CULTURE STUDIES OF THE MARINE BROWN ALGA PETALONIA FASCIA (O.F. MÜLL.) KUNTZE

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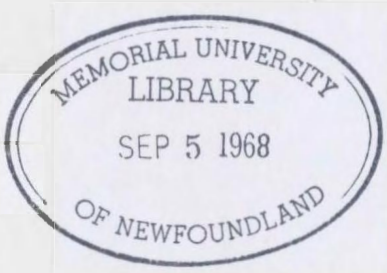

CULTURE STUDIES OF THE MARINE BROWN ALGA

PETALONIA FASCIA (O. F. MÜLL.) KUNTZE

by

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Submitted in partial fulfillment of the
requirements for the degree of
Master of Science
Memorial University of Newfoundland

November, 1967

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ABSTRACT

The morphological life cycle of the marine brown alga Petalonia fascia (O. F. Müll.) Kuntze has been investigated in artificial sea water under unialgal culture. The zoospores produced from the plurilocular sporangia of sporophytes developed directly into protonemata, plethysmothalli, and Ralfsia-like thalli. The appearance and fertility of various morphological types of thalli of P. fascia might be controlled by iodine concentrations, light and temperature. The last two factors, interrelated with each other, were discussed. Protonemata and/or plethysmothalli survived in the most extreme experimental culture conditions, such as lowest and highest temperatures, intensities of light, and total iodine deficiency. However, Ralfsia-like thalli and sporophytes required more suitable environments.

Optimum concentrations of inorganic micronutrient requirements (I, Br, Mo, Fe, Mn, Cu, Zn, and Co) and growth-regulating substances (indoleacetic acid and vitamin B_{12}) for the growth, development, and/or reproduction of P. fascia were determined. Iodine appears to be an essential element for growth, morphogenesis and reproduction of P. fascia and it cannot be replaced by bromine. There is an antagonistic effect between iron and manganese in the growth of the experimental alga.

The low concentrations of indoleacetic acid stimulate growth and accelerate zoospore production of P. fascia. This plant hormone may counteract the zinc deficiency. Vitamin B_{12} is a good stimulant for the growth of this alga, but it does not substitute for cobalt.

CONTENTS

ACKNOWLEDGEMENTS

I wish to express my thanks to Dr. Robert K. S. Lee, of the National Museum of Canada, for suggesting this investigation and for the identification of specimens. I am most grateful to Dr. George Moskovits, who assumed supervision of the thesis problem when Dr. Lee left, for his guidance, particularly at a crucial point in the research, and for his help in bringing this thesis to its final form. Thanks are also due to Dr. G. R. South who helped with advice and criticism during the writing-up period. Dr. F. A. Aldrich, former Head of the Biology Department, and Dr. Marshall Laird, present Head of the Biology Department, assisted in many ways to make this thesis possible and to see it to a successful conclusion. My thanks is extended to them for all they did.

I am particularly grateful to Memorial University for Fellowship aid given me from 1965 to 1967.

Thanks are offered to my friends, Mr. W. F. Lee and Maynard Y. Chen for transportation to collect culture material, to Mr. Christopher Fong and Mr. L. Moores for help with photographic work, to Mr. C. W. Huang, Mr. C. S. Liu and Mr. F. R. Smith for the translation of a few papers into English. I would especially like to thank Miss Lillian Sullivan for typing the final draft of this thesis.

INTRODUCTION

Petalonia fascia (O. F. Müll.) Kuntze is a very widely distributed marine brown alga in the Family Scytosiphonaceae, Order Scytosiphonales (Parke & Dixon, 1964). The species has frequently been described under the following synonyms: Phyllitis fascia (Müll.) Kützing (Farlow, 1881; Saunders, 1898; Yendo, 1919; Newton, 1931) and Ilea fascia (Müll.) Fries (Smith, 1944).

The plant consists of a discoid holdfast, a short filiform stipe and a flattened blade. There are many small tufts of multicellular hairs on the surface of the blade. A cross section of the blade shows a central medulla composed of large, colorless cells interspersed with hyphae. The cortex consists of small, colored cells which develop into plurilocular sporangia. The latter are uniseriate and are crowded together on the surface of the blade (Newton, 1931; Fritsch, 1945; Chapman, 1962). They were described by Taylor (1937) as gametangia.

Although the life cycle of P. fascia has been studied by several workers (Yendo, 1919; Dangeard, 1963, 1964; Caram, 1965; Nakamura, 1965), its details remain uncertain. However, the closely related species Colpomenia sinuosa(=C. peregrina) and Scytosiphon lomentaria in the Scytosiphonaceae have an isomorphic and heteromorphic alternation of generations, respectively (Kunieda & Suto, 1938; Tatewaki, 1966; Lund, 1966).

Culture investigations on the development of P. fascia, usually starting with zoospores from the plurilocular sporangia of the

sporophytes, have been made by a number of workers. In most instances zoospores gave rise directly to protonemata[1] (Yendo, 1919; Dangeard, 1963; Caram, 1965), plethysmothalli[2] (Dangeard, 1964; Caram, 1965) and/or Ralfsia-like thalli[3]. From these, new sporophytes developed. In some cases the protonemata produced plurilocular sporangia, which liberated zoospores that gave rise to new protonemata (Dangeard, 1963). Dangeard (1964) found that plethysmothalli of P. zosterifolia produced unilocular sporangia whose zoospores formed new plethysmothalli directly. More recently, Nakamura (1965) discovered that the zoospores derived from unilocular sporangia of the crustaceous Ralfsia-like thallus in both S. lomentaria and P. fascia developed in culture into thalli which produced plurilocular sporangia. Caram (1965) suggested that no meiotic division took place in the life cycle of P. fascia and thus considered it to be a completely asexual reproduction.

All previous workers had employed undefined media in their investigations into the life cycle of P. fascia. The purpose of the present study was to investigate further the life cycle of P. fascia

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[1]Protonemata: a filamentous stage derived from zoospores from the plurilocular sporangia of P. fascia. Either an entirely filamentous structure, or formed as a pseudo-disc which gives rise to new sporophytes.

[2]Plethysmothallus: similar to protonema and likewise derived from zoospores from the plurilocular sporangia of P. fascia. Constructed from radiating branches which give rise to unilocular sporangia.

[3]Ralfsia-like thallus: formed from zoospores from plurilocular sporangia of P. fascia. Morphologically similar to thalli of the brown alga Ralfsia. Gives rise directly to sporophytes of P. fascia.

by culturing the organism under a number of conditions using both artificial sea water and natural sea water with and without enrichment. Subsequently, examinations were made of the effects of concentrations of trace elements (I, Br, Mo, Fe, Mn, Cu, Zn, and Co) and growth-regulating substances (indoleacetic acid and vitamin B_{12}) and of variations in pH, light intensities, photoperiods and temperatures on the growth, morphology and/or reproduction of the experimental alga.

MATERIALS AND METHODS

I. Development of Culture Media

In order to study the growth, morphology, reproduction and life cycle of *P. fascia* in unialgal culture, attempts were made to find a suitable culture medium. The use of synthetic sea water permits uniformity in repetitive cultures and precise detection of mineral and organic nutritional requirements. A synthetic sea water based on Lyman & Fleming's (1940) formula and incorporating the P 1 metal mix of Provasoli & McLaughlin (1955) and Provasoli, McLaughlin & Droop (1957) as modified by Moskovits (1961), was therefore adopted. Since the synthetic sea water did not include the trace elements molybdenum, vanadium and iodine, which might be essential for the growth of the experimental alga, these were added in the form of sodium molybdate, sodium metavanadate and potassium iodide. Nitrate was added as Ketchum & Redfield's solution A (Ketchum & Redfield, 1938). Phosphate was added as a sodium phosphate solution. A suitable starting medium for nutritional studies was thus developed. This will be referred to as *Petalonia* medium.

The *Petalonia* medium was prepared as follows:

(1) Each salt was added and completely dissolved in the order listed to prevent possible precipitation[4]. All reagents were of analytical grade and were weighed on an analytical balance.

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[4]When precipitation occurred, the medium was discarded. Precipitation would cause mineral deficiencies in the final medium.

	NaCl	23.476 g
	$MgCl_2.6H_2O$	10.629 g
	Na_2SO_4 (anhydrous)	3.917 g
	$CaCl_2.2H_2O$	1.459 g
	KCl	0.664 g
	$NaHCO_3$	0.192 g
	KBr	0.096 g
	KI	0.025 g
	Distilled Water	1,000.0 ml
(2)	Tris (hydroxymethyl) aminomethane	1.0 g
(3)	Ketchum & Redfield's Solution A[5]	2.0 ml
(4)	Medium adjusted to pH 7.0 with 1 N HCl before the addition of Na_2HPO_4 to prevent precipitation of the phosphate.	
(5)	Sodium phosphate Solution[6]	1.0 ml
(6)	P 1 metal mix (modified)[7]	30.0 ml
(7)	The final pH of medium adjusted to pH 7.8 with 1 N KOH	

[5]Ketchum & Redfield's solution A: 20.2 g KNO_3 in 100 ml distilled water.

[6]Sodium phosphate solution: 3.56 g Na_2HPO_4 (anhydrous) in 100 ml. distilled water.

[7]Composition of P 1 metal mix (modified) (continued)

Six enriched natural seawaters were also prepared and examined (Appendix 1).

A. Media for Nutritional Studies

(a) Inorganic mineral nutrient solutions

A number of nutrient solutions, based on the Petalonia medium, were prepared in order to determine the concentrations of various micronutrients required for growth and/or reproduction. Controls consisted of the Petalonia medium from which a given micronutrient was omitted. The same element was then added to the deficient medium by a 10-fold series dilution technique to provide a range of concentrations of the micronutrient. In a set of concentrations only the

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[7] Metal mix (continued)

Salts	Amounts*
$Na_2EDTA.2H_2O$	1.0 g
H_3BO_3	1.14 g
$FeCl_3.6H_2O$	0.049 g
$MnSO_4.H_2O$	0.124 g
$ZnSO_4.7H_2O$	0.022 g
$CuSO_4.5H_2O$	0.00016 g
$CoSO_4.7H_2O$	0.00048 g
$Na_2MoO.2H_2O$	0.01667 g
$NaVO_3$	0.00007 g

*The amounts are for 1 liter of distilled water.

tested micronutrient was varied while the others were kept constant. The composition of concentration ranges for each micronutrient are given in Appendix 2.

(b) Organic nutrient solutions

1) Indoleacetic acid

Media for studying the effect of indoleacetic acid (IAA) on the growth of this alga were prepared by the addition of IAA with and without zinc in Zn-deficient Petalonia medium. Indoleacetic acid was added to the media with and without zinc in the following concentrations: 0, 10, 50, 100, 200, and 400 µg per liter. The Zn-containing medium was prepared by adding 0.023 g $ZnSO_4.7H_2O$ per liter to the Zn-deficient Petalonia medium.

2) Vitamin B_{12}

The influence of various concentrations of vitamin B_{12} on the growth of P. fascia was determined using two culture media: A and B. Medium A was the Petalonia medium without cobalt. Medium B contained cobalt. Vitamin B_{12} was added to both media in the following concentrations: 0, 0.01, 0.1, 1.0, 10, 100, 1000, and 10,000 µg per liter.

B. pH of Media

For the nutritional studies, the pH of the media was adjusted to 7.8. However, for the study of pH effects on growth, the Petalonia

medium was made up in large quantities, then subdivided and dispensed in 1-liter Pyrex bottles. The medium in each bottle was brought to the desired pH value (5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 7.8, 8.0 and 8.5) by the addition of 1 N HCl or 1 N KOH.

II. Preparation of Culture Vessels and Glassware

Pyrex storage dishes (100 mm x 80 mm) with glass covers were employed as culture containers throughout the experiments. Microscope slides, which could be removed from time to time for microscopic examination, were placed in each culture dish. All glassware was cleaned by scrubbing in an Alconox solution followed by ten rinses with hot tap water and five rinses with distilled water. This was followed by rinsing with a 0.5% EDTA solution (pH 7.0) in order to remove adsorbed metal ions. A final rinse in distilled water completed the cleaning process. Heating at 350°C for three hours served to remove organic material from the glassware and to sterilize it.

III. Culture Conditions

A. Illumination

Two types of culture apparatus were used. Both types utilized overhead illumination from fluorescent tubes.

Culture apparatus 1 was a Psycrotherm incubator (New Brunswick Scientific Co., New Brunswick, N.J.) illuminated with eight 20 watt

Westinghouse F24 T12 high output, cool white, fluorescent tubes. Light intensities of 240, 480, 600, 900, 1020, and 1140 ft-c were obtained by adjusting the shelves to distances of 50, 36, 29, 25.5, 21.5, and 18.5 cm below the tubes, respectively.

Culture apparatus 2 was a constant temperature walk-in incubator illuminated by three 30 watt General Electric F30 T12 cool white, fluorescent tubes providing 240, 300, 360, 480, 600, and 900 ft-c at distances of 33, 27, 23, 20.5, 18, and 11.5 cm, respectively.

Experiments on the effects of light intensity were carried out at three different temperatures (6, 16 and 26 ± 1°C) under varied light intensities. Cultures for nutritional studies were illuminated at 480 ft-c.

A photoperiod of 16 hours of light and 8 hours of dark was used throughout the present study, with the exception of one experiment conducted under continuous illumination to determine the effect of photoperiod on the growth and reproduction of P. fascia.

All culture containers were kept covered and were agitated by hand twice daily. The positions of the culture dishes within the incubator were changed at daily intervals to eliminate, as far as possible, the effects of unequal illumination.

B. Temperature

Experiments on the effects of temperature were carried out at 1, 6, 16, 20, 26, and 30 (± 1) °C. All experiments, excepting those at 16 ± 1°C, were performed in the Psycrotherm incubator, in which the temperatures were changed, as desired, at different culture periods. The constant temperature walk-in incubator was maintained at 16 ± 1°C throughout the experiments as a control. Experiments involving nutritional studies were maintained at 16 ± 1°C.

IV. Source and Preparation of algal Material

Mature sporophytes were collected intertidally from February 1966 to March 1967 at St. Phillips, on Conception Bay, Newfoundland. Fertile sporophytes were selected by such criteria as dark-brown color, smooth surface and lack of epiphytes. Surface contaminants were wiped off with a soft brush, after which the plants were thoroughly rinsed in synthetic seawater. This procedure was repeated until no epiphytes were observed microscopically on the sporophytes. The clean, fertile, sporophytes were again quickly rinsed three times and allowed to dry in air for 10-30 minutes. They were then placed in a 100 ml covered beaker, a small amount of the Petalonia medium was added, and allowed to stand overnight. The zoospores were discharged in large numbers during the time of imbibition or by the following morning, if fully mature sporophytes had been selected.

Upon discharge, the zoospores swam about for 24-36 hours, then settled to the bottom of the culture dish and germinated. Actively motile zoospores were drawn off the surface of the medium into a 125 ml Erlenmeyer flask which was then vigorously shaken by hand. After shaking, a drop of suspension was examined in a Levy haemocytometer to determine zoospore numbers. These were then adjusted to give approximately 10^6 cells per ml in the Petalonia medium.

All inoculations of culture media were made within 24 hours after zoospore liberation. Each culture was inoculated with approximately 2×10^5 zoospores per 200 ml of nutrient solution.

V. Maintenance of Cultures

All experiments were set up as duplicate cultures with the exception of the triplicate culture series for iodine and IAA experiments and the quadruplicate culture series for the temperature effect experiments.

Following inoculation, the media in the culture containers were changed on the 14th and 24th days of each experimental period of 30-34 days. The majority of the thalli remained attached to the walls of the culture dish and to the slide placed in the bottom of the dish. Free-floating thalli filtered off under vacuum (using Whatman No. 1 filter paper) were replaced in their original dishes.

VI. Estimation of Growth

Growth of P. fascia was estimated from the increase in length of the sporophytes and from the increase in total dry weight during the experimental period. The slides were removed from the cultures for observation. Twenty of the largest sporophytes present on a standard area of slide were selected for length measurements and recorded at 28 days from the time of inoculation. Measurements were recorded in millimeters (mm). Dry weight was estimated by vacuum-filtering algal material from the cultures through Whatman No. 1 filter paper, followed by a rapid washing with distilled water. The washed thalli were then scraped from the filter paper and quickly transferred to an aluminum weighing dish and dried for 24 hours at 100°C. After drying the weighing dishes were allowed to cool in a desiccator for 1 hour and were weighed as quickly as possible.

RESULTS

I. Experiments to determine the suitability of natural sea water media and enrichments for the gorwth of P. fascia in culture.

Growth of experimental alga in natural sea water with and without enrichment is shown in Table I. These results indicate that the best growth was obtained at 16 ± 1°C and 480 ft-c with the following media: FE, SW I, and SW II.

Table I. Growth of P. fascia (expressed by dry weights) in natural sea water with and without enrichment at two temperatures and two light intensities for 16 hours daily.

Culture Conditions		Growth expressed by average dry weight of thalli (mg)						
		Media						
		NSW	BA	HS	vSt	FE	SWI	SWII
6 ± 1°C	240 ft-c	0.5	0.3	3.9	1.5	8.3	9.5	8.4
	480 ft-c	1.2	0.7	9.6	2.8	18.9	21.4	20.8
16 ± 1°C	240 ft-c	1.0	0.6	9.8	2.3	18.8	19.9	18.6
	480 ft-c	2.2	1.1	17.4	5.1	35.6	40.3	38.3

NSW = Aged natural sea water
BA = Boalch "A" medium (1961)
HS = Haxo & Sweeney (1955)
vSt = von Stosch (1964)
FE = Føyn's Erd Schreiber (1934)
SW I = Iwasaki (1961)
SW II = Iwasaki (1961)

Thirty days after inoculation, zoospores developed into protonemata only in natural sea water and Boalch "A" medium. Media FE, SW I and SW II, produced all four morphological types of thalli. Media HS and vST produced all types of thalli except plethysmothalli (Table II).

Table II. Morphological types of thalli of *P. fascia* formed in natural sea water with and without enrichment. Temperature: 16 ± 1°C. Illumination: 480 ft-c for 16 hours daily. Culture period: 30 days.

Culture Media	Types of Thalli			
	Protonema	Plethysmothallus	*Ralfsia*-like thallus	Sporophyte
NSW	+	-	-	-
BA	+	-	-	-
HS	+	-	+	+
vSt	+	-	+	+
FE	+	+	+	+
SW I	+	+	+	+
SW II	+	+	+	+

II. Experiments to determine the effects on growth of P. fascia of certain synthetic sea water components.

A. Effects of sodium molybdate, sodium metavanadate and potassium iodide on growth of P. fascia.

The results of the experiments are given in Table III.

Table III. Growth of P. fascia (expressed by dry weights) with different additions of sodium molybdate, sodium metavanadate and potassium iodide to synthetic sea water. Temperature: 16 ± 1°C. Illumination: 480 ft-c for 16 hours daily. Culture period: 21 days.

Addition	Average dry weight of thalli (mg)
None	4.05
Sodium molybdate (2×10^{-6}M)	4.65
Sodium metavanadate (1.6×10^{-8}M)	4.45
Sodium molybdate (2×10^{-6}M) + Sodium metavanadate (1.6×10^{-8})	5.1
Potassium iodide (1.5×10^{-4}M)	18.3
Sodium molybdate (2×10^{-6}M) + Sodium metavanadate (1.6×10^{-8}M) + Potassium iodide (1.5×10^{-4}M)	20.4

Zoospores developed into protonemata and plethysmothalli which exhibited slightly better growth in the synthetic sea water

containing sodium molybdate and/or sodium metavanadate than in the synthetic sea water alone. When these salts were both present, plethysmothalli and protonemata still did not produce sporophytes. Three weeks after inoculation, decolorization of some of the protonemata and plethysmothalli occurred in synthetic sea water with and without molybdenum and vanadium.

When potassium iodide was added alone or with sodium molybdate and sodium metavanadate to the synthetic sea water, two weeks after inoculation, protonemata and Ralfsia-like thalli produced sporophytes. Although plethysmothalli were found here, no sporophytes developed from them until the end of the experiment (21 days).

B. Effects of KNO_3 and Na_2HPO_4 on growth of P. fascia.

Growth of P. fascia was not markedly affected by an increasing concentration of KNO_3 in the synthetic sea water containing molybdenum, vanadium and iodine (Table IV).

Table IV. Growth of P. fascia (expressed by dry weights) with the various amounts of KNO_3 in the synthetic sea water containing Mo, V and I. Temperature: 16 ± 1°C. Illumination: 480 ft-c for 16 hours daily. Culture period: 30 days.

KNO_3 Concentration	Average dry weight of thalli (mg)
3.7×10^{-3}M	43.5
5.6×10^{-3}M	43.9
7.4×10^{-3}M	43.1

When the concentration of Na_2HPO_4 increased up to 2.5×10^{-4}M in the synthetic sea water containing 3.7×10^{-3}M KNO_3 with molybdenum, vanadium and iodine, maximum growth was obtained. Beyond this concentration, thallus growth was slightly inhibited (Table V).

Table V. Growth of P. fascia (expressed by dry weights) with different additions of Na_2HPO_4 in the synthetic sea water containing KNO_3 with Mo, V and I. Temperature: 16 ± 1°C. Illumination: 480 ft-c for 16 hours daily. Culture period: 30 days.

Na_2HPO_4 Concentration	Average dry weight of thalli (mg)
1.1×10^{-4}M	38.6
1.7×10^{-4}M	39.7
2.2×10^{-4}M	41.2
2.5×10^{-4}M	42.8
2.8×10^{-4}M	41.9

III. Nutritional Studies

A. Inorganic micronutrients

(a). Iodine

1) Effects of iodide concentration on the growth, morphology and development of P. fascia.

The growth of the experimental alga in media containing varying concentrations of iodide is shown in Table VI.

Table VI. Growth of P. fascia (expressed by thallus lengths and dry weights) with different additions of potassium iodide-deficient Petalonia medium. Culture period: 33 days.

Medium Nos.	KI concentrations	Iodide concentrations µg/l	Length of thalli* (mm)	Average dry weight of thalli (mg)
1	Control (-KI)	0	0.335	1.9
2	4×10^{-9}M	50.76×10^{-2}	0.379	4.3
3	4×10^{-8}M	50.76×10^{-1}	0.407	6.8
4	4×10^{-7}M	50.76	0.526	8.5
5	4×10^{-6}M	50.76×10^{1}	0.922	14.7
6	4×10^{-5}M	50.76×10^{2}	2.251	40.5
7	1.38×10^{-4}M	17.58×10^{3}	2.621	46.8
8	4×10^{-4}M	50.76×10^{3}	3.540	52.5
9	4×10^{-3}M	50.76×10^{4}	3.708	54.9
10	20×10^{-3}M	25.37×10^{5}	3.597	53.5

*In media 1 to 5, protonemata lengths were measured, while in media 6 to 10, sporophyte lengths were measured.

The zoospores grew into protonemata and plethysmothalli in all media. In medium 7, zoospores also developed into Ralfsia-like thalli in a week, whereas this development occurred after a week in

media 6, 8, 9, and 10. Two weeks after inoculation of zoospores, Ralfsia-like thalli produced sporophytes (Fig. 1) in media 7 to 10. However, in medium 6 the sporophytes were produced a week later. By the end of the experiment (33 days) no Ralfsia-like thalli were found when the KI concentration was less than 4×10^{-5}M (media 1 to 5).

During the first two weeks after inoculation, some of the protonemata and plethysmothalli in media 1 to 4 became decolorized and portions of their thalli disintegrated. This phenomenon increased progressively with the duration of the culture periods. By the 24th day, nearly all of the thalli in media 1 to 4 were decolorized and deaths had occurred, although the mortality decreased as the KI concentrations increased in media 1 to 4. The surviving thalli ceased to grow by the end of the experiment. However, two weeks after inoculation in media 6 to 10, the protonemata and plethysmothalli remained in a healthy condition and developed into sporophytes. When inoculated into medium 5, however, the protonemata and plethysmothalli produced a few sporophytes by the end of the experiment. All three kinds of thalli became increasingly darker as the concentration of KI increased.

No sporophytes were produced in media having a KI concentration of less than 4×10^{-6} M (media 1 to 4). Increasing the concentration of KI in media 5 to 9 produced a corresponding increase in sporophyte length and dry weight of thalli (Table VI, Figs. 2 and 3). However, thallus growth was slightly inhibited in medium 10. The results

obtained from this experiment nonetheless indicate a direct correlation between growth and the amount of added iodide.

2) Effect of iodide concentration in the reproduction of P. fascia.

The third group of cultures from the triplicate series was used to observe the effect of iodide concentration on the reproduction of the alga. Cultures in media 1 to 4 did not form sporophytes or reproductive structures. The sporophytes in media 5 to 10 produced zoospores with progressively shorter culture periods as the concentration of KI was increased (Table VII). The results obtained indicate that an increase in the addition of iodide accelerates the process leading to maturation and to the production of zoospores.

Table VII. Effect of potassium iodide concentration on the reproduction of the sporophytes of P. fascia.

Medium Nos.	5	6	7	8	9	10
KI CONCENTRATIONS	$4x10^{-6}M$	$4x10^{-5}M$	$1.38x10^{-4}M$	$4x10^{-4}M$	$4x10^{-3}M$	$20x10^{-3}M$
Days of zoospore production after inoculation.	48	42	39	37	36	36

Further observations were made on the development, morphology and reproduction of protonemata, plethysmothalli and Ralfsia-like thalli. Some of these three kinds of thalli were isolated from media 7 to 10. These were transferred into fresh media (containing the same KI concentrations as those in which development had taken place) and into medium 7. The observations are described as follows:

(i) Protonemata:

Some protonemata were isolated from a week-old culture in medium 10 and transferred to fresh medium 10 and to medium 7. In medium 10, some of these formed very short filaments (Fig. 4) and/or long, creeping and branching filaments (Fig. 5) from which sporophytes developed. These filaments did not produce pseudodiscs. In medium 7, protonemata with pseudodiscs gave rise directly to many sporophytes (Fig. 6). Sometimes, free branching filaments of the protonemata without pseudodiscs became fertile, producing plurilocular sporangia whose zoospores might give rise to many successive generations of filamentous protonemata, but not to plethysmothalli. These plurilocular sporangia were sessile and discharged zoospores through a terminal pore (Fig. 7).

(ii) Plethysmothalli:

Dark, irregular spheres with radiating filaments arising from their peripheries (Fig. 8) were found in media

8 and 9. The spheres ranged from 100-500 μ in diameter. When squashed between cover glass and slide, these separated from one another and appeared as pinnately-branched plethysmothalli (Fig. 9). Thirty days after inoculation into the original media, they were transferred into fresh media having the original KI concentration and into medium 7. Zoospores were produced from the unilocular sporangia of the plethysmothalli (Fig. 10) in media 8 and 7, thirty-nine days after inoculation, but appeared two days earlier in media 9 and 7. These results show that the higher concentrations of iodide stimulate and accelerate zoospore formation and release.

Plethysmothalli from both media 8 and 9 when transferred to medium 7 produced zoospores at different times. It is possible that the zoospores had been formed before transfer. In medium 7, the zoospores produced a few filaments which became attached to the slide in and to the walls of the culture dishes. A week later, these filaments were identified as plethysmothalli. These might produce several generations reproducing by zoospores from unilocular sporangia. In medium 9, new sporophytes (Fig. 11 and 12) were produced from the freshly transferred plethysmothalli, four days earlier than from corresponding plethysmothalli in medium 8. These sporophytes produced zoospores again from plurilocular sporangia, 5 to 6 weeks later.

(iii) Ralfsia-like thalli:

Ten Ralfsia-like thalli of similar size (1.0 mm diameter) were isolated from a four week old culture (medium 7). Five were transferred to medium 10; the other five were transferred to fresh medium 7. Forty-four and forty-six days after inoculation into medium 10 and medium 7, respectively, zoospores were produced from the unilocular sporangia of the Ralfsia-like thalli. These zoospores did not undergo fusion but gave rise directly to protonemata and to Ralfsia-like thalli (Fig. 13). Sporophytes developed from both of these.

After the release of zoospores, the Ralfsia-like thalli were transferred to other culture dishes containing fresh media having the same KI concentrations as those in which they originally developed. This was done in order to observe their further development. It was found that the fertile Ralfsia-like thalli occurred more frequently in medium 10 than in medium 7. In contrast, the sporophytes were more frequent in medium 7. The sporophytes were formed on the Ralfsia-like thalli either before or after the formation of unilocular sporangia, in both media. However, several sporophytes were observed growing from the central part, but not from the margin of the Ralfsia-like thalli (Fig. 1).

Hard, black spheres (Fig. 14) resembling those composed of plethysmothalli were also found in medium 10.

Their sizes ranged from 150-600 μ in diameter. Viewed from the surface, they appeared different from those found in media 8 and 9. They were fertile Ralfsia-like thalli bearing unilocular sporangia (Fig. 15). These thalli did not become attached to the slide in and to the walls of the culture dishes as seen in the culture in medium 7. The zoospores were observed discharging through a small opening at the distal end of the unilocular sporangia of the Ralfsia-like thalli (Fig. 16). The zoospores were isolated and inoculated into media 10 and 7 in which they germinated and developed into protonemata and Ralfsia-like thalli, but not to plethysmothalli (Fig. 17). The growth of thalli in medium 10 was more rapid than in medium 7 (Figs. 18 and 19). In medium 10, new sporophytes were produced from the protonemata and Ralfsia-like thalli three days earlier than in medium 7. These results confirm those from the previous experiment in which higher concentrations of iodide stimulated thallus growth.

3) Determination of the essentiality of iodide as a growth requirement for P. fascia.

Zoospores inoculated into 20 culture dishes of iodide-deficient Petalonia medium developed into protonemata and plethysmothalli, but not into Ralfsia-like thalli in ten days. The growth of these structures was slightly retarded and showed decolorization.

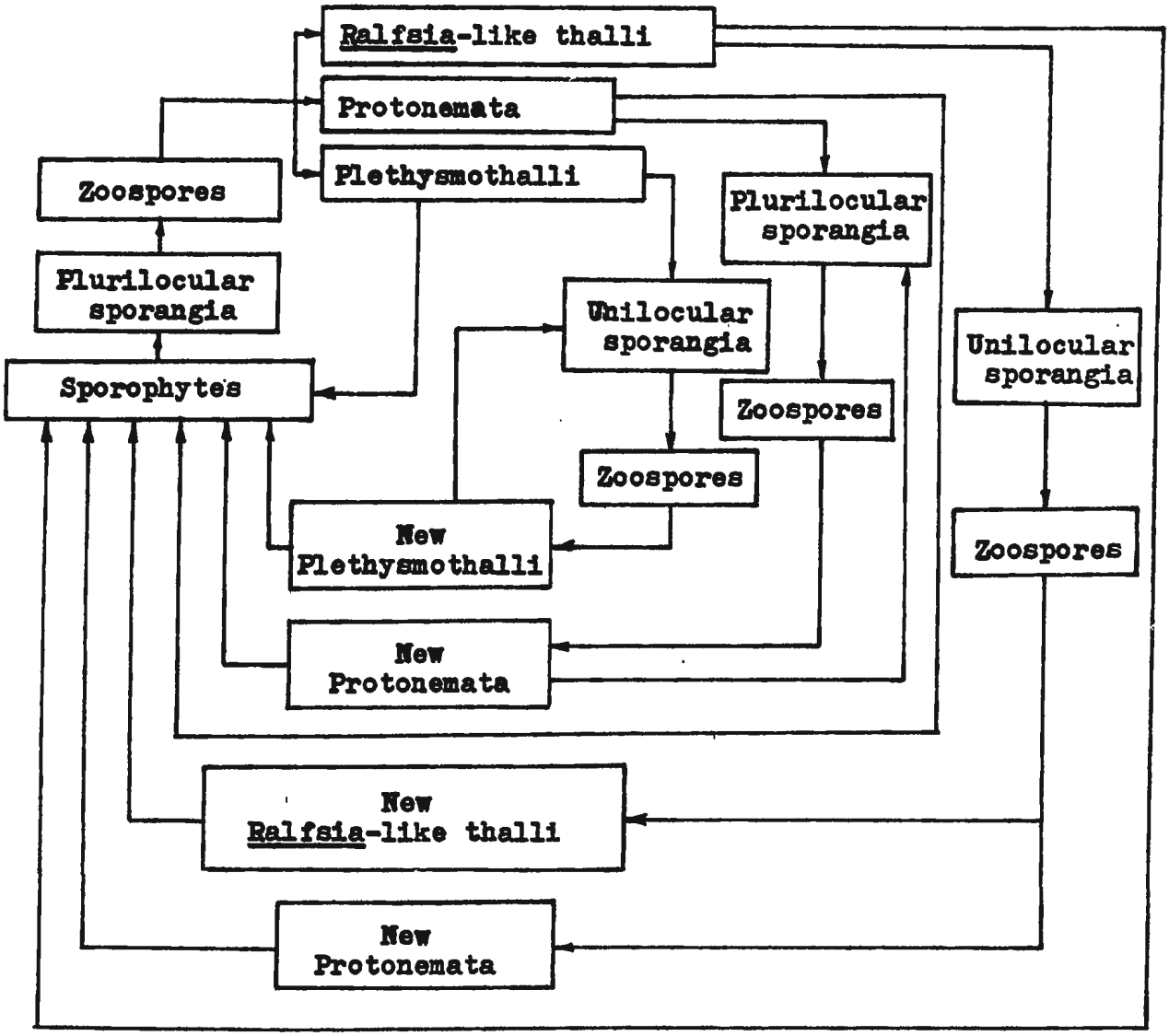
Ten days after inoculation of zoospores, two cultures were harvested and dry weights were determined. To ten of the remaining cultures, KI was added to give a concentration of 1.38×10^{-4}M. In these cultures, normal growth was resumed, and a week later sporophytes developed. From the remaining cultures, at 10-day intervals, two cultures each of algae in iodide-enriched and iodide-deficient Petalonia media were harvested and dry weights were determined. At each such interval, fresh iodide-enriched or iodide-deficient Petalonia media was added to the remaining algal cultures. Fifty days after inoculation, only protonemata and plethysmothalli were present in the iodide-deficient Petalonia medium. Nearly all of these thalli which had failed to produce sporophytes had become decolorized and, by the last harvest most were dead. The thalli in the cultures to which iodide was added increased in dry weight throughout the culture period. In the last two cultures, in iodide-enriched Petalonia medium (52 days after inoculation), zoospores were produced by the sporophytes. The dry weights of these cultures were not measured since the zoospores had already been released, thus reducing thallus weight.

The results of the experiments, given in Table VIII and Fig. 20, show that the addition of iodide is absolutely essential for growth, morphogenesis and reproduction of P. fascia in unialgal culture.

Table VIII. Growth of P. fascia (expressed by dry weight) with and without iodide. Inocula were deprived of iodide for 10 days. Iodide was then added to give 1.38×10^{-4}M KI.

Days after Inoculation	Average dry weight of thalli (mg)	
	No addition	1.38×10^{-4}M KI added
10	0.4	-
20	1.2	10.7
30	1.6	22.5
40	0.7	31.5
50	0.2	45.2

According to the morphological observations previously described, the stages in the life cycle of P. fascia in iodide cultures, may be illustrated as shown below.



(b) Bromide

1) Effect of varying concentrations of bromide on the growth of P. fascia.

During the frist two weeks after inoculation of zoospores, sporophytes were produced from protonemata, plethysmothalli and Ralfsia-like thalli in media 1 to 8. In media 9 to 10, the sporophytes were formed a week later. This indicates that the sporophytes grow in both bromide-deficient and bromide-enriched Petalonia media.

The results of the experiments, given in Table IX and Figs. 21 and 22, show that both sporophyte length and dry weight of thalli increase as the concentration of potassium bromide is increased up to the level of 7.72×10^{-4}M. Beyond this concentration, thallus growth is inhibited.

Table IX. Growth of P. fascia (expressed by sporophyte lengths and dry weights) with different additions of potassium bromide to bromide-deficient Petalonia medium. Culture period: 33 days.

Medium Nos.	Potassium Bromide Concentrations	Bromide Concentration mg/l	Length of Sporophyte (mm)	Average dry Weight of Thalli (mg)
1	Control (-KBr)	0	1.273	25.2
2	$8x10^{-9}M$	$63.9x10^{-5}$	1.289	30.0
3	$8x10^{-8}M$	$63.9x10^{-4}$	1.386	32.4
4	$8x10^{-7}M$	$63.9x10^{-3}$	1.767	34.5
5	$8x10^{-6}M$	$63.9x10^{-2}$	1.820	37.8
6	$8x10^{-5}M$	$63.9x10^{-1}$	2.234	40.5
7	$7.72x10^{-4}M$	61.8	2.630	47.4
8	$8x10^{-4}M$	63.9	2.620	45.6
9	$8x10^{-3}M$	$63.9x10^{1}$	1.260	27.0
10	$4x10^{-2}M$	$31.96x10^{2}$	0.913	22.8

2) Effect of substituting bromide for iodide in the experimental medium.

The results obtained when zoospores were inoculated into Petalonia medium with iodide and/or bromide omitted are shown in Table X. The protonemata and plethysmothalli which developed were poorly formed. No sporophyte formation occurred either in bromide- and iodide- deficient Petalonia medium or in the iodide-deficient Petalonia medium. The best growth of P. fascia was obtained with the addition of 7.72×10^{-4}M KBr to the bromide-deficient Petalonia medium which contained 1.38×10^{-4}M KI. The dry weight of the thalli grown in bromide-deficient Petalonia medium containing iodide was approximately 14 times that of thalli grown in iodide-deficient Petalonia medium containing bromide (Table X). These results apparently indicate that bromide does not substitute for iodide and further indicate that iodide is required for the growth of P. fascia.

Table X. Growth of P. fascia (expressed by dry weights) in Petalonia medium with iodide and/or bromide omitted. Culture period: 33 days.

Addition		Sporophyte Formation	Average dry Weight of Thalli (mg)
KBr	KI		
0	0	-	1.1
7.72×10^{-4}M	0	-	1.7
0	1.38×10^{-4}M	+	24.3
7.72×10^{-4}M	1.38×10^{-4}M	+	48.2

(c) Molybdenum

Effect of molybdenum concentration on the growth and morphology of P. fascia.

P. fascia grew in molybdenum-deficient Petalonia medium. When sodium molybdate at a concentration of 5×10^{-9}M was added to the medium, growth was slightly stimulated. A tenfold increase in molybdenum concentration produced a corresponding increase in the length and dry weight of the thalli. Maximum growth was obtained with 5×10^{-6}M sodium molybdate. Further tenfold increases in concentration (5×10^{-5} to 5×10^{-3}M) appeared to be inhibitory (Table XI and Figs. 23 and 24).

Sporophytes were formed in all the experiments in which molybdenum was added, as well as in those to which no molybdenum was added. Visual estimation of the number of sporophytes indicated an increase with increasing molybdenum concentration up to 5×10^{-6}M; beyond this, sporophyte numbers sharply decreased. In the highest concentration of sodium molybdate (5×10^{-3}M) about half of the thalli remained at the protonemata, plethysmothalli and Ralfsia-like thalli stages.

Table XI. Growth of P. fascia (expressed by sporophyte lengths and dry weights) with different additions of sodium molybdate to molybdenum-deficient Petalonia medium. Culture period: 30 days.

Medium Nos.	Sodium Molybdate Concentration	Molybdenum Concentration µg/l	Length of Sporophyte (mm)	Average dry Weight of Thalli (mg)
1	Control ($-Na_2MoO_4.2H_2O$)	0	1.528	31.4
2	$5x10^{-9}M$	$4.8x10^{-1}$	1.787	33.8
3	$5x10^{-8}M$	4.8	1.959	39.8
4	$5x10^{-7}M$	$4.8x10^{1}$	2.046	42.8
5	$1.97x10^{-6}M$	$18.9x10^{1}$	2.174	43.5
6	$5x10^{-6}M$	$4.8x10^{2}$	2.329	44.5
7	$5x10^{-5}M$	$4.8x10^{3}$	2.032	40.5
8	$5x10^{-4}M$	$4.8x10^{4}$	1.554	36.4
9	$5x10^{-3}M$	$4.8x10^{5}$	1.346	30.3

(d) Iron

Effect of iron concentration on the growth of P. fascia.

Two weeks after inoculation of zoospores, sporophytes were produced from the protonemata, plethysmothalli and Ralfsia-like thalli in all media containing varying concentrations of iron, and also in the iron-deficient control. The growth of the experimental alga indicated that an increase in the concentration of iron was accompanied by a corresponding increase in the length of sporophytes and in dry weight of the thalli (Table XII and Figs. 25 and 26). The color of the thalli deepened from light to dark brown as the concentration of iron increased.

Table XII. Growth of P. fascia (expressed by sporophyte lengths and dry weights) with different additions of ferric chloride to iron-deficient Petalonia medium. Culture period: 30 days.

Medium Nos.	Ferric Chloride Concentration	Iron Concentration µg/l	Length of Sporophyte (mm)	Average Dry Weight of Thalli (mg)
1	Control (-$FeCl_3$)	0	2.014	32.7
2	$4x10^{-9}M$	$2.233x10^{-1}$	2.143	34.0
3	$4x10^{-8}M$	2.233	2.305	37.6
4	$4x10^{-7}M$	$2.233x10^{1}$	2.414	41.5
5	$4x10^{-6}M$	$2.233x10^{2}$	2.578	46.0
6	$5.18x10^{-6}M$	$2.89\ x10^{2}$	2.588	47.0
7	$4x10^{-5}M$	$2.233x10^{3}$	2.683	49.7

(e) Manganese

Effect of manganese concentration on the growth of P. fascia.

One week after inoculation of zoospores into manganese-containing media and into the manganese-deficient control, germination occurred in all media, except medium 9, to give protonemata, plethysmothalli and Ralfsia-like thalli (Table XIII). In medium 9, the zoospores died and disintegrated. Sporophytes were produced from protonemata, plethysmothalli and Ralfsia-like thalli in media 1 to 7, at 14 days after inoculation. In medium 8, however, sporophytes were not produced until three weeks after inoculation.

The growth of P. fascia in response to the addition of various concentrations of manganous sulfate was very striking (Table XIV, and Figs. 27 and 28). The optimal concentration was 1.8×10^{-5}M. When the concentration was increased to 1.8×10^{-4}M, growth was inhibited.

Table XIII. Growth of *P. fascia* (expressed by sporophyte lengths and dry weights) with different additions of manganous sulfate to manganese-deficient *Petalonia* medium. Culture period: 30 days.

Medium Nos.	Manganous sulfate Concentration	Manganese Concentration µg/l	Length of Sporophyte (mm)	Average Dry Weight of Thalli (mg)
1	Control ($-MnSO_4$)	0	1.069	37.5
2	1.8×10^{-9}M	9.89×10^{-2}	1.177	39.0
3	1.8×10^{-8}M	9.89×10^{-1}	1.198	40.1
4	1.8×10^{-7}M	9.89	1.203	40.5
5	1.8×10^{-6}M	9.89×10^{1}	1.290	41.3
6	1.8×10^{-5}M	9.89×10^{2}	1.437	48.0
7	2.1×10^{-5}M	11.51×10^{2}	1.310	46.4
8	1.8×10^{-4}M	9.89×10^{3}	0.874	36.7
9	1.8×10^{-3}M	9.89×10^{4}	-*	-*

*Indicates that zoospores were unable to survive.

(f) Copper

Effect of copper concentration on the growth of *P. fascia*.

The results of this experiment are given in Table XIV and Figs. 29 and 30. It appears that low concentrations of copper sulfate have a stimulating effect on the growth, up to a concentration of 3×10^{-7}M. Beyond this concentration, growth is inhibited. Sporophytes were produced in all concentrations of copper as well as in copper-deficient *Petalonia* medium.

Table XIV. Growth of P. fascia (expressed by sporophyte lengths and dry weights) with different amounts of copper sulfate added to copper-deficient Petalonia medium. Culture period: 30 days

Medium Nos.	Copper sulfate Concentration	Copper Concentration µg/l	Length of Sporophyte (mm)	Average Dry Weight of Thalli (mg)
1	Control (-$CuSO_4$)	0	2.088	36.0
2	$3x10^{-10}M$	$19.7x10^{-3}$	2.216	39.7
3	$3x10^{-9}M$	$19.7x10^{-2}$	2.293	40.5
4	$1.6x10^{-8}M$	$10.2x10^{-1}$	2.339	41.9
5	$3x10^{-8}M$	$19.7x10^{-1}$	2.395	42.8
6	$3x10^{-7}M$	19.7	2.487	44.5
7	$3x10^{-6}M$	$19.7x10^{1}$	2.240	36.4
8	$3x10^{-5}M$	$19.7x10^{2}$	1.496	22.8

(g) Zinc

Effect of zinc concentration on the growth of *P. fascia*.

The results of this experiment are shown in Table XV and Figs. 31 and 32. Zinc sulfate showed a stimulatory effect, evident at low concentration of 8×10^{-11} M and increasing at higher concentrations up to 8×10^{-7} M. However, growth was progressively inhibited with further increases in the concentration of zinc. Sporophytes occurred in all media, two weeks after inoculation of zoospores. In zinc-deficient *Petalonia* medium, many "slender" sporophytes were produced as compared with those in "complete" *Petalonia* medium (Figs. 33 and 34). These sporophytes were light brown in color and showed numerous, colorless hairs. The average length and width of sporophytes cultivated in the zinc-deficient and in zinc-sufficient (8×10^{-7} M) *Petalonia* media were 1.263 mm x 0.074 mm and 1.836 mm x 0.165 mm, respectively.

Table XV. Growth of P. fascia (expressed by sporophyte lengths and dry weights) with different amounts of zinc sulfate added to zinc-deficient Petalonia medium. Culture period: 30 days.

Medium Nos.	Zinc Sulfate Concentration	Zinc Concentration µg/l	Length of Sporophyte (mm)	Average Dry Weight of Thalli (mg)
1	Control ($-ZnSO_4$)	0	1.263	35.1
2	$8x10^{-11}M$	$5.23x10^{-3}$	1.394	38.0
3	$8x10^{-10}M$	$5.23x10^{-2}$	1.457	40.7
4	$8x10^{-9}M$	$5.23x10^{-1}$	1.562	42.0
5	$8x10^{-8}M$	5.23	1.728	45.0
6	$8x10^{-7}M$	$5.23x10^{1}$	1.836	46.8
7	$2.2x10^{-6}M$	$14.28x10^{1}$	1.757	44.1
8	$8x10^{-6}M$	$5.23x10^{2}$	1.722	43.0
9	$8x10^{-5}M$	$5.23x10^{3}$	1.125	34.2

(h) Cobalt

Effect of cobalt concentration on the growth of P. fascia.

Growth of P. fascia in different cobalt concentrations is shown in Table XVI and Figs. 35 and 36. Sporophytes were formed in all media, two weeks after inoculation of zoospores. In the highest concentration of cobalt sulfate (1.7×10^{-4}M), nearly all of the protonemata developed into sporophytes by the end of the experiment (30 days). These sporophytes were loosely attached to the slides and to the walls of the culture dishes, or were free-floating in the medium. When the concentration of cobalt was increased up to 1.7×10^{-8}M, maximum growth was obtained. However, when the concentrations of cobalt sulfate were greater than the optimum, an inhibitory effect was observed, and the dry weight and length of the thalli were low in comparison with those obtained at the optimum concentration for growth.

Table XVI. Growth of *P. fascia* (expressed by sporophyte lengths and dry weights) with different amounts of cobalt sulfate added to cobalt-deficient *Petalonia* medium. Culture period: 30 days.

Medium Nos.	Cobalt Sulfate Concentration	Cobalt Concentration µg/l	Length of Sporophyte (mm)	Average Dry Weight of Thalli (mg)
1	Control ($-CoSO_4$)	0	1.533	39.5
2	1.7×10^{-11}M	1×10^{-3}	1.882	40.8
3	1.7×10^{-10}M	1×10^{-2}	2.129	41.8
4	1.7×10^{-9}M	1×10^{-1}	2.230	43.5
5	1.7×10^{-8}M	1.0	2.403	49.3
6	4.6×10^{-8}M	2.73	2.308	47.0
7	1.7×10^{-7}M	1×10^{1}	2.185	43.7
8	1.7×10^{-6}M	1×10^{2}	1.862	41.6
9	1.7×10^{-5}M	1×10^{3}	1.646	40.2
10	1.7×10^{-4}M	1×10^{4}	1.461	37.8

B. Organic Nutrients

(a) Indoleacetic acid (IAA)

1) Effect of indoleacetic acid, with and without zinc, on the growth of P. fascia.

Nineteen days after inoculation of zoospores into the two highest concentrations of IAA (200 and 400 µg/L), some of the sporophytes which had developed were found to be disintegrating. The degree of disintegration increased with increase in IAA concentration and was more extensive in the presence of zinc (8×10^{-7}M zinc sulfate) than in its absence. Sporophytes grown in medium with zinc and having 400 µg IAA/L were more swollen than those in the same medium with 200 µg IAA/L (Figs. 37 and 38). No swollen sporophytes occurred in the highest concentration of IAA without zinc (Fig. 39).

Another effect involved the numbers of hairs produced on the sporophytes. The lowest concentration of IAA (10 µg/L) both with and without zinc stimulated hair formation. The largest numbers of hairs were produced here. In contrast, the higher levels of IAA inhibited hair formation. The fewest hairs were produced in a concentration of 400 µg IAA/L with zinc.

The results of this experiment (Table XVII and Figs. 40, 41 and 42), indicate that total dry weight of thalli grown in zinc-deficient Petalonia medium containing 10 µg IAA/L was as great as in

the zinc-sufficient (8×10^{-7}M $ZnSO_4$) Petalonia medium without IAA. The lengths and widths of sporophytes in the former medium were approximately 0.9 and 0.6 times as great, respectively, as those in the latter medium. When 50 µg IAA/L was added to the zinc-deficient Petalonia medium, the approximate length but not the width of sporophytes was similar to that in the zinc-sufficient Petalonia medium without IAA. Maximum length and width of sporophytes, and maximum total dry weight of thalli were obtained when the media contained 100 µg IAA/L both with and without zinc. Higher concentrations of IAA resulted in an inhibition of growth. However, any addition of IAA to the zinc-deficient Petalonia medium does not result in the same width of sporophytes as that grown in the zinc-sufficient Petalonia medium without IAA.

Table XVII. Growth of P. fascia (expressed by lengths and widths of sporophytes and dry weights) in response to indoleacetic acid with zinc-deficient and zinc-sufficient (8×10^{-7}M $ZnSO_4$) Petalonia medium. Culture period: 34 days. Temperature: 16 ± 1°C. Illumination: 360 ft-c for 16 hours daily.

IAA added µg/L	Length of sporophyte (mm)		Width of sporophyte (mm)		Average Dry Weight of Thalli (mg)	
	-Zn	+Zn	-Zn	+Zn	-Zn	+Zn
0	1.135	1.975	0.071	0.149	36.3	40.3
10	1.717	2.629	0.083	0.162	40.9	52.6
50	1.900	2.700	0.101	0.188	45.5	59.4
100	2.116	2.914	0.116	0.202	52.6	66.3
200	1.302	1.428	0.082	0.128	35.4	41.6
400	0.883	0.979	0.047	0.079	23.0	27.9

2) Effect of indoleacetic acid, with and without zinc, on the reproduction of *P. fascia.*

The third group of cultures from the triplicate series was used to observe the effect of IAA, with and without zinc, on reproduction. The results of this experiment are shown in Table XVIII. The optimal concentrations of IAA promoting zoospore formation were 50-100 µg/L. The presence of zinc appears to reduce the amount of time needed to produce zoospores. Higher concentrations of IAA (with or without zinc) increase this time.

TABLE XVIII. Effect of various concentrations of IAA, with and without zinc, on the reproduction of *P. fascia.*

IAA Concentration µg/L	Number of days from inoculation to liberation of zoospores from the sporophytes	
	Zinc Omitted	8×10^{-7}M $ZnSO_4$ added
0	42	39
10	39	36
50	37	35
100	37	35
200	39	40
400	44	46

(b) Vitamin B_{12}

Effect of vitamin B_{12}, with and without cobalt, on the growth of P. fascia.

The results of this experiment are shown in Table XIX and Fig. 43. When vitamin B_{12} was added in concentrations up to 1µg/L in media with and without cobalt, growth was nearly proportional to the vitamin concentration. Beyond this concentration, thallus growth was slightly inhibited.

Table XIX. Growth of P. fascia (expressed by dry weights) in response to vitamin B_{12}, with and without cobalt. Culture period: 30 days. Temperature: 16 ± 1°C. Illumination: 300 ft-c for 16 hours daily.

Vitamin B_{12} added µg/L	Average dry weight of thalli (mg)	
	Medium A (Without Cobalt)	Medium B (4.6×10^{-8}M $CoSO_4$ added)
0	15.1	27.5
0.01	19.4	31.2
0.1	20.2	34.0
1.0	22.4	38.9
10.0	18.0	34.5
100.0	16.9	32.1
1,000.0	15.4	27.3
10,000.0	14.8	25.6

Growth obtained in the cobalt-enriched Petalonia medium (containing 4.6×10^{-8}M $CoSO_4$) without vitamin B_{12} was better than any growth resulting from the addition of vitamin B_{12} to the cobalt-deficient Petalonia medium. This indicates that cobalt is an essential element for the growth of P. fascia. Vitamin B_{12} may behave as a growth stimulant, but not as an indispensable growth factor for this alga.

IV. Physical Conditions

A. pH

Effects of pH on the growth and morphology of P. fascia.

The growth of P. fascia at different pH values in Petalonia medium is shown in Table XX and Fig. 44. Zoospores did not survive at pH values as low as 5.0. Growth improved with an increase in pH, the optimum pHs occurring between 7.8 and 8.0. Beyond pH 8.0, growth decreased.

Two weeks after inoculation, zoospores developed into protonemata at all the pH values above 5.0. At pH 5.5 only protonemata were formed. At pH 6.0 only protonemata and Ralfsia-like thalli were formed. At pH 6.5 and higher, all four morphological types of thalli were produced (Table XXI). However, sporophytes developed from protonemata and Ralfsia-like thalli, but not from plethysmothalli.

Table XX. Growth of *P. fascia* (expressed by dry weights) at different pH values in *Petalonia* medium at 16 ± 1°C, 480 ft-c and 16 hours of light. Culture period: 30 days.

pH	Average dry weight of thalli (mg)
5.0	0*
5.5	1.0
6.0	4.0
6.5	10.8
7.0	23.9
7.5	31.5
7.8	47.8
8.0	46.5
8.5	42.7

*No growth

Table XXI. Morphological types of thalli formed at different pH values in Petalonia medium, two weeks after inoculation of zoospores. Temperature: 16 ± 1°C. Illumination: 480 ft-c for 16 hours daily.

pH	TYPES OF THALLI			
	Protonema	Ralfsia-like thallus	Plethysmothallus	Sporophyte*
5.5	+	-	-	-
6.0	+	+	-	-
6.5	+	+	+	+
7.0	+	+	+	+
7.5	+	+	+	+
7.8	+	+	+	+
8.0	+	+	+	+
8.5	+	+	+	+

*Indicates that sporophytes developed from protonemata and Ralfsia-like thalli.

In a three-week old culture, Ralfsia-like thalli appeared at pH 5.5. These did not give rise to sporophytes until the end of the experiment (30 days). At pH 6.0, plethysmothalli were observed, but they did not develop into sporophytes. At pHs higher than 6.5, protonemata, Ralfsia-like thalli and plethysmothalli gave rise to sporophytes, the numbers of which increased with increasing pH values up to 8.0 and decreased beyond 8.0.

With respect to color, the thalli appeared greenish-brown at pH 5.5. This color deepened to a light brown with increasing pH up

to 7.5. At pH values higher than 7.5, the thalli showed the normal color, a dark brown.

B. Light Intensity and Photoperiod

(a) Effects of light intensity on the growth, morphology and reproduction of P. fascia.

Fig. 45 represents the growth of P. fascia at two different temperatures (6 and 16 ± 1°C) under various light intensities in Petalonia medium. The growth occurs in linear proportion to the light intensity up to 480 ft-c. Although growth was somewhat inhibited at light intensities above 480 ft-c, all four morphological types of thalli were formed.

The relationship between dry weight and light intensity for each temperature is shown in Table XXII. Both experiments showed that the optimum light intensity for growth was 480 ft-c.

Further observations were made on the experimental alga under varied light intensities at 26 ± 1°C. It was found that nearly all of the zoospores died when they were illuminated at intensities above 600 ft-c. The surviving zoospores developed into malformed protonemata and plethysmothalli. Thirty-three days after inoculation, all these thalli died. However, the cultures which were exposed at 240 and 480 ft-c, remained as protonemata and plethysmothalli. These

Table XXII. Growth of P. fascia (expressed by dry weights) under different light intensities using Petalonia medium at temperatures of 6 and 16 ± 1°C and 16 hours of light. Culture period: 30 days.

Light intensity ft-c	Growth express by average dry weight of thalli (mg)	
	6 ± 1°C	16 ± 1°C
240	20.5	29.5
300	24.0	-*
360	25.8	-
480	33.5	43.9
600	29.6	42.4
900	27.1	40.6
1020	-	39.3
1140	-	38.8

*Indicates that no experiments were done at the corresponding light intensities, because of inability to adjust light-to-culture distance, to obtain the desired light intensities.

thalli did not develop into sporophytes even sixty days after inoculation under such illumination. Thereafter, when they were transferred to a temperature of 16 ± 1°C, and illuminated at 480 and 240 ft-c for one and two weeks, respectively, sporophytes were produced. These sporophytes produced zoospores four weeks after illumination at 480 ft-c and a week later at 240 ft-c, at 16 ± 1°C.

(b) Effects of photoperiod on the growth and liberation of zoospores from sporophytes of P. fascia.

The growth of the experimental alga with 16-and 24-hour photoperiods is shown in Table XXIII. Results in Table XXIV indicate that better growth was obtained under continuous light than with 16 hours of light.

Table XXIII. Growth of P. fascia (expressed by dry weights) at two different photoperiods with Petalonia medium at 16 ± 1°C and 480 ft-c. Culture period: 30 days.

Photoperiod hour/day	Average Dry Weight of Thalli (mg)
16	43.7
24	55.2

With respect to morphology, there were no marked differences due to the length of photoperiods, except that no plethysmothalli were formed in continuous light.

It was also found that the liberation of zoospores from sporophytes occurred a week earlier in cultures under continuous as compared with those under 16 hours of light (Table XXIV).

Table XXIV. Number of days required for the liberation of zoospores from the sporophytes of P. fascia cultivated in Petalonia medium at two different photoperiods, 16 ± 1°C and 480 ft-c.

Photoperiod hour/day	Number of days from inoculation to liberation of zoospores
16	39
24	32

C. Temperature

(a) Effects of temperature on the growth and morphology of P. fascia.

The results are given in Table XXV and Fig. 46. In the latter, each point is the average of two cultures, except at 16 ± 1°C which represents an average of ten cultures. These data closely indicate that temperature has an appreciable effect on the growth of the experimental alga.

The slowest growth occurred at 1 ± 1°C. Zoospores developed only into protonemata; no other morphological forms of thalli were observed at this temperature. Better growth was obtained at 6 ± 1°C. Sporophytes were produced from protonemata and Ralfsia-like thalli. Although plethysmothalli were found here, no sporophyte developed from them. The optimum temperature for growth was at

Table XXV. Growth of P. fascia (expressed by dry weights) in Petalonia medium at various temperatures, 480 ft-c and 16 hours of light. Culture period: 30 days.

Temperature ±1°C	Average dry weight of thalli (mg)
1	0.5
6	34.2
16	45.5
20	42.1
26	10.5
31	0*

*Indicates lethal temperature.

16 ± 1°C, at which sporophytes were produced from the other three morphological types of thalli. At 20 ± 1°C, sporophytes were formed, as at 16 ± 1°C. When the temperature was increased above 20 ± 1°C, growth decreased sharply. However, when the temperature was raised to 26 ± 1°C, the zoospores developed into protonemata and plethysmothalli but not into Ralfsia-like thalli. No sporophyte formation was observed at this temperature. All zoospores died at 31 ± 1°C, 24 to 48 hours after inoculation.

Temperature effects on the morphology of P. fascia are summarized in Table XXVI. When zoospores were originally inoculated at 1 ± 1°C for two weeks, only protonemata were developed. When cultures were then transferred to 16 ± 1°C for about two weeks, all four morphological

types of thalli were observed.

Table XXVI. Morphological types of thalli of P. fascia formed at different temperatures in Petalonia medium. Illumination: 480 ft-c for 16 hours daily. Culture period: 30 days.

Temperature ±1°C	Types of Thalli			
	Protonema	Plethysmothallus	Ralfsia-like thallus	Sporophyte
1	+	-	-	-
6	+	+	+	+
16	+	+	+	+
20	+	+	+	+
26	+	+	-	-
31	L.T.*	L.T.	L.T.	L.T.

*L.T. = Lethal Temperature

(b) Effect of temperature on the reproduction of P. fascia.

The other two replicate cultures were retained in order to observe the zoospore production from sporophytes. The results are presented in Table XXVII. Liberation of zoospores occurred sooner at 20 ± 1°C than at 16 ± 1°C. The longest time needed for zoospore liberation occurred at 6 ± 1°C. A temperature of 26 ± 1°C resulted in poor growth of protonemata and plethysmothalli. These thalli did

Not produce sporophytes even sixty days after inoculation of zoospores at such temperature. However, growth was resumed when cultures were transferred to a temperature of 16 ± 1°C for a week. Sporophytes were observed and produced zoospores after four weeks incubation at 16 ± 1°C.

Table XXVII. Numbers of days required for zoospores production from the sporophytes of P. fascia cultivated in Petalonia medium under the fixed temperatures of 480 ft-c and 16 hours daily light.

Temperature ±1°C	Days of zoospore liberated after inoculation
1	-*
6	52
16	40
20	38
26	-
31	0**

*Indicates no measurement; sporophytes were not produced here.

**Indicates lethal temperature.

DISCUSSION

The suitable media for the studies on the life cycle of Petalonia fascia were found to be "Erd-Schreiber" (Föyn, 1934), SW I (Iwasaki, 1961) and SW II (Iwasaki, 1961). The others, BA (Boalch, 1961), HS (Haxo and Sweeney, 1955) and vSt (von Stosch, 1964) were not successful. The reasons for this are very difficult to explain. The aged natural sea water used as a basic medium for this, had the disadvantage that the nutrients, particularly the microelements, were already present in variable concentrations. Harvey (1957) noted that N, P, Fe, and Mn, had very variable concentrations in Atlantic waters. Therefore, several workers devised enriched sea water by adding different amounts of these elements and/or soil extract to natural sea water for growing marine algae. The latter varied greatly with the sample of soil utilized.

Further, while natural sea water is used as the basis for culture media, it is impossible to ascertain whether or not the alga has a requirement for a particular nutrient since so many inorganic and organic nutrients are present in sea water. Therefore, for nutritional studies, synthetic sea water should be employed for repeatability and precision in detecting mineral and organic nutritional requirements.

When molybdenum and vanadium were added to synthetic sea water, zoospores developed only into protonemata and plethysmothalli. All four morphological types of thalli were formed when iodide was added.

These thalli grew better in the synthetic sea water containing molybdenum, vanadium and iodine than in synthetic sea water alone.

There is no evidence to indicate any possible function of vanadium in growth of P. fascia, although Arnon (1958) showed that vanadium played an essential role in photosynthesis for the fresh-water green alga, Scenedesmus obliquus.

Iodine and molybdenum will be discussed in later sections.

The nitrate and phosphate experiments showed that the requirements for good growth of P. fascia was 3.7 mM nitrogen and 0.25 mM phosphorus, giving a N/P ratio of 14.8/1. Cooper (1937) found a ratio of 16.3/1 for marine phytoplankton and 20/1 in the sea, while Boalch (1961) gave a ratio of 5/1 for Ectocarpus confervoides. These figures indicate that the optimum concentration of nitrogen and phosphorus supply is quite different for different species.

Iodine

The iodine experiments indicated that in iodide concentrations in culture corresponding to those in natural sea water (4×10^{-7}M KI (50.76 µg iodide/L)) Ralfsia-like thalli and sporophytes were not produced. Much higher concentrations of iodide were found to be necessary in culture than in the field to stimulate algal growth and reproduction. The growth of the experimental alga was very slightly inhibited in the highest concentration of iodide, i.e. 25.37×10^5 µg

iodide/L, which was 50,000 times greater than in sea water.

Results of the present studies showed that growth of P. fascia was stimulated at iodide concentrations of 50.76×10^{2} µg/L; slight inhibition occurred at 25.37×10^{5} µg/L. Comparisons with previous workers' findings are given in Table XXVIII. It indicates that small additions of iodide to natural sea water with and without enrichment promote algae growth; higher concentrations have an inhibitory effect.

Von Stosch (1964) found that iodine was required for vegetative development of Asparagopsis sp. He further cultivated Ectocarpus confervoides in iodine-depleted sea water medium in which Asparagopsis had previously been grown. He found that E. confervoides suffered unusual damage or nearly death. At that time he added 49.8×10^{-2} µg iodide/L, i.e. less than 1/100 amount of iodine content in natural sea water, to iodine-depleted medium. Their growth showed a remarkable improvement. Fries (1966) found that inorganic iodine and organically bound iodine were utilized by a red alga, Polysiphonia urceolata. He found the best growth of P. urceolata was brought about by the addition of inorganic iodine, 50.76×10^{1} µg iodide/L.

More recently Tatewaki (1966) studied the formation of sporophytes from the zygote of Scytosiphon lomentaria. He used a medium containing 20 µg iodine/L. He did not report the influence of iodine on the development of this alga. However, Iwasaki (1967) showed that

Table XXVIII. A comparison of iodide concentrations stimulating and inhibiting the growth of certain marine algae.

Species	Authors	Media*	Concentrations of iodide µg/L Stimulation	Inhibition	Remarks
Green algae					
Bryopsis plumosa	Duggar (1906)	NSW	-	19.0×10^5	At Cette
B. plumosa	"	"	-	31.7×10^5	At Naples, Italy
Red algae					
Callithamnion roseum	"	"	-	31.7×10^5	At Cette
C. roseum	"	"	-	63.4×10^5	At Naples
Ceramium sp.	"	"	-	19.0×10^5	At Cette
Ceramium sp.	"	"	-	31.7×10^5	At Naples
Nitophyllum punctatum	"	"	-	31.7×10^5	At Cette
N. punctatum	"	"	-	63.4×10^5	At Naples
Asparagopsis sp.	Von Stosch (1964)	ESW	12.6×10^2	-	

(continued)

Table XXVIII. (Continued)

Species	Authors	Media*	Concentrations of iodide µg/L		Remarks
			Stimulation	Inhibition	
Asterocystis ramosa	Fries (1966)	ASP 6F	50.76	-	
Polysiphonia urceolata	"	"	50.76×10^{1}	-	
Porphyra tenera	Iwasaki (1967)	$ASP_{12}NTA$	10	50	Conchocelis phase
Brown algae					
Ectocarpus confervoides	Von Stosch (1964)	ESW	49.8×10^{-2}	-	
Laminaria hyperborea	Harries (1932)	NSW	50.8	50.8×10^{3}	

*NSW = Natural Sea Water ESW = Enriched Sea Water

ASP 6F = Fries (1963) $ASP_{12}NTA$ = Provasoli (1963)

"-" indicates no measurement

the range of iodine concentration between requirement (10 µg/L) and toxicity (50 µg/L) for the growth of the Conchocelis phase of Porphyra tenera was extremely narrow. He suggested that iodine was essential for the growth of Conchocelis.

Under the culture conditions used, iodine was essential for the growth, morphology, development and reproduction of P. fascia: the minimal concentration required for development of Ralfsia-like thalli and sporophytes were 50.76×10^2 µg/L and 50.76×10^1 µg/L, respectively. The other two morphological types of thalli, protonemata and plethysmothalli, were formed even in iodide-deficient Petalonia medium. Growth of all four types of thalli improved with an increase in iodide concentration up to 50.78×10^4 µg/L. Beyond this concentration, some of the plethysmothalli and Ralfsia-like thalli tended to roll up their thalli. A reason for this reaction may have been that the rolling up was induced by too high an iodide concentration. Yendo (1919) studied Phyllitis fascia (= Petalonia fascia) in culture, using filtered natural sea water, found only protonemata. Caram (1965) growing plants in Erd-Schreiber culture medium, found protonemata, plethysmothalli and sporophytes in the life cycle of P. fascia. These two authors did not observe Ralfsia-like thalli. Nakamura (1965) first demonstrated that zoospores, produced from unilocular sporangia of Ralfsia-like thalli, developed into the thalli of Petalonia fascia and Scytosiphon lomentaria in culture with ES medium (Provasoli's enriched sea water). The same observations were made by Tatewaki (1966) on zoospores from the unilocular

sporangia of the Ralfsia-like thallus (sporophyte) in the closely related species S. lomentaria, which alternated with a gametophyte which produced plurilocular sporangia in culture. From his observations of Danish S. lomentaria in the field, Lund (1966) disagreed with Nakamura's (1965) and Tatewaki's (1966) suggestions that the Ralfsia-like thallus of Japanese S. lomentaria is sporophytic. He supposed that Microspongium gelatinosium with unilocular sporangia could represent the sporophyte in the life cycle of Danish S. lomentaria. This controversy indicates either that the morphology of cultured S. lomentaria differs from that in nature or that there are a number of possible life cycles within the genus. In the present investigation with P. fascia the whole range of morphological types known in the life cycle was obtained. One possible reason why previous workers did not observe all morphological types of thalli during the course of thier studies might be that the media used were unsuitable. Since it is shown here that iodine appears to be essential as a governing factor in the normal morphogenesis of P. fascia in culture, previous discrepancies in results obtained by earlier workers might possibly be accounted for by the varying concentrations of iodine in the media used. However, the life cycles of P. fascia and that of the closely related S. lomentaria would both appear to require a considerable amount of further investigation.

Jacques (1937) first reported that iodide penetrated into the protoplasm of Valonia macrophysa and accumulated in the cell sap (Jacques and Osterhout, 1938). Kelly and Baily (1951; 1955) demonstrated

uptake of radioactive iodine by Ascophyllum nodosum through an ion exchange process in which all radioactive iodine was reduced to iodide, not oxidized to iodate. They concluded that the exchange process took place only in living cells. Kelly (1953) found that iodine uptake by A. nodosum was related to respiration. However, Tong and Chaikoff (1955), using radioactive iodine, radioactive iodide and filter paper chromatography, showed that the tissues of Nereocystis luetkeana took up radioiodide, but not radioiodine. They found that iodide uptake was in the form of active ion transport which depended upon respiration function. This finding confirmed Kelly's (1953) result. However, Shaw (1959) suggested that iodide uptake in Laminaria digitata proceeded in aerated sea water by the oxidation of iodide to I_2; this I_2 either diffused or hydrolyzed to HIO which penetrated into the tissues. Within the tissues they were reduced back to iodide. This alga took up each iodide ion approximately consuming between three and six molecules of O_2 (Shaw, 1960). Therefore, he presumed that iodide absorption was connected with the oxidation of a carbohydrate-like compound or compounds.

According to Shaw's hypothesis, iodide added to aerated sea water was oxidized to iodine only when iodide oxidase was present in the algae. Grimm (1952) reported a negative test for iodine content in Ilea fascia (=Petalonia fascia). Iodide oxidase seems to be absent in the cells of P. fascia. One problem arising from the above discussions is that of determining why higher iodide concentrations

promote growth and accelerate zoospore production in culture. This phenomenon may be attributed to media with changed physico-chemical conditions which are more suitable for this alga. The real reasons are as yet unknown in the present studies.

A problem arising from the above discussions is that of determining why more iodine is required for growth, development and reproduction of P. fascia in culture than in the field.

No data of iodine content of sea water are available from St. Phillips, Newfoundland, where the culture material was collected. According to Young, Smith and Langille's (1959) determination, the iodine content of sea water in the vicinity of the Atlantic Provinces of Canada is 6 to 53 µg iodine/L. The seasonal variation in iodine content of sea water is still unknown, but the quantities of iodine forms, iodide and iodate, seem to vary with pHs of sea water. Sonstadt (1872) considered that iodide could not permanently exist in a slightly alkaline medium such as sea water, but that all the iodine present must be in the form of iodate. Sugawara (1955) found that there was about one-third of the total iodine in the form of iodide and the remaining two-thirds were in the form of iodate in Western Pacific waters. Barkley and Thompson (1960) found that one- to two-thirds of total iodine was present as iodate in North Pacific waters.

Iodine uptake in the form of iodide by marine algae has been verified. The rate of iodide uptake by the green alga, Valonia macrophysa was proportional to the external concentration of iodide,

but was little affected (or not at all) by the pHs of sea water between pH 7.0 and pH 9.0 (Jacques, 1937). Klemperer (1957) showed radioiodide uptake by Fucus ceranoides with various iodide concentrations in the medium. He found the uptake decreased as the iodide concentration increased over 20 μM, but failed to give the pH of his sea water. Although there is no direct evidence from the present investigation, the results indicate that better growth of P. fascia was obtained with increase in iodide concentrations even 10,000 times more than that in natural environment. This fact may be attributed to the lower pH (7.8) used in the nutrient medium. Beyond this pH growth decreased.

Further evidence may be found in the nitrate experiment where growth of P. fascia was not promoted by an increasing concentration of nitrate in the synthetic sea water. Klemperer (1957) found that nitrate acted as a competitive inhibitor of iodide uptake by Fucus ceranoides.

From the present experiments and in the light of work with iodine by other workers with algae, a number of further lines of work can be suggested here:

(1) Comparative studies in field and in culture may indicate whether more iodine is absorbed by plants in culture or not, and to what extent this element is used.

(2) If iodide is absorbed by this experimental alga, further studies should be made to determine whether an enzyme system is concerned with iodide uptake.

Bromine

Maximum growth of P. fascia was obtained with 7.72×10^{-4}M KBr (61.8 mg bromide/L). This concentration almost corresponds to the bromine content of natural sea water. Beyond this concentration growth was inhibited. All four morphological types of thalli were formed in both bromide-deficient and bromide-enriched Petalonia medium.

Iwasaki (1967) found that optimum concentration of bromine for the best growth of Conchocelis phase of Porphyra tenera was 5 mg/L; some inhibition was found at 10 mg/L. Fries (1966) reported that the growth of many species of red algae was inhibited by a bromide concentration of 8.14×10^{-4}M, though this amount is in agreement with the bromine content of the natural environment. He found bromide concentration as low as 5×10^{-7}M slightly stimulated the growth of Polysiphonia urceolata and Goniotrichum elegans in axenic culture. Much higher or lower concentrations of bromide did not give obvious response. Therefore, he suggested that bromine did not play any essential role in algal metabolism.

Thompson and Korpi (1942) in estimation of bromine concentration in sea water, found that their results were affected by the presence of iodide and iodate. They also found that bromine-chlorinity ratios remained constant in the water of the Bering Sea and the Northeast Pacific.

Results of the present studies indicate that bromide does not substitute for iodide. In a fresh water alga, Nitella clavata, bromine

and chlorine were exchanged or displaced each other in their cells: the prior absorption of KCl or KI by this alga reduced the subsequent uptake of bromide (Hoagland, et al., 1926; 1928). There is no other evidence as to the function of bromine in algal metabolism. However, in a higher plant (e.g. potato discs, Solanum tuberosum) there is evidence to show that bromine may have some connection with protein synthesis (Steward and Preston, 1940; 1941).

Molybdenum

Molybdenum is necessary for nitrate utilization (Walker, 1953; Arnon, et al., 1955) and for nitrogen fixation (Eyster, 1959) in algae.

The quantitative requirement for molybdenum to sustain vigorous growth of P. fascia was determined to be 5×10^{-6}M Na_2MoO_4 (4.8×10^2 µg Mo/L) when the nitrate was the sole source of nitrogen. Much higher concentrations of molybdenum inhibited thallus growth and sporophyte formation.

Essentiality of molybdenum (20 µg/L) with nitrate as nitrogen source was shown in Chlorella pyrenoidosa by Walker (1953). He found, however, that if urea was used as the sole nitrogen source or was present in the medium together with nitrate, no molybdenum requirement could be detected. Molybdenum requirement for the fresh water alga, Scenedesmus obliquus was abolished when plants were grown with ammonia or urea instead of nitrate (Ichioka and Arnon, 1955). Nicholas (1961)

confirmed that Scenedesmus required molybdenum only when it was cultured in nitrate nitrogen. Eyster (1959) determined that critical concentrations of molybdenum for nitrogen fixation and for nitrate utilization by Nostoc muscorum were 1×10^{-7}M (9.6 µg Mo/L) and 1×10^{-10}M (9.6×10^{-3} µg Mo/L), respectively. No molybdenum was required when ammonium ion served as the sole source of nitrogen.

Arnon, Ichioka, Wessel, Fujiwara and Wolley (1955) indicated that molybdenum was essential for growth and cell division of Scenedesmus obliquus. Quantitative requirement for molybdenum by S. obliquus was found to be extremely low in the range between 10^{-7} and 10^{-6} g Mo per liter (0.1 - 1.0 µg Mo/L) of nutrient solution. Good growth occurred with 3,000 atoms of Mo per cell (47.8×10^{-20} g Mo/cell). When the supply was reduced to 1,500 - 1,700 atoms (23.9 - 27.1×10^{-20} g Mo/cell), no cell division took place.

Any possible function of molybdenum in metabolism of P. fascia is at present unknown, although evidence for other species would suggest that it plays an important role in nitrate utilization.

Iron

Iron, while not a constituent of chlorophyll molecules, is indispensable for the synthesis of chlorophyll (Price and Carell, 1964). Deficiency of this element might result in poor chlorophyll development and showed light brown color in P. fascia. The color of the thalli deepened with increasing iron concentrations. The addition of ferric

chloride to iron-deficient Petalonia medium stimulated growth of the experimental alga up to the highest concentration used, 4×10^{-5}M (2.233×10^{3} µg Fe/L). The growth curve was an approximately linear function of iron concentration in media which contained 1.151×10^{3} µg Mn/L (Figs. 27 and 28). Therefore, soluble iron is essential for this alga.

Many workers prefer to chelate the iron with citrate (Hopkins, 1930c; Kylin, 1943) or with EDTA (Provasoli, et al., 1957). Although EDTA accentuated the need for more Zn, Mn and Ca in the nutrient solution for Chlorella, it did not affect its iron requirement (Walker, 1953; 1954). In my investigation, $Na_2EDTA.2H_20$ (28.57 mg/L) was added to the nutrient media. The greatest problem of iron nutrient was associated with its availability to the algae. On standing, the dissolved iron changed to a colloidal form which reduced its availability. Fresh iron solutions were required for improved culturing conditions. Myers (1951) and Gerloff, et al. (1950) showed that aged nutrient media containing iron were less effective than media freshly prepared for the growth of Chlorella, and Coccochloris. This change might be due to the aggregation of colloidal particles which were only sluggishly reversible and biologically unavailable.

The solubility of iron in alkaline solution is very low. Cooper (1935) found that less than 10^{-12} µg per liter of free ferric ion was present in the sea. When iron, in concentrations greater than 4×10^{-5}M (2.233×10^{3} µg Fe/L), was added to iron-deficient Petalonia

medium precipitation occurred and mineral deficiencies resulted. Such medium cannot be used in nutritional studies of P. fascia, although marine diatoms are able to assimilate ferric hydroxide or ferric phosphate in colloidal and/or particulate forms, and utilize it for their growth (Cooper, 1935; Harvey, 1937).

Goldberg (1952), using radioactive iron, found that the marine diatom, Asterionella japonica utilized only particulate and/or colloidal iron as a growth nutrient, whereas ionically complexed ferric ion as the citrate, ascorbate or synthetic humate was not available for uptake. Kylin (1943) reported that the addition of 0.000001% iron citrate (iron ion concentration was calculated to be 2.34 µg/L) to natural sea water was enough to stimulate the growth of Ulva lactuca. When the iron ion concentration was increased up to 2.34×10^4 µg/L, further stimulation was not obtained, nor could any toxic effect be observed. He attributed this to the fact that the highest concentration of iron citrate caused precipitation. However, Boalch (1961) observed no effect on the growth of Ectocarpus confervoides by the addition of 2.7-10.8 mg $FeCl_3.6H_2O$/L (5.58×10^2 - 2.23×10^3 µg Fe/L) to enriched sea water. He also experimented with various artificial sea waters and found that E. confervoides grew quite well in Droop's medium (Droop, 1955) containing 0.7 mg $FeSO4.7H_2O$/L (141 µg Fe/L) when salinity was doubled and brought nearer to that of natural sea water.

Iwasaki and Matsudaira (1957) showed that the highest

photosynthetic activity of Porphyra tenera was obtained by adding the concentration of 500 µg ferric ion (Fe-EDTA) per liter. Ten years later, Iwasaki (1967) found that the addition of as low as 50 µg iron/L supported the best growth of Conchocelis phase of P. tenera in the $ASP_{12}NTA$ medium (Provasoli, 1963).

From the above discussions it is shown that the algae utilize iron in relation to the chelators, pHs and salinity of media used. Results of the present investigation show that the best growth of P. fascia was obtained at an iron concentration of 2.233×10^3 µg Fe/L chelated with $Na_2EDTA.2H_20$ (28.57 mg/L) at pH 7.8 and salinity 32.48 °/oo.

Manganese

The best growth of P. fascia was brought about by the addition of 1.8×10^{-5}M $MnSO_4$ (9.89×10^2 µg Mn/L to manganese-deficient Petalonia medium when the nitrate was the sole source of nitrogen.

Evidence in the literature indicates that manganese utilization in algae is related to nitrogen metabolism. Manganese stimulated the growth of Ulva lactuca only when nitrogen was supplied as nitrate, but not when supplied as ammonium (Kylin, 1943). However, Harvey (1947) found that manganese stimulated growth rate of Chlamydomonas when either nitrate or ammonium was supplied as a source of nitrogen.

Kessler (1955) found that manganese exclusively affected photosynthesis, and it did not influence chlorophyll formation and respiration in a green alga, Ankistrodesmus braunii. He concluded

that the role of manganese in photosynthesis was mainly concerned with oxygen evolution. He also found that the reduction of nitrate by molecular hydrogen was increased in the presence of manganese.

Hopkins (1930a and b) found that manganese was not replaced by iron; both were essential, and he suggested that manganese functioned physiologically in an indirect manner by its action on the state of oxidation of iron, i.e. manganese tended to control the ratio $(Fe^{++}):(Fe^{+++})$ in the culture media or in the cells. According to his hypothesis when manganese in excess of the normal requirement (9.89×10^2 µg Mn/L) is contained in the media, the reduction of ferric to ferrous ion is prevented. This will result in either too high a concentration of ferric ions or the prevention of its reduction by experimental algae. Therefore, the high concentration of manganese might cause toxicity when accompanied by large amounts of iron. However, Somers and Shive (1942) found that manganese played the role of such an oxidizing agent and that an excess of manganese might induce symptoms of iron deficiency by converting the available iron into the physiologically inactive ferric condition. In the present investigation, the thalli did not show any symptoms of iron deficiency and were not injured by the higher concentrations of manganous sulfate (9.89×10^3 µg Mn/L). Although the highest concentrations of manganese (9.89×10^4 µg Mn/L) did not cause precipitation, zoospores did not survive in such a medium. From these facts, it is inferred that the toxicity may be caused directly by the high concentration of manganese and indirectly by the high concentration of ferric ions.

The interrelationship between iron and manganese has an effect on the growth of P. fascia, as shown in Table XXIX. These results are extrapolated from experimental results in sections d and e. The approximate range of values of iron to manganese ratio, which produced good growth of the experimental alga, was found to be 0.3-2.0. Variation in the ratios either above or below this effective range resulted in poor growth. However, Somers and Shive (1942) found that the ratios of iron to manganese in a nutrient solution conducive to good growth and development of soybeans fluctuated within a narrow range above 2.0, regardless of total concentrations of these elements within the limits employed.

Kylin (1943) found that the addition of 0.0001-0.001% manganous sulfate (2.53×10^2-2.53×10^3 µg Mn/L) to natural sea water acted as a good stimulant for the growth of Ulva lactuca; a slightly toxic effect was observed at the concentration of 0.01% manganous sulfate (2.53×10^4 µg Mn/L). Boalch (1961) showed that the growth-stimulating concentration of manganese for Ectocarpus confervoides was 4×10^{-6}M $Mncl_2$ (2.2×10^2 µg Mn/L). Maximum growth of Conchocelis phase of Porphyra tenera was obtained 4×10^2 µg Mn/L, beyond this concentration, an inhibitory effect occurred (Iwasaki, 1967).

From the above discussions, it is shown that more manganese is required for the growth of P. fascia than that in other species of algae examined experimentally. Since it has been shown in culture that different species require various amounts of manganese, it could be

Table XXIX. Growth of P. fascia in relation to the ratios of iron to manganese in culture media.

Culture media and numbers	Concentrations in nutrient solution mg/L		Average dry weight of thalli (mg)	Ratio of Fe/Mn in nutrient solution
	Fe	Mn		
Iron Expt.				
1	0	1.151	32.7	0
2	0.0002233	1.151	34.0	0.000194
3	0.002233	1.151	37.6	0.00194
4	0.02233	1.151	41.5	0.0194
5	0.2233	1.151	46.0	0.194
6	0.289	1.151	47.0	0.251
7	2.233	1.151	49.7	1.94
Manganese Expt.				
1	0.289	0	37.5	0
2	0.289	0.0000989	39.0	2922.0
3	0.289	0.000989	40.1	292.2
4	0.289	0.00989	40.5	29.22
5	0.289	0.0989	41.3	2.922
6	0.289	0.989	48.0	0.2922
7	0.289	1.151	46.4	0.251
8	0.289	9.89	36.7	0.02922
9	0.289	98.9	-	0.002922

suggested that the differences lie in differences between the compositions of the various media used, and differences would be related to the chelators, nitrogen sources and iron concentrations used in the media.

Copper

The optimum concentration of copper sulfate for the maximum growth of P. fascia was 3×10^{-7}M (19.7 µg Cu/L). This concentration corresponds to the copper content (13-22 µg/L) of the sea water in the vicinity of the Atlantic Provinces of Canada found by Young, Smith and Langille (1959). The inhibitory effect in culture occurred at ten times higher than this concentration. The growth-promoting and -inhibiting actions of copper for P. fascia were remarkable in that the range between optimal and inhibitory levels was extremely narrow.

The effect of chelation on toxicity of a heavy metal ion or, alternately, the effects of chelation on the growth-promoting activity of an essential trace element in the marine unicellular alga, Phaeodactylum tricornutum was considered by Spencer (1957). In either case the biological activity might be expected to be related to the concentration of the particular form or forms in which it was available to the alga. In his experiment he found the cupric ion was increasingly toxic to P. tricornutum at concentrations in excess of about 2×10^{-6}M (12.7×10^{1} µg Cu/L). The Cu-EDTA complex did not become toxic until the concentrations in excess of 5×10^{-3}M (3.2×10^{5} µg Cu/L) were reached.

He showed that the toxicity of cupric chelate was probably accounted for by the equilibrium concentrations of cupric ions which, under these conditions, were of the order required to cause a comparable growth-inhibition in the absence of EDTA.

Iwasaki and Matsudaira (1957) found that the photosynthetic activity of Porphyra was stimulated remarkably even in high copper ion concentrations (5×10^2 µg Cu/L) when the metal chelate compound (EDTA) was present in the medium. They attributed this effect to the fact that the copper ion was taken up as its stable chelate compounds with EDTA, thus stimulating photosynthesis. This apparently conforms with Spencer's finding. In my investigation, no experiments were done to compare the effect of the absence and presence of EDTA on copper requirements of P. fascia.

Kylin (1943) reported that sea water (Kristineberg, west coast of Sweden) itself contained enough copper for the growth of Ulva lactuca. A toxic effect resulted from the addition of $CuSO_4$ higher than 0.000001% (4.1 µg Cu/L) to the enriched sea water. Iwasaki and Matsudaira (1957) found that the addition of copper ion up to 10 µg/L to filtered natural sea water accelerated the photosynthesis of Porphyra tenera. The higher concentrations strongly inhibited it.

Greenfield (1942) studied Chlorella photosynthesis by manometric methods at five different light intensities in different concentrations of copper sulfate. He found that photosynthesis of Chlorella cells was inhibited in concentrations as low as 10^{-7}M $CuSO_4$ (6.36 µg Cu/L). He

also found that the inhibition occurred chiefly at low light intensity (below 1,600 lux, i.e. 148.6 ft-c) and to a lesser extent at the high value (above 1,600 lux up to 22,000 lux, i.e. 2,043.8 ft-c). The treatment with high concentration of $CuSO_4$ (5×10^{-6}M; 3.2×10^2 µg Cu/L) reduced photosynthesis of Chlorella vulgaris to about 50% of the control. Walker (1953), however, has shown that a lower concentration of copper (30 µg/L, approximately 1/10 the concentration found to be toxic by Greenfield (1942)) is essential for growth of Chlorella pyrenoidosa.

Zinc

Low concentrations of zinc sulfate stimulated the growth of Petalonia. The maximum effect was obtained in a concentration of 8×10^{-7}M (52.3 µg Zn/L); the growth rate declined with a further increase of zinc concentration.

Zinc is known to be a component of the enzyme carbonic anhydrase (Keilin and Mann, 1940). Steeman, et al. (1949) identified carbonic anhydrase activity in Elodea and Fontinalis spp., possibly in the gaseous exchange of carbon dioxide. Osterlind (1950) reported that carbonic anhydrase occurred in Scenedesmus quadricauda and Chlorella pyrenoidosa. This enzyme was also present in Nostoc muscorum (Brown Eyster, 1955). Higher plants (tomato) grown in Zn-deficient medium showed low carbonic anhydrase activity (Wood and Sibly, 1952). Wiessner (1962) suggested that zinc might participate in photosynthesis at the level of carbon dioxide fixation through carbonic anhydrase in algae.

Millar and Price (1960) and Price (1962) showed that zinc deficiency in Euglena gracilis did not cause early death, but that the growth rate was sharply decreased. Since lactate dehydrogenase activity was reduced in zinc-deficient medium, concentrations of zinc were perhaps sufficiently low to inhibit growth, through their effect on lactate dehydrogenase activity.

Zn-deficient Euglena gracilis was measured by Price and Millar (1962). They found that the oxygen uptake of Euglena in Zn-deficient was lower than that in Zn-sufficient medium. The growth of Zn-deficient Euglena was limited by the activity of oxidative enzymes of respiration. Price and Vallee (1962) confirmed that zinc alone was a limiting growth factor in Euglena.

Bachman and Odum (1960), using six species of marine benthic algae, found a direct relationship between Zn^{65} uptake and photosynthesis. Their finding was confirmed by Gutknecht (1961). He suggested that Zn^{65} uptake was mainly by the adsorption-exchange process which may be related to photosynthesis via the pH increases. These findings were also verified by himself (1963, 1965).

Protein and RNA synthesis in E. gracilis were markedly reduced in Zn-deficiency while there was a concomitant increase in amino acids and polyphosphate. In addition, zinc deficiency led to mitotic arrest as indicated by a doubling of DNA content of the deficient cells in Euglena (Wacker, 1962). In view of the occurrence of zinc as an essential component of many respiratory enzymes (Vallee, 1959) and controlling the nucleic acids and protein metabolism (Wacker, 1962),

the growth of Petalonia seemed to be controlled by the amounts of zinc. Although no further experiments have been done, it is inferred from this that the type of growth in Petalonia is intercalary growth (Chapman, 1962; Taylor, 1937). This type of growth takes place by mitotic division which results in the formation of new cells and tissues. When this alga is cultured in Zn-deficient Petalonia medium, mitotic division is retarded and poor growth results.

Zinc requirements for the growth of Chlorella pyrenoidosa in two different media were approximately 4.5 µg Zn per gram dry weight of Chlorella for nitrate medium and 6.5 µg Zn/g for urea medium. If EDTA was present in the media, much larger quantities of zinc were required (Walker, 1954). Krauss and Porter (1954) reported that absorption of Zn by C. pyrenoidosa was directly proportional to the concentration of the ions in the nutrient solution over remarkably wide ranges of concentration. Only with phosphorus and sulfur were limiting values approached at high concentrations.

Kylin (1943) found that the best growth of Ulva lactuca was obtained by the addition of zinc (41.5 µg/L) to the natural sea water. If higher concentrations were used, a toxic effect was observed. Recently Iwasaki (1967) determined that the optimal concentration of zinc for the best growth of Conchocelis phase of Porphyra tenera was 30 µg Zn/L; inhibition occurred at a concentration of 80 µg Zn/L.

From the above discussion, zinc appears to be an essential component of respiratory enzymes and has a function in photosynthesis, protein and RNA synthesis. The quantities of zinc required for algae vary with the presence of other elements (such as P and S) and chelators used in the media. Such variations may explain why higher concentrations of zinc inhibited the growth of P. fascia. This could be expected, assuming that photosynthesis and respiration were inhibited through enzymatic activities.

Zinc is also related to auxin synthesis as discussed in a later section.

Cobalt

The optimum concentration of cobalt for supporting the maximum growth of P. fascia was 1.7×10^{-8}M $CoSO_4$ (1 µg Co/L). Much higher concentrations had an inhibitory effect.

Cobalt is known as a constituent of vitamin B_{12} (Hutner, et al. 1950). Marston (1952) also indicated that cobalt is an indispensable part of the vitamin B_{12} molecule.

Cobalt requirement for axenic cultures of blue-green algae was demonstrated by Holm-Hansen, Gerloff and Skoog (1954). They found that the nutritional function of cobalt seemed to be associated with the role of vitamin B_{12}; the cobalt requirement of Nostoc muscorum could be satisfied by the addition of 0.075 µg vitamin B_{12} in cobalt deficient

medium, obtaining in this way as much growth as with 0.2-0.4 µg Co/L.

Cobalt metabolism in Rhodymenia palmata was studied by Scott and Ericson (1955). They found that the uptake rate of radioactive cobalt in the light was greater and more rapid than in the dark. They also found that uptake of radioactive cobalt depended on the supply of carbon dioxide, and indicated that certain products of photosynthesis might form a stable complex with cobalt ions. They concluded that there was no direct experimental evidence for the cobalt incorporation into vitamin B_{12}, and suggested vitamin B_{12}, originating from bacterial synthesis, was subsequently accumulated by marine algae.

Spencer (1957) demonstrated the cobalt requirement of a marine unicellular alga, Phaeodactylum tricornutum; no deficiency of cobalt occurred at an ionic concentration of 10^{-14}M (5.9×10^{-7} µg Co/L). The photosynthesis of Porphyra tenera was accelerated by the addition of cobalt (50 µg/L) to the filtered natural sea water, though 100 µg Co/L produced an inhibition (Iwasaki and Matsudaira, 1957). Iwasaki (1967) found that maximum growth of the Conchocelis phase of Porphyra tenera in axenic culture required 5 µg Co/L containing in the ASP_{12}NTA medium of Provasoli (1963).

Indoleacetic Acid (IAA)

Skoog (1940) demonstrated that zinc is required for synthesis

of IAA. It was later shown by Wildman, et al. (1947) that tryptophan is a precursor in auxin synthesis, while Tsui (1948) indicated that it is in tryptophan synthesis that zinc is utilized. Subsequently, the quantity of auxin produced is proportional to the quantity of zinc.

The data from the present investigation clearly indicated that concentrations of 50-100 µg IAA/L in Zn-deficient Petalonia medium caused an increase in the length of the sporophytes up to about the same length but not the same width as in Zn-sufficient Petalonia medium without IAA. A possible explanation of this fact is that the role of IAA is generally concerned with cell elongation. However, the total dry weight of thalli was increased by the small addition of IAA (10 µg/L) in Zn-deficient Petalonia medium. It is possible that IAA may counteract the zinc deficiency.

A concentration of 400 µg IAA/L strongly inhibited the growth of P. fascia in length and width of thalli, and total dry weight. Davidson (1950) cultured the sporelings of Fucus evanescens in 0.01 mM indoleacetic acid (1,752 µg IAA/L). He determined that the length of thalli and holdfasts, and numbers of holdfasts of sporelings increased 200%, 50% and 21%, respectively, over control plants in natural sea water.

The toxic effect of relatively high concentrations of IAA in P. fascia may be interpreted by the results obtained by Van Overbeek (1949) and Williams (1949). Van Overbeek found that brown algae formed

high concentrations of IAA. Williams showed that Laminaria agardhii produced a sufficient concentration of IAA by itself and it was injured by the amounts of exogenous auxin (100-10,000 µg IAA/L) which could be innocuous or stimulating to low-auxin algae. This toxic effect may result in derangement of metabolism.

Vitamin B_{12}

The addition of 1 µg of vitamin B_{12}/L to cobalt-deficient Petalonia medium produces increased growth. Vitamin B_{12} does not appear to completely substitute for cobalt in the growth of P. fascia. This result contradicts the findings by Holm-Hansen, et al. (1954) in the blue-green algae. They found that a small amount of vitamin B_{12} (0.075 µg/L) could completely replace the cobalt required (0.20-0.40 µg/L) to obtain maximum growth. However, the growth of P. fascia was much better in the medium containing vitamin B_{12} with cobalt than without cobalt. This may suggest that the physiological activity of vitamin B_{12} is many times greater when incorporated with cobalt than that of vitamin B_{12} alone.

Ericson and Lewis (1953) grew many species of marine red, green and blue-green unicellular algae under sterile conditions, and vitamin B_{12} was added to the culture media. Growth of these algae was not significantly stimulated by the addition of vitamin B_{12}. Iwasaki and Matsudaira (1957) obtained an increase in CO_2 assimilation in

Porphyra tenera when they added vitamin B_{12}, but only with material collected in early spring. This fact could be connected with seasonal variations of vitamin B_{12} content of sea water or variations in the bacterial flora on this alga. In comparison with my investigations on P. fascia in unialgal culture, growth was stimulated by the addition of 1 µg vitamin B_{12}/L. This stimulatory effect could be directly caused by the uptake of vitamin B_{12} and indirectly increased the efficiency of utilization of mineral elements, especially cobalt, since the number of bacteria in the culture did not increase considerably.

pH

The optimum pH for growth of P. fascia was at about 7.8-8.0. Retardation of growth was observed at pH's lower than 7.8 and higher than 8.0. Owing to precipitation, experiments were not conducted at a pH higher than 8.5.

The experimental establishment of pH-effect on growth and nutrition is complicated, since the pH of culture media may change considerably during the course of culturing. It is frequently impossible to separate the direct effects of pH from other contributing factors (the solubility and availability of trace elements and the ratios of H_2CO_3, HCO_3^- and $CO_3^=$ vary with pH).

According to the suggestion by Emerson and Green (1938), the concentrations of carbonate, bicarbonate and free carbon dioxide all

varied with the pH values. The free carbon dioxide in the medium decreased from 100% at pH 4 to vertually zero concentration at 8. The carbonate concentration increased from zero at a pH of about 8 to 100% at pH 12. The bicarbonate was at a maximum around pH 8. The pH of natural sea water, in which about 90% of the total carbon dioxide is in the form of bicarbonate ions, is about 8 (Blink, 1963).

Emerson and Green (1938) made an important contribution to knowledge of the influence of pH on Chlorella photosynthesis. They showed that the rate of photosynthesis remained constant in pH range between 4.6-8.9 if both carbon dioxide and light were saturated. According to Thomas (1955), pH may affect photosynthesis indirectly by changing the amount of available carbon dioxide. Blink (1963) investigated the effect of pH on the rate of photosynthesis of several littoral marine algae. A low photosynthetic rate was obtained at high pH among many brown algae such as Laminaria, Desmarestia, Dictyoneurum, Egregia and Macrocystis, which apparently required free CO_2 as a carbon source, and only rarely used bicarbonate ions. He attributed this to the fact that the bicarbonate ions could not readily penetrate into the cells and hence were not available for photosynthesis. However, he also found that other brown algae, Pelvetia (and possibly Fucus), readily utilized bicarbonate ions.

Light Intensity

The experimental data indicate that the growth and reproduction of Petalonia are approximately proportional to light intensity up to

480 ft-c at two different temperatures (6 and 16 ± 1°C). The higher light intensities are slightly inhibitory, but all four morphological types of thalli were formed. Ralfsia-like thalli and sporophytes of Petalonia were not observed under the light intensities between 240 and 1,140 ft-c at 25-27°C. When the cultures were incubated at 15-17°C, all four morphological types of thalli were formed and produced zoospores from the sporophytes. It seems reasonable to conclude that the light intensity strongly influences the growth, morphology, and reproduction of Petalonia in connection with temperature.

Comparison can be made between the observations by Segi and Kida (1957) in Undaria undarioides and Boalch (1961) in Ectocarpus confervoides. Boalch found that light intensities between 125 and 1,500 ft-c had no marked effect on growth but did affect the lag phase, the optimum for this being 600-700 ft-c. He attributed this effect to the fact that the growth process of this alga was light saturated at intensities as low as 125 ft-c and that light intensities as high as 1,500 ft-c did not have any inhibitory effect. Segi and Kida showed that the gametophytes of U. undarioides grew faster in 2,200-3,200 lux (204-297 ft-c) than in 4,500 lux (418 ft-c). The development of gametophytes was poor when the light intensity was below 2,200 lux (204 ft-c); the survival rate of gametophytes was decreased with increasing light intensity and temperature. However, in 1958 they also showed that the suitable light intensities for the growth of sporophytes were about 1,500-2,000 lux (139.4-185.8 ft-c) at 14°C. These apparently indicated that the development of gametophytes and

sporophytes of U. undarioides required different light intensities.

From the above discussions, it could be inferred that production of the four morphological types of thalli in the life cycle of P. fascia may depend on different light intensities.

Photoperiod

It has been found that the sporelings of Petalonia grow more rapidly in continuous light than in 16 hours photoperiod per day. The dry weight was increased by about 26% in condinuous light cultures over than in 16 hours daily light. The liberation of zoospores was accelerated in the continuous light condition.

Kurogi (1959) found that photoperiods of 10 and 12 hours daily light induced an abundant formation of monosporangia and caused the discharge of a great number of monospores, while 15 hours of light daily did not enhance the formation of monosporangia and liberation of monospores in the Conchocelis phase of Porphyra tenera. He also found that the effect of day length on the liberation of monospores was associated with change of water temperature. Iwasaki (1961) confirmed Kurogi's work. The thalli of Porphyra tenera derived from monospores grew well and normally in artificial media at 13-18°C and in high intensity (400-500 ft-c) incandescent light of 8-11 hours daily, but not in fluorescent light. Kurogi, et al. (1962) further found that the Conchocelis-thallus of P. kuniedai discharged the

greatest number of monospores in 4-7 hours and very few in 13-15 hours of light period daily at temperatures of 18-23°C. This has clearly indicated that the Conchocelis-thallus of P. kuniedai behaves as a short-day plant. Kurogi and Sato (1962a and b) suggested that adequate photoperiod for the growth, the formation of monosporangia as well as the liberation of monospores was 8-10 hours of light daily, although water temperature, light intensity and other factors were to be also considered. Therefore, it may be concluded that the length of photoperiod affects algal growth, morphology, formation and liberation of spores and even their geographical distribution.

Temperature

Temperature may be a controlling or a lethal factor for algae. As a controlling factor, temperature controls metabolism, growth, morphology and reproduction. The optimum temperatures for the last three processes are different in the present investigation of P. fascia.

Optimum temperature for sustained maximum growth was 16 ± 1°C. Thallus growth decreased with increasing temperature up to 26 ± 1°C, while the lethal temperature in culture was 31 ± 1°C. The relation between growth and temperature is shown in Fig. 46.

There is a close relationship between temperature and morphology of P. fascia as shown in Table XXVI. Protonemata grew in a wide temperature ranging from 0-27°C; plethysmothalli were observed at 5-27°C; Ralfsia-like thalli and sporophytes appeared in the temperatures ranging from 5-21°C.

The optimal temperature for zoospore production from sporophytes of P. fascia was between 16 ± 1°C and 20 ± 1°C; 6 ± 1°C was suboptimum. Beyond these temperatures no reproductive thalli were produced.

It could be concluded that P. fascia survives in unfavorable temperature conditions by vegetative growth of protonemata and/or plethysmothalli, but not in Ralfsia-like thalli and sporophyte stages. When protonemata and plethysmothalli were transferred to a suitable temperature, their growth and reproduction were rapidly resumed. Therefore, temperature may have a marked influence on the growth, morphology, types of sporangia and the rate of zoosporogenesis. These rate and types vary, in addition, according to other culture conditions.

From Table XXX, it is seen that temperature influences the growth and reproduction of many marine brown algae in culture. A general conclusion from this is that reproduction occurs over a narrower temperature range than that at which growth will occur. Variations occur in different species and in different intensities of illumination within the same species.

Myers (1928) cultivated Egregia menziesii at 10-16°C obtaining good growth and reproduction of both gametophytes and sporophytes, while at temperatures increasing up to 20°C these plants grew well but failed to reproduce. In Nereocystis luetkeana the vegetative growth of gametophytes and sporophytes progressed well at 14°C, but the gametophytes

Table XXX. Comparison with certain marine brown algae in response to temperatures.

Species	Temperature °C				Author	Remarks
	Growth		Reproduction			
	Optimum	Lethal	Stimulation	Inhibition		
Phyllitis fascia (=Petalonia fascia)	6-14	-	6-14	-	Yendo (1919)	Protonema
Petalonia fascia	14	-	14	-	Nakamura (1965)	Ralfsia-like thallus and sporophyte
Petalonia fascia	12-15	-	12-15	-	Caram (1965)	Protonema, plethysmothalli and sporophyte
Alaria esculenta	14	20	-	-	Boney (1966)	Sporophyte
Ectocarpus confervoides	15-20	30	-	-	Boalch (1961)	Gametophyte and sporophyte
Egregia menziesii	10-16	-	10-16*	16-20*	Myers (1928)	Gametophyte and sporophyte *Gametophyte
Nereocystis luetkeana	14-18	-	6-9	14	Kemp & Cole (1961)	Gametophyte
Laminaria religiosa	6-9	24.8	6.5-11.3*	12.4	Ueda (1929)	Gametophyte and sporophyte *Gametophyte

(continued)

Table XXX. (continued)

L. saccharina	-	-	16-18	-	Parke (1948)	Gametophyte
L. hyperborea	5-17	20	5-17	19	Kain (1964)	Gametophyte
Laminaria sp.	12-15	-	12-15*	20-21*	Wang and Hsueh (1959)	Gametophyte and sporophyte *Gametophyte

"-" Indicates no measurement.

became fertile only when temperature was reduced to 6-9°C (Kemp and Cole, 1961). In the case of Eisenia arborea an abundance of fruiting gametophytes and young sporophytes developed in culture at 17-22°C (Hollenberg, 1939). Hollenberg could not induce fertility in gametophytes by temperature reduction.

Ueda (1929) noted that both gametophytes and sporophytes of Laminaria religiosa developed well at temperatures between 6-9°C. However, he found that gametophytes became fertile at temperatures approaching 11.3°C, while this temperature inhibited sporophyte formation. Alternatively, Wang and Hsueh (1959) found that the sorus formation and zoospore liberation of Laminaria sp. were stimulated at 12-15°C. They were inhibited at 20-21°C, while this temperature supported good growth of sporophytes.

From the above discussions, it is clear that different temperatures are required for the vegetative growth and the development of reproductive structures of gametophytes and sporophytes of many marine brown algae in culture. Other environmental factors, beyond the scope of the present work, may also play an important role in development.

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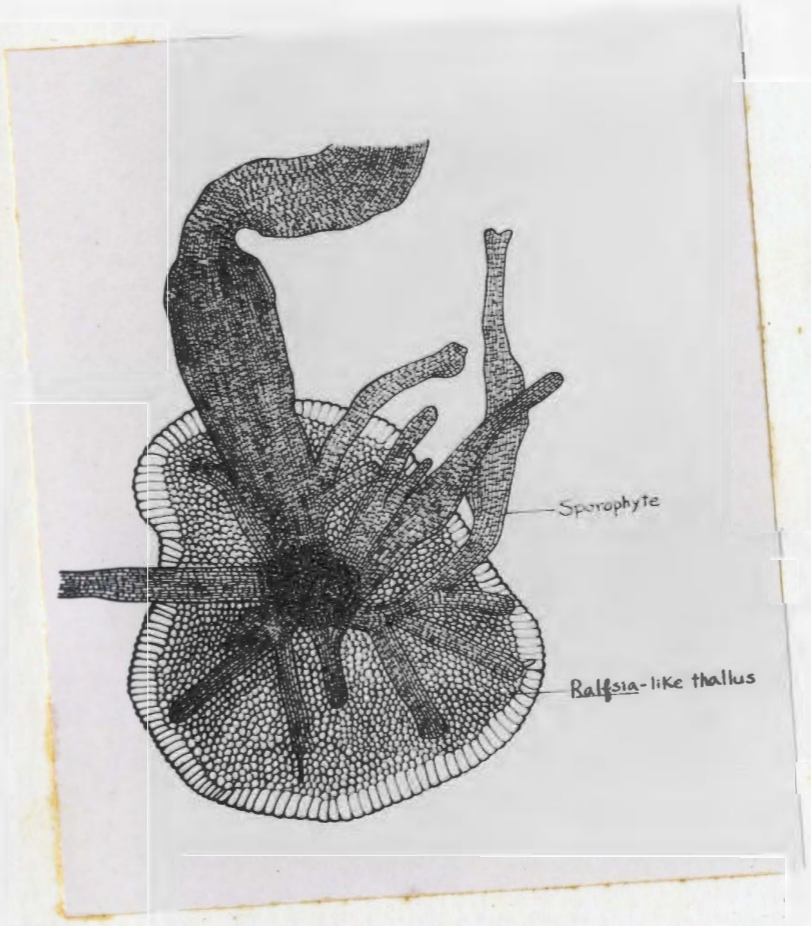

Fig. 1. A Ralfsia-like thallus of P. fascia, showing many sporophytes growing out from the central region, x 500.

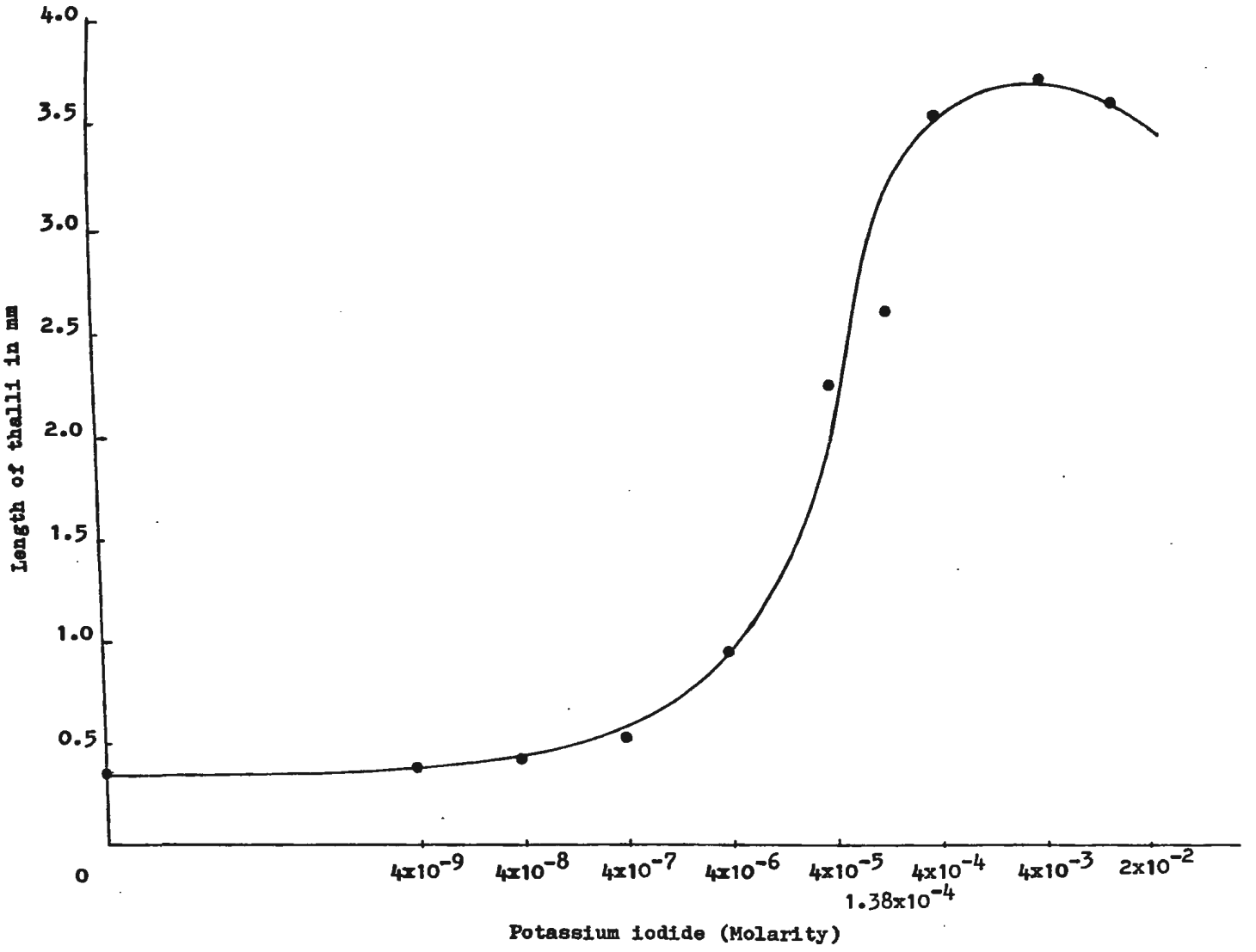

Fig. 2. Influence of varying iodide concentrations on the growth of Petalonia fascia as expressed by the length of the thalli.

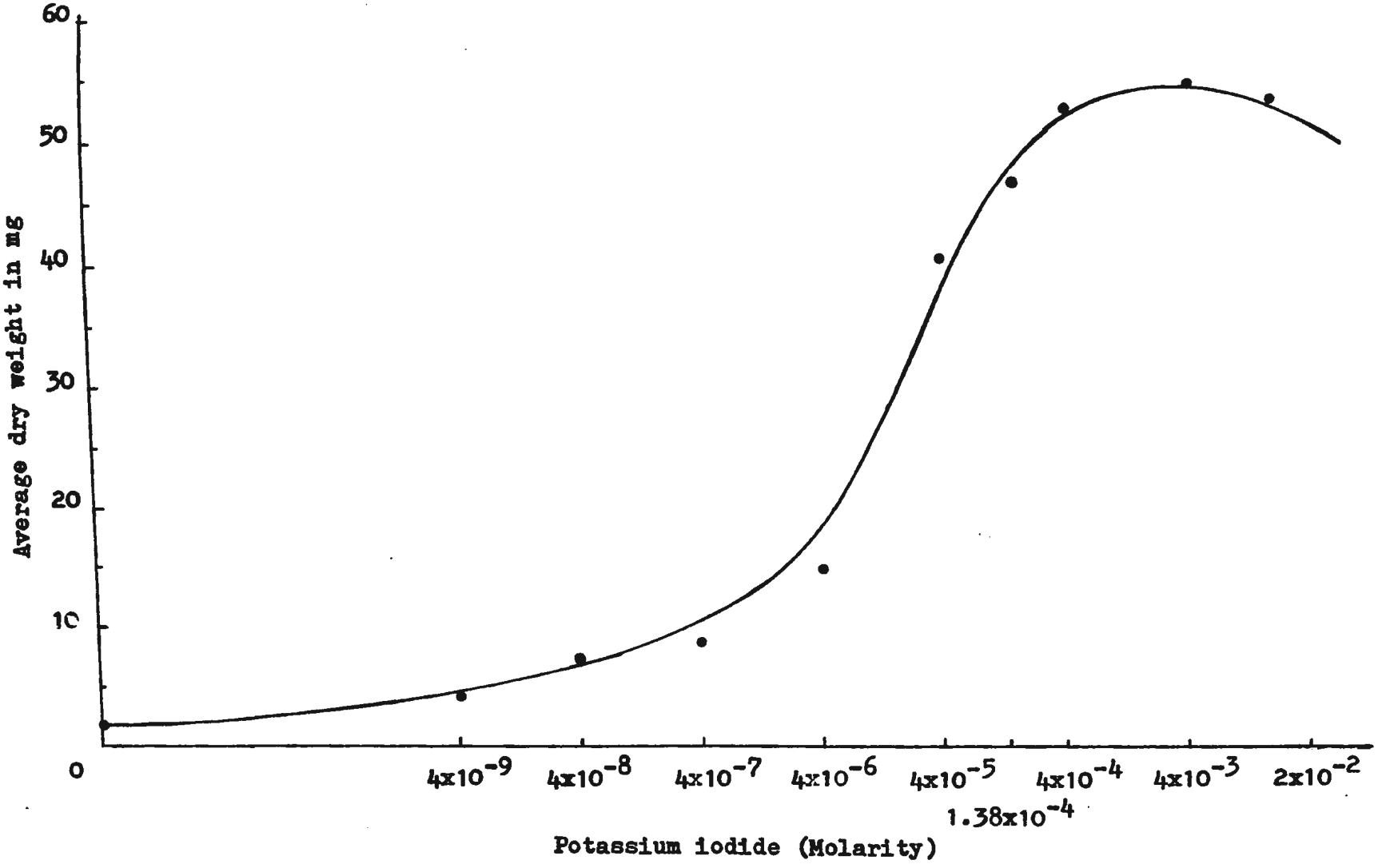

Fig. 3. Influence of varying iodide concentrations on the growth of Petalonia fascia as expressed by dry weight.

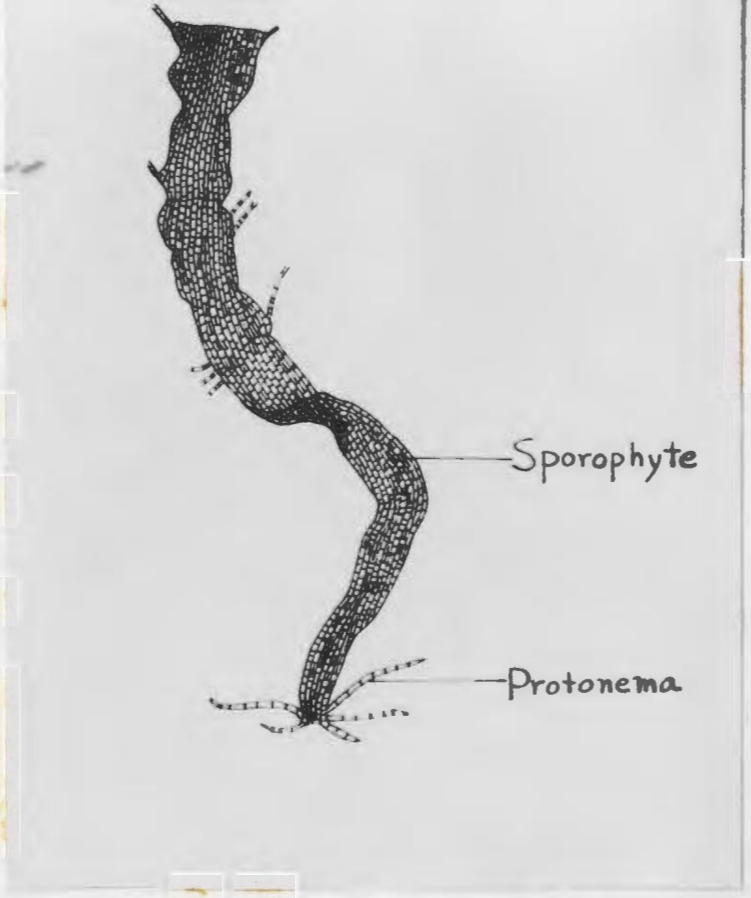

Fig. 4. A short filament of a protonema with a young sporophyte in the iodide series medium 10, x 500.

Fig. 5. A long, creeping and branching filament of a protonema producing a young sporophyte in the iodide series medium 10, x 500.

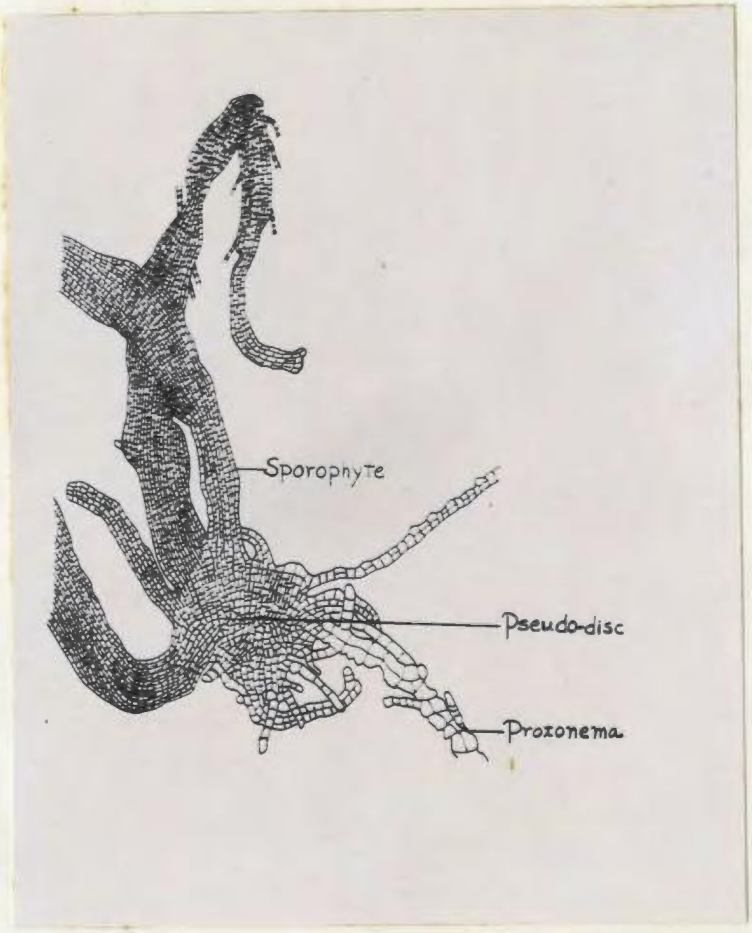

Fig. 6. A pseudodiscoid protonema giving rise to many sporophytes in iodide medium 7, x 510.

Fig. 7. Portion of the fertile filamentous protonemata, showing plurilocular sporangia, grown in iodide series medium 7, x 500.

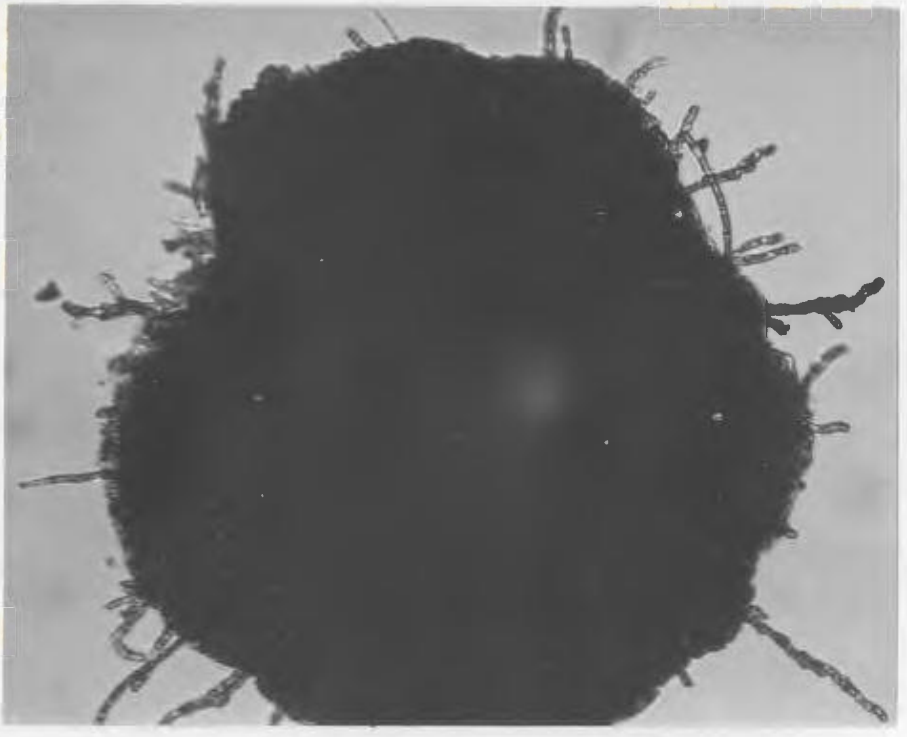

Fig. 8. Clumped plethysmothalli, grown in iodide series medium 9, showing radiating filaments, x 215.

Fig. 9. A plethysmothallus squashed from clumped plethysmothalli, showing irregular branches, x 220.

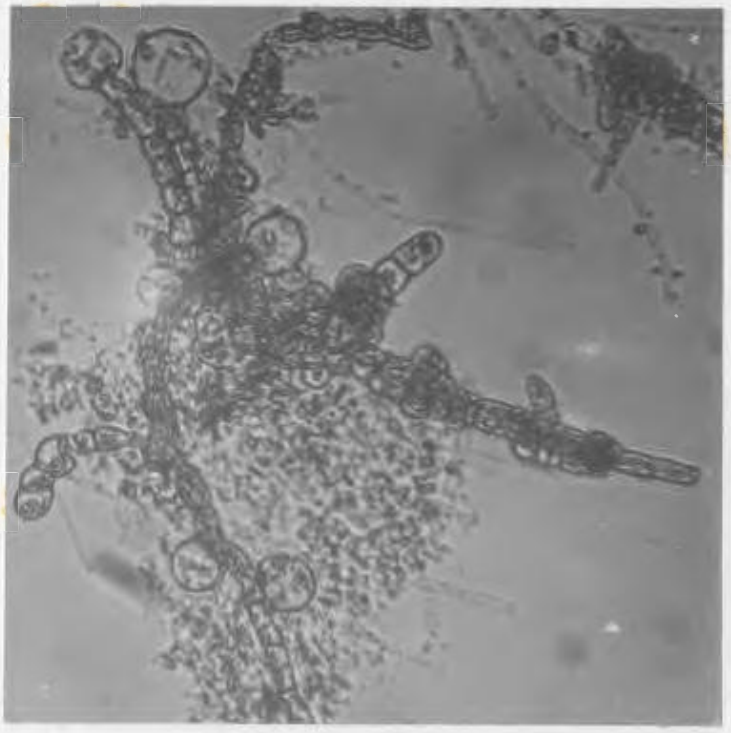

Fig. 10. Portion of the fertile branches of plethysmothalli, showing unilocular sporangia in iodide series medium 8, x 500.

Fig. 11. A plethysmothallus with a number of sporophytes in iodide series medium 9, x 210.

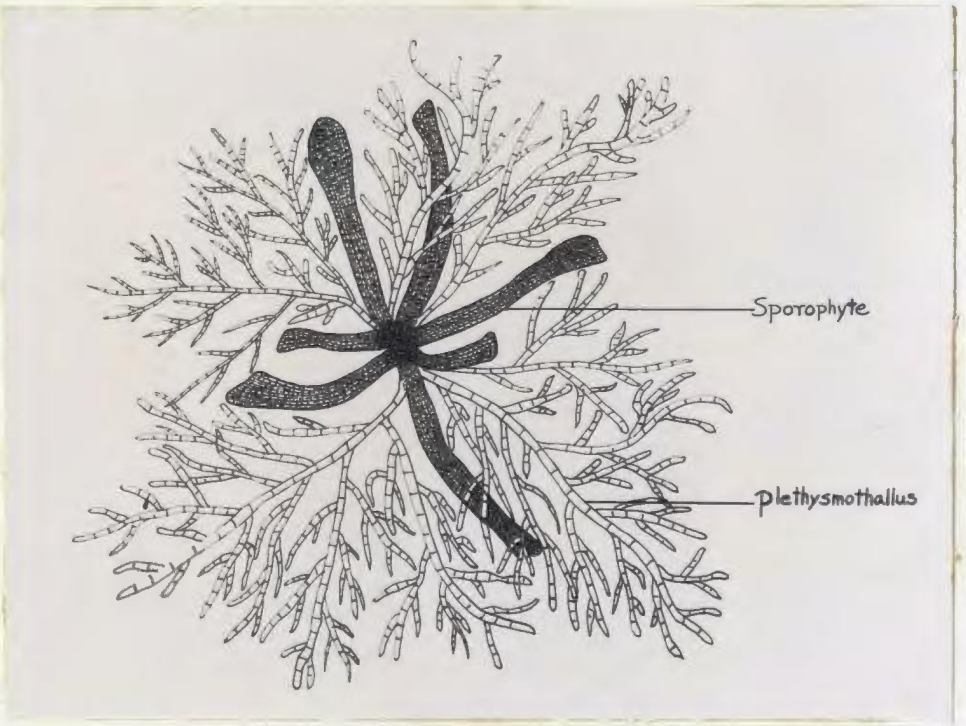

Fig. 12. A cluster of new sporophytes growing out from the plethysmothallus in iodide series medium 8, x 200.

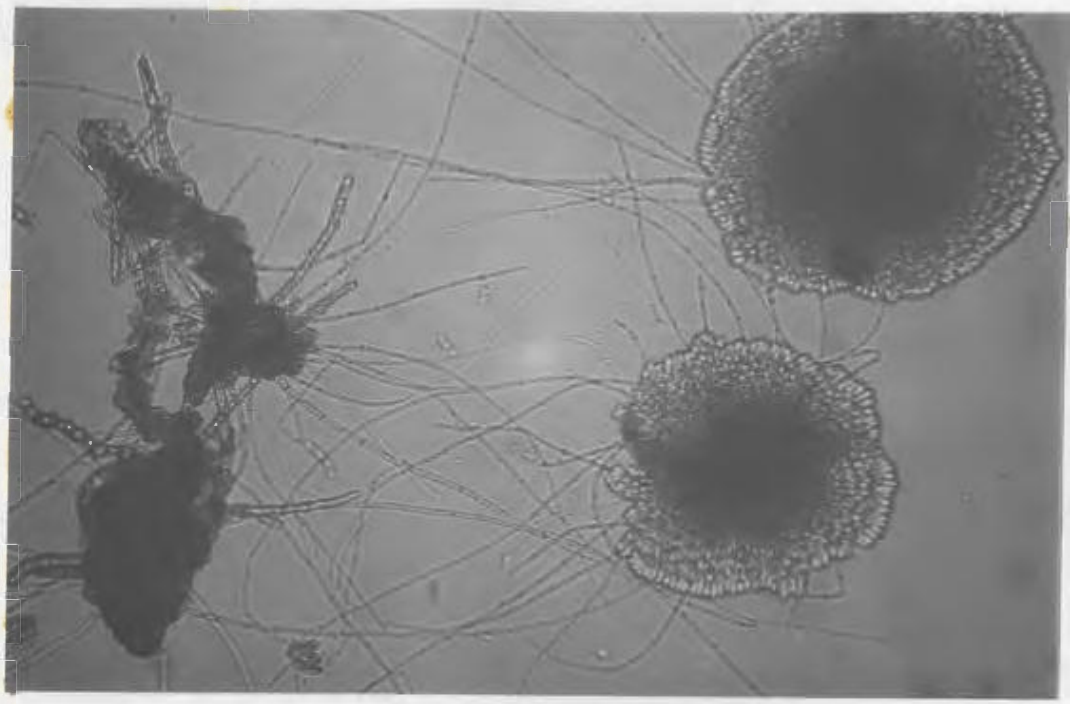

Fig. 13. Two young pseudodiscoid protonemata (left) and two young Ralfsia-like thalli (right) developed from the zoospores of Ralfsia-like thalli, grown in iodide series medium 7, x 500.

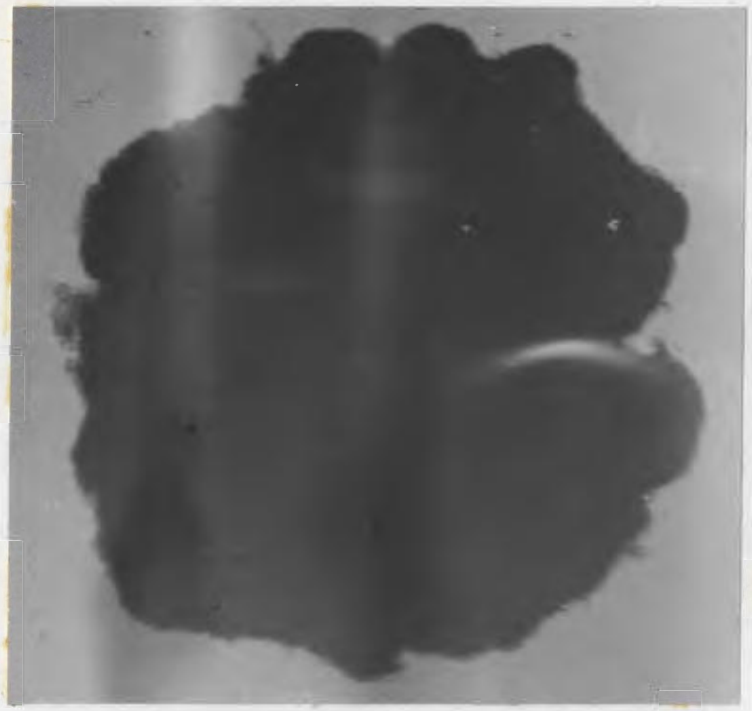

Fig. 14. A hard black sphere, grown in iodide series medium 10, x 210.

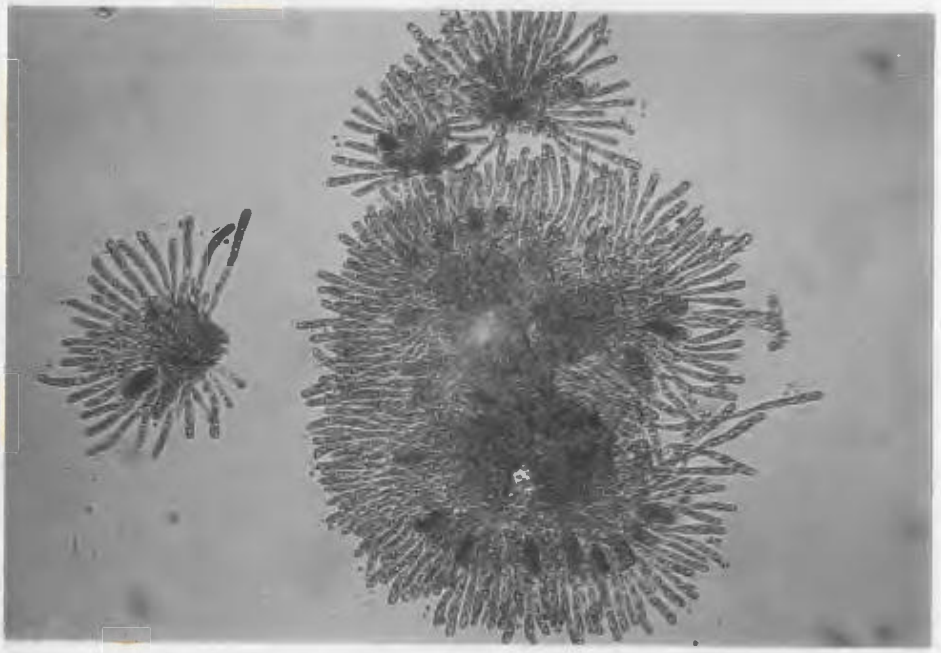

Fig. 15. Fertile Ralfsia-like thalli, showing unilocular sporangia in iodide series medium 10, x 165.

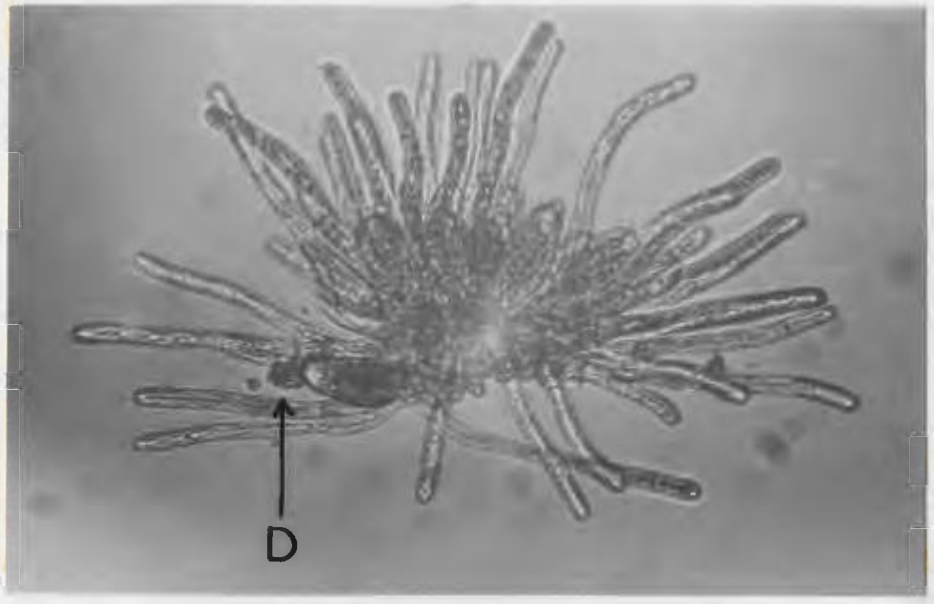

Fig. 16. The unilocular sporangia of a Ralfsia-like thallus, showing the zoospores being discharged (D) in iodide series medium 10, x 500.

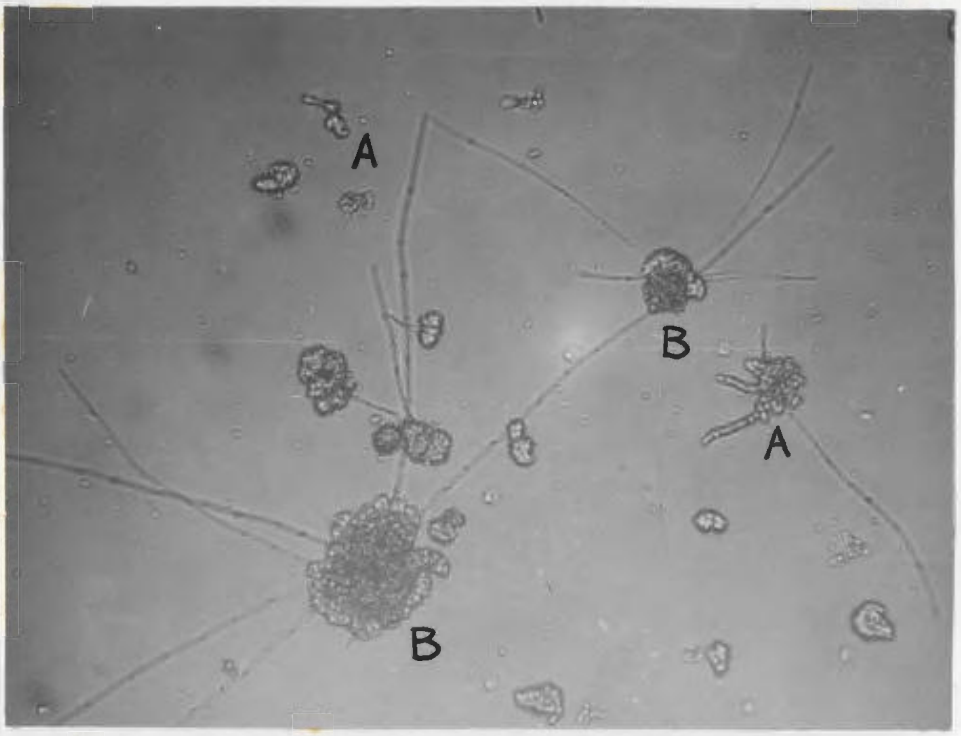

Fig. 17. Young protonemata (A) and Ralfsia-like thalli (B) developed from zoospores from Ralfsia-like thalli, x 500.

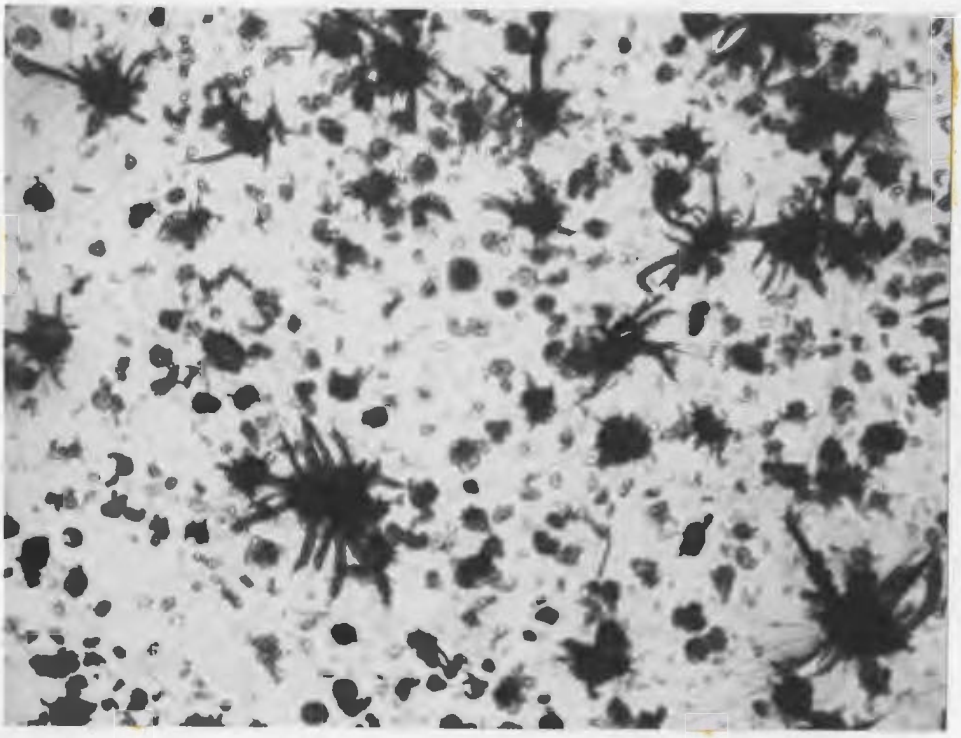

Fig. 18. The zoospores from Ralfsia-like thalli cultivated in iodide series medium 10, x 200.

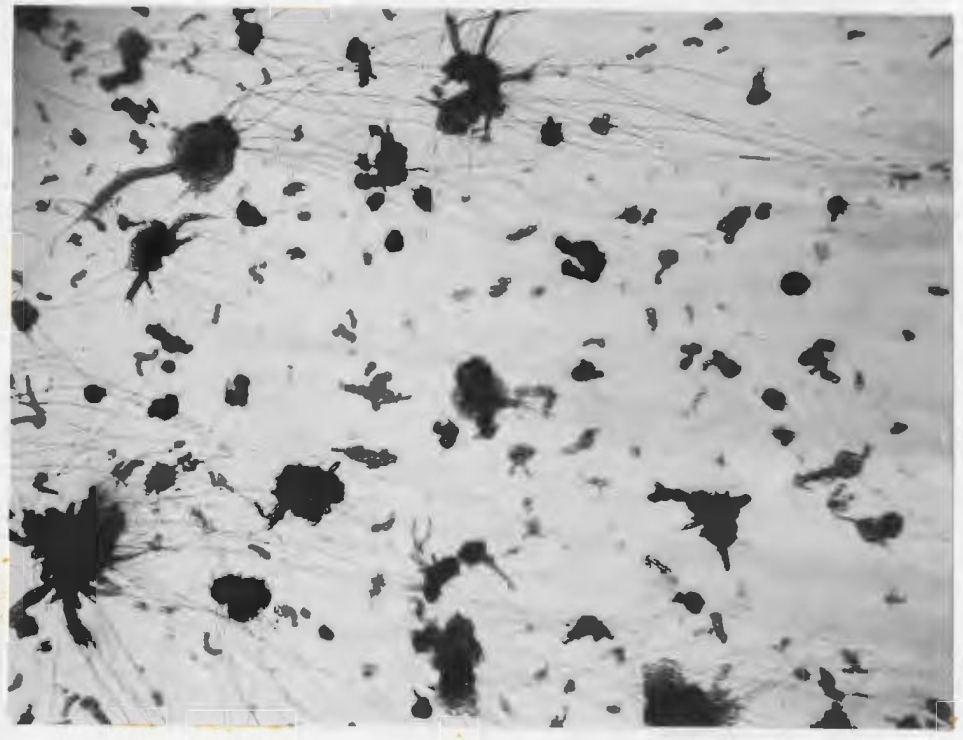

Fig. 19. The zoospores from Ralfsia-like thalli grown in iodide series medium 7, x 200.

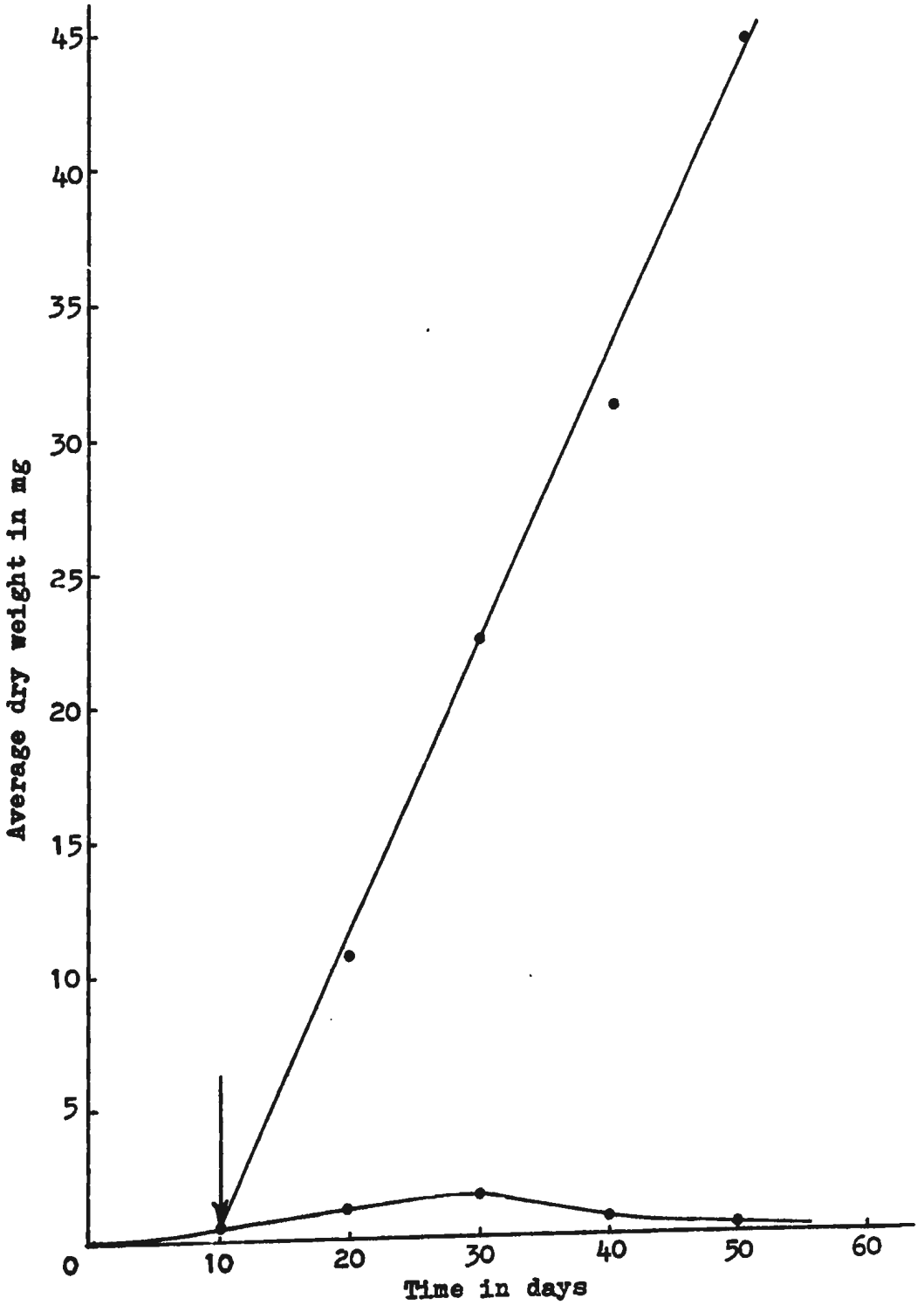

Fig. 20. Effect of added iodide on the growth of iodide-deficient Petalonia fascia cultures. At the time indicated by the arrow, 23 mg KI/L were added.

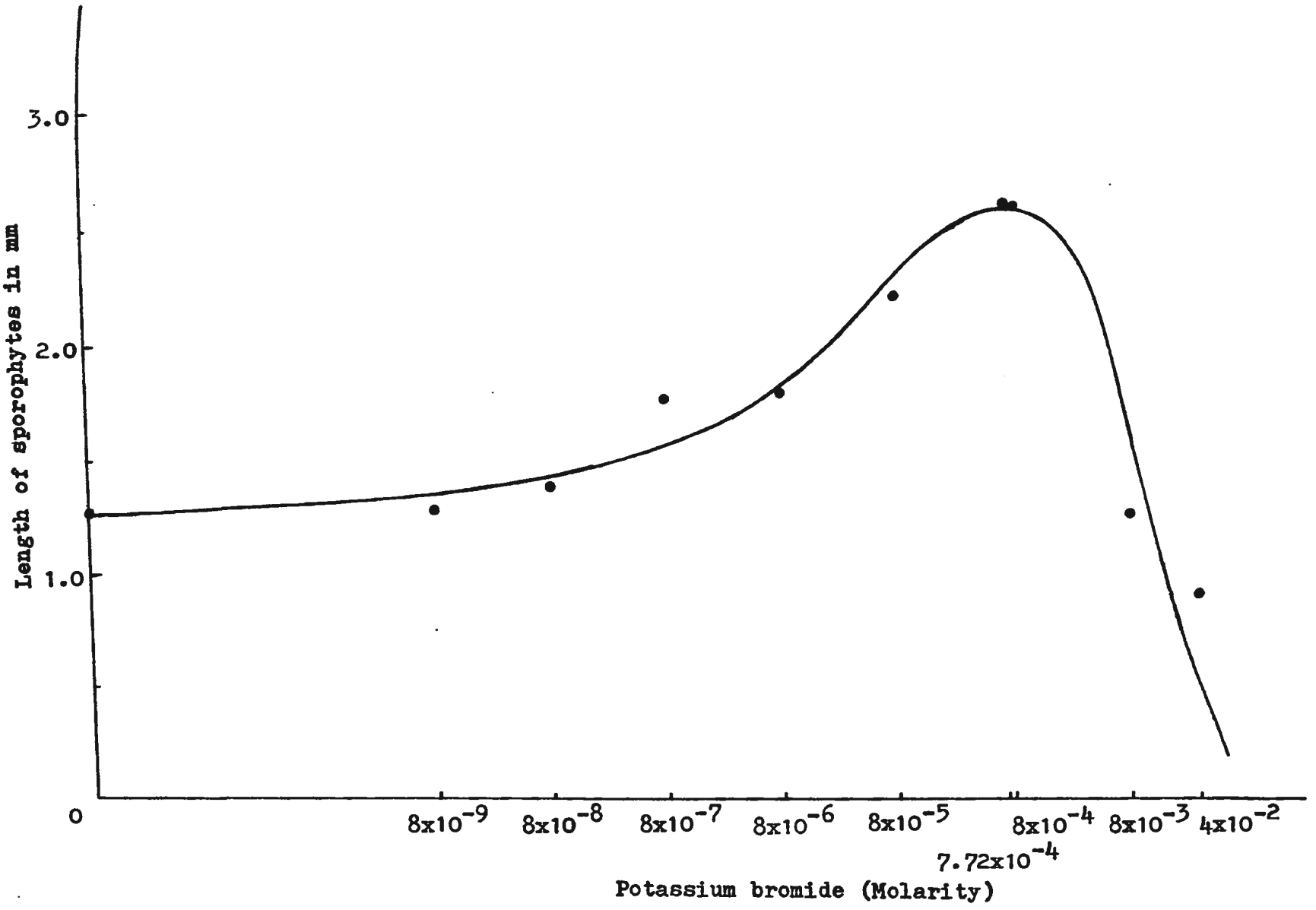

Fig. 21. Effect of varying concentrations of bromide on the growth of Petalonia fascia as expressed by the length of sporophytes.

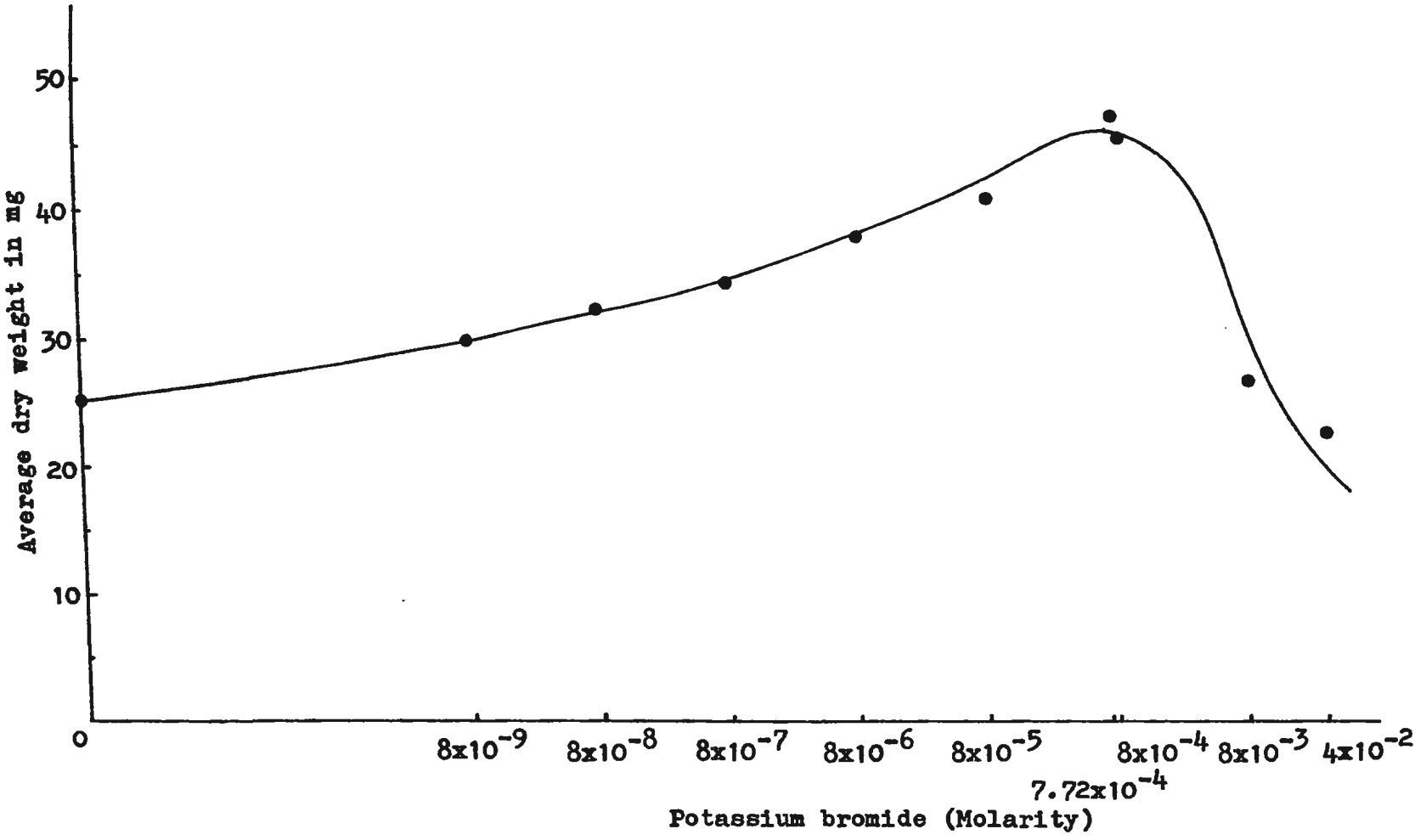

Fig. 22. Effect of varying concentrations of bromide on the growth of Petalonia fascia as expressed by dry weight.

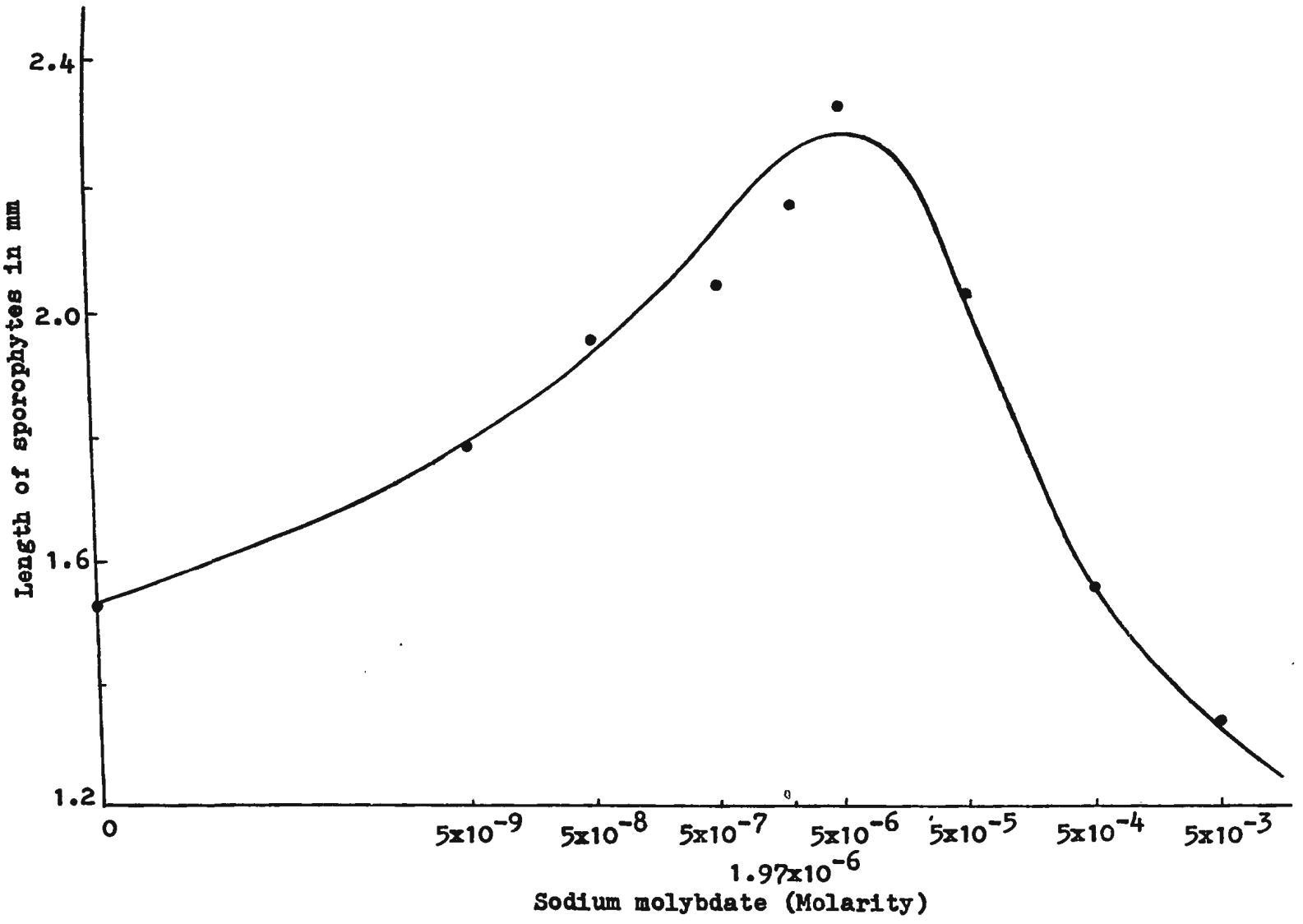

Fig. 23. Effect of varying concentrations of molybdenum on the growth of Petalonia fascia as expressed by length of sporophytes.

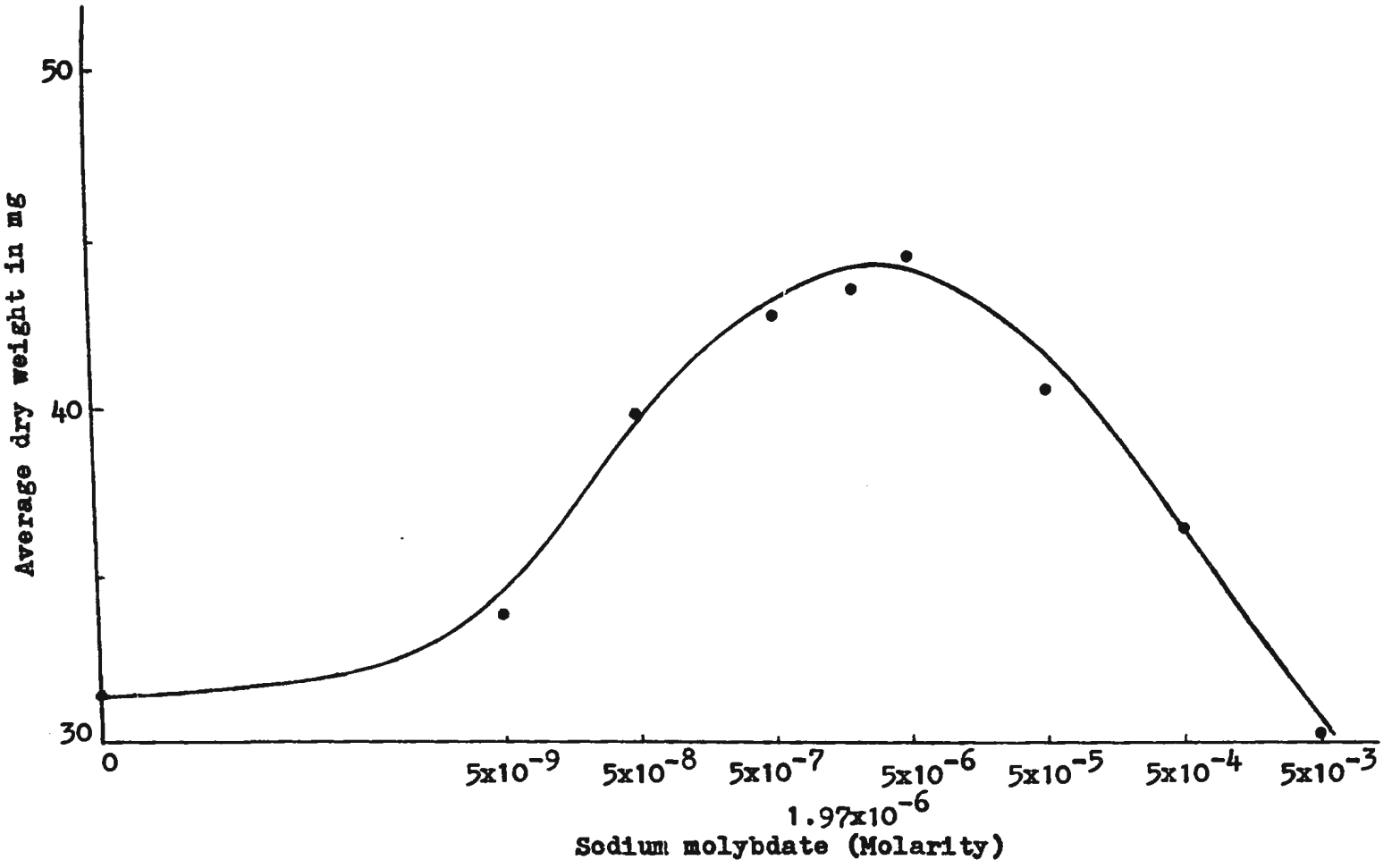

Fig. 24. Effect of varying concentrations of molybdenum on the growth of Petalonia fascia as expressed by dry weight.

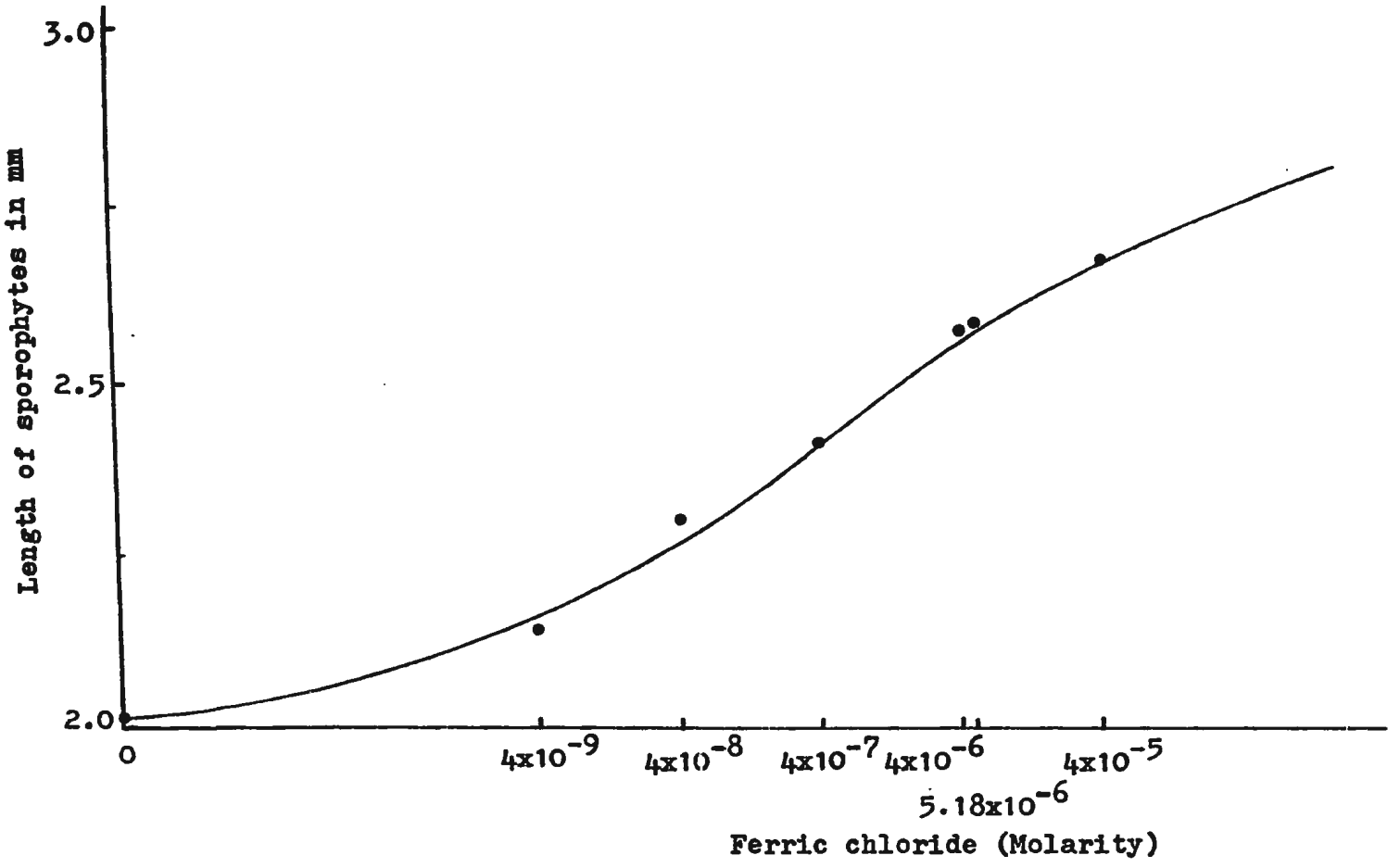

Fig. 25. Effect of varying iron concentrations on the growth of Petalonia fascia as expressed by length of sporophytes.

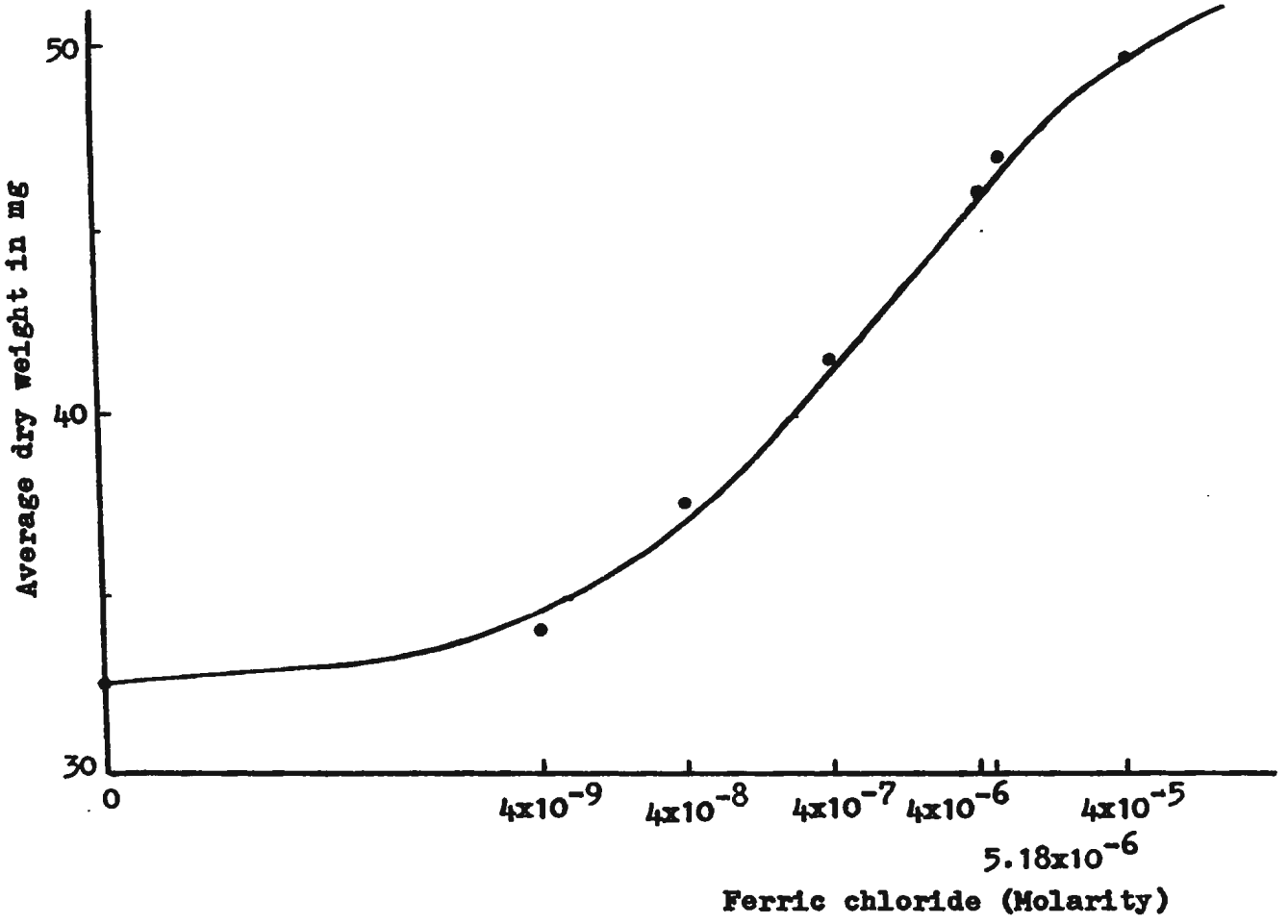

Fig. 26. Effect of varying iron concentrations on the growth of Petalonia fascia as expressed by dry weight.

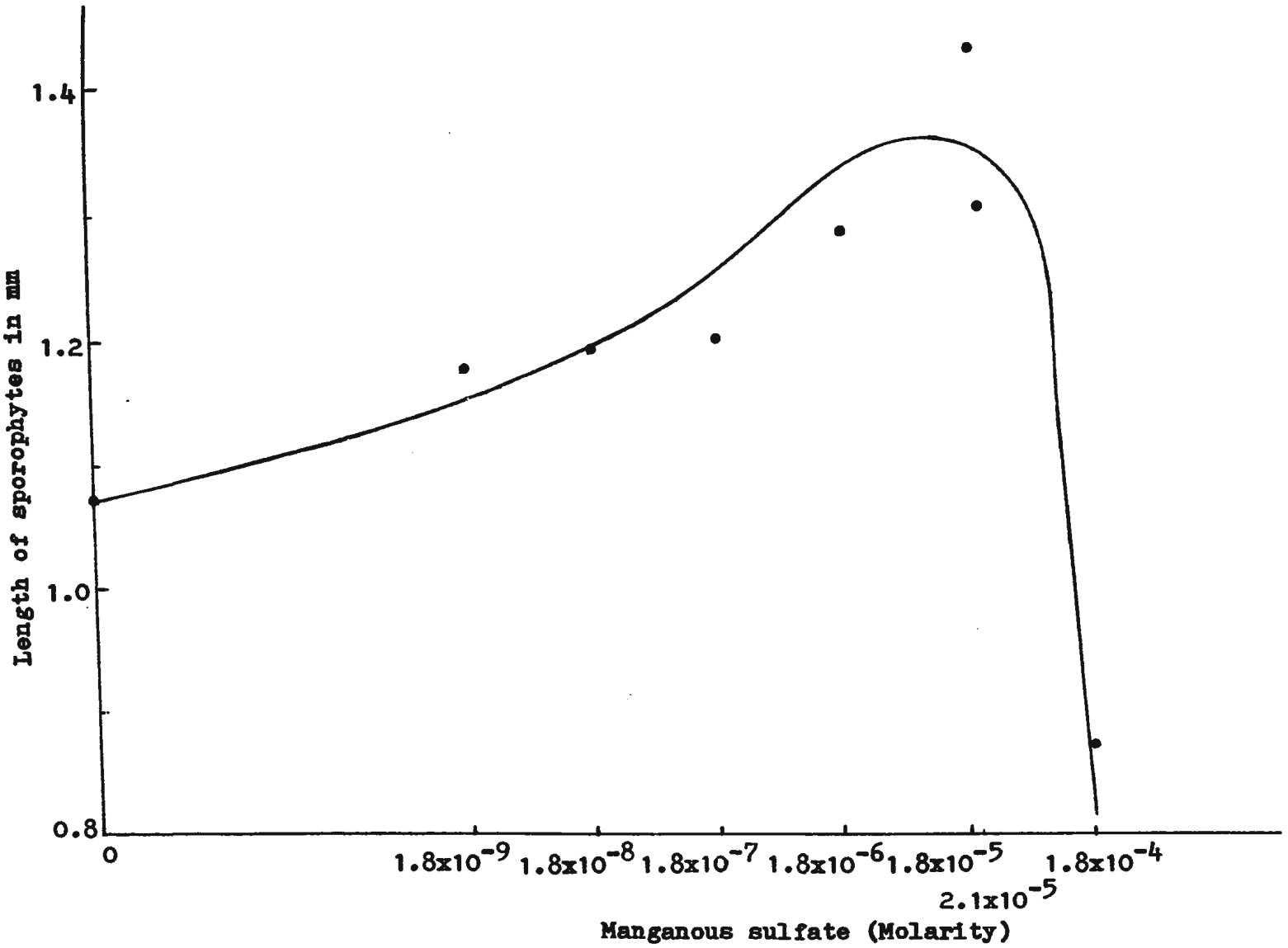

Fig. 27. Effect of varying manganese concentrations on the growth of Petalonia fascia as expressed by length of sporophytes.

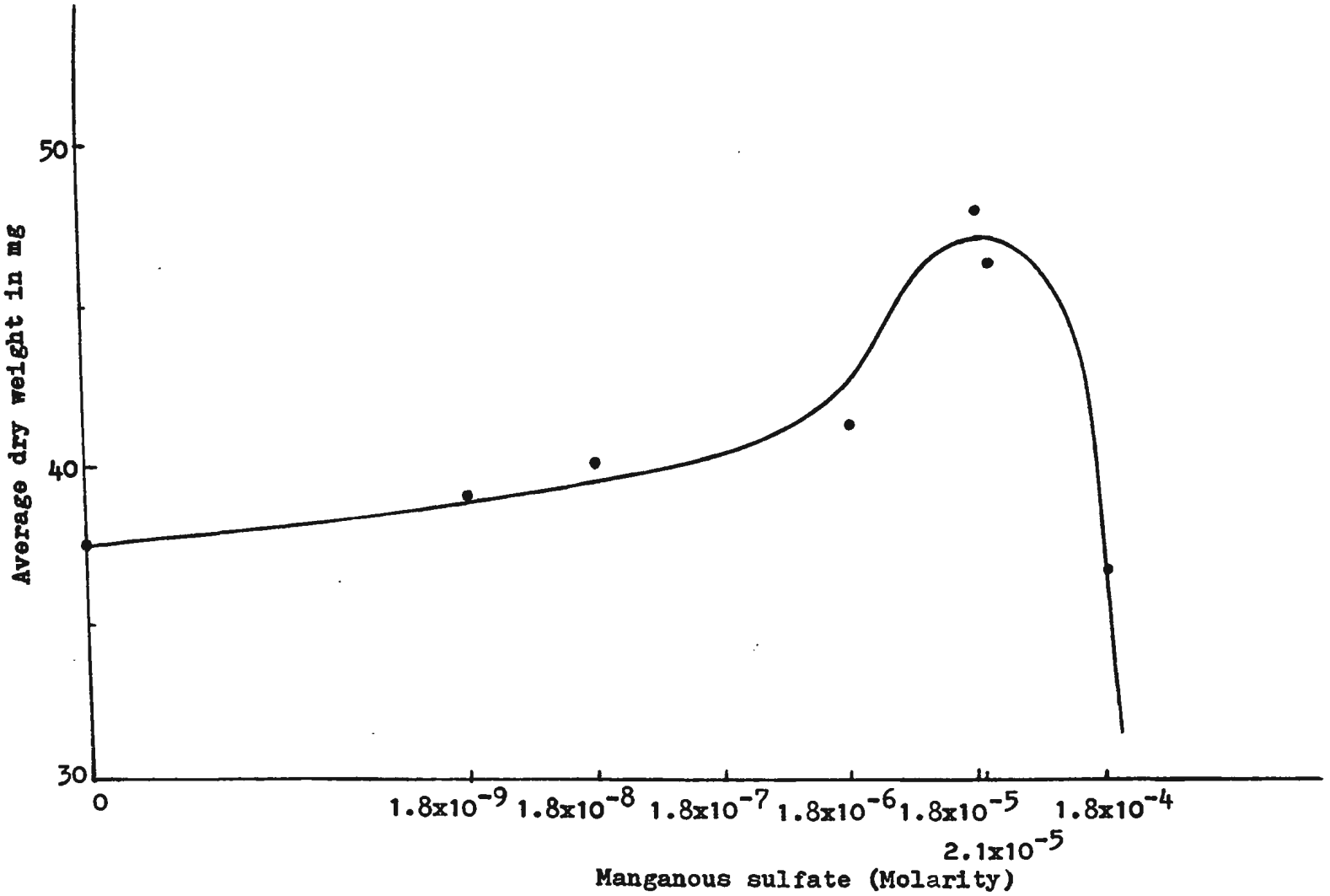

Fig. 28. Effect of varying manganese concentrations on the growth of Petalonia fascia as expressed by dry weight.

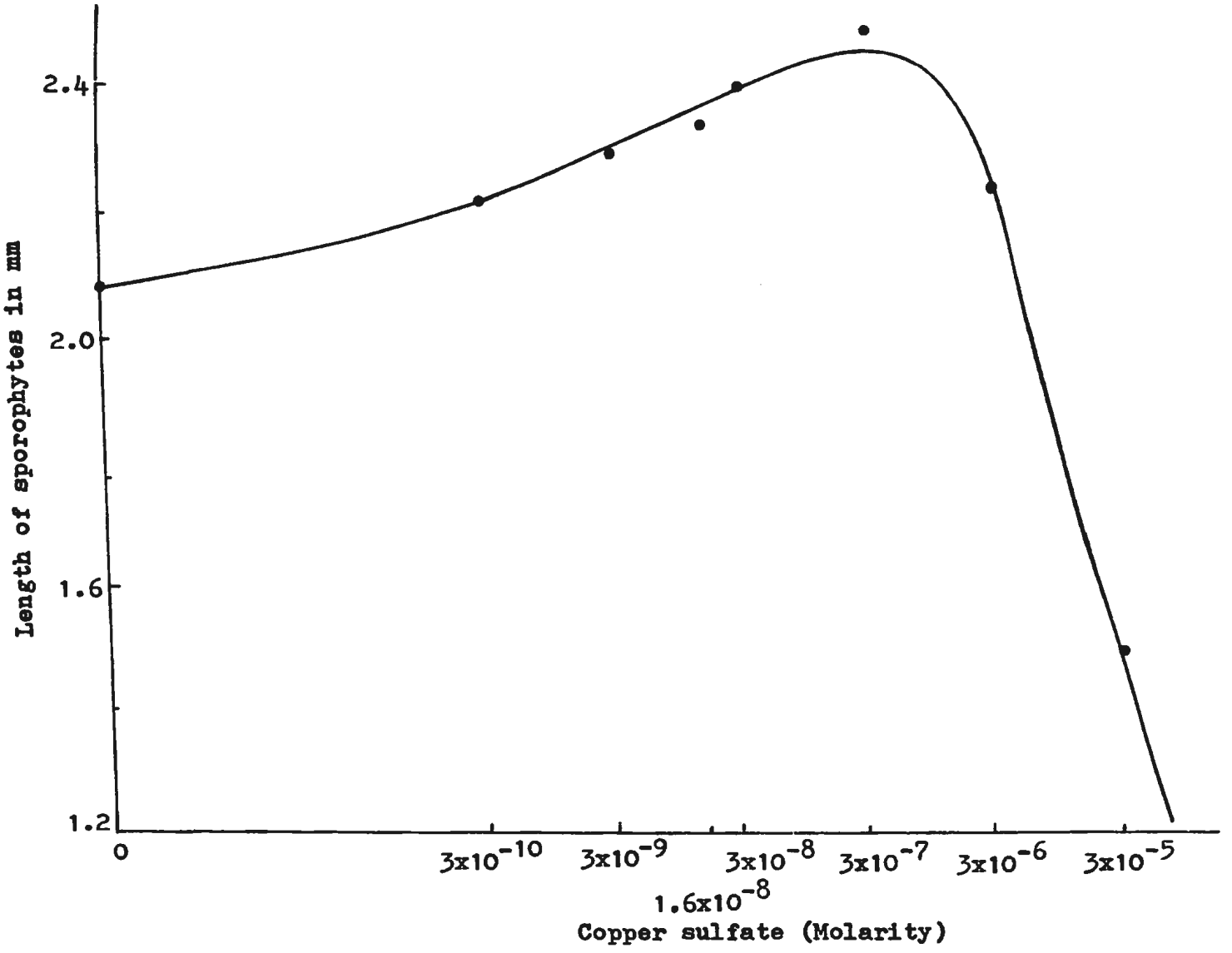

Fig. 29. Effect of varying concentrations of copper on the growth of Petalonia fascia as expressed by length of sporophytes.

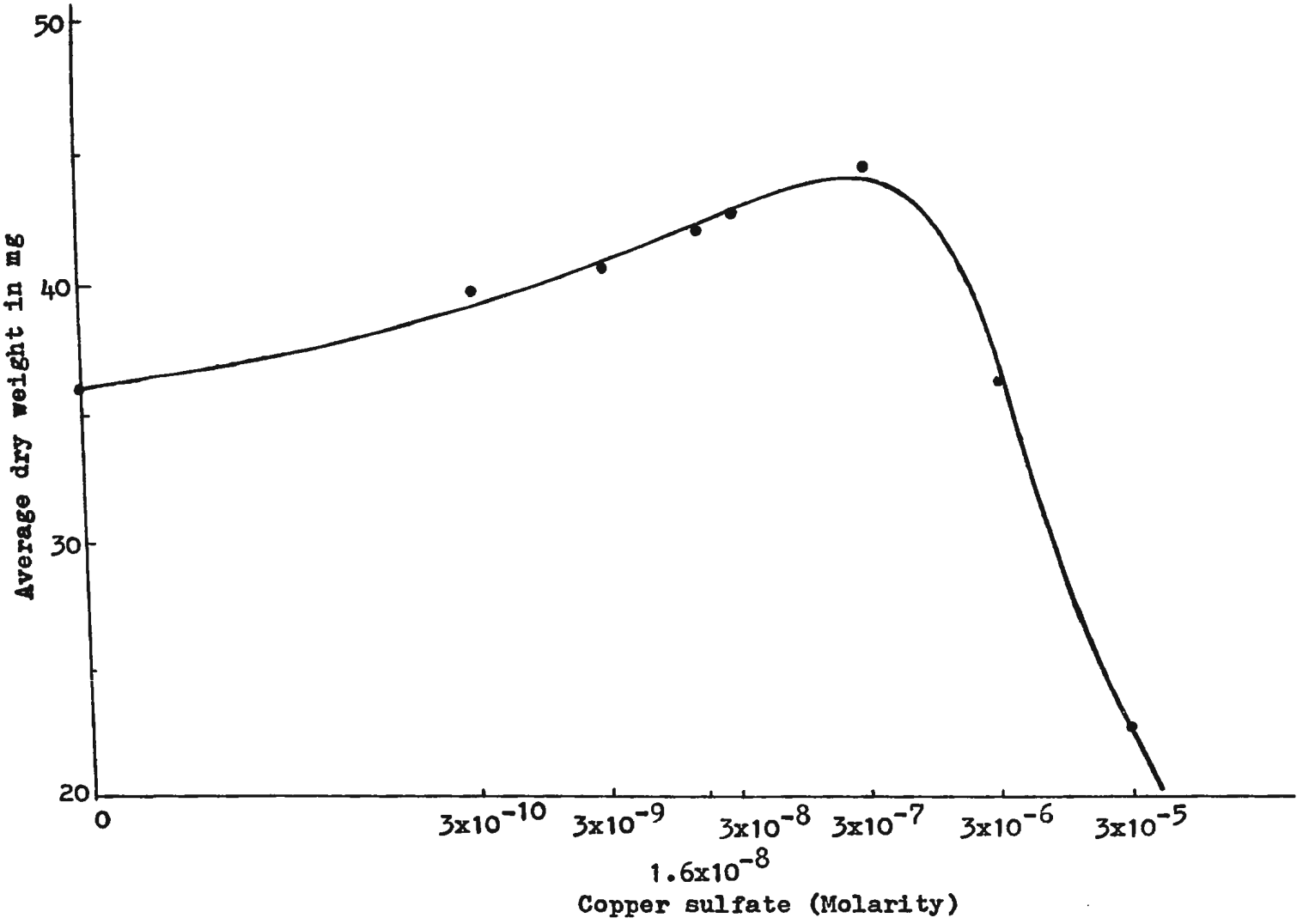

Fig. 30. Effect of varying concentrations of copper on the growth of Petalonia fascia as expressed by dry weight.

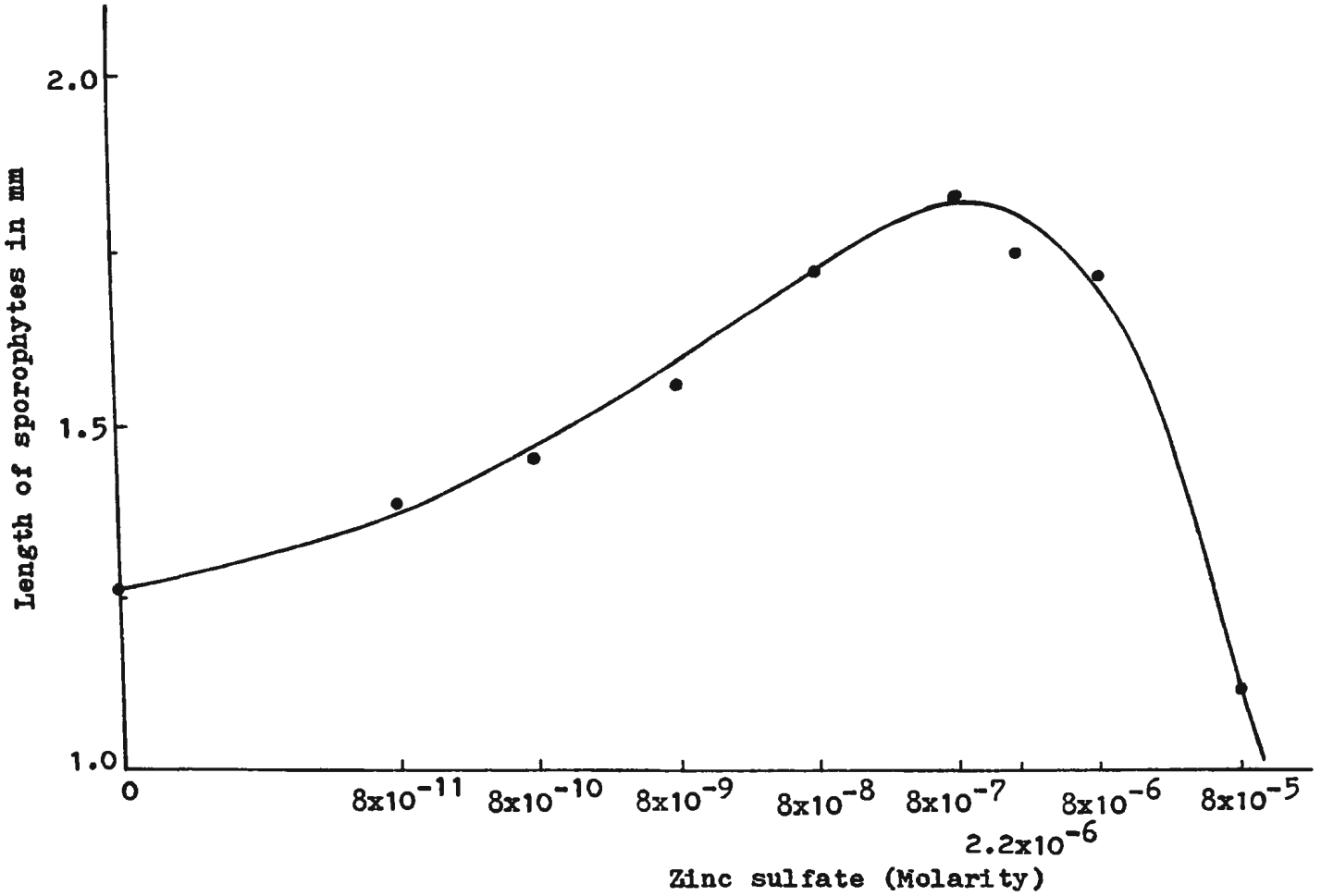

Fig. 31. Effect of varying concentrations of zinc on the growth of Petalonia fascia as expressed by length of sporophytes.

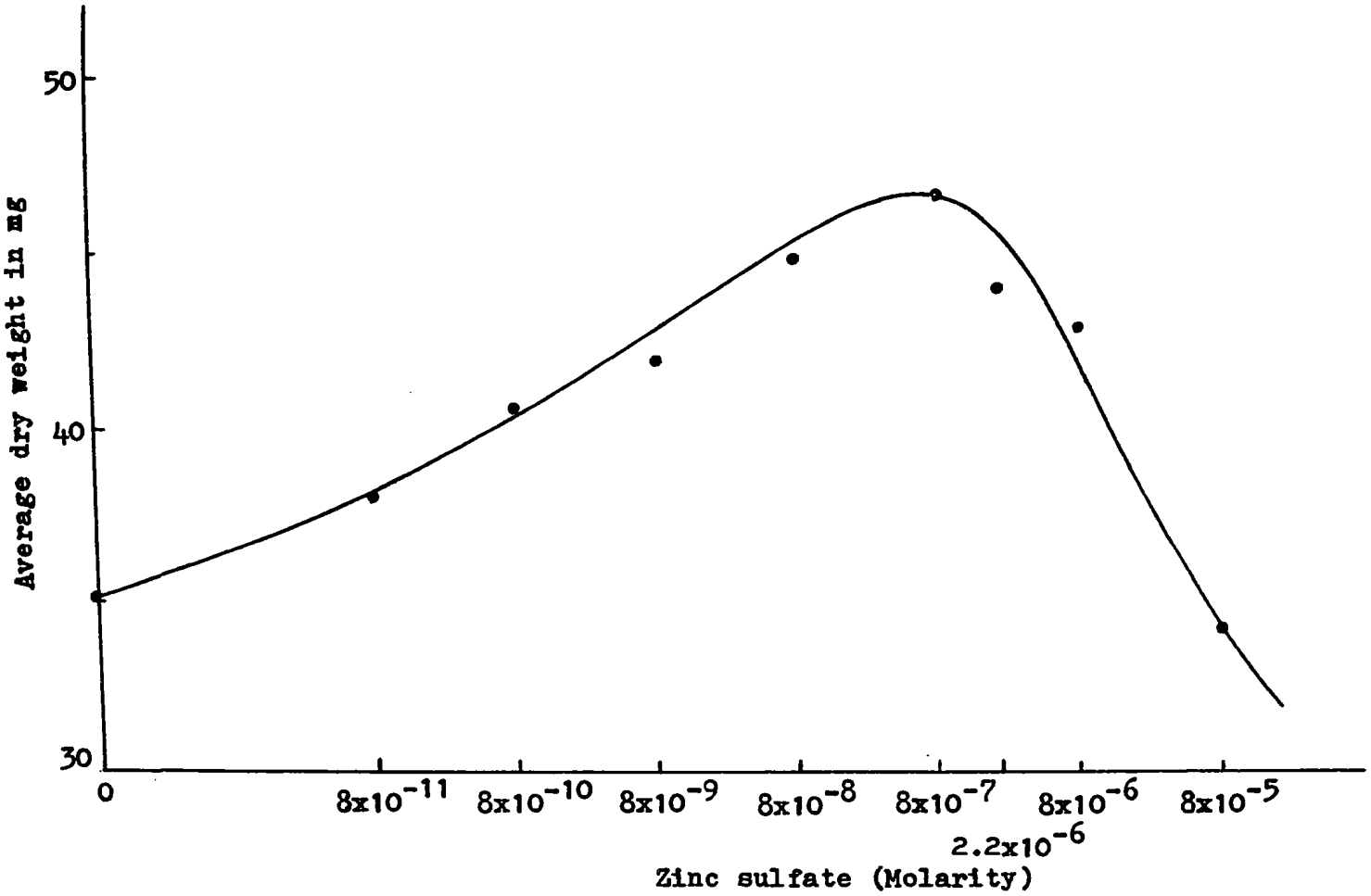

Fig. 32. Effect of varying concentrations of zinc on the growth of Petalonia fascia as expressed by dry weight.

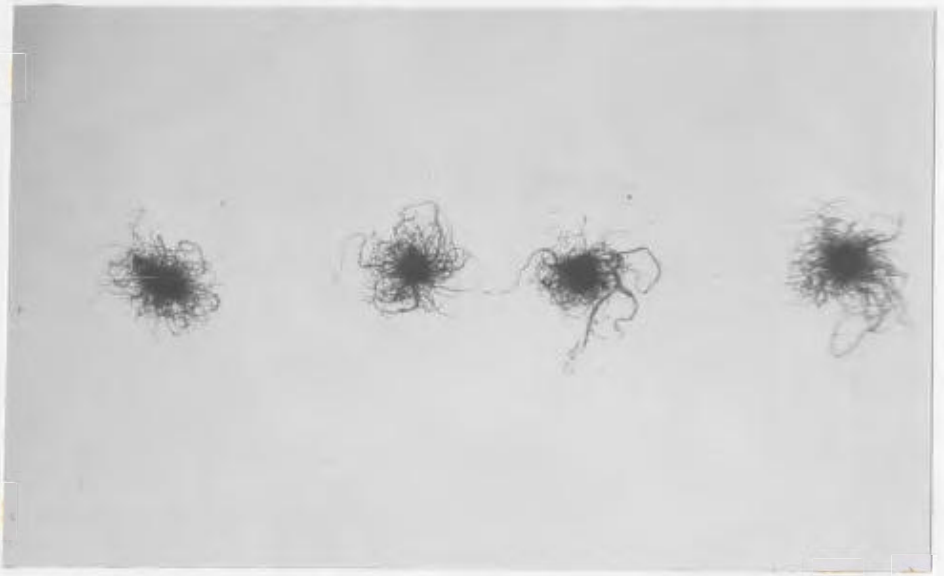

Fig. 33. Sporophytes grown in Zn-deficient Petalonia medium, x 4.

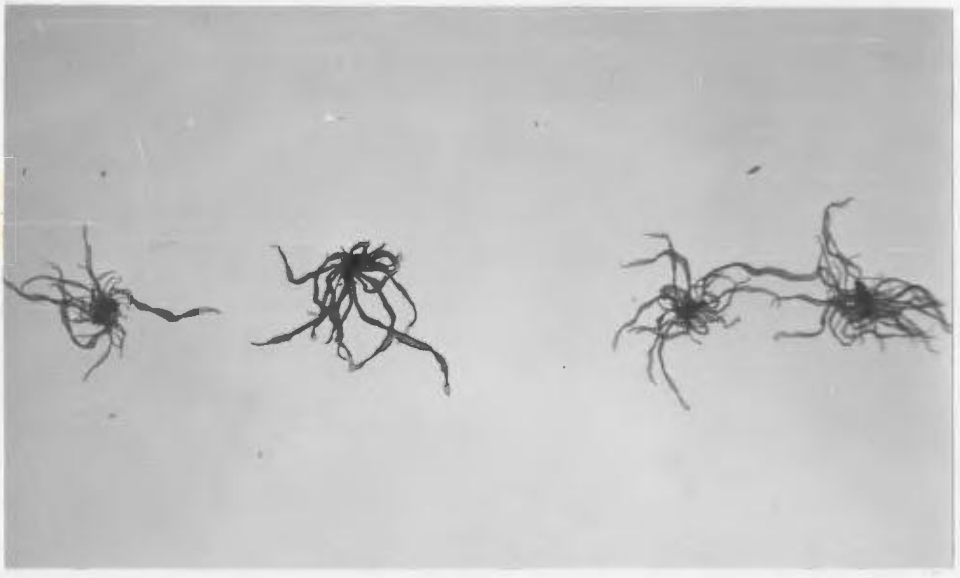

Fig. 34. Sporophytes cultivated in Zn-sufficient Petalonia medium, x 4.

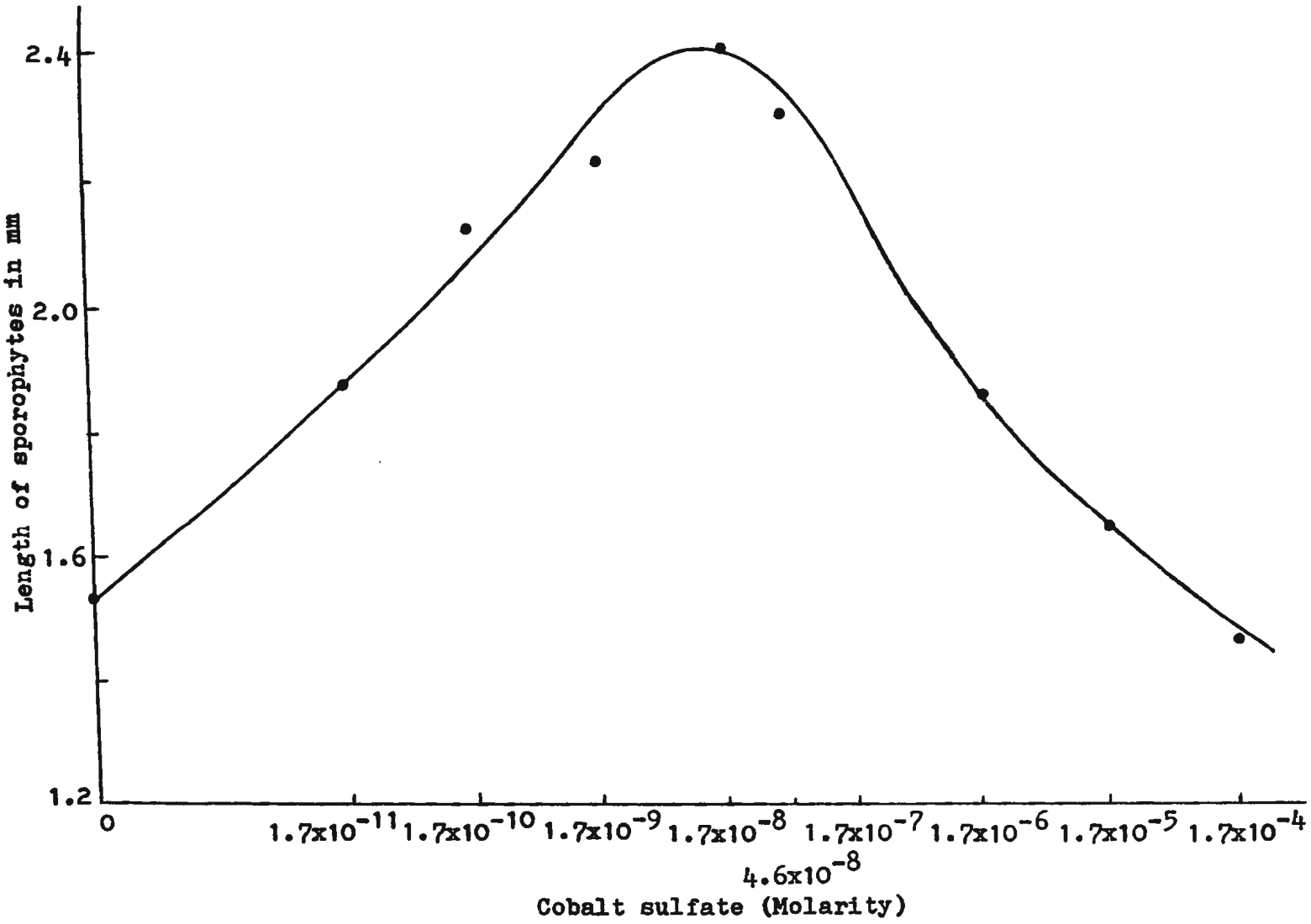

Fig. 35. Effect of varying cobalt concentrations on the growth of Petalonia fascia as expressed by length of sporophytes.

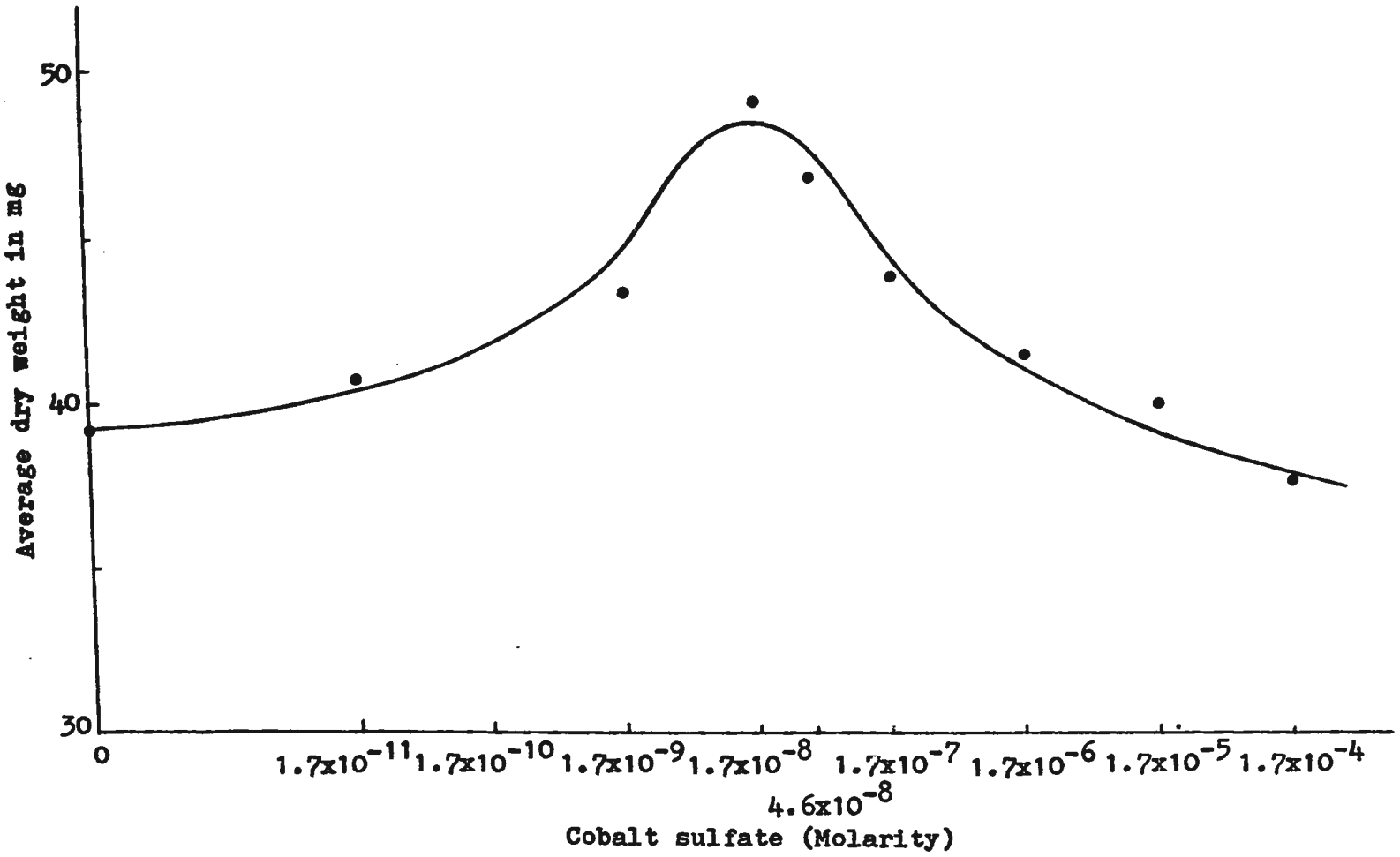

Fig. 36. Effect of varying cobalt concentrations on the growth of Petalonia fascia as expressed by dry weight.

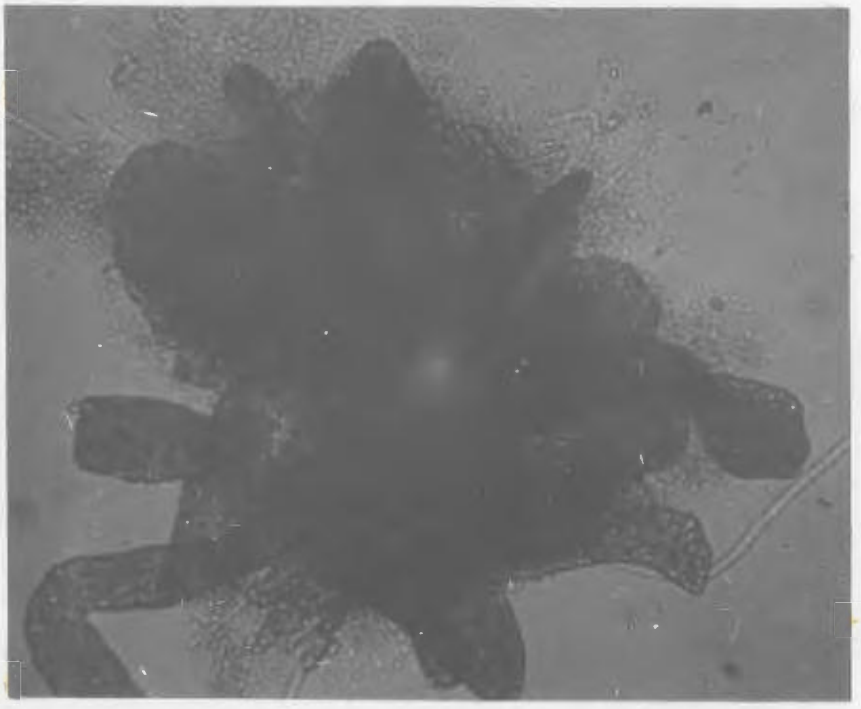

Fig. 37. Sporophytes grown in zinc-sufficient Petalonia medium (52.3 µg Zn/L) with 400 µg IAA/L, showing disintegration and swelling, x 210.

Fig. 38. Sporophytes cultivated in zinc-sufficient Petalonia medium (52.3 µg Zn/L) with 200 µg IAA/L showing disintegration and swelling, x 210.

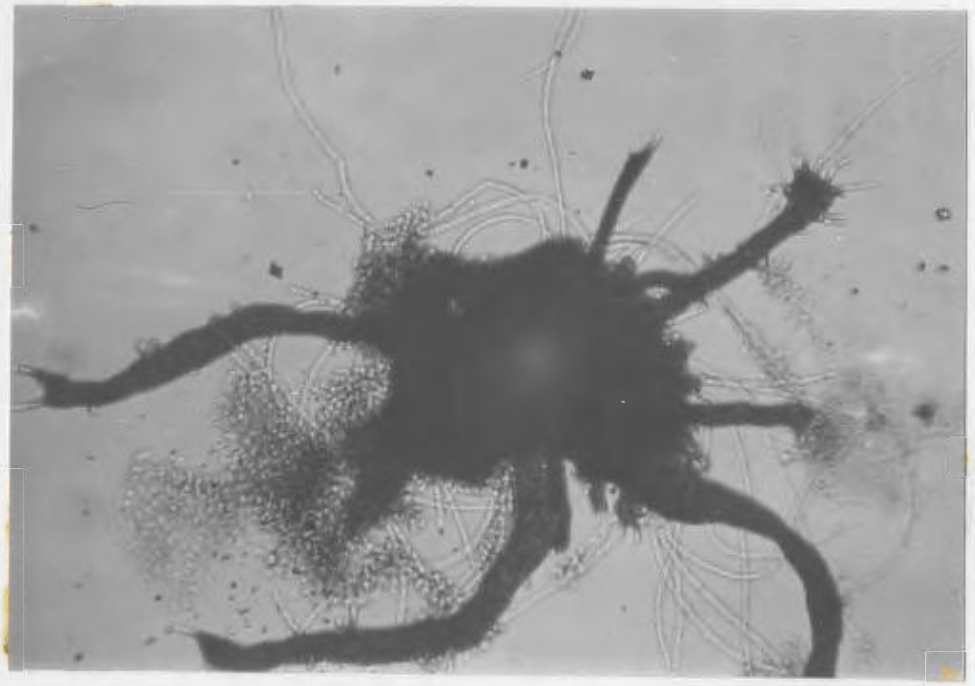

Fig. 39. Sporophytes grown in zinc-deficient Petalonia medium with 400 µg IAA/L, showing disintegration but no swelling, x 210.

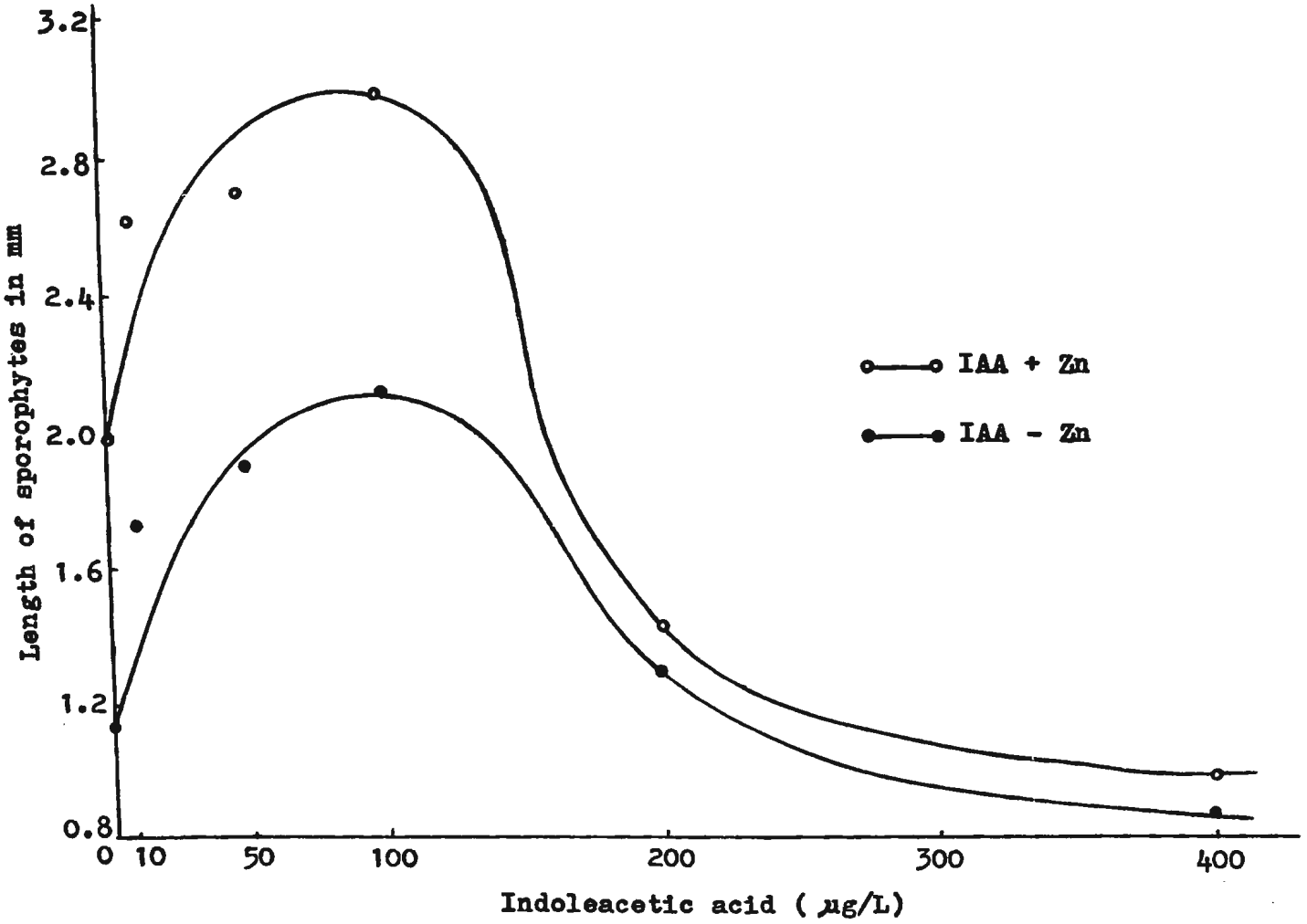

Fig. 40. Growth of Petalonia fascia with varying concentrations of indoleacetic acid (with and without zinc) as expressed by length of sporophytes.

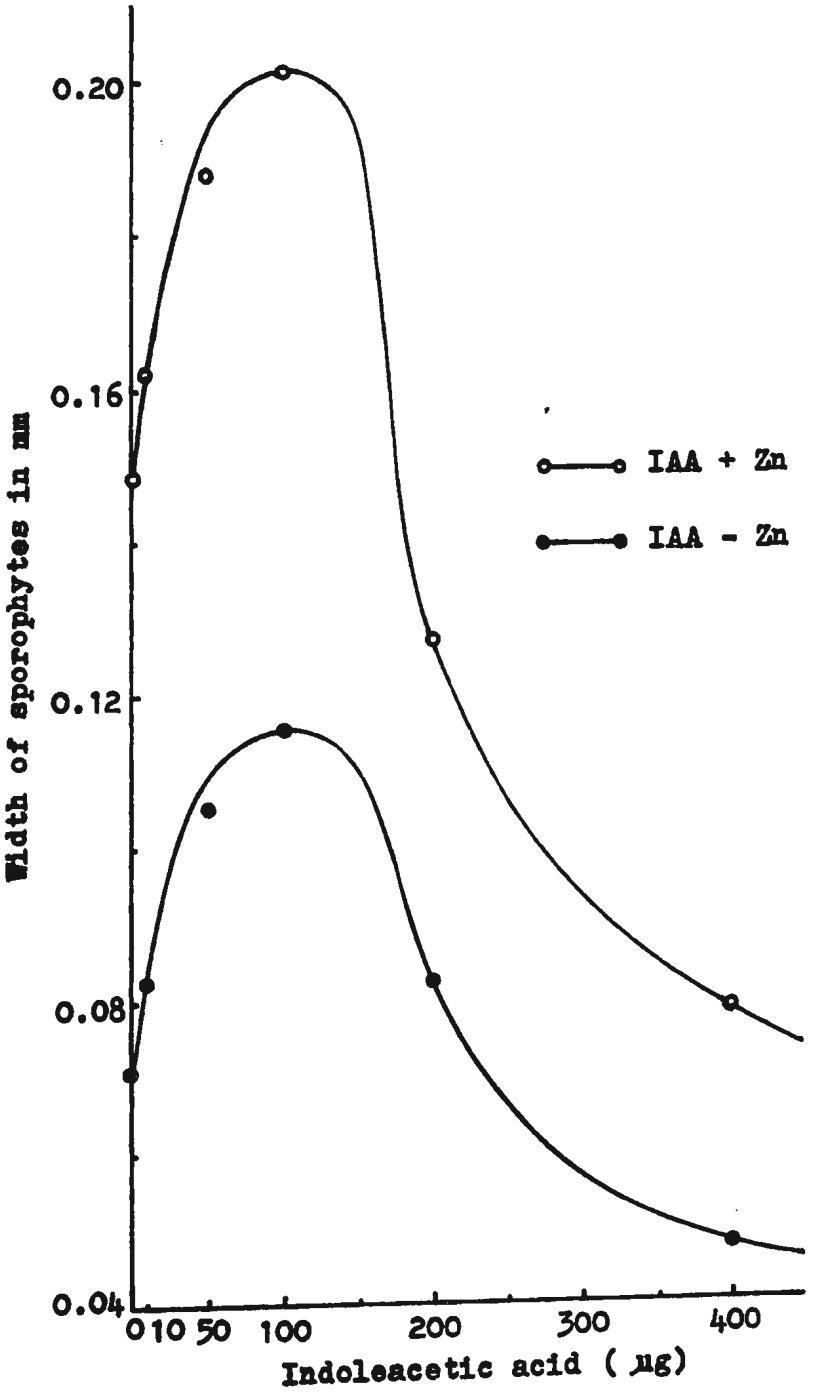

Fig. 41. Growth of Petalonia fascia with varying concentrations of indoleacetic acid (with and without zinc), as expressed by width of sporophytes.

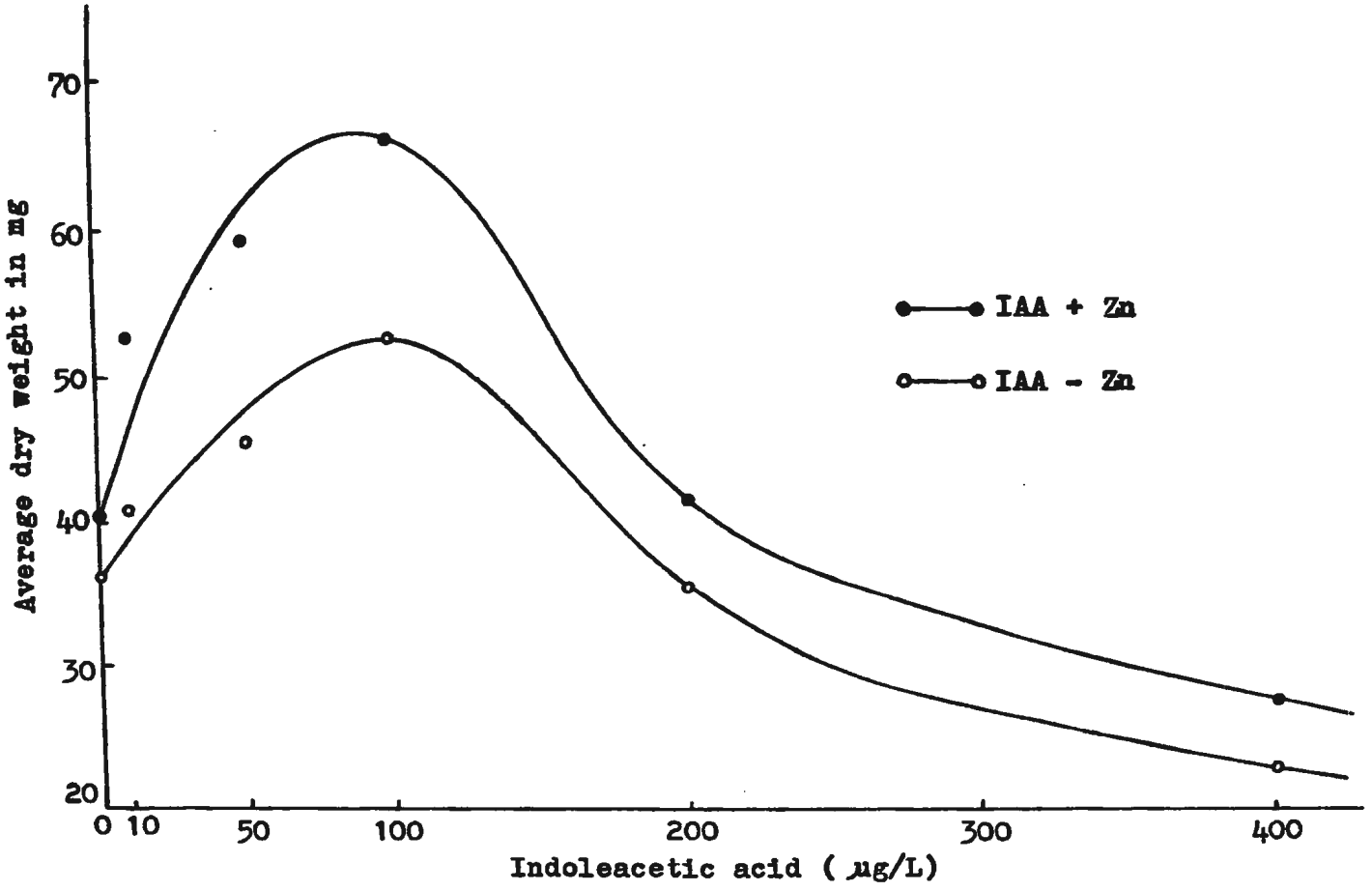

Fig. 42. Growth of Petalonia fascia with varying concentrations of indoleacetic acid (with and without zinc) as expressed by dry weight.

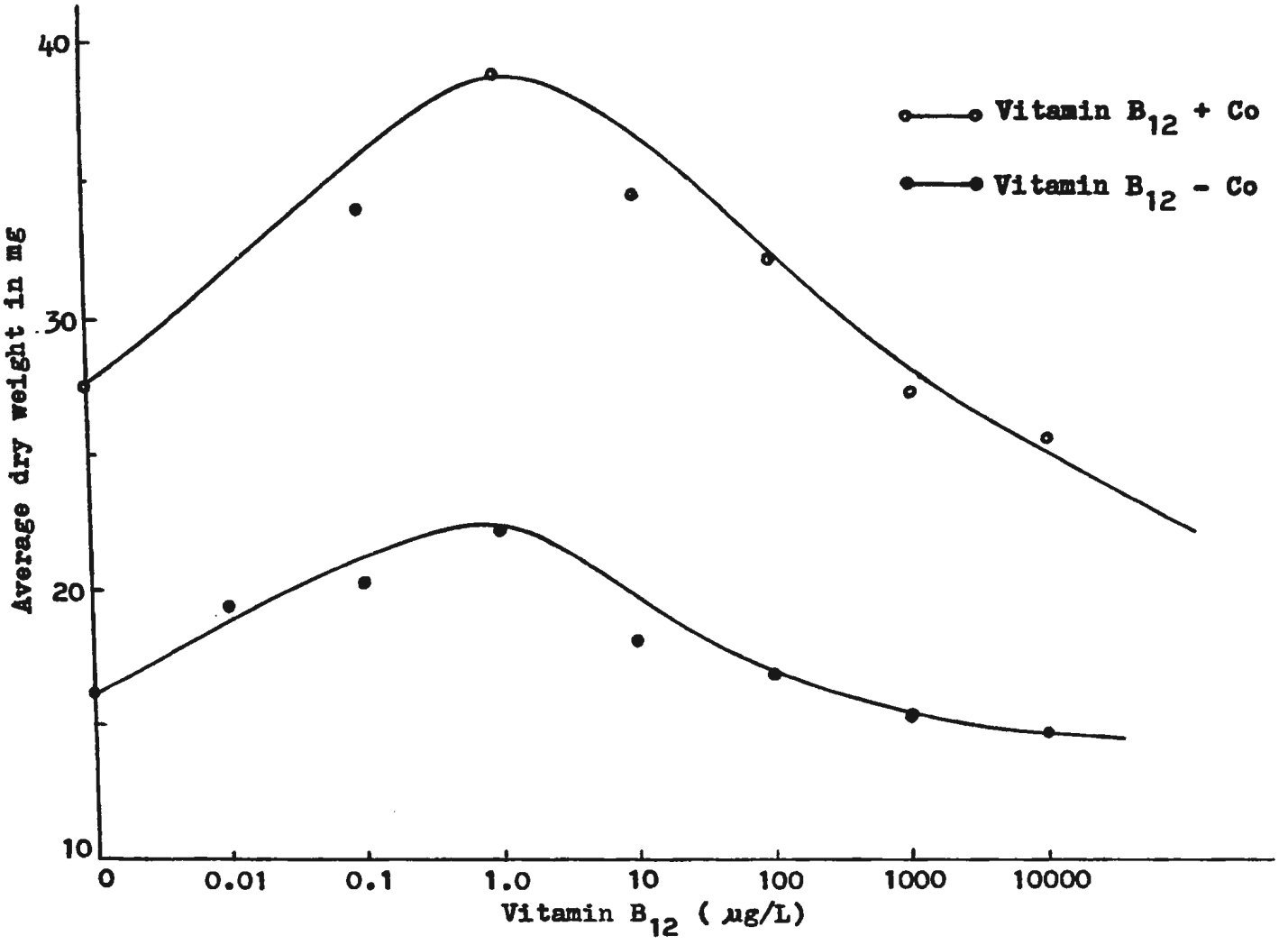

Fig. 43. Growth of Petalonia fascia in response to vitamin B_{12} (with and without cobalt) as expressed by dry weight.

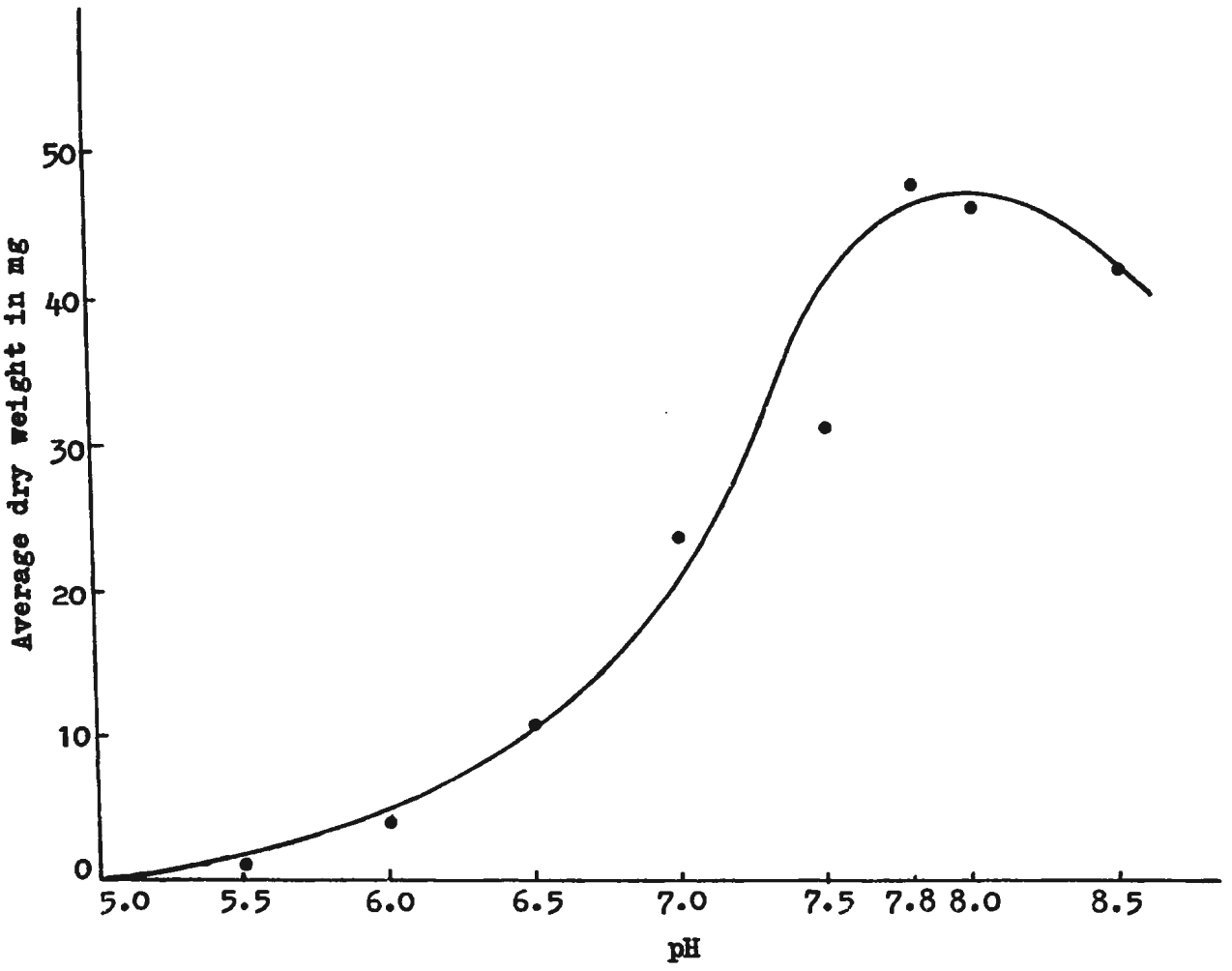

Fig. 44. Growth of Petalonia fascia at varying pHs as expressed by dry weights.

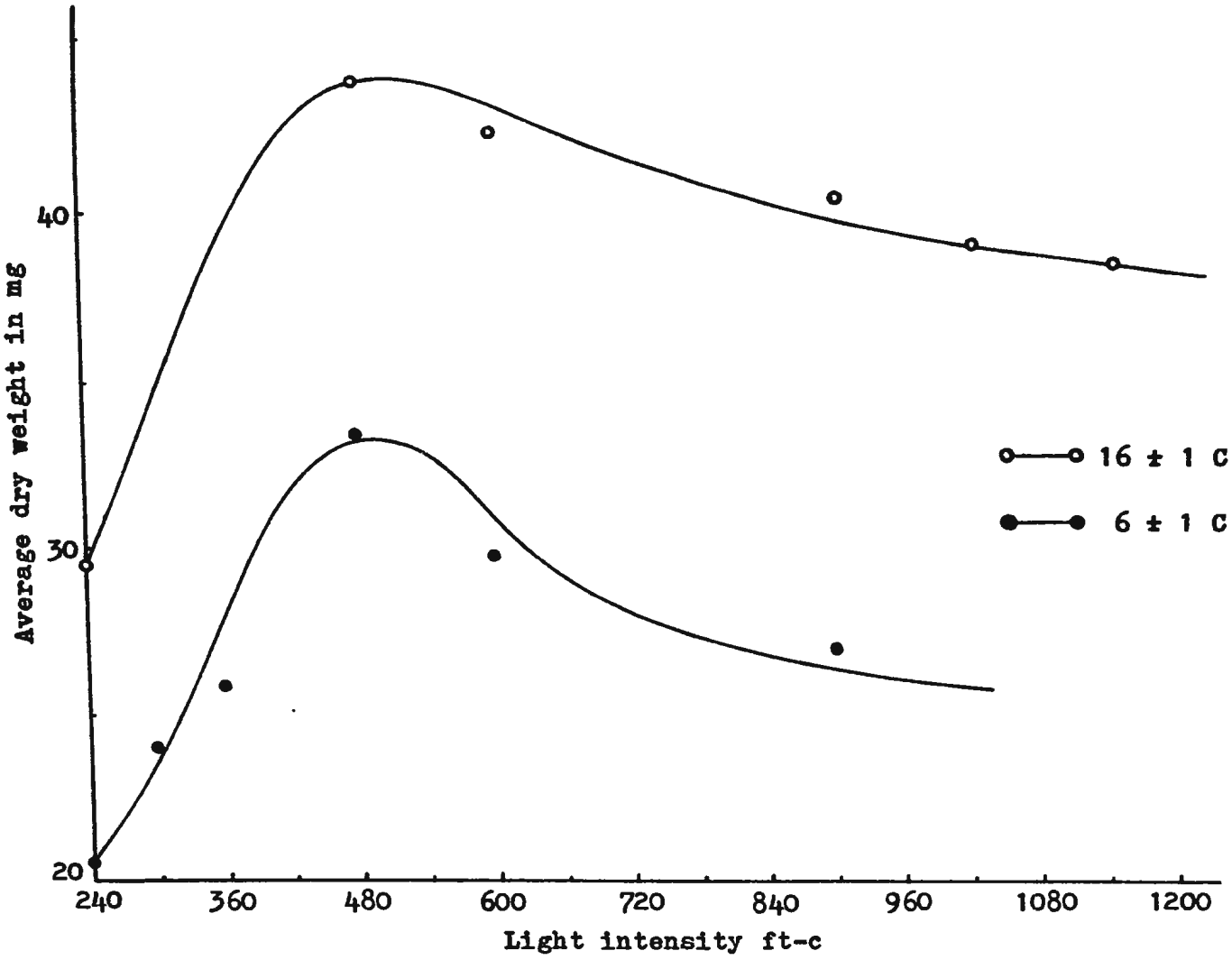

Fig. 45. Effect of light intensity on the growth of Petalonia fascia at two different temperatures (6 and 16 ± 1°C), as expressed by dry weight.

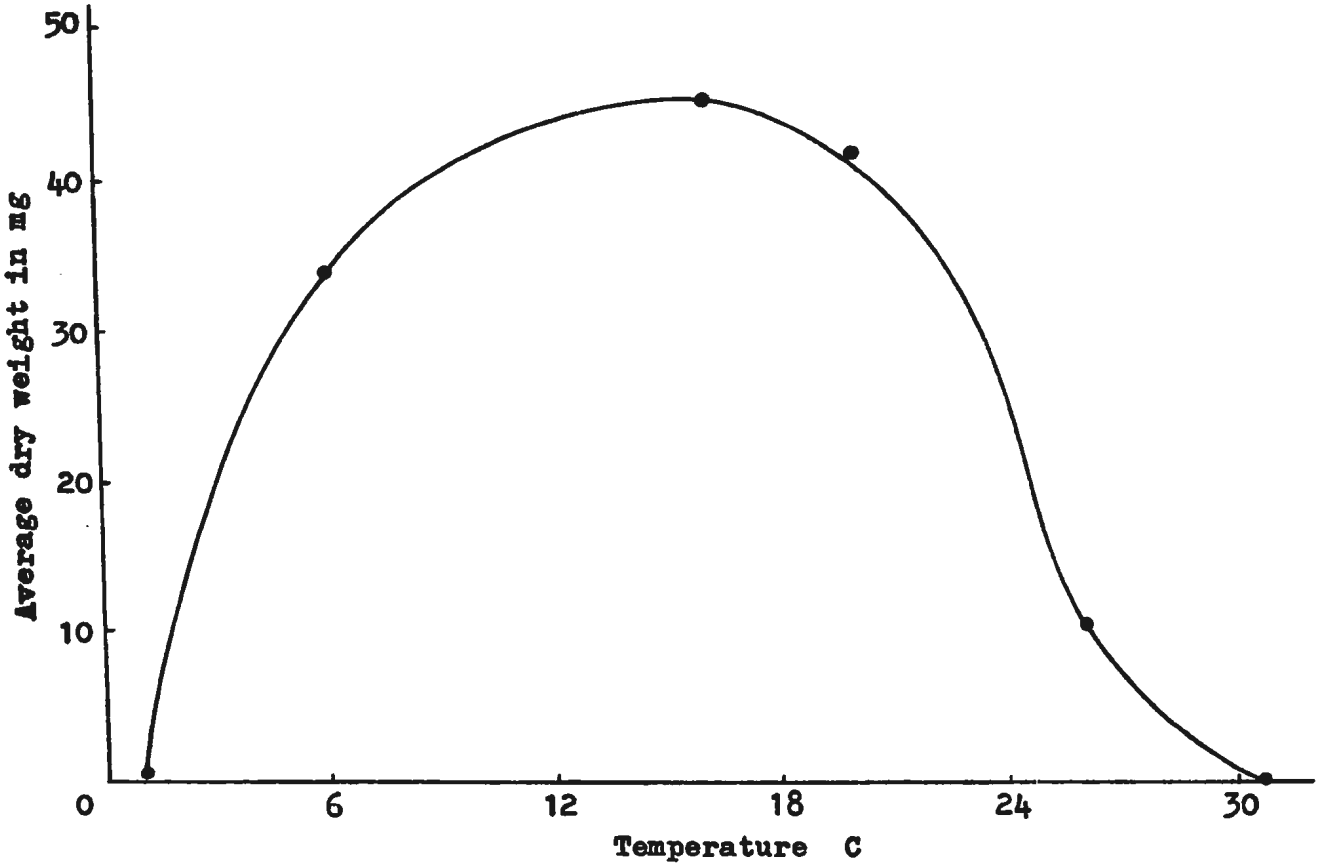

Fig. 46. Effect of varying temperatures on the growth of Petalonia fascia in Petalonia medium at 480 ft-c, as expressed by dry weight.

Appendix 1. Sea water enrichments. Weights are recorded in mg unless otherwise indicated.

	BA	HS	vSt	FE	SW I	SW II
Aged natural sea water (L.)[1]	1	0.75	1020.0 (g)	1	1	1
Distilled water (L.)	-	0.25	-	-	-	-
NaCl	-	-	-	-	-	-
$MgCl_2.6H_2O$	-	-	-	-	-	-
Na_2SO_4 (anhydrous)	-	-	-	-	-	-
$CaCl_2.2H_2O$	-	-	-	-	-	-
KCl	-	-	-	-	-	-
$NaHCO_3$	-	-	-	-	-	-
KBr	-	-	-	-	-	-
KI	-	-	-	-	-	-
KNO_3	202.0	202.0	-	-	72.2	72.2
Na_2HPO_4	-	-	4.26	7.93	-	-
$Na_2.EDTA.2H_2O$	-	10.0	3.72	-	-	-
H_3BO_3	-	-	-	-	-	-
$FeCl_3.6H_2O$	2.7	0.97	-	-	-	-
$MnSO_4.H_2O$	-	-	-	-	-	-
$ZnSO_4.7H_2O$	-	-	-	-	-	-
$CuSO_4.5H_2O$	-	-	-	-	-	-
$CoSO_4.7H_2O$	-	-	-	-	-	-
$Na_2MoO_4.2H_2O$	-	-	-	-	-	-
$NaVO_3$	-	-	-	-	-	-
Tris-buffer	-	-	-	-	500.0	500.0
KH_2PO_4	-	-	-	-	8.8	4.5
Na_2-glycerophosphate.$5H_2O$	-	-	-	-	-	10.5
K_2HPO_4	34.8	35.0	-	-	-	-
Fe-EDTA (1:1 chelation)[2]	-	-	-	-	0.5 (as Fe)	0.5 (as Fe)
$FeSO_4.7H_2O$	-	-	0.278	-	-	-

(continued)

Appendix 1 (continued)

$MnCl_2.4H_2O$	0.2	0.075	0.0198	-	-	-
$NaNO_3$	-	-	42.5	100.0	-	-
Vitamin B_{12} (ug)	-	-	1.0	-	-	-
Soil extract[3] (ml)	-	20.0	-	50.0	-	-
pH	7.25	7.1	7.3	7.05	8.1 (adjusted)	8.1 (adjusted)
Salinity $^o/_{oo}$	31.25	23.09	31.44	29.94	31.68	31.38

BA = Boalch "A" medium (1961) HS = Haxo & Sweeney (1955) vSt = von Stosch (1964)
FE = Føyn's Erd-Schreiber (1934) SW I = Iwasaki (1961) SW II = Iwasaki (1961)'

[1]Sea water was taken from St. Phillips on October 1, 1965. The enriched sea water was prepared on the basis of 6-month aged natural sea water which was filtered through Whatman No. 1 filter paper and then heated to 73°C on two successive days. Salinity 31.09 $^o/_{oo}$, pH 7.45.

[2]Fe-EDTA (1:1 chelation): dissolved 70 mg of $Fe(NH_4)_2.6H_2O$ + $Na_2EDTA.2H_2O$ 66 mg in distilled water and brought to 100 ml; 1 ml = 0.1 mg Fe.

[3]Soil extract: 1 kg finely sieved garden soil from Memorial Greenhouse to 2 liters distilled water; autoclave at 121°C and 15 lb. for 30 minutes. Allow soil to settle and then filter the clean supernatant..

Appendix 2. The composition of concentration ranges for each micronutrient.

(a) Iodine

1) Control (without KI)
2) 4×10^{-9}M KI (50.76×10^{-2} µg I/L)
3) 4×10^{-8}M KI (50.76×10^{-1} µg I/L)
4) 4×10^{-7}M KI (50.76 µg I/L)
5) 4×10^{-6}M KI (50.76×10^{1} µg I/L)
6) 4×10^{-5}M KI (50.76×10^{2} µg I/L)
7) 1.38×10^{-4}M KI (17.58×10^{3} µg I/L)---*Petalonia* medium
8) 4×10^{-4}M KI (50.76×10^{3} µg I/L)
9) 4×10^{-3}M KI (50.76×10^{4} µg I/L)
10) 2×10^{-2}M KI (25.37×10^{5} µg I/L)--- Additional preparation

(b) Bromine

1) Control (without KBr)
2) 8×10^{-9}M KBr (63.9×10^{-5} mg Br/L)
3) 8×10^{-8}M KBr (63.9×10^{-4} mg Br/L)
4) 8×10^{-7}M KBr (63.9×10^{-3} mg Br/L)
5) 8×10^{-6}M KBr (63.9×10^{-2} mg Br/L)
6) 8×10^{-5}M KBr (63.9×10^{-1} mg Br/L)
7) 7.72×10^{-4}M KBr (61.8 mg Br/L)---*Petalonia* medium
8) 8×10^{-4}M KBr (63.9 mg Br/L)
9) 8×10^{-3}M KBr (63.9×10^{1} mg Br/L)
10) 4×10^{-2}M KBr (63.9×10^{2} mg Br/L)---Additional preparation

(c) Molybdenum

1) Control (without $Na_2MoO_4.2H_2O$)

2) 5×10^{-9}M Na_2MoO_4 (4.8×10^{-1} µg Mo/L)

3) 5×10^{-8}M Na_2MoO_4 (4.8 µg Mo/L)

4) 5×10^{-7}M Na_2MoO_4 (4.8×10^{1} µg Mo/L)

5) 1.97×10^{-6} M Na_2MoO_4 (18.9×10^{1} µg Mo/L)---Petalonia medium

6) 5×10^{-6}M Na_2MoO_4 (4.8×10^{2} µg Mo/L)

7) 5×10^{-5}M Na_2MoO_4 (4.8×10^{3} µg Mo/L)

8) 5×10^{-4}M Na_2MoO_4 (4.8×10^{4} µg Mo/L)

9) 5×10^{-3}M Na_2MoO_4 (4.8×10^{5} µg Mo/L)

(d) Iron

1) Control (without $FeCl_3.6H_2O$)

2) 4×10^{-9}M $FeCl_3$ (2.233×10^{-1} µg Fe/L)

3) 4×10^{-8}M $FeCl_3$ (2.233 µg Fe/L)

4) 4×10^{-7}M $FeCl_3$ (2.233×10^{1} µg Fe/L)

5) 4×10^{-6}M $FeCl_3$ (2.233×10^{2} µg Fe/L)

6) 5.18×10^{-6}M $FeCl_3$ (2.89×10^{2} µg Fe/L)---Petalonia medium

7) 4×10^{-5}M $FeCl_3$ (2.233×10^{3} µg Fe/L)

(e) Manganese

1) Control, (without $MnSO_4.H_2O$)

2) 1.8×10^{-9}M $MnSO_4$ (9.89×10^{-2} µg Mn/L)

3) 1.8×10^{-8}M $MnSO_4$ (9.89×10^{-1} µg Mn/L)

4) 1.8×10^{-7}M $MnSO_4$ (9.89 µg Mn/L)

5) 1.8×10^{-6}M $MnSO_4$ (9.89×10^{1} µg Mn/L)

6) 1.8×10^{-5}M $MnSO_4$ (9.89×10^{2} µg Mn/L)

7) 2.1×10^{-5}M $MnSO_4$ (11.51×10^{2}µ g Mn/L)---Petalonia medium

8) 1.8×10^{-4}M $MnSO_4$ (9.89×10^{3} µg Mn/L)

9) 1.8×10^{-3}M $MnSO_4$ (9.89×10^{4} µg Mn/L)

(f) Copper

1) Control (without $CuSO_4.5H_2O$)

2) 3×10^{-10}M $CuSO_4$ (19.7×10^{-3} µg Cu/L)

3) 3×10^{-9}M $CuSO_4$ (19.7×10^{-2} µg Cu/L)

4) 1.6×10^{-8}M $CuSO_4$ M (10.2×10^{-1} µg Cu/L)---Petalonia medium

5) 3×10^{-8}M $CuSO_4$ M (19.7×10^{-1} µg Cu/L)

6) 3×10^{-7}M $CuSO_4$ M (19.7 µg Cu/L)

7) 3×10^{-6}M $CuSO_4$ M (19.7×10^{1} µg Cu/L)

8) 3×10^{-5}M $CuSO_4$ M (19.7×10^{2} µg Cu/L)

(g) Zinc

1) Control (without $ZnSO_4.7H_2O$)
2) 8×10^{-11}M $ZnSO_4$ (5.23×10^{-3} µg Zn/L)
3) 8×10^{-10}M $ZnSO_4$ (5.23×10^{-2} µg Zn/L)
4) 8×10^{-9}M $ZnSO_4$ (5.23×10^{-1} µg Zn/L)
5) 8×10^{-8}M $ZnSO_4$ (5.23 µg Zn/L)
6) 8×10^{-7}M $ZnSO_4$ (5.23×10^{1} µg Zn/L)
7) 2.2×10^{-6}M $ZnSO_4$ (14.28×10^{1} µg Zn/L)---Petalonia medium
8) 8×10^{-6}M $ZnSO_4$ (5.23×10^{2} µg Zn/L)
9) 8×10^{-5}M $ZnSO_4$ (5.23×10^{3} µg Zn/L)

(h) Cobalt

1) Control (without $CoSO_4.7H_2O$)
2) 1.7×10^{-11}M $CoSO_4$ (1×10^{-3} µg Co/L)
3) 1.7×10^{-10}M $CoSO_4$ (1×10^{-2} µg Co/L)
4) 1.7×10^{-9}M $CoSO_4$ (1×10^{-1} µg Co/L)
5) 1.7×10^{-8}M $CoSO_4$ (1.0 µg Co/L)
6) 4.6×10^{-8}M $CoSO_4$ (2.73 µg Co/L)---Petalonia medium
7) 1.7×10^{-7}M $CoSO_4$ (1×10^{1} µg Co/L)
8) 1.7×10^{-6}M $CoSO_4$ (1×10^{2} µg Co/L)
9) 1.7×10^{-5}M $CoSO_4$ (1×10^{3} µg Co/L)
10) 1.7×10^{-4}M $CoSO_4$ (1×10^{4} µg Co/L)