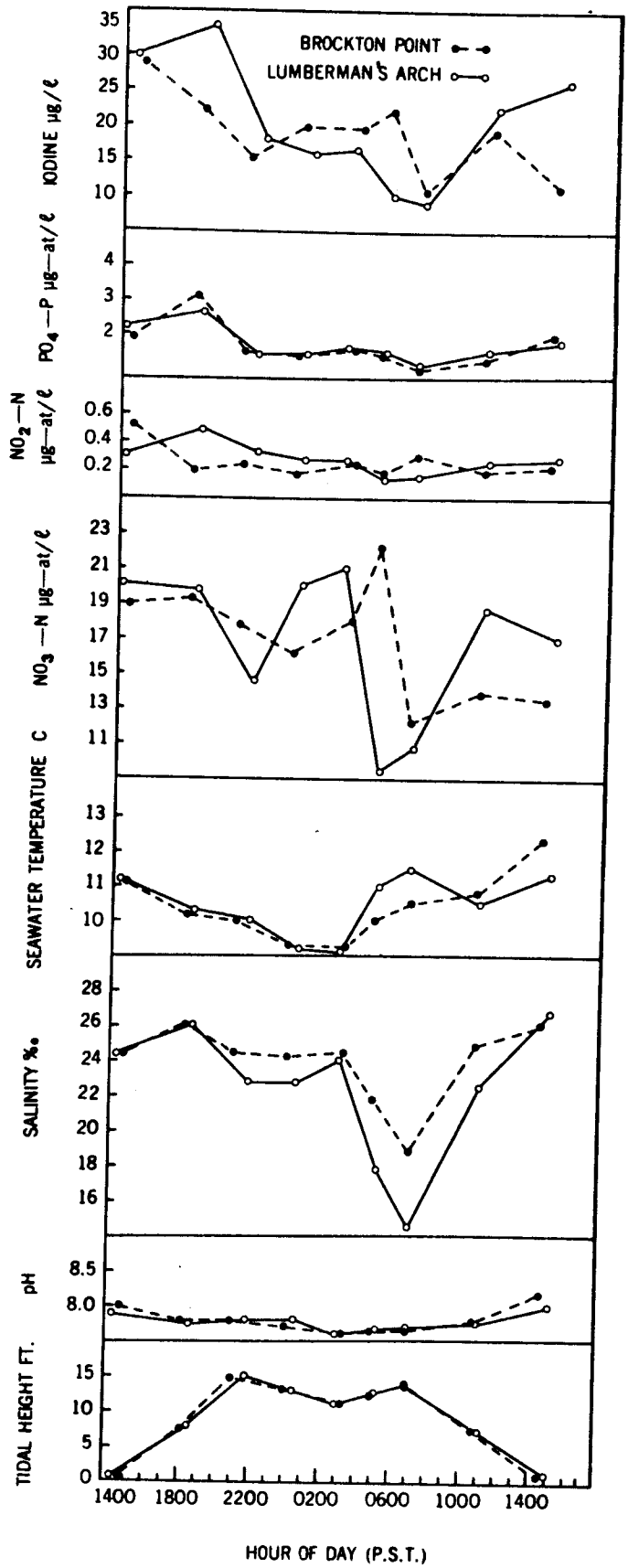

BROCKTON POINT
LUMBERMAN'S ARCH
IODINE µg/ℓ
PO4—P µg—at/ℓ
NO2—N µg—at/ℓ
NO3—N µg—at/ℓ
SEAWATER TEMPERATURE C
SALINITY ‰
pH
TIDAL HEIGHT FT.
1400 1800 2200 0200 0600 1000 1400
HOUR OF DAY (P.S.T.)

Figure 31. Average diurnal variations of pH, salinity, seawater temperature, nitrate, nitrite, phosphate, and total inorganic iodine during one complete tidal cycle for the summers of 1968-70.

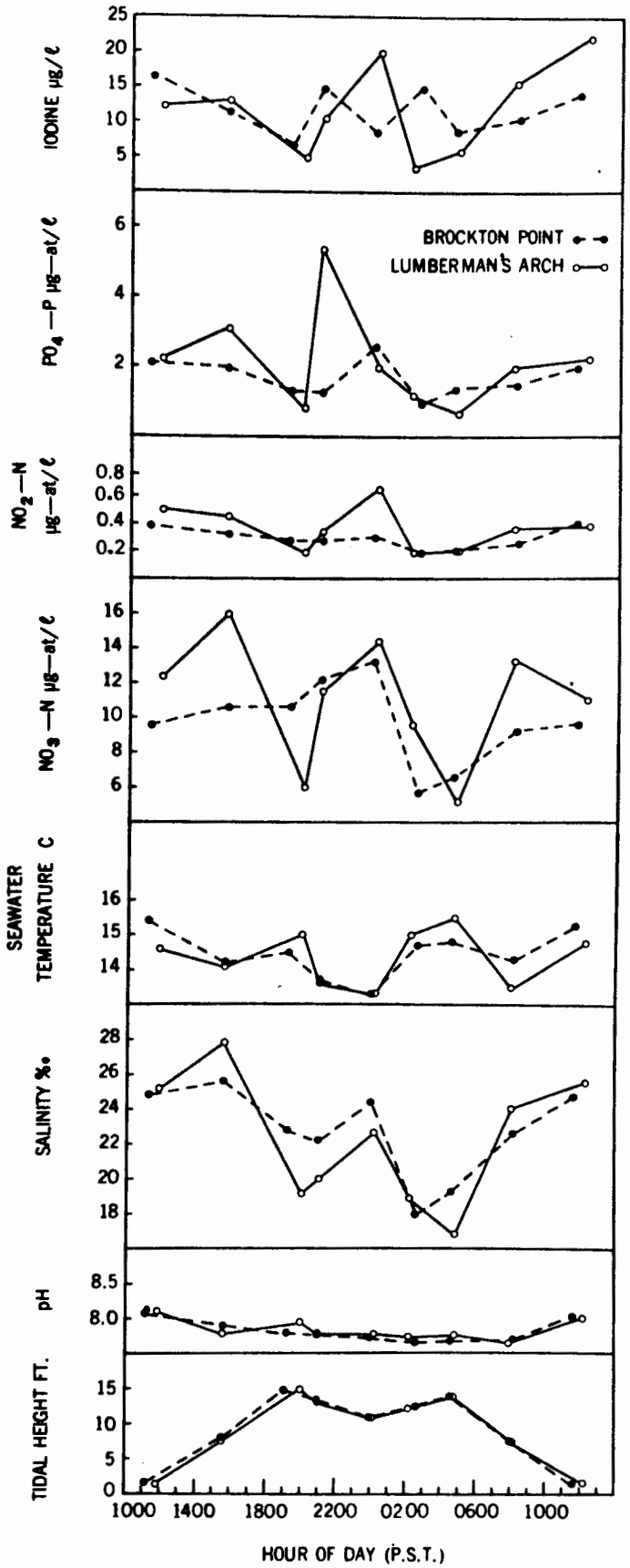
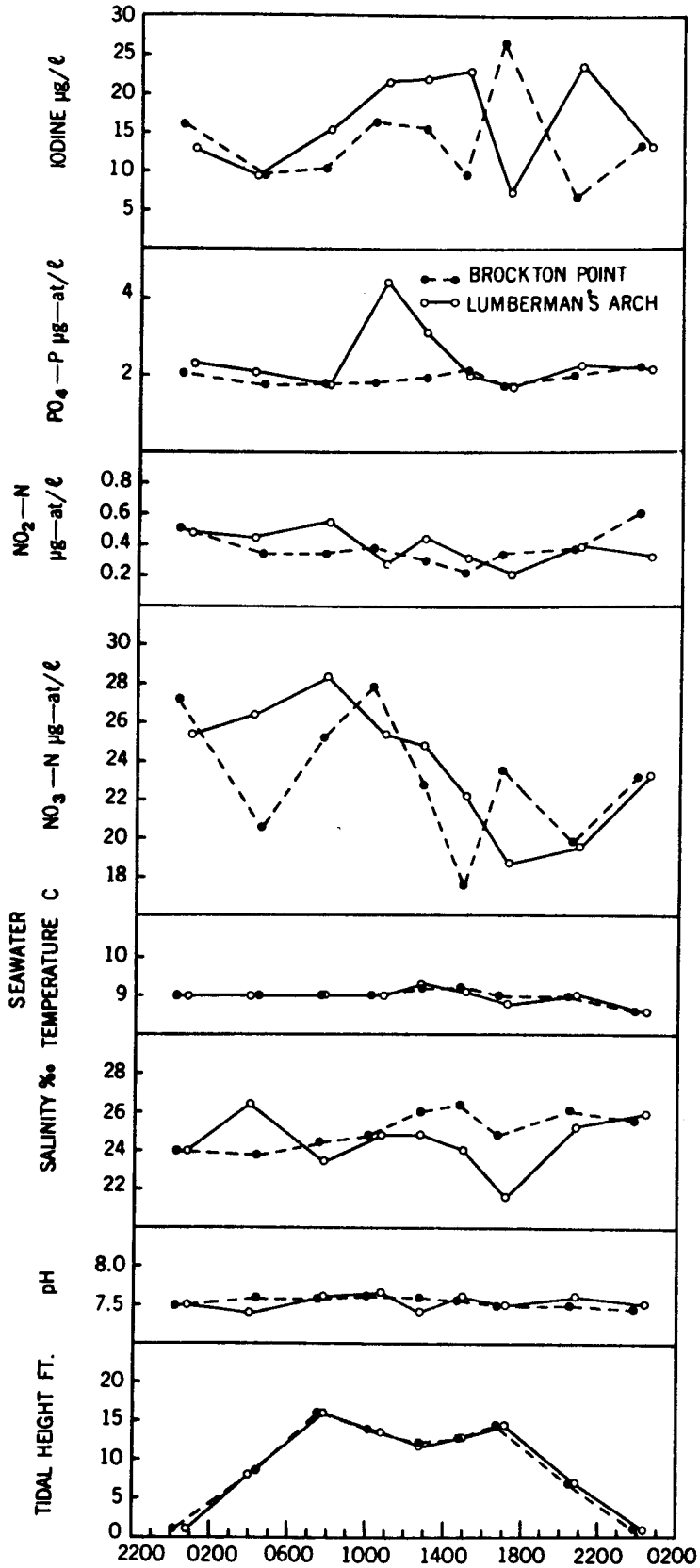

IODINE µg/ℓ
25
20
15
10
5
PO_4 —P µg—at/ℓ
6
4
2
BROCKTON POINT
LUMBERMAN'S ARCH
NO_2—N µg—at/ℓ
0.8
0.6
0.4
0.2
NO_3 —N µg—at/ℓ
16
14
12
10
8
6
SEAWATER TEMPERATURE C
16
15
14
SALINITY ‰
28
26
24
22
20
18
pH
8.5
8.0
TIDAL HEIGHT FT.
15
10
5
0
1000 1400 1800 2200 0200 0600 1000
HOUR OF DAY (P.S.T.)

Figure 32. Average diurnal variations of pH, salinity, seawater temperature, nitrate, nitrite, phosphate, and total inorganic iodine during one complete tidal cycle for the autumns of 1968-70.


SEAWATER
IODINE µg/ℓ
PO4—P µg—at/ℓ
NO2—N µg—at/ℓ
NO3—N µg—at/ℓ
TEMPERATURE C
SALINITY ‰
pH
TIDAL HEIGHT FT.
BROCKTON POINT
LUMBERMAN'S ARCH
30
25
20
15
10
5
4
2
0.8
0.6
0.4
0.2
30
28
26
24
22
20
18
10
9
28
26
24
22
8.0
7.5
20
15
10
5
0
2200
0200
0600
1000
1400
1800
2200
0200
HOUR OF DAY (P.S.T.)

the spring and summer, and around mid-night during the autumn and winter.

The following descriptions of pH, salinity, temperature, and nutrients are based on two types of data. The first is used to describe seasonal variations (Fig. 33-39). Here, monthly values were determined by averaging weekly data collected at low tide. In the second, diurnal variation is described as a function of tidal phase. Each plotted value represents a three year average for each tidal phase (Fig. 29-32).

pH

Higher pH values were found in summer (8.14) than in winter (7.52) at Lumberman's Arch (Fig. 33). In spring and summer pH values were a little higher during the day than the night (Fig. 30, 31).

Salinity

Salinity fluctuation varied with seasons and tidal phases (Fig. 34, 29-32). It increased to a maximum in winter of 26.1-27.8‰, and decreased to a minimum in summer of 19.5-20.0‰. Salinity varied considerably during the diurnal tidal cycle (Fig. 29-32). The greatest diurnal variation in salinity over a 24 hour period occurred during the spring (14.5-26.7‰).

Figure 33. Seasonal variations (monthly means) of pH in Burrard Inlet as measured weekly at low tide.

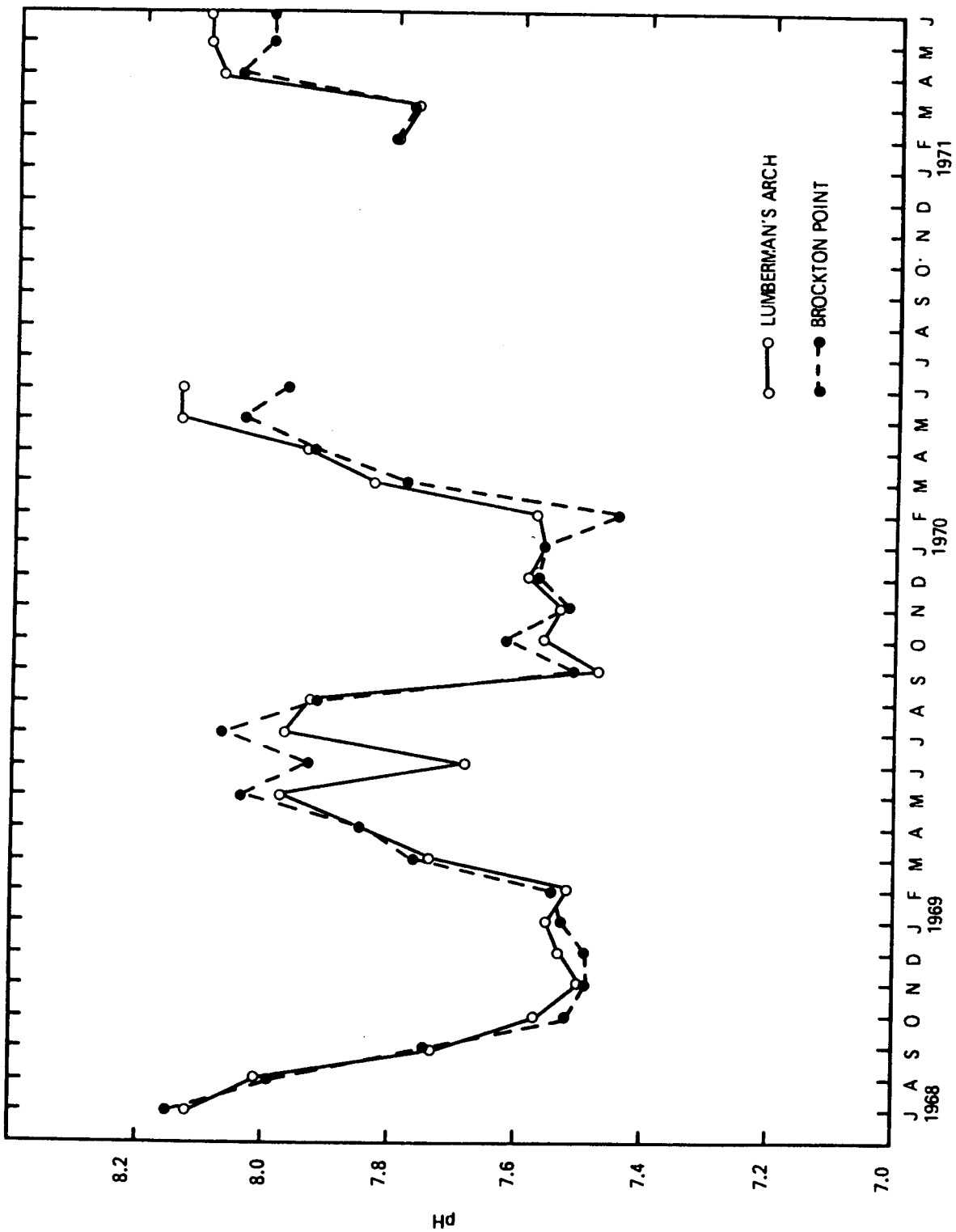
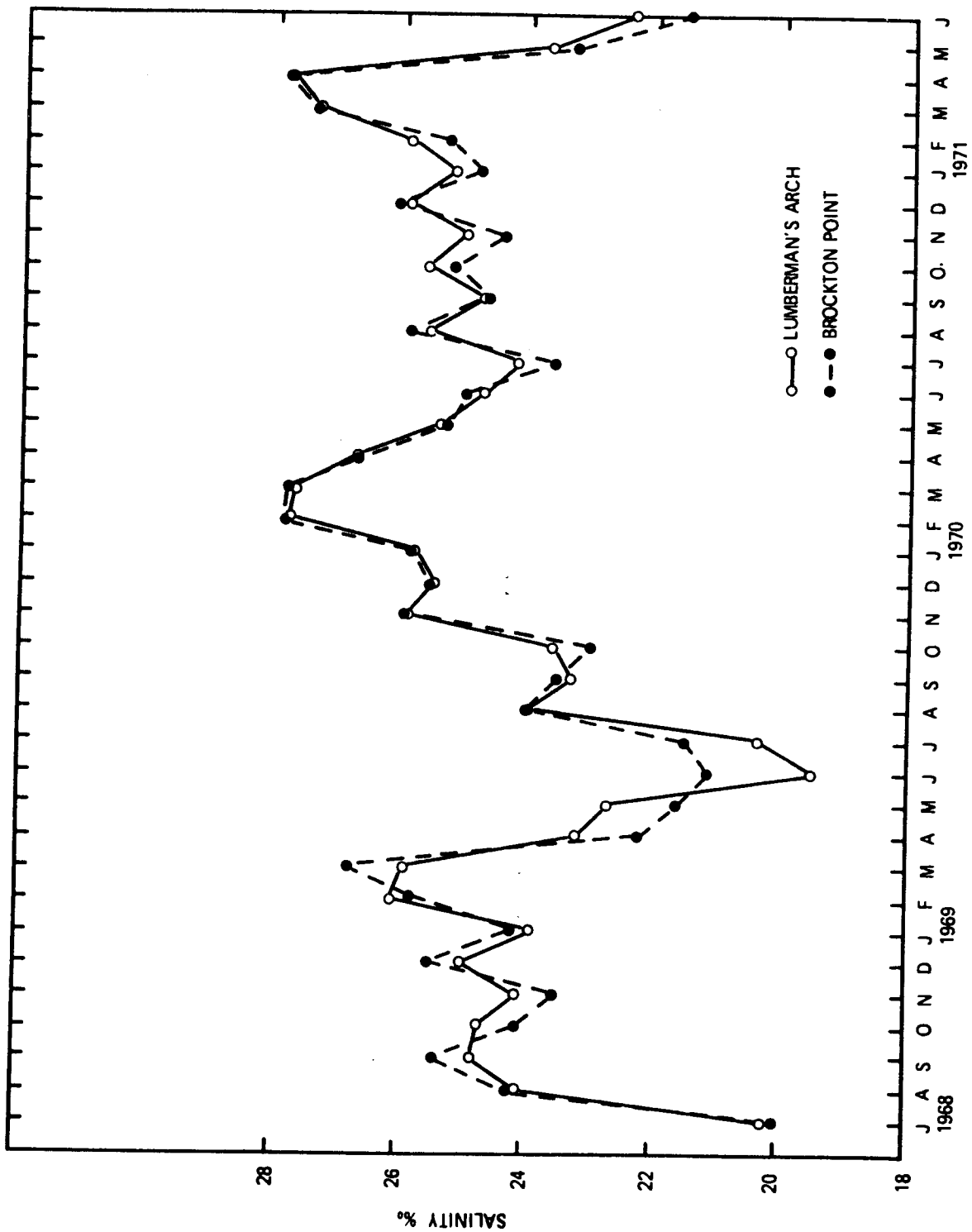

LUMBERMAN'S ARCH
BROCKTON POINT
pH
8.2
8.0
7.8
7.6
7.4
7.2
7.0
J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J
1968
1969
1970
1971

Figure 34. Seasonal variations (monthly means) of salinity in Burrard Inlet as measured weekly at low tide.


LUMBERMAN'S ARCH
BROCKTON POINT
SALINITY ‰
28
26
24
22
20
18
J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J
1968
1969
1970
1971

Temperature

The highest surface water temperatures occurred in July, 15.6-16.0 C, and the lowest temperatures in January, 4.7-7.0 C (Fig. 35). The annual mean temperature over 3 years was 10.2 C.

Surface water temperatures showed some variation with the tidal cycle during the spring and summer, but varied little during autumn and winter (Fig. 29-32). The greatest diurnal variations occurred in spring (9-11.5 C).

Nitrate

The annual variation of nitrate concentrations is shown in Fig. 36. The highest nitrate concentration occurred during January (27.1-29.6 μg-at/l). The lowest nitrate concentration occurred in July and August (6.0-7.2 μg-at/l). There were two nitrate maxima at Lumberman's Arch during the winter of 1968-1969, the first one occurred in November and the second one in January. These two peaks shifted two months later in 1970 and 1971.

There was a considerable diurnal variation in nitrate concentration for all four seasons (Fig. 29-32). Variation was greatest at Lumberman's Arch during spring (9.5-20.2 μg-at/l), and least during the winter (23.6-30.3 μg-at/l).

Figure 35. Seasonal variations (monthly means) of surface seawater temperature in Burrard Inlet as measured weekly at low tide.

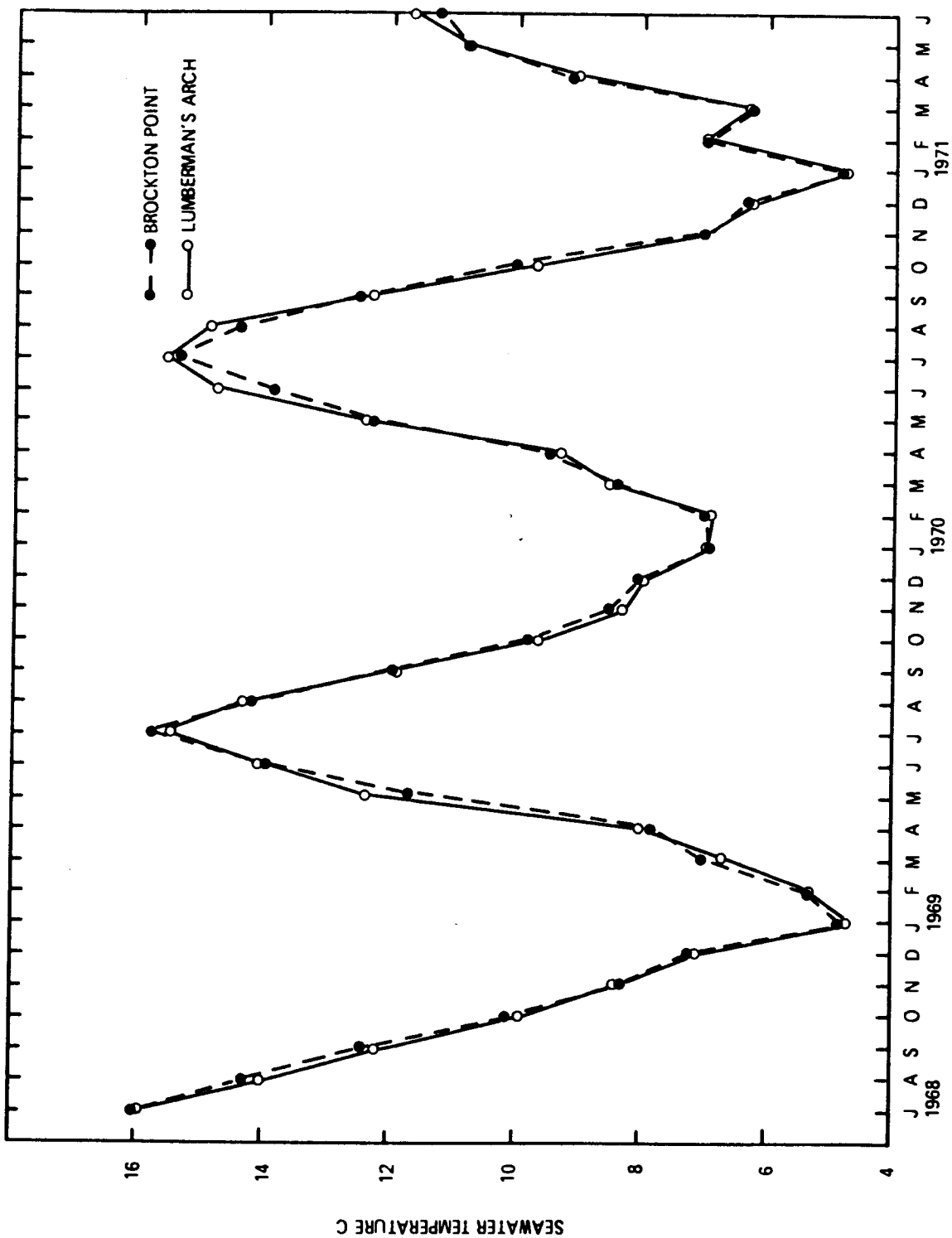
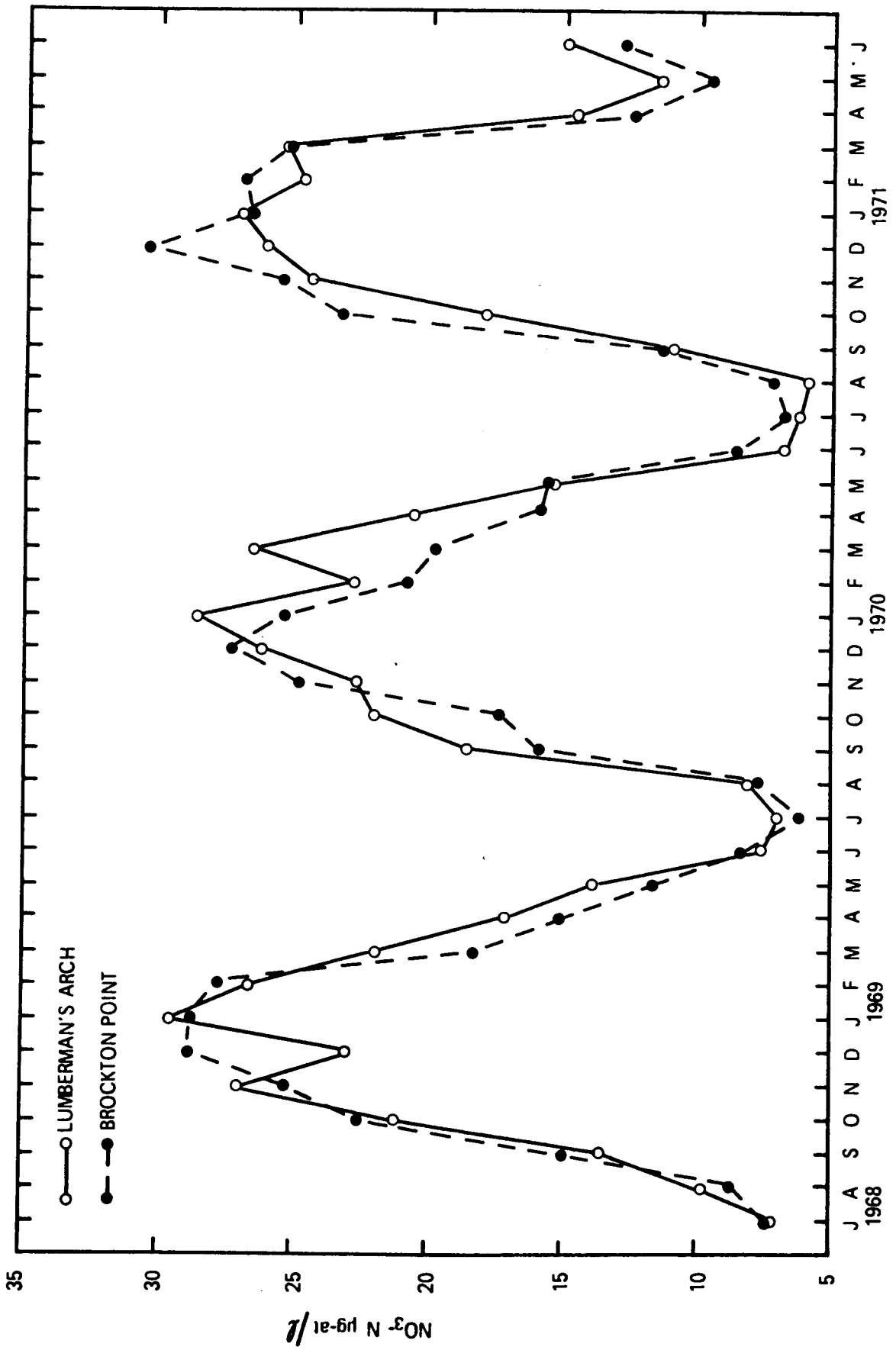

BROCKTON POINT
LUMBERMAN'S ARCH
SEAWATER TEMPERATURE C
16
14
12
10
8
6
4
J A S O N D J F M A M J J A S O N D J F M A M J J
1968
1969
1970
1971

Figure 36. Seasonal variations (monthly means) of nitrate in Burrard Inlet as measured weekly at low tide.


LUMBERMAN'S ARCH
BROCKTON POINT
NO3- N µg-at/l
35
30
25
20
15
10
5
J A S O N D J F M A M J J A S O N D J F M A M J J
1968
1969
1970
1971

Nitrite

Seasonal distribution of nitrite was irregular (Fig. 37). The highest monthly nitrite concentration of 0.74-0.79 μg-at/l occurred in different months over the 3-year period. The greatest diurnal variation (Fig. 29-32) of nitrite occurred during the summer (0.19-0.65 μg-at/l).

Phosphate

There was considerable variation in monthly mean inorganic phosphate concentrations (Fig. 38), and the occurrence of high and low peaks was irregular at Lumberman's Arch. Diurnal variation (Fig. 29-32) of phosphate was greatest during summer at Lumberman's Arch (0.7-5.4 μg-at/l).

Iodine

The total inorganic iodine concentration in surface seawater did not follow a seasonal pattern (Fig. 39). The greatest mean monthly value for Lumberman's Arch was 29.2 μg/l and the lowest was 2.9 μg/l.

There was considerable diurnal variation in iodine concentrations (Fig. 29-32). The greatest variation occurred during the spring, 9.6-34.2 μg/l, and least during the autumn, 7.6-23.8 μg/l.

Figure 37. Seasonal variations (monthly means) of nitrite in Burrard Inlet as measured weekly at low tide.

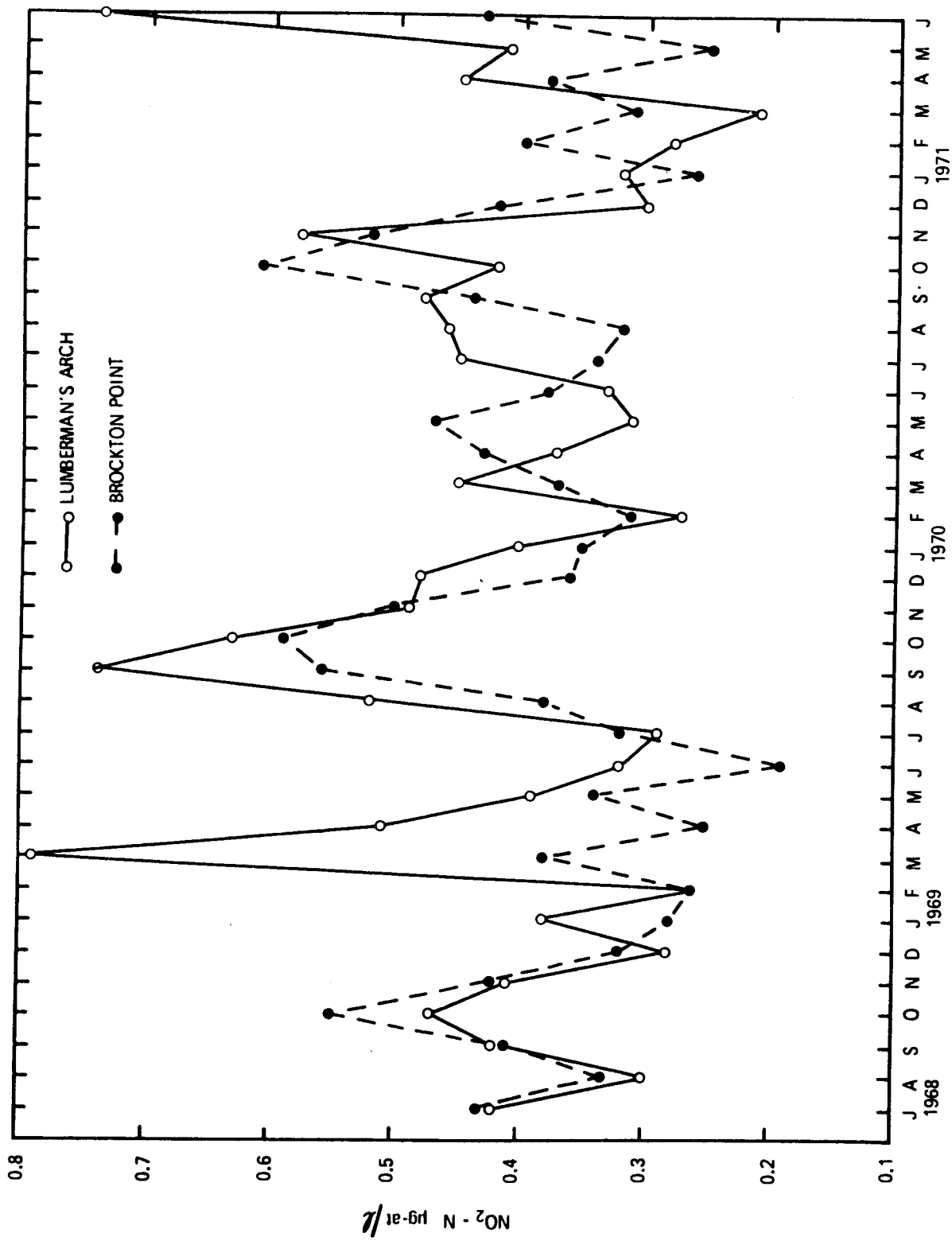

LUMBERMAN'S ARCH
BROCKTON POINT
NO2 - N µg-at/l
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J
1968
1969
1970
1971

Figure 38. Seasonal variations (monthly means) of phosphate in Burrard Inlet as measured weekly at low tide.

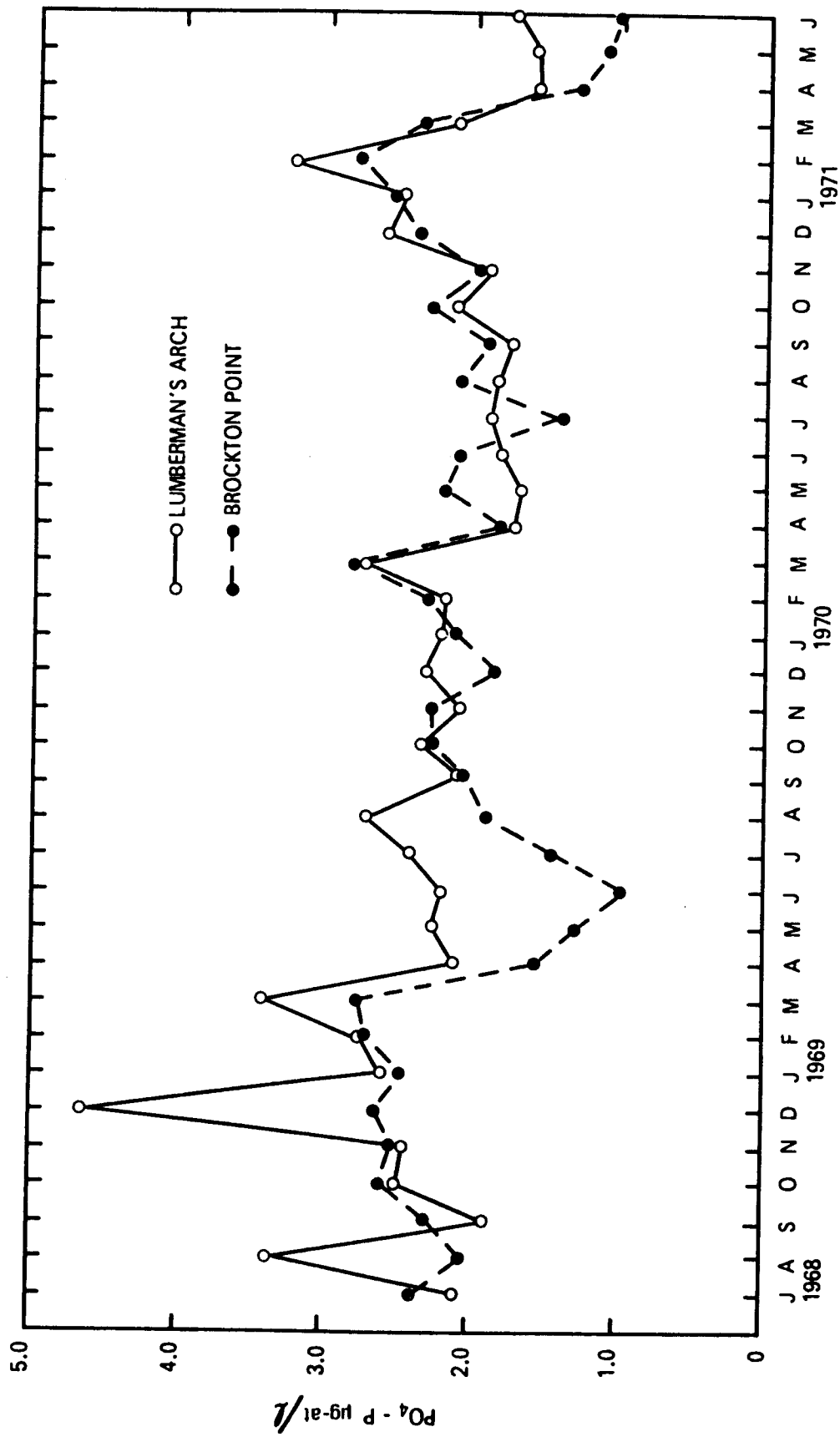
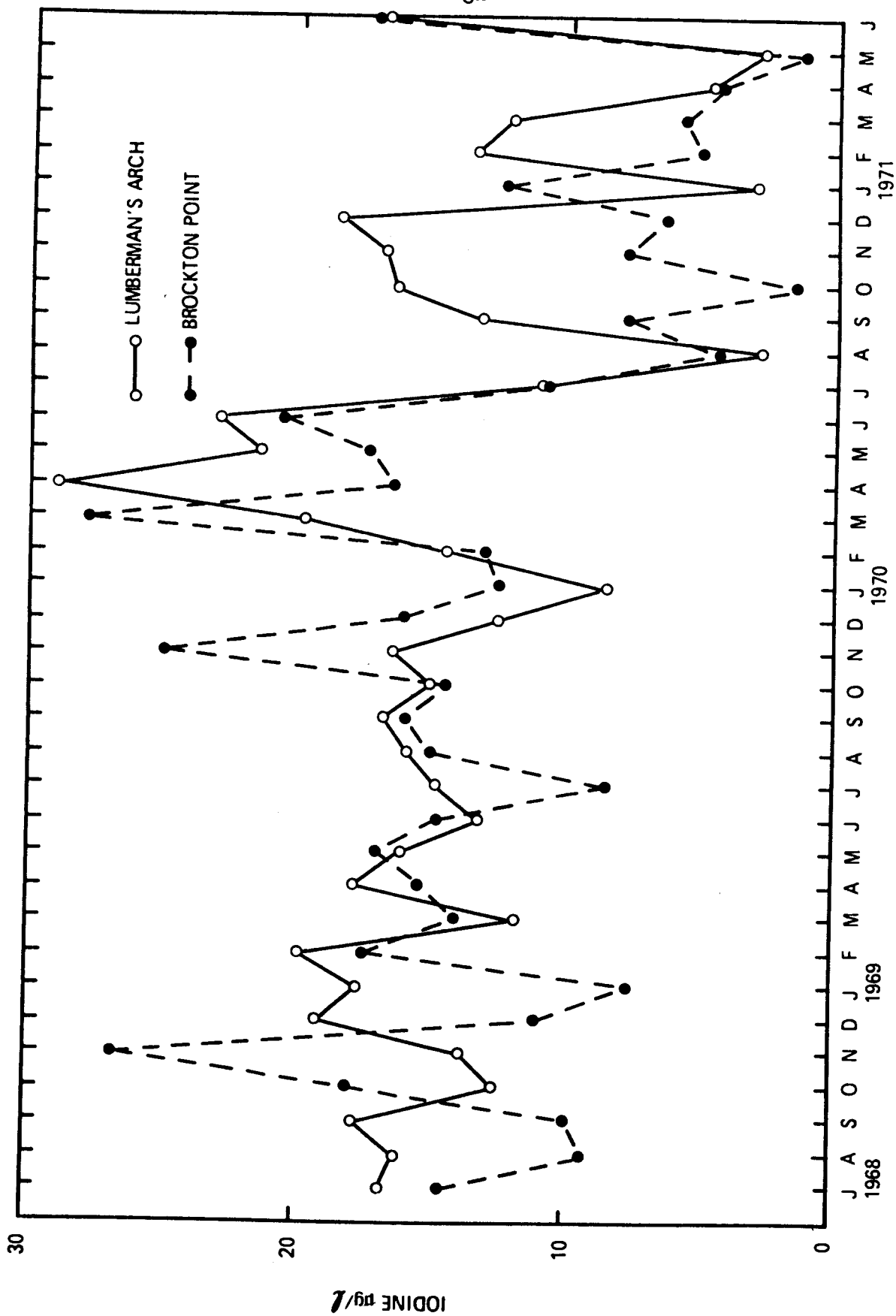

LUMBERMAN'S ARCH
BROCKTON POINT
PO_4 - P µg-at/l
5.0
4.0
3.0
2.0
1.0
0
J A S O N D J F M A M J J A S O N D J F M A M J J
1968
1969
1970
1971

Figure 39. Seasonal variations (monthly means) of total inorganic iodine in Burrard Inlet as measured weekly at low tide.


LUMBERMAN'S ARCH
BROCKTON POINT
IODINE µg/L
30
20
10
0
J A S O N D J F M A M J J A S O N D J F M A M J J
1968
1969
1970
1971

DISCUSSION

The environmental features monitored in Burrard Inlet were greatly influenced by the season and the tidal phase and were similar for Lumberman's Arch and Brockton Point.

The annual and seasonal surface seawater temperatures were similar at the two sites with minima occurring in January and maxima in July.

The summer minimum in salinity seems to be influenced by Fraser River discharges since the salinity starts to fall in early April and reaches a minimum in mid-June. Pickard and McLeod (1953) showed that the average monthly discharge of the Fraser River rose from a minimum of 0.8×10^{11} cubic feet in March to a maximum of 9×10^{11} cubic feet in late May.

The diurnal variation in pH may partly be related to high carbon dioxide during the night and low carbon dioxide during the day as a result of the plant/animal metabolism. Other factors which may cause variations in pH are, for example, seasonal variations in light intensity which would affect photosynthesis, and the amount and chemical character of run-

off water.

Nutrient cycles in Burrard Inlet are similar to those in shallow coastal waters, but the influence of river discharge and sewage are more apparent. Nitrate and phosphate concentrations were relatively high compared to Indian Arm, the Strait of Georgia and Pacific Ocean (Table 13). Relatively low concentrations of nitrate and phosphate were found in summer at both stations. This may be attributed to their removal by phytoplankton blooms and the rapid growth of the macroalgae. Kelps which grew in the intertidal during the spring, died in the summer, but subtidal plants continued to grow. Plant decay would regenerate nitrate and phosphate.

The regeneration of phosphate is favored in estuaries (Carritt and Goodgal, 1954), and its rate is more rapid than nitrate (Riley, 1967). Nitrate and phosphate began to increase in the autumn and continued to do so throughout the winter months at both stations. Nitrate concentrations at Brockton Point and Lumberman's Arch reached their highest levels in December and January respectively. Phosphate concentrations at Brockton Point reached their highest levels over the 3 years in February and March, and at Lumberman's Arch in December, February, March and August. During these same months the

Table 13. Comparison of surface nitrate and phosphate concentrations in Burrard Inlet with adjacent regions.

Location	Concentration of nutrients µg-at/l NO_3-N	PO_4-P	Reference
Lumberman's Arch	18.02*	2.34*	This study
Brockton Point	17.49*	2.09*	"
Indian Arm			Gilmartin (1962)
Head	--	1.84	
Mouth	--	1.49	
Strait of Georgia	15.7	0	Fulton et al (1968)
Northeast Pacific Ocean (West of Vancouver Island)	2.2	--	Anderson et al (1969)

*Average of three year data

salinity was higher, corresponding to a general reduction in Fraser River discharge (Pickard and McLeod, 1953).

In estuaries, the release of phosphorus from the silt occurs with an increase in pH (Carritt and Goodgal, 1954). Such an effect is important in an inlet, where pH varies with the season, state of the tides and biological activity. Frolander (1964) showed that nitrate and phosphate varied with biological activity and depth. He also showed that the phosphate concentration of surface water tends to be highest at night, when photosynthetic activity is absent. With increased turbidity and/or increased depth (and subsequent limiting of photosynthetic activity), phosphate concentration rises.

The three year average of nitrite at Lumberman's Arch was 0.42 μg-at/l and Brockton Point was 0.38 μg-at/l. These were much higher than values for the Strait of Georgia, 0.13 μg-at/l (Bishop et al, 1966). Nitrite concentration was slightly higher in the autumn at Brockton Point than at Lumberman's Arch, but the opposite occurred during the summer.

Iodine concentrations did not vary significantly from season to season between the two stations. Slightly higher

iodine concentrations occurred at Lumberman's Arch which is closer to the sea than Brockton Point. Iodine concentrations at both stations were lower than in the Strait of Georgia which had a concentration of 25 μg/l (Cameron, 1922), and lower than for the northeast Pacific Ocean, 60 μg/l (Barkley and Thompson, 1960) and 40 μg/l for western north Pacific (Sugawara and Terada, 1957). These findings are in close agreement with Vinogradov's (1953) data which showed that more iodine is in the open sea than near the shore. He also found that this variation parallels the differences in the chlorine concentration.

The natural habitat of the studied L. saccharina population may be described as variable. All of the monitored parameters displayed considerable diurnal variations and some seasonal variation. No consistent difference in the monitored parameters were observed between the two study sites.

CHAPTER V

IN SITU STUDIES OF THE DEVELOPMENT OF GAMETOPHYTES AND VERY YOUNG SPOROPHYTES OF L. SACCHARINA IN BURRARD INLET

INTRODUCTION

Gametophytes of L. saccharina are perennial and become fertile during all months of the year on the Devon and Argyll coasts (Parke, 1948). Druehl (1965) noted young macroscopic sporophytes of L. saccharina in Burrard Inlet during February-March and again in early autumn.

The gametophytes and very young sporophytes of the four laminarialean species present at Lumberman's Arch (see Chapter IV) could not be distinguished by their morphology until they were approximately 5 cm long. Therefore, to study the development of the gametophytes of L. saccharina, it was necessary to mark them.

The purpose of the present study was to determine the relationship between the various environmental factors and seasonal development of in situ L. saccharina gametophytes. Meiospores were labelled with a fluorescent brightener, Calcofluor White (American Cyanamid Co.) so as to distinguish them from other species of Laminariales.

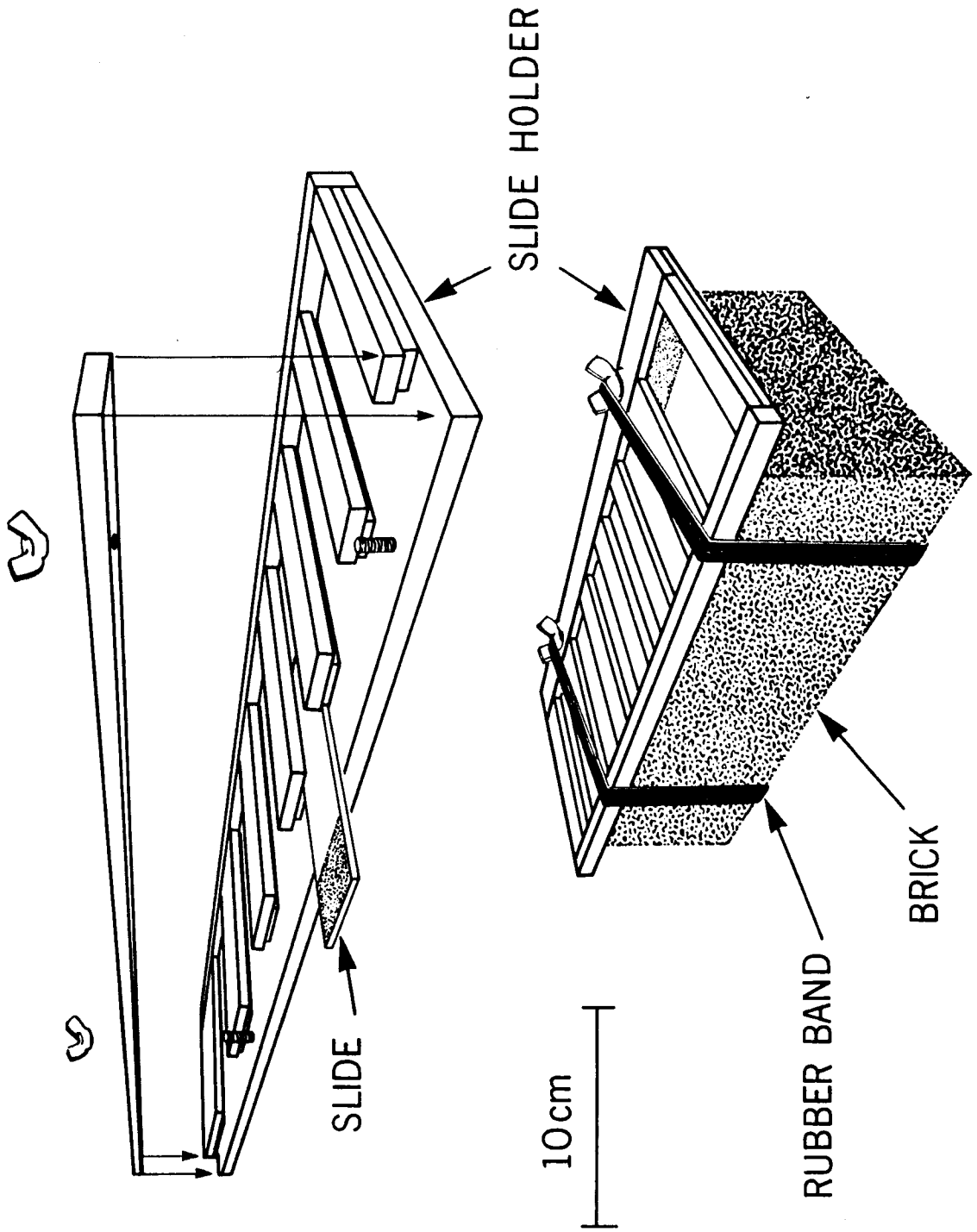
Fluorescent brighteners were first used by Darken (1961, 1962) as vital stains and markers for genetical and developmental studies of microorganisms. They were used as tracers for ecological studies of soil microorganisms (Eren and Pramer, 1968), for host-parasite relationship studies (Patton and Johnson, 1966; Wilson, 1966) and for morphogenetical studies with cellular slime molds (Harrington and Raper, 1968). Cole (1964) first applied the use of fluorescent brighteners to the gametophytes of Laminariales, and found that 0.01% Calcoluor White PMS was stable, intensely fluorescent at pH 7.8, nontoxic, quickly and permanently absorbed by the cellulosic cell walls, and transported in subsequent gametophytic growth. She suggested that fluorescent brighteners could be applied in cytological, genetical, developmental and ecological studies of marine algae in the field and laboratory. Nakazawa et al (1969) labelled Fucus eggs with Calcofluor White to study rhizoid differentiation. They found that Calcofluor White was nontoxic for cell growth and stained only the cell wall.

MATERIALS AND METHODS

Mature sori were collected from Lumberman's Arch, Burrard Inlet at the 0.1 foot tide level during all months of the year. A meiospore suspension was obtained according to Druehl and Hsiao's technique (1969), and adjusted to about 1.2×10^5 meiospores/ml. Meiospores were labelled in the laboratory with 0.01% Calcofluor White ST solution (v/v autoclaved seawater). After 24 hours they settled on glass slides (single frosted) which were then placed in a plexiglass slide holder (Fig. 40), and rinsed several times with autoclaved natural seawater. The holder was then secured to a brick with rubber bands, and placed at the 0.1 foot tide level at Lumberman's Arch.

Each month from July, 1968 to June, 1971 30 slides were placed in the field. Five to ten slides were retrieved every ten days or more frequently. During periods of neap tides a scuba diver collected the slides. They were examined under both bright-field white light and ultraviolet contrast fluorescent light. The time of development for oogonia and antheridia was recorded from the dates they were first observed.

Figure 40. Slide holder used to outplant meiospores in phenological studies.


SLIDE HOLDER
SLIDE
10cm
RUBBER BAND
BRICK

The fluorescent was detected using a Reichert Zetopan phase contrast microscope equipped with a high pressure mercury vapour lamp HBO 200W as the ultraviolet light source, an E4 exciter filter, a red filter RG ½mm, a UV absorption filter SP 1 and a contrast fluorescence condenser. This set-up emitted a wavelength of approximately 362 nm.

Photographs were taken using a Reichert photo-automatic camera with Kodak panatomic x film. Exposure times ranged from 0.5 to 4 seconds for white light and from 60 to 300 seconds for ultraviolet light, depending on the specimens and the magnifications employed.

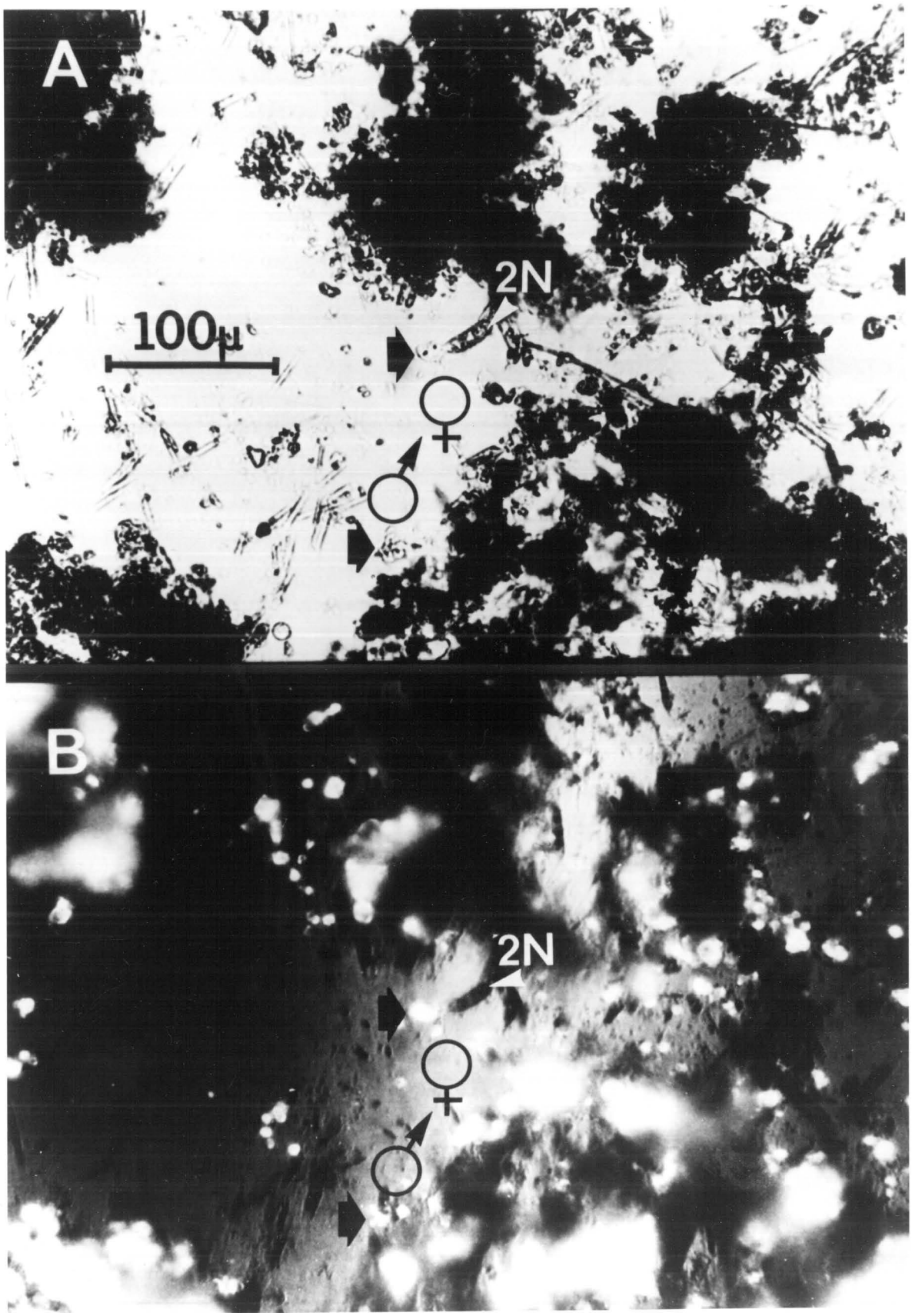
RESULTS

The natural population of L. saccharina sporophytes produced sori at Lumberman's Arch throughout the year. The greatest number of plants became fertile between May and June, and between October and December. The labelled meiospores developed into gametophytes ranging from single cell female plant to filamentous plants usually male with many vegetative cells, which produced oogonia, antheridia and gametes at all times of the year (Table 14, Fig. 41). The maximum time

Table 14. Maximum time in days for gamete production by labelled *in situ* *Laminaria saccharina* gametophytes in Burrard Inlet.

Month	1968		1969		1970		1971		Average	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
January			16	22	15	23	15	23	15.3	22.7
February			12	17	--	18	14	17	13.0	17.3
March			10	14	--	15	11	15	10.5	14.7
April			10	12	14	14	11	15	11.7	13.7
May			10	13	12	16	--	14	11.0	14.3
June			14	17	11	16	12	18	12.3	17.0
July	14	--	14	19	15	20			14.3	19.5
August	--	--	14	19	14	20			14.0	19.5
September	14	18	14	17	--	18			14.0	17.7
October	--	18	18	18	11	18			14.5	18.0
November	16	20	--	22	15	20			15.5	20.7
December	16	24	18	26	--	24			17.0	24.7

Figure 41. Meiospores labelled with Calcofluor White ST of *in situ* *Laminaria saccharina* developed into gametophytes and a microscopic sporophyte (small arrow) as viewed by conventional (A) and fluorescence (B) microscopy. Only labelled meiospores fluoresce intense white (large arrow), and they are readily differentiated from the gametophytes and microscopic sporophytes of other 3 laminarialean species.


A
2N
100μ
B
2N

required for gamete production by L. saccharina is shown in Table 14. Oogonia and antheridia were produced in the shortest time during late winter and spring. The longest time required was in late autumn and early winter. Gametophytes produced antheridia a few days earlier than oogonia. Microscopic sporophytes were observed through the year. However, naturally growing young macroscopic sporophytes were observed only during February-March and September-October.

DISCUSSION

The gametophytes of L. saccharina developed from meiospores labelled with Calcofluor White, and produced antheridia and oogonia during all months of the year in Burrard Inlet at the 0.1 foot tide level. The labelled plants were always covered at low tide. The average low tide level at the study site was 3.4 feet over 3 years, and even the lower low water of the large tide was 0.3 foot (Canadian Hydrographic Service 1968-1971).

The regular production of gametes throughout the three year study period demonstrated that in situ conditions did not limit gametogenesis. Generally, gametogenesis took

longer from November to January than during the remainder of the year. This indicates that some factor or factors may be suboptimal during late autumn and early winter and likely would be temperature and/or light, rather than nutrients, as they were at high levels during the above mentioned period.

Gametophytes became fertile in April and August when phosphate and nitrate were at their lowest concentrations, and so at other times of the year these two nutrients would not limit fertility. In fact the actual concentration of nutrients might be higher in the microhabitats, occupied by the gametophytes, than in the overlying waters where nutrient concentrations were measured. Frequently, L. saccharina gametophytes did produce gametes during April and August in Burrard Inlet, a time when surface concentration of nutrients, especially phosphate, were low. However, the microhabitat nutrient concentrations might well have been higher and together with natural light and temperature conditions, made gamete production possible. I demonstrated that gametophytes of L. saccharina in axenic culture with optimal light and temperature required at least 5 μg-at NO_3-N/l and 10 μg-at PO_4-P/l (Chapter II). Harries (1932) found that the fertility of L. saccharina varied with nitrate concentration when the

plants were illuminated with a suitable light intensity and supplied with sufficient phosphate.

However, fertility also varied with light intensity, photoperiod (Hsiao and Druehl, 1971) and temperature (Tseng et al, 1962; Kain, 1969). Generally, during the period between late winter and spring, and in early autumn, these parameters plus the nutrients promoted most rapid for oogonial and antheridial production. During late autumn and early winter the seawater temperature ranged from 4.7 to 8.4 C (Chapter IV), and light had lower intensity and shorter photoperiods. Although there were sufficient nutrients, the gametophytes took a longer time to produce gametes. Burrows (1961) found that the gametophytes of L. saccharina became fertile almost equally well at 5 and 10 C under culture conditions using enriched Erdschreiber medium, 18 h light and 300 ft-c. Harries (1932) showed that the fertility of L. saccharina varied with light intensity when nitrate and phosphate were in sufficient quantity.

These earlier studies support my conclusion that some aspect of light and/or temperature are the limiting factors in gametogenesis of L. saccharina in Burrard Inlet.

The iodine concentration of seawater in Burrard Inlet

did not change significantly from season to season. The gametophytes of L. saccharina did not require iodine for gametogenesis, but small additions had a stimulatory effect (Chapter III).

When one considers the facts that (1) gametogenesis occurs throughout the year and (2) new macroscopic sporophytes arise only in late winter and early autumn the following conclusion is reached. The establishment of a new generation of macroscopic sporoplytes is not limited in situ by gametogenesis but by the response of newly produced microscopic sporophytes to their environment. Of the environmental parameters monitored (Chapter IV) nitrate concentration, salinity and temperature showed greatest seasonal variation. The nitrate concentration and salinity were lowest and temperature highest during the summer period when new macroscopic sporophytes were not produced. Further, light intensity and photoperiod were greatest during this period (Druehl, 1965). Druehl (1967) showed that L. saccharina was eurythermal and euryhaline. Nitrate might be limiting but established macroscopic sporophytes grow actively during this time (Druehl, 1965). Therefore I believe that light is the limiting factor in macroscopic sporophyte production. The effect being that during the summer there is too

much insolation - a function of photoperiod and intensity. However, there is also a possibility of some detrimental synergism existing between light and some other parameter - perhaps temperature - which might determine time of macroscopic sporophyte production.

CHAPTER VI

SUMMARY

A study was made of the effects of selected inorganic nutrients (nitrate, phosphate and iodine) on meiospore germination, morphology, gametogenesis and metabolites of Laminaria saccharina gametophytes in axenic cultures. Further, the phenology of gametophytes was followed in situ and related to natural variations in the concentrations of these selected inorganic nutrients.

All nutritional experiments were conducted twice, once in the late winter-early spring and once in the autumn. The results of these experiments were essentially the same, indicating that the meiospore prehistory did not affect the response of the subsequent gametophytes.

Generally, the gametophytes reflected optimal culture conditions by having a higher percentage germination, by producing more gametangia per plant, by having a greater percentage fertility and producing more metabolites (excepting lipids) per cultured meiospore.

Optimal levels of nitrate, phosphate and iodine were considerably higher than those found in the overlying water.

The availability of nitrate and phosphate to gametophytes in their natural habitats is not known. However, levels of these nutrients are most likely higher than in the overlying waters monitored in this study.

In all the culture conditions the male gametophyte had a greater percentage fertility than the female gametophyte, and antheridia were produced through a greater range of nutrient conditions than oogonia. From this it might be inferred that the female is the limiting agent in sexual fusion. In situ observations demonstrated that the male produced antheridia earlier than the female, thus adding further weight to this conclusion.

Iodine was not essential for gametogenesis, but at levels 4-5 times that found in the overlying water a stimulatory effect was observed. Larger quantities were ineffective and high concentrations had an inhibitory effect which was diminished by increasing chloride concentrations. The natural chlorinity of seawater might counteract adverse iodine effects on the plants thus allowing them to accumulate high concentrations of iodine.

In situ gametophytes, which developed from labelled meiospores, produced gametes and microscopic sporophytes

during all months of the year. There was a tendency for gametogenesis to take longer during the period between late autumn and early winter than the remainder of the year. The greatest concentration of nitrate and phosphate were found during the period mentioned above, and iodine remained essentially constant throughout the year. This indicates that some other factor or factors might be responsible for limiting growth of the new sporophyte generation. Most likely these factors are temperature and/or light intensity and photoperiod. Druehl (1965) observed that young macroscopic sporophytes of L. saccharina appeared during February-March and again in early autumn. My observations (Chapter V) agree with Druehl's (1965) description. When I consider that gametes and microscopic sporophytes were produced throughout the year and that macroscopic sporophytes arise during two fairly well defined seasons I am led to the conclusion that gametogenesis does not limit the new generations of sporophytes but rather the response of microscopic sporophytes to their environment is the limiting action.

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