THE REARING OF OYSTER LARVAE ON AN ALGAL DIET

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From the Marine Biological Station, Port Erin

(Text-figs. 1–3)

CONTENTS

INTRODUCTION

The cultivation of the European flat oyster, *Ostrea edulis* L., has been the subject of numerous experiments on the Continent and in Britain. These experiments and their varying but usually ephemeral success, have been fully reviewed in several publications (Kändler, 1930; Gaarder & Bjerkan, 1934; Orton, 1937), and it is not proposed to go over this ground in the present report which deals purely with experimental work.

It is fair criticism of most of this previous work to state that ultimate failure, or at any rate only partial success, was associated with imperfect knowledge of one or other phase of the oyster's life history, and its special and varying requirements in the way of food and physical conditions at the successive stages of growth and development. Much greater significance therefore attaches to the most recent contribution to the oyster-breeding problem—that of the Ministry of Agriculture and Fisheries—an investigation in which the importance of a full knowledge of the biological and physical factors, and their incidence at all stages of the oyster's life is adequately realized, and applied so far as practical limitations permit; with the result that Cole (1938) is now able to claim, as the outcome of several years' success,

that "the production of oyster-spat on a commercial scale in tanks may be reasonably expected".

Among the various factors recognized as significant, that of food and feeding, especially in the free-swimming larval stage, stands out pre-eminent; and some of the preliminary work undertaken in the course of the Ministry's experiments is described by Cole (1937). This work, carried out at the Conway Mussel Cleansing Station, was necessarily conditioned by the physical environment there obtaining, and it was realized by the Ministry's staff that certain aspects of the work, notably the detailed study of unicellular algal cultures as potential sources of food supply for the developing larvae, could better be studied at the Port Erin Biological Station, where facilities were available for small-scale experiments under the control of an algologist.

The experiments in the present series have extended so far over five seasons. It is not justifiable to apply to these experiments the criterion of percentage settlement alone, which on a commercial or semi-commercial scale, would naturally afford a measure of relative success. The work has been to a great extent exploratory, and the "success" of an experiment has been appraised on the total contribution which it has made to the solution of the general problem in view.

At the same time, certain positive, occasionally spectacular, results have been achieved; for example, in some experiments 90–99% of the larvae initially introduced completed their metamorphosis. Side by side, however, with these encouraging successes there must be recorded a not inconsiderable number of unexplained failures—that is, failures to repeat results in consecutive and even occasionally in parallel experiments where all observable external conditions were as closely identical as possible. Such seeming inconsistencies appear to be a commonplace among the observations of oyster-workers, and lead to the conclusion that certain factors—some of them conceivably genetic—remain unrecognized, and may assume significant proportions under certain conditions.

The content of the report calls for brief notice. Primarily devoted to an account of a series of feeding experiments on oyster larvae on a laboratory scale, that aspect of the work and especially the results of the 1938 season, takes pre-eminence in point of amount and importance. At the same time, the production of the necessary larvae, whether on a large scale in the semi-natural ponds of the Station, or under controlled conditions in tanks and trays, calls for some description, since we have reason to believe that the viability of oyster larvae depends in some measure upon the factors influencing the adult breeding stock prior to spawning and liberation.

The final report must of necessity omit reference to much essential preliminary work.

The authors have great pleasure in placing on record their indebtedness to Dr R. J. Daniel, Director of the Port Erin Biological Station, 1933–9. Dr Daniel has been closely associated with the work here recorded. He was

largely responsible for its initiation, and throughout the whole period now under review has maintained a close interest. His critical advice and constructive suggestions have been of great value.

It is fitting to record here also our sense of appreciation of the willing co-operation and cordial good-will of those members of the Conway staff of the Ministry of Agriculture and Fisheries who are more directly associated with oyster culture—Mr H. P. Sherwood, Mr H. A. Cole and (during the earlier years of the work) Dr R. W. Dodgson, at that time Director of the Conway Laboratory. Throughout the investigation there has been a free interchange of experience and reciprocal personal contact between the two laboratories.

It is a pleasure to acknowledge the valued advice of Prof. J. H. Orton, D.Sc., whose specialized knowledge of oyster problems has been placed freely at our disposal.

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MAINTENANCE OF LARVAL SUPPLY

It is known that a female-functioning oyster may release anything up to a million larvae, but in the experiments under review, only relatively few could be used at a time. It was therefore a matter of some moment to extend the spawning period in order to have larvae available for as long a period as possible. It was of equal importance that the larvae should be of the highest viability.

To meet these needs the spawning stock was kept under the widest possible range of conditions.

The Spawning Stock. Four main sources of supply were used, namely: the Fal Estuary, the Yealm Oyster Fisheries, Lochryan and the Blackwater River. From these localities a sufficient stock was obtained in April of each year, and to this was added a number of "survivor" stocks which had spent the previous season in the Port Erin ponds and tanks.

The samples obtained through commercial channels contained no very small oysters, and were for the most part three years old and more, with occasional large specimens up to 11·5 cm. in diameter. These were augmented, in the last three seasons, by a number of oysters bred in the Port Erin Station; the numbers of these were never large but definite proof was obtained that some, at least, were female-functioning when two years old.

Spawning Stock kept in Outdoor "Ponds"

Three ponds used in winter and spring for other purposes, and known respectively as the "East", "West" and "Lobster" ponds, have been available during the summer for housing the oysters. In the first three or four seasons, they served also as the locus of large-scale experiments in the

rearing of larvae, and in some years a moderate spatfall occurred in them. The ponds were, however, too readily subject to varying conditions to give consistent results, and latterly their use has been restricted to that of accommodating the parent oysters.

The ponds lie west of the Station, are rock-cut and sunk to the level of the ground in the Manx slate, but differ from each other in detail.

(*a*) "*Lobster*" pond, capacity 15,000 gallons, 50 ft. × 13 ft. × 5 ft. 6 in., floored by bare rock. This was the subject of an experiment in reducing illumination with the object of keeping the *p*H value of the water low, but it involved a serious temperature loss which negatived any possible gain from change in the light factor.

(*b*) "*West*" pond, capacity 51,000 gallons, 39 ft. × 30 ft. × 9 ft. Floor and walls cement-covered, but continuous use over a long period may be assumed to have made the cement innocuous to larval life.

(*c*) "*East*" pond, capacity 73,000 gallons, 52 ft. × 31 ft. × 8 ft. 9 in., part natural rock, part cement, used only in 1938.

All ponds are filled by pumping from Port Erin Bay and the water is delivered by wooden chutes. The spawning oysters in the ponds are accommodated on light wooden trays and covered by sheets of asbestos to reduce algal growth. The number of oysters was such as never to exceed 1 per 100 gallons of sea water. Whether destined for ponds or smaller vessels, all oysters were carefully examined and "cleaned" to avoid the introduction of undesirable organisms.

Physical Conditions in the Ponds

The physical conditions in the ponds are not susceptible to any considerable degree of control. In the one instance in which control of light was attempted, no useful result emerged. The usefulness of the ponds in any given season is largely dependent upon such meteorological conditions as temperature and sunshine.

Chemical factors such as *p*H, phosphorus content and oxygen content are intimately bound up with the phytoplankton. Some of these factors have been continuously observed, but in view of the relatively small share in the ultimate result played by the ponds, it is not proposed to discuss them in detail in this report. Abstracts of the data are given in Table I.

Biological Conditions in the Ponds

The biological conditions in the ponds and particularly the character of its included plankton are of some importance. Analyses of the number and nature of organisms present in the pond waters have been made at regular intervals.

During the first year of experiment, samples of pond water were examined by Mr H. A. Cole of the Conway Experimental Station, using the "Buwa"

TABLE I. USE OF THE SPAWNING PONDS DURING THE SEASONS 1934–8. WITH NOTES ON CONDITIONS, LARVAL COUNT, SETTLEMENT OF SPAT, ETC.

Year	Weekly mean temperature			Other factors	Maximum larval count	Spat-fall	Remarks
	10° or above	15° or above	Maximum				
				West pond			
1934	May 6–Oct. 18	June 8–Aug. 28	July 16, 19°·8	*p*H 8·4–8·8	July 9–July 31 885 per gal.	18,000	*Gymnodinium simplex* very abundant. Healthy larvae, of which the earlier liberated gave best spat-fall
1935	(June)*–Oct. 15	June 15–Aug. 29	Aug. 13, 18°·6	*p*H 8·6–8·8 O_2 94–110 % Sat. P. 4–5 mg./m.^{3}	June 28–July 28 236 per gal.	3,400	*G. simplex* again abundant in early July. Spat-fall confined to July. Green unicells dominant later
1936	May 7–Oct. 10	June 21–Sept. 24	July 4, 18°·2	*p*H 8·4–8·6 O_2 102–109 % Sat. P. nil	June 29 113 per gal. July 22–Sept. 30 259 per gal.	2,780	The earlier larvae included many prematurely spawned. Spat-fall mid-Aug. only, at 16°·5
1937	May 1–(Sept.)	May 29–Sept. 7	July 24, 17°·7 Aug. 7, 17°·7	*p*H 8·5–8·8 O_2 101–118 % Sat. P. 12·5 mg./m.^{3} in July, nil in Aug.	Mid-July and mid-Aug., few	Nil	Many larvae again premature. None reached "eyed" stage
1938	(May)–(Oct.)	June 18–Aug. 20	Aug. 13, 17°·5	*p*H 8·75–8·85 early, but 8·5 in Aug. Plaice in pond	Negligible	Nil	Larvae small and probably premature
				Lobster pond			
1934	May 26–Oct. 22	July 3–Aug. 26	July 9, 17°·7	*p*H 8·3–8·5	July 11–Aug. 12 2080 per gal.	1,770	Pond covered mid-May to end of August
1935	(June)–Oct. 10	June 22–Aug. 26	July 6, 16°·7	*p*H 8·6–8·8	July, 217 per gal. Aug. 3, 40 per gal.	Nil	Pond covered for 1 week only
1936	May 10–Oct. 26	June 23–July 12: Aug. 10–Sept. 7	July 27, 16°·6	..	..	..	A few larvae used for a culture-house experiment
				East pond			
1938	(May)–(Oct.)	June 18–Aug. 27	Aug. 13, 18°·8	*p*H 8·65–8·8	Negligible	Nil	Larvae small, premature

* A date in brackets is estimated by extrapolation.

filter method. Qualitative records were kept at Port Erin. From 1935 onwards, however, all analyses, quantitative and qualitative, have been made at Port Erin. After some preliminary experiments a satisfactory method of sampling was adopted. 10 c.c. samples of sea water were centrifuged for 10 min. and 9 c.c. were removed. Sample drops of the remaining 1 c.c. were examined on a haemacytometer slide. Eight counts were made for each sample of water and the average value recorded. The first analyses were based on size alone and the plankton divided into groups of under and over 5 μ. From 1937 onwards, however, much greater discrimination was exercised. Five groups of organisms were established and used as the basis of analysis:

(i) Organisms of 2μ or less.

(ii) Flagellate forms of 2–5 μ.

(iii) Non-motile forms of 2–5 μ.

(iv) Organisms of 5–10 μ.

(v) Organisms over 10μ.

The results of the analyses are recorded in Table II.

TABLE II. MICROPLANKTON COUNTS AND ANALYSES OF POND WATERS

			Monthly averages—No. per mm.³				Maximum peak	
Pond	Year	Month	Total count	No. of organisms over 5 μ in size	No. of organisms less than 5 μ in size	Flagellates 5 μ or less in size	Date	No. of organisms per mm.³
Lobster pond	1934	Aug.	25	..	..	..	..	..
	1935	July	110	55	55	..	July 9	150
		Aug.	89	43	46	..		
	1936	Aug.	71	28	43	31	Aug. 10	92
West pond	1934	July	91	..	..	..	Aug. 2	213
		Aug.	119	..	..	..		
	1935	July	121	58	63	..	Aug. 21	175*
		Aug.	100	45	55	..		
	1936	June	35	10	25	22	Aug. 26	78†
		July	50	17	33	32		
		Aug.	68	37	31	31		
		Sept.	56	21	35	20		
	1937	June	110	27	83	61	July 12	220
		July	164	63	101	84		
		Aug.	130	21	109	80		
	1938	June	635	32	603	16	July 6	1093‡
		July	939	47	892	118		
		Aug.	546	49	497	65		
East pond	1938	June	151	14	137	60	July 27	211
		July	176	19	157	70		
		Aug.	187	28	159	47		

* At the 2 ft. level maximum count of 244 per mm.³ on August 21.
† Lower counts due to frequent additions of fresh sea water.
‡ Very high counts due to presence of fish (plaice) in pond.

Spawning Stock kept in Tanks and Small Vessels

In each of the five seasons under review, an increasing number of the spawning stock was kept indoors in small tanks and trays. In contrast to the results experienced in the "ponds" a reasonably consistent output of larvae was maintained in indoor vessels. In some years the whole experimental programme was concerned with larvae liberated under these conditions. This method brought several advantages. When the oysters were few in number in each tray, an individual emitting larvae could be readily detected and the larvae collected. The latter were all of one age, in marked contrast to the condition of those in the ponds, and when used in experiment gave opportunity for more trustworthy comparisons. In maintaining oysters in small vessels, running sea water or twice-daily changes effected with minimum temperature change is a factor in success. Sea water from the storage tanks of the Station was used. Since this water is unfiltered even a brief period of storage affects its planktonic content, and compared with freshly pumped sea water, certain flagellates sometimes appear in greatly augmented numbers. There is thus a probability that sufficient food was always present in the water supplied to the spawning oysters. Nevertheless, on a few occasions, deliberate additions of food organisms were made to the vessels, but no enrichment with nutrient materials intended artificially to raise the plankton content was attempted, nor were the vessels placed under lighting conditions suitable for algal development.

In general no attempt was made to control temperature, but in 1937 and 1938 a certain number of oysters were kept at a raised temperature in the culture-house for a period of time before and during actual liberation. It must be recorded that in the "Hatchery" trays larvae of high viability, yielding a high percentage of spat-fall, have been produced at temperatures (12°–13°·5 C.) well below the level hitherto regarded as minimum for such production, i.e. about 15°·5 C.

It would appear as if a slight but sudden rise of temperature were essential to initiate the act of liberation, but the extent of such rise and the actual temperature are dependent upon the temperature level at which, up to that stage, development has proceeded.

EXPERIMENTS ON A LABORATORY SCALE

During the four years 1934–7 understanding of the various factors involved in larval culture accumulated slowly, but the experience gained formed a foundation for the 1938 experiments in which significant results were obtained and which demand full description. The work of 1934–7 forms, therefore, a historical background to that of 1938 and only brief reference to it need be given. Each of the earlier years contributed its share of information upon which experimental methods were revised and control gained over the numerous factors affecting the larvae. By the end of 1937, from the point of

view of experimental technique, the most convenient layout and procedure was reached. All technical improvements were incorporated in the organization of the culture-house of which a description follows.

THE CULTURE-HOUSE

After one season of purely exploratory work, using a variety of vessels under such conditions as could be maintained in the existing laboratories at the Biological Station, it was realized that a properly equipped "culture-house" with facilities for temperature control and a variety of sources of water supply was essential. Plans were drawn and a building erected (see Fig. 1).

The building is of lean-to form, 15 ft. × 8 ft. It is 7 ft. high at the front and 11 ft. high at the back and is glazed on the roof and on the sides above a 3 ft. timbered skirting. Within it are two benches to accommodate culture vessels. Two heaters of common horticultural pattern are fitted under the back and front benches respectively. Together they maintain a temperature level of 20°–22° C.

Exposure to light is not uniform over the whole structure. To mitigate the possible ill-effects of too strong overhead lighting a roof-screen, made of hessian, was in use. The effect of this screen was to reduce the mean light intensity to 38 % of its full value as roughly determined in five directions by a photo-electric exposure meter. Even with this screen constantly in use there was inequality of illumination over the benches. For a variety of local reasons less light is received by the middle of the front than by the ends of the building. Sunlight falls predominantly on the southern end of the culture-house in the forenoon and on the northern end late in the afternoon. The distribution of mean daily light intensity, so far as it was incident on the exposed surfaces of the culture vessels, was determined by electric photometer for front and back benches. The results which were purely relative indicate that the average illumination falling on the front and back benches respectively, calculated on a whole day's readings with a wide range of overclouding, stood in the ratio of 100 : 76·5. The inequality between the ends and middles of the benches varied greatly with the altitude of the sun; on the daily mean (front and back benches) the illumination at the south end exceeded that in the middle of the benches in the ratio of 100 to 70. To attempt to rectify this inequality of illumination the glass of the south side was covered by coarse muslin. These arrangements were intended to secure as nearly as possible uniform illumination to all jars so that comparisons exclusive of the light factor could be made, but despite the precautions taken, the differences in illumination could not be completely eliminated and the "end" positions of the benches have frequently yielded results different in character from those of experiments occupying positions nearer the middle of the bench.

It was realized that the light intensity even if it were made uniform was not necessarily at its optimum value. The central two-light sash in the middle

of the front bench was glazed with "vita-glass", but in view of the diffused character of the internal lighting, it is not felt that this fact is of special significance.

Experimental Equipment

The principal equipment consists of experimental vessels in the form of seventeen glass bell-jars, of 20 l. capacity, arranged along the front and back benches as shown on the plan. Three additional vessels not forming part of the main series can also be accommodated along the south side. The volume of sea water used in these vessels is 16 l., and when continuously changing water is called for, this is effected by a drip-feed from a distributing system of glass tubes with screw-clipped jets which is carried round the culture-house at a level slightly above the top of the bell-jars. Batteries of carboys containing different kinds of sea water are linked to the distributing system in such a way that any vessel can be fed from either or both of two sources. To prevent loss of larvae the outflow tubes are screened with bolting silk (Wydler's No. 12). Arrangements are made for cleaning this filtering surface when necessary. The reservoirs of water are filled from cans of 6 or 8 gal. capacity in which the water is brought in from the sea. The cans are fitted with a removable lid which can be clamped down on to a soft rubber gasket which forms an effective seal. A hose connexion is then made between the carboy and a tube passing through the lid to the bottom of the can and, on air being pumped into the can by means of a hand-operated motor-tyre inflator, the water is forced up into the carboy, whence an initial act of suction serves to fill the distributing tubes and jets.

Stirring and aeration is provided for by glass plunger plates, suspended from a light braced framework of timber, hinged to the roof as shown in the figure, and maintained in slow oscillation by a mechanism based on the Scott-Dannevig Tipping Bucket. (Fig. 1.)

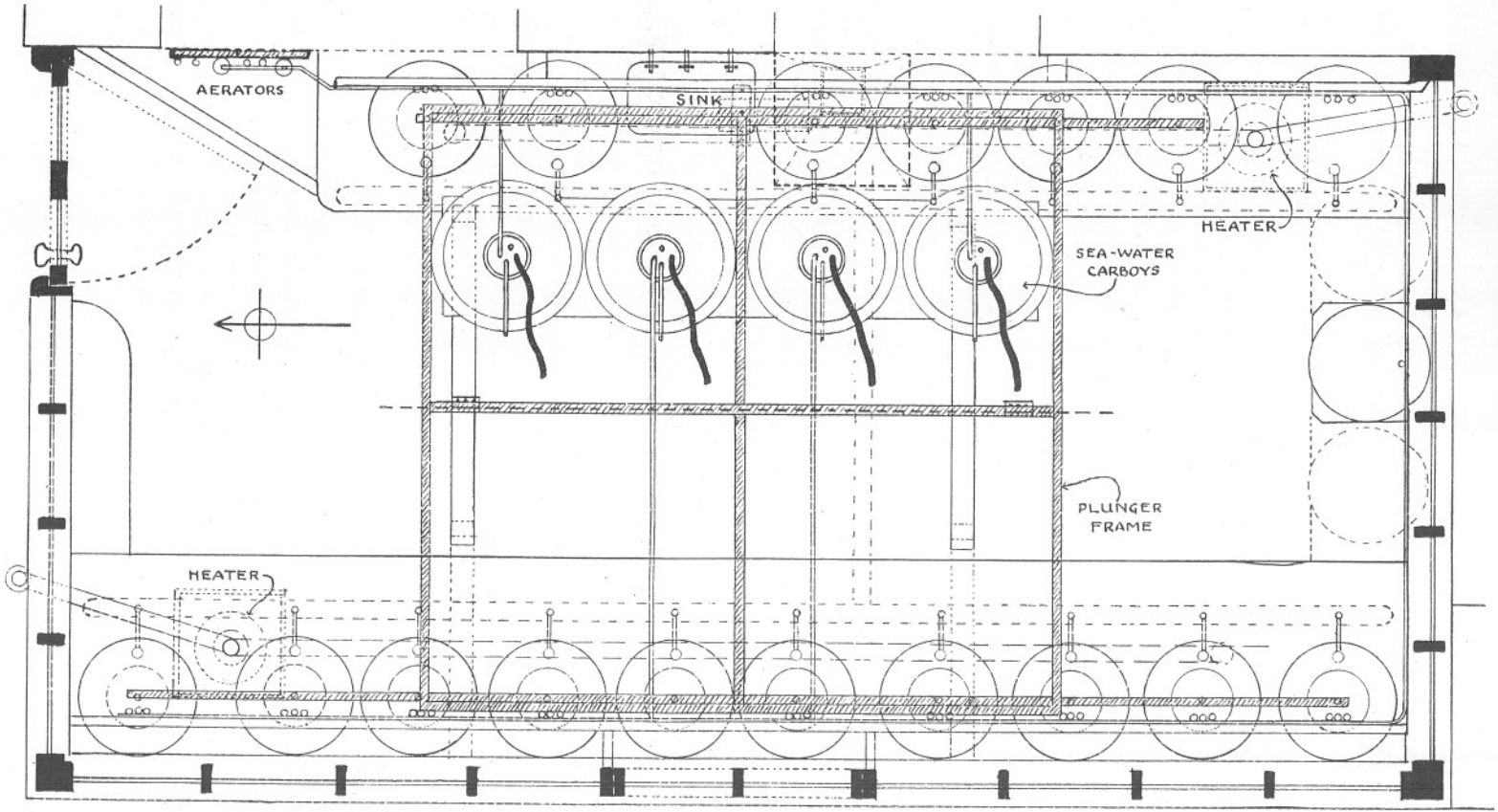
Aeration by actual bubbling has been made use of in all experiments in substitution for or along with plunger stirring. For this purpose two large Gemmill aerators, connected in parallel are fitted as shown in Fig. 1. Compressed air from these is led by glass tubing round the culture-house and thence by capillary jets to each vessel.

Biological Data

The experience of previous workers supports the assumption that oyster larvae feed on a diet consisting largely of plant organisms. The object of the present enquiry was to determine the identity and nature of those organisms which give most successful results in rearing larvae.

The utilization or otherwise by the larvae of a given algal organism is obviously determined, in the first instance, by the size of the organism in relation to the feeding mechanism of the larvae. A diameter of 10 μ is generally

PLAN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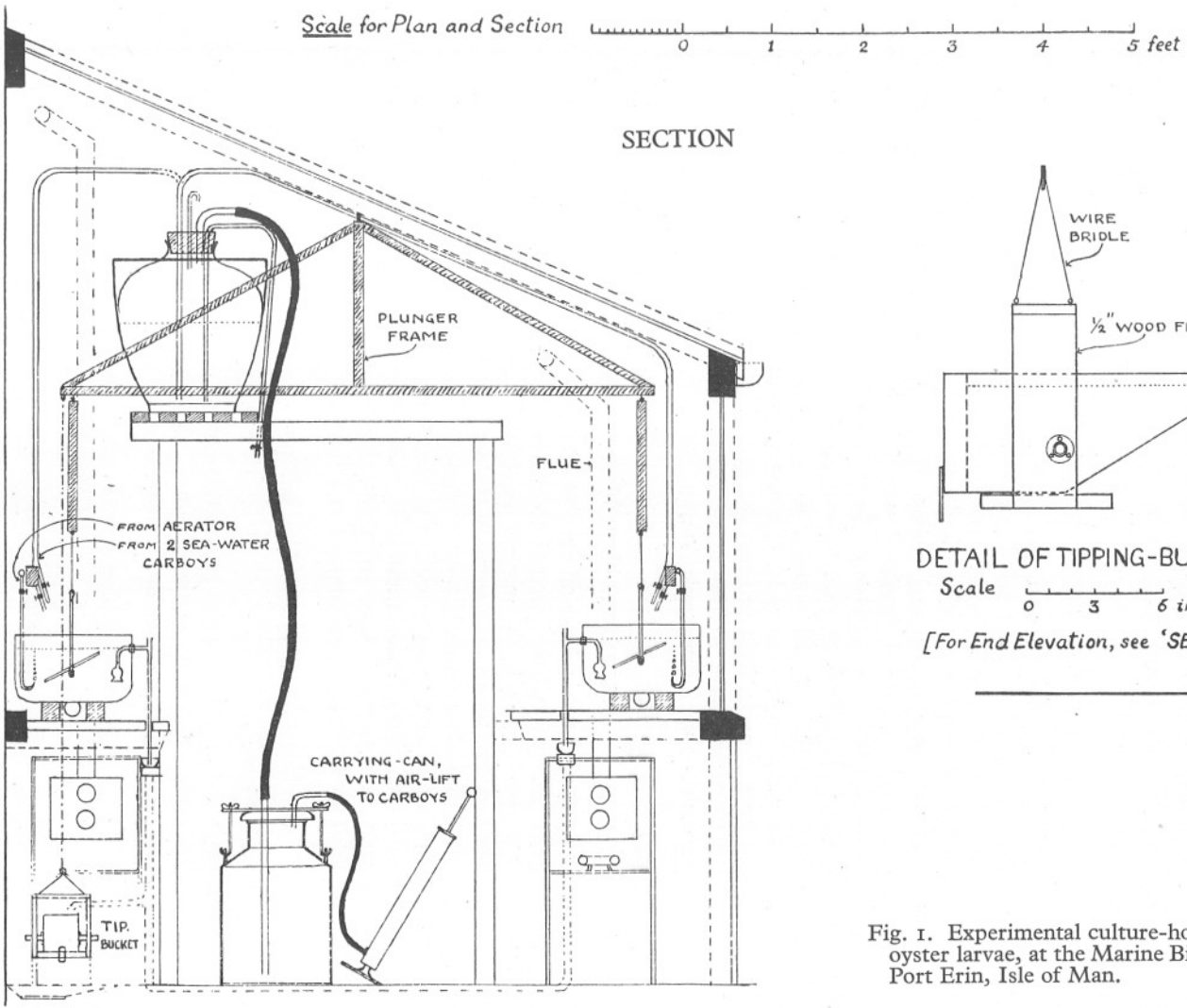
HEATER
SEA-WATER CARBOYS
PLUNGER FRAME
HEATER



DETAIL OF TIPPING-BUCKET

Scale 0 3 6 ins.

[For End Elevation, see 'SECTION']

Fig. 1. Experimental culture-house for work on oyster larvae, at the Marine Biological Station, Port Erin, Isle of Man.

accepted as the limit of size above which a particle cannot be ingested by a newly liberated larva, and in consequence, the possible variety of plant cells available is somewhat restricted.

Many of the reproductive zooids of larger algae, which might conceivably equally well serve the same purpose as unicellular microphyta, lie within the 10 μ limit. Both these types of organisms were used in the experiments, but after a little experience, it was found that the unicellular microphyta were more successful, and latterly were exclusively used.

Experiments with Algal Zooids

The difficulties arising from the use of zooids of larger algae were several. Primarily, the irregularity of release and the uncertainty of securing an adequate daily supply made precise experiment difficult. Further, the zooids were sensitive to changes in media and were liable to lose motility rapidly when added to the experimental vessels. This fact was important as it is necessary that food material should be evenly distributed.

Zooids of *Enteromorpha compressa* Grev., *E. Linza* J. Ag. var. *lanceolata* (Kütz.), and *Chorda filum* Stackh. were tried and discarded. Eggs of *Fucus* mashed into particles small enough to be ingested with their included glycogen and also spermatozooids were tried. But when it was found that the larvae in the ponds, feeding upon the natural plankton, were developing more rapidly than those in the experimental vessels, it was decided to concentrate on the use of microplankton as the source of food supply.

Experiments with Microplankton

Naturally the microplankton of the "ponds" suggested itself for the first trial, but attention was later directed to the use of sea water from the open sea.* At a later stage still the natural planktonic content was augmented in the culture jars, but finally, as a result of the experience so gained, the method was adopted of adding pure cultures of organisms to sea water whose natural plant population had been previously removed by filtration through a Berkefeld filter. Though this method is now accepted as likely to lead to good results, something of value emerged from the earlier methods used and the results may be briefly summarized as follows:

(*a*) *Using Sea Water without addition of Microplankton*

(i) "*Pond*" *Water.*

Larvae from the pond, in the pre-settlement stage were brought into the laboratory and induced to settle in small glass vessels under controlled conditions. During this phase the development of the rudimentary foot of

* Referred to subsequently as "outside" sea water.

the larva into a long tongue-like process could be readily observed. In 1936 as many as 60% of such larvae settled as spat in culture vessels at a higher temperature than that of the "pond" in which less than 1% of settlement occurred. Experiments with larvae at an earlier stage of development also gave some measure of success. A small percentage reached the settlement stage.

(ii) "*Outside*" *Sea Water.*

Better results were obtained when "outside" sea water with its naturally included plankton was used as a culture medium for larvae which had been liberated into a dish in the Hatchery. In this experiment 4·5% of the larvae achieved settlement. Further experiments on the same lines confirmed the results obtained and emphasized the need for close scrutiny of the nature of the naturally occurring plankton. Special attention was paid to the types and sizes of the micro-organisms present in the sea water supplied to the larvae. Small flagellate forms were cultured for use as additions to sea water.

(*b*) *Sea Water with Augmented Plankton*

One of the first flagellate organisms made use of in this way was *Gymnodinium simplex* (Lohmann). Indications had been given in earlier experiments that the larvae were taking in this organism from the "pond" water. It was therefore isolated, developed in pure culture and added to unfiltered (and later to filtered) sea water, though without any great success. The explanation of its failure lies possibly in the fact that it increases in size in culture and the naturally occurring small stages which might have been useful as food for larvae were absent.

(*c*) *Filtered Sea Water with added Pure Cultures of Flagellates*

The use of unfiltered sea water, though it gave certain promise of success, involved the considerable labour of determining its planktonic content before adding a culture of organisms. It also constituted an uncontrollable factor. Clearly, for experimental purposes, the use of sea water from which the natural plankton had been filtered provided better opportunity of comparing relative values of added organisms, but unfiltered sea water continued to be used when comparison between pure and mixed diet was desired.

As early as 1935 experiments in which *Coccomyxa* (a non-motile green unicell, 3–4 μ) was added to filtered sea water, gave promise of success. Some growth of the larvae was obtained; a few reached the "eyed" stage though no settlement occurred.

Gymnodinium simplex was also tried in filtered sea water but without success. It served a useful purpose, however, in directing attention to the use of flagellates as alternative to non-motile organisms in feeding experiments.

Belief that the former might serve a useful purpose was strengthened when analysis of the microplankton of "outside" sea water and "pond" water revealed the presence, in both, of flagellate as well as non-motile forms in the size-groups of under 5 μ as well as over 5 μ. Several of the smaller flagellates were therefore isolated and developed in pure culture. They proved to be more stable in size than *Gymnodinium*. Their use served as the basis for experiment in 1937 and was extended and developed in 1938. In pure culture they have been added to filtered sea water and their usefulness tested against controls in which unfiltered sea water, with and without the addition of flagellates, has been used.

These minute organisms used in culture have been referred to their appropriate algal group but they have not yet been precisely identified. The literature relating to minute algal flagellates in sea water is scanty and a considerable field of research is open to exploration.

In all, six flagellates, temporarily labelled "B", "C", "D", "F", "H" and "I" have been isolated and made the basis of experiment. The size-range, colour of the culture and tentative classification (according to Fritsch) of each species are tabulated below:

"B"	4–5 μ (golden brown)	Chrysophyceae, Chrysomonadales
"C"	6–7 μ (golden brown)	Chrysophyceae, Chrysomonadales
"D"	4–7 μ (red)	Cryptophyceae, Cryptomonadales
"F"	1·5–2 μ (greenish yellow)	Unclassifiable
"H"	5–6 μ (yellowish green)	Chlorophyceae, Polyblepharidaceae
"I"	3–5 μ (golden brown)	Chrysophyceae, Chrysomonadales

Some of these species were isolated by one of the authors (M. W. P.) but assistance in this matter was also received from Dr F. Gross while working at the Plymouth Laboratory. Those isolated at Port Erin were taken from the water of the "ponds" or from sea water collected outside Port Erin Bay, and all are maintained in "Erdschreiber"* culture solution. Two stock cultures of each species are always kept, in volumes of 1000 and 100 c.c. respectively, each in a wide-necked conical flask capable of holding twice the volume. All flasks are illuminated by north light only and the contents are subcultured monthly. The seasons 1937 and 1938 were devoted to this aspect of the work. In 1937 very little experimental work could be done owing to the failure of the stock oysters to produce viable larvae, either in the pond or in indoor trays. Only two apparently normal broods of larvae were liberated. These were used in experiment but after a few days, the larvae sank to the bottom of the culture vessels, showing signs of degeneration of the velum; in many cases the larger velar cilia had been cast off. The data here recorded are therefore largely drawn from the experiments of 1938.

In the following account the number given to a bell-jar refers to the

* "Erdschreiber" culture solution: $NaNO_3$, 0·1 g.; Na_2HPO_4, 0·02 g.; soil extract, 50 c.c.; sea water, 1000 c.c.

positions in the culture-house, numbered 1–20 in Fig. 2, below. Since the incidence of light varied according to the position of the jar and also the mechanical limitations of the culture-house prevented the inclusion of jars 17, 18, 19 and 20 in the plunger-stirring system and of 18, 19 and 20 in the drip-feed supply mechanism, the numbers given to the jars define their position and are of some significance.

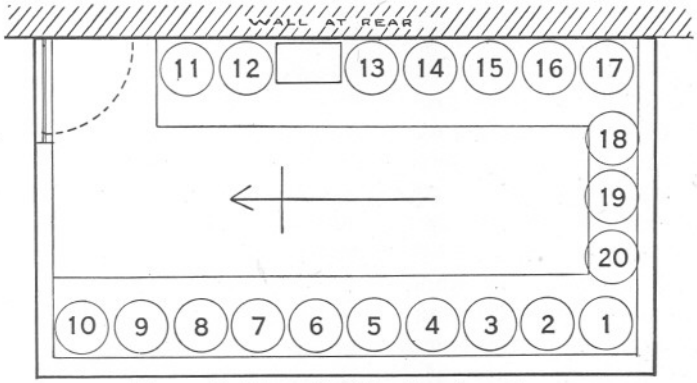


Fig. 2. Position of bell-jars in culture-house.

The Experiments of 1938

The experiments of 1938 differ from earlier ones in that they were carried out with newly emitted larvae of uniform age. All the experiments are therefore comparable one with another except for the fact that larvae liberated by different oysters may show differences in size and viability. The larvae were liberated either in the culture-house tanks, or in the Hatchery trays. The water supplying these tanks and trays had a *p*H value approximately equal to that of the sea water outside Port Erin Bay, which was used exclusively in all the 1938 experiments. Readings of the *p*H value of the sea water in the culture vessels were recorded at the beginning of an experiment and periodically while it was in progress. Temperature readings, recorded twice daily, at 10 a.m. and 6 p.m. were, in practice, taken more frequently, since changes in the weather had to be counteracted by the opening and closing of the culture-house windows in order to maintain the water in the bell-jars at a steady temperature between 20° and 22° C.

Qualitative observations on the microplanktonic organisms either occurring naturally in the sea water or added to it in the course of experiment, were made periodically and the number of organisms per cubic millimetre of sea water recorded. When unfiltered "outside" sea water was used, analyses were carried out using the same grouping of organisms as that adopted in the estimation of the pond microplankton (p. 342). In experiments in which pure cultures of flagellates were tried as the sole supply of food for the larvae, a quantity of a rich culture was added every other day, the volume being

proportioned to the count of organisms present, a count of approximately 50 per mm.^{3} being accepted as standard.

In all experiments in which filtered sea water was used, the larvae, before being added to the culture vessels, were washed very thoroughly in changes of filtered sea water to remove extraneous organisms. The number of larvae put into each jar was only roughly estimated but at the close of the experiment exact counts of all spat, surviving larvae and empty shells were made, so that the actual initial number of larvae added could be known and the true percentage of settlement calculated. To facilitate the measurement of growth, larvae were divided into size groups differing by 15 μ and in any sample the percentage of larvae in each group was recorded. Comparison of analyses of samples at various times in the course of an experiment and at the end gave data upon which the rate of growth could be calculated. The ages of the larvae were recorded as days after liberation from the parent.

As soon as spat-fall occurred the numbers of spat were counted at frequent intervals with a view to obtaining a curve representing the rate of spat-fall. In order to avoid the recounting of thousands of spat at frequent intervals, shells were replaced by fresh ones as soon as approximately a hundred spat had settled on them. Valves of *Pecten maximus* were used mainly as spat-collectors, but oyster, mussel and limpet shells were occasionally substituted. Comparison of spat-fall on upper and lower surfaces and on individual shells in different positions in the culture vessels showed inequality of settlement, but the factors controlling this distribution are not yet apparent.

Serial Record of the Experiments

The laboratory experiments carried out in 1938 fell into twelve consecutive groups; ten were devoted to the main problem of investigating the feeding and growth of the larvae, and the remaining two were short-period experiments intended to solve subsidiary problems.

Group I (Table III) was set up to test the efficiency of Berkefeld-filtered "outside" sea water plus added organisms as contrasted with "outside" unfiltered sea water. It was found that the larvae failed to develop with filtered "outside" sea water. This must have been due either to the filtering of the sea water or to the unsuitable nature of the organism, flagellate "D", which had been added. To test this point, groups II and III (Tables IV and V) were set up, with different broods of larvae respectively. In these groups the following were contrasted as media for larval culture:

(*a*) Filtered sea water.
(*b*) Filtered sea water plus flagellate, "F".
(*c*) Filtered sea water plus flagellate, "I".
(*d*) Unfiltered sea water.
(*e*) Unfiltered sea water plus flagellate, "I".
(*f*) Filtered sea water gradually replaced by unfiltered water.

At the end of these experiments it was found that the greatest success had been obtained in culture vessels to which flagellate "I" had been added irrespective of whether filtered or unfiltered sea water had been used. This appeared to indicate that the successful settlement was connected with the presence of flagellate "I". The same type of result was obtained in both groups II and III.

In group V (Table VII) relative values of filtered and unfiltered sea water plus flagellate "I" and unfiltered sea water without addition were tested. The results of these experiments confirmed the view that the addition of flagellate "I" was a significant factor, for excellent settlement results were obtained with filtered sea water plus flagellate "I" (99 %) and unfiltered sea water plus flagellate "I" (84 %), while no settlement occurred in unfiltered sea water alone. Similar experiments with flagellate "I" were repeated later in the season in groups VIII, XI and XII (Tables X, XIII and XIV) but gave less uniform results.

Groups VI, VII and IX (Tables VIII, IX and XI) were directed towards ascertaining the relative values of "B", "C", "D", "H" and "I" as food material. Of these "H" and "I" were superior to the rest.

The other groups of experiments were intended to solve subsidiary problems. Group IV (Table VI) was set up to test the effect of the addition of culture solution ("Erdschreiber") *per se* on the larvae. It was found to be negligible.

The experiments in group X (Table XII) demonstrated the extent to which the larvae removed the organisms from the sea water in the culture vessels. A series of counts were made and checked against a control. It was found, by comparison, that in the vessel provided with the larvae, food organisms disappeared from the fluid at an average rate of 24,000 per larva per day.

An experiment was brought to an end when it was judged that no further growth or settlement was likely to take place. The usual signs indicating that such a time had been reached were the loss of active motility on the part of the larvae and their congregation in heaps on the bottom of the jar. Examination of such larvae showed that many were obviously deteriorating and that numbers of the shells were empty. When observations on successive days showed that such deterioration was progressive and that most of the larvae had become affected the experiment was brought to an end.

A detailed synopsis in tabular form (Table XV) of each group of experiments and a summary table embodying the results of all the groups (except IV and X) follow. In compiling the serial records the following abbreviations are used:

C.H., culture-house;
A., air-stirred;
P., plunger-stirred;
D.–F., drip-fed;
U.O.S., unfiltered "outside" sea water;
F.O.S., filtered "outside" sea water.

TABLE III. GROUP I. JUNE 23–JULY 27 1938. TO COMPARE THE SURVIVAL OF LARVAE IN CULTURE-HOUSE IN UNFILTERED SEA WATER AND FILTERED SEA WATER WITH ADDED FLAGELLATE "D"

Conditions of Experiment. Jars were A., P. and D.-F. Larvae liberated in culture-house from "Yealm" oyster, June 23 and "Lochryan" oyster, June 25.

	U.O.S., D.-F. with U.O.S.		F.O.S. with Flagellate "D", D.-F. with F.O.S.	
Position of jar in C.H.	1	3	2	4
No. of larvae in jar	11,000	8721	8000	11,000
Source of parent	Lochryan	Yealm	Yealm	Lochryan
Food supply	Micro-organisms present in U.O.S.	Micro-organisms present in U.O.S.	Flagellate "D"	Flagellate "D"
No. of micro-organisms per mm.^{3}	June 25 18 28 20 July 5 36 14 61 21 26	18 21 36 38 19	100 140 151	50 74 90 .. ..
Analysis of organisms	Flagellate, 55 % (under 2 μ common) Non-mobile, 45 % 5 μ or less, 85 % Over 5 μ, 15 %	Flagellate, 53 % (under 2 μ rare) Non-mobile, 47 % 5 μ or less, 89 % Over 5 μ, 11 %	Flagellate "D" only	Flagellate "D" only
*p*H of water	8·07–8·22	8·07–8·20	8·08–8·25	8·07–8·20
Development of larvae	Age in days 1 165–180 μ, 4 % 1 180–195 μ, 96 % 11 210–260 μ 13 Umbonate stages 16 210–225 μ, 16 % 16 225–240 μ, 54 % 16 240–255 μ, 30 % 18 "Eyed" stages present 20 1 spat 26 318 spat 32 745 spat	Age in days 1 180–195 μ, 32 % 1 195–210 μ, 68 % 13 210–270 μ 15 Young "eyed" stages 18 210–225 μ, 14 % 18 225–240 μ, 27 % 18 240–255 μ, 18 % 18 255–270 μ, 18 % 18 270–285 μ, 23 % 18 "Eyed stages" present 20 5 spat 22 129 spat 28 1883 spat 34 3121 spat	Age in days 1 180–195 μ, 32 % 1 195–210 μ, 68 % 7 180–210 μ 13 Degenerating 15 Degenerating or dead	Age in days 1 165–180 μ, 4 % 1 180–195 μ, 96 % 5 180–195 μ 11 Degenerating 13 Degenerating or dead
Final condition of larvae	180–225 μ, 32·0 % 225–255 μ, 41·2 % "Eyed" stages, 20·0 % Settled as spat, 6·8 %	195–225 μ, 14·1 % 225–255 μ, 38·1 % "Eyed" stages, 12·0 % Settled as spat, 35·8 %	(No growth) 180–210 μ Settled as spat, Nil	(No growth) 180–195 μ Settled at spat, Nil

TABLE IV. GROUP II. JULY 2–AUGUST 8 1938. TO COMPARE THE SURVIVAL OF LARVAE IN THE CULTURE-HOUSE IN U.O.S. AND F.O.S. WITH AND WITHOUT THE ADDITION OF FLAGELLATES "I" AND "F"

Conditions of Experiment. Larvae liberated on July 2 in culture-house by a "Yealm" oyster. Sizes of larvae, 180–195 μ, 96 %; 195–210 μ, 4 %. Jars were A., P. and D.-F.

	F.O.S.			U.O.S.	
	Drip-fed with F.O.S.			Drip-fed with U.O.S.	Drip-fed with U.O.S.
Position of jar in C.H.	5	6	9	8	7	10
No. of larvae in jar	17,000	17,000	17,000	17,000	17,600	19,389
Food supply	Nil	Flagellate "F"	Flagellate "I"	Micro-organisms in U.O.S. alone	Micro-organisms in U.O.S. alone	Micro-organisms in U.O.S. plus Flagellate "I"
No. of micro-organisms per mm.^{3} July 8	Nil	41	22	41	51	35
15		34	20	28	23	31
22		..	17	19	31	17
29		..	37	..	60	111
Aug. 4		..	72	..	..	..
Analysis of organisms	Nil	Flagellate "F" only	Flagellate "I" only	Flagellate, 70 % (under 2 μ common) Non-motile, 30 % 5 μ or less, 95 % Over 5 μ, 5 %	Flagellate, 48 % (under 2 μ common) Non-motile, 52 % 5 μ or less, 92 % Over 5 μ, 8 %	Flagellate, 63 % (35 % "I") Non-motile, 37 % 5 μ or less, 82 % Over 5 μ, 18 % (*Phaeocystis Poucheti* abundant July 29)
*p*H of water	8·06–8·20	8·05–8·19	8·08–8·24	8·07–8·20	8·08–8·22	8·05–8·38
Development of larvae	Age in days 5 180–195 μ 9 180–210 μ 18 180–210 μ 23 Degenerating	Age in days 5 180–210 μ 9 180–225 μ 18 180–240 μ 23 Degenerating	Age in days 5 180–220 μ 9 180–240 μ 18 180–260 μ 20 Young "eyed" stages present 23 "Eyed" stages present 24 65 spat 27 535 spat 33 901 spat	Age in days 5 180–210 μ 9 180–210 μ 18 180–225 μ 23 Degenerating	Age in days 5 180–210 μ 9 180–240 μ 18 180–260 μ 24 "Eyed" stages present 27 2 spat 33 6 spat	Age in days 5 180–215 μ 9 180–270 μ 13 Young "eyed" stages present 16 "Eyed" stages present 17 400 spat 18 1597 spat (50 % "eyed" larvae) 23 11,335 spat 31 15,689 spat
Final condition of larvae	(No growth) 180–210 μ Settled as spat, Nil	180–210 μ, 44 % 210–225 μ, 48 % 225–240 μ, 8 % Settled as spat, Nil	180–225 μ, 49·7 % 225–255 μ, 34·0 % "Eyed" larvae, 11·0 % Settled as spat, 5·3 %	180–195 μ, 26 % 195–210 μ, 30 % 210–225 μ, 44 % Settled as spat, Nil	180–225 μ, 47·07 % 225–255 μ, 40·00 % "Eyed" stages, 12·00 % Settled as spat, 0·03 %	180–225 μ, 6 % 225–255 μ 5 % "Eyed" stages, 8 % Settled as spat, 81 %

TABLE V. GROUP III. JULY 4–AUGUST 9 1938. DUPLICATE OF GROUP II, USING LARVAE FROM ANOTHER PARENT

Conditions of Experiment. Larvae liberated on July 3 into Hatchery tray by "Port Erin" oyster (2 years old). Size of larvae, 150–165 μ, 4 %; 165–180 μ, 38 %; 180–195 μ, 54 %; 195–210 μ, 4 %. Jars were A., P. and D.-F.

	F.O.S.				U.O.S.	
	Drip-fed with F.O.S.			Drip-fed with U.O.S.	Drip-fed with U.O.S.	
Position of jar in C.H.	11	12	15	14	13	16
No. of larvae in jar	2000	3300	3498	2296	3306	1824
Food supply	Nil	Flagellate "F"	Flagellate "I"	Micro-organisms in U.O.S. alone	Micro-organisms in U.O.S. alone	Micro-organisms in U.O.S. plus Flagellate "I"
No. of micro-organisms per mm.^{3}	July 13 Nil 21 Aug. 3 5	72 37 35 ..	59 24 .. 41	47 41 .. 22	83 20 17 ..	68 31 .. 27
Analysis of organisms	..	July 13 and 21 Flagellate "F" only Aug. 3 Flagellate "F", 75 % Green Flagellate 25 % (15 μ)	Flagellate "I" only	Flagellates, 53 % (under 2 μ common) Non-motile, 47 % 5 μ or less, 89 % Over 5 μ, 11 %	Flagellates, 60 % (under 2 μ very common) Non-motile, 40 % 5 μ or less, 89 % Over 5 μ, 11 %	Flagellates, 60 % (40 % "I") Non-motile, 40 % 5 μ or less, 90 % Over 5 μ, 10 %
*p*H of water	8·13–8·22	8·12–8·20	8·12–8·23	8·12–8·20	8·12–8·20	8·12–8·22
Development of larvae	Age in days 10 {180–195 μ, 33 % {195–210 μ, 67 % 23 Majority dead	Age in days 10 {180–195 μ, 25 % {195–210 μ, 70 % {210–225 μ, 5 % 27 195–275 μ 32 "Eyed" larvae present 37 47 spat	Age in days 10 {210–225 μ, 35 % {225–240 μ, 40 % {240–255 μ, 25 % 19 "Eyed" larvae present 23 371 spat 27 1980 spat 32 2766 spat 36 3178 spat	Age in days 10 {195–210 μ, 15 % {210–225 μ, 75 % {225–240 μ, 10 % 19 "Eyed" larvae present 23 4 spat 27 67 spat 32 191 spat 37 296 spat	Age in days 10 {195–210 μ, 25 % {210–225 μ, 75 % 27 195–270 μ 32 "Eyed" larvae present 37 6 spat	Age in days 10 {210–225 μ, 35 % {225–240 μ, 40 % {240–255 μ, 20 % {255–270 μ, 5 % 19 "Eyed" larvae present 23 142 spat 27 818 spat 32 1463 spat 36 1587 spat
Final condition of larvae	(No growth) 180–210 μ Settled as spat, Nil	195–225 μ, 27·0 % 225–255 μ, 59·5 % "Eyed" stages, 12·0 % Settled as spat, 1·5 %	195–225 μ, 2 % 225–255 μ, 1 % "Eyed" stages, 6 % Settled as spat, 91 %	195–225 μ, 32 % 225–255 μ, 43 % "Eyed" stages, 12 % Settled as spat, 13 %	195–225 μ, 50·0 % 225–255 μ, 49·8 % Settled as spat, 0·2 %	195–225 μ, 2·5 % 225–255 μ, 8·74 % "Eyed" stages, 1·75 % Settled as spat, 87·01 %

TABLE VI. GROUP IV. JULY 6–16 1938. TO ASCERTAIN THE EFFECT OF CULTURE SOLUTION ("ERDSCHREIBER") *PER SE* ON DEVELOPING LARVAE

Conditions of Experiment. Larvae liberated in Hatchery tray on July 6 from "Yealm" oyster. Size of larvae, 165–180 μ, 8 %; 180–195 μ, 88 %; 195–210 μ, 4 %. Jars A. and P. (except 17), D.-F. 10,000 larvae in each jar.

	F.O.S., D.-F. with F.O.S.	U.O.S., D.-F. with U.O.S.	
Position of jar in C.H.	2	4	17
Food supply	Nil, 150 c.c. "Erdschreiber" added daily	Micro-organisms in U.O.S.; 150 c.c. "Erdschreiber" added daily	Micro-organisms in U.O.S.; 150 c.c. "Erdschreiber" added daily plus Flagellate "F"
*p*H of jars	8·12–8·20	8·13–8·24	8·11–8·23
No. of micro-organisms per mm.^{3} July 15	Nil	28	82
Analysis of organisms	..	Flagellate, 72 % (under 2 μ very common) Non-motile, 28 % 5 μ or less, 97 % Over 5 μ, 3 %	Flagellate, 60 % (mostly under 2 μ) Non-motile, 40 % 5 μ or less, 85 % Over 5 μ, 15 %
Final condition of larvae	Growth nil, still alive, normal in appearance, pale in colour	Some growth; normal in shape and colour	Some growth; normal in shape and colour

TABLE VII. GROUP V. JULY 12–AUGUST 3 1938. TO ESTIMATE THE VALUE OF FLAGELLATE "I" AS FOOD FOR DEVELOPING LARVAE

Conditions of Experiment. Larvae liberated July 12 into Hatchery tray by "Port Erin" oyster (3 years old). Size of larvae: 180–195 μ, 64 %; 195–210 μ, 36 %. Jars air-stirred and water changed by siphon.

	F.O.S.	U.O.S.	
Position of jar in C.H.	19	18	20
No. of larvae in jar	12,240	9853	10,000
Food supply	Flagellate "I"	Micro-organisms in U.O.S. plus Flagellate "I"	Micro-organisms in U.O.S.
No. of micro-organisms per mm.^{3}	July 19 60 28 34	54 65	21 21
Analysis of organisms	Flagellate "I" only	Flagellate, 70 % ("I", 60 %) Non-motile, 30 % 5 μ or less, 95 % Over 5 μ, 5 %	Flagellate, 51 % (under 2 μ abundant) Non-motile, 49 % 5 μ or less, 98 % Over 5 μ, 2 %
*p*H of water	8·19–8·30	8·18–8·26	8·18–8·25
Development of larvae	Age in days 6 240–260 μ 8 "Eyed" larvae present 9 1 spat 10 22 spat 13 10,667 spat 15 11,316 spat 17 11,778 spat 21 12,104 spat	Age in days 6 240–255 μ 8 "Eyed" larvae present 9 1 spat 10 31 spat 13 4415 spat 15 5287 spat 17 6148 spat 22 8302 spat	Age in days 6 180–210 μ 13 180–225 μ 15 180–270 μ 17 "Eyed" larvae present 22 Larvae degenerating
Final condition of larvae	180–225 μ, 0·1 % 225–255 μ, 0·1 % "Eyed" stages, 0·8 % Settled as spat, 99·0 %	240–255 μ, 5·7 % "Eyed" stages, 10·0 % Settled as spat, 84·3 %	180–195 μ, 5 % 195–225 μ, 93 % "Eyed" stages, 2 % Settled as spat, Nil

TABLE VIII. GROUP VI. JULY 27–SEPTEMBER 9 1938. TO ASCERTAIN THE RELATIVE VALUES OF FLAGELLATES "B", "C", "H" AND "I" AS FOOD FOR DEVELOPING LARVAE

Conditions of Experiment. Larvae liberated in Hatchery tray on July 27 from "Yealm" oyster. Size of larvae: 165–180 μ, 8 %; 180–195 μ, 64 %; 195–210 μ, 28 %. All jars filled with F.O.S.; and A., P. and D.-F. with F.O.S.

Position in C.H.	2	5	6	3	11
No. of larvae in jar	15,389	10,079	17,107 (but 11,704 transferred to jar 3 on Aug. 24)	11,704 (transferred from jar 6 on Aug. 24)	11,105
Food supply	Flagellate "B"	Flagellate "I"	Flagellate "H"	Flagellates "H"+"I"	Flagellate "C"
No. of micro-organisms per mm.^{3}	Aug. 1 19 12 37 22 44	19 24 31	30 30 42	(Aug. 28) 47	14 33 45
Analysis of organisms	Flagellate "B" only	Flagellate "I" only	Flagellate "H" only	Flagellate "H", 34 % Flagellate "I" 66 %	Flagellate "C" only
*p*H of water	8·20–8·25	8·18–8·25	8·13–8·24	8·20–8·22	8·18–8·22
Development of larvae	Age in days 7 210–255 μ 9 210–270 μ 12 "Eyed" larvae present, 285 μ 14 "Eyed" larvae present, 300 μ 16 2 spat 20 972 spat 24 2391 spat 28 4683 spat 30 5023 spat 33 7529 spat	Age in days 7 195–255 μ 9 195–270 μ 12 195–270 μ 14 "Eyed" larvae present, 285 μ 17 3 spat 20 159 spat 24 384 spat 27 392 spat	Age in days 7 195–240 μ 9 195–255 μ 17 195–270 μ 23 "Eyed" larvae present, 285 μ 28 Two-thirds of larvae removed to jar 3 30 60 spat 35 1157 spat 40 4103 spat	Age in days (Transferred from jar 6) 28 225–285 μ (mostly "eyed") 30 No spat 31 151 spat 34 5025 spat 35 5085 spat 38 7124 spat 45 11,151 spat	Age in days 7 210–255 μ 12 210–270 μ 17 "Eyed" larvae present, 285 μ 20 60 spat 24 941 spat 26 990 spat 27 994 spat
Final condition of larvae	210–225 μ, 2 % 225–255 μ, "Eyed" stages, 15 % Settled as spat, 34 % 49 %	195–225 μ, 37 % 225–255 μ, "Eyed" stages, 23 % Settled as spat 34 % 6 %	195–225 μ, 1 % 225–255 μ, "Eyed" stages, 6 % Settled as spat, 17 % 76 %	225–255 μ, "Eyed" stages, 1·5 % Settled as spat, 3·2 % 95·3 %	210–225, 10·5 % 225–255 μ, "Eyed" stages, 25·0 % Settled as spat, 53·5 % 11·0 %

TABLE IX. GROUP VII. AUGUST 1–SEPTEMBER 6 1938. TO ASCERTAIN THE RELATIVE VALUES OF DIFFERENTLY COLOURED FLAGELLATES AS FOOD FOR DEVELOPING LARVAE

Conditions of Experiment. Larvae liberated in Hatchery tray, July 30 from "Lochryan" oyster. Size of larvae: 180–195 μ, 76 %; 195–210 μ, 24 %. All jars filled with F.O.S.; A., P. and D.-F. with F.O.S.

Position of jar in C.H.	1	3	4
No. of larvae in jar	11,107	11,000	11,000
Food supply	Flagellate "D"	Flagellate "I"	Flagellate "H"
*p*H of water	8·20–8·24	8·18–8·24	8·14–8·22
Development of larvae	Age in days 4 180–210 μ 14 195–240 μ 20 195–255 μ (many dead) 32 Few "eyed" larvae present 38 7 spat	Age in days 4 180–210 μ 14 195–245 μ (many dead) 17 Degenerating 20 Degenerating or dead	Age in days 4 180–210 μ 14 195–240 μ 20 195–270 μ (many dead) 24 Few "eyed" larvae present 28 Degenerating or dead
Final condition of larvae*	195–225 μ, 39·94 % 225–255 μ, 32·00 % "Eyed" stages, 28·00 % Settled as spat, 0·06 %	200–245 μ, 100 % Settled as spat, Nil	195–225 μ, 48 % 225–255 μ, 40 % "Eyed" stages, 12 % Settled as spat, Nil

* Lack of success almost certainly due to sub-normal larvae; majority showed little activity and died off at early stage.

TABLE X. GROUP VIII. AUGUST 4–SEPTEMBER 6 1938. TO CONFIRM RESULTS OF GROUP V

Conditions of Experiment. Larvae liberated in Hatchery tray on August 4, from "Yealm" oyster. Size of larvae: 165–180 μ, 8 %; 180–195 μ, 77%; 195–210 μ, 15 %. Jars air-stirred and water changed by siphon.

	U.O.S.	F.O.S.	
Position in C.H.	19	18	20
No. of larvae in jar	13,500	13,500	13,578
Food supply	Micro-organisms in U.O.S. plus Flagellate "I"	Flagellate "I"	Flagellate "I"
No. of micro-organisms per mm.^{3} on Aug. 18	52	23	41
Analysis of organisms	Flagellate, 80 % (62 % "I") Non-motile, 20 % 5 μ or less, 98 % Over 5 μ, 2 %	Flagellate "I" only	Flagellate "I" only
*p*H of water	8·23–8·25	8·22–8·26	8·22–8·24
Development of larvae*	Age in days 6 195–220 μ (abnormal) 14 Deformed 18 Majority dead	Age in days 6 210–225 μ (abnormal) 11 "Eyed" larvae present, 225 μ With foot, 232 μ 14 20 spat 18 215 spat 22 341 spat 27 485 spat 33 698 spat	Age in days 6 195–225 μ (abnormal) 11 "Eyed" larvae present, 225 μ 15 7 spat 18 117 spat 22 690 spat 27 801 spat
Final condition of larvae	195–225 μ, 96 % 225–240 μ, 4 % Settled as spat, Nil	195–225 μ, 64 % "Eyed" stages, 31 % Settled as spat, 5 %	195–225 μ, 71 % "Eyed" stages, 18 % Settled as spat, 11 %

* After a few days larvae showed signs of shell irregularity. The edge of the shell appeared to have grown inwards, constricting and deforming the velum. The "eyed" stage was recorded at 225–250 μ.

TABLE XI. GROUP IX. AUGUST 8–29 1938. TO ASCERTAIN THE RELATIVE VALUES OF FLAGELLATES "B" AND "I" AS FOOD FOR DEVELOPING LARVAE

Conditions of Experiment. Larvae liberated in Hatchery tray on August 6, from "Port Erin" oyster (3 years old). Size of larvae: 180–195 μ, 53%; 195–210 μ, 47%, and from "Yealm" oyster August 7. Size of larvae: 180–195 μ, 44%; 195–210 μ, 56%. All jars filled with F.O.S. A. and P., D.-F. with F.O.S.

Position in C.H.	7	8	9	10
No. of larvae in jar	9000	9000	11,877	8699
Source of parent	Port Erin	Yealm	Port Erin	Yealm
Food supply	Flagellate "B"	Flagellate "B"	Flagellate "I"	Flagellate "I"
No. of micro-organisms per mm.^{3}	Aug. 16 59 22 29	45 31	22 31	111 61
Analysis of organisms	Flagellate "B" only	Flagellate "B" only	Flagellate "I" only	Flagellate "I" only
*p*H of water	8·18–8·21	8·18–8·21	8·16–8·21	8·22–8·24
Development of larvae	Age in days 4 195–210 μ 9 195–240 μ 11 195–260 μ 13 195–270 μ 17 Degenerating 23 Majority dead	Age in days 3 195–210 μ 8 195–230 μ 10 195–270 μ 12 195–270 μ 17 Degenerating 22 Majority dead	Age in days 4 195–210 μ 9 225–255 μ 11 "Eyed" larvae present, 285 μ 13 9 spat 15 3458 spat 16 6495 spat 17 8418 spat 18 9621 spat 21 10,536 spat 23 11,051 spat	Age in days 3 195–210 μ 8 195–255 μ 10 "Eyed" larvae present, 300 μ 11 6 spat 12 120 spat 14 558 spat 17 859 spat
Final condition of larvae	195–225 μ, 52% 225–255 μ, 44% 255–270 μ, 4% Settled as spat, Nil	195–225 μ, 36% 225–255 μ, 44% 255–270 μ, 20% Settled as spat, Nil	225–255 μ, 2·32% "Eyed" stages, 4·64% Settled as spat, 93·04%	210–225 μ, 10% 225–255 μ, 40% "Eyed" stages, 40% Settled as spat, 10%

TABLE XII. GROUP X. AUGUST 8–12 1938. TO DEMONSTRATE THE RATE OF INGESTION OF FLAGELLATES BY DEVELOPING LARVAE

Conditions of Experiment. Larvae liberated in Hatchery tray, August 7, by "Yealm" oyster. Size of larvae: 180–195 μ, 44%; 195–210 μ, 56%. Jars filled with F.O.S. A., P. No change of water.

Position of jar		15	16
No. of larvae in jar		Nil	10,000
Food supply		Flagellate "I" added to give 78 per mm.^{3} at beginning of experiment	Flagellate "I" added to give 76 per mm.^{3} at beginning of experiment
No. of micro-organisms per mm.^{3}	Aug. 8	78	76 (larvae added)
	9	75	56
	10	76	52
	11	65	32
	12	72	15
*p*H of water		8·20 (constant)	8·20–8·23

Comment. Jars 15 and 16 received equal illumination as measured by photometer. The decrease of Flagellates in 16 amounts to 24,000 organisms per larva per day.

TABLE XIII. GROUP XI. AUGUST 9–19 1938. TO REPEAT PREVIOUS GROUPS USING FLAGELLATE "I"

Conditions of Experiment. Larvae used were liberated on August 9 into Hatchery tray by Port Erin oyster (4 years old). Size of larvae: 180–195 μ, 96%; 195–210 μ, 4%. Number of larvae per jar, 10,000. All jars were supplied with F.O.S., A., P. and D.-F. with F.O.S.

Position of jar	12	13	14
*p*H of water	8·19–8·22	8·21–8·22	8·21–8·22
Food supply	Nil	Flagellate "I"	Flagellate "I"
No of micro-organisms per mm.^{3} on Aug. 17	Nil	27	20
Development of larvae Age in days			
6	180–210 μ	195–225 μ	195–225 μ
8	180–210 μ	Majority degenerating	Majority degenerating
10	Degenerating	Velum abnormal	Velum abnormal
*Final condition of larvae	180–210 μ, 100% Settled as spat, Nil	195–225 μ, 100% Settled as spat, Nil	195–225 μ, 100% Settled as spat, Nil

* Lack of success almost certainly due to sub-normal larvae.

TABLE XIV. GROUP XII. AUGUST 29–SEPTEMBER 26 1938. TO REPEAT GROUP XI WITH LARVAE LIBERATED LATE IN THE SEASON

Conditions of Experiment. Larvae liberated in Hatchery tray, August 27, by "Blackwater" oyster. Size of larvae: 165–180 μ, 4%; 180–195 μ, 92 %; 195–210 μ, 4 %. Jars filled with F.O.S. A., P., D.-F. with F.O.S. Supplied with Flagellate "I".

Position of jar	7	9	10
No. of larvae in jar	13,400	24,000	3400
No. of micro-organisms per mm.^{3}	Sept. 5 35 11 31 15 28	30 21 15	40 45 41
Analysis of organisms	Flagellate "I" only	Flagellate "I" only	Flagellate "I" only
*p*H of water	8·10–8·25	8·10–8·25	8·20–8·25
Development of larvae	Age in days 12 180–225 μ 16 180–230 μ 18 180–255 μ 23 "Eyed" larvae present 24 A few spat 30 522 spat	Age in days 12 180–225 μ 16 180–260 μ 18 180–270 μ "Eyed" larvae, 260 μ With foot, 270 μ 21 A few spat 23 Over 100 spat 24 277 spat 30 1192 spat	Age in days 12 180–225 μ 16 180–240 μ 23 "Eyed" larvae present, 24 A few spat 30 484 spat
Final condition of larvae	180–225 μ, 35 % 225–255 μ, 23 % "Eyed" stages, 38 % Settled as spat, 4 %	180–225 μ, 46 % 225–255 μ, 38 % "Eyed" stages, 11 % Settled as spat, 5 %	180–225 μ, 20·9 % 225–255 μ, 56·8 % "Eyed" stages, 8·0 % Settled as spat, 14·3 %

TABLE XV. SYNOPTIC TABLE OF LABORATORY EXPERIMENTS (EXCLUDING GROUPS IV AND X) 1938

Group and date	Position of jar	Source of parent	Date of liberation	Size range of larvae	Water supply*	Food supply	Growth of larvae	Settlement %
I	1	Lochryan	June 25	165–195 μ	U.O.S.	Micro-organisms in sea water	Fair	6·8
June 25–July 27	2	Yealm	23	180–210 μ	F.O.S.	Flagellate "D"	None	Nil
	3	Yealm	23	180–210 μ	U.O.S.	Micro-organisms in sea water	Fairly good	35·8
	4	Lochryan	25	165–195 μ	F.O.S.	Flagellate "D"	None	Nil
II	5	Yealm	July 2	180–210 μ	F.O.S.	Nil (control)	None	Nil
July 2–Aug. 5	6	Yealm	2	180–210 μ	F.O.S.	Flagellate "F"	Slight	Nil
	7	Yealm	2	180–210 μ	U.O.S.	Micro-organisms in sea water	Fair	0·03
	8	Yealm	2	180–210 μ	F.O.S. to U.O.S.	Micro-organisms in sea water	Slight	Nil
	9	Yealm	2	180–210 μ	F.O.S.	Flagellate "I"	Fair	5·3
	10	Yealm	2	180–210 μ	U.O.S.	Micro-organisms in sea water plus "I"	Very good	81·0
III	11	P.E. (2 yr. old)	July 3	150–210 μ	F.O.S.	Nil (control)	None	Nil
July 4–Aug. 9	12	P.E. (2 yr. old)	3	150–210 μ	F.O.S.	Flagellate "F"	Fair	1·5
	13	P.E. (2 yr. old)	3	150–210 μ	U.O.S.	Micro-organisms in sea water	Slight	0·2
	14	P.E. (2 yr. old)	3	150–210 μ	F.O.S. to U.O.S.	Micro-organisms in sea water	Fairly good	13·0
	15	P.E. (2 yr. old)	3	150–210 μ	F.O.S.	Flagellate "I"	Excellent	91·0
	16	P.E. (2 yr. old)	3	150–210 μ	U.O.S.	Micro-organisms in sea water plus "I"	Very good	87·01
V	18	P.E. (3 yr. old)	July 12	180–210 μ	U.O.S.	Micro-organisms in sea water plus "I"	Very good	84·3
July 12–Aug. 3	19	P.E. (3 yr. old)	12	180–210 μ	F.O.S.	Flagellate "I"	Excellent	99·0
	20	P.E. (3 yr. old)	12	180–210 μ	U.O.S.	Micro-organisms in sea water	Slight	Nil
VI	2	Yealm	July 27	165–210 μ	F.O.S.	Flagellate "B"	Good	49·0
July 27–Sept. 10	5	Yealm	27	165–210 μ	F.O.S.	Flagellate "I"	Fairly good	6·0
	6	Yealm	27	165–210 μ	F.O.S.	Flagellate "H"	Very good	76·0
	3	Yealm	27	225–285 μ	F.O.S.	Flagellates "H" and "I"	Excellent	95·3
	11	Yealm	27	165–210 μ	F.O.S.	Flagellate "C"	Very good	11·0
VII	1	Lochryan	July 30	180–210 μ	F.O.S.	Flagellate "D"	Fair	0·06
Aug. 1–Sept. 6	3	Lochryan	30	180–210 μ	F.O.S.	Flagellate "I"	Slight	Nil
	4	Lochryan	30	180–210 μ	F.O.S.	Flagellate "H"	Fair	Nil
VIII	18	Yealm	Aug. 4	165–210 μ	F.O.S.	Flagellate "I"	Shell deformed	5·c
Aug. 4–Sept. 6	19	Yealm	4	165–210 μ	U.O.S.	Micro-organisms in sea water plus "I"	Shell deformed	Nil
	20	Yealm	4	165–210 μ	F.O.S.	Flagellate "I"	Shell deformed	11·0
IX	7	P.E. (3 yr. old)	Aug. 6	180–210 μ	F.O.S.	Flagellate "B"	Fair	Nil
Aug. 8–Aug. 29	8	Yealm	7	180–210 μ	F.O.S.	Flagellate "B"	Fair	Nil
	9	P.E. (3 yr. old)	6	180–210 μ	F.O.S.	Flagellate "I"	Excellent	93·04
	10	Yealm	7	180–210 μ	F.O.S.	Flagellate "I"	Good	10·0
XI	12	P.E. (4 yr. old)	Aug. 9	180–210 μ	F.O.S.	Nil (control)	None	Nil
Aug. 9–Aug. 19	13	P.E. (4 yr. old)	9	180–210 μ	F.O.S.	Flagellate "I"	Very slight	Nil
	14	P.E. (4 yr. old)	9	180–210 μ	F.O.S.	Flagellate "I"	Very slight	Nil
XII	7	Blackwater	Aug. 27	165–210 μ	F.O.S.	Flagellate "I"	Fairly good	4·0
Aug. 29–Sept. 26	9	Blackwater	27	165–210 μ	F.O.S.	Flagellate "I"	Fair	5·0
	10	Blackwater	27	165–210 μ	F.O.S.	Flagellate "I"	Fair	14·3

Groups I and II carried out with larvae liberated in culture-house.
Groups III to XII carried out with larvae liberated in Hatchery trays.
Groups VII, VIII and XI, on account of abnormality of larvae, are disregarded in subsequent discussion.

* U.O.S. Unfiltered "outside" sea water; F.O.S. Filtered "outside" sea water.

REVIEW OF RESULTS

In the groups of experiments dealing with the growth of larvae, forty individual experiments were carried out; in nine of these, belonging to groups VII, VIII and XI, the larvae were found to be non-viable, or showed abnormalities at an early stage of the experiment. Since no reliable deductions could be made from the use of such larvae, these nine experiments have been excluded from general discussion.

Thirty-one experiments therefore come under review. Of these, two were controls in filtered sea water without added food. In twenty-two of the remaining twenty-nine, settlement of larvae was obtained, the average settlement being 39·5 %.

As the main object of these experiments was to test the food value of certain small flagellates, the greater number of culture vessels were supplied with filtered sea water to which flagellate cultures were added. Some of the experiments, however, were carried out with the use of unfiltered "outside" sea water with and without the addition of flagellates. Two experiments were also set up with filtered sea water which was subsequently changed to unfiltered sea water. A summary of results grouped according to water and food supply is given in Tables XVI and XVII.

TABLE XVI. DETAILS OF SETTLEMENT WITH VARIOUS TYPES OF FOOD SUPPLY IN BERKEFELD-FILTERED AND IN UNFILTERED "OUTSIDE" SEA WATER

Sea water ...	Filtered								Unfiltered	
	Flagellates							Unfiltered "outside" sea water added		
Food supply ...	"I"	"H"	"H" and "I"	"B"	"C"	"F"	"D"		None added	Flagellate "I"
Percentage settlement	99·0	76·0	95·3	49·0	11·0	1·5	0·0	13·0	35·8	87·01
	93·04	..	..	0·0	..	0·0	0·0	0·0	6·8	84·3
	91·0	..	..	0·0	..	..	..	..	0·2	81·0
	14·0	..	..	..	..	..	..	..	0·03	..
	10·0	..	..	..	..	..	..	..	0·0	..
	6·0	..	..	..	..	..	..	..	..	..
	5·3	..	..	..	..	..	..	..	..	..
	5·0	..	..	..	..	..	..	..	..	..
	4·0	..	..	..	..	..	..	..	..	..

From the results obtained, the food value of the different flagellates can be roughly assessed as follows:

(i) Good to very good, "I" and "H".
(ii) Fair, "B" and "C".

The highest figures for percentage settlement (99 %, 91 %) were reached in experiments in which flagellates were added to filtered sea water, but the figures for parallel and comparable experiments in which flagellates were

added to unfiltered sea water were not far behind (84%, 87%). The number of such comparable pairs of experiments is probably too small to give valuable data, but, taking the average of their results, the percentage settlement is found to be 84% in unfiltered and 65% in filtered sea water, in each case with the addition of flagellate "I". The further fact emerges that both gave vastly superior results to comparable experiments in which "outside" unfiltered sea water, *without addition*, was used (0·03%, 0·2%).

TABLE XVII. COMPARISON OF SETTLEMENTS WITH VARIOUS TYPES OF FOOD SUPPLY

		No. of jars and result			Average settlement in all jars
Sea water	Added food	Total	Settlement	No settlement	%
F.O.S.	Nil	2	0	2	0
	"B"	3	1	2	16·3
	"C"	1	1	0	11·0
	"D"	2	0	2	0
	"F"	2	1	1	0·75
	"H"	1	1	0	76·0
	"I"	9	9	0	36·4
	"H" and "I"	1	1	0	95·3
	"Outside" sea water	2	1	1	6·5
U.O.S.	Nil	5	4	1	8·6
	"I"	3	3	0	84·1

One experiment was tried in 1938 using a mixed diet of flagellates "H" and "I" as food for the larvae, "I" being added when the larvae were well advanced. The 95·3% settlement obtained suggests that the suitability of "mixed" as opposed to "pure" diet should be the subject of further investigation. It has been observed that in the majority of the experiments in which flagellate "I" has been used, settlement has proceeded almost to completion within twenty days, whereas in the two experiments in which flagellate "H" was used a comparable settlement occurred only after the lapse of thirty-five days.

Scrutiny of Table XVII shows inconsistency of result that demands consideration. After eliminating such differences of percentage settlement as may be associated with the factors of food materials and character of water supply, there still remains a number of discrepant results which cannot be satisfactorily explained by either of these factors.

Divergent results are seen in comparable experiments in which the only variable factor is that associated with the origin of the larvae. Broods of larvae differ in quality one from another and their viability is undoubtedly a factor of importance in experiment. It is not, however, always possible to assess the physiological state of the larvae by inspection at the liberation stage, although as soon as signs of deterioration make their appearance, an experiment can be discarded.

In other experiments variation in result appears to be associated with the position, in relation to illumination, of the bell-jars in the culture-house (see p. 351). Reviewing the entire experimental series, jars which gave settlements of over 80 % were those situated in the comparatively well-lighted positions at the ends of the benches, whereas a low average settlement coincided in the main with the positions of lower light intensity. Much remains to be done to disentangle so many coincident factors, but of the reality of the effect of these factors in larval culture there is now little doubt.

In 1938, as in earlier years, considerable variation was noted in the time-relations of larval settlement under which heading may be included not only

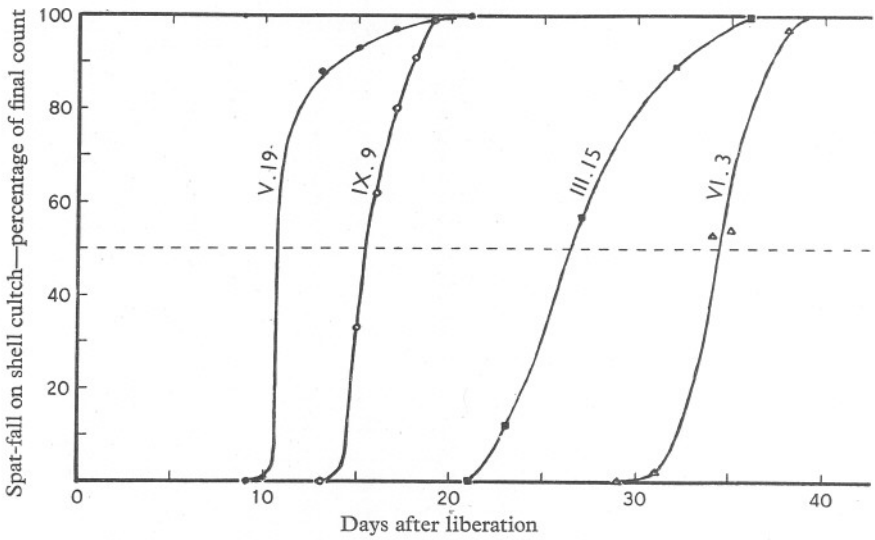


Fig. 3. Variations in the time-relations of larval settlement in four representative experiments. (Numbers on curves indicate group and position.)

the duration of larval life prior to settlement, but also the rate of settlement in its vigorous initial phase, and the total period over which spat-fall may be extended. These points are illustrated by Fig. 3, in which are embodied data from four representative experiments, all of which yielded a spat-fall exceeding 90% of the initial larval count.

In the figure, the progress of spat-fall (expressed as a growing percentage of the final spat-fall realized) is plotted against time, in "days after liberation". The four curves shown are similar in general form, and indicate that spat-fall has an initial phase of greater or less rapidity, followed by a "tailing-off", as maximum spat-fall (whatever its absolute value) is approached. Apart, however, from general similarity of type, the spat-fall curves show a wide range of variation. It will be seen that (*a*) the onset of substantial settlement (say 10 % of the final settlement realized) may occur at any time within

11–33 days after liberation, and (*b*) the rate of settlement, as indicated by the slope of the curve, over the first 50 %, may vary as 1 to 3.

Settlements exceeding 95 % were realized at either end of the time-scale, and since all experiments were carried out at a temperature of 20°–22° C., the factor of temperature cannot, in this instance, be directly associated with the length of larval life before settlement.

As to the actual duration of larval life under the conditions of experiment in the culture-house, a minimum duration was observed in group V (jars 18 and 19), in which two larvae were found to have settled after 9 days, and the vast majority after 11–14 days. In other experiments 20–25 days elapsed before the greater part of the settlement was achieved, while in certain experiments of group VI, there was no considerable settlement until the 32nd day, and some larvae were still unmetamorphosed after 40 days. This appears to be an exceptional figure and due to special causes.

Of the total spat-fall in the experiments, 85 % was on shells (chiefly *Pecten maximus*), 13 % on glass surfaces (jars, plunger plates and aerator tubes), and 2 % of the spat had metamorphosed without attachment. Out of the spat-fall occurring on shells, irrespective of whether the convex or concave surface of the valves was uppermost, 47 % were found to be on the upper, i.e. the illuminated surface, and 53 % on the lower shaded surface.

DISCUSSION

In order to understand fully the significance of the data provided by the series of investigations recorded above, it may be of advantage to compare some of the results obtained with the findings of other investigators who have made observation on the behaviour and growth of oyster larvae in relatively small volumes of water.

Kändler (1930), working in Heligoland, obtained a small settlement of spat in large vessels supplied with a continuous flow of sea water. It is assumed that small organisms present in the inflowing sea water served as food for the larvae. Erdmann (1933), using a technique devised by Hagmeier in 1932 in which minute organisms present in sea water were developed in pure culture by the Schreiber method and used as food by the larvae, reports successful settlement experiments. He speaks of 15,000 spat obtained in twelve vessels (Tongefässe). This work was carried out in a specially built culture-house.

It is also known that samples of *Ostrea edulis* sent over to America there produced larvae, a very large proportion of which developed and later settled as spat. The rich planktonic content of the natural sea water clearly supplied sufficient food material for the larvae.

Two lines of enquiry were thus indicated. The first relates to the maintenance of oyster larvae in sea water in which the natural plankton supplied the food material; and the second is concerned with experiments in which

addition of cultures of organisms suitable as food material for larvae, is made to the sea water.

Despite the comparative paucity of settlement obtained in the earlier experiments cited, the fact that settlement occurred at all, showed that the conditions obtaining in vessels of small size are not wholly inimical to the culture of larvae. With refinement of technique, it has now been proved possible to conduct critical experiments on a laboratory scale, and so to control or vary the conditions as to afford a close analysis of the separate factors governing larval life.

The high rate of mortality among developing larvae, which has been the experience of many workers in the field, may be considered to be attributable to two sets of factors which are inseparably associated with the fact of isolation of any small body of water. These factor-groups may be described as "biological" and "biophysical"—the one concerns the presence or absence of the appropriate food material, and the conditions leading to the further production of that material; the other involves the delicate physical balance, or dissociation-equilibrium, upon which the continued usefulness of sea water as a medium for growth and development so greatly depends. Experimentally, it is relatively easy to control the grosser physical conditions such as temperature and illumination, and even to observe their effects, and a considerable body of knowledge has been accumulated by ourselves, and by other workers in the field, upon which we were able to draw when making plans for the culture-house in 1935. This culture-house provided, as nearly as resources would permit, an optimal environment for that more intimate enquiry into food requirements and the character of water supply which we have indicated.

Taking first the latter problem, the obvious approach to the question lies in avoiding, so far as possible, any procedure, such as storage, undue illumination, or chemical addition, which is likely to promote or increase disturbance of internal equilibrium. To a certain extent this has been achieved by the use of sea water collected well outside the limits of littoral influence, transporting it as rapidly as possible to the culture-house, and using it (by way of a drip-feed system) in such a way as to minimize the period of its sojourn in any experimental vessel. Experiments on these lines (other factors, of course, being suitable) have yielded a high degree of success, but the authors do not on that account claim that "outside" sea water is the only appropriate medium for larval cultivation. Continued growth and settlement of larvae has been realized at Port Erin, Conway and elsewhere, under conditions far removed from those associated with open waters. In such experiments, it is our belief that the large volumes of the enclosures concerned, or special experimental safeguards if on the laboratory scale, have contributed to the maintenance of the essential biotic and physical equilibrium, and we are still convinced that, as a factor in the success of *experiments on the laboratory scale*, the use of natural water from the open sea, untreated otherwise than by filtration, is of paramount importance.

Turning to the biological aspect of the problem, from which it would appear that failure to settle might be due to insufficiency or unsuitability of food material, the line of experiment indicated was simply to add cultures of organisms to the sea water used in experiment. Using growth and percentage settlement as criteria of success, it could then be determined which organisms in culture, alone or in conjunction with others, provided the best food material for the larvae.

Perusal of the records of 1938 shows that the percentage settlement therein recorded for experiments in which certain flagellates have been used as food for the larvae, so greatly exceeds any previous record for similar experiments, that the conclusion is inescapable, that for these particular experiments the demand for suitable food supplies and a reasonably normal biophysical environment have been adequately satisfied. In this connexion, attention may be drawn to the results of an experiment recorded in Table VII in which 12,104 spat were obtained from 12,240 larvae originally introduced into one bell-jar.

The feasibility of rearing oyster larvae by artificial feeding has now been definitely established and this field of enquiry is open to further exploration. Up to the present, six separate flagellate organisms have been tested as food material for larvae. One of the most interesting facts that has emerged most clearly from the results already obtained, is that the six organisms differ markedly in their usefulness. The settlement-ratio is from 0·06 to 99%. The six organisms may be grouped in pairs, giving respectively good, medium and poor results, but with all of them some settlement was obtained.

The question naturally offering itself for solution is the reason why, other factors being equal or as nearly equal as experimental conditions permit, the values of these flagellates as food for larvae should differ so markedly one from another.

In actual experiment, not one of the organisms, even those classed as good, gave uniform results, but the inconsistency shown is possibly due, in part at least, to the supervention of factors outside experimental control. One of the chief sources of trouble lies in the fact that larvae from different oysters are not equally viable (see groups V, VIII and XI, Tables VII, X and XIII). At the present stage of experiment there is no evidence that the length of larval life is affected by the point in time within the spawning period at which they are liberated.

Leaving aside, however, these imposed and uncontrollable factors, differences between the flagellates themselves were detectable and almost certainly played a discriminating part in their service as food for larvae. Among these differences size is undoubtedly important. All the flagellates used in the experiments lie below the limit of size at which ingestion is possible, but the six organisms cover a range of 1·5–7 μ and the question arises, which size of organism provides the best source of food material for the larvae. Equal numbers of organisms of different size do not constitute equivalent bulk, and,

on general principles the larger organisms, provided they can be ingested, should supply the better food material for the larvae; but the matter is not quite so simple as that, since the flagellates differ from one another in motility. Under the microscope, they are distinguishable from one another by character as well as by rate of movement, and the ease with which they can be taken in by the larvae depends upon the effective sweeping action of the ciliary feeding mechanism relative to the bulk and movement of the food organism. It is probable, but this is a point for future investigation, that the organisms which are most readily taken in are not those of maximum ingestible size.

The greatest difference which these organisms show among themselves is that of colour. From the point of view of the larvae this is probably of major importance. Flagellates "B", "C" and "I" are golden brown, but "I" is clearly distinguishable from the other two by a quality which is hard to define. Not necessarily deeper in tint, it has a "shining" quality that the others lack. "D" is very definitely pinkish red; its colour is very deep and unmistakable in cultures of advanced development, but it forms a somewhat turbid fluid giving the appearance of a "shot" effect of grey and red. "H" is a green with a strong yellow cast, and "F" is yellow with a tinge of green.

Amongst algae in general a correlation between the colouring matter of the chromoplast and the nature of the food material stored has been clearly established. Among smaller forms both characters play their part in the problem of classification. It appears probable that the varying usefulness exhibited by these differently coloured organisms, depends directly upon the degree to which the included algal food reserves, glycogen, oil, etc. serve the immediate needs of the developing larvae. In support of this view it may be mentioned that certain preliminary histological enquiry into food reserves of the larvae themselves, demonstrated the presence of oil globules in all stages of normal larvae from the white-sick stage to the well-developed "eyed" stage. The globules were confined mainly to the epithelial cells of the alimentary tract but they do also occur in other tissues. Similar tests for glycogen gave negative results. The desirability of further research in this field is clearly indicated.

SUMMARY

The work described in this paper is a contribution to the general problems of rearing the larvae, and growing the spat of the European flat oyster, *Ostrea edulis* L.

The specific aspect of the problem dealt with in detail is the place taken by minute motile organisms (naked dinoflagellates and other unicellular algae) in the food requirements of the developing veliger larvae.

Six such flagellate organisms, ranging in size from 1·5 to 7 μ in diameter, have been isolated from sea water, and are maintained in pure culture, under conditions described. Pending specific identification, these are denoted by

letter-labels only. These organisms have been used, over a period of years, in feeding experiments with oyster larvae.

The experimental work has taken place on a laboratory scale, on which scale pure culture technique and rigorous control of conditions are alone possible.

An essential preliminary to valid feeding experiments has been the stabilization of the biophysical environment (including water conditions) at an optimum level. This has involved the construction of a culture-house, with full experimental control, the details of which are described. The use of uncontaminated sea water, collected well off-shore, is strongly advocated for small-scale experiments of this character. A temperature range of 20°–22° C., an avoidance of considerable or sudden *p*H change, screening from direct sunlight, plunger stirring and/or aeration by bubbling and slow water change, are all contributory factors on the physical side.

Given good environmental conditions, much yet depends on the inherent viability of the liberated larvae. Larvae liberated in small vessels at temperatures as low as 13°–15° C., have proved to be quite as viable as those produced at 20°–21° C., in large or semi-natural enclosures at Port Erin and elsewhere.

Feeding experiments, extending in all over five seasons, have demonstrated the special suitability of several of the flagellates, held in pure culture, and given either separately or mixed, to serve as food capable of maintaining the growth of larval oysters from liberation stage up to and including settlement. The most successful of these (denoted "H" and "I") are of a greenish yellow or golden brown colour, and measure 3–6 μ in diameter.

In the most successful experiments, the number of larvae carried through from liberation to settlement, under appropriate physical conditions, and with a diet of the organisms "H" and "I", or both together, exceeds 90 %, in one case 99 %, of the number introduced into the experimental vessel, and indicates the measure of success on the experimental scale.

An appreciation of the major physical factors, and of the feeding problems associated with larval development on the laboratory scale has now been attained. The inherent quality of "viability" and certain responses as yet obscure still demand investigation. When this is done, a sound experimental basis should be available for an attack on the ultimate problem of the consistent production of oyster larvae and spat on a commercial scale.

REFERENCES

BRUCE, J. R., 1935. *48th Rep. Mar. Biol. Sta., Port Erin.*

COLE, H. A., 1937. Experiments on the Breeding of Oysters (*Ostrea edulis*) in Tanks, with special reference to the food of the Larvae and Spat. *Min. Agric. and Fish., Fishery Invest.*, Ser. II, Vol. XV, No. 4 (1936).

—— 1938. A System of Oyster Culture. *Journ. Cons. Int. Explor. Mer*, Vol. XIII, pp. 221–38.

ERDMANN, W., 1933. Aufzucht von Austernlarven. *Der Fischmarkt*, N.F. Bd. 1, pp. 340–1.

FRITSCH, F. E., 1935. *The Structure and Reproduction of the Algae*, Vol. 1, Cambridge.

GAARDER, T. & BJERKAN, P., 1934. *Østers og Østerkultur i Norge*. Bergen.

KÄNDLER, R., 1930. Die Kultur der Auster. *Handb. der Biol. Arbeitsmethoden* (*Abderhalden*), Abt. IX, Teil 5, Heft 5.

ORTON, J. H., 1937. *Oyster Biology and Oyster Culture* (Buckland Lectures for 1935). London.

ORTON, J. H., PARKE, M. W. & SMITH, W. C., 1933. Breeding of Oysters (*O. edulis*) at Port Erin. *Nature*, Vol. CXXXI, p. 26.